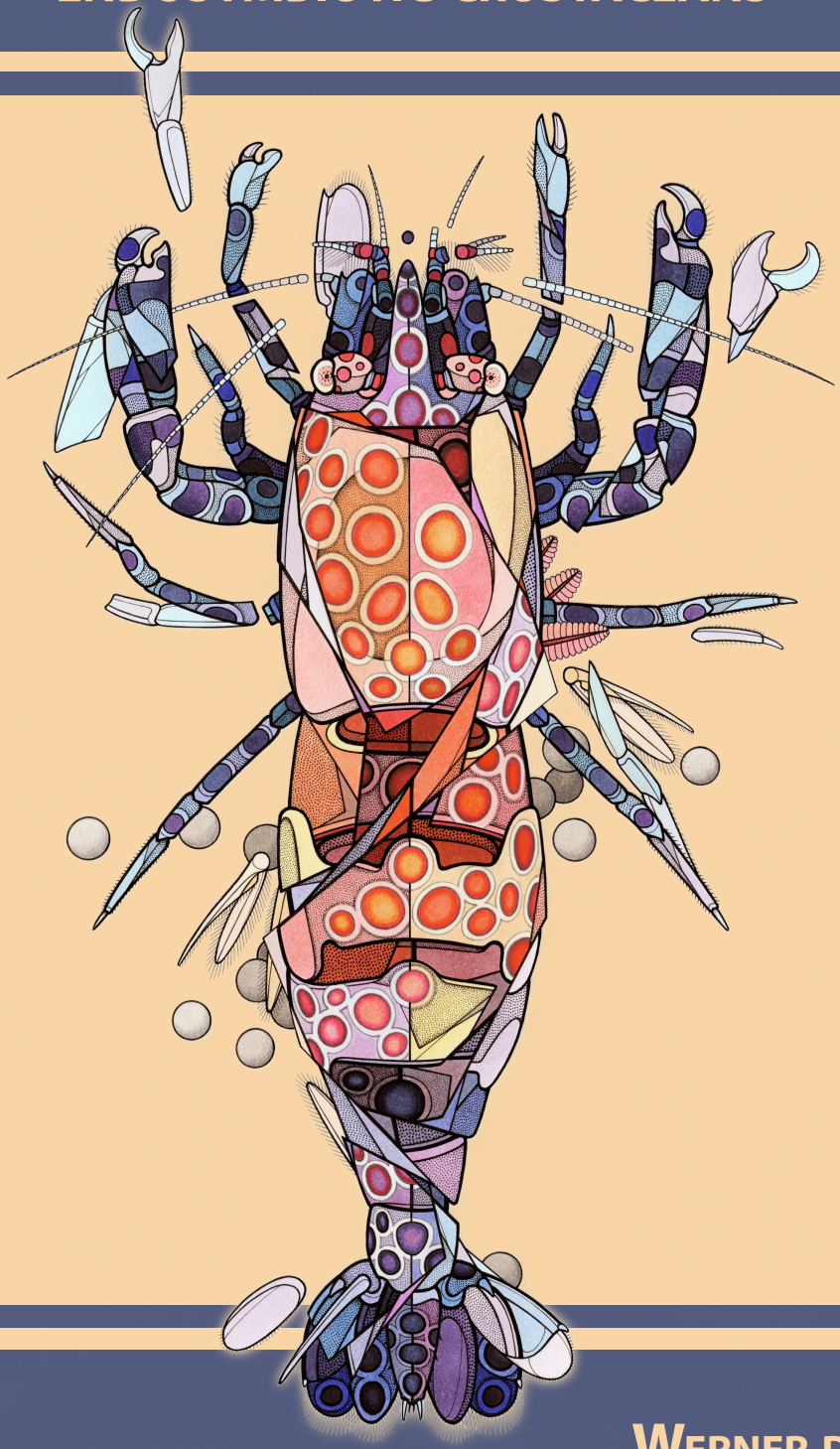
Ecomorphological aspects in the evolution of endosymbiotic crustaceans



Werner de Gier

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2024

PhD Thesis, Rijksuniversiteit Groningen, The Netherlands

Front cover image:

Artist rendition of the bivalve-associated symbiotic shrimp *Neoanchistus cardiodytes* Bruce, 1975. Artist: “Teuthis”, commissioned for this thesis.
@_Teuthis_, Le Havre, France.

Layout by Werner de Gier, with the help of Jeroen Hubert. Chapter title illustrations made by Werner de Gier, with vectors from Phylopic.

Printed by: Ridderprint, Ridderkerk.
Deposited in the online repository of the Rijksuniversiteit Groningen library.

This PhD project was made possible with the help of the former Taxonomy and Systematics (& Geodiversity), and the Plankton Diversity / Marine Evolution and Ecology research groups at Naturalis Biodiversity Center, Leiden, The Netherlands.

Taxonomic statement:

The scientific names for the taxa mentioned in this thesis follow the valid names listed in the World Register of Marine Organisms (WoRMS), at the time of writing the parts of this thesis (2019-2023).

rijksuniversiteit groningen

Ecomorphological aspects in the evolution of endosymbiotic crustaceans

Proefschrift

ter verkrijging van de graad van doctor aan de
Rijksuniversiteit Groningen
op gezag van de
rector magnificus prof. dr. ir. J.M.A. Scherpen
en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op

Dinsdag 19 maart 2024 om 14:30 uur

door

Werner de Gier

geboren op 3 maart 1995

Promotor

Prof. Dr. Bert W. Hoeksema	Rijksuniversiteit Groningen & Naturalis Biodiversity Center

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To those who came before me and whom I never got to meet

Lipke Bijdeley Holthuis (1921 – 2008)

Alexander James “Sandy” Bruce (1929 – 2022)

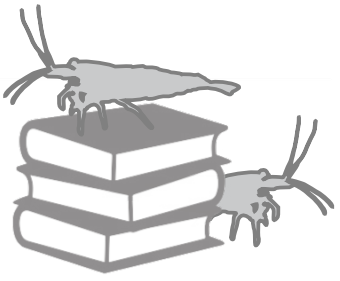
Holthuis (left) and Bruce (right), at the front entrance of the former building of the Rijksmuseum voor Natuurlijke Historie (now Naturalis Biodiversity Center), at Raamsteeg, Leiden, the Netherlands. Picture from 1982, taken by Helmut Debelius (now in the Naturalis archives).

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1

General introduction



From parasites to roommates

In ecosystems, biological communities interact with their physical environment, whereas co-existing organisms in these communities interact with each other. While most interactions between organisms are short-lasting (e.g., predation, competition), long-lasting interactions and collaborations can be evolved over long periods of time and selection pressures. This persistent association between two (or more) organisms is defined using the broad term 'symbiosis', from the Greek συμ (sym) for 'together' and βίωσις (biosis) meaning 'living'. Symbioses evolved in many different ways and along various evolutionary branches of life. In general, symbiosis involves a larger 'host', and a smaller 'symbiont' (Oliver & Russell, 2016). Different types of symbioses have been classified based on the nature of the association, and the effects the interaction has on the host's fitness (Martin & Schwab, 2012). The most elementary classification features three main types of interactions: 'mutualism', in which both the host and the symbiont gain an increase in overall fitness; 'commensalism', in which the host is not positively or negatively affected, and only the symbiont benefits; and 'parasitism', in which the symbiont (here called 'parasite') is negatively affecting the host for its own benefit (Martin & Schwab, 2012). Within the third category, some species keep the host alive (e.g., lice, fleas, and most other blood-sucking parasites), while others kill the host, in which the symbionts are called 'parasitoids'. In some rare cases the symbiont does not benefit from the interaction, which is the case for 'amensalism', in which the host's fitness decreases, while the symbiont is not positively or negatively affected (Veiga, 2016). Alternatively, in 'synnecrosis', both parties die at the end of their interaction (Veiga, 2016).

Symbioses can further be classified based on the symbiont's dependence on the interaction, and on the symbiont's position relative to the host. If the symbiont can leave and survive without the host, the symbiosis may be classified as 'facultative'. In long-lasting 'obligatory' symbioses, the symbiont and the host both need to survive for long periods of time (Nguyen & van Baalen, 2020). When the symbiont is attached to the host outer surface or moving over it, it is usually regarded an 'ectosymbiont'. In contrast, if the symbiont infects the inside of the host's body, it is considered an 'endosymbiont' (Wood-Charlson, 2013). The term 'endosymbiosis' has been used in various contexts, making a proper classification difficult. Some examples of definitions are: micro-organisms moving inside large cells as part of the 'endosymbiosis theory' (Wood-Charlson, 2013); symbionts living inside the tissue of the host (e.g., in the case of zooxanthellate-coral interactions; Wood-Charlson, 2013); or larger-sized symbionts living inside the organs of the host (but still being part of the external system; as in Baeza, 2015). The position of a symbiont can also be

next to its host, in which the host's burrow or 'home' is utilised by the symbiont. In this case, the symbiosis is called 'inquilism' (e.g., Frolová et al., 2022).

Symbiotic crustaceans

Symbiosis, in all forms, has evolved various times in the evolution of invertebrate animals. One phylum in particular, the Arthropoda (such as insects, crustaceans, arachnids, and myriapods) has evolved symbiosis in many different ways, arguably most extremely in the clade of the crustaceans. Crustaceans include the decapods, some of which can be found on our plate in seafood restaurants (e.g., shrimps, crabs, and lobsters), as well as lesser-known, sometimes very inconspicuous animals (e.g., barnacles, isopods, amphipods, krill, ostracods, and copepods; WoRMS, 2023). Symbiotic crustaceans are found in many of these groups; the only taxonomic groups without symbiotic representatives are the (marine cave-dwelling) remipedes (Remipedia) and horseshoe shrimps (Cephalocarida) (Baeza, 2015). Baeza (2015) presented an overview of all known crustacean symbiotic lineages, sometimes limited to some closely related species and in other cases entire families. Describing a full overview is not feasible for this introduction, so a brief summary of the diversity of symbiotic crustaceans, including a few examples, is given below.

Within the speciose group of the Peracarida, various lineages of amphipods ('sand fleas', or 'scuds') and isopods ('pill bugs') have adapted to a life on the inside or outside of a host. For example, in amphipods, hyperiids have evolved to live inside jellyfish (fig. 1.1A), and cyamids ('whale lice') live and breed parasitically on the outer surface of whales, sometimes covering large portions of the host's skin (Baeza, 2015). Leucothoid amphipods live commensally with a wide range of invertebrate hosts in coral reefs, occasionally even in symbiosis with larger crustaceans (Vader & Tandberg, 2015). Various families of isopods (most notably the Cymothoidae) bury themselves in the skin of fishes, in extreme cases replacing the host's tongue (Martin et al., 2022). Other hosts are utilised by the Bopyridae, which live parasitically in the abdominal and branchial chambers of larger crustaceans (Williams & Boyko, 2012). In contrast, some isopods only use their host commensally: members of Sphaeromatidae live in the body chambers of sponges, displaying intricate mating systems and other social behaviour (Shuster & Wade, 1991). In addition, members of the Santiidae are known to cultivate brightly-coloured cyanobacteria on their dorsal surface (fig. 1.1B), from which they feed (Linquist et al., 2005). This association also acts as a repulsion of potential predators, which possibly also benefits their sponge hosts (Naumann, 2017).

Within the Maxillopoda (barnacles, copepods, tongue worms, and fish lice),



1.1 Symbiotic representatives representing several crustacean taxa. A) *Hyperia galba* (Montagu, 1813), dislodged from the sea gooseberry, *Pleurobrachia pileus* (O. F. Müller, 1776); B) *Santia* sp. isopods, found on an encrusting sponge; C) *Sacculina carcini* Thompson, 1836, infecting the shore crab, *Carcinus maenas* (L., 1758); D) the coral-dwelling barnacle *Nobia grandis* Sowerby, 1839 in the scleractinian *Euphyllia glabrescens* (Chamisso & Eysenhardt, 1821). E) *Allogalathea elegans* (Adams & White, 1848), on an unidentified crinoid; F) *Trapezia rufopuncata* (Herbst, 1799), on a scleractinian coral host; G) Pistol shrimp (possibly *Alpheus thompsoni* Anker, 2022) and the shrimp goby *Amblyeleotris guttata* (Fowler, 1938) sharing a burrow; H) *Latreutes fucorum* (Fabricius, 1798), a hippolytid shrimp on a piece of *Sargassum* seaweed. Photo credits: A, C) Werner de Gier; B, D) Charles Fransen; E) Tanaka Juuyoh; F) Willem Kolvoort; G) Klaus Stiefel; H) Seabird McKeon.

symbiosis is the rule rather than the exception. Various barnacles (cirripedes) attach themselves to whales, turtles, corals (fig. 1.1D), or even other crustaceans (Chan et al., 2021). In one particular barnacle lineage (Rhizocephala; e.g., *Sacculina*, fig. 1.1C) the symbiont internally infects the decapod host as a planktonic stage, and takes over the entire body. The parasite mainly affects the host's reproductive system, rendering it infertile and even altering its behaviour when the parasite's eggs are ready to hatch (Chan et al., 2021). Tongue worms (pentastomids) have a worm-like body shape, adapted for an endoparasitic life, and infect the respiratory tracts of reptiles, birds, and mammals (including humans) (Christoffersen & De Assis, 2013). In contrast, fish lice (branchiurans) can only be found on the outer body walls of freshwater fish and amphibians, sucking blood from their host, and therefore have a streamlined body shape. Arguably the best adapted group of symbionts are parasitic copepods, where the females of various species have evolved into egg-producing shapeless entities (Bernot et al., 2021). These groups attach themselves to the soft surfaces of their host fish (often the eyeballs and gills), or invade the internal systems of invertebrate hosts, such as starfish and bivalves (Saito et al., 2020). There are even species that make galls inside hard-surfaced host echinoderms (Baeza, 2015).

Within the group with the biggest representatives, the decapods have evolved symbiosis in various ways and in many instances. The different lineages of lobsters and lobster-like crustaceans (e.g., true lobsters, crayfish, ghost shrimp, mud lobsters, and spiny and slipper lobsters) have no role as symbionts, but they are host for a wide range of symbionts, such as inquiline symbionts living in the burrows of ghost shrimp and mud lobsters, cycliophorans infecting mouthparts of true lobsters, and branchiobdellid worms infecting the outer surface of crayfish (MacGinitie, 1939; Brown et al., 2002; Baker & Giribet, 2007).

In addition to their role of a host, most other decapod groups have evolved other types of symbioses, most commonly as symbionts in coral reef ecosystems. For instance, porcelain crabs and various types of squat lobsters (part of the Anomura, or 'false crabs') have commensal, protective relationships with soft and hard corals, and in some cases even with echinoderms (Cabezas et al., 2011). Here, they use camouflage and are well-protected from any predator (fig. 1.1E). In addition, some hermit crabs (also anomurans) live mutualistically with self-collected anemones, and even carry them from the old to the new shells they inhabit. There are also coral-associated hermit crabs, living in old worm tubes which are wedged in coral tissue (e.g., Hoeksema et al., 2019).

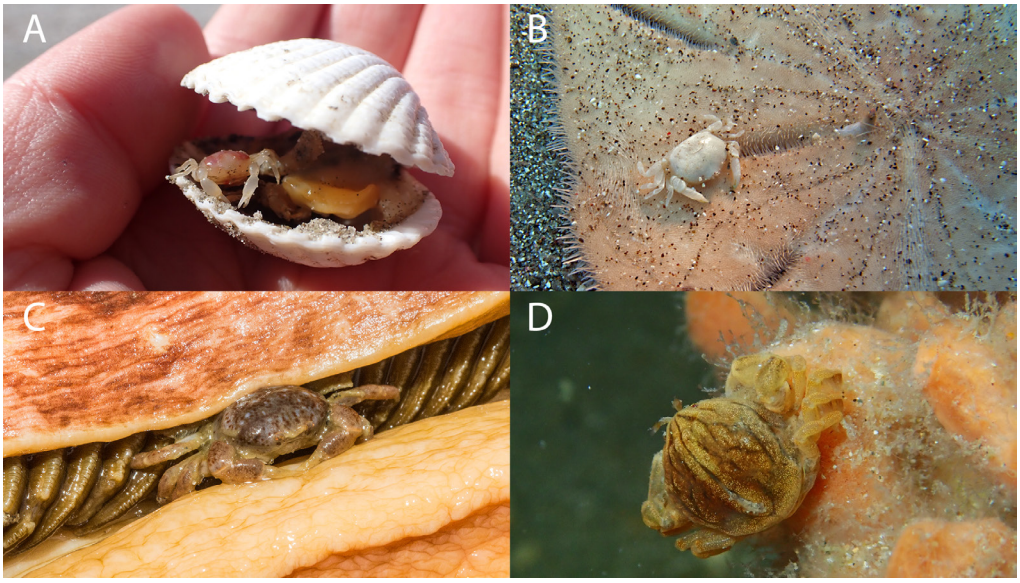
True crabs (Brachyura) have evolved many instances of symbiosis: members

of the superfamily Trapezioidea may protect their scleractinian hosts from predators like parrot fish, showcasing a true mutualistic relationship (fig. 1.1F), while various representatives of the subfamily Eumedoninae (part of the Pilumnidae) are notorious parasites of echinoderm hosts (Castro, 2015). In addition, cryptically coloured swimming- and spider crab species live commensally on various species of cnidarian hosts and sea cucumbers, in some cases even using host tissue as camouflage as part of carrying behaviour (Guinot & Wicksten, 2015). Most extreme lifestyle adaptations can be found among gall crabs (family Cryptochiridae), in which females infect host corals, and never leave their host afterwards (Van der Meij, 2015). A similar lifestyle can be found in the species-rich pea crab family (Pinnotheridae), which will be discussed in the next section.

Lastly, various groups of shrimps live with, or in a host. For example, commensal boxer shrimps (Stenopodidea: Spongicolidae and Stenopodidae) sometimes get stuck in their deep-water glass-sponge after the sponge opening closes due to growing host tissue (Baeza, 2015). Pistol shrimps have also evolved intricate collaborations with their hosts: some live in burrows together with gobies, where they depend on the hunting abilities and the surveillance of the host goby, while in return they clean and maintain the burrow (fig. 1.1G; Baeza, 2015). The only eusocial systems in crustaceans can also be found in this group: *Synalpheus* species live in large colonies in sponges, protecting their 'queen' (Duffy et al., 2000). Various members of the other lineages within Caridea (e.g., families Gnathophyllidae, Hippolytidae, Lysmatidae, Thoridae, and Pandalidae) showcase a wide variety of hosts, usually living commensally with invertebrate hosts, such as corals, echinoderms, and sponges, seaweeds (fig. 1.1H), or even as cleaners in association with fish (e.g., the genus *Lysmata*; Baeza, 2015). Members of the most speciose symbiotic family, the Palaemonidae, utilise hosts in a similar way. The diversity of this family will be discussed in a later section.

Pea crabs – a brief overview

Pinnotheridae, the 'pea crab family', currently features around 300 recognised species, sorted in three subfamilies: Pinnixinae, Pinnixulalinae, and Pinnotherinae (WoRMS, 2023). Recently, the families Tetriasidae and Parapinnixidae were split from this group (Tsang & Naruse, 2023), leaving the Pinnotheridae to be almost exclusively made up of symbiotic species. With the exception of a few species, the Pinnixinae and Pinnixulalinae inhabit the burrows and tubes as inquilines of various taxa of worms, mud lobsters, and ghost shrimps (Palacios Theil et al., 2016; Palacios Theil & Felder, 2020). The Pinnotherinae, however, utilise a wide range of different invertebrate hosts, predominantly consisting of



1.2 Various pinnotherine pea crab representatives. A) *Pinnotheres pisum* (L., 1767), inside a common cockle, *Cerastroderma edule* (L., 1758); B) *Dissodactylus mellitae* (Rathbun, 1900), on an unidentified sand dollar; C) *Opisthopus transversus* Rathbun, 1894, living between the mantle folds of a gumboot chiton (*Cryptochiton stelleri* Von Middendorff, 1847); D) *Austrotheres pregenzeri* Ahyong, 2018, presumably outside of its ascidian host. Photo credits: A) Werner de Gier; B) Marco Faasse, from Hoeksema (2015); C) Marlin Harms, also used in Chapter 2; D) Nick Shaw.

endosymbiotic bivalves (fig. 1.2A; Schmitt et al., 1973). Other endosymbiotic species inhabit the internal organs of sea cucumbers and sea urchins, or live between the soft internal folds of gastropods and ascidians (also called sea squirts, or tunicates). Some species also inhabit the shells of brachiopods, or the lateral mantle folds of chitons (fig. 1.2C; Chapter 2). One lineage consisting of about 13 species of the so-called '*Dissodactylus* complex' have a unique ectosymbiotic association with irregular sea urchins and sand dollars occurring on both sides of the Panama Isthmus. These species are known as parasites, feeding from the hosts' spines, while hopping from one host to another (e.g., Telford, 1982; Jossart et al., 2014). Species within the '*Dissodactylus* complex' also exhibit almost no sexual dimorphism and have a hard, flattened carapace (fig. 1.2B). In contrast, the other members of the Pinnotherinae showcase extreme sexual dimorphism, where the females are almost unable to move due to their inflated abdomen, which is used to carry eggs. Interestingly, this feature can also be found in bivalve-associated species from one of the other subfamilies, the Pinnixinae (e.g., some *Scleroplax* species; Zmarzly, 1992). The female 'post-hard' stages of pinnotherines are usually soft, while the males have smaller, harder bodies, and often hop between hosts to look for mates. Most pea-crab species are thought to be parasites, but only a few studies have

proven that these symbionts cause damage to the host or inhibit the host's growth (Jones & Mahadevan, 1965; Kruczyski, 1972; Bierbaum & Ferson, 1986).

Most published (pinnotherine) pea-crab studies resolve around the taxonomy and systematic placements of new species or species complexes. Historically, the first pea crabs were described by Linnaeus from European waters. i.e., the common *Pinnotheres pisum* and *Nepinnotheres pinnotheres*, after which other taxonomists described additional new species and revised the known genera. Well-known carcinologists, most notably Otto Bürger (1865–1945), Guiseppe Nobili (1877–1908), Mary Rathbun (1860–1943), Raymond Manning (1934–2000), and Tune Sakai (1903–1986) have done their fair share of pea crab taxonomy, and left us with valuable collection materials and ecological notes accompanying their descriptions, illustrations, and photographs (Bürger, 1895; Nobili, 1906; Rathbun, 1918; Sakai, 1939; Manning, 1993). More recently, using new insights on the host associations, larval stages, genetic codes, and mating systems of enigmatic species, experts have been doing much more than revising the existing genera. Teams led by Shane Ahyong and Peter Ng (Ng & Ahyong, 2022), and Emma Palacios Theil and Darryl Felder (Palacios Theil et al., 2016) made steps to build a naturally sound classification based on new morphological insights and genetic markers. Moreover, experiments have been performed by teams all over the world to clarify the behaviour and ecology of pea crabs (Ocampo et al., 2017; Souza et al., 2018; Jossart et al., 2020). These developments add to the idea that pea crabs can be feasible model organisms for evolutionary studies on symbiosis. Recent studies (Hultgren et al., 2022; Chow et al., 2023), as well as parts of this thesis, are dedicated to a rather new field of pea crab research: ecomorphology. In order to find out how endosymbiotic pea crabs have adapted to a life within their host, first an overview of all possibly adaptive characters was needed, after which statistical and phylogenetic tests can help us understand on how selection pressures affect the symbiont's body.

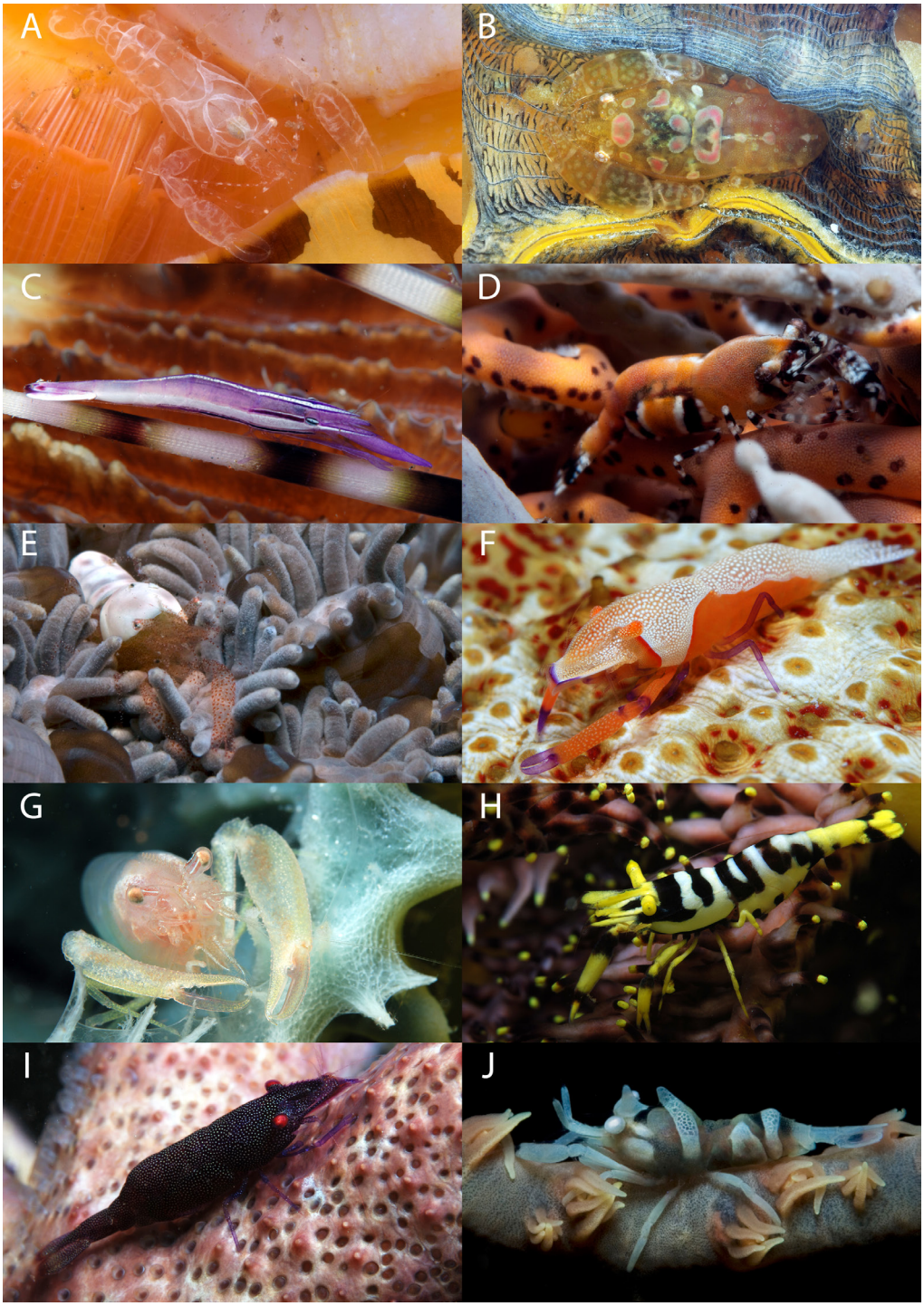
Commensal shrimp

Palaemonidae is a species-rich shrimp family: including around 1150 species in 160 genera (WoRMS, 2023). Historically, the family was split up in two subfamilies, Palaemoninae and Pontoniinae. Palaemoninae included mostly freshwater and economically important species of the genus *Macrobrachium*, and fairly common *Palaemon* species from brackish and saltwater habitats. The Pontoniinae, however, were made up of almost exclusively much-smaller marine endo- and ectosymbionts (De Grave & Fransen, 2011). Now, estimates

of the fraction of symbiotic species among the marine Palaemonidae reaches up to 70% (Chow et al., 2022), with additional species being discovered every year (Fransen et al., 2022; Neves, 2022; Komai et al., 2023). This group is now colloquially called the group of the 'commensal shrimps'. The types of symbioses within this shrimp family range from ectosymbioses with hard and soft corals, hydroids, sponges, sea stars, sea cucumbers, sea urchins, ophiuroids, crinoids, and nudibranch gastropods (fig. 1.3; Horká et al., 2016). Some associates of scleractinians even induce the forming of galls inside their host (Van der Meij et al., 2023). In contrast, some lineages have evolved an endosymbiotic relationship with various types of bivalves and ascidians, living within the folds of the mantle or branchial basket (fig. 1.3A, B), or even as inquilines in the burrows of spoon worms (Echiura), fish, and pistol shrimps (Fransen, 1994; Frolová et al., 2022). Similar to various lineages within the Lysmatidae, some groups of palaemonid shrimp are known as mutualistic fish cleaners, dancing as signal to their 'host' fish as invitation for approach (Horká et al., 2018).

The description of the first species of symbiotic Palaemonidae kickstarted a very broad field of science, starting around the time of Rumphius (1705) who reported on a few species living inside bivalve molluscs (Bruce, 1995). In the late 18th century, the Swedish naturalist Peter Forskål (1732–1763) described the first species of commensal shrimps (e.g., *Cancer custos*, presently known as *Ensiger custos*; Forskål, 1775). European authors at the start of the 19th century described 'exotic' species which were sampled from a wide range of different hosts during far-away travels. For example, Otton Paulson (1837–1886) from Ukraine published about Red Sea shrimps; Wilhelm Peters (1815–1883), from Prussia described a few species of tropical endosymbionts; and Wilhelm de Haan (1801–¬1855) from the Netherlands, published in the Fauna Japonica about crustaceans collected by Von Siebold (Paulson, 1875; Peters, 1852; de Haan, 1844).

In the late 19th century and the early 20th century, various carcinologists from different parts of the world published beautifully illustrated standard works, which are still in use by scientists to this date. In Europe, by the German Heinrich Balss (1886–1957), the Italian Guiseppe Nobili (1877–1908), and the Dutchman Johannes de Man (1850–1930); in India, the Irish scientist Stanley Kemp (1882–1945); in the United States by Lancelot Borradaille (1872–1945); and in Japan by Itsuo Kubo (1909–1968). More recently, Fenner Chace (1908–2004) in the USA and Lipke Holthuis (1921–2008) in the Netherlands have left their mark on the systematics of symbiotic crustaceans, using collection materials from their home range, or elaborate marine travels like the Albatross,



1.3 Various representatives of symbiotic palaemonid shrimps. A) *Pontonia manningi* Fransen, 2000, in the spiny oyster *Spondylus americanus* Hermann, 1781; B) *Odontonia katoi* (Kubo, 1940), in the ascidian *Polycarpa aurata* (Quoy & Gaimard, 1834); C) *Stegopontonia*

(1.3 *Cont.*) *commensalis* Nobili, 1906 on the sea urchin *Echinothrix calamaris* (Pallas, 1774); D) *Periclimenes perryae* Chace, 1942, on the basket star *Astrophyton muricatum* (Lamarck, 1816); E) *Hamopontonia corallicola* Bruce, 1970, on the scleractinian coral *Goniopora stokesi* Milne Edwards & Haime, 1851; F) *Zenopontonia rex* (Kemp, 1922), on the sea cucumber *Thelenota anax* Clark, 1921; G) *Thaumastocaris streptopus* Kemp, 1922, on the sponge *Callyspongia* sp.; H) *Laomenes* sp., on an unidentified crinoid; I) *Zenopontonia soror* (Nobili, 1904), on the sea star *Echinaster luzonicus* (Gray, 1840); J) *Dasycaris zanzibarica* Bruce, 1973, on the sea whip *Stichopathes* sp. Photo credits: Charles Fransen.

Siboga, and Snellius expeditions (Holthuis, 1950; Chace & Bruce, 1993). As seen above, the centre of symbiotic-shrimp taxonomy is traditionally located in the natural history museum at Leiden (Naturalis Biodiversity Center), partly due to the earlier work by De Haan, De Man, and Holthuis. The current symbiotic crustacean expert in Leiden, Charles Fransen, collaborates with other experts from all over the world, and has published extensively on the evolution and systematics of palaemonid shrimps. These foreign experts are, among others, Junji Okuno and Tomoyuki Komai from Japan; Sammy De Grave from the United Kingdom, Ivan Marin from Russia, Xinzheng Li from China, and Zdeněk Ďuriš from the Czech Republic. Alexander 'Sandy' Bruce, from Australia, sadly passed away after a life-long work on the symbiotic shrimps of East Africa, the Indo-West Pacific, and Australia.

Now, with the inclusion of more observation data from the field and the lab, as well as integrative methods in taxonomy such as Scanning Electron Microscopy (SEM) and DNA-barcoding, more studies could have been done on the ecology, anatomy, and the phylogenetic placements of the symbiotic Palaemonidae (Dobson et al., 2014; Ashelby, 2015; Horká et al., 2016). It was argued that symbiotic shrimp could play an important role as model organisms for symbiosis in evolutionary sciences (Baeza et al., 2015). Moreover, some studies also focused on the functional morphology of certain anatomical features, and linked this to convergent evolution within the clades. For instance, the pistol-claw, characteristic for pistol shrimps, evolved multiple times in the Palaemonidae as well (e.g., in *Coralliocaris*, *Harpiliopsis*, and *Periclimenaeus*), possibly even with the same function (Kaji et al., 2018). Convergence was also studied by Chow et al. (2021), who tested for similar dactylus and mandible shapes across the fully known phylogenetic tree of symbiotic palaemonids, with a special interest in the various host associations. In this thesis, this study on convergence is expanded by focusing on two separate clades of endosymbionts living in bivalve molluscs and ascidians that were defined by Horká et al. (2016) and Chow et al. (2021). To do this, the number of species included in the available phylogeny reconstructions was increased, with a special focus on these two clades. The first clade includes *Ascidonia*, *Conchodytes*, *Dactylonia*, *Odontonia*,

and *Pontonia*, as well as a few smaller (sometimes monotypic) genera, from now on referred to as 'the *Conchodytes* clade', and the other clade includes *Anchistus* (s.l.), *Paranchistus* (s.l.), *Neoanchistus*, and *Dasella*, and how also supplemented by three newly (re)erected genera, *Ensiger*, *Polkamenes*, and *Tympanicheles*. The latter clade is from now on referred to as 'the *Anchistus* clade'. Why, and how these clades were used to study convergent evolution of the adaptive, functional morphology, is discussed below.

Ecomorphological adaptations to an endosymbiotic life

Symbiotic crustaceans utilise a wide range of different hosts. Some of the crab and shrimp species mentioned above are generalists, housed by multiple different species, genera, families, or even phyla. While true specialists, also found in the two above mentioned clades, are hosted by only one, or a few closely related species. These specific habitats would expectedly have their own specific selection pressures, adapting the symbiont to a life inside the host or on its surface. When comparing endosymbiotic clades with their ectosymbiotic, or even free-living relatives, it can be seen that selection pressures seem to have affected mostly species with an endosymbiotic life (Fransen, 1994). Both the endosymbiotic pea crabs and palaemonid shrimps evolved so-thought specific 'ecomorphological' adaptations. For the shrimp species in the '*Anchistus*' and '*Conchodytes*' clade, these adaptations include (after Chapter 4): reduced frontal, dorsal and lateral protrusions for easier movement within the host (e.g., shortening of the rostrum, reduction of the number of rostral teeth, absence or reduction of the antennal and hepatic spines); (2) development of scales, (hooked) teeth, and microsetae on the dactyls of ambulatory pereiopods to increase grip; (3) adaptive (often cryptic) colouration; (4) a roughly cylindrical body shape with a body size that is adapted to the size of the body cavity of the host; and (5) differences in eye morphology (Bruce, 1976, 1995; Fransen, 1994; Dobson et al., 2014). Among pinnotherine pea crabs some of these adaptations can also be observed, to some degree: most endosymbiotic species have a soft, roundly shaped carapace, without any ornamentations (with the exception of a few species, e.g. in the genera *Tridacnatheres*, *Xanthasia*, *Durckheimia*, *Serenotheres*, and *Austrotheres*; fig. 1.2D); most species of the genus *Arcotheres* seem to have microstructures on the distal ends of their last walking leg dactyli (Ng & Ahyong, 2022); and most species have under-developed eyes, with the exception of a few ectosymbiotic members of the clade (Chapter 2). In both the shrimps, as well as the pea crabs, female specimens often also exhibit an extremely inflated abdomen, used to carry larger eggs than their ectosymbiotic relatives (Fransen, 1994; 2002). A few of these adaptations can also be seen in hyper-specialised endoparasitic isopods, copepods, and tongue worms, as well as other endosymbionts, such

as echinoid-dwelling eumedonine crabs (e.g., *Echinoecus*), and gall-forming species from the shrimp genus *Paratypton*, and the crab family Cryptochiridae (Baeza, 2015).

The adaptations mentioned above are present in the two endosymbiotic shrimp clades, but not all to the same degree, and the same is probably true for the pea crabs. There is much variation between and within the studied clades. For instance, the microstructures on the walking legs of some of the bivalve-associated *Conchodytes* species do not resemble the walking legs of the ascidian-associated, but closely related, genus *Odontonia*. However, the microstructures of certain members of *Conchodytes* and *Pontonia* do match quite well (studied in Chapter 7). Some of the groups studied in this thesis have developed complex, unique features. For instance, in the genera *Anchistus*, *Neoanchistus*, and *Tympanicheles*, the major claws bear a drum-like organ, which is not yet found in any other genus within the family Palaemonidae (Chapter 5).

These sometimes minute, sometimes huge differences between morphologies, and the evolution of unique features, are possibly all related to the nature of the species' symbiosis. The host specificity of the symbiont, physical attributes of the host, and their ecological abundance might all play a role in the evolution of the endosymbiotic clades. Experts have even found that endosymbionts appear to colonise new host groups (even entire phyla) at a faster rate, and more frequently, than their ectosymbiotic parallel lineages (Horká et al., 2016). How these species do that, is explained below.

Host switching in an evolutionary history

The initial colonization of a host, and therefore the start of a new lineage with a symbiotic life style, frequently took place in crustacean evolution. With respect to these host-switching events in their evolutionary history, the symbiotic Palaemonidae is arguably the best studied non-parasitic clade (Kou et al., 2015; Horká et al., 2016; Chow et al., 2021), with some studies done on the similar host-switching evolutionary histories of zoantharian cnidarians (Kise et al., 2023), and symbiotic bivalves (Goto et al., 2012). Furthermore, host-switching events have, together with coadaptation and -speciation, been argued to be the main drivers of diversification within the Palaemonidae (Horká et al., 2016). As Horká et al. (2016) mention, in general, host switching events happen between closely related host species (e.g., the same genus, or family), and host switches between distantly related taxa are infrequent. This is also the case for the symbiotic Palaemonidae, among which most host switches have happened between host species within the same phylum. Most between-phylum

host switches have happened in ectosymbiotic lineages, possibly because of the similarity in microhabitats (e.g., ectosymbiosis between cnidarian and echinoderm hosts; Horká et al., 2016; Chow et al., 2021). In addition, some ectosymbiotic species went back to a free-living state or a facultative symbiosis (Chow et al., 2021; Frolová et al., 2022).

Host switching events between similar hosts seem obvious, since morphological, behavioural and physiological specializations allow a symbiont to efficiently exploit a single host, but this makes it difficult to exploit new, phylogenetically distant hosts (Horká et al., 2016). Some lineages, however, made the step to colonise an extremely different microhabitat from their presumed ancestral state. These clades shifted from ectosymbiosis towards an endosymbiotic lifestyle, and this is thought to have happened at least four times in the evolution of symbiotic Palaemonidae: (1) in the '*Conchodytes* clade', and studied in this thesis (Horká et al., 2016: Clade 6), in which the coral-associated gall-shrimp *Paratypton* may also be included (Van der Meij et al., 2023); (2) in the '*Anchistus* clade' studied in this thesis (Horká et al., 2016: part of Clade 5); (3) in the clade including the genera *Anisomenaeus*, *Nippontonia*, *Orthopontonia*, *Onycocaris*, *Periclimenaeus*, *Thaumastocaris*, and *Typton* (and possibly more genera; Horká et al. 2016: part of Clade 5); and (4) in the clade (defined by Odijk & Fransen, 2017) including the genera *Apopontonia*, *Climeniperaeus*, *Paraclimenaeus*, and *Unesconia* (unpublished data). The latter two clades are hosted by colonial ascidians and sponges, or sponges exclusively, respectively.

Increasing the number of included species in the studied shrimp clades usually results in new insights in host-switching and in initial colonization events (Frolová et al., 2022), but also in the two phylogenetic studies of this thesis. What makes the '*Anchistus* and *Conchodytes* clade' unique, is the way in which they seemingly have switched between host phyla, in a relatively short time. For example, Horká et al. (2016) and Chow et al. (2021) already suggested that the '*Conchodytes* clade' had an ancestral state reconstruction with a bivalve association, after which multiple lineages switched towards a symbiosis with an ascidian (in *Ascidonia*, *Dactylonia*, and *Odontonia*). How these clades can switch between such distantly related groups is unknown, but it may have something to do with the interior microhabitat of the host clades (Horká et al., 2016). Bivalve (and gastropod) molluscs, as well as all types of ascidians, produce nutritious mucus, which may be used as a food source. Moreover, both host groups possess folds in their internal organs, creating a perfect habitat for the shrimps: in ascidians this is the branchial basket; and in bivalves these are the mantle folds (Fransen, 1994). These similarities between the host groups may make it less difficult to adapt to a new host after colonization. Rapid host

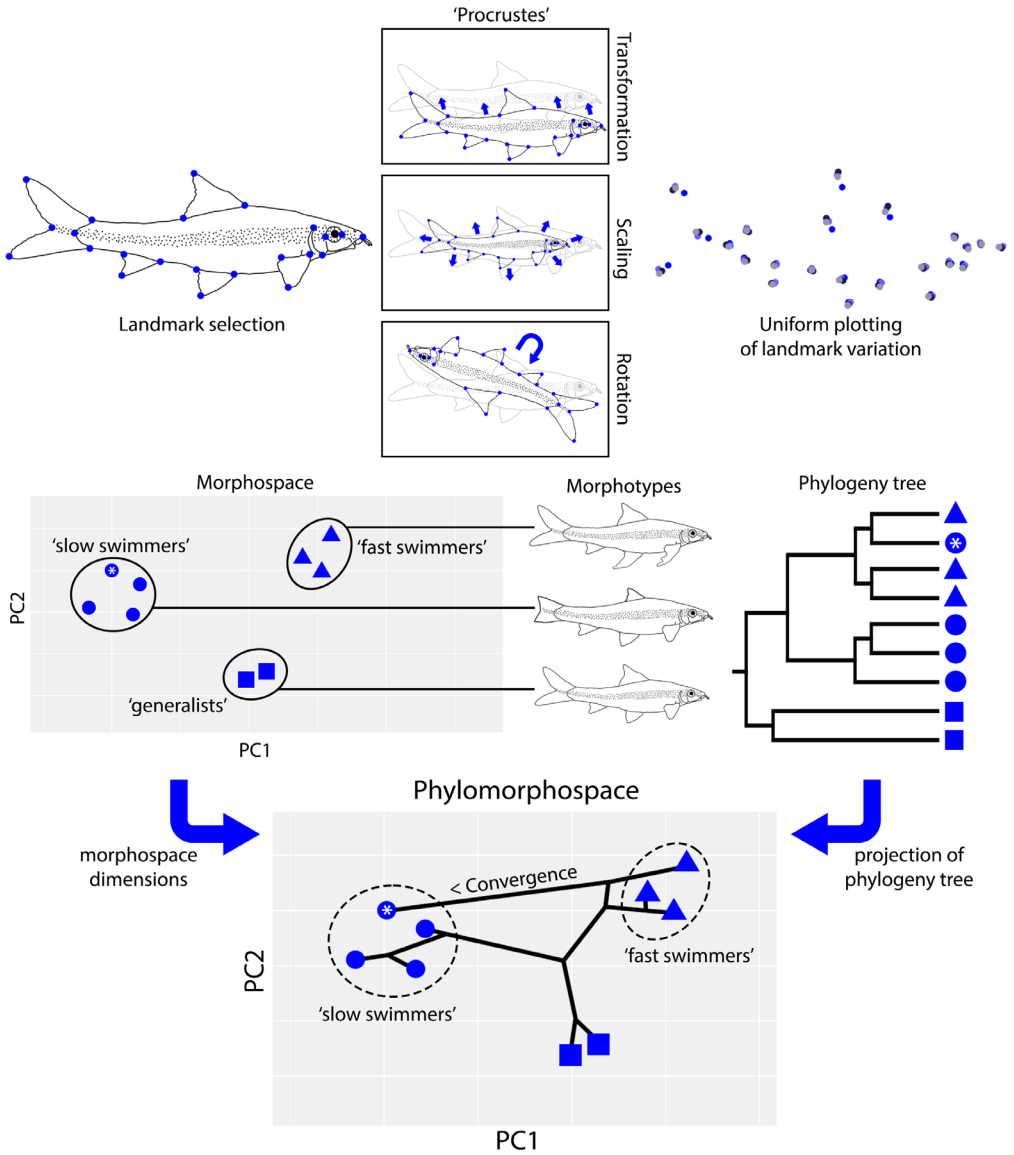
switches may also result in small- or large-scale morphological convergence, owing to similar (but possibly not identical) selection pressures for both host groups (Chapter 7).

In phylogenetic studies focusing on pea crabs, host-switching events have not been studied in detail. However, the phylogeny reconstructions by Palacios Theil et al. (2016) can be supplemented, and used to map host associations (Hultgren et al., 2022; Chow et al., 2023). Studies have found that the endosymbiotic pea crabs (Pinnotherinae) are descendants of an ancestral inquiline, tube- and burrow-dwelling species (now represented by the Pinnixinae and Pinnixulalinae). According to the phylogenetic models by Palacios Theil et al. (2016), it appears that bivalve-associated lineages have switched multiple times to ascidian, gastropod, and echinoderm hosts (once also a switch to ectosymbiosis in the '*Dissodactylus* complex'). As mentioned above, two confirmed species of Pinnixinae have also switched back to endosymbiosis, in the genus *Scleroplax* (Hultgren et al., 2022). The number of species associated with ascidians is not yet high enough for comparisons with the two studied shrimp clades. However, the number of seemingly generalist species that live both in bivalves as well as in ascidians (e.g., *Fabia subquadrata*, *Nepinnotheres pinnotheres*), and the fact that the exclusively ascidian-associated species are nested next to bivalve-associated species (*Tunicotheres* and *Tumidotheres*, resp.), gives the impression that host switching between these two host groups is perhaps easier than between an echinoderm and a bivalve host (Palacios Theil et al., 2016).

In this thesis, the new placement of species within the '*Conchodytes* clade' and the '*Anchistus* clade' will highlight new host-switching events (Chapters 4 and 5), and morphometric analyses will dive deep in the way these species have adapted to new hosts, with a special focus on species that are placed next to host-switching events (Chapter 7). For pea crabs, host-switching will be studied in more detail on a morphological level, in order to find patterning in the way the carapace changes during host shifts (Chapter 6).

Morphometrics, a way to study ecomorphology and convergent evolution

Almost all studies focusing on ecomorphology, either in a micro- or macro-evolutionary setting, get their raw data from measuring and mapping their specimens. Morphometry encompasses a broad scale of measuring methods to gather quantitative data from size and shape of an object, and is often the only method to do so. In case of ecomorphological research, morphometrics is used as a proxy for shape and size, and therefore for ecological function



1.4 Overview of a geometric morphometric workflow to study convergence of morphotypes in *Crossocheilus oblongus* Kuhl & Van Hasselt, 1823, the Siamese algae-eater. Study and data are hypothetical. Landmarks are selected on 2D images, after which a Procrustes analyses transforms, scales, and rotates the linked landmarks to a uniform projection. Plotting of the variation in landmarks is performed to see which morphological features are variable. A morphospace is constructed, which shows morphological separation of the three morphotypes, in this case three morphotypes were defined by their movement speed. A phylogeny tree (based on molecular data) is built to see if the morphotypes are separate clades, or the result of convergent evolution. In this case, one (sub)species has convergently evolved from a 'fast swimming' clade towards the 'slow swimmers'. This was also evident in the phylomorphospace plot, showing a clear convergence event in the projection of the phylogenetic tree.

and performance (Feilich & López-Fernández, 2019). Morphometrics comes in three different forms (Zelditch et al., 2004): Traditional morphometrics, geometric morphometrics and the recently acknowledged functionally informed measurements (Feilich & López-Fernández, 2019).

Traditional morphometrics have been widely used in the last 30 to 40 years and uses measurements (lengths and weights) of body parts of the studied subject. Examples of these measurements might be body weight, length and width, appendage length and specific bone or organ lengths (Zelditch et al., 2004). Instead of taking actual measurements, geometric morphometrics use coordinates or outlines in a 2D or 3D environment to describe and quantify shape and size and keep their geometry (Klingenberg, 2016). Using manually placed 'landmarks' and estimated 'semi landmarks' (including so-called 'sliding semi landmarks', or 'sliders') a shape can be plotted onto a graph/coordinate system and statistically analysed (fig. 1.4). Variations of these landmarks within a sample can be analysed and plotted as well, using so-called 'deformed grids' or 'warps' (see below; Rohlf, 1998). While geometric and traditional morphometrics provide information about the size and shape of an object, they do not include insights about the potential functionality of the structure.

However, so-called functionally informed measurements provide morphometric approximations to biomechanics and functional morphology (Feilich & López-Fernández, 2019). In ecomorphological studies, for all three forms of data-acquisition a certain amount of knowledge about the functional anatomy of the study object is needed (Feilich & López-Fernández, 2019).

If two-dimensional measurements are used, data can be directly plotted and analysed, but using three-dimensional wrapping methods, or two- or three-dimensional landmarks, the data has to be generalised using a Procrustes analysis. Using geometric data (landmarks) or wrapping-grid information (Horstmann et al., 2018), Procrustes analyses remove any variation in the rotation, position and size of studied subjects, resulting in comparable 'shape alterations' (fig. 1.4; Horstmann et al., 2018). The visualization of this shape variations is usually done with the aid of vectors of the relative landmark displacements and consequently the before-mentioned deformed grids, or warps (Rohlf, 1998; Zelditch et al., 2004).

After the variation of the shape has been plotted and visualised, it needs to be statistically analysed. A Principal Component Analysis (PCAs) ordination method is normally used for this (fig. 1.4). The statistical analysis converts a large number of possibly correlated variables to multiple dimensions (or

components), where the first component accounts for ('explains') the largest part of the variability of the datapoints. Using character loadings and vector scores, the variables responsible for the variation of the datapoints can be deduced. Datapoints in these so-called 'morphospaces' can be linked to individual observations and therefore to different classifications based on ecology (ectomorphs or morphotypes; fig. 1.4). Some examples of multivariate statistics in ecomorphological contexts can be found in Von Rintelen et al. (2010), Trontelj et al. (2012), Claverie & Wainwright (2014), Sherratt et al. (2019), Dugo-Cota et al. (2019).

PCA analyses and their counterparts can differentiate data and explain the variability within a sample, but in an evolutionary context, multivariate statistics needs to be combined with some additional phylogenetic information. If necessary phylogenetic trees and distances between the taxa are already available, a rather easy way for visualization might be the use of a 'phylomorphospace' (fig. 1.4; Stayton, 2011, Dugo-Cota et al., 2019, Sherratt et al., 2019). Using this visualization-method of both shape and phylogeny in one set of axes, the distances between the datapoints can tell us something about convergent evolution, based on the idea that convergence results in two taxa evolving to become more similar to each other than their ancestors were (Stayton, 2015). Values (C_1 to C_4, in Stayton, 2015, 2018) coming from a so-called convergence analysis, which analyses the phylogenetic distances between samples with the same morphological features and their common ancestor, can be statistically analysed to find out which morphological groups are the result of convergent evolution in evolutionary lineages (Dugo-Cota et al., 2019).

Thesis outline

Before any ecomorphological analyses focusing on symbiont-host adaptations and convergent evolution can be performed, a proper literature review is needed to acquire all relevant ecological and morphological notes for a clade of symbionts. While this knowledge is available for the studied clades of shrimps, for the pea crabs this was not so. Therefore, a literature review on the ecomorphological features of pinnotherine pea crabs is presented (Chapter 2).

After the collection of materials and ecological notes, phylogenetic trees should be built as preparations for extensive phylomorphometric analyses. In order to perform the necessary phylogenetic analyses, material was examined and subsampled for both clades of endosymbiotic shrimps introduced above. DNA barcoding of the sampled shrimps resulted in the discovery and description of a new species, *Odontonia kerangcaris*, featuring an aberrant host choice (Chapter 3). The new species was added to the phylogeny reconstruction of

the '*Conchodytes* clade'. This analysis, based on four molecular markers, as well as morphological characters can be found in Chapter 4. Using a so-called 'Total Evidence' (TE) approach, all species of the clade without molecular data could be added. In Chapter 5, the TE approach resulted in the discovery of two polyphyletic genera in the '*Anchistus* clade' and in the (re)erection of three new genera: *Ensiger*, *Polkamenes*, and *Tympanicheles*. The species in the new genera, and in the other genera of the '*Anchistus* clade' were described, accompanied by illustrations, photographs, and SEM- and µCT-captures of phylogenetically relevant morphological features.

Using the phylogeny reconstructions from previous works and from chapters 4 and 5, phylomorphometric studies could be done on some ecomorphological features of the pinnotherine pea crabs and two clades of shrimps, respectively. In Chapter 6, a landmark-based morphometric, and consequently phylomorphometric analysis was performed on the carapace shapes of pea crabs, mainly focusing on species of the Pinnotherinae. Special interest was given to morphological convergence, which was proven by statistical tests in the phylomorphospace. In Chapter 7, similar tests were done to find convergence between and within the '*Anchistus* and *Conchodytes* clades'. Classical morphometrics were used to map the post-orbital carapace lengths on the phylogeny reconstructions, and 2D morphometrics were used to show convergence in the walking-leg dactyli shapes, which are thought to be adaptive for their hosts. In addition, SEM-captures were used to inspect the microstructures on the walking leg dactyli, and seek for possible convergence herein.

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2

A review of the ecomorphology of pinnotherine pea crabs (Brachyura: Pinnotheridae), with an updated list of symbiont-host associations

Werner de Gier & Carola Becker

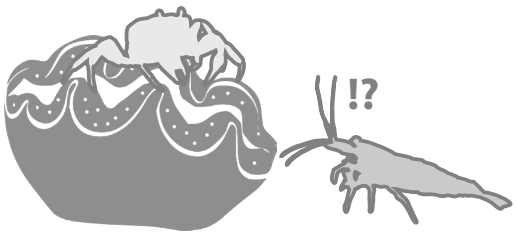
This chapter is published as:

de Gier, W. & Becker, C. (2020) A review of the ecomorphology of pinnotherine pea crabs (Brachyura: Pinnotheridae), with an updated list of symbiont-host associations. Diversity, 12(11): 431.

Abstract

Almost all pea crab species in the subfamily Pinnotherinae (Decapoda: Brachyura: Pinnotheridae) are considered obligatory endo- or ectosymbionts, living in a mutualistic or parasitic relationship with a wide variety of invertebrate hosts, including bivalves, gastropods, echinoids, holothurians, and ascidians. While the subfamily is regarded as one of the most morphologically adapted groups of symbiotic crabs, the functionality of these adaptations in relation to their lifestyles has not been reviewed before. Available information on the ecomorphological adaptations of various pinnotherine crab species and their functionality was compiled in order to clarify their ecological diversity. These include the size, shape, and ornamentations of the carapace, the frontal appendages and mouthparts, the cheliped morphology, the ambulatory legs, and the reproductive anatomy and larval characters. The phylogenetic relevance of the adaptations is also reviewed and suggestions for future studies are made. Based on an updated list of all known pinnotherine symbiont–host associations and the available phylogenetic reconstructions, it is concluded that, due to convergent evolution, unrelated species with a similar host interaction might display the same morphological adaptations.

Keywords: Decapoda; micro-computed tomography; morphology; parasitism; Pinnotherinae; symbiosis; symbiotic fauna



An Introduction to Pea Crabs

Symbiotic lifestyles, whether they are considered parasitic, commensal, or mutualistic, can be found in species of almost all major crustacean taxa. Only the remipedes (Remipedia) and horseshoe shrimps (Cephalocarida) form an exception, including no apparent taxa living in or on host organisms (Baeza, 2015). The true crabs (Decapoda: Brachyura) encompass 14 families with symbiotic species (Castro, 2015). One of these families, the Pinnotheridae or 'pea crabs', currently holds around 320 recognised species (Ng et al., 2008; WoRMS, 2020), which can almost all be classified as obligatory endo- or ectosymbiotic (Baeza, 2015). The family is currently split into four subfamilies: Pinnotherinae, Pinnixinae, Pinnothereliinae, and Pinnixulalinae (Palacios Theil et al., 2016). Members of Pinnotherinae are usually defined as small symbiotic crabs, living commensally or parasitically as endosymbionts between the branchial organs of bivalve, gastropod, and chiton molluscs, inside the pharyngeal basket of ascidians, inside the intestinal or respiratory system of holothurians and echinoids, and ectosymbiotic on the outer surface of various echinoids (Schmitt et al., 1973). In addition, there are exceptional cases of pinnotherines living in brachiopods, on asteroids, and supposedly in decapod burrows and worm tubes (Schmitt et al., 1973; Manning, 1993a). The complicated multi-staged life history of only a few pinnotherines has been well studied (Campos, 1989a; Ocampo et al., 2017), but remains unknown for most other species. Pinnotherine species which have been identified as free-living are usually described from single specimens and one of the sexes only (Schmitt et al., 1973). Although hard stage males and females are known to leave their (intermediate) host for numerous possible reasons (e.g., copulation during swarming; Derby & Atema, 1980), it is most likely that soft staged individuals collected outside another invertebrate have been dislodged from their hosts (McDermott, 2009), or are just venturing shortly outside their hosts (Ahyong, 2018).

Most members of the subfamilies Pinnixinae, Pinnothereliinae, and Pinnixulalinae (Palacios Theil et al., 2016) can be found as commensal symbionts living inside the holes and tubes of living annelid and sipunculid worms, and inside mud shrimp burrows (Decapoda: Axiidae and Upogebiidae). Although pinnixine, pinnothereliine, and pinnixulaline crabs are known for their co-inhabiting behaviour, around 19 species are still considered to be free-living, whereas seven species are known as obligatory endo- or ectosymbionts. Closer inspection of the free-living species and their habitat is needed in order to confirm whether they are indeed free-living or if their host was simply not found and therefore not collected (Werding & Sanchez, 1989).

While pea crabs are regarded as one of the most specialised groups of symbiotic crabs (Sèrene, 1961), only few authors succeeded in testing or observing the functionality of their ecomorphological adaptations (Becker & Türkay, 2017). In the taxonomic literature, morphological adaptations are commonly only mentioned as part of species descriptions (Manning, 1993a; Campos, 1996a), while review papers mainly focus on the correlation between the sizes of the host and the symbiont (Watanabe & Hemni, 2009), and on the morphology of the anatomical features associated with feeding habits and host choice, which are both thought to drive speciation (Becker & Türkay, 2017).

Due to their small size and cryptic way of living, the adaptations pinnotherines have evolved in order to live in and on their host are barely understood (Palacios Theil & Felder, 2020). This study aims to review the anatomy and hypothesised functional roles of the anatomical structures in pinnotherines, and to illustrate a number of these anatomical features. In this way, we hope to shed more light on the host specificity of the morphological adaptations and whether they have any phylogenetic relevance in the evolution of the whole subfamily. An updated, more extensive list of known symbiont-host relationships of the Pinnotherinae is also given (see table 2.1), partly based on earlier works (Schmitt et al., 1973; McDermott, 2009; Baeza, 2015).

Studying Pea Crab Morphology

Traditionally, the morphological features of pea crabs were only illustrated using camera lucida illustrations (Ahyong & Ng, 2007a) or photographs (Rathbun, 1918). Most of the morphological features we can study using the previous literature is limited to only the third maxillipeds and the dorsal view of the entire female crab, whereas later, the (available) male crabs were also illustrated. More recent taxonomic works also included illustrations of the details of the ambulatory legs (especially the dactyli), chelae, and frontal view of the head region (Ahyong & Ng, 2007a). In more recent morphological papers, scanning electron microscopy (SEM) was used to capture the minute details on the claws (Becker & Türkay, 2015). In the present review, we aim not only to include the traditional methods in order to show the morphological features, but also a relatively new way to study both the internal and external morphology of pea crabs, by micro-computed tomography (µ-CT) scanning.

Three specimens from the Naturalis Biodiversity Center decapod collection (Leiden, the Netherlands; formerly Rijksmuseum van Natuurlijke Historie, RMNH) were selected for their distinct overall morphology, one representing the Pinnixinae (*Pinnixa cyllindrica* (Say, 1818)), and two representing the variety within the Pinnotherinae (*Nepinnotheres pinnotheres* (Linnaeus,

1758) for its basic pinnotherine body shape and *Xanthasia murigera* White, 1846 for its abnormal carapace ornamentations). The three specimens were illustrated using 3D models based on µ-CT: 3D models were made in the Naturalis Biodiversity Center CT-scanning and imaging facilities (Leiden, the Netherlands), using Avizo 9.5.0 volume-rendering software (FEI, 2018) and a Zeiss Xradia 520 Versa 3D X-ray microscope (CT-scanner), of specimens in 70% ethanol. The following settings were used: Optical magnification of 0.39, a scanning current of 87.0 µA, a scanning voltage of 80.0 kV, an exposure time ranging from 1.3 to 1.5 ms, and pixel sizes ranging from 23.5 to 27.6.

The line drawings in this review were traced from previous literature. SEM pictures of claw morphologies and ornamentations were made at the Senckenberg Research Institute and Natural History Museum (Frankfurt, Germany), from the same samples and using the same methodology as described by Becker and Türkay (2015).

To highlight the adaptive evolution of various anatomical features, we have organised the review into the five following sections: carapace shape, size, and ornamentation; frontal appendages and mouthparts; cheliped morphology; ambulatory leg adaptations; and sexual anatomy and larval characters. In addition, we have provided an updated list of all known pinnotherine symbiont–host associations (see table 2.1).

Adaptations in Pinnotherine Morphology

Carapace Shape, Size, and Ornamentation

Most pinnotherine crabs are known for their strong sexual dimorphism, in which the females reach larger sizes than the conspecific males. This is most likely linked to their mating systems, in which the trait ‘pure-search polygynandry of sedentary females’ occurs (Christensen & McDermott, 1958; Yanagisawa & Hamaishi, 1968; Baeza & Thiel, 2007). This is, however, not apparent in all pinnotherine genera, where a different mating strategy is used. Both sexes of the species in the ectosymbiotic *Dissodactylus* complex (genera *Dissodactylus* and *Clypeasterophilus*) share a similar size and shape of the carapace and appendages. These genera are thought to use ‘pure-search polygynandry of mobile females’ as mating strategies (Baeza & Thiel, 2007; Alves et al., 2017). The very subtle sexual dimorphism is thought to be the result of both sexes living on their host, rather than in their host, being able to leave their host and not being restricted to the space in the host’s cavities Soong, 1997). Similarly, female members of *Ostracotheres tridacnae* (Rüppell, 1830) and *Xanthasia murigera*, which inhabit giant clams (Tridacninae), are of the same size and shape as their male counterparts (Ahyong & Ng, 2005, 2008). It is thought that

males reach similar sizes as their female conspecifics due to their spacious *Tridacna* hosts allowing them to grow larger (Ahyong, 2018). Furthermore, male and female members of the holothurian-associated genera *Alain* and *Holotheres* share a similar shape and size of the carapace, but males possess relatively stouter chelae and are only slightly (10–20%) smaller in carapace width and length than females (Ahyong & Ng, 2008a; Ng, 2018). In many pinnotherine species, the morphology of only one sex is known, resulting in limited knowledge on sexual dimorphism in those species (Schmitt et al., 1973). In addition, although sexual dimorphism is most extreme in mollusc-inhabiting pea crabs (e.g., the genera *Pinnotheres*, *Fabia*, *Arcotheres*), it can be found all over the family tree, in association with almost all possible hosts (see table 2.1).

Size differences between crab species is thought to be linked to specific morphological traits of their hosts, such as microhabitat space (Becker & Türkay, 2017). The largest species of pinnotherine crab, *Pinnaxodes gigas* Green, 1992, has a carapace width of 36 mm as is reported from the siphon of a large geoduck, the mudburrowing bivalve *Panopea* sp. (Campos, 2016). The smallest pea crab species, *Nannotheres moorei* Manning & Felder, 1996, can be found in narrow hammer oysters (*Malleus candeanus* (d'Orbigny, 1853)) barely reaching a carapace width of 1.5 mm (Manning & Felder, 1996). The smallest *Arcotheres* species (*A. pollus* Ahyong & Ng, 2020) also lives inside a hammer-oyster (*M. albus* Lamarck, 1819) (Ahyong & Ng, 2020). Host size does not just explain the interspecific size differences, but also intraspecific variation in the crabs. Cuesta et al. (2019) studied the correlations between crabs of both sexes (*Pinnotheres bicristatus* García Raso & Cuesta, 2019) and one of their host bivalves, *Anomia ephippium* Linneaus, 1758. A strong positive correlation was found between the sizes of the hosts and the sizes of the soft-shelled (post-hard) females, with larger hosts harbouring larger post-hard females. The larger size of the females can be explained by their sedentary lifestyle, not having to leave their host ever again. Additionally, being larger is also advantageous for reproductive purposes: larger body sizes can produce larger broods (Hines, 1992). A similar, but weaker, positive correlation was found between the sizes of male crabs and their hosts; male crabs were always smaller than females in the same host size (Cuesta et al., 2019). It is thought that smaller males looking for mates have access to a larger size range of host individuals (Baeza & Thiel, 2007). In *Pinnotheres pisum* (Linnaeus, 1767), *P. taichungae* K. Sakai, 2000, and probably many more bivalve inhabitants, the size of the host is also positively correlated with the infestation rates within and between host species (Haines et al., 1994; Hseuh, 2003).

In addition to the study mentioned above (Cuesta et al., 2019), no correlation

was found between the size of hard stage females and the size of their newly invaded hosts (Marco-Herrero et al., 2020). This suggests that intruding crabs do not select the biggest host available, but they will be limited in their growth by the size of the specific host. Similar results (Becker & Türkay, 2017) were found in populations of other bivalve-associated pea crabs from all over the world: *Afropinnotheres monodi* Manning, 1993 (Drake et al., 2014), *Arcotheres sinensis* (Shen, 1932) (Sun et al., 2006), *Austinotheres angelicus* (Lockington, 1877) (Salas-Moya et al., 2014), *Calyptraeotheres garthi* (Fenucci, 1975) (Ocampo et al., 2012), *Pinnotheres pisum* (Atkins, 1926a), and *Pinnotheres tsingtaoensis* Shen, 1932 (Soong, 1997). In addition to these mollusc-inhabiting species, Ahyong (Ahyong, 2018) found that *Austrotheres holothuriensis* (Baker, 1907) has a larger maximum size in spacious holothurians than in the mostly smaller ascidian host species. The specialist congeneric *A. pregenzeri* Ahyong, 2018, however, grows to similar sizes in its comparable ascidian hosts. Similarly, Becker and Türkay (2010) found larger *Nepinnotheres pinnotheres* specimens infesting shells of *Pinna nobilis* Linneaus, 1758, compared those from ascidian hosts. In general, larger hosts are thought to offer greater food resources than smaller hosts (Becker & Türkay, 2017).

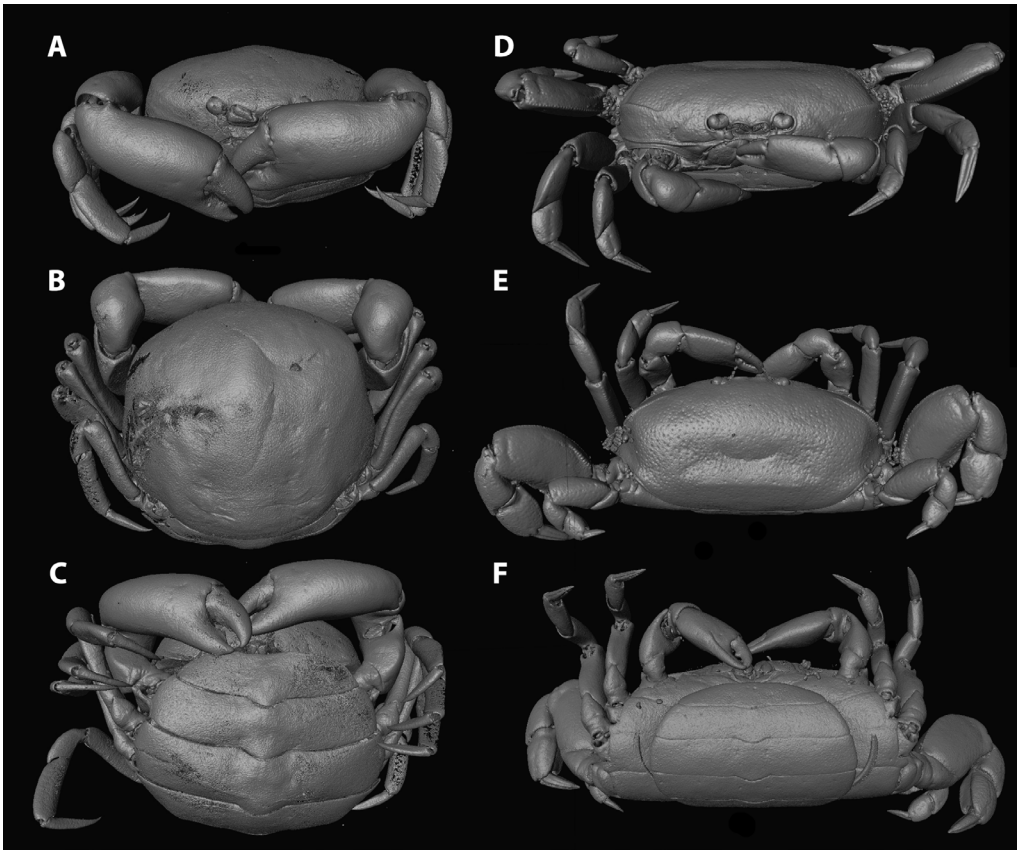
Based on their reproductive strategies, the shape and rigidness of the carapace can change throughout the multi-staged lifecycle of both female and male pea crabs. Campos (1989a) suggests two different ontogenetic pathways, based on his own observations and previous literature. In the first strategy, male and female crabs moult into their hard stages prior to host invasion and copulate after invasion of the host. After copulation, the female moults into her more globular post-hard (soft) stages and remains in the host. The hard stage male, characterised by having a well-calcified carapace, is fit for entering bivalve hosts (McDermott, 2009) and is suggested to leave the host again (Christensen & McDermott, 1958) and copulate with other host-inhabiting hard stage females to increase its reproductive success (Watanabe & Hemni, 2009). Becker and Türkay (2017) confirmed this theory for hard stage males of *Pinnotheres pisum*, observing the lack of distal segments in their ambulatory legs, likely due to them having been squashed by the closing of their bivalve hosts. During their time between hosts, male crabs of the same species might even use vectors like detached egg-cases of whelk snails (*Buccinum*) to cover greater distances (Hoeksema, 1981).

This first strategy is found in most pinnotherine genera, but many details remain unknown for almost all species. The second strategy is similar, but differs in a few ways: juvenile crabs infest their (intermediate) hosts in the first post-planktonic stage and moult into a male or female hard stage crab. In this stage,

morphological adaptations for swimming develop (e.g., hard carapace and ambulatory leg morphology, discussed later), and both male and female crabs leave their host for copulation in open water (often called swarming behaviour). Afterwards, females infest their terminal host and moult into more globular post-hard stages, while males might still switch between hosts in their terminal hard-stage. This strategy is thought to follow a seasonal pattern (Pearce, 1966; Campos, 2016) and is known from members within the genera *Austrotheres* (Ahyong, 2018), *Calyptraeotheres* (Ocampo et al., 2017), *Fabia* (Pearce, 1966), and *Tumidotheres* (Ocampo, 1989; Campos, 2016). It is worth noting that swarming behaviour has been observed in *Fabia subquadrata* Dana, 1851, and *Tumidotheres maculatus* (Say, 1818) using 'night-light' fishing of a few swarming individuals, as described in Pearce (1966). Another unrelated species, *Tritodynamia horvathi* Nobili, 1905, which was transferred from the Pinnotheridae to another family (Ng et al., 2008), is known for its excessive swarming behaviour (Takahashi et al., 1999) and might have contributed to the theory that some pinnotherids swarm in a similar way (Pearce, 1966).

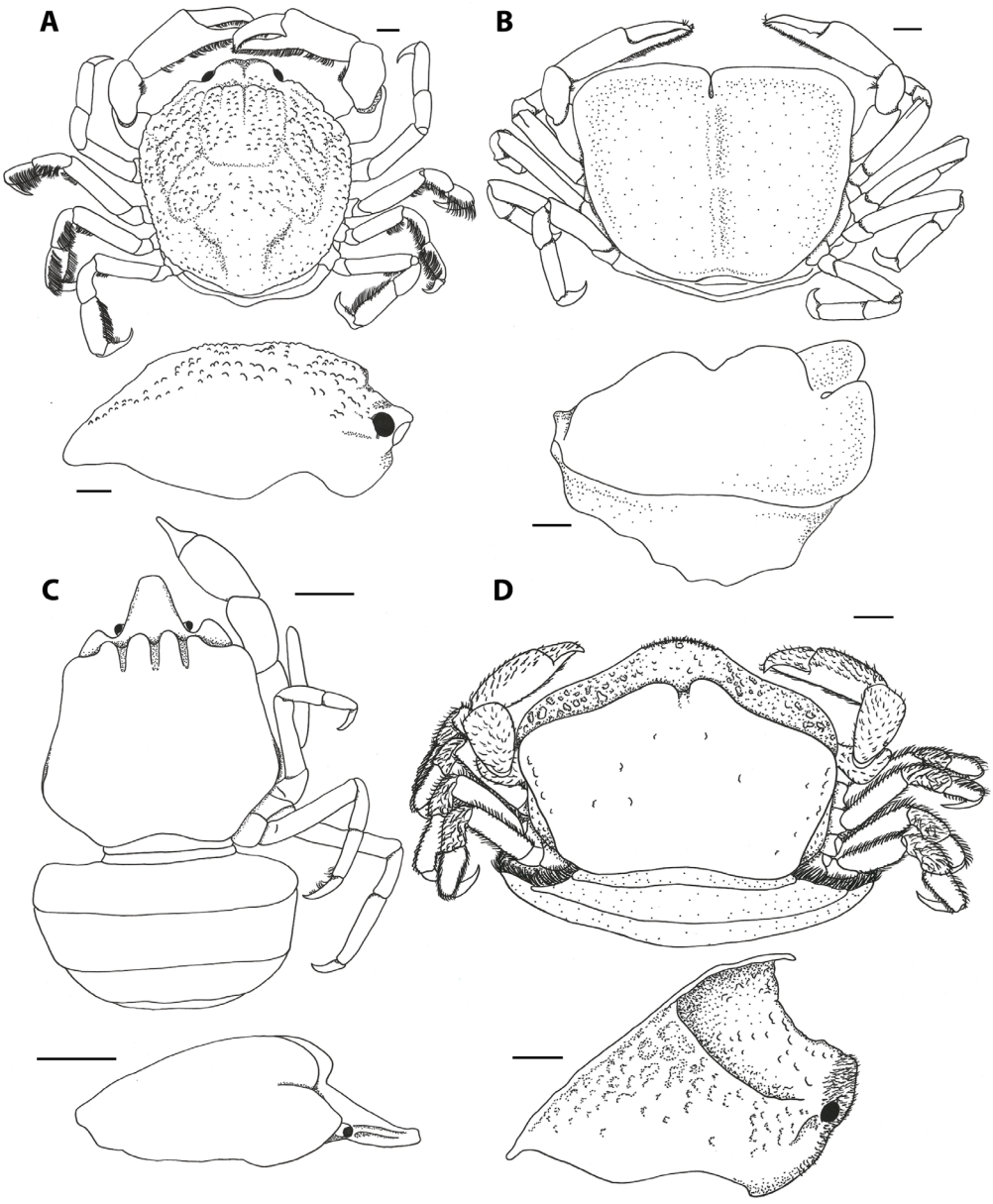
Interspecific differences in carapace shape and rigidness of post-hard females can be traced back to their specific host range. Endosymbiotic pea crabs known from echinoderms and geoducks (e.g., genera *Alain*, *Buergeres*, *Holotheres*, *Holothuriophilus*, *Pinnaxodes*; see table 2.1) share a firm, round to subangular carapace (Campos, 2016). Similarly, members of the ectosymbiotic genera *Dissodactylus* and *Clypeasterophilus*, all known from the outer surface of flattened sea urchins such as sand dollars, share a flattened, extremely calcified, and somewhat widened carapace, which is thought to be useful for manoeuvering between the spines of sea urchins (De Bruyn, 2010). This somewhat flattened and wide carapace resembles that of the hard stage males and females of other genera associated with bivalves, like *Fabia subquadrata* and *Zaops ostreum* (Say, 1817) (Pohle & Telford, 1982). Most crabs of the remaining pinnotherine genera known from molluscs and ascidians (with the exception of a few genera discussed below) live securely inside their host and share a globular soft-shelled carapace in the terminal female stages. This feature is usually accompanied by an enlarged pleon for egg development (fig. 2.1A–C) (Watanabe & Hemni, 2009; see below). In a few cases, the carapace might be more calcified in specimens infesting certain bivalve groups, like the Arcidae (Komai et al., 2020). The reason for this aberrant post-hard stage morphology is not known as for now.

In contrast to the morphological variation within the Pinnotherinae, members within the Pinnixinae, Pinixulalinae, and Pinnothereliinae all share a similar body shape. All representatives of these taxonomic groups have a flattened,



2.1 Three-dimensional models of two representatives of Pinnotheridae. A–C) A typical (female) bivalve- and ascidian-inhabiting pinnotherine, *Nepinnotheres pinnotheres* (Linnaeus, 1758) (RMNH.CRUS.D.36): carapace width 1.8 mm; D–F) A typical tube-dwelling pinnixine crab, *Pinnixa cyllindrica* (Say, 1818) (RMNH.CRUS.D.10104): carapace width 1.5 mm. Videos of the 3D models can be found in the Supplementary Materials.

wide carapace shape, and usually a third ambulatory leg that is larger in size than the other ones (fig. 2.1D–F) (Palacios Theil & Felder, 2020). This body shape is thought to be the result of the symbiotic lifestyle of these crabs within the tubes and burrows of worms and decapods such as mud shrimps (Zmarzly, 1992). Although the crabs from these three subfamilies appear to be morphologically similar, Manning and Felder (1989) discuss very slight intraspecific ecophenotypic variation, resulting from the crabs living in burrows from related but separate species of *Callianassa* mud shrimps. In addition, Palacios Theil and Felder (2020) mentioned that the diversity of body shapes is the result of convergent evolution, resulting from host choices, rather than shared synapomorphies. Furthermore, a few non-pinnotherine pea crabs are known from atypical hosts: living inside and on hosts usually inhabited by pinnotherines. Other than their habitat preferences, none of these species resemble pinnotherines in their general morphology.



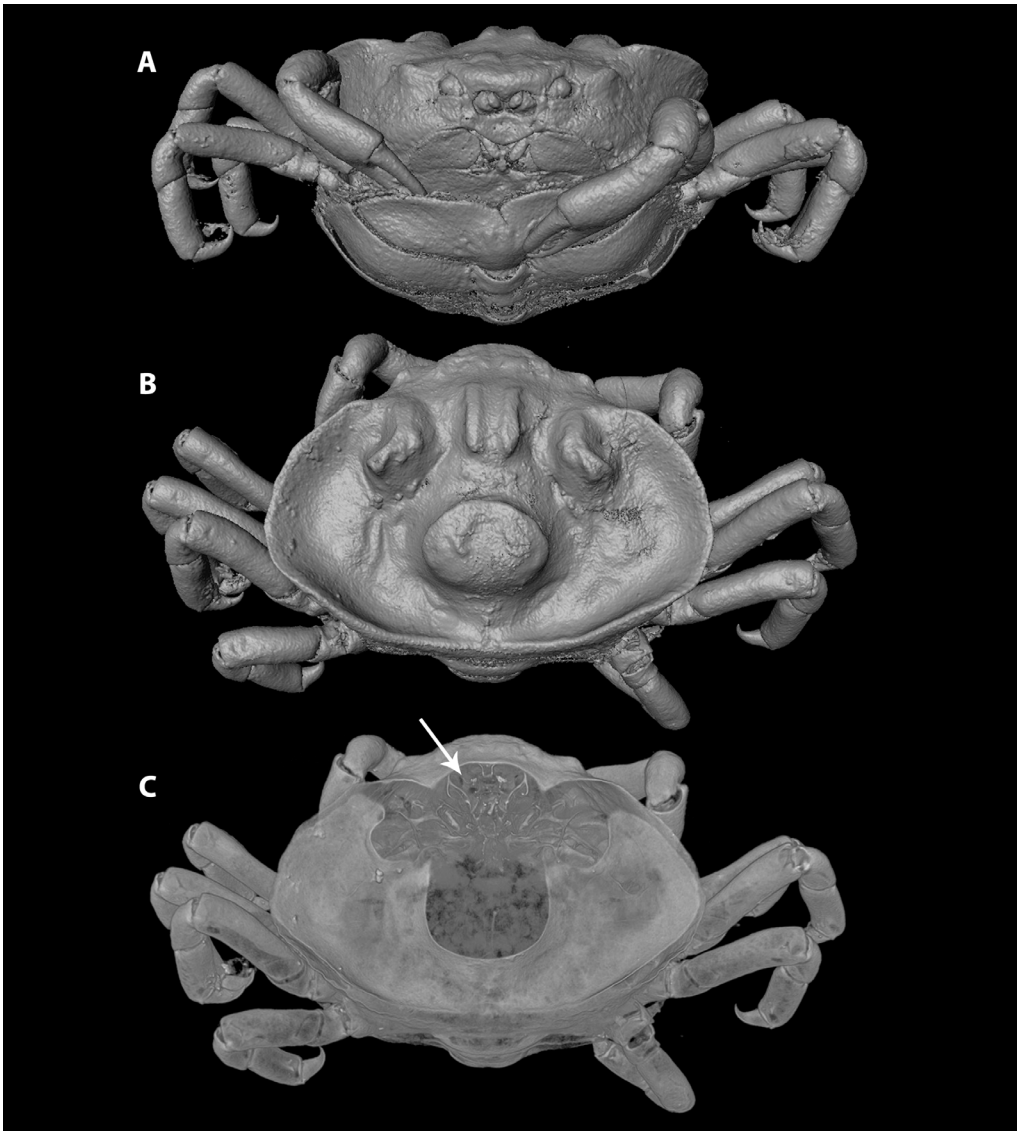
2.2 Dorsal and lateral views of representatives of Pinnotherinae with ornamented carapace morphologies. A) Austrotheres pregenzeri Ahyong, 2018, after Ahyong (2018); B) Durckheimia lochi Ahyong & Brown, 2003, after Ahyong and Brown (2003); C) Limotheres nasatus Holthuis, 1975, after Holthuis (1975); D) Serenotheres besutensis (Serène, 1967) after Ahyong and Ng (2005). Scale A–D = 1 mm.

A few, presumably not closely related, pinnotherine genera share various structural ornamentations on their carapaces. These ornamentations are described in the taxonomic literature as tubercles, plates, lamellae, and upturned

margins. The functionality of these ornamentations is still unknown (Ng & Meyer, 2016), but these structures might be the result of adaptive evolution (Ahyong & Ng, 2005). Both species of the genus *Austrotheres* have a subhexagonal carapace shape, with a distinct (in *A. pregenzeri*) to weak (in *A. holothuriensis*) epigastric ridge, which is covered with tubercles in *A. pregenzeri* (fig. 2.2A) (Ahyong, 2018). Members of *Durckheimia* display two upturned margins: one medial plate and one anterior plate, often with a sharp medial notch, continuing into two lateral margins (fig. 2.2B) (Ahyong & Brown, 2003; Ahyong & Ng, 2005. Similarly, crabs of the monospecific genus *Visayeres* share the medial plate of the supposedly related species of *Durckheimia*, showing a conical dorsal surface (Ahyong & Ng, 2007b). Members of the genera *Serenotheres* and *Limotheres* share a somewhat pentagonal carapace shape, with a pronounced rostrum (more pronounced in *Limotheres*) and an angled dorsal surface, which forms a weak (*Limotheres*) or strong (*Serenotheres*) eave-like (overhanging) structure anteriorly with the 'true' frontal margin that is much lower than the front of the dorsal margin (fig. 2.2C, D) (Holthuis, 1975; Ahyong & Ng, 2005; Ng & Meyer, 2016).

Lastly, both members of the monotypic genera *Tridacnatheres* and *Xanthasia* share a unique ornamentation of the carapace: a sharp, upturned (in *Xanthasia*) or weak, folded (in *Tridacnatheres*) ridge at the carapace margin, which terminates anteriorly in the hepatic region, in addition to a strong (in *Xanthasia*) or weak (in *Tridacnatheres*) rostro-dorsal and medial mushroom-like tubercle (fig. 2.3) (Ahyong & Ng, 2005). Virtual sections of CT-scan volumes of *X. murigera* reveal that ornamentations have a well-calcified outer surface, but no associated tissues were identified underneath. The stomach of the crab is partly calcified and is obviously attached to the inner surface of the rostro-dorsal tubercle (fig. 2.3C). Using this imaging method, no other organs were apparently associated in a similar way with the other ornamental structures. The carapace of *Xanthasia* (and, to a lesser extent, that of *Tridacnatheres*) resembles those found in various unrelated leucosiids (purse crabs, such as *Alox*, *Ebelia*, and *Ixa*), hymenosomatids (pillbox crabs, such as *Halicarcinus*), and epialtids (symbiotic spider crabs, such as *Oxypleurodon*).

Although the functionality of this wide range of morphological features is currently unknown, patterns in carapace ornamentation can be linked to host specificity. Most of the above-mentioned species live in various, often spacious, hosts: members of *Austrotheres* live in holothurians and (large) ascidians, but are known to venture outside their hosts (Ahyong, 2018); members of *Durckheimia* and *Limotheres* live in scallops of the family Limidae; all species of *Serenotheres* and *Visayeres* live inside boring mussels (Lithophaginae); and the members of



2.3 Three-dimensional model of a female *Xanthasia murigera* White, 1846 (RMNH. CRUS.D.27677): carapace width 1.2 mm. A) Dorsal view; B) Lateral view; C) Volume horizontally cut through carapace showing the stomach inside the most rostro-dorsal tubercle (arrow). Videos of this 3D model can be found in the Supplementary Materials.

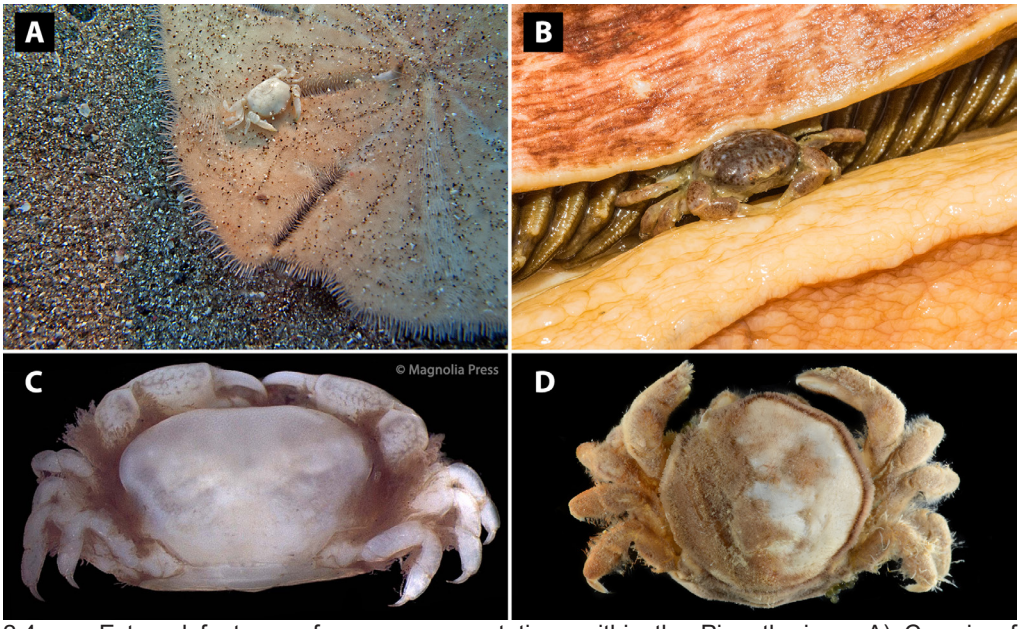
Xanthasia and *Tridacnatheres* live inside giant clams (genus *Tridacna*). The bivalve hosts mentioned above are not necessarily inhabited exclusively by these pea crab genera (see table 2.1). The unique ornamentations on the carapaces can play parts in structural and/or chemical mimicry to confound the host. For example, host mucus may stick to the carapace of the crab easily due to its crevices. Owing to the presence of host mucus on the crab, the crab may not be perveived as a foreign object. While both passive and active mimicry

as camouflage have been studied in crustaceans in detail (Guinot & Wicksten, 2015), their use of structural and chemical mimicry to avoid being noticed by a host has not received detailed examination hitherto. Other crustaceans possibly utilizing similar strategies might be found in the palaemonid shrimp genera associated with bivalves like *Anchistus*, *Conchodytes*, and *Pontonia* (Fransen, 1994): these genera possess less spines on their carapaces than their ectosymbiotic relatives, probably evolved to be smoother due to their endosymbiotic lifestyle (Fransen, 2002). In addition, cleaning shrimp of the species *Ancylomenes pedersoni* (Chace, 1958) and other cleaning shrimp symbiotic to anemones might use a similar strategy: in order to not get stung and devoured by the anemone, the shrimps need to acclimate themselves by acquiring host tissue, a phenomenon, which is also well known from clownfish (Amphiprioninae) (Mebs, 2009).

The variation in body shape is also translated into the variation of rostrum shape and size. Although the functionality is unknown, species of some pea crab genera possess an elongated rostrum, like *Austrotheres* (Ahyong, 2018), *Limotheres* (Holthuis, 1975), *Serenotheres* (e.g., Ahyong & Ng, 2005) and, to a lesser degree, in *Abyssotheres* (Komatsu & Ohtsuka, 2009) and *Nepinnotheres* (e.g., Manning, 1993a). Members of the (paraphyletic) genus *Fabia* and the related genus *Bonita* possess an extension of the rostrum towards the midline of the carapace: two longitudinal sulci split the anterior side of the carapace in three portions (Campos, 1996a, 2009).

Although other symbiotic crab families are known for their host-specific and cryptic lifestyle using camouflage (e.g., Pilumnidae, Eumedoninae, such as *Ceratocarcinus*, *Harrovia*, *Zebrida*) (Castro, 2015; Humann & Deloach, 2017), most pinnotherines do not display intricate camouflage patterns. Most species have evolved to be clear, transparent or unicoloured (mostly white, yellowish, or brown, purple to black in some species of *Arcotheres*) (Caro, 2018). Adult female individuals of some endosymbiotic species are so translucent that the inner organs shine through, most conspicuously the orange-coloured mature ovaries (such as in *Nepinnotheres*, *Pinnotheres*, and *Zaops*) (Campos, 1996a; Becker & Türkay, 2017; Cuesta, 2019).

Additionally, males of *Nepinnotheres pinnotheres* (as *Pinnotheres veterum* Bosc, 1801) were reported to change their colour at night (Atkins, 1926b). A few cases in which crypsis seems obvious, concern the genera *Dissodactylus* and *Clypeasterophilus*, which are thought to mimic shell fragments or coral rubble in soft sediments (De Bruyn et al., 2016). The white colouration might also mimic shell fragments attached to the host, as some sea urchins (e.g., sand dollars)



2.4 External features of some representatives within the Pinnotherinae. A) Crypsis of *Dissodactylus mellitae* (Rathbun, 1900) on a sand dollar; B) Redmottled colouration of *Opisthopus transversus* Rathbun, 1894 living in the folds of a gumboot chiton (*Cryptochiton stelleri* Von Middendorff, 1847); C) Dense setation at the lateral carapace margins in *Holothuriophilus trapeziformis* Nauck, 1880; D) Overall setation within *Nepinnotheres edwardsi* (De Man, 1887) (SS-4433), setae removed on the right side. Photo credits: A) Marco Faasse, from Hoeksema (2015); B) M. Harms; C) reproduced from Campos et al. (2012); D) reproduced from The Biodiversity of Singapore database, Arthur Anker. Photographs reproduced with permission from the respective photographers and copyright holders.

cover themselves in rubble (fig. 2.4A; Humann et al., 2013) and some regular echinoids hold debris over their test using tube feet. The Caribbean species *Clypeasterophilus rugatus* (Bouvier, 1917) even has black-and-white coloured bands on its ambulatory legs (Humann et al., 2013), similar to Indo-West Pacific *Zebrida* crabs (Pilumnidae: Eumedoninae) (Humann & Deloach, 2017). More elaborate colourations can be found in the males of *Pinnotheres bicristatus* (Cuesta et al., 2019), *Pinnaxodes gigas* and *P. floridensis* Wells & Wells, 1961, and *Opisthopus transversus* Rathbun, 1894 (fig. 2.4B) (Campos, 2016). While the cause or potential function of the colouration in *Pinnotheres bicristatus* is not mentioned in the description (Cuesta et al., 2019), the colouration of the other three species is discussed in taxonomic works. The species display orange-red spots on the dorsal surface of their ambulatory legs and carapace, while the ventral side of these structures display orange-grey spots, which may be caused by carotenes derived from their host (Hopkins, 1964). *Pinnotheres gigas* is known from various geoduck species while *P. floridensis* has only been found in a single species of holothurian. In contrast, *O. transversus* is known

from a wide range of hosts, including holothurians (see table 2.1). Although the species might partly share a similar microhabitat (geoducks siphons somewhat resemble the digestive organs of holothurians) and may have a similar diet (as demonstrated in the third maxillipeds, see below; Campos, 2016), this does not fully explain their colouration, because there are other species living inside holothurians with similar mouthparts that lack such colour patterns (e.g., *Holotheres*). The holothurian-associated pinnixine crab species *Pinnixa barnharti* Rathbun, 1918, is known to have a similar orange-red colouration, which may also be linked to its diet. This crab species is known to compete with O. transversus for shelter, so probably also for food sources (Zmarzly, 1992).

Setal coverage can be found in many crustacean lineages and, similarly, a wide range in different setal coverage patterns can be found in the Pinnotherinae. Most species are glabrous or only have a sparsely setose integument, in combination with some setae for feeding practises (see below: Cheliped Morphology and Ambulatory Leg Adaptations). A few exceptions are the conspicuously tomentose holothurian-associated genera *Alain*, *Holotheres*, *Holothuriophilus* (fig. 2.4C), and *Trichobezoares*, which possess a very setose carapace or carapace margins (Ng & Manning, 2003; Campos et al., 2012; Ng, 2018a). Ahyong (2018) mentions that since these genera do not appear to be related, the setation may be an adaptation for holothurian infestation. Few other representatives with setose carapaces belong to *Arcotheres* (e.g., *A. pollus*), *Afropinnotheres* (e.g., *A. monodi*), *Mesotheres* (e.g., *M. barbatus* (Desbonne, in Desbonne & Schramm, 1867)), *Nepinnotheres* (e.g., *N. pinnotheres*, *N. edwardsi* (De Man, 1887) fig. 2.4D), and *N. villosulus* (Guérin, 1832)), *Pinnotheres* (e.g., *P. pilulus* Tai, Feng, Song & Chen, 1980), and *Tumidotheres* (*T. maculatus*) (Miers, 1886; De Man, 1887; Kruczynski, 1974; Tai & Yang, 1991; Manning, 1993a; Becker & Türkay, 2017; Ng et al., 2019; Ahyong & Ng, 2020). The actual function of full or partial coverage with setae remains unknown, but Becker and Türkay (2017) suggest that *Nepinnotheres pinnotheres* uses the short setae to collect mucus from the body walls of ascidian hosts, since it lacks the setal comb on the chelipeds (see below: Cheliped Morphology). Similarly, Kruczynski (1974) observed individuals of *Tumidotheres maculatus* continuously cleaning their carapaces to collect bivalve gill mucus. The setose pinnixine crab *Glasella leptosynaptae* (Wass, 1968) has been reported from the body of holothurians, with the original description stating that it usually occurs near the anterior end, but never near the mouth of the holothurian. Wass (1968) mentioned that the ridges and setae on the carapace may enable the crab to cling to rough-surfaced holothurians, since the crab was always found with its dorsal surface pressed against the body wall of the host (Wass, 1968). Long setae on the dactylus and propodus of the third maxillipeds of this species

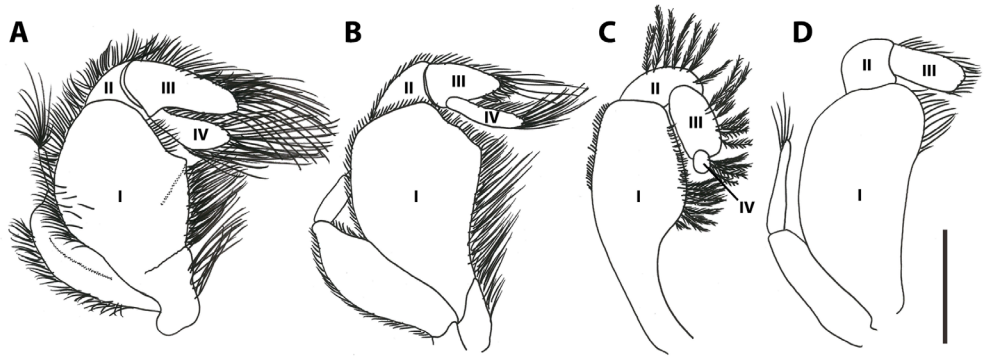
indicate a filter-feeding diet, but no observations were made. The full body setation of the previously mentioned pinnotherine species might also play a role in chemical mimicry or defense: host mucus might attach to the short setae in order to conceal the crab, or to make the crab less palatable when venturing outside of the host (Luckenback & Orth, 1990).

Frontal Appendages and Mouthparts

The process of host recognition is one of the most studied subjects in symbiotic crustacean research (Jossart et al., 2020). Studying this process is necessary to understand the evolution, ecology, but also the functional morphology of symbiotic crustaceans. The morphological features thought to be linked to host recognition in pinnotherines are all located anteriorly, namely the eyes for visual cues, and both antennulae and antennae for picking up and emitting chemical cues. The eyes were at first considered to play a role in host recognition; however, the ectosymbiotic *Dissodactylus primitivus* Bouvier, 1917, was shown to find its host using only chemical cues (see below) (Jossart et al., 2020). Although related species within the genera *Dissodactylus* and *Clypeasterophilus* are known to hop on and off their hosts and are therefore atypical within the Pinnotherinae (De Bruyn et al., 2010), the lack of functionality of their relatively small eyes remains unexplained (Jossart et al., 2020). Most pinnotherine species have small eyes, but there is quite a lot of variation in their placement and size, which may be linked to their specific host range (variation in general eye shape can also be found in other symbiotic crustaceans, such as palaemonid shrimp; Dobson et al., 2014, 2016). The placement of the eyes and their visibility in dorsal view have been used as taxonomic characters, although size is usually only briefly mentioned. One species stands out, since it hints to evolutionary processes known from animals in caves and deep-sea environments: *Arcotheres latifrons* (Bürger, 1895) is an eyeless species (Ahyong & Ng, 2007a). Since the host of this species is unknown, it is impossible to say if the host plays a role in the reduction and eventual disappearance of the eyes. The species, however, is known from a single specimen only, which supports the idea that the lack of eyes in this specimen is an anomaly. The larval development of other *Arcotheres* species has been studied before and no larval stage is known to lack eyes (e.g., Watanabe & Hemni, 2009).

Species within the *Dissodactylus* complex are commonly used as model organisms to examine host recognition in pinnotherids (Souza et al., 2018; Jossart et al., 2020), but more species have been studied in this regard (Becker & Türkay, 2017). The antennulae were identified as the principal structures of chemoreception in all studied species (Derby & Atema, 1980; Becker & Türkay, 2017) and no variation among different pinnotherine lineages is known. In

addition to the setae on the antennulae, other setae types have a chemoreceptive function in brachyuran crabs as well (Davie et al., 2015) and male crabs often possess elongated setae near the eyes, such as in *Austinotheres angelicus* (Campos, 2002) and *Dissodactylus primitivus* (Jossart et al., 2020). Located near the antennulae are the antennae, which emit chemical (excretory) cues. Some pinnotherine species are attracted to conspecifics (e.g., T*unicotheres moseri* (Rathbun, 1918) Becker & Türkay, 2017), which is likely due to chemical cues emitted from the antennal glands (green glands). The morphology of antennae was discussed by previous authors for their supposed taxonomic relevance (Ng et al., 2019; Campos & Hernández-Aguilera, 2020).



2.5 Morphology of the third maxillipeds in pinnotherines. A) *Pinnaxodes floridensis* Wells & Wells, 1961, after Wells and Wells (1961); B) *Afropinnotheres ratnakara* Ng & Kumar, 2015, after Ng and Kumar (2015); C) *Dissodactylus schmitti* Griffith, 1987, exopod not illustrated, after Griffith (1987a); D) *Ostracotheres cynthiae* Nobili, 1906, after Ahyong (2018). Roman numerals indicate different segments: I: fused ischium and merus, II: carpus, III: propodus, IV: dactylus. Scale A, B, D = 1 mm; C = 0.5 mm.

The third maxillipeds cover the other mouthparts and are also located anteriorly. These structures are thought to play a major role in feeding and are among the most important structures mentioned in studies on pinnotherid taxonomy and evolution. The pinnotherid third maxillipeds evolved to display two distinct features that most other crab families do not display and appear to be heavily modified for symbiotic life (Ahyong & Ng, 2009): (1) the ischium and merus are fused into an ischiomerus, with a suture only visible in *Pinnaxodes* (fig. 2.5A; Takeda & Masahito, 2000), but hardly apparent in all other genera; and (2) the dactylus is reduced in various species, leaving a two-segmented palp (fig. 2.5D; Campos, 1996b), or dislocated to the base of the propodus forming a ‘subchelate’ third maxilliped (Manning, 1993a). The features of the third maxilliped have been used as characters to distinguish species and genera (Campos, 1990; Manning, 1993a; Ahyong & Ng, 2005; Ahyong, 2018), but the systematic relevance of the third maxilliped morphology was recently questioned,

because of the high intrageneric variation in the genera *Nepinnotheres* (Ahyong & Ng, 2020), *Calyptraeotheres*, and *Dissodactylus*. Additionally, the third maxilliped appears to provide little significance in recognising phylogenetic lineages (Campos & Hernández-Aguilera, 2020). The three-segmented palp (consisting of a carpus, propodus and dactylus, articulated with a fused ischiomerus) is known from most genera and is thought to be plesiomorphic. A two-segmented palp (consisting of a carpus and propodus) is known from a few genera (*Austrotheres*, *Calyptraeotheres*, *Discorsotheres*, *Dissodactylus*, *Gemmotheres*, *Latatheres*, *Nannotheres*, *Ostracotheres* (fig. 2.5D), and *Tunicotheres*) and is thought to be an apomorphic character (Ahyong, 2018; Campos & Hernández-Aguilera, 2020). Additionally, a three-segmented palp has been observed in one specimen of *Discorsotheres spondyli* (Nobili, 1905) (a species with a known two-segmented palp) and is thought to be an anomaly (Ahyong, 2018).

Although the palp might not have the once-thought systematic significance (Manning, 1993a), it may be relevant for studies focusing on functional morphology. The palps are usually covered with long (feathery) setae and are thought to be used for various feeding strategies: they may be used by bivalve-associated pea crabs, enabling them to grasp host mucus from their own ambulatory legs or chelae, or directly from the hosts' gills (Becker & Türkay, 2017). Another strategy would be to filter planktonic food from the bypassing water, as suggested for some holothurian-associated genera (such as *Pinnaxodes* (fig. 2.5A), *Holotheres*, and *Holothuriophilus* (Wells & Wells, 1961; Ng & Manning, 2003; Ocampo, 2012). Species of the bivalve-associated *Afropinnotheres* are known for their disproportionately large dactyli of the third maxillipeds (fig. 2.5B) and might use the third maxillipeds in a similar way (Manning, 1993a). Similarly, Christensen and McDermott (1958) suggested that pea crabs living in the atrial cavities of ascidians (in this case *Pinnotheres pugettensis* Holmes, 1900, *P. taylori* Rathbun, 1918, and *Nepinnotheres pinnotheres*) use similar strategies for feeding. On the other hand, species of the ascidian-associated *Tunicotheres* bear no dactyli on the third maxillipeds, so this is likely not the case (Campos, 1996b). The authors also mentioned that immature crabs of *Zaops ostreum* possess feathery mouthparts and loose them in later stages, while switching feeding strategy (see below: Ambulatory Leg Adaptations; Christensen & McDermott, 1958). Most species within the tube- and burrow-dwelling subfamilies Pinnixinae, Pinnixulalinae, and Pinnothereliinae have extremely long setae on their dactyli of the third maxillipeds, thought to be used for feeding (Christensen & McDermott, 1958). More evidence for an ecomorphological role of the palp of the third maxilliped can be found in some species lacking a dactylus (or having a seemingly dysfunctional dactylus):

species of *Dissodactylus* and *Clypeasterophilus* bear very small dactyli on their third maxillipeds (fig. 2.5C) and are known to feed on the spines and tube feet of their sea urchin hosts (see below), instead of eating planktonic material and/or mucus (Griffith, 1987b). Similarly, members of the bivalve-and ascidian-associated *Calyptraeotheres* and gastropod-associated *Orthotheres* also appear to possess very small dactyli on their third maxillipeds (Griffith, 1987a), whereas most other mollusc- and ascidian-associated genera would possess well-developed dactyli.

Pea crabs have a wide range of epipod shapes for internal grooming of the gills, but their morphologies are probably not directly related with their host choice and dietary habits (Pohle, 1989). Pohle (1989) found groups of anchor-shaped outgrowths (setules) in setae on the epipods of the maxilla, maxillulae, and maxillipeds, in members of the genera *Opisthopus*, *Dissodactylus*, *Pinnaxodes*, and the unrelated (non-pinnotherine) *Pinnotherelia* (1989). Pohle did not only study the epipods of pinnotherines, but also the number of gills (Pohle & Marques, 1998). Pohle and Marques (1998) found that the number of gill pairs in pinnotherid crabs could vary between species, while the number is constant in most other brachyuran families. Representatives from the genera *Opisthopus*, *Pinnaxodes*, *Calyptraeotheres*, *Tumidotheres*, *Orthotheres*, *Tunicotheres*, and *Nepinnotheres* appear to have four pairs of gills, while members of *Durckheimia*, *Ostracotheres*, *Xanthasia*, *Limotheres*, *Arcotheres*, and *Zaops* appear to have three pairs of gills. The genera *Dissodactylus*, *Clypeasterophilus*, and *Pinnotheres* have three or four gill pairs, depending on the species. Pohle and Marques (1998) mentioned that this low number of gills is probably the result of a symbiotic lifestyle, rather than the crabs' size: the smaller species within the genus *Aphanodactylus* (Pinnotheroidea: Aphanodactylidae) were found to have more gill pairs than the larger bivalve-associated pinnotherines.

Although they are seldomly illustrated, the other five pairs of mouthparts (mandibles, maxillae, maxillulae, and first and second pair of maxillipeds) may possess phylogenetically significant anatomical characters (as in palaemonid shrimps; Fransen, 2002). In addition, they may be linked to dietary preferences: symbiotic amphipods appear to have specialised mouthparts, depending on their host and dietary preferences (Vader, 1983). Similarly, crabs feeding on bivalve mucus may possess other mouthpart characters than crabs feeding on sea urchin spines.

Cheliped Morphology

While crabs from other brachyuran lineages may use their chelipeds for feeding, defense, intraspecific aggression, and/or courtship (Davie et al., 2015), the

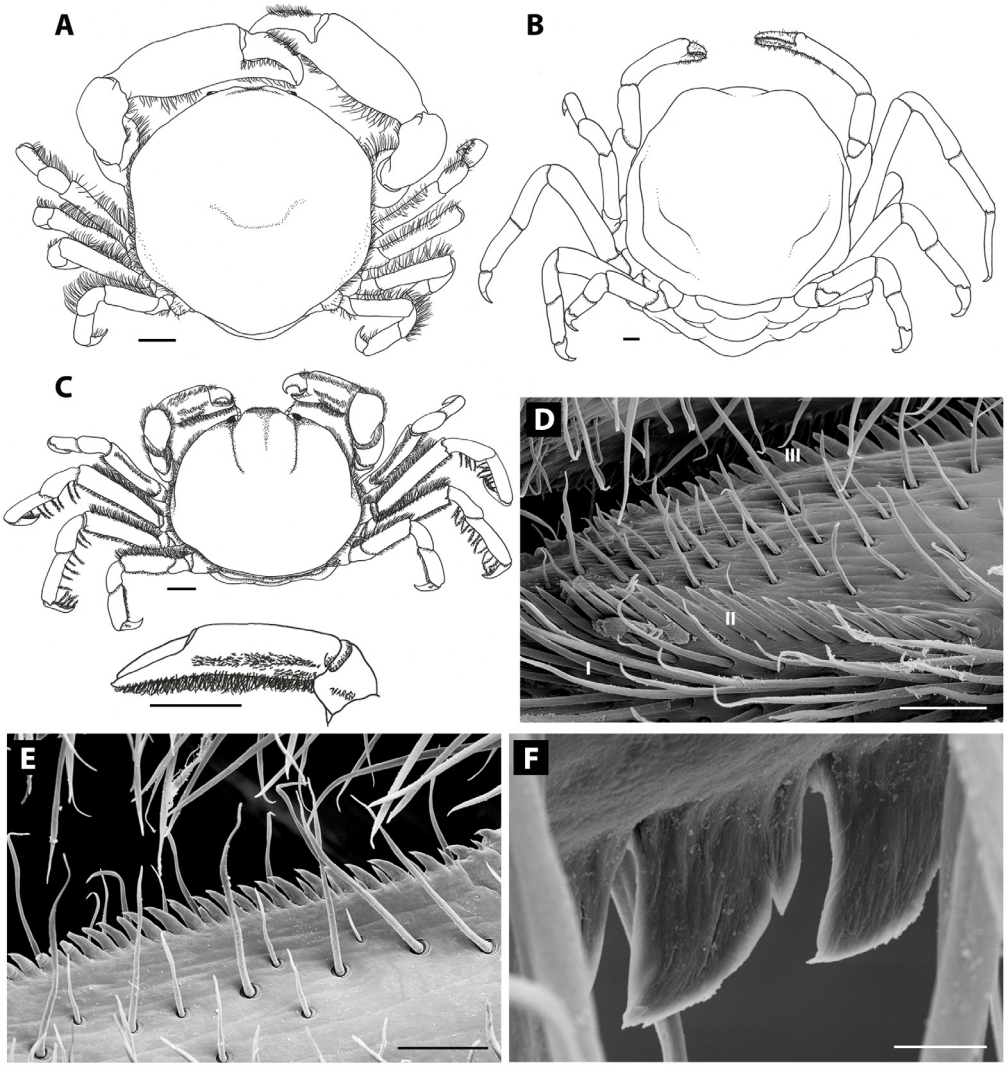
chelipeds of pinnotherine species were previously believed to only play a role in feeding strategies (Becker & Türkay, 2017). Similar to the morphology of the carapace, the chelae display a wide range of shapes and sizes, including ornamentations like setation and specialised feeding structures. For instance, the relatively largest (relative to body size) and most robust chelae (robustness: chela circumference/length; Telford, 1982) can be found in species associated with holothurians and hosts with a similar internal morphology. The robust chelae are most pronounced in members of *Austrotheres*, *Holothuriophilus*, *Holotheres* (fig. 2.6A), *Buergeres*, *Pinnaxodes*, and *Trichobezoares* (e.g., Ng & Manning, 2003; Campos, 2016). Similar robust chelae, however, can also be found in the free-living genus *Hospitotheres*, the tunicate-associated genus *Tunicotheres*, and a few members of the bivalve-associated genera *Tumidotheres* and *Nepinnotheres* (Campos, 1996b; Manning, 1993a; Palacios Theil & Felder, 2019) The function of the robust chelae of the before-mentioned genera is not well understood, but the specialised third maxillipeds and position within the host of the holothurian-associated genera (see above: Frontal Appendages and Mouthparts) suggest that the chelae do not play a major role in the feeding strategies (Wells & Wells, 1961). In support of this hypothesis, it is worth noting that *Buergeres deccanensis* (Chopra, 1931) is known to inflict damage to its host, by piercing the body wall with its chelae while inhabiting the respiratory system (Jones & Mahadevan, 1965).

The somewhat robust chelipeds of the species within the ectosymbiotic sea urchin-associated genera *Dissodactylus* and *Clypeasterophilus* have been studied in detail (Telford, 1982). The species within these two genera display a range of different sizes of the chelipeds and morphologies of the cutting edges of both fingers, which is thought to be linked to the dietary habits (Telford, 1982) and the ability to attach themselves to the hosts (Bell, 1984; De Bruyn, 2010). Telford (1982) stated that the porosity of the urchin's spines is directly linked to the robustness and cutting morphology of the associated crabs' chelae. For example, the species *Dissodactylus mellitae* (Rathbun, 1900) possesses very robust chelae, which are perfectly adapted for clipping more porous spines. Another species, *Clypeasterophilus rugatus* (mentioned by Telford (1982) as *D. calmani* Rathbun, 1918), has comparatively slender chelae, thought to be adapted for feeding on soft tube-feet (podia). Telford (1982) mentioned that the most common host of *C. rugatus*, the echinoid *Clypeaster rosaceus* (Linnaeus, 1758), is the host with the least porous spines, which are the most difficult to clip. In addition, *D. primitivus* was thought to be the least adapted and most evolutionarily primitive of the studied species (Telford, 1982), and *C. rugatus* the species with the most derived (or adapted) traits (Griffith, 1987a), but these hypotheses are rejected in recent molecular analyses (Palacios Theil et al.,

2016), placing *C. rugatus* at a basal position of the clade.

Very slender chelipeds can be found in most of the bivalve-associated genera, reaching most extreme shapes in *Amusiotheres* (fig. 2.6B), *Durckheimia*, *Discorsotheres*, *Solenotheres*, and *Tacitotheres* (Ahyong & Ng, 2005, 2007a; Ng & Ngo, 2010, Ng & Ho, 2016a; Ahyong, 2018). The lack of prominent teeth on the cutting surfaces of the chelae, and the elongated mani in most of these species, suggest that chelae are not used for cutting, but for brushing mucus and grooming (e.g., *Pinnotheres pisum*; Becker & Türkay, 2017). A common associated feature with such elongated chelae is a setal ornamentation of the inner surface of the palm and pollex. This brush-like row of setae can be found in female specimens of many genera associated with bivalves: *Abyssotheres*, *Afropinnotheres*, *Amusiotheres*, *Arcotheres*, *Austrotheres*, *Bonita*, *Fabia* (fig. 2.6C), *Gemmotheres*, *Discorsotheres*, *Durckheimia*, *Latatheres*, *Nannotheres*, *Nepinnotheres* (but not N. pinnotheres), *Pinnotheres* (fig. 2.6D), *Sindheres*, *Tacitotheres*, *Viridotheres*, *Visayeres*, *Xanthasia*, *Waldotheres*, and *Zaops* (Manning, 1993a, 1996; Manning & Felder, 1996; Campos, 1996a, 2009, 2016; Campos & Manning, 2000; Kazmi & Manning, 2003; Ahyong & Ng, 2005, 2007a, 2007b; Komatsu & Ohtsuka, 2009; Ng & Ho, 2016a; Becker & Türkay, 2017; Ahyong, 2018). This adaptive feature can also be found in two genera associated with gastropods, *Ernestotheres* and *Calyptraeotheres* (Manning, 1993a; Campos, 1990), and in the sea urchin-associated *Dissodactylus latus* Griffith, 1987 (Griffith, 1987b). After being mentioned in taxonomic papers several times, Becker and Türkay (2017) showed the setae row for the first time in detail, using SEM, and found the setae to be of the long regularly orientated pappo-serrate type in *Pinnotheres pisum* (fig. 2.6D). The same species was observed and even photographed feeding from strands of nutrient-rich mucus hanging from the gills of their bivalve hosts, using the setal comb. Similarly, the pinnixine crab *Scleroplax faba* (Dana, 1851) is also known to feed from mucus strands from bivalve hosts, similar to bivalve-inhabiting pinnotherines (Zmarzly, 1992). This species possesses a setose surface on the inner surface of the chelae, but lacks the specialised setal comb discussed above.

Additionally, rows of soft denticles, accompanied by soft setae on both sides of the claw, were found on the cutting edges of both the pollex and the movable finger of *Pinnotheres pisum* (Becker & Türkay, 2017), *P. pectunculi* Hesse, 1872 (fig. 2.6E, F), and *Nepinnotheres pinnotheres* (Becker, pers. obs.). The mechanical properties of the denticles were revealed during preparation for SEM (fig. 2.6D–F), as the denticles appeared soft during preparation, making the preservation and study difficult (Becker, pers. obs.). These three species were also found to possess a short row of similar, but longer, denticles on the



2.6 Morphology of the chelipeds and associated ornamentations in pinnotherines. A) Enlarged chelae in *Holotheres danielae* Ahyong, 2010, after Ahyong (2010); B) *Amusiotheres obtusidentatus* (Tai et al., 1980), after Ng and Ho (2016a); C) *Fabia subquadrata* Dana, 1851, note the setal comb on the inner side of the claw, after Campos (1996a); D) *Pinnotheres pisum* (Linneaus, 1767), note the pappo-serrate setal comb (I), the short row of soft denticles on the inner surface of the pollex tip (II), and the similar denticles on the cutting edges of the claw (III); E) *Pinnotheres pectunculi* Hesse, 1872, with a row of soft denticles; F) *Pinnotheres pectunculi* Hesse, 1872, detail of one of the scales, note the rough surface and the serrated tips. Scale A–C = 1 mm; D, E = 100 µm; F = 10 µm.

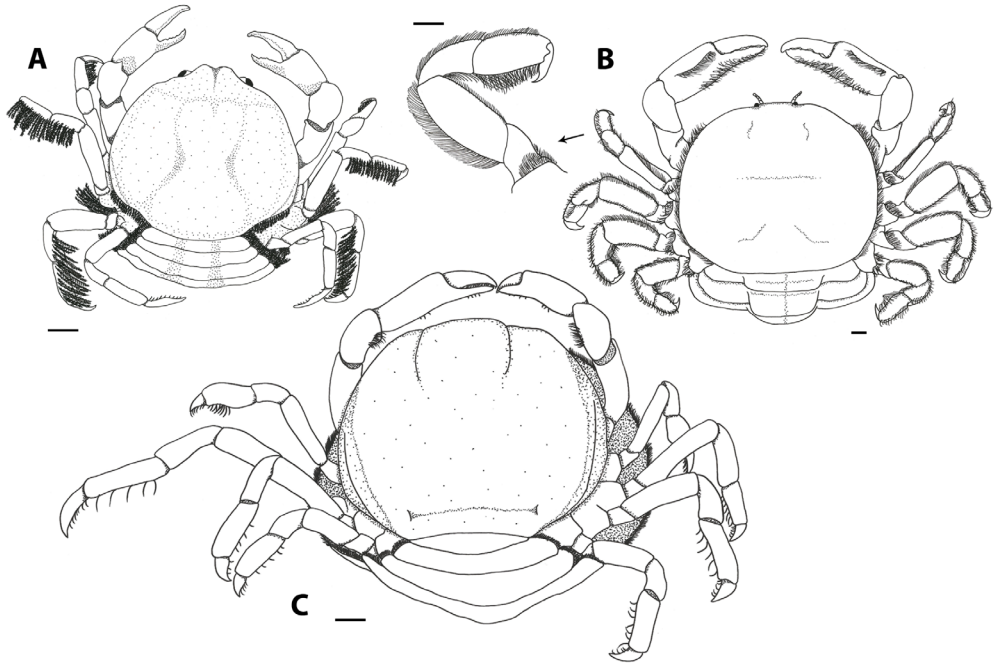
inner side of the tip of the pollex (fig. 2.6D). A quick survey of the available taxonomic literature reveals more species that possess the small denticles on the cutting edges of the chelae: *Pinnotheres haiyangensis* Shen, 1932,

P. dilatatus Shen, 1932, and *P. luminatus* Tai et al., 1980, were all illustrated by Tai and Yang (1991) with small denticles on the inner surface of both the pollex and the movable finger. More recently, *Sindheres karachiensis* Kazmi & Manning, 2003, was illustrated and described with special attention to the denticles, looking similar to those mentioned above (Kazmi & Manning, 2003). A thorough survey of these and other species is needed to confirm if the row of denticles is homologous to the row found in *Pinnotheres pisum*, *P. pectunculi* and *Nepinnotheres pinnotheres*, and whether this character is present in more pinnotherine species. The function of these denticles is not known, but the position and the softness of the structures suggest that they are not used for scraping host mucus (Becker, pers. obs.). The soft denticles might, however, play a role in chemoreception, where the crabs use their chelae's soft denticles to 'taste' their food before digesting it. Similar soft denticles can be found in many more crab species and this feature is not limited to pinnotherids (Becker, pers. obs.). The denticles in the studied pinnotherids can be observed to have a rough surface and serrate tips, potentially bearing pores similar to the ones found on the chelae of the hermit crab *Pagurus hirsutiusculus* (Dana, 1851) (Mesce, 1993). This row of denticles resembles structures found on the first chelipeds of some palaemonid shrimp species, living in association with bivalves and ascidians (Fransen, pers. comm.).

Ambulatory Leg Adaptations

In all symbiotic brachyuran crab lineages, most adaptive features can be found in the morphology of the ambulatory legs (Castro, 2015). A few examples are the last pair of ambulatory legs of sponge crabs (Dromiidae) and carrier crabs (Dorippidae), the subchelate ambulatory legs of zebra crabs (Pilumnidae: Eumedoninae), and flexible dactylo-propodal articulation of coral-clinging crabs (Tetraliidae) (Castro, 2015). The Pinnotheridae form no exception, since the most apparent feature of the tube-dwelling pinnixine, pinnixulaline, and pinnothereliine crabs are the wide third pair of ambulatory legs for gripping the walls of shared burrows and tubes (Palacios Theil & Felder, 2020). The Pinnotherinae have more subtle morphological adaptations of the ambulatory legs, which are discussed below.

The most apparent ontogenetic changes can be seen in the morphology of the ambulatory legs. In both reproductive strategies (Campos, 1989a), the hard stage males possess long plumose swimming setae, usually on the second and third ambulatory legs (e.g., described from *Pinnotheres pisum* (in Hartnoll, 1972) and *Zaops ostreum* (in Stauber, 1945)). The hard stage crabs swim between hosts and use their long setae for swimming by "bending their chelae slightly inward and by holding the first and fourth ambulatory legs stationary in



2.7 Morphology of the ambulatory legs in pinnotherines. A) *Tumidotheres margarita* (Smith, 1869), after Campos (1989a); B) *Ernestotheres conicola* (Manning & Holthuis, 1981), note the flattened ambulatory legs, after Manning (1993a); C) *Fabia carvachoi* (Campos, 1996), after Campos (1996a). Scale A–C = 1 mm.

an inverted V-shape, and by fast stroking both sides of the second and third ambulatory legs back and forth sequentially" (Hsueh & Huang, 1996). In some species, swarming of post-hard staged males and females is known, even after the initial infestation. In this case, the crabs also develop new swimming setae (known from members of *Calyptraeotheres* (Ocampo et al., 2017), *Tumidotheres* (fig. 2.7A) (Campos, 1989a, 2016), *Austrotheres* (Ahyong, 2018), *Fabia* (Pearce, 1966), and seemingly from species of *Afropinnotheres* (Manning, 1993a), *Ostracotheres* (Ahyong, 2018), *Nepinnotheres* (Ahyong & Ng, 2020), and *Pinnotheres* (Hseuh & Huang, 1996)). In addition, some species are known to develop similar secondary swimming setae, but in a later moulting stage: Watanabe and Henmi (2009) found that one female crab (an unidentified species within the genus *Arcotheres*) developed swimming setae in a post-hard stage, after forming simple setae at first. A similar development was found in post-hard stages of *Pinnotheres pisum* (Atkins, 1958), but the author does not mention whether the setae are of simple or plumose type (Watanabe & Hemni, 2009). The secondary development of plumose swimming setae in post-hard stages might be a strategy for crabs to leave their host when circumstances are unfavourable (e.g., when starving; Watanabe & Hemni, 2009).

Similar to the overall shape and size of the chelipeds, the ambulatory legs of pinnotherines also display a wide range of shapes and sizes. The widest legs among pinnotherines, just like the most robust chelae, are again found in holothurian- and geoduck-associated genera like *Pinnaxodes* and *Holothuriophilus* (Campos, 2016). Members of the gastropod-associated genera *Mesotheres*, *Ernestotheres* (fig. 2.7B), and to some extent *Orthotheres*, have flattened, broad ambulatory legs (Manning, 1993a; Ng & Ho, 2016b; Ng et al., 2019), probably to cling to their large, mobile hosts. In contrast, members of *Waldotheres*, *Amusiotheres*, *Tacitotheres*, *Zaops*, and most other bivalve-associated genera have elongated, slender, and feeble ambulatory legs. This indicates that they do not leave their sedentary host, and rarely move around within the host (Campos, 1989a). Members of *Zaops* might form an exception in having swollen propodi of the ambulatory legs, similar to the ambulatory legs of *Raytheres* (Campos, 2002). It remains unknown whether the swollen propodi are an adapted feature.

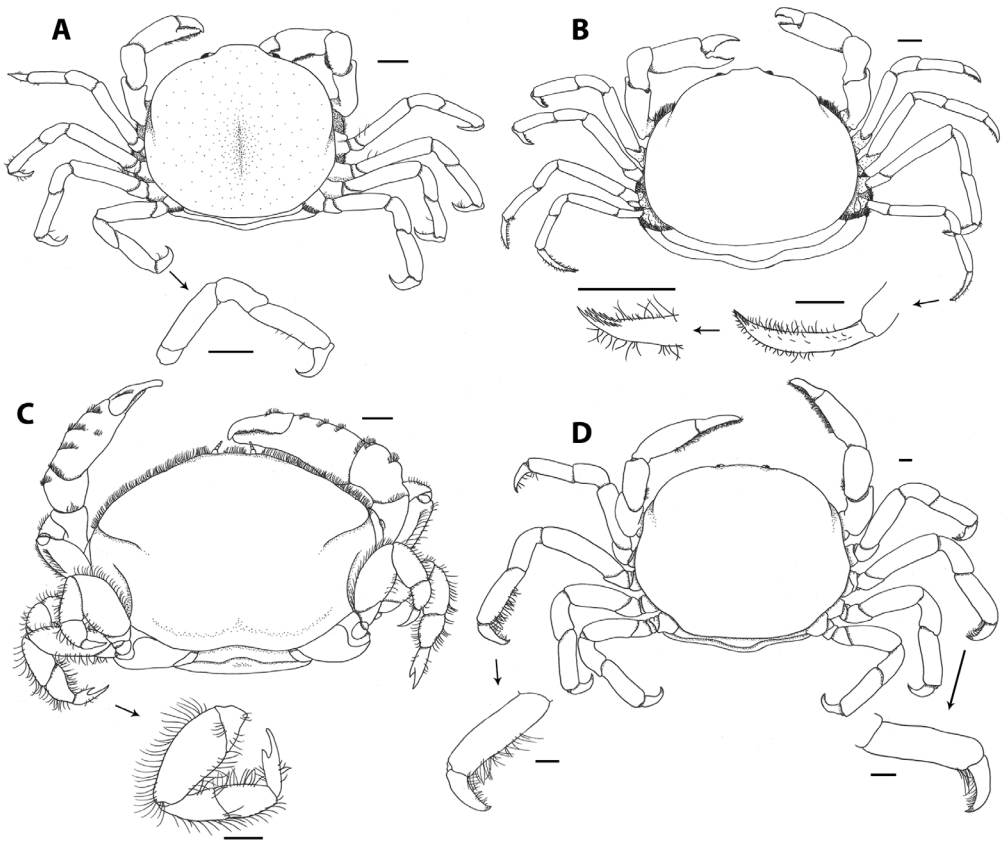
The different sizes of ambulatory legs in pinnotherines have also been studied in detail, with special focus on the elongation of just one leg after the hard stages (Gordon, 1936; Griffin & Campbell, 1969; Campos & Manning, 2001; Campos, 2001, 2013). This asymmetry of the ambulatory legs is thought to be linked to the feeding habits and the initial settlement of the female crabs inside the host (Becker & Türkay, 2017). In laboratory experiments, Watanabe and Henmi reared a member of the genus *Arcotheres* and found that the longer ambulatory leg of this species developed on the side of the crab which was directed to the opening of the bivalve host (Watanabe & Henmi, pers. comm. in Becker & Türkay, 2017). While the elongation of the single leg segments may vary between species and genera, in most cases, the dactylus and propodus of the elongated ambulatory leg possess morphological adaptations, seemingly for ‘reeling in’ mucus strands (discussed below), similar to the modified cheliped mentioned above. Asymmetry of the ambulatory legs is not limited to, but is most apparent in the bivalve-associated genera *Amusiotheres*, *Discorsotheres*, *Fabia* (fig. 2.7C), *Solenotheres*, *Tacitotheres*, and *Zaops* (Campos, 2013; Ahyong, 2018; Ng et al., 2019). Extremely asymmetrical legs can also be found in the limpet-associated *Enigmatheres* (Campos, 2009).

Most variation in the ambulatory legs can be found in the most distal segment, the dactylus. For instance, the previously mentioned ectosymbiotic genera *Dissodactylus* and *Clypeasterophilus* have bifurcate (‘forked’) dactyli in their first, second, and third pair of ambulatory legs (fig. 2.8C), which are thought to aid in moving between the spines of their host urchins and sand dollars (Rathbun, 1918; Bell, 1984; Griffith, 1987a; Campos & Griffith, 1990). Similarly,

one species within *Abyssotheres* (A. abyssicola (Alcock & Anderson, 1899)) has an "obtuse projection on the dorsal surface of the dactylus of the walking legs", but this seems to be a unique feature, even within the genus (Manning & Galil, 2000). Morphological adaptations in the dactyli of other species can also be linked to their host choice and position inside the host: the holothurian-associated *Holotheres halingi* (Hamel et al., 1999) and its congeners possess falcate, sharp dactyli in all ambulatory legs, used to cling to the inner surface of the host. The description of *Holotheres halingi* mentions the species to be favouring lateral contact more than bottom contact, and the species seems to be unable to walk due to its enlarged pleon and modified ambulatory legs (Hamel et al., 1999). Morphologically similar falcate dactyli can be found in a wide range of pinnotherine genera, not limited to holothurian symbionts: *Discorsotheres*, *Durckheimia*, *Latatheres*, *Orthotheres*, *Ostracotheres*, *Serenotheres*, *Solenotheres*, *Tridacnatheres*, *Visayeres* (fig. 2.8A), *Xanthasia*, and some species of *Nepinnotheres* (Ahyong & Ng, 2005, 2007a, 2007b; Ng & Ngo, 2010; Ahyong, 2018).

Members of the bivalve-associated genus *Arcotheres* are unique with regard to a few morphological features (Campos, 2001), most obvious in the form of the dactyli of the last pair of ambulatory legs. The dactyli are described as 'sword-shaped' (Campos, 2001), being straighter and more elongated (longer or of equal length as the attached propodus) than the dactyli of the other ambulatory legs (fig. 2.8B) (Ahyong & Ng, 2007a). The dactyli of the last pair of ambulatory legs are often ornamented with rows of short, simple setae (e.g., *A. ridgewayi* (Southwell, 1911); illustrated in Ng et al. (2017)) and a row of denticles (e.g., most conspicuous in *A. placunae* (Hornell & Southwell, 1909), illustrated in Trivedi et al. (2018), and *A. vicajii* (Chhapgar, 1957) in Trivedi et al. (2020).

The functions of these setae and denticles are not known, but since the last pair of ambulatory legs is generally shorter than the third pair in *Arcotheres*, it is improbable that crabs use these legs to 'reel in' host mucus. The denticles, however, resemble those on the chelipeds' cutting edges mentioned above (see Cheliped Morphology), and may be used to scrape or groom their host, or even their own bodies for gathering mucus: some species are illustrated with their last pair of ambulatory legs being folded up against the dorsal side of their carapace (e.g., *A. borradailei* (Nobili, 1906); Ng et al., 2017). In addition, the denticles might be used for chemoreception, by 'tasting' food with its ambulatory legs, as oberved in *Zaops ostreum* (Stauber, 1945), or for providing grip inside the host: similar rows of scales can be found on the dactyli of the ambulatory legs of some palaemonid shrimp species, also living in bivalves and ascidians (Fransen, 1994, 2002; de Gier & Fransen, 2018). Furthermore,



2.8 Morphology of the ambulatory legs and dactyli in pinnotherines. A) *Visayeres acron* Ahyong & Ng, 2007, with falcate ambulatory leg dactyli, after Ng & Ahyong (2007b); B) *Arcotheres similis* (Bürger, 1895), with sword-shaped last ambulatory leg dactyli, with microstructured dactylus tip, after Ng & Ahyong (2007a); C) *Dissodactylus schmitti* Griffith, 1987, with bifurcate second and third ambulatory leg dactyli, after Griffith (1989b); D) *Discorsotheres spondyli* (Nobili, 1905), with asymmetrical setation on the propodus and dactylus of the second pair of ambulatory legs, after Ahyong (2018). Scale A–D = 0.5 mm.

all members of the gastropod-associated *Calypraeotheres*, except *C. garthi*, also possess sword-like, setose dactyli on their last ambulatory legs (Campos, 1990, 1999; Hernández-Ávila & Campos, 2006; Campos & Hernández-Ávila, 2010; Ayón-Perente & Hendrickx, 2014). The function and microstructure of the appendages remain unknown. Moreover, some species of *Pinnotheres* also possess a similar dactylus on both their last ambulatory legs (see table 2.1).

In addition to *Arcotheres* and *Calyptraeotheres*, species in many other genera possess inconspicuous ornamentations on their leg segments, but the arrangement may vary between genera. For example, simple setae are found on the dactyli of the fourth ambulatory legs of species within *Gemmotheres* and

Tunicotheres (Campos, 1996b), and similar setae are found on the propodus and dactylus of members of the genus *Discorsotheres*, with some species even showing asymmetry between the leg setation (fig. 2.8D) (Ahyong, 2018). The functionality of this specific setation is probably linked to the feeding strategy of these species, although this has only been observed in *Zaops ostreum* (Stauber, 1945; Christensen & McDermott, 1958). The observed crabs feed in a similar way as *Pinnotheres pisum* (Becker & Türkay, 2017) by gathering mucus strings, but Stauber (1945) observed that they "catch newly formed mucus with the (distally setose) ambulatory legs, then reach underneath the pleon with their chelipeds, comb the legs, and pass the food on to the mouth" (Christensen & McDermott, 1958).

The detailed illustrations and ecological information provided by Zmarzly (1992) allow for a quick survey of the potentially adapted morphology of symbiotic pinnixine species. Both the pinnixine crab species *Scleroplax faba* and *S. littoralis* (Holmes, 1895) are known as endosymbionts in holothurians and bivalves respectively, and possess falcate dactyli on their ambulatory legs, thought to aid in attaching themselves to their host. *Scleroplax faba* also appears to possess uniform ambulatory leg lengths, atypical for members of the Pinnixinae, Pinnothereliinae, and Pinnixulalinae.

Sexual Anatomy and Larval Characters

The reproductive strategies of Pinnotherinae, their larval development and sexual anatomy show several traits that seem important with regard to their symbiotic lifestyle. It is, however, hard to distinguish between adaptations that are characteristic for small-sized crabs in general and those that are specific to symbiotic lifestyles. Hines (1991) has shown that the investment in egg production (body weight/brood weight) in *Zaops ostreum* (as *Pinnotheres ostreum*) and *Fabia subquadrata* is highly increased compared to free-living crab species. Hartnoll (2006) reviewed the reproductive investment among a range of brachyuran crabs and concluded that metabolic costs drive trade-offs between growth (body size) on the one hand and reproductive investment (relative brood size) on the other. A large body size reduced the risk of predation, but may lead to reaching sexual maturity later in ontogeny and, also, to producing smaller broods (in relation to body size) as more energy resources go into growth (Hartnoll, 2006). Female bivalve-dwelling pinnotherines with a life cycle similar to *Pinnotheres pisum*, remaining solely within their host after starting metamorphosis, are not exposed to predators. Such species can, thus, 'afford' to invest a greater deal of energy resources in reproduction. By not being very mobile, they also save on metabolic costs for locomotion and do not need to search for food as they directly obtain it from their host.

Egg production, however, is also constrained by female body size in general and particularly by the space that is available for yolk accumulation inside the body. This may explain that pinnotherids, despite being generally small in order to be able to enter and fit inside their hosts, have a preference for large- over small-sized host species within their specific host range, as the same species reaches larger body sizes in more spacious hosts (Becker & Türkay, 2017). This again has an effect on the fecundity (eggs per brood) which is positively correlated with body size (carapace width) within a species (e.g., *Dissodactylus primitivus*, *D. crinitichelis* Moreira, 1901 in Telford (1978); *Austinotheres angelicus* in Salas-Moya et al. (2014)).

Another important adaptation regarding brood size is the large pleon of female pinnotherids which functions in incubating the eggs until larvae hatch. The pleon is extremely enlarged in bivalve-associated Pinnotherinae resembling *Pinnotheres pisum*: the pleon covers the whole ventral side of the crab, reaches the mouthparts anteriorly and even covers the proximal segments of the ambulatory legs (Becker, 2010). In fact, the pleon is enlarged to a degree that it seriously hampers locomotion of adult female *P. pisum* (Becker, pers. obs.). Due to spending their whole adult life inside the host, where females are protected from predators and have plenty of food, locomotion is not crucial for survival. A comparison of brood sizes (mass of brood and number of eggs), pleon width, and body size (body mass and carapace width) among various species may yield insights into the degree of reproductive investment, and the adaptations in relation to different hosts and life history strategies of Pinnotherinae. Unfortunately, the current knowledge on pleon sizes among pea crabs is very limited as no study has focused on this character so far, and only few taxonomic descriptions show the female in ventral aspect or details of the pleon in the presented line drawings (but see *Pinnaxodes floridensis*; Wells & Wells, 1961). It is, however, obvious that the female pleon of ectosymbiotic pinnotherine genera (*Dissodactylus* and *Clypeasterophilus*) is not as wide as in most endosymbiotic taxa. As in all brachyuran crabs, a sexual dimorphism in pleon width is also obvious in these genera, with the male having a narrow pleon and the female possessing a wide pleon for breeding. The female pleon in *Dissodactylus* and *Clypeasterophilus* is, however, not enlarged to a degree that it is visible from the dorsal view (George & Boone, 2003) and does not appear to prevent locomotion. The members of the ectosymbiotic genera need to retain the ability to move around on the host and escape predators.

Although the size relations and the outer morphology of the pleon have barely been studied, the internal morphology has caught interest in the past. In most brachyuran crabs, the ovaries are restricted to the cephalothorax and

do not extend into the pleon (McClay & Becker, 2015). In the bivalve-dwelling *Pinnotheres pisum* and *P. pectunculi*, and the ascidian and bivalve-dwelling *Nepinnotheres pinnotheres*, ovaries extend into the pleon and run along both sides of the digestive system (Becker et al., 2011). To date, it is unknown which other pinnotherine genera show the same extension of ovaries, but it is very likely the case for many endosymbiotic species with an extremely wide pleon. A study of male *P. pisum* and *N. pinnotheres* has shown that a corresponding adaptation is present in males: parts of the vas deferens, where gametes develop and seminal plasma is produced, reach into the narrow male pleon as well (Becker et al., 2013). This shows that the large size of the female pleon alone may not explain the adaptation of extending reproductive organs beyond the cephalothorax.

Interestingly, another symbiotic group of crabs shows the same adaptation as Pinnotherinae, at least in the females: several species of Cryptochiridae (gall crabs) associated with stony corals have ovaries extending into the pleon to a varying degree (Vehof et al., 2016). Also, in the free-living mangrove crab *Goniopsis cruentata* (Latreille, 1803), mature ovaries extend into the first pleomers (de Souza & Silva, 2009). This species is relatively small sized with females reaching maturity (L50) at 22.6 mm carapace width (Cobo & Fransozo, 2005). The trait of reproductive organs being extended from the cephalothorax into the pleon shows how hard it is to identify the responsible driver for evolutionary changes. Small body sizes and symbiotic/parasitic lifestyles similarly lead to peculiar sexual adaptations and an increase of the investment in reproduction (Strathmann & Strathmann, 1982; Bush et al., 2001). Most endosymbionts have smaller body sizes than their free-living relatives, thus, adaptations cannot be linked to body size or symbiotic lifestyle alone.

Other characters which may have significance with regard to the pinnotherid's symbiotic lifestyle can be found in the larval development and morphology: most brachyuran zoea larvae possess paired lateral spines, and a dorsal and rostral spine (Martin, 2014), which are either regarded as buoyancy structures for planktonic dispersal or as an antipredatory adaptation. Within the Pinnotherinae, larvae of *Pinnaxodes chilensis* (H. Milne Edwards, 1837) (Gonzales-Canales et al., 2018), *Clypeasterophilus rugatus* (Pohle, 1984), *Nepinnotheres pinnotheres* (as *Pinnotheres veterum* Bosc, 1801) (Lebour, 1928), and *Afropinnotheres monodi* (Marco-Herrero et al., 2016) also possess these spines, and confirm to the general brachyuran larval morphology. Several other species and genera of Pinnotherinae are known to lack the dorsal spine, such as *Pinnotheres pisum* and *P. pectunculi* (Becker, 2010), some are even completely spineless (e.g., *Zaops ostreum*; Morgan, 1987).

In the latter species, behavioural experiments revealed a specific behaviour when exposed to predators: zoea larvae of *Z. ostreum*, quickly sank to the sea floor when the pleon was flexed tightly against the body (Morgan, 1987). This behaviour was interpreted as an antipredatory defence (Morgan, 1987), but may also have benefits for pea crab larvae settling in a habitat with suitable hosts or in a large aggregation of hosts as in mussel or oyster beds. It it worth noting that pinnotherines are not the only group in which some species show a reduction of spines in zoeal stages; this adaptation is also found in species of Leucosiidae and Hymenosomatidae (Lebour, 1928; Martin, 2014), and may have evolved for similar reasons, either as an antipredatory strategy or for the control of larval settlement in suitable habitats (in both cases by being able to sink faster and more directional).

Some species of Pinnotherinae show an abbreviated larval development or even parental care. The larval development tends to be abbreviated in pinnotherines, by having only two to four zoeal stages, while most brachyuran crabs have five. In *Tunicotheres moseri*, symbiotic with ascidians (Goodbody, 1960), and *Mesotheres barbatus* (as Orthotheres barbatus (Desbonne in Desbonne & Schramm, 1867); Bolaños et al., 2004), only two zoea stages were found. *Tunicotheres moseri* also shows brood care: after the larvae hatch from the eggs, they are not dispersed, but remain under the female pleon until they reach the first crab stage (Bolaños et al., 2004).

Updated List of Symbiont-Host Associations

an updated symbiont-host association list for all recognised (valid) species within the Pinnotherinae is shown in table 2.1.

Table 2.1 Updated symbiont-host association list for all recognized (valid) species within the Pinnotherinae (previous lists include McDermott (2009), Soong (1997), and Silas & Alagarswami (1965)). Pinnotherine taxonomy follows WoRMS (2020) and the Systema Brachyurorum (Ng et al., 2008), unless stated otherwise in the subscripts (see below). References including the most complete information about host specificity can be found for all species. If recent works list new host species, but neglect the already known hosts from older literature (Schmitt et al., 1973), both the older and more recent references are included. Host nomenclature follows identifications provided in the references, also updated with WoRMS (2020). Where host groups or host species are unknown, a question mark is provided; where identifications of host species were unable to link with a recognized species, identifications are provided with a question mark between parentheses: (?). Notes on the host specificity or taxonomy can be found below, indicated with superscript numbers. Distribution abbreviations: IWP) Indo-West Pacific; EP) East Pacific; ATL) Atlantic Ocean (please note that Atlantic and East Pacific distributions also include non-tropical waters).

Table 2.1

Species	Host	Host Species	Distr.	References
Abyssotheres[1]				
A. abyssicola (Alcock & Anderson, 1899)	Bivalvia	*Acesta indica* (Smith, 1899)	IWP	Manning & Galil, 2000
A. acesticola Komatsu & Ohtsuka, 2009	Bivalvia	*Acesta philippinensis* (Bartsch, 1913)	IWP	Komatsu & Ohtsuka, 2009
Afropinnotheres				
A. crosnieri Manning, 1993	?	?	ATL	Manning, 1993a
A. dofleini Lenz, 1914[1]	Bivalvia, Ascidia	*Atrina squamifera* (Sowerby, 1835), *Perna perna* (Linnaeus, 1758), *Pinna* sp.; *Ascidia sydneiensis* (Stimpson, 1855), *Pyura stolonifera* (Heller, 1878)	ATL/ IWP	Ng, 2018a
A. guinotae Manning, 1993	Bivalvia	'unnamed bivalve mussel'	ATL	Manning, 1993a
A. larissae (Machkevskiy, 1992)	Bivalvia	*Crassostrea tulipa* (Lamarck, 1819)	ATL	Manning, 1993a
A. monodi Manning, 1993	Bivalvia	*Cerastoderma edule* (Linnaeus, 1758), *Cerastoderma glaucum* (Bruguière, 1789), *Chamelea gallina* (Linnaeus, 1758), *Donax trunculus* Linnaeus, 1758, Eastonia rugosa (Helbling, 1779), *Mactra stultorum* (Linnaeus, 1758), Mytilus galloprovincialis Lamarck, 1819, *Polititapes aureus* (Gmelin, 1791), *Ruditapes decussatus* (Linnaeus, 1758), *Scrobicularia plana* (da Costa, 1778), *Spisula solida* (Linnaeus, 1758), *Venerupis corrugata* (Gmelin, 1791)	ATL	Pérez-Miguel et al., 2019
A. ratnakara Ng & Kumar, 2015	Bivalvia	*Atrina vexillum* (Born, 1778), *Perna perna* (Linnaeus, 1758)	IWP	Ng & Kumar, 2016; Ng et al., 2017
Alain				
A. crosnieri Manning, 1998	Holothuria	*Molpadia* sp.	IWP	Manning, 1998

Table 2.1 *Cont.*

A. raymondi Ahyong & Ng, 2008	Holothuria	'unidentified deep-water holothurian'	IWP	Ahyong & Ng, 2008
Alainotheres				
A. leloeuffi (Crosnier, 1969)[2]	?	?	ATL	Manning, 1993a
Amusiotheres				
A. hanumantharaoi Devi & Shyamasundari, 1989	Bivalvia	*Amusium pleuronectes* (Linnaeus, 1758)	IWP	Devi & Shyamasundari, 1989
A. obtusidentatus (Tai in Tai, Feng, Song & Chen, 1980)	Bivalvia	*Amusium pleuronectes* (Linnaeus, 1758), *Ylistrum japonicum* (Gmelin, 1791)	IWP	Ng & Ho, 2016a
Arcotheres				
A. alcocki (Rathbun, 1909)	Bivalvia	*Atrina vexillum* (Born, 1778), *Meretrix* sp., *Meretrix casta* (Gmelin, 1791), *Mytilus* sp., *Pinna atropurpurea* (Sowerby, 1825), *Tegillarca granosa* (Linnaeus, 1758)	IWP	Schmitt et al., 1973; Kazmi et al., 2016
A. arcophilus (Bürger, 1895)	Bivalvia	*Arca* sp.	IWP	Schmitt et al., 1973
A. atrinae (T. Sakai, 1939)	Bivalvia	*Atrina japonica* (Reeve, 1858)	IWP	Sakai, 1939
A. boninensis (Stimpson, 1858)	Bivalvia	*Saccostrea echinata* (Quoy & Gaimard, 1835)	IWP	Schmitt et al., 1973
A. borradailei (Nobili, 1906)	Bivalvia	*Mya* sp., *Pinna* sp.	IWP	Schmitt et al., 1973
A. coarctatus (Bürger, 1895)	Bivalvia	*Polymesoda* spp.	IWP	Ahyong & Ng, 2007a
A. cyclinus (Shen, 1932)	Bivalvia	*Barbatia virescens* (Reeve, 1844), *Cyclina sinensis* (Gmelin, 1791), *Meretrix meretrix* (Linnaeus, 1758)	IWP	Tai & Yang, 1991
A. exiguus (Bürger, 1895)	?	?	IWP	Ahyong & Ng, 2007a
A. guinotae Campos, 2001	Bivalvia	*Barbatia* sp.	IWP	Campos, 2001

Table 2.1 *Cont.*

A. latifrons (Bürger, 1895)	?	?	IWP	Ahyong & Ng, 2007a
A. latus (Bürger, 1895)[3]	Bivalvia	*Atrina vexillum* (Born, 1778), *Pinna* sp.	IWP	Ahyong & Ng, 2007a
A. modiolicola (Bürger, 1895)	Bivalvia	*Mactra violacea* (Gmelin, 1791), *Marcia opima* (Gmelin, 1791), *Modiolus philippinarum* (Hanley, 1843)	IWP	Schmitt et al., 1973; Ahyong & Ng, 2007a
A. nudifrons (Bürger, 1895)	?	?	IWP	Ahyong & Ng, 2007a
A. obesus (Dana, 1852)	Bivalvia	*Arca* sp., *Cytherea* sp., *Gafrarium* spp., *Sunetta subquadrata* (Sowerby, 1851)	IWP	Schmitt et al., 1973; Komai et al., 2020
A. ocularius Komai, Kawai & Ng, 2020	Bivalvia	*Andara* spp.	IWP	Komai et al., 2020
A. palaensis (Bürger, 1895)	Bivalvia	*Anadara antiquata* (Linnaeus, 1758), *Arca* sp., *Barbatia foliata* (Forsskål in Niebuhr, 1775), *Mactra grandis* Gmelin, 1791, *Placuna ephippium* (Philipsson, 1788), *Tegillarca granosa* (Linnaeus, 1758)	IWP	Schmitt et al., 1973
A. pernicola (Bürger, 1895)	Bivalvia	*Perna* sp.	IWP	Ahyong & Ng, 2007a
A. placunae (Hornell & Southwell, 1909)	Bivalvia	*Placuna placenta* (Linnaeus, 1758)	IWP	Schmitt et al., 1973
A. placunicola Ng, 2018	Bivalvia	*Placuna ephippium* (Philipsson, 1788)	IWP	Ng, 2018b
A. pollus Ahyong & Ng, 2020	Bivalvia	*Malleus albus* (Lamarck, 1819)	IWP	Ahyong & Ng, 2020
A. purpureus (Alcock, 1900)	Bivalvia	*Ostrea* sp., *Protapes gallus* (Gmelin, 1791)	IWP	Mohanty et al., 2018
A. rayi Ahyong & Ng, 2007	Bivalvia	?	IWP	Ahyong & Ng, 2007a
A. rhombifer (Bürger, 1895)	Bivalvia	*Tucetona auriflua* (Reeve, 1843)	IWP	Ahyong & Ng, 2007a
A. ridgewayi (Southwell, 1911)[3]	Bivalvia	*Pinna atropurpurea* (Sowerby, 1825)	IWP	Ahyong & Ng, 2007a

Table 2.1 *Cont.*

A. rotundatus (Bürger, 1895)	Bivalvia	*Circe* sp.	IWP	Ahyong & Ng, 2007a
A. shahi Trivedi, Campos & Vachhrajani, 2018	Bivalvia	*Magallana bilineata* (Röding, 1798)	IWP	Trivedi et al., 2018
A. similis (Bürger, 1895)	Bivalvia	*Ostrea* sp., *Placuna placenta* (Linnaeus, 1758)	IWP	Schmitt et al., 1973
A. sinensis (Shen, 1932)	Bivalvia	*Alectryonella plicatula* (Gmelin, 1791), *Chlamys nipponensis* (?), *Magallana angulata* (Lamarck, 1819), Magallana gigas (Thunberg, 1793), *Meretrix lusoria* (Röding, 1798), *Modiolus auriculatus* (Krauss, 1848), *Mytilus unguiculatus* Valenciennes, 1858, *Mytilus edulis* Linnaeus, 1758, *Ruditapes philippinarum* (Adams & Reeve, 1850), *Venerupis aspera* (Quoy & Gaimard, 1835)	IWP	Schmitt et al., 1973; Kuo et al., 2018
A. spinidactylus (Gordon, 1936)	Bivalvia	*Modiolus philippinarum* (Hanley, 1843), *Modiolus* sp.	IWP	Schmitt et al., 1973
A. tivalae (Gordon, 1936)	Bivalvia	*Tivela stefaninii* (Nardini, 1933)	IWP	Schmitt et al., 1973
A. vicajii (Chhapgar, 1957)	Bivalvia	*Marcia recens* (Holten, 1802), *Mercenaria* sp., *Meretrix casta* (Gmelin, 1791), *Perna viridis* (Linnaeus, 1758)	IWP	Kazmi et al., 2016; Trivedi et al., 2020
A. winckworthi (Gordon, 1936)	Bivalvia	*Protapes gallus* (Gmelin, 1791)	IWP	Schmitt et al., 1973
Austinotheres				
A. angelicus (Lockington, 1877)	Bivalvia	*Modiolus capax* (Conrad, 1837), *Ostrea angelica* Rochebrune, 1895, *Saccostrea palmula* (Carpenter, 1857)	EP	Schmitt et al., 1973; Salas-Moya et al., 2014
Austrotheres				
A. holothuriensis (Baker, 1907)	Holothuria, Ascidia	*Herdmania grandis* (Heller, 1878), 'holothurians'	IWP	Ahyong, 2018
A. pregenzeri Ahyong, 2018	Ascidia	*Herdmania grandis* (Heller, 1878)	IWP	Ahyong, 2018

Table 2.1 *Cont.*

Bonita				
B. mexicana Campos, 2009	Bivalvia	*Pseudochama exogyra* (Conrad, 1837)	EP	Campos, 2009
Buergeres				
B. choprai Ahyong & Ng, 2020	Holothuria	*Actinopyga echinites* (Jaeger, 1833)	IWP	Ahyong & Ng, 2020
B. deccanensis (Chopra, 1931)	Holothuria	*Holothuria scabra* Jaeger, 1833	IWP	Jones & Mahadevan, 1965
B. holothuriae (Semper, 1880)	Holothuria	*Stichopus horrens* Selenka, 1867	IWP	Ng & Manning, 2003
B. ortmanni (Bürger, 1895)	Holothuria	*Holothuria fursocinerea* Jaeger, 1833	IWP	Ng & Manning, 2003
Calyptraeotheres				
C. camposi Ayón-Parente & Hendrickx, 2014	Gastropoda	*Crepidula striolata* Menke, 1851	EP	Ayón-parente & Hendrickx, 2014
C. garthi (Fenucci, 1975)	Gastropoda	*Bostrycapulus odites* Collin, 2005, *Crepidula argentina* Simone, Pastorino & Penchaszadeh, 2000, *Crepidula cachimilla* Cledón, Simone & Penchaszadeh, 2004, *Crepidula plana* Say, 1822, *Crepidula protea* (d'Orbigny, 1841), *Crepidula unguiformis* Lamarck, 1822, *Crepidula* sp., *Trochita pileus* (Lamarck, 1822)	ATL	Campos, 1999; Ribeiro et al., 2020
C. granti (Glassell, 1933)	Gastropoda	*Bostrycapulus odites* Collin, 2005, *Crepidula cachimilla* Cledón, Simone & Penchaszadeh, 2004, *Crucibulum spinosum* (Sowerby, 1824), *Crepidula striolata* Menke, 1851, *Lottia mesoleuca* (Menke, 1851)	EP	Campos, 1990; Ocampo et al., 2012
C. hernandezi Hernández-Ávila & Campos, 2006	Gastropoda	*Crucibulum auricula* (Gmelin, 1791)	ATL	Hernández-Ávila & Campos, 2006
C. pepeluisi Campos & Hernández-Ávila, 2010	?	?	EP	Campos & Hernández-Ávila, 2010

Table 2.1 *Cont.*

C. politus (Smith, 1870)	Gastropoda	*Calyptraea* sp., *Crepipatella dilatata* (Lamarck, 1822)	EP	Campos, 1999
Clypeasterophilus				
C. juvenilis (Bouvier, 1917)	Echinoidea	*Clypeaster subdepressus* (Gray, 1825), *Meoma ventricosa* (Lamarck, 1816)	ATL	Filho et al., 2014
C. rugatus (Bouvier, 1917)	Echinoidea	*Clypeaster rosaceus* (Linnaeus, 1758), *Encope michelini* Agassiz, 1841	ATL	Filho et al., 2014
C. stebbingi (Rathbun, 1918)	Echinoidea	*Clypeaster subdepressus* (Gray, 1825)	ATL	Filho et al., 2014
C. ususfructus (Griffith, 1987)	Echinoidea	*Clypeaster europacificus* Clark, 1914, *Clypeaster speciosus* Verrill, 1870	EP	Filho et al., 2014
Discorsotheres				
D. camposi Ahyong, 2018	Bivalvia	*Spondylus* spp.	IWP	Ahyong, 2018
D. spondylia (Nobili, 1905)	Bivalvia	*Spondylus exilis* cf. (Sowerby, 1895)	IWP	Ahyong, 2018
D. subglobosus (Baker, 1907)	Bivalvia	*Equichlamys bifrons* (Lamarck, 1819), *Modiolus areolatus* (Gould, 1850), *Pecten fumatus* Reeve, 1852, *Spondylus tenellus* Reeve, 1856	IWP	Ahyong, 2018
D. subquadrata (T. Sakai, 1939)	Bivalvia	*Magallana nippona* (Reki, 1934), *Mytilus edulis* Linnaeus, 1758, *Spondylus squamosus* Schreibers, 1793	IWP	Ahyong, 2018
Dissodactylus[4]				
D. crinitichelis Moreira, 1901	Echinoidea	*Clypeaster rosaceus* (Linnaeus, 1758), *Clypeaster subdepressus* (Gray, 1825), *Encope emarginata* (Leske, 1778), *Encope michelini* Agassiz, 1841, *Leodia sexiesperforata* (Leske, 1778), *Mellita quinquiesperforata* (Leske, 1778), *Mellita* sp., *Meoma ventricosa* (Lamarck, 1816)	ATL	Filho et al., 2014
D. glasselli Rioja, 1944	Echinoidea	*Encope micropora californica* Verrill, 1870, *Lanthonia grantii* (Mortensen, 1948), *Lanthonia longifissa* (Michelin, 1858), *Mellita kanakoffi* Durham, 1961	EP	Filho et al., 2014

Table 2.1 *Cont.*

D. latus H. Griffith, 1987	Echinoidea	*Clypeaster subdepressus* (Gray, 1825), *Encope michelini* Agassiz, 1841, *Leodia sexiesperforata* (Leske, 1778)	ATL	Filho et al., 2014
D. lockingtoni Glassell, 1935	Echinoidea	*Encope grandis* Agassiz, 1841, *Encope micropora californica* Verrill, 1870, *Leodia sexiesperforata* (Leske, 1778), *Encope* spp., *Lanthonia grantii* (Mortensen, 1948), *Lanthonia longifissa* (Michelin, 1858), *Mellita kanakoffi* Durham, 1961	EP	Filho et al., 2014
D. mellitae (Rathbun, 1900)	Echinoidea	*Clypeaster subdepressus* (Gray, 1825), *Encope michelini* Agassiz, 1841, *Leodia sexiesperforata* (Leske, 1778), *Mellita isometra* Harold & Telford, 1990, *Mellita quinquiesperforata* (Leske, 1778)	ATL	Filho et al., 2014
D. nitidus Smith, 1870	Echinoidea	*Encope grandis* Agassiz, 1841, *Encope micropora californica* Verrill, 1870, *Encope* spp., *Lanthonia longifissa* (Michelin, 1858)	EP	Filho et al., 2014
D. primitivus Bouvier, 1917	Echinoidea	*Meoma ventricosa* (Lamarck, 1816), *Plagiobrissus grandis* (Gmelin, 1791)	ATL	Filho et al., 2014
D. schmitti H. Griffith, 1987	?	?	EP	Griffith, 1987b
D. xantusi Glassell, 1936	Echinoidea	*Clypeaster subdepressus* (Gray, 1825), *Encope grandis* Agassiz, 1841, *Encope micropora californica* Verrill, 1870, *Encope michellini* Agassiz, 1841, *Encope* spp., *Leodia sexiesperforata* (Leske, 1778), *Mellitella stokesii* (Agassiz, 1841), *Lanthonia longifissa* (Michelin, 1858)	EP	Filho et al., 2014
Durckheimia				
D. caeca Bürger, 1895	Bivalvia	*Chama pacifica* Broderip, 1835, *Lima lima* (Linnaeus, 1758), *Lima vulgaris* (Link, 1807)	IWP	Ahyong & Ng, 2005
D. carnipes De Man, 1889	?	?	IWP	Ahyong & Ng, 2005
D. lochi Ahyong & Brown, 2003	Bivalvia	*Ctenoides ales* (H. J. Finlay, 1927), *Lima vulgaris* (Link, 1807)	IWP	Ahyong & Ng, 2005
Enigmatheres				
E. canfieldi (Rathbun, 1918)	Gastropoda	*Megathura crenulata* (Sowerby, 1825)	EP	Campos, 2009

Table 2.1 *Cont.*

Ernestotheres				
E. conicola Manning, 1993	Gastropoda	*Conus* sp.	ATL	Manning, 1993a
Fabia[5]				
F. byssomiae (Say, 1818)	Bivalvia	*Hiatella arctica* (Linnaeus, 1767), *Anadara notabilis* (Röding, 1798)	ATL	Campos, 1996a
F. carvachoi Campos, 1996	Bivalvia	*Semele flavescens* (Gould, 1851)	EP	Campos, 1996a
F. concharum (Rathbun, 1894)	Bivalvia	*Cryptomya californica* (Conrad, 1837), *Donax gouldii* Dall, 1921, *Leukoma staminea* (Conrad, 1837), *Modiolus capax* (Conrad, 1837), *Modiolus modiolus* (Linnaeus, 1758), *Mya arenaria* Linnaeus, 1758, *Parapholas californica* (Conrad, 1837), *Pholadidea loscombiana* Turton, 1819, *Tapes* sp., *Tivela stultorum* (Mawe, 1823)	EP	Campos, 1996a
F. emiliai (Melo, 1971)	Bivalvia	*Anadara brasiliana* (Lamarck, 1819), *Glycymeris longior* (Sowerby, 1833), *Glycymeris* sp.	ATL	Campos, 2013
F. felderi Gore, 1986	?	?	ATL	Campos, 2013
F. hemphilli (Rathbun, 1918)	?	?	ATL	Campos, 2013
F. malaguena (Garth, 1948)	?	?	EP	Campos & Manning, 1998
F. subquadrata Dana, 1851	Bivalvia	*Cyclocardia ventricosa* (Gould, 1850), *Leukoma staminea* (Conrad, 1837), *Modiolus capax* (Conrad, 1837), *Modiolus modiolus* (Linnaeus, 1758), *Mya arenaria* Linnaeus, 1758, *Mytilus californianus* Conrad, 1837, *Mytilus edulis* Linnaeus, 1758, *Saxidomus gigantea* (Deshayes, 1839), *Tresus capax* (Gould, 1850), *Tivela stultorum* (Mawe, 1823), *Tresus nuttallii* (Conrad, 1837)	EP	Campos, 2016
F. tellinae Cobb, 1973	Bivalvia	*Laciolina magna* (Spengler, 1798)	ATL	Campos, 1996a

Table 2.1 *Cont.*

Gemmotheres				
G. chamae Roberts, 1975	Bivalvia	*Chama* spp.	ATL	Campos & Hernández-Aguilera, 2020
Holotheres				
H. danielae Ahyong, 2010	Holothuria	*Acaudina molpadioides* (Semper, 1867)	IWP	Ahyong, 2010
H. flavus (Nauck, 1880)	Holothuria	'unidentified holothurian'	IWP	Ng & Manning, 2003
H. halingi (Hamel, Ng & Mercier, 1999)	Holothuria	*Holothuria scabra* Jaeger, 1833	IWP	Hamel et al., 1999
H. semperi (Bürger, 1895)	Holothuria	*Holothuria fursocinerea* Jaeger, 1833, *Holothuria scabra* Jaeger, 1833	IWP	Ng & Manning, 2003
H. setnai (Chopra, 1931)	Holothuria	*Actinopyga mauritiana* (Quoy & Gaimard, 1834)	IWP	Schmitt et al., 1973
Holothuriophilus[6]				
H. pacificus (Poeppig, 1836)	Holothuria	*Athyonidium chilensis* (Semper, 1868)	EP	Schmitt et al., 1973
H. trapeziformis Nauck, 1880	Holothuria	*Holothuria inornata* (Semper, 1868)	EP	Ocampo et al., 2012
Hospitotheres				
H. powelli Manning, 1993[2]	Decapod burrows	*Balsscallichirus balssi* (Monod, 1933), *Leptalpheus* sp. nov. (?)	ATL	Manning, 1993a
Juxtafabia				
J. muliniarum (Rathbun, 1918)	Bivalvia	*Chione californiensis* (Broderip, 1835), *Chionista fructifraga* (Sowerby, 1853), *Chione tumens* Verrill, 1870, *Leukoma grata* (Say, 1831), *Tagelus affinis* (Adams, 1852), *Polymesoda inflata* (Philippi, 1851)	EP	Campos, 1993
Latatheres				
L. affinis (H. Milne Edwards, 1853)	?	?	IWP	Ahyong, 2018

Table 2.1 *Cont.*

L. tomentipes (Takeda & Konichi, 1994)	Bivalvia	'unidentified bivalve mollusc'	IWP	Ahyong, 2018
Limotheres				
L. nasatus Holthuis, 1975	Bivalvia	*Ctenoides mitis* (Lamarck, 1807)	ATL	Holthuis, 1975
Mesotheres				
M. barbatus (Desbonne, 1867)	Gastropoda	*Cittarium pica* (Linnaeus, 1758)	ATL	Schmitt et al., 1973
M. serrei (Rathbun, 1909)	Gastropoda	*Strombus* sp.	ATL	Schmitt et al., 1973
M. strombi (Rathbun, 1905)	Gastropoda	*Pleuroploca* sp., *Strombus alatus* Gmelin, 1791, *Strombus pugilis* Linnaeus, 1758, *Strombus* sp., *Triplofusus giganteus* (Kiener, 1840)	ATL	Schmitt et al., 1973; Ng et al., 2019
M. unguifalcula (Glassel, 1936)[7]	Gastropoda	*Strombus* sp., *Turbo* sp.	EP	Campos, 1989b
Nannotheres				
N. moorei Manning & Felder, 1996	Bivalvia	*Malleus candeanus* (d'Orbigny, 1853)	ATL	Manning & Felder, 1996
Nepinnotheres[5]				
N. affinis (Bürger, 1895)	Bivalvia	*Chlamys hastata* (Sowerby, 1842), *Ostrea* sp., *Pinna* sp.	IWP	Schmitt et al., 1973
N. africanus Manning, 1993	?	?	ATL	Manning, 1993a
N. androgynus Manning, 1993	Bivalvia	*Panopea glycimeris* (Born, 1778)	ATL	Manning, 1993a
N. atrinicola (Page, 1983)	Bivalvia	*Atrina zelandica* (Gray, 1835), *Austrovenus stutchburyi* (Wood, 1828), *Modiolus areolatus* (Gould, 1850)	IWP	Page, 1983
N. cardii (Bürger, 1895)	Bivalvia	*Fragum unedo* (Linnaeus, 1758), *Mactra chinensis* Philippi, 1846, *Mytilus unguiculatus* Valenciennes, 1858, *Meretrix meretrix* (Linnaeus, 1758), *Ostrea denselamellosa* Lischke, 1869, *Pinna bicolor* Gmelin, 1791, *Spisula sachalinensis* (Schrenck, 1862)	IWP	Schmitt et al., 1973

Table 2.1 *Cont.*

N. edwardsi (De Man, 1887)	Bivalvia	*Atrina vexillum* (Born, 1778), *Ostrea* sp., *Pinna* sp.	IWP	Schmitt et al., 1973
N. fulvia Ahyong & Ng, 2020	Bivalvia	*Fulvia australis* (Sowerby, 1834)	IWP	Ahyong & Ng, 2020
N. glaberrimus (Bürger, 1895)	Bivalvia	*Arca* sp., *Ctenoides suavis* Masahito, Kuroda & Habe in Kuroda & al., 1971, *Geloina coaxans* (Gmelin, 1791)	IWP	Schmitt et al., 1973
N. latipes (Jacquinot, 1846)	Bivales	*Modiolus* sp., *Pinna* sp., *Saccostrea scyphophilla* (Peron & Lesueur, 1807)	IWP	Ahyong, 2020b
N. margaritiferae (Laurie, 1906)	Bivalvia	*Mytilus* sp., *Pinctada imbricata* (Röding, 1798)	IWP	Schmitt et al., 1973
N. novaezelandiae (Filhol, 1885)	Bivalvia	*Aulacomya atra* (Molina, 1782), *Austrovenus stutchburyi* (Wood, 1828), *Magallana gigas* (Thunberg, 1793), *Mytilus galloprovincialis* Lamarck, 1819, *Paphies ventricosa* (Gray, 1843), *Perna canaliculus* (Gmelin, 1791)	IWP	Page, 1983
N. pectinicola (Bürger, 1895)	Bivalvia	*Decatopecten radula* (Linnaeus, 1758)	IWP	Schmitt et al., 1973
N. pinnotheres (Linnaeus, 1758)	Bivalvia, Ascidia	*Atrina pectinata* (Linnaeus, 1767), *Pinna nobilis* Linnaeus, 1758; *Ascidia mentula* Müller, 1776, *Halocynthia papillosa* (Linnaeus, 1767), *Microcosmos* spp.	ATL	Becker & Türkay, 2017; Pérez-Miguel et al., 2019
N. rathbunae (Schmitt, 1973)	Bivalvia	*Donax* sp.	IWP	Schmitt et al., 1973
N. rouxi (H. Milne Edwards, 1853)	?	?	IWP	Schmitt et al., 1973
N. sanqueri Manning, 1993	?	?	ATL	Manning, 1993a
N. tellinae (Manning & Holthuis, 1981)	Bivalvia	*Austromacoma nymphalis* (Lamarck, 1818)	ATL	Manning, 1993a
N. villosulus (Guérin, 1832)	Bivalvia	*Atrina chinensis* (Deshayes, 1841), *Pinctada margaritifera* (Linnaeus, 1758)	IWP	Schmitt et al., 1973

Table 2.1 *Cont.*

Opisthopus				
O. transversus Rathbun, 1894[8]	Polyplacophora, Gastropoda, Bivalvia, Polychaeta, Holothuria	*Cryptochiton stelleri* (Middendorff, 1847); *Aplysia vaccaria* Winkler, 1955, *Bulla gouldiana* Pilsbry, 1895, *Megastraea undosa* (Wood, 1828), *Megathura crenulata* (Sowerby, 1825), *Navanax inermis* (Cooper, 1862), *Neverita lewisii* (Gould, 1847); *Crassadoma gigantea* (Gray, 1825), *Dinocardium robustum* (Lightfoot, 1768), *Megapitaria squalida* (Sowerby, 1835), *Modiolus* sp., *Mytilus edulis* Linnaeus, 1758, *Nuttallia nuttalli* (Conrad, 1837), *Pholas* sp., *Platyodon* sp., *Tivela stultorum* (Mawe, 1823), *Tresus nuttallii* (Conrad, 1837), *Zirfaea pilsbryi* Lowe, 1931, *Zirfaea* sp.; *Arenicola* sp., *Chaetopterus variopedatus* (Renier, 1804); *Apostichopus californicus* (Stimpson, 1857), *Apostichopus parvimensis* (Clark, 1913), *Caudina* sp.	EP	Schmitt et al., 1973; Campos, 2016
Orthotheres				
O. bayou Ng & Ho, 2016	Gastropoda	*Haliotis asinina* Linnaeus, 1758	IWP	Ng & Ho, 2016b
O. haliotidis Geiger & Martin, 1999	Gastropoda	*Haliotis asinina* Linnaeus, 1758	IWP	Ng & Ho, 2016b
O. turboe T. Sakai, 1969	Gastropoda	*Turbo argyrostomus* Linnaeus, 1758	IWP	Ng & Ho, 2016b
Ostracotheres				
O. cynthiae Nobili, 1906	Ascidia	*Herdmania momus* (Savigny, 1816), *Herdmania* sp.	IWP	Ahyong, 2018
O. tridacnae (Rüppel, 1830)	Bivalvia	*Tridacna maxima* (Röding, 1798), *Tridacna squamosina* Sturany, 1899	IWP	Ahyong, 2018
Pinnaxodes[6]				
P. bipunctatus (Nicolet, 1849)	Echinoidea	'probably sea-urchins'	EP	Campos, 2017
P. chilensis (H. Milne Edwards, 1837)	Echinoidea	*Caenocentrotus gibbosus* (Agassiz in Agassiz & Desor, 1846), *Loxechinus albus* (Molina, 1782), *Tetrapygus niger* (Molina, 1782)	EP	Campos, 2017

Table 2.1 *Cont.*

P. floridensis H.W. Wells & M.J. Wells, 1961	Holothuria	*Holothuria princeps* Selenka, 1867	ATL	Schmitt et al., 1973
P. gigas Green, 1992	Bivalvia	*Panopea generosa* Gould, 1850, *Panopea globosa* Dall, 1898, *Pinna rugosa* Sowerby, 1835	EP	Campos, 2016
P. major Ortmann, 1894	Bivalvia, Holothuria	*Atrina pectinata* (Linnaeus, 1767), *Barnea* sp., *Crenomytilus grayanus* (Dunker, 1853), *Gregariella difficilis* (Deshayes, 1863), *Mactra antiquata* Spengler, 1802, *Meretrix lamarckii* Deshayes, 1853, *Mytilus* sp., *Ruditapes philippinarum* (Adams & Reeve, 1850); *Holothuria hilla* Lesson, 1830, *Stichopus gyrifer* Selenka, 1867	IWP	Schmitt et al., 1973; Takeda & Masahito, 2000; Marin, 2014
P. mutuensis T. Sakai, 1939	Bivalvia	*Crenomytilus grayanus* (Dunker, 1853), *Gregariella difficilis* (Deshayes, 1863), *Modiolus modiolus* (Linnaeus, 1758), *Mya arenenaria* Linnaeus, 1758, *Mytilus edulis* Linnaeus, 1758, *Mytilus galloprovincialis* Lamarck, 1819, *Mytilus unguiculatus* Valenciennes, 1858	IWP	Ng & Manning, 2003; Marin, 2014
P. tomensosus Ortmann, 1894	Bivalvia	*Anomalocardia flexuosa* (Linnaeus, 1767), *Cyrtopleura costata* (Linnaeus, 1758)	ATL	De Melo & Boehs, 2004
Pinnotheres[5]				
P. bicristatus García Raso & Cuesta, 2019	Bivalvia	*Anomia ephippium* Linnaeus, 1758, *Ostrea edulis* Linnaeus, 1758	ATL	Cuesta et al., 2019; Marco-Herrero et al., 2020
P. bidentatus T. Sakai, 1939[9]	'Free living'	?	IWP	Hsueh & Huang, 1996
P. corbiculae T. Sakai, 1939[10]	Bivalvia	*Corbicula japonica* Prime, 1864	IWP	Schmitt et al., 1973
P. coutierei Nobili, 1905	?	?	IWP	Schmitt et al., 1973
P. dilatatus Shen, 1932	Bivalvia	*Venerupis aspera* (Quoy & Gaimard, 1835)	IWP	Schmitt et al., 1973
P. excussus Tai in Tai, Feng, Song & Chen, 1980	Bivalvia	*Gafrarium* sp.	IWP	Tai & Yang, 1991

Table 2.1 *Cont.*

P. globosus Hombron & Jacquinot, 1846[3]	Bivalvia	*Meretrix* sp., *Pinna* sp., *Sunetta subquadrata* (Sowerby, 1851)	IWP	Schmitt et al., 1973
P. gordonae Shen, 1932[11]	Bivalvia	*Anomia cytaeum* Gray, 1850, *Fulvia undatopicta* (Pilsbry, 1904), *Mytilus unguiculatus* Valenciennes, 1858, *Ruditapes philippinarum* (Adams & Reeve, 1850), *Venerupis aspera* (Quoy & Gaimard, 1835), *Venerupis philippinarum* (Adams & Reeve, 1850)	IWP	Schmitt et al., 1973
P. guerini H. Milne Edwards, 1853	Bivalvia	'oysters'	ATL	Schmitt et al., 1973
P. haiyangensis Shen, 1932	Bivalvia	*Laternula gracilis* (Reeve, 1860)	IWP	Schmitt et al., 1973
P. hickmani (Guiler, 1950)	Bivalvia	*Eumarcia fumigata* (Sowerby, 1853), *Modiolus* sp., *Mytilus* sp., *Neotrigonia margaritacea* (Lamarck, 1804), *Plebidonax deltoides* (Lamarck, 1818)	IWP	Ahyong & Brown, 2003
P. hirtimanus H. Milne Edwards, 1853	?	?	ATL	Schmitt et al., 1973
P. kamensis Rathbun, 1909	?	?	IWP	Schmitt et al., 1973
P. kutensis Rathbun, 1909	?	?	IWP	Schmitt et al., 1973
P. lanensis Rathbun, 1909	?	?	IWP	Schmitt et al., 1973
P. laquei T. Sakai, 1961[12]	Brachiopoda	*Laqueus rubellus* (Sowerby, 1846), 'other brachiopods'	IWP	Feldmann et al., 1996
P. lithodomi Smith, 1870	Bivalvia	*Leiosolenus attenuates* (Deshayes, 1836), *Lithophaga aristata* (Dillwyn, 1817)	EP	Schmitt et al., 1973
P. luminatus Tai in Tai, Feng, Song & Chen, 1980	Bivalvia	*Asaphis violascens* (Forsskål in Niebuhr, 1775)	IWP	Tai & Yang, 1991
P. lutescens Nobili, 1905	?	?	IWP	Schmitt et al., 1973
P. mactricola Alcock, 1900	Bivalvia	*Mactra violacea* (Gmelin, 1791)	IWP	Schmitt et al., 1973

Table 2.1 *Cont.*

P. maindroni Nobili, 1905	?	?	IWP	Schmitt et al., 1973
P. nigrans Rathbun, 1909	?	?	IWP	Schmitt et al., 1973
P. obscuridentata Tai & Song, 1986	?	?	IWP	Tai & Song, 1991
P. obscurus Stimpson, 1858	?	?	IWP	Schmitt et al., 1973
P. onychodactylus Tesch, 1918	?	?	IWP	Schmitt et al., 1973
P. paralatissimus Tai & Song, 1986	?	?	IWP	Tai & Song, 1991
P. parvulus Stimpson, 1858	Bivalvia	*Pecten albicans* (Schröter, 1802), *Pinna* sp., *Saxidomus purpurata* (Sowerby, 1852), *Sunetta subquadrata* (Sowerby, 1851)	IWP	Schmitt et al., 1973
P. pectunculi Hesse, 1872	Bivalvia	*Clausinella fasciata* (da Costa, 1778), *Glycymeris glycymeris* (Linnaeus, 1758), *Venus casina* Linnaeus, 1758, *Venus verrucosa* Linnaeus, 1758	IWP	Becker & Türkay, 2017
P. perezi Nobili, 1905	Bivalvia	*Pholas* sp.	IWP	Schmitt et al., 1973
P. pholadis De Haan, 1835	Bivalvia	*Barnea manilensis* (Philippi, 1847), "*Chlamys nipponensis"* (possibly *Chlamys farreri* (K. H. Jones & Preston, 1904)), *Meretrix lusoria* (Röding, 1798), *Mimachlamys sanguinea* (Linnaeus, 1758), *Mytilus edulis* Linnaeus, 1758, *Pecten albicans* (Schröter, 1802), *Ruditapes philippinarum* (Adams & Reeve, 1850), *Spisula sachalinensis* (Schrenck, 1862)	IWP	Schmitt et al., 1973
P. pichilinquei Rathbun, 1923	?	?	EP	Schmitt et al., 1973
P. pilulus Tai in Tai, Feng, Song & Chen, 1980	Bivalvia	*Martesia* sp.	IWP	Tai & Yang, 1991

Table 2.1 *Cont.*

P. pisum (Linnaeus, 1767)	Bivalvia	*Acanthocardia echinata* (Linnaeus, 1758), *Cerastoderma edule* (Linnaeus, 1758), *Chamelea gallina* (Linnaeus, 1758), *Clausinella fasciata* (da Costa, 1778), *Donax vittatus* (da Costa, 1778), *Dosinia lupinus* (Linnaeus, 1758), *Ensis ensis* (Linnaeus, 1758), *Ensis magnus* Schumacher, 1817, *Gari fervensis* (Gmelin, 1791), *Modiolus modiolus* (Linnaeus, 1758), *Mytilus edulis* Linnaeus, 1758, *Mytilus galloprovincialis* Lamarck, 1819, *Nucula nitidosa* Winckworth, 1930, *Ostrea edulis* Linnaeus, 1758, *Spisula* spp.	ATL	Becker & Türkay, 2017
P. pugettensis Holmes, 1900	Ascidia, Bivalvia	*Halocynthia aurantium* (Pallas, 1787), *Halocynthia igaboja* Oka, 1906, *Ascidia paratropa* (Huntsman, 1912); *Mya arenaria* Linnaeus, 1758	EP	Schmitt et al., 1973
P. quadratus Rathbun, 1909	Bivalvia	*Arca* sp.	IWP	Schmitt et al., 1973
P. sebastianensis (Rodrigues da Costa, 1970)	Bivalvia	*Atrina rigida* (Lightfoot, 1786)	ATL	Schmitt et al., 1973
P. serrignathus Shen, 1932	?	?	IWP	Schmitt et al., 1973
P. shoemakeri Rathbun, 1918	?	?	ATL	Schmitt et al., 1973
P. siamensis Rathbun, 1909	?	?	IWP	Schmitt et al., 1973
P. taichungae K. Sakai, 2000[9]	'Free living'	?	IWP	Schmitt et al., 1973
P. taylori Rathbun, 1918	Ascidia	'transparent tunicates'	EP	Schmitt et al., 1973
P. trichopus Tesch, 1918	Bivalvia	*Pinctada* sp.	IWP	Schmitt et al., 1973
P. tsingtaoensis Shen, 1932	Bivalvia	*Hiatula acuta* (Cai & Zhuang, 1985), "Laternula peichiliensi" (?), *Mactra quadrangularis* Reeve, 1854	IWP	Tai & Yang, 1991; Soong, 1997

Table 2.1 *Cont.*

Raytheres				
R. clavapedatus (Glassell, 1935)	Bivalvia	*Leiosolenus attenuates* (Deshayes, 1836)	EP	Schmitt et al., 1973
Serenotheres				
S. besutensis (Serène, 1967)	Bivalvia	*Lithophaga* sp.	IWP	Ahyong & Ng, 2005
S. janus Ng & Meyer, 2016	Bivalvia	*Leiosolenus obesus* (Philippi, 1847)	IWP	Ng & Meyer, 2016
Sindheres				
S. karachiensis Kazmi & Manning, 2003	Bivalvia	*Gastrochaena* sp.	IWP	Kazmi & Manning, 2003
Solenotheres				
S. prolixus Ng & Ngo, 2010	Bivalvia	*Solen corneus* Lamarck, 1818	IWP	Ng & Ngo, 2010
Tacitotheres				
T. glaber (Bürger, 1895)	Bivalvia	*Tapes conspersus* (Gmelin, 1791), *Tapes literatus* (Linnaeus, 1758)	IWP	Schmitt et al., 1973
T. laevis (Bürger, 1895)	Bivalvia	*Coralliophaga* sp.	IWP	Schmitt et al., 1973
T. longipes (Bürger, 1895)	?	?	IWP	Schmitt et al., 1973
Trichobezoares				
T. pilumnoides (Nobili, 1906)[13]	Holothuria	*Holothuria scabra* Jaeger, 1833	IWP	Ahyong & Ng, 2008
T. villosissimus (Doflein, 1904)	Holothuria	*Actinopyga mauritiana* (Quoy & Gaimard, 1834)	IWP	Ahyong & Ng, 2008

Table 2.1 *Cont.*

Tridacnatheres				
T. whitei (de Man, 1888)	Bivalvia	*Pinna* sp., *Tridacna gigas* Linnaeus, 1758, *Tridacna squamosa* Lamarck, 1819	IWP	Ahyong & Ng, 2005
Tumidotheres				
T. carabiensis Palacios Theil & Felder, 2019	Bivalvia	*Atrina rigida* (Lightfoot, 1786), *Barbatia candida* (Helbling, 1779), *Isognomon alatus* (Gmelin, 1791), *Pinctada imbricata* Röding, 1798, *Pteria colymbus* (Röding, 1798)	ATL	Palacios Theil & Felder, 2019
T. maculatus (Say, 1818)[14]	Bivalvia	*Aequipecten tehuelchus* (d'Orbigny, 1842), *Anomia simplex* d'Orbigny, 1853, *Argopecten gibbus* (Linnaeus, 1758), *Argopecten irradians* (Lamarck, 1819), *Atrina rigida* (Lightfoot, 1786), *Atrina seminuda* (Lamarck, 1819), *Atrina serrata* (Sowerby, 1825), *Chama macerophylla* Gmelin, 1791, *Modiolus americanus* (Leach, 1815), *Modiolus modiolus* (Linnaeus, 1758), *Mya arenaria* Linnaeus, 1758, *Mytilus edulis* Linnaeus, 1758, *Mytilus platensis* d'Orbigny, 1842, *Ostrea puelchana* d'Orbigny, 1842, *Perna perna* (Linnaeus, 1758), *Placopecten magellanicus* (Gmelin, 1791)	ATL	Palacios Theil & Felder, 2019
T. margarita (Verrill, 1869)	Bivalvia	*Argopecten irradians concentricus* (Say, 1822), *Argopecten ventricosus* (Sowerby, 1842), *Barbatia reeveana* (d'Orbigny, 1846), *Crassadoma gigantea* (Gray, 1825), *Limaria pacifica* (d'Orbigny, 1846), *Pinctada mazatlanica* (Hanley, 1856)	EP	Campos, 1989a
T. orcutti (Rathbun, 1918)	?	?	EP	Schmitt et al., 1973
Tunicotheres				
T. moseri (Rathbun, 1918)	Ascidia	*Molgula occidentalis* Traustedt, 1883, *Phallusia nigra* Savigny, 1816, *Polycarpa spongiabilis* Traustedt, 1883	ATL	Campos, 1989a
Viridotheres				
V. asaphis Ahyong, 2020	Bivalvia	*Asaphis violascens* (Forsskål in Niebuhr, 1775)	IWP	Ahyong, 2020a
V. buergeri (Rathbun, 1909)	?	?	IWP	Schmitt et al., 1973

Table 2.1 *Cont.*

V. cygnus Ahyong, 2020	Bivalvia	*Mactra pura* Reeve, 1854,	IWP	Ahyong, 2020b
V. gracilis (Bürger, 1895)	Bivalvia	*Marcia opima* (Gmelin, 1791), *Solen* sp.	IWP	Schmitt et al., 1973
V. kupang Ahyong, 2019	?	?	IWP	Ahyong, 2019
V. lilliyae (Manning, 1993)	?	?	ATL	Manning, 1993a
V. marionae Manning, 1996	Bivalvia	*Europicardium caparti* (Nicklès, 1955)	ATL	Manning, 1996
V. otto Ahyong & Ng, 2007	?	?	IWP	Ahyong & Ng, 2007a
V. sanguinolariae (Pillai, 1951)	Bivalvia	*Hiatula diphos* (Linnaeus, 1771)	IWP	Schmitt et al., 1973
V. takedai Ahyong, Komai & Watanabe, 2012	Bivalvia	*Nipponoclava gigantea* (Sowerby, 1888)	IWP	Ahyong et al., 2012
V. viridis (Manning, 1993)	?	?	ATL	Manning, 1993a
Visayeres				
V. acron Ahyong & Ng, 2007	Bivalvia	*Lithophaga* sp.	IWP	Ahyong & Ng, 2007b
Waldotheres				
W. mccainae (Schmitt, 1973)	Bivalvia	*Donax rugosus* Linnaeus, 1758	ATL	Manning, 1993a
Xanthasia				
X. murigera White, 1846	Bivalvia	*Hippopus* sp., *Tridacna crocea* Lamarck, 1819, *Tridacna gigas* (Linnaeus, 1758), *Tridacna maxima* (Röding, 1798), *Tridacna squamosa* Lamarck, 1819	IWP	Ahyong & Ng, 2005
Zaops				
Z. angelae Manning, 1993	Bivalvia	*Brachidontes modiolus* (Linnaeus, 1767)	ATL	Palacios Theil & Felder, 2019

Table 2.1 *Cont.*

Z. geddesi (Miers, 1880)	Bivalvia	*Crassostrea rhizophorae* (Guilding, 1828)	ATL	Schmitt et al., 1973
Z. ostreum (Say, 1817)	Bivalvia	*Anomia peruviana* d'Orbigny, 1846, *Crassostrea rhizophorae* (Guilding, 1828), *Crassostrea virginica* (Gmelin, 1791), *Mytilus edulis* Linnaeus, 1758, *Pecten* spp.	ATL	Schmitt et al., 1973

Notes:

[1]: Ng and Kumar (2015) showed *Afropinnotheres* to be present in the Indian Ocean, with the description of *A. ratnakara*, but most other species of *Afropinnotheres* are from the west coast of Africa, with *A. monodi* even reaching as far as Europe (Pérez-Miguel et al., 2019). *Afropinnotheres dofleini* was described from South Africa, connecting the two distribution patterns (Ng & Kumer, 2015).

[2]: All twelve specimens listed in the original description of *Hospitotheres powelli* were found in the soft-soiled estuary in Bonny River (Nigeria), found in decapod burrows (Manning, 1993a). The specimens supposedly shared these burrows with *Leptalpheus* sp. nov. and *Balsscallichirus balssi* (as *Callianassa balssi*). The actual host of this species might be a bivalve (Galeommatoidea, Myidae and Lucinidae), living inside the same burrows as the decapods (Goto et al., 2014). Consequently, the hypothetical host might have been destroyed during collection. Crabs identified as *H. powelli* appear to share many features with mollusk-inhabiting pea crabs, but closer inspection is needed. Another West African species, *Alainotheres leloeuffi*, shares a similar description of the habitat and was described based on one male specimen from a sandy sublittoral habitat in Ivory Coast. The only description of the habitat from the male holotype lacks information about a potential host, but mentioned it was dredged from "the reddish-brown sea floor (depth: 20 m)". Dredging is known to destroy delicate invertebrates, including the shells of bivalves, and might have dislodged the crab from its original host (Števčić et al., 1988). Another possibility is that the male crab had left its host to search for host-infesting females.

[3]: The holotype of the first described species from Singapore, *Pinnotheres globosum* Hombron & Jacquinot, 1846 is considered lost, and the designation of a neotype will be published in 2020 (following Ng & Corbari, 2019). *Arcotheres latus* (Bürger, 1895) and *A. ridgewayi* (Southwell, 1911) were found to be synonymous with *P. globosum* (now probably in *Arcotheres*) (Ng & Corbari, 2019). Until a revision is published, we list all three species separately.

[4]: Although *Dissodactylus meyerabichi* is regarded a junior synonym of *D. nitidus*, some databases still include the species as an accepted name (Ng et al., 2008; WoRMS, 2020). Following Griffith (1987b), we do not include this species in Table 1. *Dissodactylus* may also include six additional species described as *Dissodactylus* zoea stages from Japan (Aikawa, 1933). Schmitt et al. (1973) listed the six species as 'Species incertae', provisionally in a separate genus, *Dissodactylozoea*. The identity of these specimens remains unknown.

[5]: Although *Fabia*, *Nepinnotheres*, and *Pinnotheres* have been the subject of many revisions, erecting new genera accounting for previously included species (Manning, 1993b; Campos, 1996a, b), the three genera still prove to be polyphyletic (Palacios Theil et al., 2016). The molecular phylogeny reconstruction of Palacios Theil et al. (2016) included two species of *Fabia* and *Nepinnotheres*, all being placed in different lineages. Additional molecular and morphological studies are needed to properly revise the two genera, but the distribution of the members of *Fabia* and *Nepinnotheres* can provide hints of a more natural classification. Within *Fabia*, four species are from the eastern Pacific (including the type species *F. subquadrata*) and five are from the tropical Western Atlantic (including the other analysed species). Within *Nepinnotheres*, five species are from the Atlantic coast of Africa (with *N. pinnotheres* also reaching Europe), while the remaining thirteen species can be found in the (greater) Pacific region, from India to New Zealand and the Philippines. Although Palacios Theil et al. (2016) include only one species of *Pinnotheres* in their phylogenetic analyses, the genus is (still) urgently in need of a thorough revision, as stated by previous authors (Tesch, 1918; Gordon, 1936; Manning, 1993a). Evidence for the heterogeneity of the genus is the extreme morphological variation in the currently 45 recognised species, in addition to the absence of illustrations, host-information, and collection materials. A quick review of the illustrations of some of the better-known Indo-West Pacific species suggest already four species needing to be included in *Arcotheres* due to their sword-like dactyli on the last ambulatory legs: *P. obscuridentata* (Tai & Song, 1991), *P. excussus* (Tai & Yang, 1991), and *P. parvulus* (Sakai, 1939). Proper examination is needed to refer the four (and possibly more) species to *Arcotheres* (as in Ng et al., 2017 and Ahyong & Ng, 2020).

[6]: There are some unanswered questions about the taxonomy of *Holothuriophilus* and *Pinnaxodes*, most recently highlighted by Ng and Kumar (2015). *Holothuriophilus pacificus* and *H. trapeziformis* are listed as the only species within *Holothuriophilus* (Ng et al., 2008; WoRMS, 2020), but previous authors (Takeda & Masahito, 2000; De Melo & Boehs, 2004) mentioned *Pinnaxodes mutuensis* and *P. tomentosus* to also be included in *Holothuriophilus* (see Campos et al., 2012). Jiang and Liu (2011), Marin (2014), and subsequently Ng and Kumar (2015) include the two species in *Pinnaxodes*, based on morphological differences between the two genera (see Ng & Manning, 2003). We follow Ng and Kumar (2015) in including the two species in *Pinnaxodes*. After Ng and Manning (2003) suggested it, Palacios Theil et al. (2016) showed the southeastern Pacific species *Holothuriophilus pacificus* and *Pinnaxodes chilensis* to be related. The molecular phylogeny did not include the Indo-West Pacific and Western Atlantic species of *Pinnaxodes*, which are needed to solve this taxonomic problem. While both species of *Holothuriophilus* live in holothurians in the southeastern Pacific, members of *Pinnaxodes* have been found in a wide range of hosts organisms, from the Western Atlantic, Indo-West Pacific, and eastern Pacific. *Pinnaxodes chilensis* can be found inside the rectums of several species of urchins (Takeda & Masahito, 2000; Campos, 2017), while *P. bipunctatus* was described “probably from a sea urchin” and has not been examined since (Campos, 2017). Campos (2017) placed the species in *Pinnaxodes* after detailed examination of the description, and suggested it is related to *P. chilensis*. *Pinnaxodes floridensis* can be found in western Atlantic waters, inside the respiratory system of holothurians (Takeda & Masahito, 2000). This species was described by Wells and Wells (1961) after examination of 174 specimens, and found to “live commensally, not harming the [holothurian] host”. Although the Western Atlantic distribution raises questions about the generic status of this species, Takeda and Masahito (2000) relate the species to the western Pacific

P. major. *Pinnaxodes major* was reported as an inhabitant of a holothurian (Sakai, 1965), which would be in line with the hosts of the other species of *Pinnaxodes* and *Holothuriophilus*. This species, however, can also be found in a wide range of shallow-water mussels and fan shells (Takeda & Masahito, 2000), as in *P. mutuensis* and *P. tomentosus*. In contrast, *P. gigas*, a species more recently described by Green (1992) from the northeast Pacific has been found only once in fan shells (Campos, 2016). Preferred hosts are geoduck clams. The host choice and potential switching (from a holothurian host to a geoduck within one life cycle) are discussed by Campos (2016).

[7]: *Mesotheres unguifalcula* can be found on the Pacific coasts of Mexico, in the stomachs of large gastropods from the genera *Strombus* and *Turbo*. Campos (1989b) mentioned the discovery of *M. unguifalcula*: “According to Glassell (1935) the host for this species was not determined, but he recorded for the female topotypes that were collected “on the ambulacral groove of starfish.” I consider that this needs confirmation.” (Campos, 1989b). No other specimen has been collected from sea stars after 1936, so this might be an oddity or a rare encounter of an intermediate host.

[8]: *Opisthopus transversus* can be found in a wide range of hosts (Campos et al., 1992): inside the folds and openings of chitons, gastropods, bivalves, and holothurians. Campos et al. (1992) suggested the crab to also live inside annelid worms like *Chaetopterus variopedatus*, which contrasts with the lifestyle of the above mentioned *Hospitotheres* and most pinnixine genera. Schmitt et al. [6] mentioned the species as living as a commensal symbiont inside the tubes of living *C. variopedatus*, and cite Hopkins and Scanland (1964). Hopkins and Scanland (1964) described the hosts of *O. transversus* and stated that they found the largest specimens inside large species of gastropods and bivalves, somewhat smaller specimens inside holothurians and the smallest specimens inside the small gastropod *Bulla*, and inside worm tubes of living *C. variopedatus*. Hopkins and Scanland (1964) suggested that the juvenile crabs to seek shelter until they can compete with the other crabs inhabiting the worm tubes (here *Pinnixa barnharti* and *Polynyx* sp. (Porcellanidae)). In failing to do so, the crabs will inhabit the available holothurians and gastropods. These observations might however suggest the worms to be an intermediate host for the crabs until they can move to their terminal host. In the absence of a particular obligate host choice of *O. transversus* is a derived or primitive character is not known as for now, but can be studied using molecular techniques (Palacios Theil et al., 2016).

[9]: *Pinnotheres taichungae* was originally identified as *Pinnotheres bidentatus* (Hsueh & Huang, 1996), an ambiguous species from two localities in Japan (Sakai, 2000). The specimens described by T. Sakai (both sexes) and later by K. Sakai (only males) as *P. bidentatus* have been regarded as free-living (T. Sakai, 1939; K. Sakai, 2000). Similarly, *P. taichungae* is also known as a free-living species: “Female crabs of this species may not necessary behaving as its congeners - commensal in bivalves, they may emerge into water columns during flooding tides, presumably, buried to substrata during ebb tides, since the water margin retreats up to 3 km on the shoreline at this time.” (about *P. taichungae*, as *P. bidentatus* (Hsueh & Huang, 1996)). As McDermott (2009) already stated, free living pinnotherines have probably been dislodged from their host in the collection procedure. The swimming setae on all ambulatory legs described by Hsueh and Huang (1996) suggest the specimens to be hard staged males and females, maybe leaving their hosts for copulation (Derby & Atema, 1980).

[10]: *Pinnotheres corbiculae* can only be found in the brackish-water clam *Corbicula japonica*, which makes it the only pinnotherid crab living in a brackish environment. T. Sakai (1939) described the species from 'Yamato-sizimi' clams (*C. japonica*), from the southern Sendai River (Kagoshima prefecture, Japan), and mentions another uncertain locality from Nagasaki. He mentioned the species to be the only pinnotherid living inside freshwater clams, but recent studies suggest *C. japonica* only to be found in brackish water in Japan and Korea where populations cannot live for long durations in environments with salinity greater than 21 psu or less than 0.3 psu (Yamada et al., 2014). *Pinnotheres corbiculae* has not been collected after the original description and appears to only be present in southern Japan. More specimens are needed find if *P. corbiculae* can be found in *C. japonica* in other parts of Japan, if water salinity is related to infestation rate, and if the species has evolved morphological adaptations to live in brackish environments.

[11]: Ng et al. (2008; in note 6 of the Pinnotheroidea section) changed the name *Pinnotheres gordoni* to *P. gordonae*, due to the species being named after Isabella Gordon, a woman.

[12]: The unique host-choice of this crab was described by T. Sakai in 1961 (see Sakai, 1965). Not baring any morphological adaptations, *Pinnotheres laquei* can be found in a common Japanese brachiopod, *Laqueus rubellus*, and supposedly in more species of brachiopods (Feldmann et al., 1996). Although the external morphology of this brachiopod resembles bivalves, the internal morphology is unique to the group. Feldmann et al. (1996) described the positioning and commensal lifestyle of *P. laquei* in its host, and mentions this species to be the only crab (and one of a few invertebrates) to live in association with a brachiopod.

[13]: While the original description by Nobili (1906) of *Trichobezoares pilumnoides* did not mention any hosts, Laurie (1915) describes two female specimens from holothurians and one female specimen from a sponge (Ng, 2018a). Laurie's observation is probably a rare finding of a soft-shelled female leaving the holothurian host. There is an additional (new) species that is found in sponges from the Caribbean (Humann et al., 2013), but the sex of this species is undetermined and this might also be just a male crab wandering between hosts.

[14]: *Tumidotheres maculatus* can be found in a wide range of bivalve hosts, but also in the tubes of worms and sometimes free-living in sandy substrates (Schmitt et al., 1973). All other species of *Tumidotheres* are only found in bivalves, and since *T. maculatus* is found to be closely related to its congeners (Palacios Theil et al., 2016; Palacios Theil & Felder, 2019), it is safe to say that the free-living and specimens of this species inhabiting worm tubes were collected during a swarming event, as described by Derby and Atema (1980) and Campos (1989a). We therefore chose to only list the bivalve associates.

Phylogenetic Significance of Adaptive Features and Future Perspectives

The phylogenetic significance of the morphological adaptations can be examined by linking the adaptations with recent molecular phylogenetic reconstructions (Palacios Theil et al., 2016; Palacios Theil & Felder, 2020). Most adaptive features seem to be the result of convergent evolution, rather than shared synapomorphies (Palacios Theil et al., 2016), and these are: the size, ornamentations, colour patterns, and setation of the carapace, in addition to the differences between male and female carapaces; the morphology of the eyes, rostrum, third maxillipeds (Campos & Hernández-Aguilera, 2020), and the specialised feeding structures on the chelipeds. More data are needed to confirm if the adaptive features of the ambulatory legs bear any phylogenetic significance (especially the features of the dactyli).

Although many more species need to be included in future molecular analyses, a few adaptive features could be phylogenetically relevant characters that are taxonomically important. The development of swimming setae in both males and females in their hard stages for copulation in open water (the 'second strategy' in Campos, 1989a) can be found in some genera listed within the West Atlantic and eastern Pacific 'Pinnotherinae II' group (sensu Palacios Theil et al., 2016). The swimming setae can be found in *Calyptraeotheres*, *Fabia* (specifically *F. subquadrata*), and *Tumidotheres*, and may be used by *Fabia emiliai* (de Melo, 1971) and *Juxtafabia muliniarum* (Rathbun, 1918), judging from the swimming setae seen in the presented figures (Campos, 1993, 2013). This strategy is not known from the other species clustering in the same lineage: *Tunicotheres moseri*, *Holothuriophilus pacificus* (Poeppig, 1836), *Pinnaxodes chilensis*, and the species in the *Dissodactylus* complex.

All these species have a firm to hard carapace in the 'post-hard' stages of the female, which is not the case in the other analysed branches of pinnotherine evolution and might be an adaptation associated with open-water copulation. Such 'swarming' behaviour is also known from some *Austrotheres* species, and female swimming setae are known from some members of *Afropinnotheres*, *Nepinnotheres*, *Ostracotheres*, and *Pinnotheres* (all not included in the phylogenetic reconstructions in Palacios Theil et al., 2016). Those are all species from the Indo-West Pacific and will probably be placed elsewhere on the tree later on.

Another character was found in all branches of the 'Pinnotherinae II' (Palacios Theil et al., 2016): the relatively large and robust chelae present in all species within the *Dissodactylus* complex (all members within *Dissodactylus* and

Clypeasterohilus), all species of *Tunicotheres*, *Tumidotheres*, *Holothuriophilus*, *Pinnaxodes*, *Calyptraeotheres*, and *Fabia subquadrata*, *F. emiliai*, and *Juxtafabia muliniarum*. Although the feeding strategies and the use of chelae might differ between species (e.g., strictly parasitic feeding on host tissues in *Dissodactylus* using their chelae, 'grooming' in *Fabia* using the setal comb, and filter feeding in *Pinnaxodes* using the third maxillipeds), the chelae are very different from the feeble chelipeds of other crabs included in the phylogenetic reconstruction (Palacios Theil et al., 2016) like the Vietnamese bivalve-associated *Solenotheres prolixus* Ng & Ngo, 2010, the Chinese/Thai *Amusiotheres obtusidentatus* (Tai et al., 1980), and the European *Pinnotheres pisum*.

The large, robust chelae are not limited to the 'Pinnotherinae II' species (for example, *Nepinnotheres pinnotheres*, and *Alain raymondi* Ahyong & Ng, 2008 also possess relatively large chelae), and one species with slender chelae, *Zaops ostreum*, might be more closely related to *Tumidotheres* than previously thought (Palacios Theil & Felder, 2019) and might cluster within the 'Pinnotherinae II'.

Although most recent evolutionary studies on pea crabs have been focussing on a small subgroup of the Pinnotherinae (Palacios Theil & Felder, 2019), or the other pinnotherid subfamilies (Palacios Theil & Felder, 2020), the study by Palacios Theil et al. (2016) provides a sufficiently large base for further studies on the complete pinnotherine evolution. Genetic barcodes of more species, especially those from the Indo-West Pacific, are needed to solve taxonomic problems, but also to build a complete and robust phylogeny.

A large-scale revision of Indo-West Pacific pinnotherids will be published in the near future (Komai et al., 2020). Using a combination of phylogenetic reconstructions with morphometric analyses and detailed host information, detailed insights regarding patterns of convergent evolution and adaptive radiation of morphological structures can be obtained. such studies will constitute a crucial contribution to our understanding of pinnotherid biodiversity.

Supplementary information

The following are available online at https://www.mdpi.com/1424-2818/12/11/431/s1: A video of the rotating 3D models of the CT-scanned material from fig. 2.1 and fig. 2.3.

Acknowledgements

The authors would like to thank Bert W. Hoeksema (Naturalis Biodiversity Center and University of Groningen) for his general feedback and for his assistance during the last stages of writing. Charles H. J. M. Fransen, Ronald Vonk (both Naturalis Biodiversity Center), Stephen K. Donovan, and Jeroen Hubert (Leiden University) are thanked for their helpful comments and feedback. The authors would like to show gratitude to Dirk van der Marel and Rob Langelaan for their assistance operating the CT-scanners in the imaging facilities of Naturalis Biodiversity Center. Marco Faasse (Naturalis Biodiversity Center and Coast Marine Research, Marlin Harms (via Flickr), Ang Yuchen and Jonathan Ho (Lee Kong Chian Natural History Museum) are thanked for their help by providing pictures for fig. 2.4. In addition, the authors would like to thank Peter K. L. Ng (Lee Kong Chian Natural History Museum) for providing information and literature on a few obscure pea crab species, and three anonymous reviewers for their insightful comments.

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3

Odontonia kerangcaris sp. nov., a new bivalve-associated shrimp (Crustacea: Decapoda: Palaemonidae) from East Kalimantan, revealing intrageneric host switching

Charles Fransen, Mike Groenhof, and Werner de Gier

This chapter is published as:

Fransen, C.H.J.M., Groenhof, M. & de Gier, W. (2021) *Odontonia kerangcaris* sp. nov., a new bivalve-associated shrimp (Crustacea, Decapoda, Palaemonidae) from East Kalimantan, revealing intrageneric host switching. Zootaxa, 5081(2): 275–285.

Abstract

A new species of bivalve mollusk dwelling palaemonid shrimp of the genus *Odontonia* is described from the Berau Islands, East Kalimantan, Indonesia. This is the only *Odontonia* species recorded as a symbiont of a bivalve mollusk, all other congeners are known to associate with solitary ascidians. The new species belongs to the group wherein the dactylus of the ambulatory pereiopods has an accessory tooth. It differs from these species in the absence of a forward directed proximal tooth on the flexor margin of the corpus of the ambulatory dactyli. It also lacks the small denticles posterior to the distoventral accessory tooth on the dactylar corpus.

Key words: new species; Indonesia; intrageneric host switching



Introduction

The genus *Odontonia* Fransen, 2002, currently comprises eight species (de Gier & Fransen, 2018): *O. bagginsi* de Gier & Fransen, 2018; *O. compacta* (Bruce, 1996); *O. katoi* (Kubo, 1940); *O. plurellicola* de Gier & Fransen, 2018; *O. rufopunctata* Fransen, 2002; *O. seychellensis* Fransen, 2002; *O. sibogae* (Bruce, 1972); and *O. simplicipes* (Bruce, 1996). All species are distributed in the Indo-West Pacific and living as endosymbionts in solitary ascidians (de Gier & Fransen, 2018).

A specimen of a yet unknown *Odontonia* was collected in 2003 during a marine biodiversity survey of the Berau Islands, East Kalimantan, Indonesia (Hoeksema 2004). The new species is herein described and figured, and compared with its congeners.

Materials and methods

The specimen was collected by SCUBA diving during a marine biodiversity survey of the Berau Islands organised by Naturalis Biodiversity Center and the Indonesian Institute of Sciences (LIPI) in the East Kalimantan Programme of the Royal Netherlands Academy of Arts and Sciences (KNAW). The specimen was encountered inside the bivalve mollusk *Chama lazarus* Linnaeus, 1758, and preserved on 70% ethanol.

The specimen was studied and drawn using a dissecting stereomicroscope (Zeiss Discovery.V8) and a compound microscope (Olympus BX53) both provided with a drawing tube. Drawings were scanned (Canon Canoscan 9000F) with a resolution of 600 dpi and subsequently mounted into plates using Adobe Photoshop software (Adobe Systems, USA).

Measurements (in mm) are provided for postorbital carapace length (pocl.), measured from the posterior orbital margin to the posterior margin of the carapace in the dorsal midline.

The type-specimen is deposited in the zoological collections of the Museum Zoologicum Bogoriense (MZB), Research Center for Biology, Indonesian Institute of Sciences, Cibenong, Indonesia.

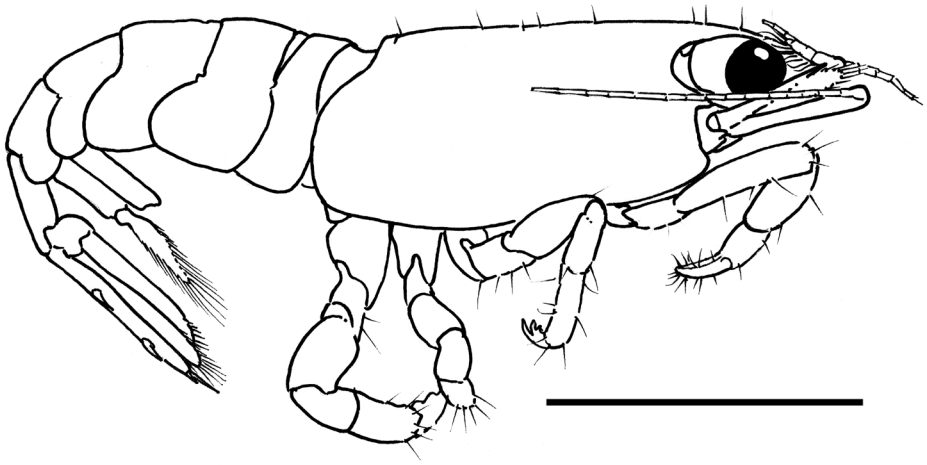
Systematic account

Family Palaemonidae Rafinesque, 1815

Genus *Odontonia* Fransen, 2002

***Odontonia kerangcaris* sp. nov.** (figs. 3.1–3.8)

Zoobank accession link: urn:lsid:zoobank.org:act:197FDE7A-BDDD-4162-8412-FEC59D128460



3.1 *Odontonia kerangcaris* sp. nov., holotype male, pocl. 2.1mm, MZB 5341, habitus, lateral view (second pereiopods detached, not drawn). Scale = 2 mm. Artwork: Charles Fransen.

Material examined. Holotype male, pocl. 2.1mm, MZB 5341, stn BER.39, Indonesia, NE Kalimantan, Berau Islands, Baliktaba reef, SW-side (N of Panjang Isl.), 02˚34'43.3"N 118˚00'48.2"E, 24.x.2003, depth 33m, SCUBA diving, in *Chama lazarus* Linnaeus, 1758, collected by C.H.J.M. Fransen.

Diagnosis. Rostrum short, not overreaching antennular peduncle, without ventral tooth. Pterygostomial angle broadly rounded, slightly produced. Basal segment of antennular peduncle with strong, acute medioventral tooth, with distolateral tooth reaching distal margin of intermediate segment. Carpocerite just falling short of distal margin of lamina of scaphocerite; distolateral tooth of scaphocerite robust, 0.15 times length of lamina (incl. distolateral tooth), almost twice as long as distal lamina; incision between distolateral tooth and lamina rather deep. Dactylus of ambulatory pereiopods with acute distoventral accessory tooth, perpendicular to flexor margin; ventral margin of corpus with one rounded protuberance at 2/3rd and one rounded protuberance proximally, without denticles in between; unguis strongly curved, 0.5 times corpus length, with large scales distally on outer margin, gradually decreasing in size proximally. Telson with two pairs of submarginal dorsal spines at 0.3 and 0.7

of telson length; distal and proximal pair of spines of equal length, 0.14 times telson length.

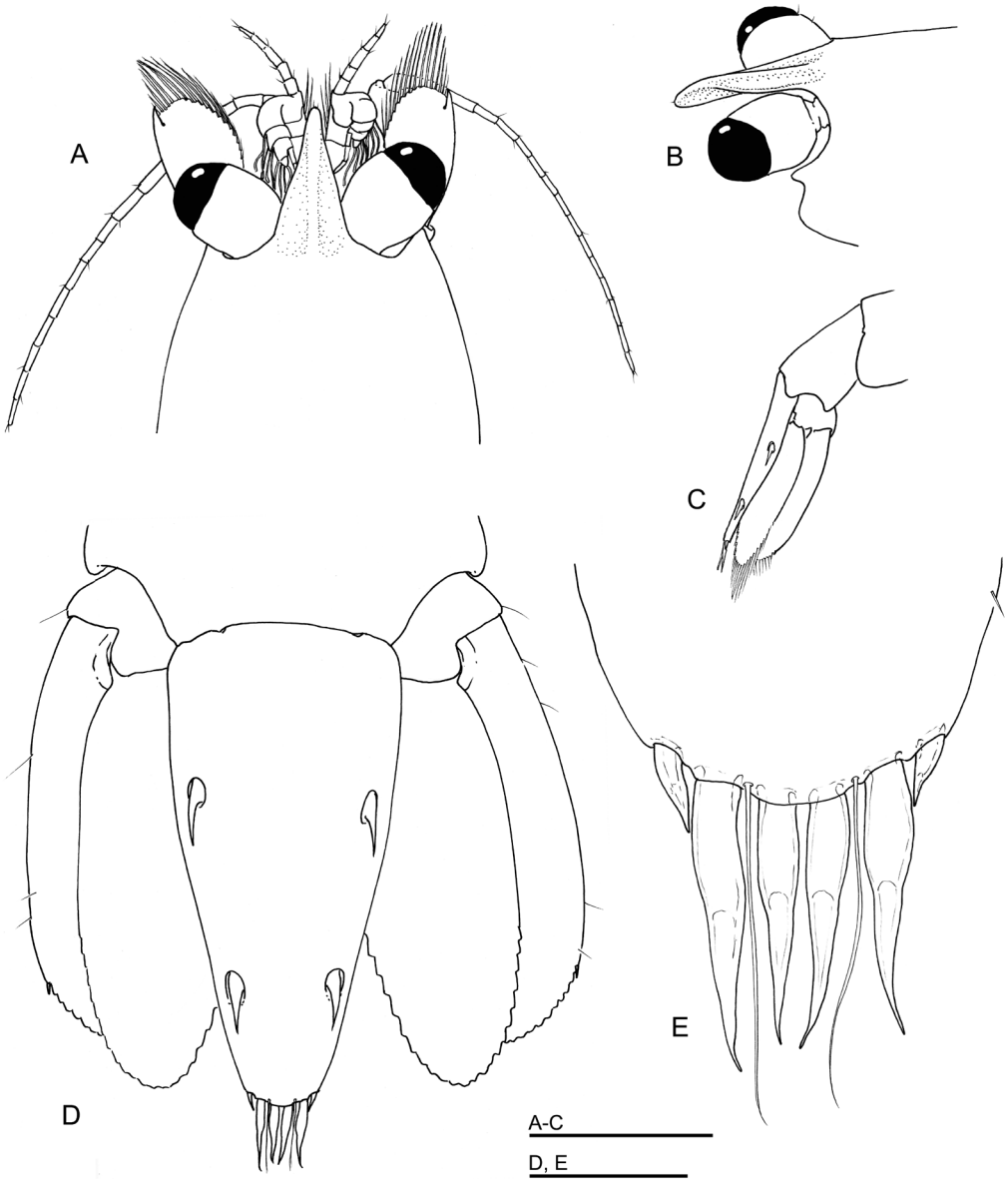
Description. Body (fig. 3.1) subcylindrical, somewhat depressed. Carapace (fig. 3.1) glabrous with a few scattered simple setae. Rostrum (fig. 3.2A, B) well developed, without dorsal teeth, reaching half of distal segment of antennular peduncle, with broad, indistinct, shallow dorsal elevation over entire length and acute lateral carinae, with slightly concave ventral carina in distal part; distal end rounded in lateral view, without subdistal ventral tooth, with few distal setae, blunt in dorsal view, broadened at base. Inferior orbital angle (fig. 3.2B) produced, directed inward. Antennal spine (fig. 3.2B) blunt, protruding rounded process, not separated by notch from inferior orbital angle. Anterolateral margin straight, pterygostomial angle slightly produced, rounded.

Abdomen (fig. 3.1) smooth; sixth segment 1.3 times longer than fifth, 1.3 times broader than long, posteroventral angle rounded, posterolateral angle feebly produced, blunt; pleura of first five segments broadly rounded.

Telson (fig. 3.2C, D) almost twice as long as sixth abdominal segment, about twice as long as proximal width; lateral margins slightly convex, almost straight, convergent posteriorly; posterior border without median process; two pairs of submarginal dorsal spines at 0.3 and 0.7 of telson length; distal and proximal pair of spines of equal length, 0.14 times telson length; posterior margin (fig. 3.2E) with three pairs of spines, lateral spines small, marginal, about 1/3 of length of intermediate spines; submedian spines slightly shorter than intermediate spines; both intermediate and submedian spines about as long as dorsal spines, but more slender.

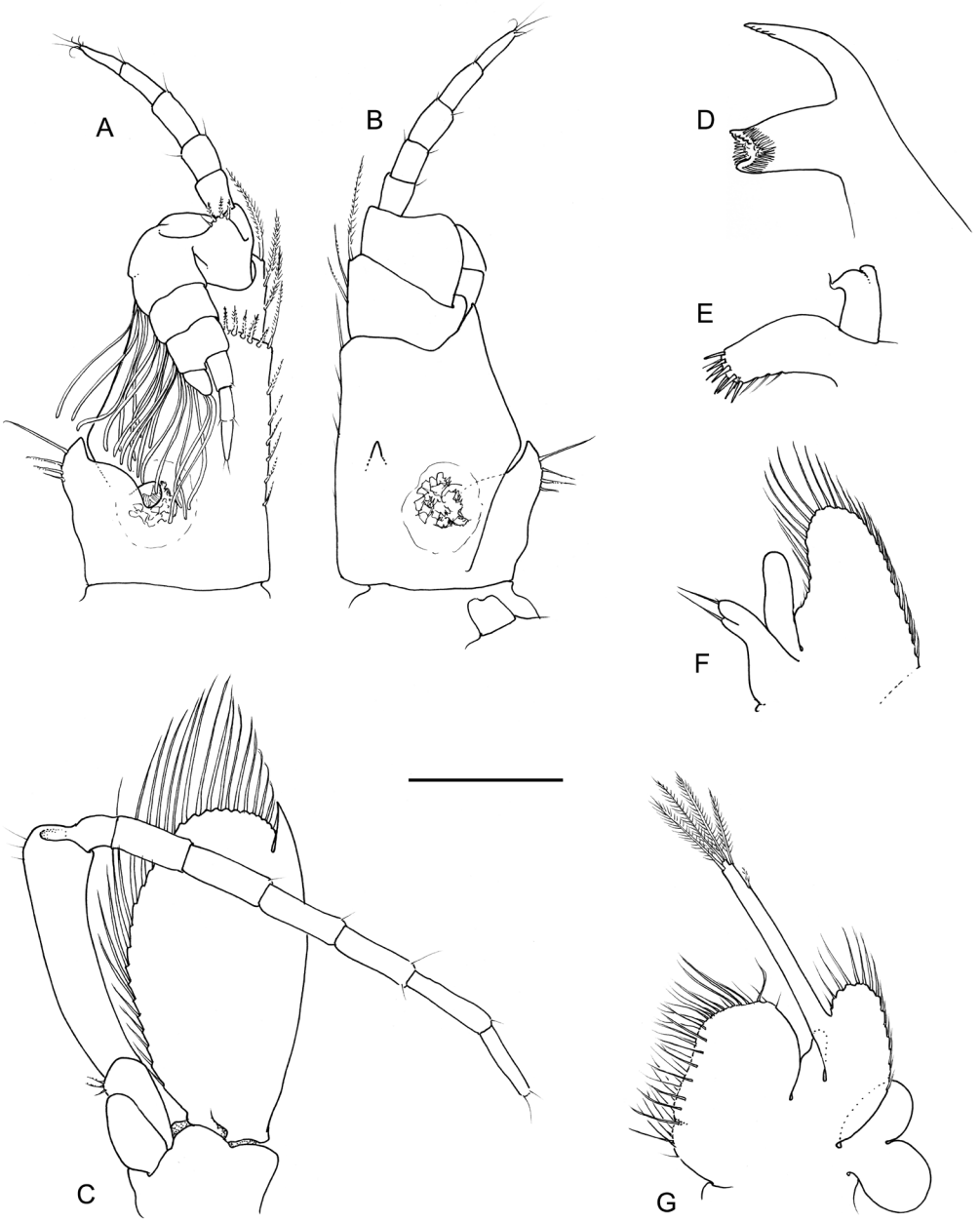
Eyestalk (fig. 3.2A, B) short, about as long as broad, broader than diameter of hemispherical cornea.

Antennula (fig. 3.3A, B) with peduncle and flagella short. Basal segment 1.4 times as long as proximal width, with acute produced distolateral tooth reaching distal margin of intermediate segment, anterior margin oblique; ventromedial tooth large, acute, at midlength of basal segment, submarginal; stylocerite short, reaching halfway basal segment, tip acute, distolateral margin with 4 short plumose setae; statocyst with statolith present in proximal part. Intermediate segment short, slightly broader than long; distal segment broader than long. Upper flagellum short, biramous, with 3 segments fused; short free ramus one-segmented; longer free ramus 3-segmented. Lower flagellum with 5 segments.



3.2 *Odontonia kerangcaris* sp. nov., holotype male, pocl. 2.1 mm, MZB 5341. A) anterior carapace and appendages, dorsal view; B) anterior part of carapace, rostrum and eyes, dorsolateral view; C) tail-fan and sixth abdominal segment, lateral view; D) telson and uropods, dorsal view; E) distal part of telson, dorsal view. Scale A–C = 1 mm; D = 0.5 mm; E = 0.125 mm. Artwork: Charles Fransen.

Antenna (fig. 3.3C) with basicerite short, laterally unarmed, with large rounded antennal gland tubercle medially; ischiocerite and merocerite normal; carpocerite just falling short of distal margin of lamina of scaphocerite, slender, 3.7 times longer than distal width; flagellum about as long as post-orbital carapace length;



3.3 *Odontonia kerangcaris* sp. nov., holotype male, pocl. 2.1 mm, MZB 5341. A) left antennula, dorsal view; B) idem, ventral view; C) left antenna, ventral view; D) left mandible; E) left maxillula (lower lacinia missing); F) left maxilla (proximal part of scaphognathite missing); G) left first maxilliped. Scale = 0.5 mm. Artwork: Charles Fransen.

scaphocerite with lamina twice as long as wide, anterior margin rounded, lateral margin broadly convex; distolateral tooth robust, 0.15 times length of lamina (incl. distolateral tooth), almost twice as long as distal lamina length, somewhat

curved inward; incision between distolateral tooth and lamina rather deep.

Epistome with blunt anterior median carina; labrum normal, oval.

Paragnaths well developed, alae with broad transverse more or less rectangular distal lobes, and small rounded more or less triangular ventromesial lobes; corpus very short, with shallow median excavation, bordered laterally by non-setose, oblique, carinae.

Second thoracic sternite with shallow indistinct rounded median elevation, without setae.

Third thoracic sternite unarmed.

Fourth thoracic sternite with low, medially notched, triangular plate formed by the fused lateral carinae.

Fifth thoracic sternite with well-developed lateral plates with shallow central slit posteromedial to second pereiopod coxae; coxae almost against each other.

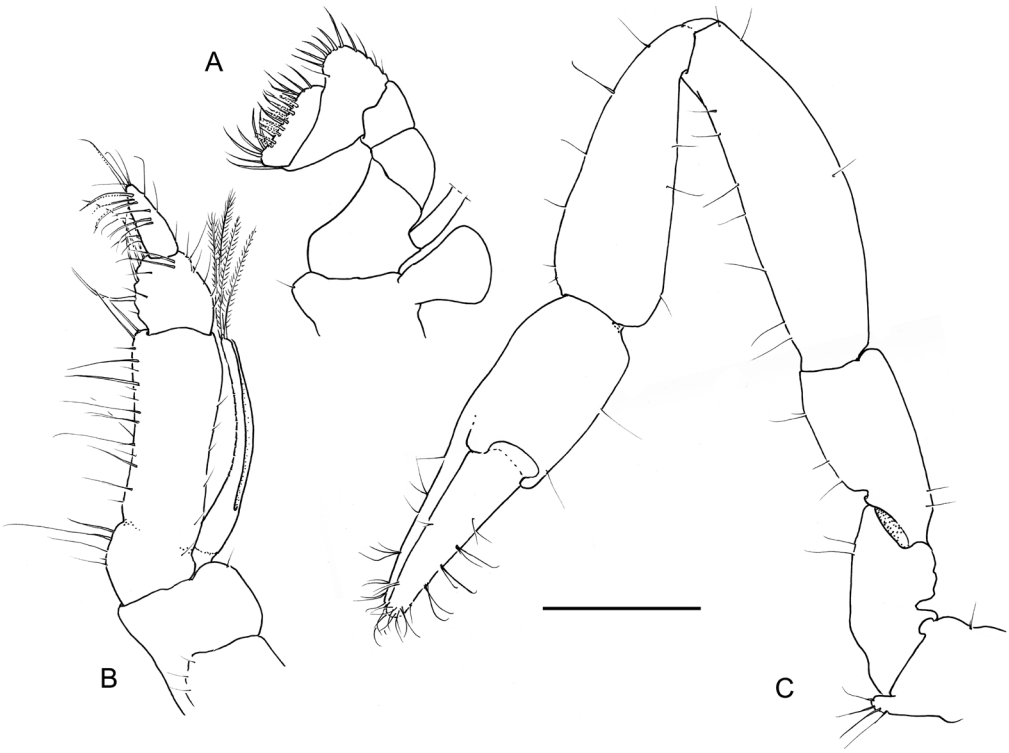
Sixth to eighth thoracic sternites unarmed, broadening posteriorly.

Mandible (fig. 3.3D) with incisor process with 5 acute distal teeth, small denticles along medioventral margin absent; molar process robust with several blunt teeth, some fringed with setal brushes.

Maxillula (fig. 3.3E) with upper and lower lacinia rather small; distal lacinia rectangular with two rows of about 8 stout distal spines, almost devoid of setae; lower lacinia slender, triangular, with few short serrulate setae in distal part; palp feebly bilobed, large lobe with small ventral tubercle with single short recurved seta.

Maxilla (fig. 3.3F) with basal endite well developed, with distal lobe and proximal lobe distinct, each with one long, simple distal seta; coxal endite obsolete, medial margin convex, non-setose; scaphognathite of moderate size; palp simple, longer than basal endite, blunt distally, non-setose.

First maxilliped (fig. 3.3G) with coxal and basal endite partly fused, broad; basal endite fringed with scattered, rather short simple and finely serrulate setae along medial and distal margins; coxal endite convex, indistinctly separated from basal endite, with few simple setae medially; exopod well developed,

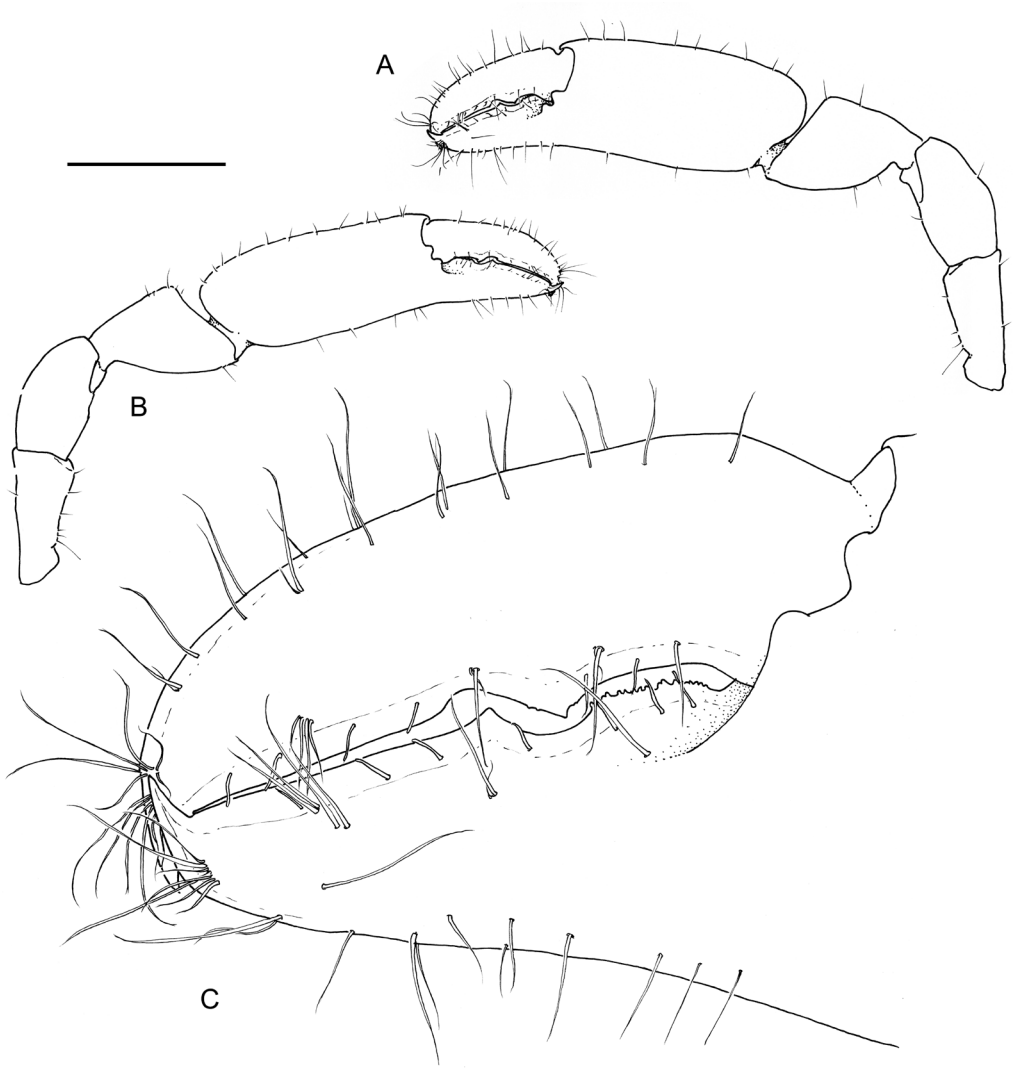


3.4 *Odontonia kerangcaris* sp. nov., holotype male, pocl. 2.1 mm, MZB 5341. A) left second maxilliped; B) left third maxilliped; C) left first pereiopod. Scale = 0.5 mm. Artwork: Charles Fransen.

flagellum with 4 long plumose setae distally; caridean lobe rather small, narrow; epipod bilobed; palp simple, rather short, non-setose.

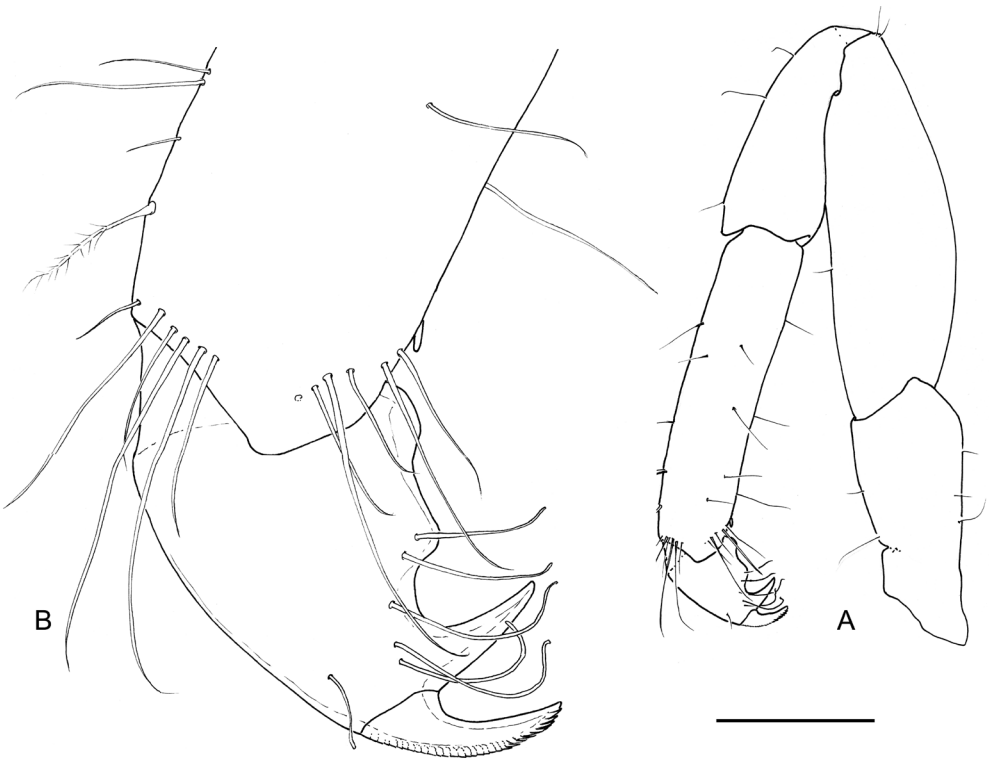
Second maxilliped (fig. 3.4A) with endopod short, stout; dactylar segment 3.0 times broader than long, fringed with short, coarsely serrulate, spiniform, and longer curled, finely serrulate setae medially; propodal segment with row of robust spines and few simple setae along expanded distomedial margin; one seta in distal part of ventrolateral margin; carpal segment short, broader than long, unarmed; meral segment without setae; ischial and basal segment completely fused, medially somewhat excavate, without setae, basal part strongly convex medially; exopod long, with 4 long plumose setae distally; coxal segment medially slightly produced, with few short simple setae, with expanded epipod laterally.

Third maxilliped (fig. 3.4B) short; with ischiomerus partly fused to basis, not broadened, 2.3 times as long as broad, not tapered distally, somewhat flattened, with row of long simple setae along medial margin, lateral margin



3.5 *Odontonia kerangcaris* sp. nov., holotype male, pocl. 2.1 mm, MZB 5341. A) right major second pereiopod, dorsomedial view; B) left minor second pereiopod, dorsomedial view; C) fingers of right major second chela, medial view. Scale A, B = 1.25 mm; C = 0.125 mm. Artwork: Charles Fransen.

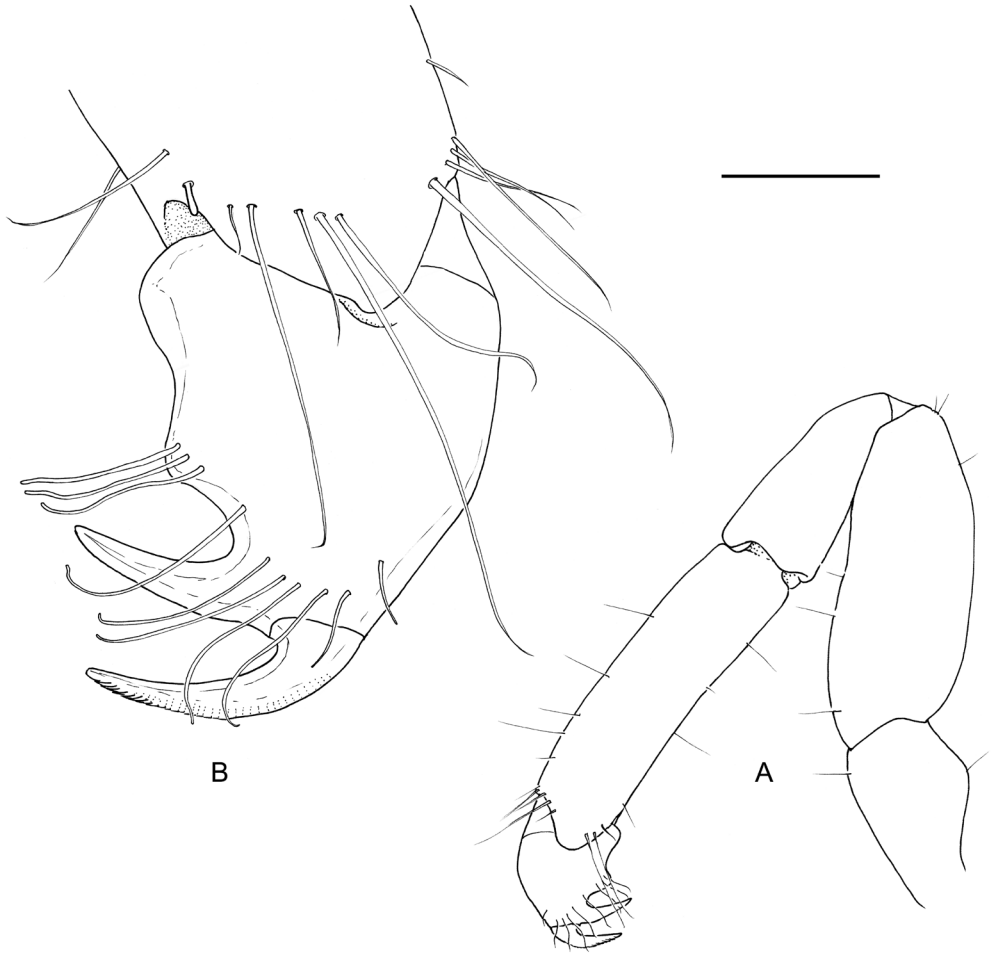
with few simple setae; basal segment medially convex with few long simple setae on medial margin; exopod well developed, reaching distal margin of ischiomerus, with 4 long plumose setae in distal part; coxal segment without medial process, with large lateral plate with one short simple seta laterally; without arthrobranch; ultimate and penultimate segments of equal length; penultimate segment as long as broad, somewhat flattened, with few long finely serrulate setae ventromedially; ultimate segment more slender, with groups of long coarsely serrulate setae ventromedially and distally.



3.6 *Odontonia kerangcaris* sp. nov., holotype male, pocl. 2.1 mm, MZB 5341. A) left third pereiopod, lateral view; B) idem, distal part propodus and dactylus. Scale A = 0.5 mm; B = 0.125 mm. Artwork: Charles Fransen.

First pereiopod (fig. 3.4C) stout, exceeding carpocerite with chela and half of carpus; chela 3.0 times longer than deep, slightly compressed; fingers as long as palm, cutting edges entire, with groups of serrulate setae, tips slightly hooked; cleaning organ absent; carpus 0.8 times length of chela, 2.4 times longer than distal width, tapering proximally, unarmed; merus as long as chela, 3.3 times longer than central width, somewhat curved, with few setae medially; ischium 0.5 times merus length, slightly expanded medially, with few setae medially; basis as long as ischium, with few setae medially; coxa with small ventral lobe with few short simple setae.

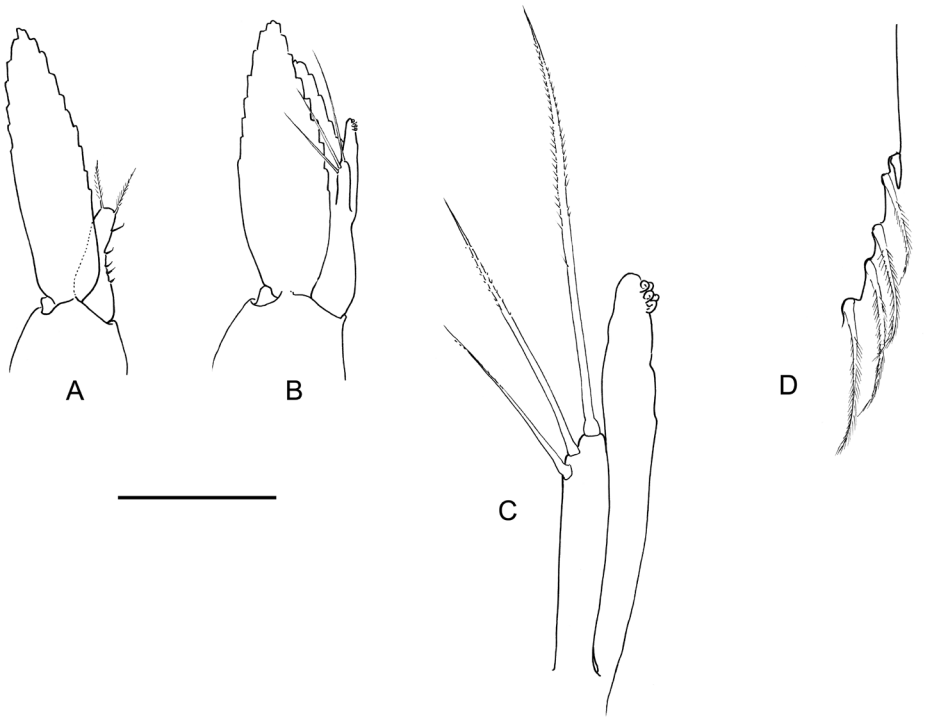
Second pereiopods (fig. 3.5A, B) subequal, similar. Major right chela (fig. 3.5C) with palm slightly compressed, without carinae, with few scattered simple setae; fingers with simple setae; dactylus 0.6 of palm length, 3.2 times longer than deep, with one large triangular tooth at 1/3 of cutting edge, distal part of cutting edge entire, straight, tip strongly hooked; fixed finger 2.1 times as long as deep, with broad flattened tooth with row of small denticles in proximal part, separated by shallow notch from triangular acute tooth at midpoint of cutting edge, distal



3.7 *Odontonia kerangcaris* sp. nov., holotype male, pocl. 2.1 mm, MZB 5341. A) left fifth pereiopod, lateral view; B) idem, distal part propodus and dactylus, medial view. Scale A = 0.5 mm; B = 0.125 mm. Artwork: Charles Fransen.

part of cutting edge entire, straight, tip strongly hooked; carpus 0.55 of palm length, 1.5 times longer than distal width, strongly tapering proximally; merus as long as carpus, 1.8 times longer than central width, distomedially excavate; ischium as long as merus, somewhat tapering proximally, with slightly protruded distomedial angle; basis and coxa without special features. Minor cheliped (fig. 3.5B) similar, dactylus slightly longer in relation to palm than in major chela.

Ambulatory pereiopods (figs. 3.6, 3.7) short, stout. Dactylus of third pereiopod (fig. 3.6A, B) with corpus moderately compressed, about 1.2 times longer than proximal width, with single row of few simple setae along ventral margin and in distal part, distoventral accessory tooth acute, perpendicular to flexor margin, ventral margin with one rounded protuberance at 2/3rd of ventral margin and



3.8 *Odontonia kerangcaris* sp. nov., holotype male, pocl. 2.1 mm, MZB 5341. A) left first pleopod; B) left second pleopod; C) idem, appendix masculina and appendix interna; D) right exopod of uropod, distolateral part. Scale A, B = 0.5 mm; C, D = 0.125 mm. Artwork: Charles Fransen.

one rounded protuberance proximally, without denticles in between; unguis strongly curved, about 0.5 of corpus length, distally with large dorsal scales, gradually decreasing in size proximally; propodus 2.8 times dactylus length, 4.7 times longer than proximal width, with one short, blunt, spine in distal part of flexor margin, with few long slender simple setae; carpus 0.64 of propodus length, 2.25 times longer than distal width, slightly tapering proximally, with indistinct distal lobe, unarmed; merus about as long as propodus, slightly swollen, about 2.8 times longer than central width, cylindrical; ischium 0.66 of merus length, 2.2 times longer than distal width; basis and coxa without special features. Fourth and fifth (fig. 3.7A, B) pereiopods similar.

Male first pleopod (fig. 3.8A) with endopod 0.4 times as long as exopod, with 2 long distal setae, with row of 5 small simple setae on lateral margin.

Male second pleopod (fig. 3.8B, C) with endopod slightly shorter than exopod, with appendix masculina, equal to about half length of appendix interna, reaching halfway endopod, with 3 very long setulose setae distally. Uropods (figs. 3.2D,

3.8D) normal, with short unarmed protopodite; exopod broad, 2.0 times longer than central width, lateral margin strongly convex, without distolateral tooth, with minute spinule distolaterally; endopod slightly exceeding exopod, reaching posterior margin of telson, 2.4 times longer than wide.

Size. This is a small sized species, pocl. is 2.1 mm.

Colouration. Not known.

Host. Mollusca, Bivalvia, Chamidae, *Chama lazarus* Linnaeus, 1758.

Distribution. Only known from the type locality: Berau Island, NE Kalimantan, Indonesia. Etymology. From the Indonesian word '*kerang*', which translates to "bivalve", combined with the Latin '*caris*' referring to shrimp.

Remarks. The species falls within the genus definition for *Odontonia* provided by Fransen (2002) and modified by de Gier & Fransen (2018). The new species has the accessory tooth on the dactyli of the ambulatory pereiopods similar to the ones found in *O. rufopunctata*, *O. bagginsi*, *O. sibogae*, *O. compacta*, and *O. katoi*. It differs from these species in the absence of a forward directed proximal tooth on the flexor margin of the corpus of the ambulatory dactyli. It also lacks the small denticles posterior to the distoventral accessory tooth on the dactylar corpus.

Species of *Odontonia* are known as endosymbionts of solitary ascidians (de Gier & Fransen 2018). *Odontonia kerangcaris* sp. nov. is the first species within the genus recorded from a bivalve mollusk. The species has affinities with species of the closely related bivalve mollusk dwelling genus *Conchodytes* Peters, 1852 (Horká et al. 2016; Chow et al. 2021). The accessory tooth and protuberances on the flexor margin of the ambulatory pereiopods resemble almost all *Conchodytes* species except for *C. chadi* (Marin, 2011) and *C. monodactylus* (Holthuis, 1952).

Other palaemonid endosymbionts known from chamid bivalves are *Pontonia pilosa* Fransen, 2002 and *Bruceonia ardeae* (Bruce, 1981). The East Atlantic *P. pilosa* has been recorded as an endosymbiont of *Pseudochama cristella* (Lamarck, 1819). *B. ardeae* has been recorded from the Great Barrier Reef, Australia, as an endosymbiont of *Chama pacifica* Broderip, 1835. Both *P. pilosa* and *B. ardeae* have the furry appearance shared with another palaemonid endosymbiont of bivalves: *Pinnotherotonia rumphiusi* Marin & Paulay, 2010, hosted by the venerid bivalve *Periglypta crispata* (Deshayes, 1854). A dense

setal coverage has also been noted for several pinnotherine crabs infesting bivalves (de Gier & Becker 2020).

The phylogenetic position of the new species seems to be at the base of the *Odontonia* clade and as sister of the *Conchodytes* clade (now in de Gier et al., 2022), revealing intrageneric host switching.

Acknowledgements

We thank Bert W. Hoeksema (Naturalis Biodiversity Center), Suharsono (PPO-LIPI), Yosephine Tuti (PPO-LIPI), and Anna E.W. Manuputty (PPO-LIPI) for organizing the survey to the Berau Islands. The survey was sponsored by the Indonesian Institute of Sciences (LIPI), the Netherlands Organization for Scientific Research (WOTRO grant WT 87–299), the Royal Dutch Academy of Sciences (KNAW), and Derawan Dive Resort. We thank Zdeněk Ďuriš for providing much appreciated feedback during review.

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4

Coming out of your shell or crawling back in: multiple interphylum host switching events within a clade of bivalve- and ascidian-associated shrimps (Caridea: Palaemonidae)

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This chapter is published as:

de Gier, W., Groenhof, M. & Fransen, C.H.J.M. (2022) Coming out of your shell or crawling back in: multiple interphylum host switching events within a clade of bivalve- and ascidian-associated shrimps (Caridea: Palaemonidae). Contributions to Zoology, 91(3): 166–198.

Abstract

Marine symbiotic Palaemonidae, comprising over 600 species, live in association with marine invertebrates of different phyla, like Cnidaria, Echinodermata, Mollusca, Porifera, and Tunicata. A phylogenetic study is performed on a clade of bivalve- and ascidian-associated endosymbiotic shrimp species (Caridea: Palaemonidae), using morphological and molecular data. A Total Evidence approach is used in order to include all currently known ingroup species in an evolutionary framework. Ancestral state reconstruction analyses are performed to identify host-switching events and ancestral ranges. The clade, including *Ascidonia*, *Conchodytes*, *Dactylonia*, *Odontonia*, and *Pontonia*, and various smaller genera, is recovered as monophyletic, with an ascidian-associated ancestral host state. At least six interphylum host switches are tentatively identified, with members of *Odontonia* and *Notopontonia* switching back to an ascidian host affiliation after the ancestral host switch of the clade including *Conchodytes*, *Odontonia* and related genera, from an ascidian- to a bivalve host. The clade including *Ascidonia* and *Pontonia* was recovered to have an ancestor with an East Pacific/Atlantic distribution. The other studied genera remained in the original ancestral Indo-West Pacific range. We hypothesise that similar internal environments of shrimp hosts from different phyla will function as hot spots for interphylum host switching in various lineages of symbionts.

Keywords: ancestral biogeography; ancestral character state analysis; morphological phylogeny; symbiosis; total evidence

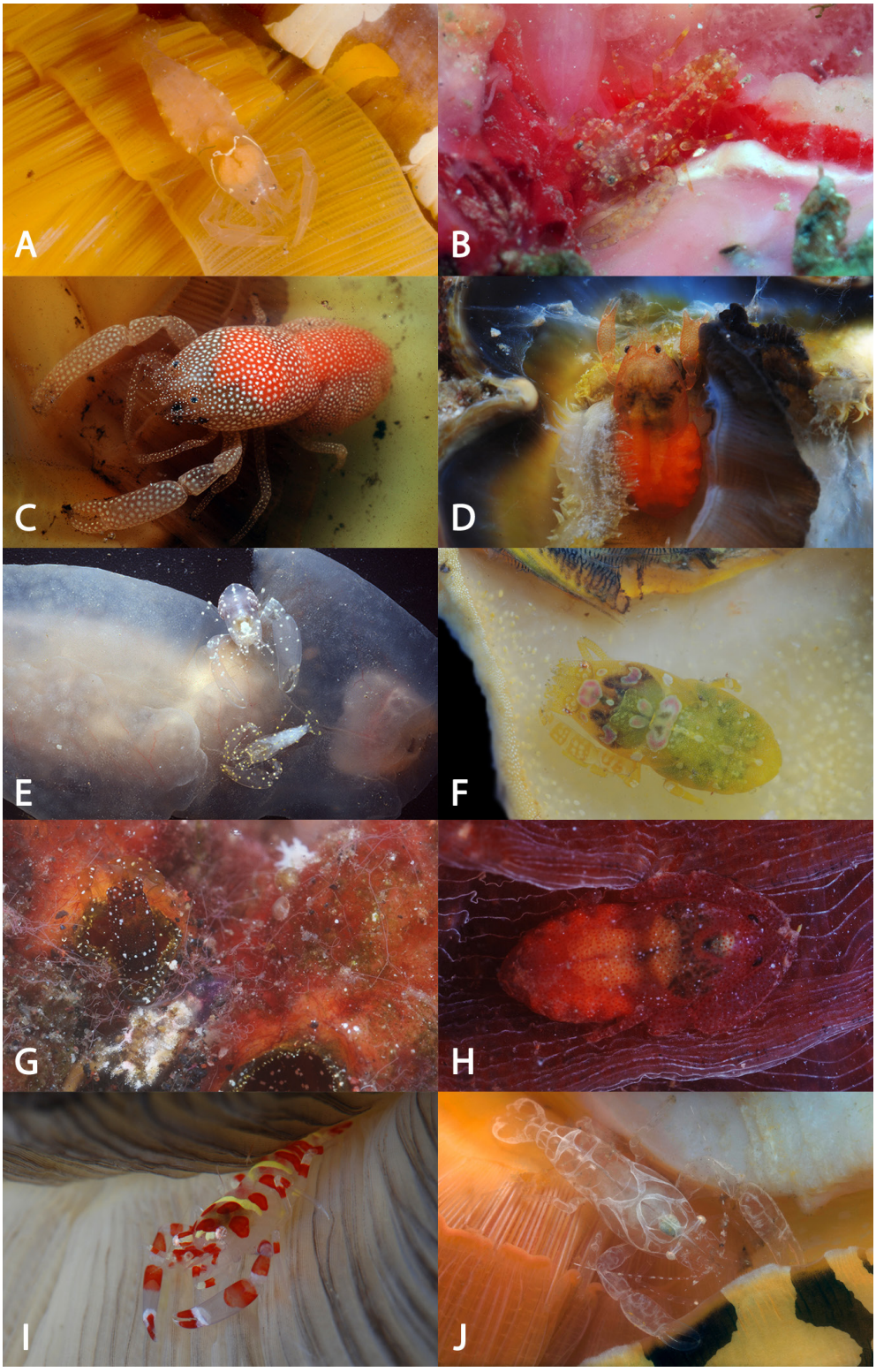


Introduction

Symbiotic palaemonid shrimp species are known to be associated with a wide variety of hosts (Bruce, 1976; Fransen, 1994a; Kou et al., 2015; Horká et al., 2016; Chow et al., 2021). Two of the currently recognised clades are known as symbionts of molluscs and solitary ascidians (Horká et al., 2016; Chow et al., 2021). The endosymbiotic shrimps of these two clades have a cryptic lifestyle and are thought to be well adapted to the organisms they inhabit, which is reflected by alterations in their morphology. This includes: (1) reduced frontal, dorsal and lateral protrusions for easier movement within the host (e.g., shortening of the rostrum, reduction of the number of rostral teeth, absence or reduction of the antennal and hepatic spines); (2) development of scales, (hooked) teeth, and microsetae on the dactyls of ambulatory pereiopods to increase grip; (3) adaptive (often cryptic) colouration; (4) a roughly cylindrical body shape with a body size that is adapted to the size of the body cavity of the host; (5) differences in eye morphology (Bruce, 1976, 1994; Fransen, 1994a; Dobson et al., 2014, 2016). Adaptations to an endosymbiotic lifestyle can also be found in other groups of crustaceans, such as pinnotherine pea crabs (Brachyura: Pinnotheridae) (de Gier & Becker, 2020).

Interphylum host switches are common within marine palaemonid shrimps (Kou et al., 2015; Horká et al., 2016; Chow et al., 2021). To obtain insight into host-switching events during the evolution of the marine Palaemonidae, Horká et al. (2016) performed a molecular phylogenetic analysis based on mitochondrial and nuclear genes. They recognised two distinct clades in which marine palaemonid shrimps radiated over bivalve mollusc and solitary ascidian hosts. From their analyses, they concluded that there were at least two lineages associated with solitary ascidians and two with bivalve molluscs, which were consistently related to each other (Horká et al., 2016). Additionally, two species of Periclimenaeus Borradaile, 1915 in the analysis were associated with compound ascidians, but clustered with sponge-endosymbionts (Horká et

4.1 An overview of the diversity in morphology, colouration, and host-associations of the studied clade. A) *Anchiopontonia hurii* (Holthuis, 1981) in the spiny oyster *Spondylus* sp.; B) *Ascidonia quasipusilla* (Chace, 1972) in a solitary ascidian; C) *Conchodytes meleagrinae* Peters, 1852 in the spiny oyster *Spondylus* sp.; D) *Conchodytes pteriae* Fransen, 1994 in the pearl oyster *Pteria loveni* (Dunker, 1879); E) male-female pair of *Dactylonia ascidicola* (Borradaile, 1898) from the solitary ascidian *Ascidia* sp.; F) *Odontonia katoi* (Kubo, 1940) in the solitary ascidian *Polycarpa aurata* (Quoy & Gaimard, 1834); G) *Odontonia plurellicola* de Gier & Fransen, 2018 in the colonial ascidian Plurella sp.; H) *Odontonia sibogae* (Bruce, 1973) in the solitary ascidian *Polycarpa* sp.; I) *Platypontonia hyotis* Hipeau-Jacquotte, 1971 in the giant honeycomb oyster *Hyotissa hyotis* (Linnaeus, 1758); J) *Pontonia manningi* Fransen, 2000 in the spiny oyster *Spondylus americanus* Hermann, 1781. Photo credits: Charles Fransen.


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al., 2016). Similar results were found by a more recent study focussing on host spectrum and morphological adaptations (Chow et al., 2021). The number of host switches found in these two studies was higher than previous estimates, which were based on smaller datasets comprising less genera and species (Kou et al., 2015).

The ancestral host association of the clade containing *Conchodytes* Peters, 1852 and related species (fig. 4.1) is unresolved at present (Horká et al., 2016: fig. 4, clade 6; Chow et al., 2021: fig. 3, upper branch of clade iiic). Previous phylogenetic analyses by Fransen & Reijnen (2012), Kou et al. (2015), Horká et al. (2016), and Chow et al. (2021) did not comprise the entire generic and species diversity of these two clades known at that time and new species have been discovered since (e.g., de Gier & Fransen, 2018; Anker & De Grave, 2021; Fransen et al., 2021). It is hypothesised that the number of host switches is underestimated due to the limited number of taxa included in previous analyses. Consequently, there is a need for a more comprehensive and detailed phylogenetic analysis combined with the reconstruction of ancestral host-associations.

In this study, we extended the molecular alignment and included a morphological dataset, similar to those of Fransen (2002) and de Gier & Fransen (2018), to include all species that are hypothesised to be part of the aforementioned clade 6 from Horká et al. (2016) (table 4.1). Our assemblage of genera thus consisted of *Anchiopontonia* Bruce, 1992, *Bruceonia* Fransen, 2002, *Cainonia* Bruce, 2005, *Conchodytes* Peters, 1852, *Pinnotherotonia* Marin & Paulay, 2010, *Platypontonia* Bruce, 1968, and *Pseudopontonia* Bruce, 1992, which are associated with bivalve molluscs, and the genera *Ascidonia* Fransen, 2002, *Colemonia* Bruce, 2005, *Dactylonia* Fransen, 2002, *Notopontonia* Bruce, 1991, and *Rostronia* Fransen, 2002, of which all species are associated with solitary ascidians.

The clade also includes *Odontonia* Fransen, 2002, and *Pontonia* Latreille, 1829, of which the included species do not all inhabit a similar host. All species of *Odontonia* inhabit solitary ascidians, except for the recently described species *O. kerangcaris* Fransen, Groenhof & de Gier, 2021, which inhabits a bivalve mollusc (Fransen et al., 2021). Most species of *Pontonia* inhabit molluscs, while *Pontonia panamica* Marin & Anker, 2008 is associated with solitary ascidians, and the host-association of *Pontonia longispina* Holthuis, 1951 is still unknown. In addition, most of the mollusc-associated *Pontonia* species have symbioses with bivalves, with the exception of *P. chimaera* Holthuis 1951, which lives inside the gastropod *Strombus galeatus* Swainson,

Table 4.1 Overview of all species within the studied clade, their general distribution range and preferred host group. Abbreviations: IWP) Indo-West Pacific; EP) East Pacific region; E Atl) East Atlantic Ocean; W Atl) West Atlantic Ocean. Detailed information and literature sources are provided in the appendices (supplementary table S1).

Ingroup species	Region	Host group(s)
Anchiopontonia Bruce, 1992		
A. hurii (Holthuis, 1981)	IWP	Mollusca: Bivalvia: Pectinida: Spondylidae
Ascidonia Fransen, 2002		
A. californiensis (Rathbun, 1902)	EP	Chordata: Ascidiacea: Phlebobranchia: Ascidiidae
A. flavomaculata (Heller, 1864)	E Atl	Chordata: Ascidiacea: Phlebobranchia: Ascidiidae
A. miserabilis (Holthuis, 1951)	W Atl	Chordata: Ascidiacea: Phlebobranchia: Ascidiidae
A. pusilla (Holthuis, 1951)	EP	Chordata: Ascidiacea: Stolidobranchia: Pyuridae - Aplousobranchia: Diazonidae
A. quasipusilla (Chace, 1972)	E + W Atl	Chordata: Ascidiacea: Solidobranchia: Pyuridae,
Bruceonia Fransen, 2002		
B. ardeae (Bruce, 1981)	IWP	Mollusca: Bivalvia: Venerida: Chamidae
Cainonia Bruce, 2005		
C. medipacifica (Edmondson, 1935)	IWP	Mollusca: Bivalvia: Pectinida: Spondylidae - Venerida: Chamidae
Colemonia Bruce, 2005		
C. litodactylus Bruce, 2005	IWP	Chordata: Ascidiacea indet.
Conchodytes Peters, 1852		
C. biunguiculatus (Paulson, 1875)	IWP	Mollusca: Bivalvia: Ostreida: Pinnidae
C. chadi (Marin, 2011)	IWP	Mollusca: Bivalvia: Ostreida: Ostreidae
C. kempoides Bruce, 2013	IWP	Mollusca: Bivalvia: Ostreida: Isognomonidae
C. maculatus Bruce, 1989	IWP	Mollusca: Bivalvia: Ostreida: Margaritidae
C. meleagrinae Peters, 1852	IWP	Mollusca: Bivalvia: Ostreida: Margaritidae
C. monodactylus Holthuis, 1952	IWP	Mollusca: Bivalvia: Ostreida: Pteriidae, Pinnidae
C. nipponensis (De Haan, 1844)	IWP	Mollusca: Bivalvia: Ostreida: Pinnidae
C. philippinensis Bruce, 1996	IWP	Unknown, probably Mollusca: Bivalvia
C. placunae (D.S. Johnson, 1967)	IWP	Mollusca: Bivalvia: Pectinida: Placunidae

Table 4.1 *Cont.*

C. pteriae Fransen, 1994	IWP	Mollusca: Bivalvia: Ostreida: Pteriidae
C. tridacnae Peters, 1852	IWP	Mollusca: Bivalvia: Cardiida: Cardiidae
Dactylonia Fransen, 2002		
D. anachoreta (Kemp, 1922)	IWP	Chordata: Ascidiacea: Stolidobranchia: Styelidae
D. ascidicola (Borradaile, 1898)	IWP	Chordata: Ascidiacea; Phlebobranchia: Ascidiidae
D. borradalei Bruce, 2005	IWP	Chordata: Ascidiacea: Phlebobranchia: Ascidiidae
D. carinicula Bruce, 2006	IWP	Unknown, probably from encrusting Chordata: Ascidiacea
D. franseni Bruce, 2003	IWP	Chordata: Ascidiacea: Phlebobranchia: Ascidiidae
D. holthuisi Fransen, 2002	IWP	Chordata: Ascidiacea: Phlebobranchia: Plurellidae
D. monnioti (Bruce, 1990)	IWP	Chordata: Ascidiacea: Phlebobranchia: Ascidiidae
D. okai (Kemp, 1922)	IWP	Chordata: Ascidiacea: Phlebobranchia: Ascidiidae
Notopontonia Bruce, 1991		
N. platycheles Bruce, 1991	IWP	Chordata: Ascidiacea: Stolidobranchia: Pyuridae
Odontonia Fransen, 2002		
O. bagginsi de Gier & Fransen, 2018	IWP	Chordata: Ascidiacea indet.
O. compacta (Bruce, 1996)	IWP	Chordata: Ascidiacea: Stolidobranchia: Pyuridae
O. katoi (Kubo, 1940)	IWP	Chordata: Ascidiacea: Stolidobranchia: Styelidae, Pyuridae
O. kerangcaris Fransen, Groenhof & de Gier, 2021	IWP	Mollusca: Bivalvia: Venerida: Chamidae
O. plurellicola de Gier & Fransen, 2018	IWP	Chordata: Ascidiacea: Phlebobranchia: Plurellidae
O. rufopunctata Fransen, 2002	IWP	Chordata: Ascidiacea: Stolidobranchia: Styelidae
O. seychellensis Fransen, 2002	IWP	Unidentified Chordata: Ascidiacea
O. sibogae (Bruce, 1973)	IWP	Chordata: Ascidiacea: Stolidobranchia: Styelidae, Pyuridae
O. simplicipes (Bruce, 1996)	IWP	Chordata: Ascidiacea: Stolidobranchia: Styelidae
Opaepupu Anker & De Grave, 2021		
O. huna Anker & De Grave, 2021	IWP	Mollusca: Bivalvia: Venerida: Trapezidae

Table 4.1 *Cont.*

Pinnotherotonia Marin & Paulay, 2010		
P. rumphiusi Marin & Paulay, 2010	IWP	Mollusca: Bivalvia: Venerida: Veneridae
Platypontonia Bruce, 1968		
P. brevirostris (Miers, 1994)	IWP	Mollusca: Bivalvia: Ostreida: Ostreidae
P. hyotis Hipeau-Jacquotte, 1971	IWP	Mollusca: Bivalvia: Ostreida: Ostreidae, Gryphaeidae
Pontonia Latreille, 1929		
P. chimaera Holthuis, 1951	EP	Mollusca: Gastropoda: Littorinimorpha: Strombidae
P. domestica Gibbes, 1850	W Atl	Mollusca: Bivalvia: Ostreida: Pinnidae
P. longispina Holthuis, 1951	EP	Unknown
P. manningi Fransen, 2000	E + W Atl	Mollusca: Bivalvia: Pectinida: Spondylidae, Pectinidae
P. margarita Smith in Verrill, 1869	EP	Mollusca: Bivalvia: Ostreida: Pinnidae, Margaritidae
P. mexicana Guérin-Méneville, 1855	W Atl	Mollusca: Bivalvia: Ostreida: Pinnidae
P. panamica Marin & Anker, 2008	EP	Chordata: Ascidiacea: Phlebobranchia: Ascidiidae
P. pilosa Fransen, 2002	E Atl	Mollusca: Bivalvia: Venerida: Chamidae
P. pinnae Lockington, 1878	EP	Mollusca: Bivalvia: Ostreida: Pinnidae
P. pinnophylax (Otto, 1821)	E Atl	Mollusca: Bivalvia: Ostreida: Pinnidae
P. simplex Holthuis, 1951	EP	Mollusca: Bivalvia: Ostreida: Pinnidae
Pseudopontonia Bruce, 1992		
P. minuta (Baker, 1907)	IWP	Chordata: Ascidiacea: Stolidobranchia: Styelidae
Rostronia Fransen, 2002		
R. stylirostris (Holthuis, 1952)	IWP	Chordata: Ascidiacea: Phlebobranchia: Ascidiidae

1823. The recently designated monotypic genus *Opaepupu* Anker & De Grave, 2021 is also included in the analysis. This genus also associates with bivalves, with the only currently recognised species living in symbiosis with *Trapezium oblongum* (Linnaeus, 1758). Although the systematic position of this genus may not be immediately clear due to its distinct unique morphology and lack of obvious similarity with other species, Anker & De Grave (2021) argued it is morphologically most similar to several IWP genera that also associate with bivalves, namely *Anchiopontonia*, *Bruceonia*, *Cainonia*, *Pinnotherotonia*, *Platypontonia* and *Pontonia*. *Opaepupu huna* Anker & De Grave, 2021 may

thus also fall into the aforementioned clade and is therefore included in the present study.

We aim to construct detailed phylogenies to determine whether the genera and our focus clade are monophyletic, to reconstruct ancestral host-associations and to identify interphylum host-switches. In addition, the ancestral ranges of the clades will also be studied. This study will help to shed light on the evolution of symbiotic relationships of a subset of marine palaemonid shrimps and to elucidate their evolutionary relatedness and biogeography. We hypothesise that similar internal environments of hosts from different phyla will function as hot spots for interphylum host switching in various lineages of symbionts. We thus expect multiple host switches from one phylum to the other and vice versa in various lineages of symbionts.

Materials and methods

Taxon Sampling

A total of 55 ingroup and five outgroup species were included in this study (supplementary appendix S1; table 4.1, supplementary table S1). Of those included in the ingroup, 39 species have their distribution in the Indo-West Pacific region (IWP), eight in the East Pacific region (EP), and another eight in either the West and/or East Atlantic Ocean (W/E Atl) (table 4.1). Five outgroup taxa were selected to span across the spectrum of hosts within the symbiotic Palaemonidae, representing echinoderm, sponge and cnidarian associates and one mostly free-living species (supplementary table S1). These are *Actinimenes inornatus* (Kemp, 1922), *Actinimenes ornatus* (Bruce, 1969), *Cuapetes tenuipes* (Borradaile, 1898), *Periclimenes colemani* Bruce, 1975 and *Typton wasini* Bruce, 1977. With the exception of *C. tenuipes*, all these outgroup species fall within Clade 5 of Horká et al. (2016), which is the sister clade of the present clade of focus. All outgroup species have an IWP distribution.

Fresh specimens were collected by scuba diving or other standard collection methods (supplementary table S1). Recently collected specimens were directly stored in 96% ethanol for DNA barcoding and identification. Subsampling was done by removing the left second and third pleopod or left third and fourth pereiopod of each specimen. After subsampling, specimens were moved to 70% ethanol for morphological analysis and long-term storage in the Naturalis Biodiversity Center decapod collection (RMNH.CRUS.D.; Leiden, The Netherlands) or the Museum Zoologicum Bogoriense (mzb.; Bogor, Indonesia). The newly sequenced material consists of 24 species, spread over seven

genera. In addition to the newly generated sequences, sequence data from GenBank (Sayers et al., 2020) was used (supplementary table S1).

DNA extraction, amplification and sequencing

Total genomic DNA was extracted from the subsampled tissue using the DNeasy Blood & Tissue Isolation Kit (qiagen), according to the manufacturer's protocols for animal tissue (insects). Elution was performed twice using 150 µl Buffer ae instead of using 200 µl Buffer ae once, to increase overall DNA yield and DNA concentration. Partial segments of four genes were amplified by PCR: mitochondrial genes for cytochrome c oxidase subunit I (COI, 658 bp) and 16S rRNA (16S, ca. 501 bp); and nuclear genes for histone 3 (H3, 310 bp) and 18S rRNA (18S, ca. 663 bp), partially based on the study of Horká et al. (2016). Polymerase chain reactions (PCRs) for the markers COI, 16S and 18S were performed in 25-µl volumes following Fransen & Reijnen (2012) and Brinkman & Fransen (2016) containing 1 µl of DNA template, 1.00 µl (10 pMol/µl) forward and reverse primers, 0.5 µl (2,5 mM) dNTPs, 0.25 µl (5 units/µl) Qiagen Taq DNA polymerase, 2.5 µl (10×) CoralLoad PCR Buffer, and 18.5 µl Ultrapure MilliQ (H2O). PCRs performed for the marker H3 differed slightly, with the addition of 5 µl Qiagen Q-solution (5×), accounting for the concentration with 13.8 µl Ultrapure MilliQ (H2O). The mitochondrial genes for COI and 16S rRNA were amplified using the universal primer pairs lco 1490/hco 2189 (Folmer et al., 1994) and 16Sar/16Sbr (Palumbi et al., 1991) respectively. The nuclear genes for H3 and 18S rRNA were amplified using the primer pairs H3F/H3R (Colgan et al., 2008) and 18Sa2.0/18S9r (Whiting, 2002). The pcr program for the amplification of the genes COI, 16S and 18S was as follows: 3 min at 96°C for initial denaturation, followed by 40 cycles of denaturing for 10 s at 96°C, annealing for 1 min at 50 °C, and extension for 1 min at 72°C, and a final extension step at 72°C for 5 min. The PCR program for the amplification of H3 was as follows: 3 min at 96°C for initial denaturation, followed by 40 cycles of denaturing for 10 s at 96°C, annealing for 1 min at 48°C, and extension for 1 min at 72°C, and a final extension step at 72°C for 5 min. PCR products lengths were checked using Thermofisher sybr Safe DNA stained 2% agarose gels. Sanger sequencing reactions were performed by BaseClear B.V., Leiden, The Netherlands.

Morphological character state analysis

All ingroup and outgroup species were scored morphologically, based on descriptions and illustrations in previous literature, as well as specimens present in the Naturalis collections (supplementary table S1). Specimens were studied with a dissecting stereomicroscope (Zeiss Discovery.V8) and

a compound microscope (Olympus bx53). The morphological data matrix contains 85 characters (supplementary appendix S2, supplementary table S2), and was formatted based on previous analyses by Fransen (2002) and de Gier & Fransen (2018) on a subset of the species in the current analysis. Multistate characters 3, 4, 12, 14, 15, 18, 19, 23, 28, 29, 36, 47, 49, 50, 64, 66, 68, 70, 76–78 and 80 were treated as ordered (Fitch, 1971), while characters 1, 2, 5, 6, 11 and 16 were considered irreversible (Camin & Sokal, 1965) and thus accompanied by a step matrix (using character states A, B, C, and D, instead of numbers, following Fransen (2002)). The remaining characters were treated as unordered (Farris, 1970). Character states A and 0 were generally attributed to the outgroup taxon *Cuapetes tenuipes*, unless in the case of an assumed linear transformation series in characters 47, 68, 70 where the species does not exhibit one of the extremes, and when the character state in *C. tenuipes* was unknown (characters 23–26, 29) or inapplicable (characters 57, 58, 60, 84, 85). Unknown character states were treated as missing data (= ?) and inapplicable characters were coded with gaps (= -). The character states, step-matrices and resulting morphological dataset can be found in the appendices (supplementary appendix S2, supplementary table S2).

Phylogeny reconstructions

Multiple sequence alignments were obtained using the ClustalW (Thompson et al., 1994) algorithm (default settings), and their ends were trimmed manually in mega v10.2.5 (Kumar et al., 2018). Alignments of protein-coding genes COI and H3 were subjected to Xia's test of nucleotide substitution saturation (Xia et al., 2003) in Dambe v7.3.0. (Xia, 2009). Only the third codon position of COI was found to be saturated and thus excluded, resulting in a total of 395 positions being included (table 2). After aligning, non-coding ribosomal 16S and 18S genes were examined for highly divergent blocks using Gblocks v0.91b (Talavera & Castresana, 2007) using default parameters but allowing gap positions, smaller finer blocks, and less strict flanking positions in both 18S and 16S. This resulted in the retention of 436 positions in 16S and 650 positions in 18S. Models for sequence evolution were calculated in mega v10.2.5 (Kumar et al., 2018) using default settings (table 4.2). Best models were chosen based on values for the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC).

A concatenated dataset containing all molecular markers was constructed in mega v10.2.5 (Kumar et al., 2018). This alignment consisted of 105 sequences and 1791 positions (table 4.2). Phylogenetic analyses were performed under the maximum likelihood (ML) and Bayesian Inference (BI) criteria using RAxML via the on-line cipres (Miller et al., 2010) with the RAxML-ng BlackBox v1.0.0

Table 4.2 Analytical methods and the used models for sequence evolution.

	RAxML model	MrBayes model	Trimmed fragment length
COI	TN93+G+I	GTR+G+I*	395 bp.
H3	K80+G**	K2+G	310 bp.
16S	TN93+G	HKY+G	436 bp.
18S	K80+G	K2+G	650 bp.
Morphology***	MULTI14_MK	Variable coding, rates=gamma	85 char.

*: MrBayes cannot implement TN93 so instead the second-best model, GTR+G+I was used. **: RAxML cannot implement K2 so instead K80 was used. ***: The mixed model for the MrBayes analysis of the morphological data in the Total Evidence analysis was coded to be gamma-distributed. In addition, the Multi4_MK model was selected to deal with the morphological dataset, with 14 being the number of different character states (0-9, A-D).

tool (Kozlov et al., 2019) and MrBayes v3.2.7 (Ronquist & Huelsenbeck, 2003), respectively. For the single-marker ML analyses default settings were used and automatic bootstrapping with a cut-off of 0.03, under an unpartitioned model. The concatenated four-marker dataset used a partitioned model with scaled branch length linkage and automatic bootstrapping with a cut-off of 0.03. Rest of the settings were default. The BI analyses were conducted using a Markov Chain Monte Carlo (MCMC) method with two independent runs and four chains. Analyses were run with a minimum of 5.000.000 generations to ensure that the average standard deviation of split frequencies (ASDSF) reached a value < 0.01, which would indicate the two runs have converged to a stationary distribution. The BI analysis on the concatenated molecular dataset did not reach this threshold after an additional increase of 15.000.000 generations (making a total of 20.000.000 generations) and was terminated with a ASDSF value of 0.010234. Trees were sampled each 500 generations. The initial 25% of trees was discarded as burn-in and the remaining trees were used to generate consensus trees and to estimate Bayesian posterior probabilities (PP). The BI analysis was consequently examined using MCMC Convergence Diagnostics in Tracer v.1.7.1 (Rambaut et al., 2018), where the 0.25 burn-in fraction was taken into account. Closer inspection of the trace plots of the log-likelihood indicated that convergence was reached, with an effective sample size (ESS) of 3477.3.

The morphological data matrix was analysed in paup v4.0a (Swofford, 2003) under the maximum parsimony (MP) criterion, using ordered characters and step matrices as mentioned above. A heuristic search was performed with 1000 repetitions. The strict consensus and the 50% majority rule consensus trees were obtained.

A total evidence (TE) analysis was done, similar to the one used in Van Der Wal et al. (2019). For this approach, concatenation of the molecular and morphological data was performed manually using a standard text editor. This alignment consisted of 66 sequences and 1876 positions (see table 4.2). All species were represented by morphological data, and where possible, with a set of sequences of all four available markers. If a species was found to be represented in multiple lineages in the previous single-markers analyses, all lineages were represented with their own sequences, in addition to a copy of the morphological data for this species. The selected sequences used in the TE analysis can be found in the appendices (supplementary table S1). Models for sequence evolution were as defined previously (table 4.2). Ordered and irreversible states were treated accordingly in MrBayes (Ronquist & Huelsenbeck, 2003), using the command ctype (table 4.2).

All trees were manually rooted by the outgroup taxon *Cuapetes tenuipes* and visualised in FigTree v1.4.4 (Rambaut, 2009). The resulting phylogenetic trees of the analyses based on single-gene markers (COI, H3, 16S, and 18S) can be found in the appendices (supplementary fig. S1), while the others can be found in the results (see below). The trees in the results were edited in Adobe Illustrator cc (Adobe Systems, USA) to match the colour scheme of the phylogenetic trees in previous studies (Horká et al., 2016; Chow et al., 2021).

Biogeographical analysis

In order to reconstruct the ancestral biogeographic ranges of the studied ingroup, broad current geographical distributions were obtained from taxonomic and ecological literature (supplementary appendix S1). Distributions were classified as mentioned above (EP, IWP, E Atl, W Atl). The resulting ML tree from the TE analysis was used as a backbone for figures, but an ultrametric version of the tree was used during the analyses. The ultrametric version of the phylogenetic tree was made using the force.ultrametric() function of the R-package phytools (Revell, 2012). Three different models of biogeographic evolution were assessed using the R-package BioGeoBEARS (Matzke, 2013), similar to other studies focussing on other marine organisms (e.g., Baraf et al., 2019). The following models were used for comparison: Dispersal-Extinction-Cladogenesis (DEC) (Ree & Smith, 2008); Dispersal-Vicariance Analysis (DiVA) (Ronquist, 1997); and Bayesian inference of historical biogeography for discrete areas (BayArea) (Landis et al., 2013). AIC scores were used to choose the best-fitting model, and can be found in the appendices (supplementary table S3). Given the recent concerns regarding the addition of the founder speciation event parameter “J” (Ree & Sanmartín, 2018), the current analyses resort to the most commonly used models for comparison without considering “jump”

(e.g., Baraf et al., 2019). For all analyses, a restricted model was used to limit the movement of ranges to only adjacent areas. This was done using a matrix file, as described in the BioGeoBEARS manual (Matzke, 2013). The resulting pie-charts displaying the ML values were edited and added to the phylogeny in Adobe Illustrator cc (Adobe Systems, USA). ML-values for all probabilities can be found in the appendices (supplementary table S4).

Ancestral state reconstruction of host associations

To reconstruct the evolution of host-associations, an ancestral character state reconstruction was performed in R using the MP criterion and the ML tree from the TE analysis as a backbone. The R-packages ape v. 5.5 (Paradis et al., 2019) and Geiger 2.0.7 (Penell et al., 2014) were used. Pie-charts displaying ML-values similar to the ones seen in comparable studies (e.g., Salis et al., 2018) were added in Abode Illustrator cc (Adobe Systems, USA). ML-values for all probabilities can be found in the appendices (supplementary table S4).

Results

Molecular phylogeny reconstructions

Three major clades can be distinguished in the ingroup, with most deeper nodes bearing support values lower than 50 (fig. 4.2). Various intrageneric branches within *Conchodytes*, *Ascidonia* and *Pontonia* were recovered with both high bootstrap and Bayesian pp support values. All outgroups were separated from the ingroup, except for one *Periclimenes colemani* specimen of which only a 18S sequence was available, which nested within a clade of *Dactylonia* (see discussion). One of two branches of *Platypontonia hyotis* Hipeau-Jacquotte, 1971 was the most basal ingroup lineage, while the other branch seems to be a basal branch within the first clade (see below). The placement of *P. hyotis* is debated in the discussion.

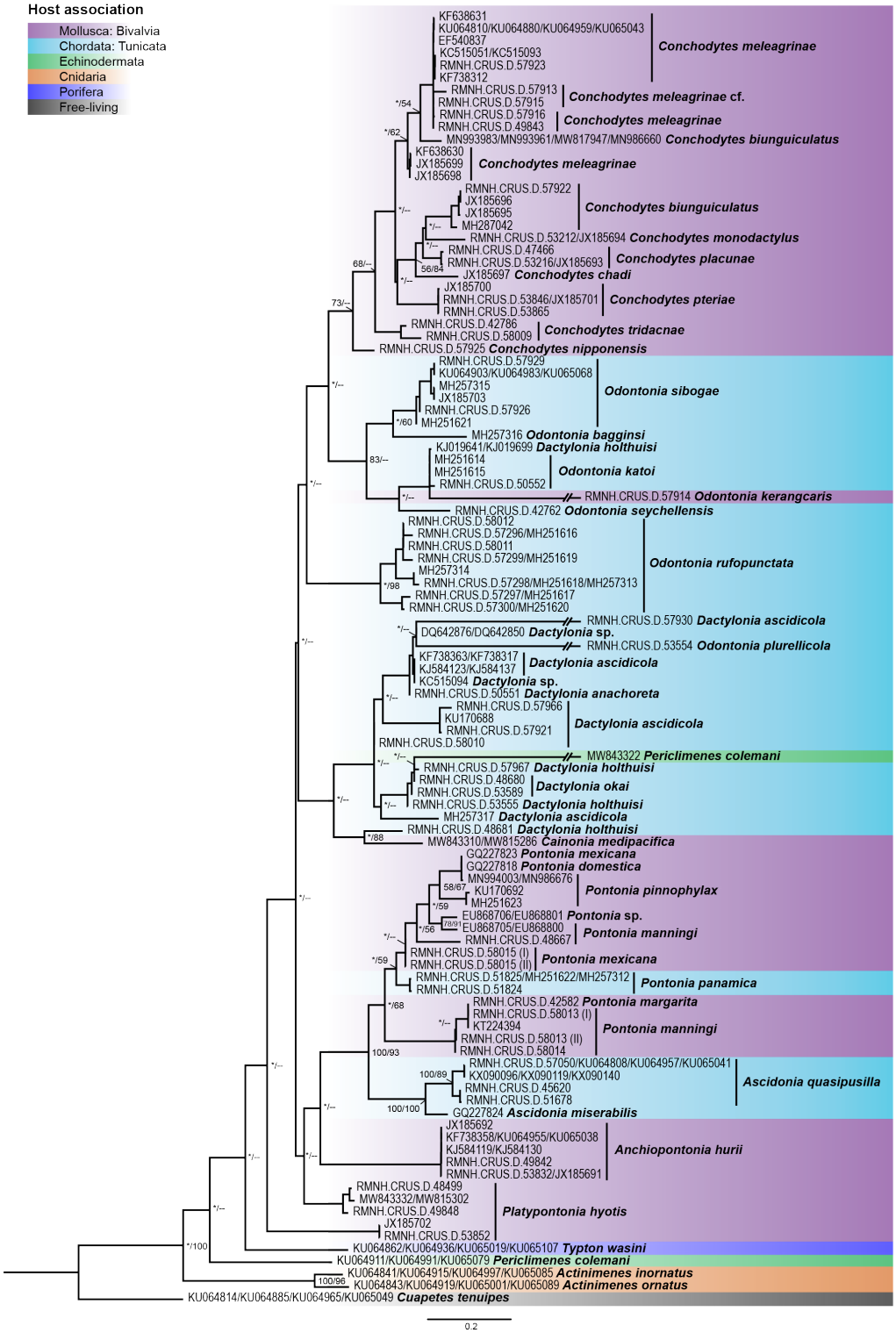
The first clade contains the Atl/EP genera *Ascidonia* and *Pontonia*, the IWP genus *Anchiopontonia*, and one branch of *Platypontonia hyotis*. Although some of the intrageneric species relations of this clade are resolved with high bootstrap or Bayesian pp values, the topology of the deeper nodes is resolved with very low bootstrap values and different topologies in the Bayesian analysis. Basally, the second branch of *Platypontonia hyotis* and a branch with five sequences of *Anchiopontonia hurii* (Holthuis, 1981) are split off from the first clade respectively, after which the monophyletic genus *Ascidonia* (consisting of *Ascidonia miserabilis* (Holthuis, 1951), and four sequences of *Ascidonia quasipusilla* (Chace, 1972)) appears to be sister of the monophyletic

genus *Pontonia*. Within *Pontonia*, various species seem to be phylogenetically paraphyletic: *Pontonia manningi* Fransen, 2000 can be found in a basal branch also containing one sequence of *Pontonia margarita* Smith, 1869, and in more derived branches, of which one is also containing an unidentified species of *Pontonia*; *Pontonia mexicana* Guérin-Méneville, 1855 can be found in two branches, one of which is grouped basally to the more derived species like *Pontonia pinnophylax* (Otto, 1821), and one sequence was grouped within the most derived branch, also containing sequences of *Pontonia domestica* Gibbes, 1850 and *P. pinnophylax*; and *P. pinnophylax* itself can be found in two groups, albeit both in the most derived branches of the genus. Whether these paraphyletic lineages can be attributed due to misidentifications, differences in distribution, or host choice is discussed below (see Discussion).

The second clade appears to be a sister to the third, much bigger clade. Species relations within these clades are not well resolved, with either low support values, different topologies in the Bayesian analysis, or both. The second clade contains all species of *Dactylonia* (except for one sequence of *Dactylonia holthuisi* Fransen, 2002) and (the monotypic genus) *Cainonia*, as well as a sequence of *Odontonia plurellicola* de Gier & Fransen, 2018 and of one specimen of the outgroup species *Periclimenes colemani*. *Cainonia* appears to be a sister clade to the rest of the species, although one sequence of *Dactylonia holthuisi* is grouped together with the only species of *Cainonia*. The other sequences of *D. holthuisi* within the second clade appear to be grouped together, with the inclusion of two sequences of *Dactylonia okai* (Kemp, 1922), the outgroup species *Periclimenes colemani*, and more basally, one sequence of *Dactylonia ascidicola* (Borradaile, 1898). The other branch of *Dactylonia* contains all other sequences of *D. ascidicola*, albeit in two to three different branches. One of these branches also contains two unidentified species of *Dactylonia*, the 18S sequence of *Odontonia plurellicola*, and one sequence of *Dactylonia anachoreta* (Kemp, 1922).

Lastly, all but one species of *Odontonia*, all of *Conchodytes* and one sequence of *Dactylonia holthuisi* form the largest third clade. Most basally, a branch containing eight sequences of *Odontonia rufopunctata* Fransen, 2002 splits

4.2 Phylogeny based on the RAxML tree topology of the concatenated molecular dataset (COI, H3, 16S, 18S). RAxML bootstrap support and Bayesian posterior probabilities expressed as percentages are indicated respectively. Dashes (--) indicate values <50; asterisk (*) indicates different topology of RAxML or MrBayes tree. Four branches are shortened for convenience. Three major clades can be recognized. Newly acquired barcodes are indicated with a collection accession number (RMNH.CRUS.D., MZB.), otherwise GenBank accession numbers are given. The selection of species can be found in the appendices (supplementary table S1). Colours indicate various host associations.


Host association
Mollusca: Bivalvia
Chordata: Tunicata
Echinodermata
Cnidaria
Porifera
Free-living
KF638631
KU064810/KU064880/KU064959/KU065043
EF540837
KC515051/KC515093
RMNH.CRUS.D.57923
KF738312
Conchodytes meleagrinae
RMNH.CRUS.D.57913
RMNH.CRUS.D.57915
Conchodytes meleagrinae cf.
RMNH.CRUS.D.57916
RMNH.CRUS.D.49843
Conchodytes meleagrinae
MN993983/MN993961/MW817947/MN986660 Conchodytes biunguiculatus
KF638630
JX185699
JX185698
Conchodytes meleagrinae
RMNH.CRUS.D.57922
JX185696
JX185695
MH287042
Conchodytes biunguiculatus
RMNH.CRUS.D.53212/JX185694 Conchodytes monodactylus
RMNH.CRUS.D.47466
RMNH.CRUS.D.53216/JX185693
Conchodytes placunae
JX185697 Conchodytes chadi
JX185700
RMNH.CRUS.D.53846/JX185701
RMNH.CRUS.D.53865
Conchodytes pteriae
RMNH.CRUS.D.42786
RMNH.CRUS.D.58009
Conchodytes tridacnae
RMNH.CRUS.D.57925 Conchodytes nipponensis
RMNH.CRUS.D.57929
KU064903/KU064983/KU065068
MH257315
JX185703
RMNH.CRUS.D.57926
MH251621
Odontonia sibogae
MH257316 Odontonia bagginsi
KJ019641/KJ019699 Dactylonia holthuisi
MH251614
MH251615
RMNH.CRUS.D.50552
Odontonia katoi
RMNH.CRUS.D.57914 Odontonia kerangcaris
RMNH.CRUS.D.42762 Odontonia seychellensis
RMNH.CRUS.D.58012
RMNH.CRUS.D.57296/MH251616
RMNH.CRUS.D.58011
RMNH.CRUS.D.57299/MH251619
MH257314
RMNH.CRUS.D.57298/MH251618/MH257313
RMNH.CRUS.D.57297/MH251617
RMNH.CRUS.D.57300/MH251620
Odontonia rufopunctata
RMNH.CRUS.D.57930 Dactylonia ascidicola
DQ642876/DQ642850 Dactylonia sp.
RMNH.CRUS.D.53554 Odontonia plurellicola
KF738363/KF738317
KJ584123/KJ584137
KC515094 Dactylonia sp.
Dactylonia ascidicola
RMNH.CRUS.D.50551 Dactylonia anachoreta
RMNH.CRUS.D.57966
KU170688
RMNH.CRUS.D.57921
Dactylonia ascidicola
RMNH.CRUS.D.58010
MW843322 Periclimenes colemani
RMNH.CRUS.D.57967 Dactylonia holthuisi
RMNH.CRUS.D.48680
RMNH.CRUS.D.53589
Dactylonia okai
RMNH.CRUS.D.53555 Dactylonia holthuisi
MH257317 Dactylonia ascidicola
RMNH.CRUS.D.48681 Dactylonia holthuisi
MW843310/MW815286 Cainonia medipacifica
GQ227823 Pontonia mexicana
GQ227818 Pontonia domestica
MN994003/MN986676
KU170692
MH251623
Pontonia pinnophylax
EU868706/EU868801 Pontonia sp.
EU868705/EU868800
RMNH.CRUS.D.48667
Pontonia manningi
RMNH.CRUS.D.58015 (I)
RMNH.CRUS.D.58015 (II)
Pontonia mexicana
RMNH.CRUS.D.51825/MH251622/MH257312
RMNH.CRUS.D.51824
Pontonia panamica
RMNH.CRUS.D.42582 Pontonia margarita
RMNH.CRUS.D.58013 (I)
KT224394
RMNH.CRUS.D.58013 (II)
RMNH.CRUS.D.58014
Pontonia manningi
RMNH.CRUS.D.57050/KU064808/KU064957/KU065041
KX090096/KX090119/KX090140
RMNH.CRUS.D.45620
RMNH.CRUS.D.51678
Ascidonia quasipusilla
GQ227824 Ascidonia miserabilis
JX185692
KF738358/KU064955/KU065038
KJ584119/KJ584130
RMNH.CRUS.D.49842
RMNH.CRUS.D.53832/JX185691
Anchiopontonia hurii
RMNH.CRUS.D.48499
MW843332/MW815302
RMNH.CRUS.D.49848
JX185702
RMNH.CRUS.D.53852
Platypontonia hyotis
KU064862/KU064936/KU065019/KU065107 Typton wasini
KU064911/KU064991/KU065079 Periclimenes colemani
KU064841/KU064915/KU064997/KU065085 Actinimenes inornatus
KU064843/KU064919/KU065001/KU065089 Actinimenes ornatus
KU064814/KU064885/KU064965/KU065049 Cuapetes tenuipes
0.2

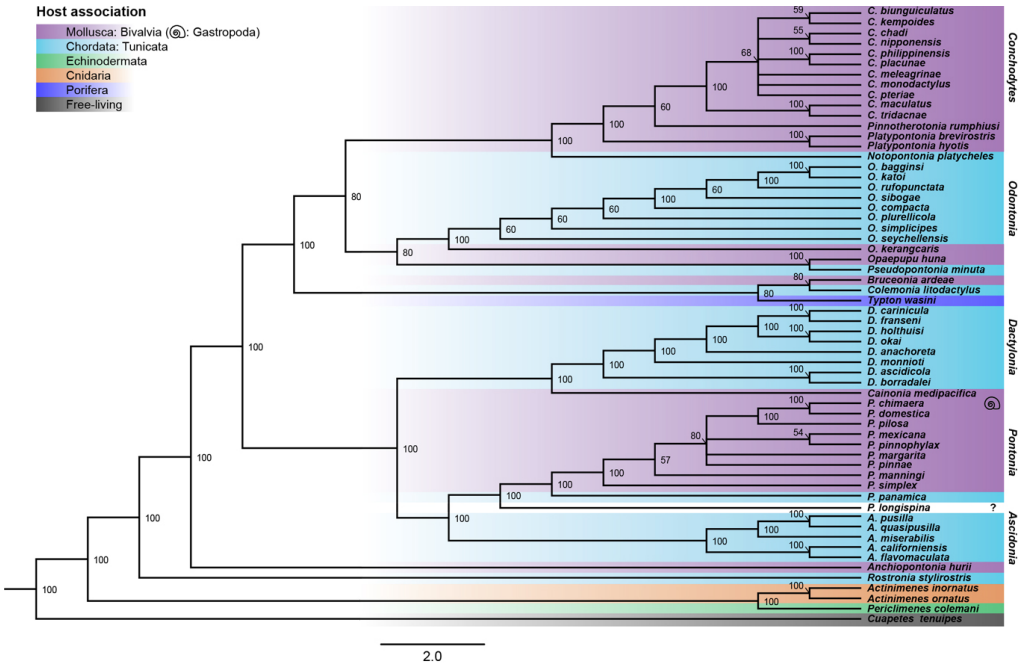
off, after which two branches appear: one containing the remaining sequences of *Odontonia* (and one sequence of *Dactylonia holthuisi* (see Discussion)), and the other containing all sequences of *Conchodytes*. *Odontonia* seems to be split up further into two main branches, one of which is containing basally *Odontonia seychellensis* Fransen, 2002, with *Odontonia katoi* (Kubo, 1940) and *O. kerangcaris* splitting up as sister species. The other branch contains *Odontonia sibogae* (Bruce, 1973), with *Odontonia bagginsi* de Gier & Fransen, 2018 as its sister species. *Conchodytes* appears to be the most well-resolved, with various branches being resolved with high bootstrap support values. *Conchodytes nipponensis* (De Haan, 1844) and *Conchodytes tridacnae* Peters, 1852 both respectively split off first, after which the genus is subdivided into two major branches: one branch containing (from basally branching off to more derived) *Conchodytes pteriae* Fransen, 1994, *Conchodytes chadi* (Marin, 2011), and *Conchodytes placunae* (D. S. Johnson, 1967). The branch ends in a split with *Conchodytes monodactylus* Holthuis, 1952, and (four sequences of) *Conchodytes biunguiculatus* (Paulson, 1875) being recovered as sister species. One other sequence of *C. biunguiculatus* can be found nested in the other major branch, between the two branches of *Conchodytes meleagrinae* Peters, 1852.

Morphological phylogeny

The morphological analysis recovered all the genera as monophyletic (fig. 4.3). *Cuapetes tenuipes*, *Actinimenes inornatus*, *A. ornatus* and *Periclimenes colemani* were recovered as outgroup species, while *Typton wasini* clustered with the ingroup species *Colemonia litodactylus* Bruce, 2005 and *Bruceonia ardeae* (Bruce, 1981) in the 50% majority consensus tree (fig. 4.3). Five distinct, well-supported clades could be recognised, with the monotypic IWP genera *Rostronia* and *Anchiopontonia* branching off basally, accompanied by high (100) support values. The first clade contains the Atl/EP genera *Pontonia* and *Ascidonia* that together form a well-supported lineage (100). Branching off this first clade is the second well-supported (100) clade, containing the IWP genera *Cainonia* and *Dactylonia*. Sister of the first two clades are the following three clades, in succession: the third well supported (100) clade containing the outgroup species *Typton wasini* basally, and the two monotypic IWP genera *Bruceonia* and *Colemonia*; the fourth well supported (100) clade contains the two IWP genera *Pseudopontonia* and *Opaepupu* basally, and all species of *Odontonia*; the fifth and last clade contains *Notopontonia* most basally, after which *Platypontonia* and later *Pinnotherotonia* branched off, the latter forming a direct sister to *Conchodytes*.

Two somewhat supported polytomies can be found in the morphological

phylogeny: one in the genus *Conchodytes*, branching off into six lineages (of which three contain two species); and one in the genus *Pontonia*, branching off to four lineages (of which one contains two species, and one three species).

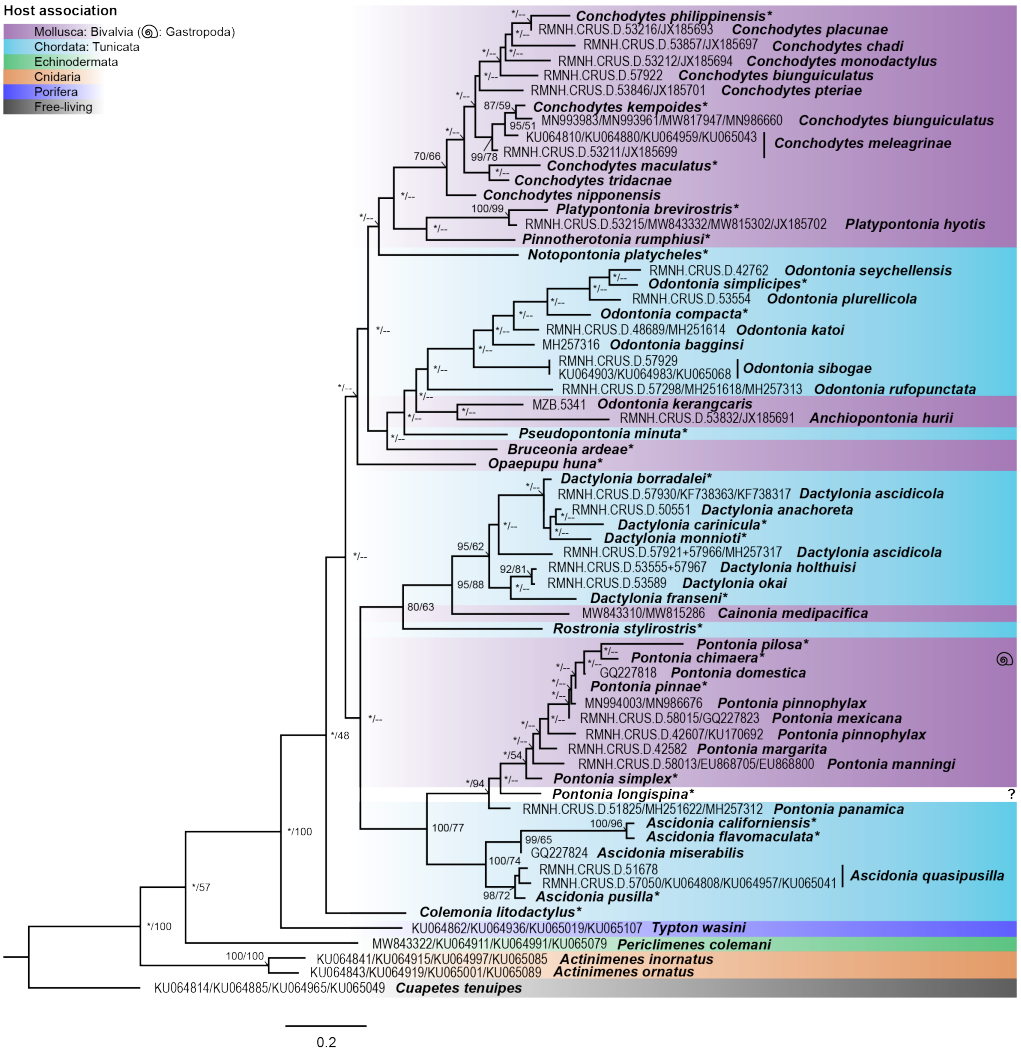


4.3 The 50% majority rule consensus tree of the morphological analysis in paup. Five distinct clades can be recognized. Support values are given at every dichotomous or polytomous branching. Colours indicate various host associations. The host association of *Pontonia longispina* Holthuis, 1951 is unknown, indicated with a question mark (?), and the known host association of *Pontonia chimaera* Holthuis, 1951 is with gastropod molluscs, which is indicated with an outline of a shell.

Total Evidence analysis

Four clades can be distinguished based on the TE approach (fig. 4.4). Unfortunately, the deeper nodes in the phylogeny had support values < 50 and inferences from these nodes should thus be made with caution. The in- and outgroup were confidently separated, illustrated by both high bootstrap and Bayesian pp support values. *Colemonia litodactylus* was the most basal ingroup lineage.

The first clade contains the Atl/EP genera *Ascidonia* and *Pontonia*. Within *Pontonia*, *Pontonia panamica* was basal to the rest of the genus. While the multiple sequences of *Ascidonia quasipusilla* clustered together nicely, the two sequences of *P. pinnophylax* were divided by a branch containing *P. mexicana*.



4.4 Phylogeny based on the RAxML tree topology of the te approach. RAxML bootstrap support and Bayesian posterior probabilities expressed as percentages are indicated respectively. Dashes (--) indicate values <50; asterisk (*) indicates different topology of RAxML or MrBayes tree. Four major clades can be recognized. Newly acquired barcodes are indicated with a collection accession number (RMNH.CRUS.D., MZB.), otherwise GenBank accession numbers are given. The selection of species can be found in the appendices (supplementary table S1), and species of which only morphological data was analysed are indicated with an asterisk (*) and no accession number. Colours indicate various host associations. The host association of *Pontonia longispina* Holthuis, 1951 is unknown, indicated with a question mark (?), and the known host association of *Pontonia chimaera* Holthuis, 1951 is with gastropod molluscs, which is indicated with an outline of a shell.

The splitting of the second clade containing *Dactylonia*, *Cainonia*, and *Rostronia* was weakly supported by both the bootstrap values, as well as the

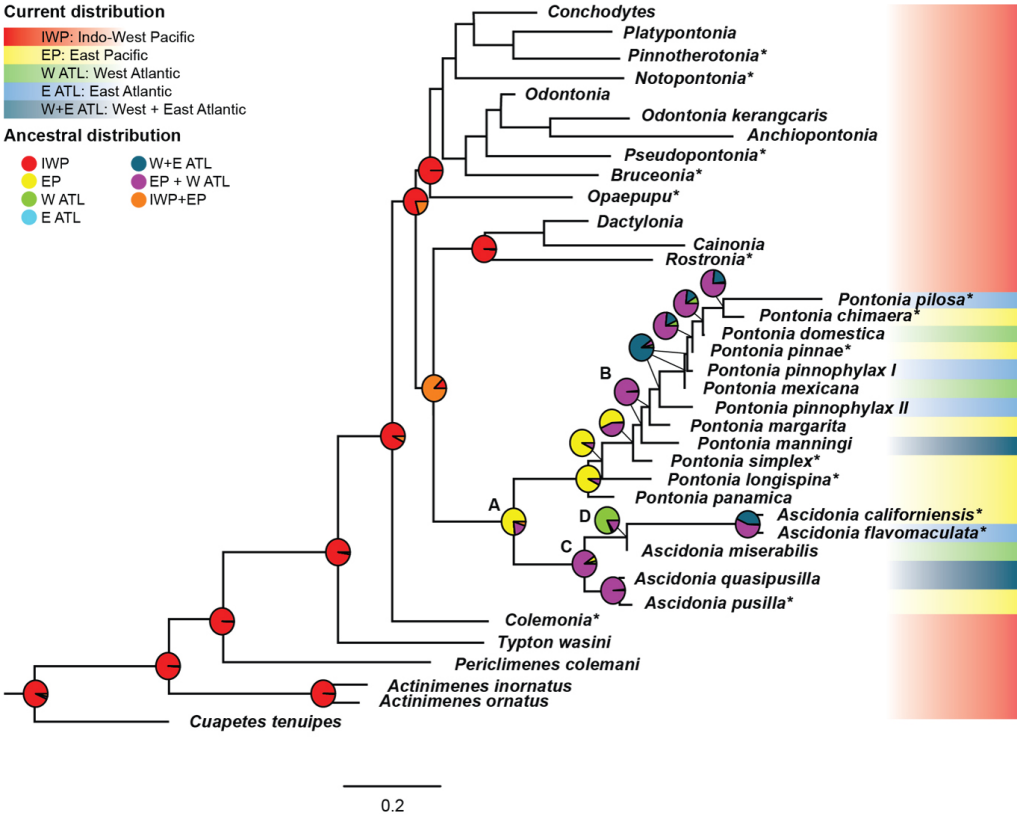
Bayesian Inference pp support values. However, the internal branches were better supported. *Rostronia* basally branching off from this clade resulted in low support values in the BI analysis, while the bootstrap support value is 80. The two sequences of *Dactylonia ascidicola* did not cluster together, but were only separated by low support values and aberrant topologies of the MrBayes analysis. The third and fourth clades were recovered as sister clades with *Opaepupu* basal to these two clades, although these relationships were only weakly supported. The third clade contains a monophyly of *Odontonia*, *Pseudopontonia*, *Bruceonia* and *Anchiopontonia*. The placement of the monotypic genera directly basal to *Odontonia* had low support values. *O. kerangcaris*, was placed most basally to the genus together with the monotypic genus *Anchiopontonia*, with low support values. There were topological incongruencies between both analyses, but the sequences of *Odontonia sibogae* clustered together as expected.

The fourth clade contains a monophyly of all species of *Conchodytes* and a basal lineage of *Platypontonia* and *Pinnotherotonia*, with *Notopontonia* being basal to this entire clade. This topology between the four genera was supported relatively weakly in both analyses. Within *Conchodytes*, support values were generally low, with some high support values within and for the clade containing *C. meleagrinae*, *Conchodytes kempoides* Bruce, 2013, and one sample of *C. biunguiculatus*. In addition, *C. nipponensis* was recovered as the most basal species in *Conchodytes*, with somewhat high support values.

Historical biogeography analysis

An ancestral state reconstruction analysis was performed to identify ancestral biogeographic ranges of the studied ingroup taxa. Of the studied models, the dec model was retrieved with the lowest AIC score (see supplementary table S3). The clade with the genera *Pontonia* and *Ascidonia* has a distribution outside Indo-West Pacific area (see table 4.1). The common ancestor of this clade has an EP/W Atl distribution (fig. 4.5: node A, P = 0.77). *Pontonia* has maintained an EP distribution until the common ancestor of *P. manningi* evolved with an ancestral range in the EP/W Atl (fig. 4.5: node B, P = 0.98).

The common ancestor of the genus *Ascidonia* was recovered to have an EP/W Atl distribution (fig. 4.5: node C, P = 0.91). This remains the ancestral range for the common ancestor of the clade containing *Ascidonia pusilla* (Holthuis, 1951) and *A. quasipusilla*. The ancestor of the other clade with sister species *Ascidonia californiensis* (Rathbun, 1902) and *Ascidonia flavomaculata* (Heller, 1864) was recovered as mainly W Atl (fig. 4.5: node D, P = 0.81).



4.5 Phylogeny based on the RAxML tree topology of the te approach (fig. 4.4), with ancestral biogeographic range reconstructions on the internal nodes (probabilities are shown as pie charts). Indo-West Pacific genera simplified as genus names, except for *Odontonia kerangcaris* Fransen, Groenhof & de Gier, 2021 due to its position outside of the genus. Colours indicate distribution ranges, both for the species as well as the ancestral distribution ranges. Species/genera of which only morphological data was analysed are indicated with an asterisk (*).

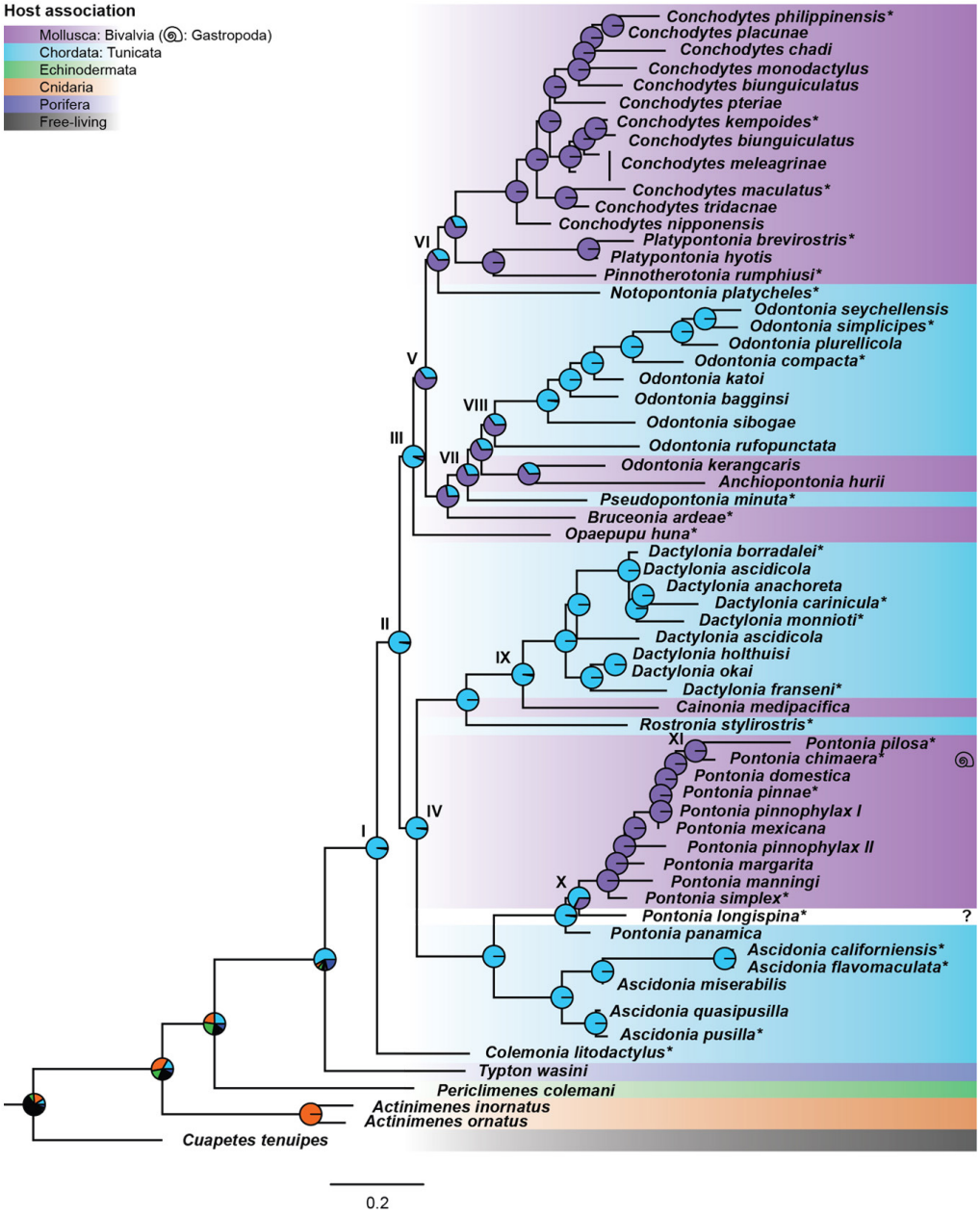
Ancestral state reconstruction of host associations

An ancestral character state reconstruction analysis resulted in Maximum Likelihood probabilities for all 127 internal nodes, which translate to the probabilities an ancestral clade of shrimp would have been associated to a certain type of host. As expected, all internal nodes of the clade containing *Conchodytes*, *Platypontonia*, *Pinnotherotonia* are fully resolved to be associated to bivalve molluscs (P = 1.00), similar to most internal nodes of *Pontonia* (excluding *P. panamica* and *P. longispina*). Similarly, the clades containing *Ascidonia*, *Dactylonia* and most of *Odontonia* are fully resolved to be associated with ascidians.

Most interesting are the ancestral character states of the entire ingroup, and nodes where interphylum host-switches seem to occur. The character state

of the ancestral node of the entire ingroup is recovered to be an ascidian-associated species, both with or without the inclusion of the basally branching *Colemonia* (fig. 4.6: node I and II, P = 0.99 and P = 1.00). While the ancestral host association of the first major clade, containing *Conchodytes*, *Odontonia* and related genera, is recovered as a bivalve associate (fig. 4.6: node V, P = 0.62), the character state of the ancestor when including *Opaepupu* is still recovered as an ascidian-associate (fig. 4.6, node III, P = 0.95). A host switch has occurred from an ascidian to a bivalve mollusc host at the base of this clade, but also separately in the branch containing *Opaepupu*. In addition, the ancestral character state of the clade including *Conchodytes*, *Platypontonia*, *Pinnotherotonia*, and *Notopontonia*, was recovered to be a bivalve-associate, even when taking the ascidian-associated *Notopontonia* into account (fig. 4.6: node VI, P = 0.64). This means that there has occurred a similar switch, albeit now from a bivalve mollusc to an ascidian host, within the branch of *Notopontonia*. Within the other part of the clade, comprised of *Odontonia*, *Anchiopontonia*, *Pseudopontonia*, and *Bruceonia*, two to three host switches, again from a bivalve mollusc to an ascidian host, can be observed. First one in the single branch of *Pseudopontonia*, and later in the branch containing *O. rufopunctata*, and in the other lineage of *Odontonia* (excluding *O. kerangcaris*).

The ancestral state of the clade containing *Pontonia*, *Ascidonia*, *Dactylonia* and related species is recovered as an ascidian-associated lineage (fig. 4.6: node IV, P = 1.00). Within this clade, two host switches from an ascidian host to a bivalve mollusc, and one within-phylum (from a bivalve mollusc to a gastropod mollusc) host switch can be found. The subclade containing *Dactylonia*, *Cainonia* and *Rostronia* can be observed to have an interphylum host switch at a basal branch of the clade (after *Rostronia* splits off, at the branching with *Cainonia*): the ancestral character state of this node is recovered as an ascidian associated species (fig. 4.6: node IX, P = 0.99). In contrast to the monotypic *Cainonia*, the various bivalve-associated *Pontonia* species form a separate clade after splitting off from their ascidian associated congener, *P. panamica*. The unknown host association of *P. longispina* causes the ancestral state reconstruction of their joint node to have a lower probability in favour of an ascidian-associated ancestor (fig. 4.5: node X, P = 0.67). The intergeneric host switch of *P. chimaera* from a bivalve to a gastropod mollusc, results in a fully resolved ancestral character reconstruction in favour of a bivalve mollusc host (fig. 4.5: node XI, P = 1.00).



4.6 Phylogeny based on the RAxML tree topology of the te approach (fig. 4.4), with ancestral character state reconstructions on the internal nodes (probabilities are shown as pie charts). Colours indicate various host associations, both for the species as well as the ancestral character states. The host association of *Pontonia longispina* Holthuis, 1951 is unknown, indicated with a question mark (?), and the known host association of *Pontonia chimaera* Holthuis, 1951 is with gastropod molluscs, which is indicated with an outline of a shell. Species of which only morphological data was analysed are indicated with an asterisk (*).

Discussion and Conclusions

Uncertain placement of ingroup species among different tree topologies

While most of the genera share a similar, monophyletic topology in all trees, there are some species with one or more questionable placements among the different tree topologies. In addition, various genera which were only represented by one species (monotypic) in the concatenated alignment, or in the TE analysis differ in placement in the various tree topologies as well. The differences in placement among the various analysis-methods can be explained by missing data in the molecular alignment (e.g., Van Der Wal, 2019), uncertain identifications (Raupach & Radulovici, 2015), or bias in the analysis methods (e.g., due to convergent evolution of adaptive morphological features; Scotland et al., 2003; Lee & Palci, 2015; Caldas & Schrago, 2018). The current placements, possible explanations of these placements, and potential solutions will be discussed below for all notable species.

Anchiopontonia hurii can be observed to be most closely related to the recently described species *Odontonia kerangcaris* in the TE analysis (fig. 4.4), while it was placed basally to *Ascidonia* and *Pontonia* in the concatenated and morphological alignment (figs. 4.2, 4.3). Bruce (1992) argued that *A. hurii* was closely related to some other species that have long dorsal telson spines, but this character can be found in various lineages of the studied ingroup. Previous phylogenetic studies have at least confirmed the basal placement of the species, but if the species is basal to some IWP genera (e.g., Fransen & Reijnen, 2013, Chow et al., 2021) or to the entire, or a part of the ingroup (e.g., Gan et al., 2015, Horká et al., 2016), remains elusive.

While the placement of *Conchodytes* as a genus is fairly well resolved in all phylogeny reconstructions, the placement of its species is far from similar in all analyses. In addition, previous literature based on morphological data does not seem to fully comply with the current results based on molecular data (e.g., Holthuis, 1952; Bruce, 1989; Fransen, 1994b; Bruce, 2013). In the current analyses, there seems to be a consensus about the basal placement of *C. nipponensis* and *C. tridacnae* (and the closely related *Conchodytes maculatus* Bruce, 1989, which is only represented by morphological data) to the rest of the genus, but the support values resulting from the TE and concatenated alignment are rather low (figs. 4.2 and 4.4). The deviation in placements might be related to the morphological similarity of *C. nipponensis* to the other members of the genus (fig. 4.3) (e.g., Fransen, 1994b). Bruce (1989) mentioned that *C. maculatus* is morphologically quite distinct, suggesting a basal placement too

in the genus. The remaining species of *Conchodytes* seem to cluster in two lineages in the TE and concatenated approach (figs. 4.2 and 4.4), similar to the previous phylogenetic studies on the genus (Fransen & Reijnen, 2012, 2013): *C. chadi*, *C. placunae*, *C. monodactylus* and (one lineage of) *C. biunguiculatus* seem to cluster together in all analyses, complemented by one species which is only represented by morphological data (*C. philippinensis* Bruce, 1996). It is worth noting that the previous phylogenetic studies of this genus used only one molecular marker (COI). Interestingly, Bruce (1996) mentions *C. philippinensis* to be most related to *C. nipponensis* and *C. maculatus*, which are here recovered as one of the basal species within *Conchodytes* (fig. 4.4). While in both analyses *C. pteriae* is represented as a basal species related to this cluster, previous literature states *C. pteriae* to be a sister to *C. meleagrinae*, a species from the other group in the present analyses.

The second group in the present analyses is a cluster of two lineages of *C. meleagrinae*, and one lineage of *C. biunguiculatus*. In addition, this cluster also includes *C. kempoides*, which is only represented by morphological data from the limited published illustrations and description (Bruce, 1989, 2013). As mentioned above, *C. meleagrinae* and *C. biunguiculatus* are represented by two lineages each, the latter lineages even found in both *Conchodytes* groups. The possible explanations for these may be deduced from the localities and host of the species (supplementary table S1): the *C. biunguiculatus* specimen from Chow et al. (2020, 2021) which does not comply with the phylogenetic position found in previous studies (Fransen & Reijnen, 2012, 2013) comes from the Red Sea (Eilat, Israel), from a *Pinctada margaritifera* (Linnaeus, 1758). The other specimens are from more Eastern waters (Indian Ocean, IWP; supplementary table S1), presumably from different species of bivalves (presumably all *Pinna* spp.). The genetic distance between the two lineages of *C. biunguiculatus* can partially be explained by the geographical distance between both populations, although a placement as sister species would be expected (as can be seen in the genus *Alpheus* Fabricius, 1798 in Williams et al., 2001). Similarly, a difference in host species would possibly not affect the genetic distance between populations, as was proven in *Z. soror* by Antokhina & Sorokin (2010). This was however just proven in one species, and is worth examining in other palaemonid shrimp species with a smaller host-range. Physical examination of this specimen is needed to find out if this was correctly identified or whether it belonged to an undescribed species of *Conchodytes*. Holthuis (1952) already noted a resemblance of *C. monodactylus* to *C. biunguiculatus*, which is herein also confirmed based on molecular data of the “true” *C. biunguiculatus* (fig. 4.4). Similarly, there are two separate lineages of *C. meleagrinae*: one more basally placed to the other in both TE and concatenated analyses. The possible

explanation for this placement is difficult to identify: the localities and host choice of all analysed specimens are similar. Only one specimen (KF638630), one of the three specimens which were included by Fransen & Reijnen (2013), was found in an aberrant host species (*Spondylus* sp.). Fransen & Reijnen (2013) found one clade of *C. meleagrinae*, but a reasonable genetic distance between the three basal specimens (KF638630, JX185699, and JX185698) and the more derived specimen (KF638631). In addition, two specimens are indicated as "*C. meleagrinae* cf." in our analyses. These specimens were found in a specimen of *Pteria* sp., and have some morphological character that are different from the other specimens. The specimens (RMNH.CRUS.D.57913 and RMNH.CRUS.D.57915) are smaller in body size (pocl of 3–5 mm), while other specimens are somewhat larger in size (pocl 5–7.5 mm). Consequently, various defining characters are underdeveloped in these specimens. They, however, share various characters on the third, fourth and fifth walking leg dactyli, and the second pair of chelae, that differ it from the original identification of *C. pteriae* (Fransen, pers. obs.). A more complete molecular alignment is needed to compare the specimens of this species, but also all other species within *Conchodytes*, correctly.

The two lineages recovered in the TE analysis (fig. 4.4) within *Dactylonia* are hard to identify in the other molecular analysis (fig. 4.2). The first lineage seems to partially correspond to the previous study by Fransen (2002) and includes *D. okai*, *D. holthuisi*, and one species that is only represented by morphological data (*Dactylonia franseni* Bruce, 2003). The second lineage seems to be represented by D. anochoreta, *Dactylonia borradalei* Bruce, 2005, *Dactylonia carinicula* Bruce, 2006, *Dactylonia monnioti* (Bruce, 1990) (the latter three only represented by morphological data), and two lineages of *D. ascidicola*. Interestingly, Bruce (2003) already thought *D. franseni* to be related to *D. okai*, but also to *D. ascidicola*, which is in part congruent with the current analyses. In a similar situation, Bruce (2006) thought *D. carinicula* to be a close relative to *D. ascidicola*, *D. anachoreta*, or *D. monnioti*, which seems to correspond as well. At least two species with uncertain placements cause the phylogeny reconstruction to be far from resolved within the genus. As mentioned above, *D. ascidicola* is represented by two specimens in two lineages in the TE analysis, and by eight specimens (as five separate lineages) in the concatenated alignment. Assuming the specimen identified as *D. anachoreta* is correctly sequenced, an indication of the true relations between the specimens identified as *D. ascidicola* can be seen. Due to both lineages being examined for their morphology, and the high genetic similarity, and therefore low intrageneric support values, the "true" lineage of *D. ascidicola* cannot be identified. However, there seems to be a consensus that *D. ascidicola* is closely related to *D. anachoreta* (and

possibly to *D. borradalei*, as this species was described by Bruce (2005) on the basis of specimens previously attributed to *D. ascidicola*). The only known COI sequence of *D. ascidicola* is located in the other *Dactylonia* lineage (fig. 4.2: MH257317) and was later used in the TE alignment (fig. 4.4), where it clustered with the above-mentioned clade. The other problematic species, *D. holthuisi*, can be observed to be represented in four lineages in the concatenated alignment: the presumably correct placement as two clustering with *D. okai*, one basally to *Dactylonia* clustering with *Cainonia*, and one within *Odontonia*. The first three lineages were correctly identified by physical examination, while the Taiwanese material from Gan et al. (2015) from the fourth lineage could not be examined. This specimen might have been mistaken for *Odontonia katoi* due to the shrimp's small size and ascidian-inhabiting behaviour (the host of Gan et al.'s (2015) specimen is however unknown). The specimen related to *Cainonia* is represented by a single 16S sequence, potentially causing this placement (supplementary fig. S1C). Undersampling of genetic markers in the available specimens, as well as marker choice, can influence the resulting placement in a phylogenetic tree (e.g., da Silva et al., 2011). Similar to *Conchodytes*, more molecular data is needed in order to correctly place the species of *Dactylonia* in a phylogenetic framework.

Although the species within *Odontonia* are placed in a different order in all three tree topologies, there seems to be some kind of consensus on which species are more basal, and which species are more related to one another. The recovered topologies are largely similar to those of Fransen (2002). *Odontonia* seems to be split up into two main lineages in the analysis using only molecular markers, with *O. rufopunctata* being placed basal to the *Odontonia* and *Conchodytes* groups (fig. 4.2). *Odontonia rufopunctata* is placed in a basal position to the other ascidian-associated *Odontonia* species in the TE analysis as well (fig. 4.4). Similarly, the bivalve-associated *O. kerangcaris* is placed basally to the other *Odontonia* species with *Anchiopontonia hurii* in the TE analysis (fig. 4.4), while it is placed in *Odontonia* next to *O. katoi* in the tree resulting from the concatenated alignment (fig. 4.2). *Odontonia kerangcaris* is also placed basally in the morphological analysis, however, *O. rufopunctata* is placed in a derived branch of *Odontonia* in this tree topology. The basal placement of *O. kerangcaris* is as expected, since the species' host and morphological features were found by Fransen et al. (2021) to resemble that of related, bivalve-associated genera such as *Conchodytes*. The placement of *O. rufopunctata* is less expected, but the placement away from the other species within the genus was also recovered in a previous study based on DNA and morphological features (de Gier & Fransen, 2018). As mentioned above, the other species seem to be grouping in two separate lineages in the concatenated analysis:

one lineage including *O. katoi*, *O. seychellensis* and *O. kerangcaris* (and one specimen of *Dactylonia holthuisi*); and one lineage including *O. bagginsi* and *O. sibogae*. The placement of the relatively basal *O. katoi* is interesting, since it here clusters with *O. seychellensis*, which was recovered to be a very derived species in the TE analysis. The potential explanation for this can be found in the only successfully sequenced marker of *O. katoi*, 16S. *Odontonia katoi* can therefore only be compared to other 16S sequences, in this case being limited to only *O. sibogae* and *O. rufopunctata* (see de Gier & Fransen, 2018). Due to *O. simplicipes* and *O. compacta* being only represented by morphological data, and *O. katoi*, *O. kerangcaris*, *O. plurellicola*, *O. bagginsi*, and *O. seychellensis* only being represented by one molecular marker, the relation between these species and the other species in *Odontonia* is far from resolved.

The inclusion of the recently described genus *Opaepupu* is only based on similarities in morphological characters, and a similar host association (with a bivalve mollusc). Anker & De Grave (2021) even remarked that this monotypic genus might not necessarily be closely related to the other bivalve endosymbionts. Nonetheless, *O. huna* seems to bear various morphological characters that resulted in the current analyses in a basal placement to *Conchodytes* (fig. 4.3) or to a clade including *Conchodytes*, *Odontonia*, and other related genera (fig. 4.4). These morphological characters include: slender, seemingly unadapted dactyli on the ambulatory legs; a well-developed, acute antennal spine and dorsal and rostral carina; and a slender third maxilliped (Anker & De Grave, 2021; supplementary appendix S2, supplementary table S2). In a similar situation the sole member of *Notopontonia*, *N. platycheles* Bruce, 1991, was also included based on the morphological similarities of the described specimens in Bruce (1991) and Berggren (1999). Based on external characters, Bruce (1991) argued *Notopontonia* to be closely related to *Pontonia*. However, Bruce (1991) did not discover a host for the species, and suggested that the species was a bivalve associate due to its depressed body shape and the horizontal disposition of the second pereiopods (Berggren, 1999). Berggren (1999) found the species in the ascidian *Herdmania momus* (Savigny, 1816), but did not mention anything about its systematic placement. The ascidian-associated habit of *N. platycheles*, as well as its previously thought relatedness with *Pontonia*, lead us to believe the species is a member of the currently studied clade.

Platypontonia hyotis is represented by five individuals in the concatenated alignment, clustered in two groups of two and three barcodes respectively. The basal placement of the first group (fig. 4.2) raises questions about the similarities of these specimens, compared to the ones being basally placed

next to *Anchiopontonia*, *Ascidonia* and *Pontonia*. The possible explanation for this aberrant placement can be found in the selection of the barcodes (supplementary table S1): the two basally placed barcodes are both COI-barcodes, while the other three clustered barcodes are a combination of 16S and H3 barcodes. Physical examinations were done on four specimens, eliminating the chance of misidentifications. If the barcodes are combined and supplemented with morphological data (fig. 4.4), *P. hyotis* clusters together with *Platypontonia brevirostris* (Miers, 1884) (represented only with morphological data), as a sister to *Pinnotherotonia* (and together with *Pinnotherotonia* with *Conchodytes*). In contrast, previous analyses by Fransen & Reijnen (2012) and Chow et al. (2021) placed *Platypontonia* respectively basal to *Conchodytes*, and next to *Anchiopontonia*. Bruce (1968) even suggested a relatedness to *Pontonia*. Based on previous analyses and our data, it is likely that the genus is at least part of a clade containing *Conchodytes*, *Odontonia* and also *Dactylonia*.

Although the recovered topologies are largely similar to those of Fransen (2002), various species within *Pontonia* cause problems when studying the phylogeny reconstructions. Most problematic placements can be traced back to potential misidentifications and limited genetic information of some species, causing low intrageneric support values. Interestingly, the genetic distances between various species of *Pontonia* is very small in the concatenated alignment. These species groups are: *P. domestica*, one lineage of *P. mexicana*, and one lineage of *P. pinnophylax*; and *P. margarita* and one lineage of *P. manningi* (fig. 4.2). The placement of these specimens and more obvious problems are discussed below. First up, the placement of the *Pontonia* species with an aberrant host association (or potentially aberrant host association) (resp. *P. panamica* and *P. longispina*) are generally placed more basally to the bivalve associated species. This is the case in the morphological, as well as the TE analysis. This is in congruence with previous remarks by Marin & Anker (2008). In the tree resulting from the concatenated alignment, a branch containing *P. margarita* and two lineages of *P. manningi* is placed basally to all other species of *Pontonia*. The possible explanation to this different topology can be found in the list of successfully sequenced molecular markers (supplementary table S1): only COI and H3 have successfully been amplified for these specimens. Therefore, these specimens could only be compared to the other species of *Pontonia* using these markers, which do not give well-resolved topologies on their own (supplementary fig. S1A, B). In addition, *P. manningi* can be represented by two other, more derived, lineages: one related to an unidentified species of *Pontonia*, and one basal to this cluster and *P. pinnophylax*, *P. domestica*, and one lineage of *P. mexicana*. As mentioned above, the reason for this aberrant placement can be found in the successfully sequenced markers (supplementary table S1),

as these two lineages of *P. manningi* are represented by the two ribosomal markers (16S and 18S). Both resulting lineages of *P. manningi* were physically examined, so misidentifications are ruled out. Similarly, *P. mexicana* is also represented by two lineages, one of which was checked for its morphology. The other specimen included in the concatenated alignment (fig. 4.2: GQ227823; Baeza, 2010) could not be checked, and is represented by only one 16S sequence.

Inclusion of unidentified species

As can be observed in the phylogenetic tree resulting from the concatenated alignment, various unidentified species were included in the study (fig. 4.2). The inclusion of the species resulted in tentative identifications to the two unidentified species of *Dactylonia* and one of *Pontonia*. Both unidentified specimens of *Dactylonia* (from Kou et al., 2013 and Mitsuhashi et al., 2007) seem to group together with a large clade of *D. ascidicola*, which seems to be the best guess to the correct identification of the species. Both specimens have a distribution indicated as “Philippines, Panglao Island” (supplementary table S1), which corresponds with the known distribution of *D. ascidicola* (see Fransen, 2002). The host of these specimens are however unknown.

The unidentified specimen of *Pontonia* groups together with a lineage of *P. manningi*. Assuming these specimens were physically checked before their inclusion in the original study (Bracken et al., 2009), the possibility both specimens are the same species is very low. Both specimens are only represented by the two ribosomal markers (16S and 18S), making comparison with the other species difficult. Because physical examination was not performed, the true identity of this specimen remains elusive.

Outgroup species placement

Cuapetes tenuipes was assigned as outgroup, followed by the recovery of *Actinimenes* as being most related to this outgroup. The other two outgroup species were correctly recovered in the TE analysis, but their placement varied in the molecular and morphological analyses. The four molecular sequences of *Periclimenes colemani* were split in two in the phylogeny reconstruction based on the concatenated alignment, due to the 16S sequence of this species being from a different sample (see supplementary table S1). The 16S sequence clusters together with members of *Dactylonia* (fig. 4.2). The 16S marker, compared to 18S, resolves the intrageneric relatedness quite well for the studied clade (supplementary fig. 4.S1C), so the placement of this sequence of *P. colemani* is not expected. Since physical examination of the misplaced

specimen used in Chow et al. (2021) was not performed, it is not possible to find an explanation for its placement in *Dactylonia*.

In both the analyses using the TE and concatenated alignment, *Typton wasini* is recovered as the outgroup species most related to the ingroup. However, in the morphological dataset, the phylogenetic analysis recovered the species as a close relative to *Colemonia* and *Bruceonia*. The possible explanation for this placement can be found in the similar morphology of the sponge-inhabiting species due to convergent evolution causing homoplasy. *T. wasini* shares the following potentially ecomorphological characters with various species of the ingroup: 2 to 4 subdistal teeth on the rostrum; an absent supra-orbital tooth; a broadly rounded or straight orbital angle; a relatively large tooth on the scaphocerite; the teeth on the flexor margin of the corpus of the dactyli; and various camouflage characters (see supplementary appendix S2 and supplementary table S2). A morphological comparison between *T. wasini* and a typical ingroup species (e.g., *Conchodytes meleagrinae*) can be done by examining the published illustrations of the female habitus and walking leg dactyli by Bruce (1977) and Fransen & Reijnen (2013). whether these morphological characters have a similar (homologous) origin, can only be discovered by examining all related species of *T. wasini* in this context.

Historical biogeography

Combining the published and previously unpublished distribution data (supplementary appendix S1; table 4.1, supplementary table S1) with the presented phylogeny reconstruction from the TE approach (fig. 4.4), gives an indication of potential evolutionary distribution patterns of the studied ingroup (fig. 4.6). Almost all studied genera can be found in tropical coral reefs in the IWP, ranging from the Red Sea to Vanuatu and Hawaii (supplementary appendix S1). The EP/Atl lineage, containing all members of *Ascidonia* and *Pontonia*, forms a monophyletic clade (fig. 4.4). This is in congruence with a previous study using morphological characters (Fransen, 2002). It reflects the combination of the Terminal Tethyan Event (TTE, 12–18 Myr ago), forming a ‘hard’ barrier, and the East Pacific Barrier (EPB, 5000 km open ocean, in effect throughout the past 65 Myr), forming a ‘soft’ barrier, separating IWP and EP populations. The internal architecture of the clade is mirrored in the subsequent closure of the Isthmus of Panama (IOP, 3.1 Myr ago) separating the EP and Atl regions, as well as the ‘soft’ barrier caused by the opening of the Atlantic Ocean (OAO, in effect throughout the past ca. 60 Myr) which can be observed in several marine groups (e.g., Fransen, 2002; Anker & Baeza, 2012; Cowman & Bellwood, 2013; Anker et al. 2017; Baraf et al., 2019). Examples of closely related paraphyletic or sister species pairs that possibly evolved

as a consequence of the iop are: *P. manningi*/*P. margarita* and *A. pusilla*/*A. quasipusilla*. This also holds for the sister species *A. flavomaculata* and *A. californiensis*, although no representative of this clade has been recorded from the W Atl. This could indicate subsequent extinction in that region or it remains to be discovered there. The oao is best reflected in the species *P. pinnophylax* and *P. mexicana*. As indicated by Fransen (2002), morphological differences between these species are minute and not reliable. The genetic distance between the W Atl populations of *P. mexicana* and those of the E Atl *P. pinnophylax* could be seen as an indication of speciation in progress.

Dispersal events from the IWP to the E Atl seem to be missing in the current results, probably due to the natural dispersal of the species around South Africa being inhibited by cold water flows. In recent times, the Suez channel in Egypt, and the fishery business (oyster and mussel farmers) are giving the opportunity for Red Sea invertebrates to become introduced in Mediterranean waters (e.g., Galil et al., 2018).

The host switch from an ascidian host in *P. panamica* to a mollusc host in the remaining *Pontonia* species parallels the iop in the biogeographical analysis (fig. 4.5). The interactions between host switching and biogeographical events have been studied in detail before (e.g., Hoberg & Brooks, 2008). While this is the only apparent matching between the two analyses, it might be worth studying this in more detail.

Host switching and notes on ecomorphological characters

The ancestral character state with regard to the host choice of the entire monophyletic ingroup was recovered to be an ascidian-associated shrimp species (fig. 4.6). Interestingly, previous studies featuring ancestral state reconstruction methods resulted in an unresolved host association (Horká et al., 2016) or a bivalve-associated shrimp species (Chow et al., 2021) for the common ancestor. The explanation for this mismatch in ancestral character state recovery should be sought in the limited number of included species in the previous studies (Horká et al., 2016: n = 4; Chow et al., 2021: n =10), causing ambiguous results for the presently studied clade.

As mentioned before, there seem to be at least six to seven different instances of host switches between phyla (from an ascidian to a bivalve host, or from a bivalve to an ascidian host), and one instance of a host switch within a phylum (from a bivalve to a gastropod mollusc). It is worth noting that the number of host switches presented in this study is based on the tree topology resulting

from the TE-approach, which unfortunately is accompanied with low support values on various branches (figs. 4.4 and 4.6). Once more (complete) molecular data becomes available, the tree topologies and support values might change, revealing a different number of host switches. Thus, the number of host switches is tentatively identified as six to seven. Most of the host switches in the studied clades are characterised as a single occurrence: the host switch from an ascidian to a bivalve host in the genus *Pontonia* happened once after the branching of the ascidian-associated *P. panamica*, or after the branching of *P. longispina*, of which a host association remains uncertain (fig. 4.6). Similarly, within the clade containing *Rostronia*, *Cainonia* and *Dactylonia*, one host switch from an ascidian to a bivalve host occurred in the branch represented by the only species of *Cainonia* (fig. 4.6). In previous morphological analyses by Fransen (2002), *Cainonia* was considered to be part of *Dactylonia*, although the genus was later founded partly based on the aberrant host-association of the species (Bruce, 2005). Within the clade containing *Conchodytes*, *Odontonia* and all other smaller related genera, a switch from an ascidian-associated ancestor to a bivalve associated ancestor can be observed basally in the phylogeny reconstruction (fig. 4.6), after *Opaepupu* splits off. The ancestral character state of the clade including *Opaepupu* is still an ascidian association, meaning that in the branch of *Opaepupu* a host-switch has occurred from an ascidian to a bivalve host. After the initial switch, several unique host switching events occur in the clade, first in the branch containing *Notopontonia*, which seems to have switched back from a bivalve- to an ascidian-associated lifestyle. Similarly, in the clade containing *Pseudopontonia*, *Anchiopontonia*, and *Odontonia*, the bivalve-associated ancestors do not switch once, but twice back to an ascidian-associated species in *Pseudopontonia*, and later in *Odontonia* (fig. 4.6). Within *Odontonia*, the ancestor of the genus (excluding *O. kerangcaris*) is recovered as a bivalve-associate, suggesting that the ascidian-associated *O. rufopunctata* has switched to an ascidian-associated lifestyle independently from the rest of the genus.

It has been hypothesised that the switches from an ectosymbiotic to an endosymbiotic host association resulted in various species-specific associations with bivalve, ascidian and sponge hosts in multiple clades within the Palaemonidae (Horká et al., 2016). This can be observed in the high level of host-specificity in the studied endosymbiotic clade, exploiting 19 confirmed families (table 4.1; Chow et al., 2021). Chow et al. (2021), however, hypothesised that these clades of endosymbiotic shrimp do make smaller taxonomic jumps than ectosymbiotic shrimp, when colonizing new hosts. This is congruent with the host choices of the species in most of the studied genera (supplementary appendix S1), but this does not explain the seemingly difficult

switch from one phylum to another. One explanation for these seemingly unexpected host switching events can be found in the internal structures of the host phyla: the studied shrimp species living inside bivalve molluscs are reported as inhabitants of the mantle cavity (e.g., Fransen, 1994), feeding from the mucus and pseudofeces which are built up near the gills (e.g., Kennedy et al., 2001; Ashelby et al., 2015; De Grave et al., 2021). This soft, mucus-producing internal cavity might be morphologically similar to the pharyngeal basket of phlebobranch and stolidobranch solitary ascidians (Horká et al., 2016, for comparison, see Monniot, 1991). A seemingly similar host switch between phyla has occurred in a lineage of the mainly sponge-inhabiting genus *Periclimenaeus*, where various species can be found in the sponge-like tunnelling systems of colonial (or compound) ascidian species (e.g., Fransen, 2006; Horká et al., 2016).

The ancestor of the gastropod-associated *Pontonia chimaera* and the closely related bivalve-associated congener was recovered to be a bivalve-associate. Although this intrageneric host switch can easily be distinguished in the currently presented phylogeny reconstruction, there are two other minor host switches present in the current study. de Gier and Fransen (2018) reported that *Odontonia plurellicola* was found in a species of *Plurella* Kott, 1973, an aggregating phlebobranch species. Similarly, *Dactylonia holthuisi* can also be found in the same and other *Plurella* species (Fransen, 2006; Fransen, pers. obs.). Members of *Plurella*, like all other members of the Plurellidae, are thought to have evolved from a solitary ancestor, resulting in an aggregating species characterised by a non-shared branchial sac. Contrary to the above-mentioned ecology of *Periclimenaeus*, this means that if individuals of *O. plurellicola* and *D. holthuisi* want to move to another ascidian in the aggregate, they have to enter it from the outside (de Gier & Fransen, 2018).

An ecomorphological question remains if the host switches, albeit within or between host phyla, result in novel morphological adaptations in the evolution of the endosymbiotic crustaceans (Fransen, 1994a; de Gier & Becker, 2020; Chow et al., 2021). Various studies have focussed on, or at least highlighted seemingly host-specific morphological features, like the reduction of spines (Fransen, 1994; 2002), adaptations of the eyes (Dobson et al., 2014, 2016), cryptic camouflage patterns (Horká et al., 2016), and the ornamentations on the walking leg dactyli (De Grave, 1999; Fransen, 2002). Hints to host-specific adaptations can also be found in previously unseen microstructures, studied with Scanning Electron Microscopy (SEM) (e.g., De Grave, 1999; Dobson et al., 2014; Ashelby et al., 2015; de Gier & Fransen, 2018) and CT scanning studies (e.g., Bagge et al., 2017), also in other groups of symbiotic Crustacea

(e.g., de Gier & Becker, 2020).

As we detected multiple host switching events from one phylum to the other and vice versa in this lineage of symbiotic shrimp, we conclude that bivalves and solitary ascidians with their similar internal microenvironments function as hot spots for interphylum host switching. It is expected that interphylum host switches will be observed more often between phyla with matching internal microenvironments than between phyla without these matching internal microenvironments in various lineages of crustaceans and other symbionts.

Future perspectives

The present study features the most complete molecular and morphological dataset of the studied mollusc- and ascidian-associated shrimp clade. Although the studied dataset resolved some of the questions about monophyly, host-switching and generic relatedness, it demonstrates that more molecular data is needed in order to obtain better supported and resolved phylogeny reconstructions. New, slowly evolving, genetic markers might be needed in order to solve the deeper phylogeny of the studied clade (e.g., Bininda-Emonds, 2007). In addition, more field observations and studies addressing the feeding habits, host choice, and current distributions of palaemonid shrimps are needed in order to complement the datasets needed for analyses focussing on host associations, ancestral state reconstructions, and present and past biogeography. Fortunately, various recent studies have been addressing these subjects (e.g., Ďuriš et al., 2011; Wood et al., 2017; Levitt-Barmats & Shenkar, 2018; Chow et al., 2021; De Grave et al., 2021). As mentioned earlier, an ecomorphological approach might give other, new insights. New imaging methods, combined with ecological and molecular data might help us understand how the endosymbiotic species have adapted to their aberrant way of life.

To test the hypothesis that interphylum host switches will be observed more often between phyla with matching internal microenvironments than between phyla without these matching internal microenvironments, similar analyses could be performed with for instance pinnotherine crabs and amphipods that inhabit the same host phyla. It is expected that for instance the matching internal microhabitats of compound ascidians and sponges will be another hot spot for inter phylum host switching. A comparative study of inter phylum host switching throughout nature could reveal more of these hot spots.

Supplementary information

Supplementary material is available online at: https://doi.org/10.6084/m9.figshare.19455893.

In addition, the dataset supporting the molecular results of this article can be found in the GenBank repository (Sayers et al., 2020) under permanent identifiers (Genbank Accession numbers; see supplementary table S1).

Acknowledgements

The authors would like to thank Prof. Dr. Bert W. Hoeksema (Naturalis Biodiversity Center, Rijksuniversiteit Groningen), Dr. Jeroen Hubert (Universiteit Leiden), Dr. Ronald Vonk and Vicky Beckers (both Naturalis Biodiversity Center) for their helpful comments and feedback during the writing phase of this project. The lab-technicians Frank Stokvis, Roland Butôt, and Marcel Eurlings (all Naturalis Biodiversity Center) are thanked for their assistance generating the DNA-barcodes which were used during this study. Niels van der Windt (Naturalis Biodiversity Center, Universiteit Leiden) is thanked for his help submitting the newly acquired molecular data to GenBank. Collection-managers Jeroen Goud, Bram van der Bijl, and Esther Dondorp (all Naturalis Biodiversity Center) are thanked for their support during the examinations of the physical specimens. In addition, we would like to thank Dr. Jeroen Hubert, Tobias Hartman, Willem van de Koot, and Larissa van Vliet for their assistance during the bioinformatic ancestral-state analyses. We thank Prof. Dr. Zdeněk Ďuriš and an anonymous reviewer for their careful reading of our manuscript and their many insightful comments and suggestions.

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Polka-dotted treasures: revising a clade of ascidian- and bivalve-associated shrimps (Caridea: Palaemonidae).

Werner de Gier and Charles Fransen

This chapter is published as:

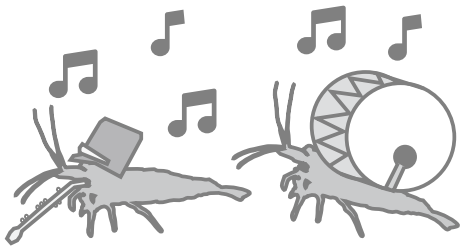
de Gier, W. & Fransen, C.H.J.M. (2023) Polka-dotted treasures: revising a clade of ascidian- and bivalve-associated shrimps (Caridea: Palaemonidae). Contributions to Zoology, 92(3): 179–282.

Zoobank accession link: urn:lsid:zoobank.org:pub:17A6B0AD-48CC-4B62-8949-12376BBF5EAB

Abstract

Most marine shrimp species of the family Palaemonidae are characterised by symbiotic associations with hosts that belong to a wide range of invertebrate phyla. One clade of related endosymbiotic species has evolved to live inside the branchial chambers of ascidians and the mantle cavities of bivalve molluscs. The phylogeny of this clade (comprising 15 bivalve-associated species in the genera *Anchistus*, *Neoanchistus*, and *Paranchistus*, and three species of ascidian-associated species in the genus *Dasella*) is the topic of the present study, which is based on both morphological and molecular data. A concatenated phylogeny reconstruction was built by using the markers COI and 16S. With the help of a total evidence approach (with a scored morphological datamatrix), species could be added for which no molecular data were available. An ancestral character state analysis was performed to detect host switches. In contrast to another endosymbiotic clade, the ancestral host state was found to be slightly in favour of bivalves. The phylogenetic relevance of morphological features is discussed with a focus on a tympanal organ located in the major chelipeds of some bivalve-associated shrimp species. This little-known anatomical structure is illustrated by SEM and µCT scans. Its possible function is discussed. In the phylogeny reconstructions, both *Anchistus* and *Paranchistus* were found to be polyphyletic. They were reclassified to obtain a more natural classification: *Anchistus custoides* and *Anchistus custos* were transferred to the resurrected genus *Ensiger*. *Paranchistus liui*, *P. nobilii*, *P. pycnodontae*, and *P. spondylis* were transferred to *Polkamenes* gen. nov. *Anchistus pectinis* and *P. ornatus* were transferred to *Tympanicheles* gen. nov. Per genus, a key to the species is provided.

Keywords: commensal shrimp; host switching; symbiosis; taxonomic revision; total evidence



Introduction

The shrimp family Palaemonidae (Crustacea: Decapoda: Caridea) includes a large number of ecto- and endosymbiotic lineages (Kou et al., 2015; Horká et al., 2016; Chow et al., 2021). Its species are known to be associated with hosts belonging to a wide variety of invertebrate taxa, including echinoderms, cnidarians, sponges, molluscs, and ascidians (e.g., Bruce, 1976a; Horká et al., 2016; Chow et al., 2021). Two lineages are known to consist of symbionts that live either inside solitary ascidians or bivalve molluscs, and in one instance of a gastropod mollusc (Bruce, 1995; Fransen, 2002). Most shrimp species within these lineages seem to be characterised by a set of morphological features that appear to be linked to their cryptic lifestyle, including: (1) reduced frontal, dorsal and lateral protrusions for easier movement within the host (e.g., shortening of the rostrum, reduction of the number of rostral teeth, absence or reduction of the antennal and hepatic spines); (2) the development of scales and microsetae on the dactyls of ambulatory pereiopods to increase grip; (3) an adaptive (often cryptic) colouration; (4) a roughly cylindrical body shape with a body size that is adapted to the dimensions of the host's body cavity; and (5) differences in eye morphology (de Gier et al., 2022 and references herein). In addition, Bruce (1972a) mentions bivalve-associated shrimps bear bigger claws on their second chelipeds, possibly caused by intraspecific competition. There are numerous other crustacean clades bearing similar morphological characters, which are thought to result from their endosymbiotic lifestyle. This is the case for the palaemonid genus *Periclimenaeus* Borradaile, 1915, associated with colonial ascidians and sponges (Fransen, 2006), and ascidian and mollusc-associated alpheid shrimps (e.g., Betaeus harfordi (Kingsley, 1878) and *Synalpheus herdmaniae* Lebour, 1938; see Lebour, 1938; Jensen, 1995). In addition, various symbiotic leucothoid amphipods (Vader, 1983) and most pinnotherine pea crabs (de Gier & Becker, 2020) show adaptive features related to a life within a host.

The two lineages mentioned above were defined by large-scale (molecular) phylogenetic studies (Kou et al., 2015; Horká et al., 2016 (parts of clades 5 and 6); Chow et al., 2021 (parts of clades iiic and iiih)). The lineage including the larger genera *Ascidonia* Fransen, 2002, *Conchodytes* Peters, 1852, *Dactylonia* Fransen, 2002, *Odontonia* Fransen, 2002, and *Pontonia* Latreille, 1829 (together with smaller related genera) was studied in detail by combining both morphological and molecular data, which showed monophyly in both the studied genera and the entire ingroup (de Gier et al., 2022). The other, smaller lineage consists of four genera, which are characterised by a conspicuous polka-dotted colouration: *Anchistus* Borradaile, 1898, *Dasella* Lebour, 1945,

Neoanchistus Bruce, 1975, and *Paranchistus* Holthuis, 1952. Members of *Anchistus*, *Neoanchistus*, and *Paranchistus* are all reported as endosymbionts, associated with bivalve molluscs, such as giant clams, fan shells, scallops, oysters and spiny oysters (Fransen, 1994a; De Grave, 1999). Species of *Dasella* are endosymbionts of large solitary ascidians (Bruce, 1981a, 2003a; Berggren, 1990). Species of these genera can be found in the Indo-West Pacific region. A revision of the taxa in this clade, including morphological diagnoses, keys, colour descriptions, host ranges, and geographical distribution is provided in the systematic account below.

Although various members of *Anchistus* and *Paranchistus* (both s.l.) have been studied for their ecological role (Johnson & Liang, 1966), mating systems and lifecycles (Baeza et al., 2013; Ghory & Kazmi, 2018), or the characterisation of their symbioses (De Grave et al., 2021), the systematic positions of its species remain elusive. While the four genera are known to be closely related (Horká et al., 2016; Chow et al., 2021), true monophyly of the clade (including all members), has not been proven yet. In addition, Chow et al. (2021) showed that the placement of species attributed to *Paranchistus* was polyphyletic. They recognised two distinct lineages, placing an unknown species of *Paranchistus* and *Paranchistus pycnodontae* Bruce, 1978 away from *Paranchistus ornatus* Holthuis, 1952. In this study, we revise the four genera and aim to construct a more robust phylogeny, parallel to the study of de Gier et al. (2022).

Host switching, an evolutionary process of a symbiotic lineage shifting from one host to another, has been a major topic in marine palaemonid shrimp phylogenetics (Horká et al., 2016; Chow et al., 2021). While lineages of ectosymbiotic palaemonids seem to switch commonly between genera or families, ascidian- and mollusc-associated species have been proven to have switched between entire phyla: the common ancestor of the before-mentioned clade containing the (mostly) bivalve-associated genera *Conchodytes* and *Pontonia*, as well as the predominantly ascidian-associated genera *Ascidonia*, *Dactylonia*, and *Odontonia*, was recovered as an ascidian-associated species. Within the clade, at least six different interphyla host switches were recognised, with *Odontonia* and *Notopontonia* Bruce, 1991 switching back to an ascidian host affiliation after an ancestral switch from an ascidian- to a bivalve host within the clade consisting of *Conchodytes*, *Odontonia*, and related genera (de Gier et al., 2022). We aim to detect possible host switches in the currently studied clade and recover the host of its common ancestor.

Materials and methods

Taxon sampling

A total of 18 ingroup and nine outgroup species were selected, all occurring in the Indo-West Pacific region (IWP) (supplementary table S1). Nine palaemonid outgroup taxa were selected across the host spectrum, representing echinoderm and cnidarian associates: *Actinimenes inornatus* (Kemp, 1922), *Actinimenes ornatus* (Bruce, 1969), *Lipkemenes lanipes* (Kemp, 1922), *Periclimenes colemani* Bruce, 1975, *Periclimenes kempi* Bruce, 1969, *Zenopontonia rex* (Kemp, 1922), and *Zenopontonia soror* (Nobili, 1904). The predominantly free-living *Cuapetes tenuipes* (Borradaile, 1898) and *Palaemonella rotumana* (Borradaile, 1898) were added to the outgroup. All associated species are part of Clade 5 of Horká et al. (2016), which is closely related to the ingroup.

Specimens were collected by scuba diving or other standard collection methods during various expeditions (supplementary table S1). For more information, field reports were published for the expeditions to Ambon (Strack, 1993), the Seychelles (Van der Land, 1994), Lombok (Hoeksema & Tuti, 2001), Berau (Hoeksema, 2004), Santo (Vanuatu; Bouchet et al., 2011), Raja Ampat (Hoeksema & van der Meij, 2008), Ternate (Hoeksema & van der Meij, 2010), and Semporna (Kassem et al., 2012). Recently sampled specimens were directly stored in 96% ethanol for DNA barcoding and identification. Subsampling was done by removing the left second and third pleopods or left third and fourth pereiopods. After subsampling, specimens were moved to 70% ethanol for morphological analysis and long-term storage in the Naturalis Biodiversity Center decapod collection (RMNH.CRUS.D.) at Leiden, The Netherlands. The newly sequenced material consists of six species (supplementary table S1). In addition, sequence data from GenBank (Sayers et al., 2020) was used (supplementary table S1).

DNA extraction, amplification and sequencing

Of the four analysed markers (COI, H3, 16S, and 18S), only two were selected (COI and 16S) for their phylogenetic relevance. DNA extraction, amplification and sequencing were done using the same methods for COI and 16S as described in de Gier et al. (2022), partially based on the protocols described by Horká et al. (2016), Fransen & Reijnen (2012), and Brinkman & Fransen (2016).

Morphological character state analysis and taxonomic study

The morphology of all in- and outgroup species was scored, based on specimens in the Naturalis collection, as well as descriptions and figures found in literature (supplementary table S1). Characters were selected based on their visibility in adult specimens, which may differ in juveniles. The resulting morphological data matrix contains 35 characters for 27 species, of which 11 multi-state characters were coded as ordered (character 1, 2, 5, 6, 9, 17, 20, and 29 to 32) (Fitch, 1971). The remaining binary and multi-state characters were coded as unordered (Farris, 1970). Character state 0 was usually attributed to the outgroup species. Unknown character states were treated as missing data, coded as question marks (?) (supplementary appendix S1, supplementary table S2).

Specimens were studied and dissected using a dissecting stereomicroscope (Zeiss Discovery.V8) and a compound microscope (Olympus BX53), both equipped with a Camera Lucida drawing tube. Of the ingroup species found in RMNH.CRUS.D., new illustrations were made for use in both the taxonomic revision and the morphological character state analysis. Illustrations were inked, scanned on 600 dpi, and subsequently mounted into plates using Adobe Photoshop software (Adobe Systems, USA).

Macro-photographs showing the life colouration of several specimens were made during field expeditions. When available, museum-collection accession numbers are given for the specimens: RMNH.CRUS.D. (Naturalis Biodiversity Center decapod collection, Leiden, the Netherlands); MNHN (Muséum national d'Histoire naturelle, Paris, France); FLMNH UF (Florida Museum of Natural History, University of Florida, USA).

Phylogeny reconstructions and ancestral character state analysis

Sequence alignments were obtained using the ClustalW (Thompson et al., 1994) algorithm (default settings) in mega v10.2.5. (Kumar et al., 2018). Ends of the COI and 16S alignments were trimmed manually in mega to 582 bp and 426 bp respectively. The alignment of the protein-coding gene COI was subjected to Xia's test of nucleotide substitution saturation (Xia et al., 2003) in dambe v7.3.0. (Xia, 2009). None of the codon positions of COI was found to be saturated and thus no data was excluded. After alignment, the non-coding ribosomal 16S gene was examined for highly divergent blocks using Gblocks

v0.91b (Talavera & Castresana, 2007) using default parameters but allowing gap positions and smaller finer blocks. Additionally, the settings were changed to allow less strict flanking positions. This resulted in the retention of all bases in the alignment.

Both COI and 16S were analysed individually, and a complement dataset was made for all specimens where both gene sequences were obtained or already available (COI+16S). A concatenated dataset containing both genes was also constructed in mega, to also include specimens where only one of the two sequences was obtained. This alignment consisted of 43 sequences and 1008 positions. A Total Evidence (TE) analysis was performed by combining all data from the concatenated alignment and the morphological dataset, to include all ingroup species in the resulting phylogenetic trees.

Models for sequence evolution were calculated in mega using default settings (table 5.1). Best models were chosen based on values for the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC).

Table 5.1 Analytical methods and models used for sequence evolution. For the alignments of the concatenated dataset and the TE analysis, partitioned models (based on the single gene and morphological analyses) were used.

Alignment	Model(s) for sequence evolution	Trimmed fragment length
Single gene COI	GTR+G+I	582 bp.
Single gene: 16S	HKY+G	426 bp.
Dual gene: COI, 16S	GTR+G+I, HKY+G	1008 bp.
Morphology*	MULTI4_MK	35 bp.

*: The mixed model for the MrBayes analysis of the morphological data in the TE analysis was coded to be gammadistributed. In addition, the Multi4_mk model was selected in the RAxML-server to deal with the morphological dataset, with 4 being the maximum number of character states per character (0–3).

Phylogenetic analyses were performed under the maximum likelihood (ML) and Bayesian Inference (BI) criteria using RAxML via the on-line cipres (Miller et al., 2010) with the RAxML-NG BlackBox v1.0.0 tool (Kozlov et al., 2019) and MrBayes v3.2.7 (Ronquist & Huelsenbeck, 2003), respectively. For the single-gene ML analyses default settings were used and automatic bootstrapping with a cut-off of 0.03, under an unpartitioned model. The dual-gene alignment used a partitioned model for both genes, with scaled branch length linkage and automatic bootstrapping with a cut-off of 0.03. Consequently, the concatenated dataset used a similar partitioned method. The BI analyses were conducted using a Markov Chain Monte Carlo (MCMC) method with two independent runs and four chains. Analyses were run with a minimum of 5,000,000 generations

to ensure that the average standard deviation of split frequencies (ASDSF) reached a value < 0.01, which would indicate that the two runs have converged to a stationary distribution. Trees were sampled each 500 generations. The initial 25% of trees was discarded as burn-in and the remaining trees were used to generate consensus trees and to estimate Bayesian posterior probabilities (PP). In all instances, the topology of the BI analyses was displayed in the results, with annotations to the Bayesian posterior probabilities and ML RAxML values at each intraspecific node.

The analysed sequences resulted in four phylogeny reconstructions: the single gene (COI, 16S) and dual gene (COI+16S) phylogeny reconstructions can be found in the appendices (supplementary figs. S1–S3); the concatenated alignment resulted in the phylogeny reconstruction presented in the results section below.

The Total Evidence analyses were performed similar to the phylogenetic analyses of the concatenated alignment, but with the addition of morphological data. The integrative dataset consisted of 52 taxa and 1043 codon/character positions. The following species were only represented by morphological data: *Anchistus gravieri* Kemp, 1922, *Tympanicheles pectinis* (Kemp, 1925) comb. nov. (was *Anchistus pectinis*), *Dasella ansoni* Bruce, 1983, *Dasella brucei* Berggren, 1990, *Neoanchistus cardiodytes* Bruce, 1975, *Neoanchistus nasalis* Holthuis, 1986, *Polkamenes liui* (Li, Bruce & R.B. Manning, 2004) comb. nov. (was *Paranchistus liui*), *Polkamenes nobilii* (Holthuis, 1952) comb. nov. (was *Paranchistus nobilii*), and *Polkamenes spondylis* (Suzuki, 1971) comb. nov. (was *Paranchistus spondylis*). All sequences were accompanied by conspecific morphological data, assuming absence of intraspecific morphological variations in the chosen characters and specimens. To check the results of our combined dataset, an analysis only using morphological characters was performed, based on the methods used by Fransen (2002), de Gier and Fransen (2018), and de Gier et al. (2022), using paup v4.0a (Swofford, 2003) under the maximum parsimony (MP) criterion, with ordered characters and step matrices as mentioned above. A heuristic search was performed with 10.000 repetitions, with a number of trees that was automatically increased with 100. The strict consensus and the 50% majority rule consensus trees were obtained.

To reconstruct the evolution of host associations, an ancestral-character-state reconstruction was performed in R using the MP criterion and the ML tree from the TE analysis as a backbone. The R-packages ape v. 5.5 (Paradis & Schliep, 2019), Geiger 2.0.7 (Penell et al., 2014) and phytools (Revell, 2012) were used. To get probability values for polytomous branches, the phytools function

'rerootingMethod()' was used, following Yang et al. (1995). The host-choice of *Zenopontonia rex* was changed from 'Echinoderm and Mollusc (nudibranch/gastropod) associate' to 'Echinoderm associate', due to technical limitations. Pie-charts displaying ML-values similar to the ones seen in comparable studies (e.g., Salis et al., 2018) were added in Abode Illustrator cc (Adobe Systems, USA). The actual ML-probabilities are presented in supplementary table S3 and supplementary fig. S4.

Phylogenetic trees were initially rooted and sorted in FigTree v1.4.4 (Rambaut, 2009), and consequently edited in Adobe Illustrator (cc).

Additional figures

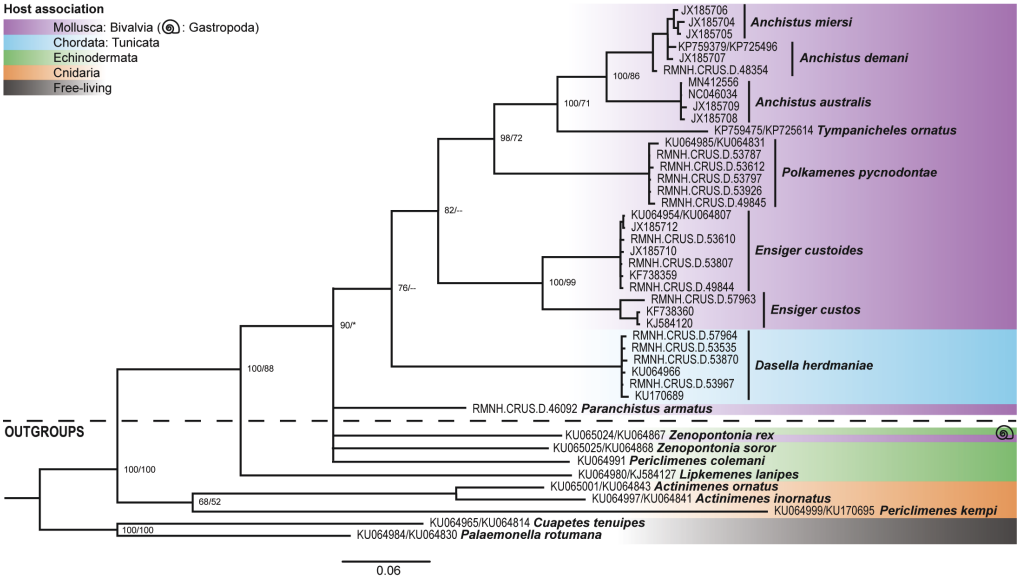
Captures of one of the major claws of *Anchistus miersi* (De Man, 1888) (taken from specimen RMNH.CRUS.D.58030, found in a *Tridacna* squamosa Lamarck, 1819), and of *Tympanicheles ornatus* (Holthuis, 1952) comb. nov. (taken from RMNH.CRUS.D.41491, from an unknown bivalve host) were made using a Scanning Electron Microscope (SEM) (jeol jsm 6480LV; Naturalis Imaging Facilities) according to the protocol by de Gier and Fransen (2018). After chemical dehydration (twice 15 min in 80% ethanol, followed by twice 15 min in 96% ethanol, followed by twice 30 min in 100% acetone) the samples were dehydrated using Critical Point Drying-methods (cpd) with a Leica em cpd300. Additional captures were made of a claw of *Anchistus australis* Bruce, 1977 (RMNH.CRUS.D.53540 found in *T. squamosa*) with a Zeiss Xradia 520 Versa 3D X-ray microscope (μCT-scanner) (Naturalis Imaging Facilities). A specimen was mounted in a 1.5 ml tube filled with 70% ethanol. Staining was performed for 1 day in a 1% pta solution. Settings were used as follows: Optical magnification 0.396; Scanning current 87 μA; Scanning voltage 80 kV; Exposure time 3 ms. Reconstruction (3D) data was edited in Avizo 9.5.0 (ThermoScientific).

Results

Molecular phylogeny reconstructions

The phylogenetic analysis using the concatenated alignment was resolved with moderately high support values (fig. 5.1). Most outgroup species were recovered as basal to the ingroup, with the tree being rooted with the two free-living species (*Cuapetes tenuipes* and *Palaemonella rotumana*). This is the case for three Cnidaria associates (two species of *Actinimenes* and *Periclimenes kempi*), and one Echinodermata associate (*Lipkemenes lanipes*). The other echinoderm-associated (and gastropod-associated) outgroups (two species of *Zenopontonia* Bruce, 1975, and *Periclimenes colemani*) were recovered in a

large polytomy together with *Paranchistus armatus* (H. Milne Edwards, 1837) and a clade of all other ingroup species. Because of the polyphyly found in *Paranchistus* and *Anchistus*, a number of species from these genera were designated to three new genera, in both the molecular and combined (TE) phylogeny reconstructions. The species are formally transferred to their new genera in the systematic account below.



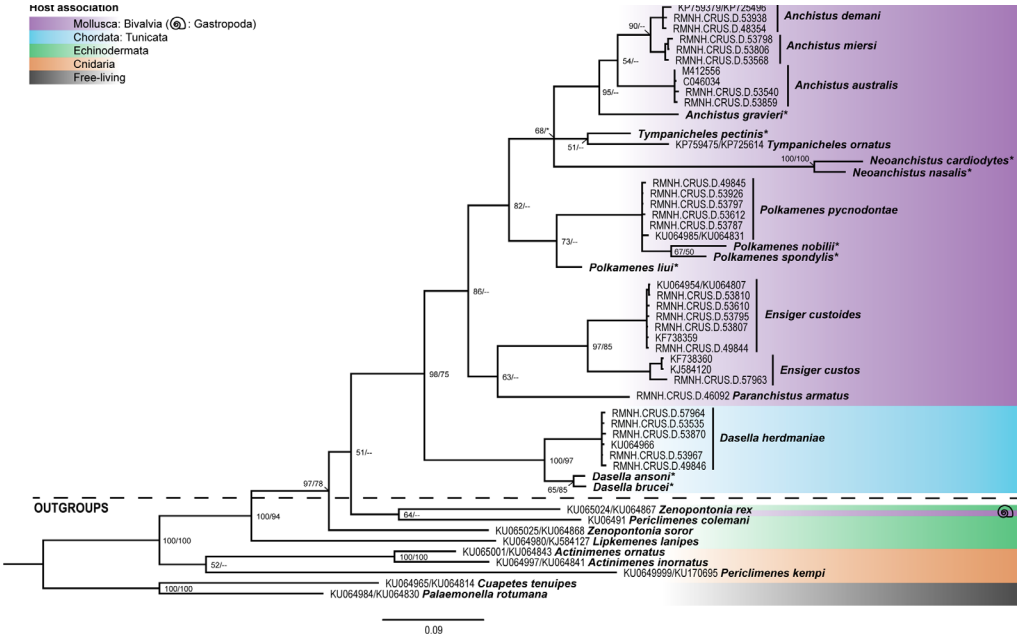
5.1 Phylogeny reconstruction based on the MrBayes tree topology of the concatenated molecular dataset (COI, 16S). RAxML bootstrap support and Bayesian posterior probabilities expressed as percentages are indicated respectively. Dashes (--) indicate values <50; asterisk (*) indicates different topology of RAxML or MrBayes tree. Barcodes are indicated by a collection accession number (RMNH.CRUS.D.) or a GenBank accession number. Species are listed in the appendices (supplementary table S1). Colours indicate various host associations. *Zenopontonia rex* (Kemp, 1922) is represented as a symbiont with two kinds of host associations: holothurians and nudibranchs.

The ingroups consists of three main clades. The ascidian-associated genus *Dasella*, represented by *Dasella herdmaniae* (Lebour, 1938) in the molecular phylogeny reconstruction, is placed basal to the other (bivalve-associated) ingroup species (except for *P. armatus*), followed by a clade of *Ensiger custoides* (Bruce, 1977) comb. nov. and *Ensiger custos* (Forskål, 1775) comb. nov. Two species with now newly described genera, *Polkamenes pycnodontae* (Bruce, 1978) comb. nov. and *Tympanicheles ornatus*, are branching off respectively, after which an *Anchistus* clade consisting of three species is recovered. This clade is represented by one basal branch of *A. australis*, and one branch including *Anchistus demani* Kemp, 1922 and *A. miersi*. *Anchistus demani* is manifested as paraphyletic, with one species branching off earlier (RMNH.

CRUS.D.48354). The placement of this specimen is discussed below.

Total Evidence approach

Four ingroup clades can be recognised in the phylogeny reconstruction resulting from the Total Evidence approach (fig. 5.2). The combined dataset using both morphology, as well as molecular data, recovered the outgroup in a similar way as the above mentioned topology (fig. 5.1). *Zenopontonia rex* and *Periclimenes colemani* were recovered as the outgroups being most related to the ingroup. This ingroup was recovered with low support values (51/--). The first clade containing the three species of *Dasella* was split off with high support values (98/75). *Dasella ansoni* and *D. brucei*, only represented by morphological data, were recovered as a sister clade of *D. herdmaniae*. The next clade branches off with somewhat low support values (86/--), containing three species: *Paranchistus armatus*, *Ensiger custos*, and *E. custoides*, of which the last two were recovered as most related to each other. The remaining ingroup species

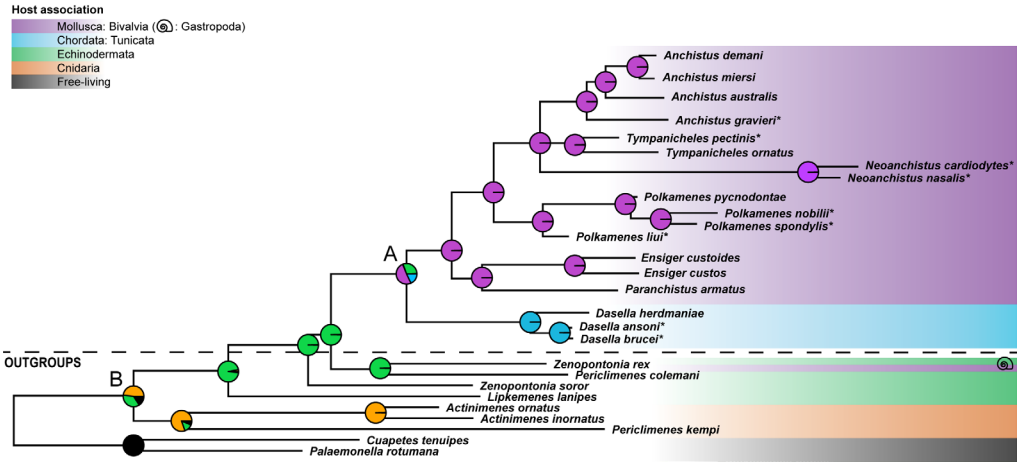


5.2 Phylogeny reconstruction based on the MrBayes tree topology of the te approach. RAxML bootstrap support and Bayesian posterior probabilities expressed as percentages are indicated respectively. Dashes (--) indicate values <50; asterisk (*) indicates different topology of RAxML or MrBayes tree. Barcodes are indicated by a collection accession number (RMNH. CRUS.D.) or a GenBank accession number. Species are listed in the appendices (supplementary table S1). Species of which only morphological data was analysed are indicated with an asterisk (*) and no accession number. Colours indicate various host associations. *Zenopontonia rex* (Kemp, 1922) is represented as a symbiont with two main host associations: holothurians and nudibranchs.

were recovered in two clades, one of which only includes species previously attributed to *Paranchistus*, but now to *Polkamenes* gen. nov.: *P. liui* (only represented by morphological data) branches off first in this clade, after which a polytomy of *P. pycnodontae*, *P. nobilii*, and *P. spondylis* was recovered. The latter two species were only represented by morphological data. The remaining ingroup species were recovered as a part of a large polytomy including three, now monophyletic genera: *Tympanicheles* gen. nov., *Neoanchistus*, and *Anchistus*. *Tympanicheles pectinis* (only represented by morphological data) is grouped together with *T. ornatus* with somewhat low support values (51/--), while the two species of *Neoanchistus* are recovered as closely related sister species (although both species are only represented by morphological data). A branch containing four species of *Anchistus* (*A. miersi*, *A. demani*, *A. australis*, and *A. gravieri*) is recovered with higher support values, with *A. gravieri* (being represented by morphological data) as the most basally placed species.

Ancestral character state analysis

An ancestral character state reconstruction analysis resulted in Maximum Likelihood probabilities for all 24 internal nodes, which translate to the probabilities that an ancestral shrimp clade would have been associated to a certain host (fig. 5.3). One internal node was recovered as polytomous, but did not interfere with the resulting probabilities (see fig. 5.3). As expected, all



5.3 Phylogeny reconstruction based on the MrBayes tree topology of the te approach (fig. 2), with ancestral character state reconstructions on the internal nodes (probabilities are shown as pie charts). Colours indicate various host associations, both for the species as well as the ancestral character states. *Zenopontonia rex* (Kemp, 1922) is represented as a symbiont with two kinds of host associations (holothurians and nudibranchs), but was only classified as an echinoderm associate in the analysis. Species of which only morphological data was analysed are indicated with an asterisk next to the species names (*).

internal nodes of the bivalve-associated clade containing all ingroup genera (except for *Dasella*) are fully resolved as associates of bivalve molluscs (P = 0.99 to 1.00). Similarly, the internal nodes in the clade containing the three species of *Dasella* are fully resolved as symbionts of ascidians.

The common ancestor of the entire ingroup is recovered as a bivalve associate, but the probability for this character state barely reaches 51% (P = 0.508) (fig. 5.3A). The chances of this common ancestor being an ascidian (P = 0.180) or echinoderm associate (P = 0.299) are much smaller. The common ancestor of a clade including the ingroup and the two most related outgroup species (*Zenopontonia rex* and *Periclimenes colemani*) is recovered as an echinoderm associate (P = 0.991). The ancestral character state of the clade described by Horká et al. (2016) (thus, also including the echinoderm associated *Z. soror* and *Lipkemenes lanipes*, and the cnidarian-associated species of *Actinimenes* and *Periclimenes kempi*) is recovered to most likely be cnidarian-associated (P = 0.483) (fig. 5.3B).

Systematic account

Genera and species within the '*Anchistus* clade' are revised with remarks on their synonymy. Diagnoses, identification keys, geographical distributions, and host ranges are provided. Drawings of diagnostic characters and colour photographs are added based on available material. Special attention is given to novel features of the ingroup species, mostly concerning mouthparts.

Abbreviations

coll.	collected by
don.	donated by
fcn.	field collection number
pocl.	postorbital carapace length, measured from the posterior orbital margin to the posterior margin of the carapace in the dorsal midline
R	rostral formula indicated by the number of dorsal rostral teeth/number of ventral rostral teeth
Stn	field station; measurements are in mm

Family Palaemonidae Rafinesque, 1815

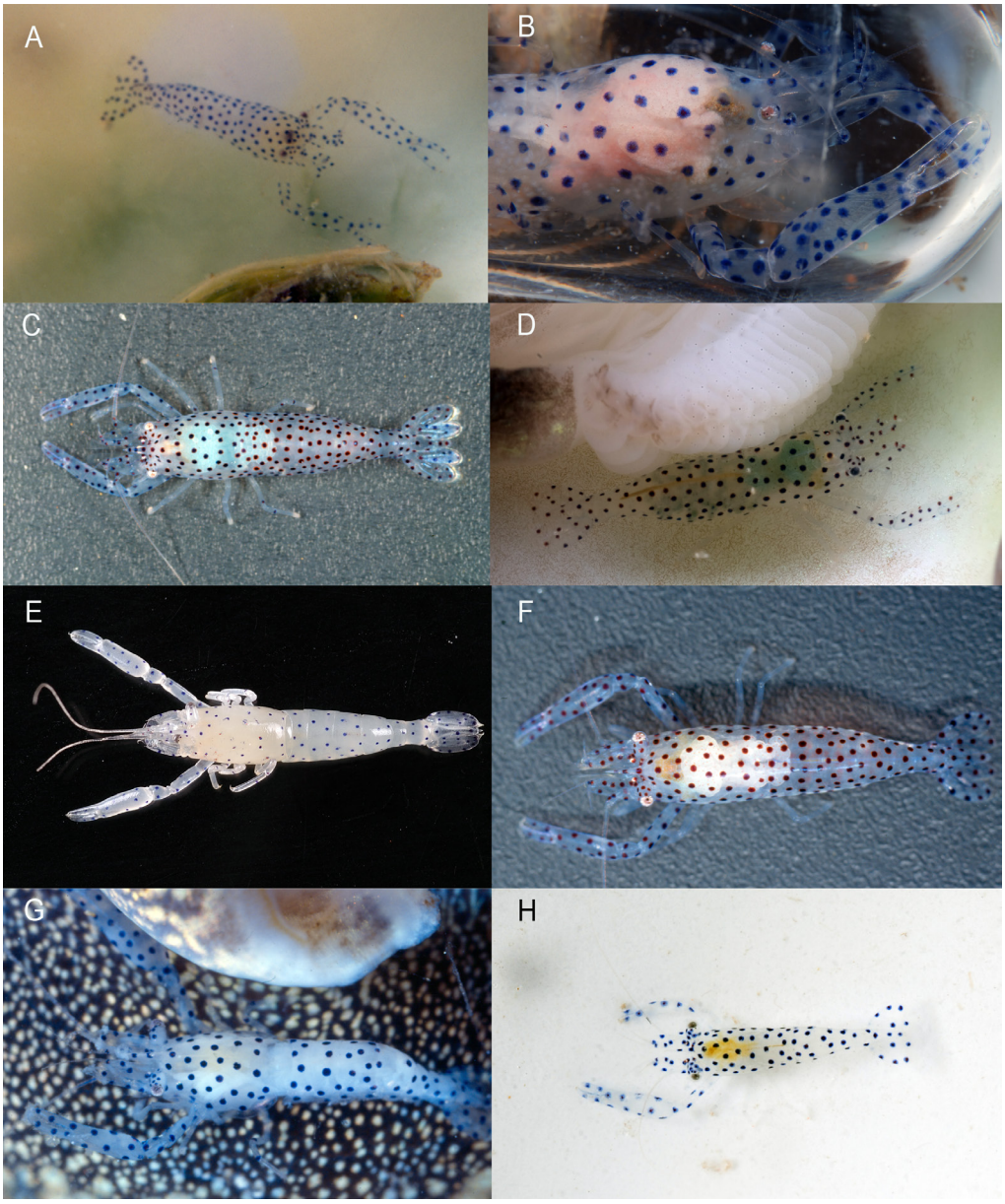
Genus *Anchistus* Borradaile, 1898

Anchistus Borradaile, 1898: 387. Type species, by original designation: *Harpilius* Miersi De Man, 1888. Gender: masculine. Name placed on the

Official List of Generic Names in Zoology in Opinion 470, in 1957.
Tridacnocaris Nobili, 1899: 234. Replacement name for *Anchisus* Borradaile, 1898. Type species therefore: *Harpilius Miersi* De Man, 1888. Gender: feminine. Name placed on the Official Index of Rejected and Invalid Generic Names in Zoology in Opinion 470, in 1957.

Diagnosis (emended from Bruce, 1995: 29). Small-sized shrimps of subcylindrical body form. Rostrum well developed, compressed, with teeth in distal part, lateral carinae feebly developed. Carapace smooth, glabrous; orbit feebly developed, inferior orbital angle distinct, antennal spine present or absent, supraorbital, hepatic and epigastric spines absent, anterolateral margin not produced. Abdomen smooth, glabrous, third segment not posterodorsally produced, anterior pleura rounded, posterior blunt. Telson with two pairs of small dorsal spines, three pairs of posterior spines. Eye small, cornea globular. Antennula normal, upper flagellum biramous, short ramus reduced. Antenna with basicerite unarmed, scaphocerite well developed with large distolateral tooth. Epistome unarmed. Mandible normal, without palp, molar process robust, incisor process dentate; maxillula with feebly bilobed palp, laciniae broad; maxilla with simple palp, basal endite broad, bilobed, coxal endite obsolete, scaphognathite broad; first maxilliped with slender palp, basal endite broad, fused with coxal endite, exopod well developed, caridean lobe broad, flagellum with numerous plumose setae distally, epipod bilobed; second maxilliped with normal endopod, dactylar segment small, exopod well developed, coxa feebly medially produced, epipod rounded, without podobranch; third maxilliped with endopod slender, ischiomerus and basis fused, exopod with numerous plumose setae distally, coxa with oval lateral plate, with rudimentary arthrobranch. Fourth thoracic sternite without median process. First pereiopods slender, chelae with fingers spatulate, non-cannulate, cutting edges minutely pectinate. Second pereiopods well developed, similar, unequal, dactylus with large acute triangular tooth in proximal half, fixed finger with series of teeth in proximal part; propodus with ventral oval tympanal organ proximally. Ambulatory pereiopods with simple or biunguiculate dactyli, without basal protuberance; unguis with dorsal pad of fine scale-like spinules. Uropod with protopodite posterolaterally acutely produced or rounded, exopod with small mobile distolateral spine, without distolateral tooth.

Four *Anchistus* species are recognised, all from bivalve hosts: *Anchistus australis* Bruce, 1977, *Anchistus demani* Kemp, 1922, *Anchistus gravieri* Kemp, 1922, and *Anchistus miersi* (De Man, 1888). *Anchistus pectinis* Kemp, 1925 is herein transferred to the new genus *Tympanicheles*.



5.4 A) *Anchistus australis* Bruce, 1977, RMNH.CRUS.D.58048, stn BER.28, in *Tridacna squamosa* Lamarck, 1819; B) *A. australis*, RMNH.CRUS.D.53540, stn ter.12, in *T. squamosa*; C) *Anchistus demani* Kemp, 1922, RMNH.CRUS.D.42792, stn SEY.792, in *Tridacna maxima* (Röding, 1798); D) *A. demani*, RMNH.CRUS.D.58049, stn THU.07, in *T. maxima*; E) *Anchistus gravieri* Kemp, 1922, RMNH.CRUS.D.58052, stn FR15, in *Hippopus hippopus* (L., 1758); F) *Anchistus miersi* (De Man, 1888), RMNH.CRUS.D.42970, stn SEY.720, in *T. squamosa*: G) *A. miersi*, RMNH.CRUS.D.46473, SW Sulawesi, in *T. squamosa*; H) *A. miersi*, RMNH. CRUS.D.53568, stn TER.19, in *T. squamosa*. Photo credits: Charles Fransen.

Key to the species of *Anchistus*

1. - Carapace without antennal spine; protopodite of uropod rounded **2.**
- Carapace with antennal spine; protopodite of uropod acute **3.**

2. - Rostrum with two dorso-distal teeth, ventrodistal margin rounded; fine scale-like spinules on unguis of ambulatory dactyli in transverse lines *A. demani*
- Rostrum with 4-8 dorsal teeth in distal part of rostrum, ventral margin with one small subdistal tooth; fine scale-like spinules on unguis of ambulatory dactyli in longitudinal lines *A. australis*

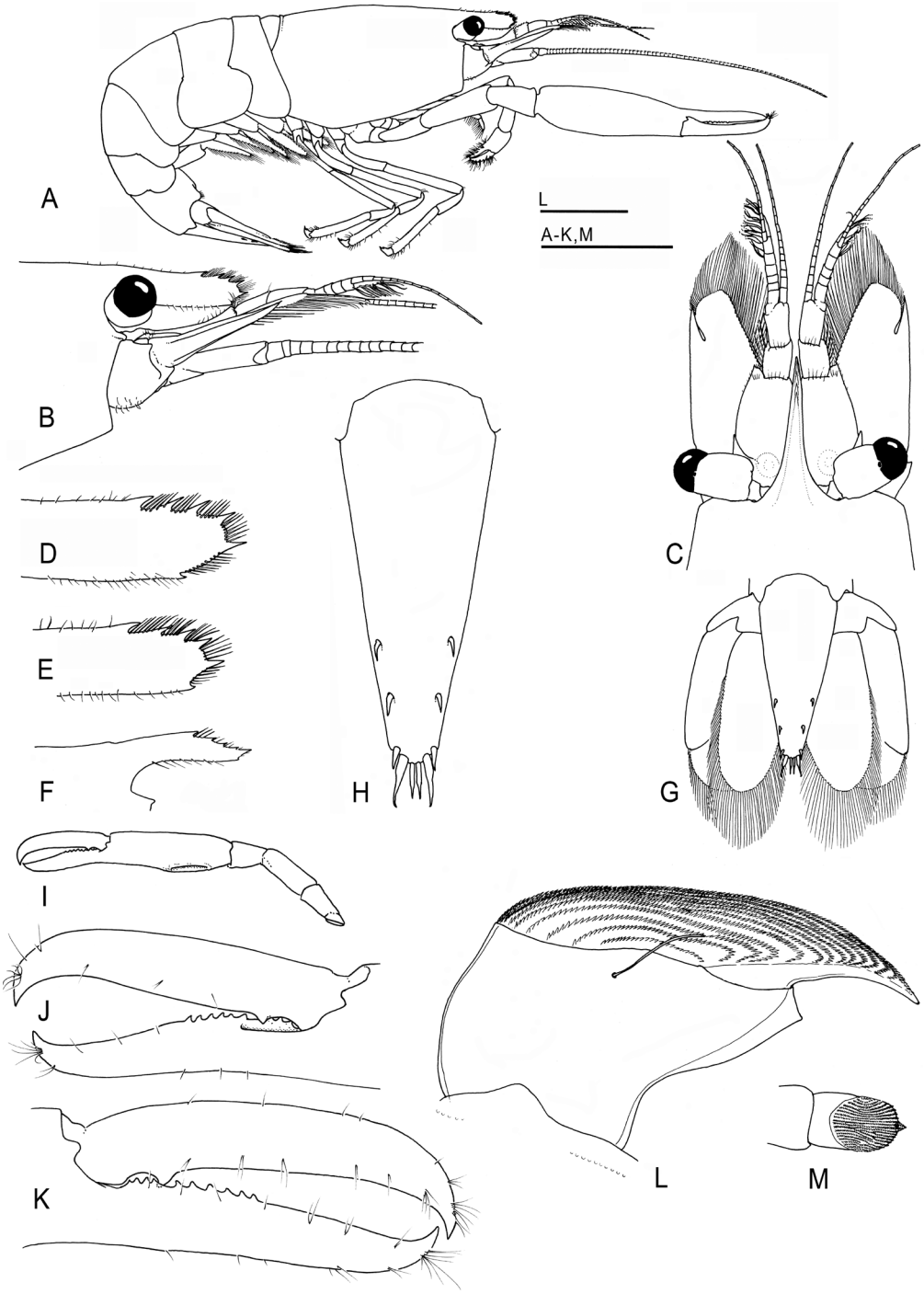
3. - Ambulatory dactyli with corpus-unguis suture perpendicular to ventral margin; corpus without accessory tooth; lateral pair of posterior telson spines as small as dorsal spines, marginal *A. gravieri*
- Ambulatory dactyli with corpus-unguis suture strongly oblique; corpus with accessory tooth; lateral pair of posterior telson spines distinctly larger than dorsal spines, submarginal *A. miersi*

***Anchistus australis* Bruce, 1977**

(fig. 5.4A–B, 5.5, 5.39E–F)

Anchistus australis f. typica Bruce, 1977a: 56, figs. 7-9 [type locality: Capre Cay, Swain's Reefs. Unavailable under Art. 15.2].
Anchistus australis f. dendricauda Bruce, 1977a: 62, figs. 10 [type locality: West Cay, Diamond Islets. Unavailable under Art. 15.2].
Anchistus australis Bruce, 1981b: 389, 395, fig. 3A; Bruce, 1983a: 43-44; Bruce, 1983b: 892, fig. 10A; Bruce, 1983c: 199 (listed); Bourdon, 1983: 867 (listed); Devaney & Bruce, 1987: 222; Bruce, 1990: 15, 18 (listed); Bruce, 1991a: 258-259, 275; Bruce, 1993: 55; Chace & Bruce, 1993: 71 (listed); Müller, 1993: 7 (listed); Strack, 1993: 53; Fransen, 1994a: 106, fig. 1; Bruce & Coombes, 1995: 106; De Grave, 1999: 126, fig. 1; Li, 2000: 6-7, fig. 6 (listed); Hayashi, 2002: 221, figs. 432a, b, 434a, b, 435a, b; Bruce, 2003b: 210 (listed); Hayashi, 2006: 510; Marin & Savinkin, 2007: table 2 (listed); De Grave & Fransen, 2011: 340 (listed); Fransen & Reijnen, 2012: 47; Hosie et al., 2015: 283 (listed); Liu, 2020: 312-313.

Material examined. INDONESIA: 1 male pocl. 5.2 mm (photo by M.S.S. Lavaleye 24/22-24), 1 female pocl. 7.00 mm (photo by M.S.S. Lavaleye 24/22-24) (RMNH.CRUS.D.41436), stn RBE.20, Ambon Island, Hitulama, 20.xi.1990, depth 15 m, scuba-diving, in *Tridacna squamosa* Lamarck, coll. C.H.J.M. Fransen and J. van Egmond. – 1 male pocl. 2.14 mm, 1 female pocl. 3.83 mm, 1



5.5 *Anchistus australis* Bruce, 1977. A–H, L, M) RMNH.CRUS.D.41438; I–K) RMNH. CRUS.D.41436. A) habitus, lateral view, B) anterior appendages, lateral view; C) anterior appendages, dorsal view; D) rostrum, lateral, female, pocl. 7.0 mm; E) idem, male, pocl. 5.2 mm;

(5.5 *Cont.*) F) idem, juvenile male, pocl. 1.1 mm; G) tail fan; H) telson; I) left major second pereiopod; J–K) idem, chela; L) dactylus left third pereiopod, medial view; M) idem, ventral view. Scale A, I = 4 mm; B, C, F, G = 2 mm; D, E, H, J, K, M = 1 mm; L = 0.1 mm. Artwork: Charles Fransen.

ovigerous female pocl. 2.92 mm (RMNH.CRUS.D.41437), stn RBE.16, W-side of Pombo Island, E of Ambon Island, 16.xi.1990, snorkeling, in *T. squamosa*, coll. J.C. den Hartog. – 1 male pocl. 2.99 mm, 20 juveniles pocl. 1.10-1.95 mm (RMNH.CRUS.D.41438), stn RBE.20, Ambon Island, Hitulama, 20.xi.1990, depth 15 m, scuba-diving, in large *Tridacna* sp. (Ø 39 cm), coll. C.H.J.M. Fransen & J. van Egmond. – 1 female pocl. 4.55 mm (RMNH.CRUS.D.41439), Nanaka, 7.ii.1980, in *Tridacna* sp., don. PPPO. – 1 ovigerous female pocl. 7.5 mm (RMNH. CRUS.D.53540, GenBank JX185709; CT-scanned), stn TER.12, Ternate, Tanjung Tabam, 0°50'5.1"N 127°23'10"E, 30.x.2009, depth 8 m, scuba-diving, in *T. squamosa*, coll. C.H.J.M. Fransen, fcn. cf-00044, photo TER.12.0073-79. – 1 non ovigerous female pocl. 5.0 mm (RMNH.CRUS.D.58048), stn BER.28, NE Kalimantan, Berau Islands, Maratua Island, N-side, lagoon near entrance, 02°14'51.0"N 118°37'47.9"E, 17.x.2003, depth 8 m, scuba diving, in *T. squamosa*, coll. C.H.J.M. Fransen, film 16. – 1 ovigerous female pocl. 3.5 mm, 1 male pocl. 3.0 mm (RMNH.CRUS.D.27919), stn RAJ.28, W Papua, Raja Ampat Islands, N Batanta, N pulau Yarifi, 00°46.779'S 130°42.711'E, 28.xi.2007, depth 8 m, scuba diving, in *T. squamosa*, coll. C.H.J.M. Fransen, fcn. cf102. **MALAYSIA**: 1 ovigerous female pocl. 7.01 mm (RMNH.CRUS.D.41287), West Malaysia, Tioman Island, Telek Dalam, in *T. squamosa*; depth 10 feet, 22.vi.1983, coll. P. Ng. – 1 ovigerous female pocl. 5.3 mm, with bopyroid on left side under carapace (RMNH.CRUS.D.53859; GenBank JX185708), stn SEM.18, Sabah, Semporna area, Ligitan Isl., 04°14'06.5"N 118°48'26.5", 4.xii.2010, depth 5 m, scuba-diving, in *T. squamosa*, coll. dive guide Jerry.

Diagnosis. Rostrum with 4–7 dorsal distal and 0–1 ventral distal tooth, reaching just beyond the basal segment of the antennal peduncle. Sixth abdominal segment with posterior ventral angle rounded. Telson with small dorsal spines at 0.67 and 0.81 of telson length, with lateral pair of terminal spines small, situated next to intermediate spines. Antennal spine absent. Anterior margin between ventral orbital angle and antero-lateral angle almost straight. Basal segment of antennular peduncle with convex lateral margin and distolateral tooth. Deep slit present between distolateral spine and lamina of scaphocerite. Stylocerite acute. Chela of first pereiopod spatulate, non-cannulate, with cutting edges laterally minutely pectinate in distal 2/3rd, medially entire. Dactylus of chela of second pereiopod with one large triangular acute tooth in proximal half; fixed finger with 4–10 small teeth in proximal half. Palm with oval tympanal organ on medio-ventrally in proximal part. Dactylus of pereiopod 3–5 biunguiculate;

flexor margin of corpus carinate with small triangular subdistal accessory tooth; unguis flattened with fine scale-like spinules in longitudinal lines. Protopod of uropod rounded.

Remarks. In one *Tridacna squamosa* specimen with a maximum width of 39 cm, one small male specimen (pocl. 2.99 mm) and 20 juveniles (RMNH. CRUS.D.41438) with carapace lengths between 1.10 and 1.95 mm were found. This indicates that part of one brood of a female remains in the host. Larval development may be somewhat abbreviated and planktonic larval stages remain in the host mantle cavity until molting in megalopa and subsequent juvenile stages. One can assume that the juveniles leave the host when they become adults. The smallest specimens show the rostrum more similar to certain *Periclimenes* O.G. Costa, 1844 species. The dorsal teeth are spread over the distal half of the rostrum and a rudiment of an epigastric spine is present in these youngsters. This implicates that the dorsal rostral spines 'move' forward after each moult, clustering together in the distal part in fully grown specimens.

The material corresponds well with the type description of *A. australis f. typica*. The characteristics of *A. australis f. dendricauda*, intermediate posterior terminal spines of telson terminating in series of short blunt processes, and dorsal rostral margin with eight teeth, were not encountered. The distolateral tooth of the basal segment of the antennular peduncle is more prominent than in the specimen figured by Bruce (1977a: fig. 7d and e).The number of teeth in the fixed finger of the second pereiopods usually is 10 in the adult specimens of the present material. In some, 7 teeth are present, and in the juveniles, sometimes even less than 7. Bruce (1977a) noted 4–5 teeth in the type-series.

Colour (fig. 5.4A–B). Large dark blue and/or red-brown dots on carapace, abdomen, pereiopods, scaphocerite, antennal peduncles and caudal fan.

Hosts. Recorded from Cardiidae, Tridacninae: *Hippopus hippopus* (L., 1758) (De Grave, 1999; Hayashi, 2006); *Tridacna derasa* (Röding) (Bruce, 1977a; Bruce, 1991a; Hayashi, 2002, 2006); *Tridacna gigas* (L., 1758) (De Grave, 1999; Hayashi, 2006); *Tridacna maxima* (Röding, 1798) (Hayashi, 2006); *Tridacna squamosa* Lamarck, 1819 (Bruce, 1977a, 1981b, 1983a, 1991a, 1993; Strack, 1993; De Grave, 1999; Hayashi, 2006; Fransen & Reijnen, 2012; Liu, 2020); *Tridacna* sp. (Bruce, 1983b; Bruce & Coombes, 1995; Hayashi, 2006).

Distribution. Queensland, Australia (Bruce, 1977a), Great Barrier Reef, Australia (Bruce, 1981b), Port Essington, Australia (Bruce, 1983a); Cobourg

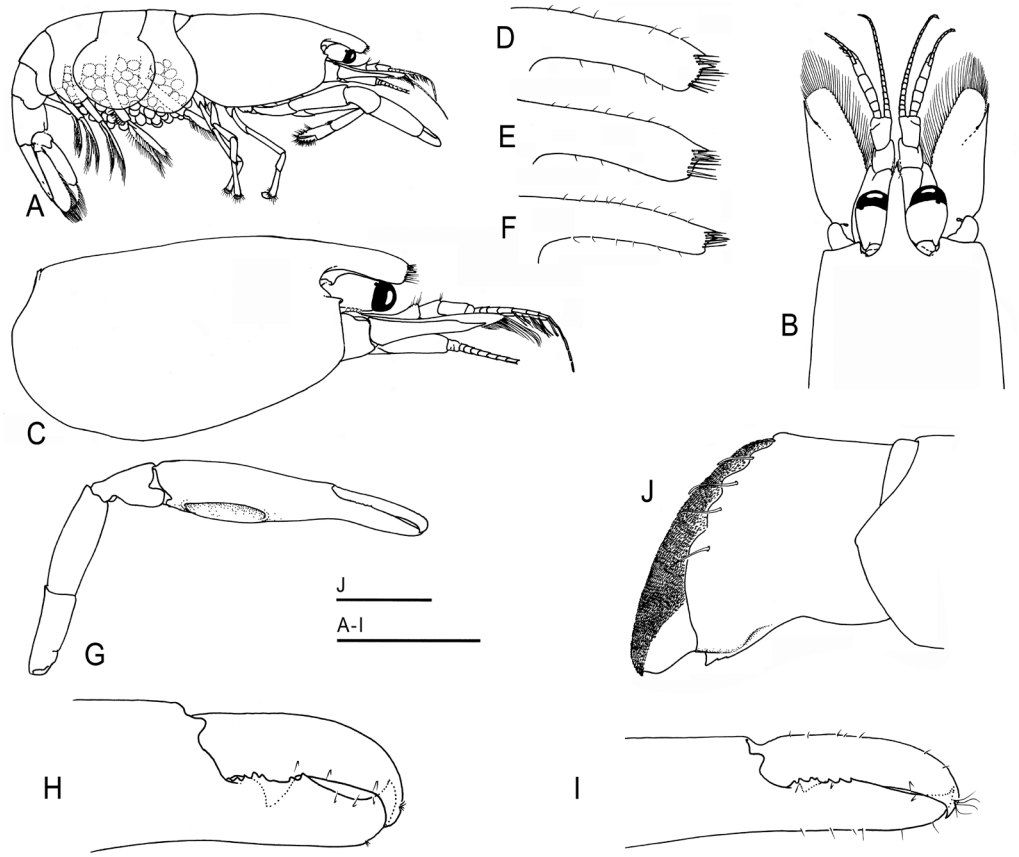
Peninsula, Australia (Bruce & Coombes, 1995); Fiji (Bruce, 1981b); Hainin Province, China (Liu, 2020); Seram Island, Indonesia (cf. Bruce, 1983b; Bourdon, 1983); Ambon Island, Indonesia (Strack, 1993; Fransen, 1994a); Ternate, Indonesia (Fransen & Reijnen, 2012); Sabah, Malaysia (Fransen & Reijnen, 2012); Tre Island, Vietnam (Bruce, 1993); Hansa Bay, Papua New Guinea (De Grave, 1999); New Caledonia (cf. Bruce, 1991a); Enewetak Atoll (Devaney & Bruce, 1987); Japan (Hayashi, 2002, 2006). Now for the first time recorded from Tioman island, West Malaysia, and from three new localities in Indonesia: Raja Ampat; NE Kalimantan; and Nanaka, Sulawesi.

Anchistus demani Kemp, 1922

(figs. 5.4C–D, 5.6–5.9)

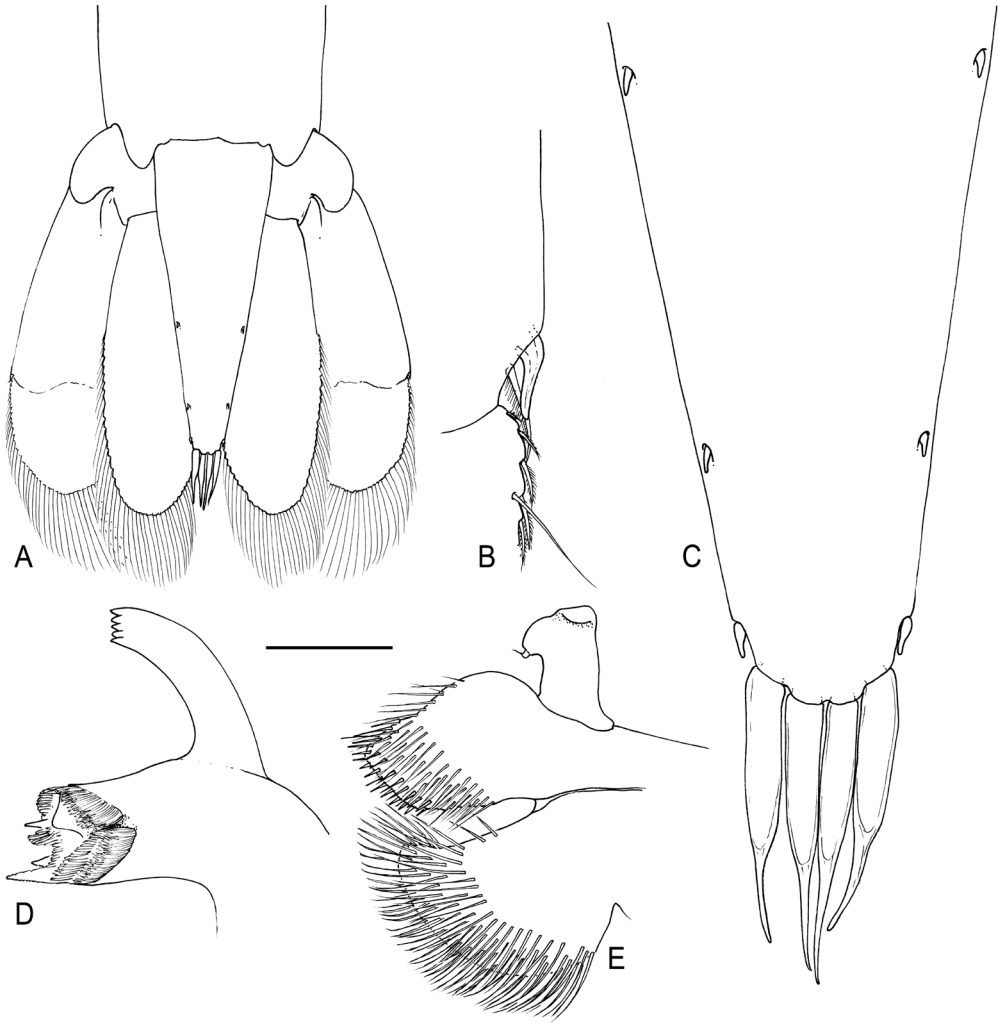
Anchistus demani Kemp, 1922: 256, figs. 86–88 [Type locality: Aberdeen, Port Blair, Andamans, low tide]; Holthuis, 1952: 13 (listed); Holthuis, 1953: 56; Bruce, 1967: 568 (listed); Bruce, 1972a: 219 (listed); Hipeau-Jacquotte, 1972b: 9; Hipeau-Jacquotte, 1973a: 104, fig. 5; Bruce, 1974: 200–201; Bruce, 1975a: 150, 162 (listed); Bruce, 1975b: 26 (listed); Bruce, 1976b: 147 (listed); Bruce, 1976c: 22; Bruce, 1976d: 464; Bruce, 1977a: 50; Bruce, 1978a: 119; Bruce, 1978b: 287 (listed); Bruce, 1979a: 232; Bruce, 1981c: 3; Bruce, 1982: 276 (listed); Bruce, 1983b: 892; Bruce, 1983c: 200 (listed); Bruce, 1984: 147 (listed); Bruce, 1990: 15, 18 (listed); Bruce, 1991a: 259–260, 275, fig. 22; Bruce, 1993: 57; Müller, 1993: 8 (listed); Chace & Bruce, 1993: 72 (listed); Britayev & Fachrutdinov, 1994: 124, fig. 2A-C; Fransen, 1994a: 107, 110, 111, fig. 2d; Fransen, 1994b: 88, pl. 1C; Poupin, 1998: 12; De Grave, 1999: 132, text-fig. 4; Li, 2000: 9 fig. 9 (listed); Hayashi, 2002: 225; Bruce, 2003b: 211 (listed); Li et al., 2007: 177, fig. 74 (listed); Marin & Savinkin, 2007: table 2, 3 (listed); Bruce, 2010a: 63 (listed); De Grave & Fransen, 2011: 340 (listed); Radhakrishnan et al., 2012: table 1 (listed); Aznar-Cormano et al., 2015: 202 (listed); Samuel et al., 2016: table 1 (listed).
Anchistia cf. *demani* Rosewater, 1965: 381, 386.
Anchistus deseani – Strack, 1993: 46 [erroneous spelling].

Material examined. INDONESIA: 1 ovigerous female pocl. 3.81 mm (photo by M.S.S. Lavaleye 8/38a), 1 female pocl. 4.07 mm, 1 male pocl. 2.60 mm (RMNH.CRUS.D.41457), stn RBE.11, Ambon Island, Nusaniwe, 12.xi.1990, depth 2 m, snorkeling, found together in one *Tridacna* sp., coll. C.H.J.M. Fransen. – 1 female pocl. 2.21 mm (RMNH.CRUS.D.41458), stn RBE.16, Pombo Island E of Ambon Island, NW side, 16.xi.1990, snorkeling, in *Tridacna squamosa* Lamarck, coll. J.C. den Hartog. – 1 non ovigerous female (R = 3/0) pocl. 2.5 mm (RMNH.



5.6 *Anchistus demani* Kemp, 1922. A–G, I, J) RMNH.CRUS.D.41457; H) RMNH. CRUS.D.41480. A) habitus, lateral view, ovigerous female pocl. 3.8 mm; B) anterior appendages, lateral view, ovigerous female pocl. 3.8 mm; C) idem, dorsal view, ovigerous female pocl. 3.8 mm; D) rostrum, female pocl. 4.1 mm; E) idem, ovigerous female pocl. 3.8 mm; F) idem, male pocl. 2.6 mm; G) left second pereiopod; H, I) idem, chela; J) right third pereiopod dactylus, medial view. Scale A = 4 mm; B, C, G, = 2 mm; D–F, H, I, = 1 mm. Artwork: Charles Fransen.

CRUS.D.46481), SW Sulawesi, Spermonde Archipelago, Kudingareng Keke, S side, depth 3-5 m, snorkeling, 26.ix.1994, from *Tridacna deresa* (Röding) (Ø 20 cm), coll. J.C. den Hartog. – 1 female pocl. 2.7 mm with pair of abdominal bopyroids, rostrum damaged (RMNH.CRUS.D.46482), stn SUL.04, N Sulawesi, Selat Lembeh, Pulau Lembey, Pantai Parigi, 01°28'N 125°14'E, small fringing reef, gently sloping from beach to 10 m, depth 1-3 m, snorkeling, 15.x.1994, in *T. squamosa*, coll. J.C. den Hartog. – 1 male 2.1 mm (R = 2/0) (RMNH. CRUS.D.46483), stn SUL.06, N Sulawesi, Selat Lembeh, Pulau Lembey, Pantai Parigi, 01°28'N 125°14'E, small fringing reef, gently sloping from beach to 10 m, depth 1-3 m, snorkeling, 15.x.1994, in *T. squamosa*, coll. J.C. den Hartog. – 1 ovigerous female pocl. 4.0 mm (R =2/0) (RMNH.CRUS.D.46484), stn SUL.06, N Sulawesi, Selat Lembeh, Pulau Lembey, Pantai Parigi, 01°28'N

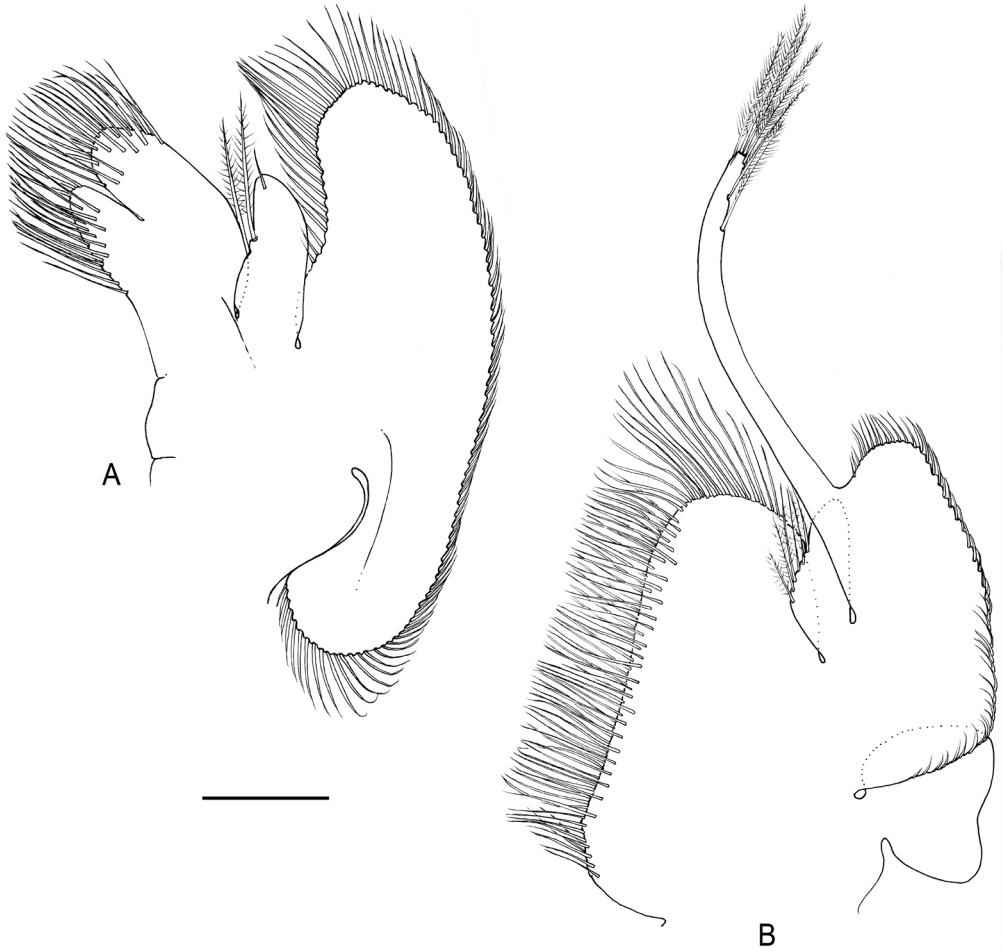


5.7 *Anchistus demani* Kemp, 1922, RMNH.CRUS.D.41457, female pocl. 4.1 mm. A) tailfan, dorsal view; B) distolateral part exopod uropod, dorsal view; C) telson, dorsal view; D) left mandible, ventral view; E) left maxillula, ventral view. Scale A = 1.25 mm; B, 0.125 mm; C = 0.25 mm; D, E = 0.5 mm. Artwork: Charles Fransen.

125°14'E, small fringing reef, gently sloping from beach to 10 m, depth 1–3 m, snorkeling, 15.x.1994, in *Tridacna maxima* (Röding) (Ø 20 cm), coll. J.C. den Hartog. – 1 male pocl. 1.9 mm (R = 2/0), 1 ovigerous female, pocl. 2.3 mm (R = 3/0), RMNH.CRUS.D.57965), stn BER.10, NE Kalimantan, Berau Islands, Samama Island, 02˚07'31.6"N 118˚20'09.7"E, 7.x.2003, depth 5 m, scuba diving, in *Tridacna crocea* Lamarck (Ø 10 cm), coll. C.H.J.M. Fransen, film 2/25-29. **PHILIPPINES**: 1 male pocl. 2.2 mm (R = 2/0), 1 female pocl. 4.0 mm (R = 3/0) (RMNH.CRUS.D.48351), stn CEB.11, Cebu Strait, W of Bohol, E side of Cabilao Island, S of Cambacis, 9°52.92'N 123°47.37'E, patchy reef

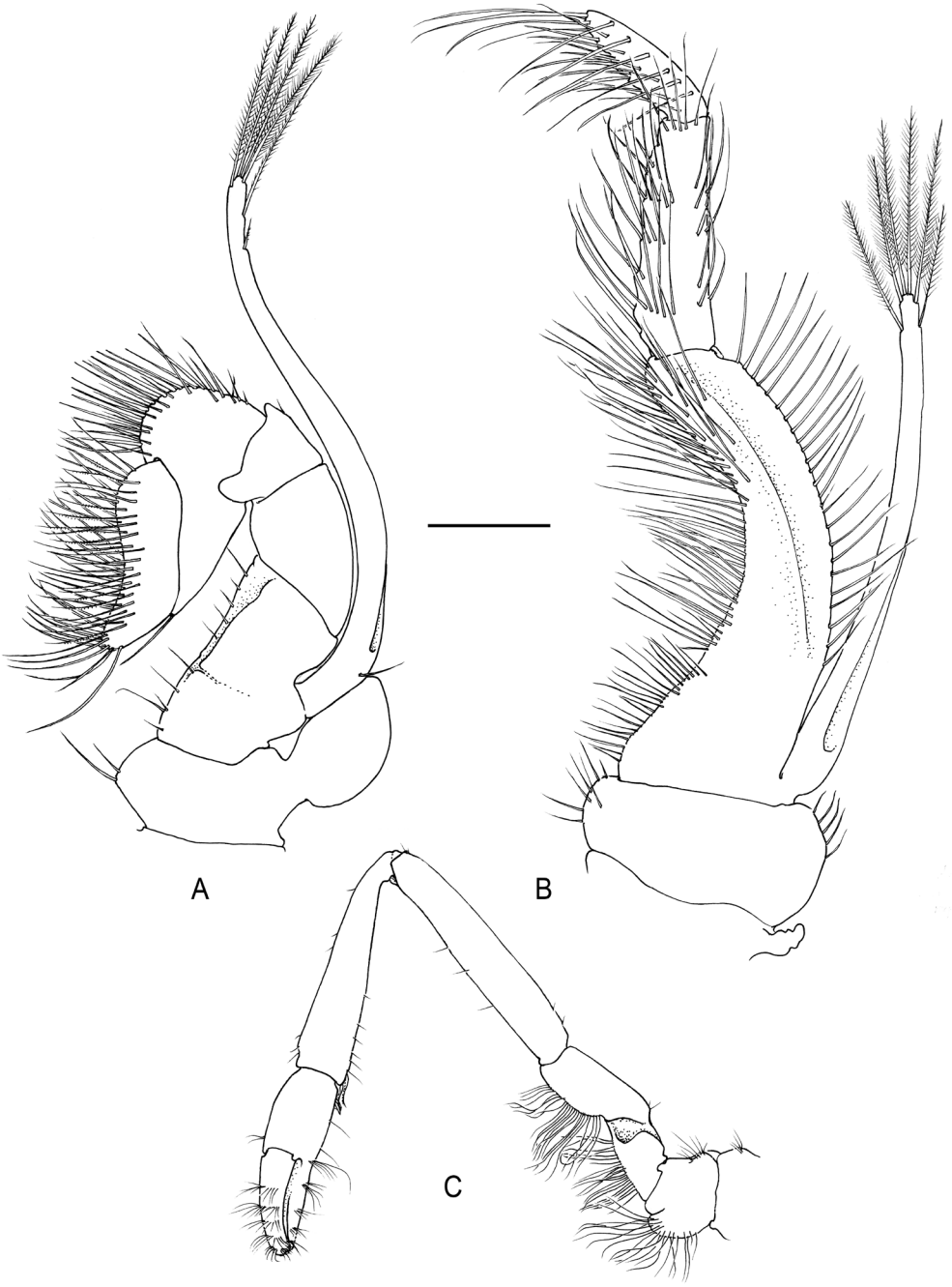
with algae, 14.xi.1999, depth 2-5 m, snorkeling, in *T. maxima*; coll. J.C. den Hartog. – 1 male pocl. 3.0 mm, 1 ovigerous female pocl. 4.9 mm (RMNH.CRUS.D.48354; GenBank OQ600396 (16S)), stn CEB.06, Cebu Strait, W of Bohol, N side of Cabilao Island, near Looc, 9°53.49'N 123°46.53'E, 9.xi.1999, depth 1 m, snorkeling, sandy reef flat with seagrass, in *Hippopus hippopus* (L.) (Ø 15 cm), coll. J.C. den Hartog. **SEYCHELLES**: 1 male pocl. 1.56 mm (RMNH.CRUS.D.42791), niop-E stn SEY.754, St Joseph Atoll, NW rim, lagoon, 5°24'S 53°19'E, 26.xii.1992, depth 1 m, reef flat at low tide, snorkeling, in *Tridacna* sp. (Ø 18 cm); coll. J.C. den Hartog, photo 17/1517. – 1 ovigerous female pocl. 3.44 mm, 4 specimens pocl. 1.44, 2.25, 2.94, 3.06 mm (RMNH.CRUS.D.42792), niop-E stn SEY.792, St François Atoll, W rim, 7°05'S 52°44'E, 5/6.i.1993, outer slope down to 27 m depth, scuba diving, in *T.*? *maxima* coll. C.H.J.M. Fransen, photo 33/512. **RED SEA**: 1 ovigerous female pocl. 3.38 mm (RMNH.CRUS.D.28583), Sudan, E of Port Sudan, Wingate reef, 5.ix.1970, on top of reef in shallow water, in *Tridacna* sp., coll. N.V.C. Polunin, no. 12. – 1 ovigerous female pocl. 4.16 mm (RMNH.CRUS.D.28584), Egypt, Gulf of Akaba, Sinai Peninsula, Marsa Murach, 22.viii.1967, coll. L. Fishelson, no. ns 2412. – 2 males pocl. 2.79 mm and 3.18 mm, 1 female pocl. 2.60 mm, 1 ovigerous female pocl. 4.81 mm (RMNH.CRUS.D.41480), SW of Umm Aabak, near Nocra Island, in *Tridacna* sp., 22.iii.1962, depth 0-2 m, Israel Red Sea Expedition no. E62/1378. – 1 male pocl. 3.12 mm (RMNH.CRUS.D.41481), Landing Bay, Entedibir Island, 31.iii.1962, in *Tridacna* sp., depth 2 m, Israel Red Sea Expedition no. E62/1432. – 1 male pocl. 2.79 mm (RMNH.CRUS.D.41482), Umm Aabak, 25.iii.1962, Israel Red Sea Expedition no. E62/2220. – 1 female pocl. 2.73 mm (RMNH.CRUS.D.41483), no further locality information; 1962, Israel Red Sea Expedition no. E62/1503. – 2 ovigerous females pocl. 2.0 and 2.5 mm, 1 male pocl. 1.5 mm, 1 juvenile pocl. 1.0 mm (RMNH.CRUS.D.58049), stn THU.07, Fsar, 22°13'46.4"N 39°01'44.5"E, 10.xi.2015, depth 12 m, in 3 small *T. maxima*, coll. C.H.J.M. Fransen. – 1 ovigerous female pocl. 2.8 mm (RMNH.CRUS.D.58050), stn THU.14, Tahlah, 22°16'25.9"N 39°03'01.1"E, 13.xi.2015, depth 4 m, in small T. crocea, coll. C.H.J.M. Fransen. **MICRONESIA**: few specimens (RMNH.CRUS.D.28934), Palau Group, Ngemelis, S of Koror, -.iv.1971.

Diagnosis. Rostrum reaching distal end of basal segment of the antennular peduncle, distally typically bidentate (seldom only one tooth present). Antennal spine absent. Ventral orbital angle slightly produced. Antero-lateral angle of carapace rounded. Anterior margin of carapace between orbital ventral angle and antero-lateral margin straight. Sixth abdominal segment with posterior ventral angle rounded. Telson with dorsal spines minute, at 0.61 and 0.87 of telson length, with lateral pair of terminal spines small, situated subdistal to



5.8 *Anchistus demani* Kemp, 1922, RMNH.CRUS.D.41457, female pocl. 4.1 mm. A) left maxilla, ventral view; B) left first maxilliped, ventral view. Scale = 0.5 mm. Artwork: Charles Fransen.

intermediate spines. Basal segment of antennular peduncle with distolateral angle rounded. Scaphocerite with straight lateral margin and deep slit between distolateral tooth and lamina. Third maxilliped with antepenultimate segment slightly broader than penultimate segment. First pereiopod with chela spatulate, non-cannulate; lateral cutting edges minutely pectinate in distal 2/3, medially entire. Second pereiopods with chela equal or unequal, dactylus with one large triangular tooth in proximal half; fixed finger with 4–11 small teeth in proximal half; palm with oval tympanal organ medio-ventrally in proximal half. Ambulatory pereiopods with dactylus biunguiculate; accessory tooth blunt and inconspicuous; dorsal flattened surface of unguis with fine scale-like spinules. Protopod of uropod rounded.



5.9 *Anchistus demani* Kemp, 1922, RMNH.CRUS.D.41457, female pocl. 4.1 mm. A) left second maxilliped, ventral view; B) left third maxilliped, ventral view; C) left first pereiopod, lateral view. Scale A, B = 0.5 mm; C = 1.25 mm. Artwork: Charles Fransen.

Remarks. Specimens correspond closely with previous descriptions. The first pereiopods have minutely pectinate cutting edges like the other members of the genus. The species seems close to *A. australis* in sharing the absence of an antennal spine, in having a deep slit between de distolateral tooth and the lamina of the scaphocerite, and in having similar ambulatory dactyli.

Colour (fig. 5.4C–D). The colour pattern is similar to that of *A. miersi*, e.g., transparent with numerous circular dark blue dots over body, antennae, chela of second pereiopods and caudal fan. Kemp (1922: 259) noted the dots to be pale green or red: “Two of the specimens, which were found together in a large *Tridacna*, were transparent when alive and dotted all over with pale green chromatophores. The female bore green eggs. The third specimen also found in *Tridacna*, was transparent with red chromatophores …”.

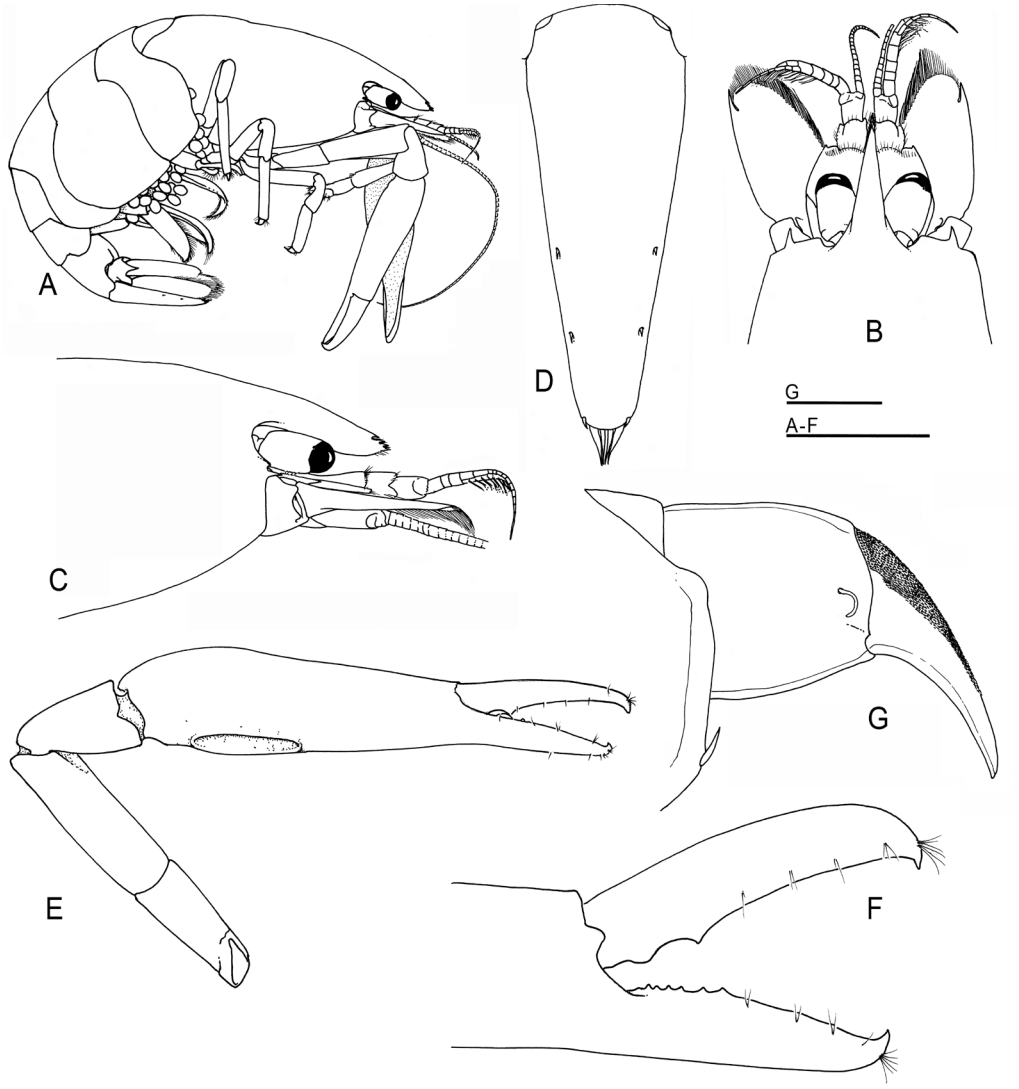
Hosts. Recorded from Cardiidae, Tridacninae: *Tridacna*? *crocea* Lamarck, 1819 (Bruce, 1979a); *Tridacna gigas* (L., 1758) (Holthuis, 1953); *Tridacna maxima* (Röding, 1798) (Bruce, 1974, 1976c, 1976d, 1977a, 1978a; 1993; Britayev & Fachrutdinov, 1994; Poupin, 1998); *T.*? *maxima* (Fransen, 1994b); *Tridacna squamosa* Lamarck, 1819 (Rosewater, 1961; Bruce, 1976d; Britayev & Fachrutdinov, 1994); *Tridacna* sp. (Holthuis, 1953; Bruce, 1981c; 1983b; 1991a; Fransen, 1994a). Newly reported from *Hippopus hippopus* (L., 1758); *Tridacna derasa* (Röding, 1798); and confirmed from *T. crocea*.

Distribution. Port Blair, Andaman Islands (Kemp, 1922); Marshall Islands (Holthuis, 1953; Rosewater, 1961); Gilbert Islands (Holthuis, 1953); Thailand (Rosewater, 1965); Perhantian Islands, Malaysia (Bruce, 1979a); Zanzibar and Kenya, East Africa (Bruce, 1976c, 1976d); Seychelles (Bruce, 1976c; Fransen, 1994b); Aldabra Island, Comoro Islands (Bruce, 1978a); Farquhar, Seychelles (Bruce, 1974); Capricorn group, Australia (Bruce, 1977a); Heron Island, Australia (Bruce, 1981c); Seram Island, Indonesia (Bruce, 1983b); Ambon, Indonesia (Fransen, 1994a); New Caledonia (cf. Bruce, 1991a); Tre Island, Vietnam (Bruce, 1993); South Vietnam (Britayev & Fachrutdinov, 1994); Tuamotu (Poupin, 1998). Here reported for the first time from East Kalimantan and Sulawesi, Indonesia; the Red Sea; Palau; and Cebu Strait, the Philippines.

***Anchistus gravieri* Kemp, 1922**

(figs. 5.4B, 5.10–5.15)

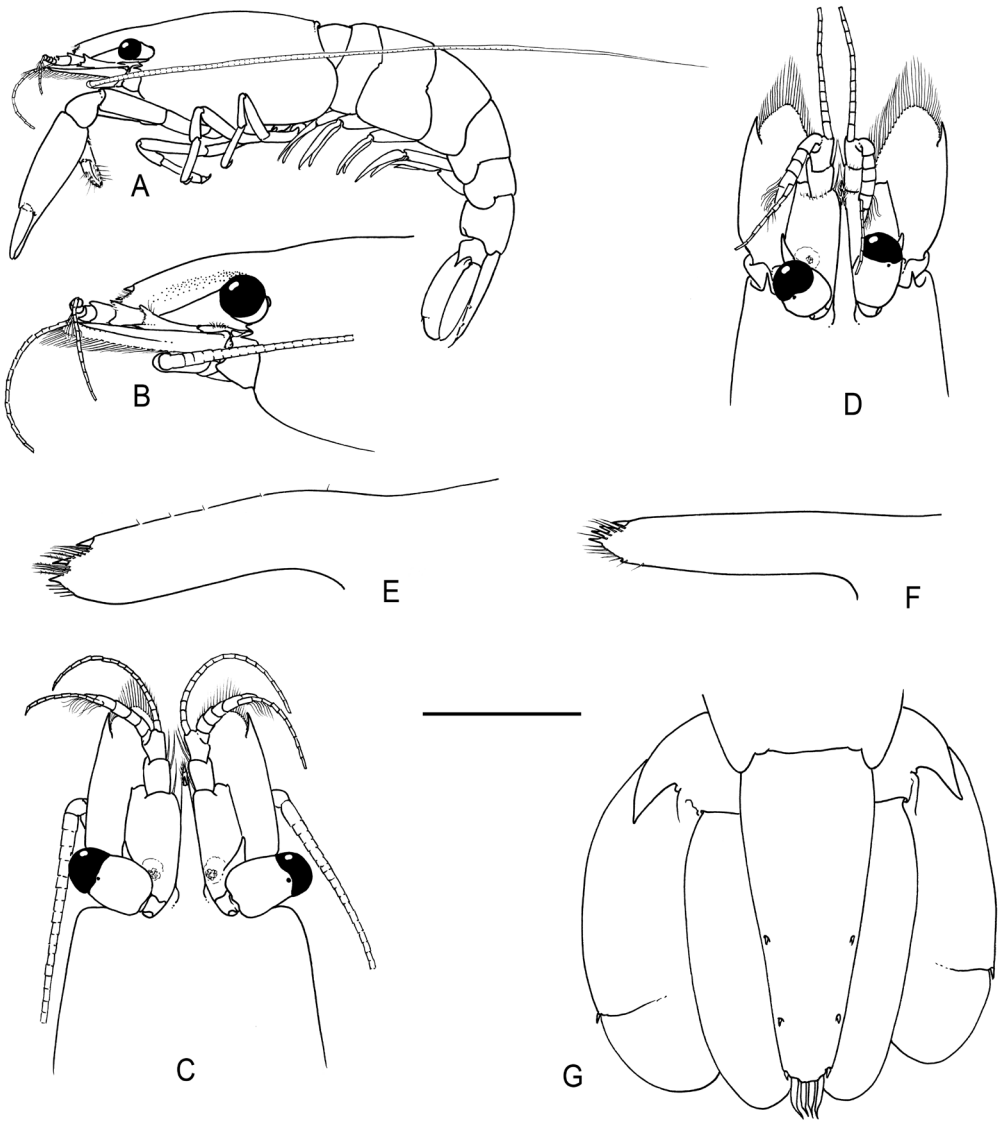
Anchistus gravieri Kemp, 1922: 249 (key), 252-255, figs. 82–84 [Type locality: Vanikoro, Santa Cruz Islands, Polynesia]; Holthuis, 1952: 13 (listed); Bruce,



5.10 *Anchistus gravieri* Kemp, 1922, RMNH.CRUS.D.58051, Nathrang, ovigerous female, pocl. 6.9 mm. A) habitus, lateral; B) anterior appendages, dorsal view; C) idem, lateral view; D) telson, dorsal view; E) left second pereiopod; F) idem, chela; G) right third pereiopod dactylus. Scale A = 4 mm; B, C, E = 2 mm; D, F = 1 mm; G = 0.1 mm. Artwork: Charles Fransen.

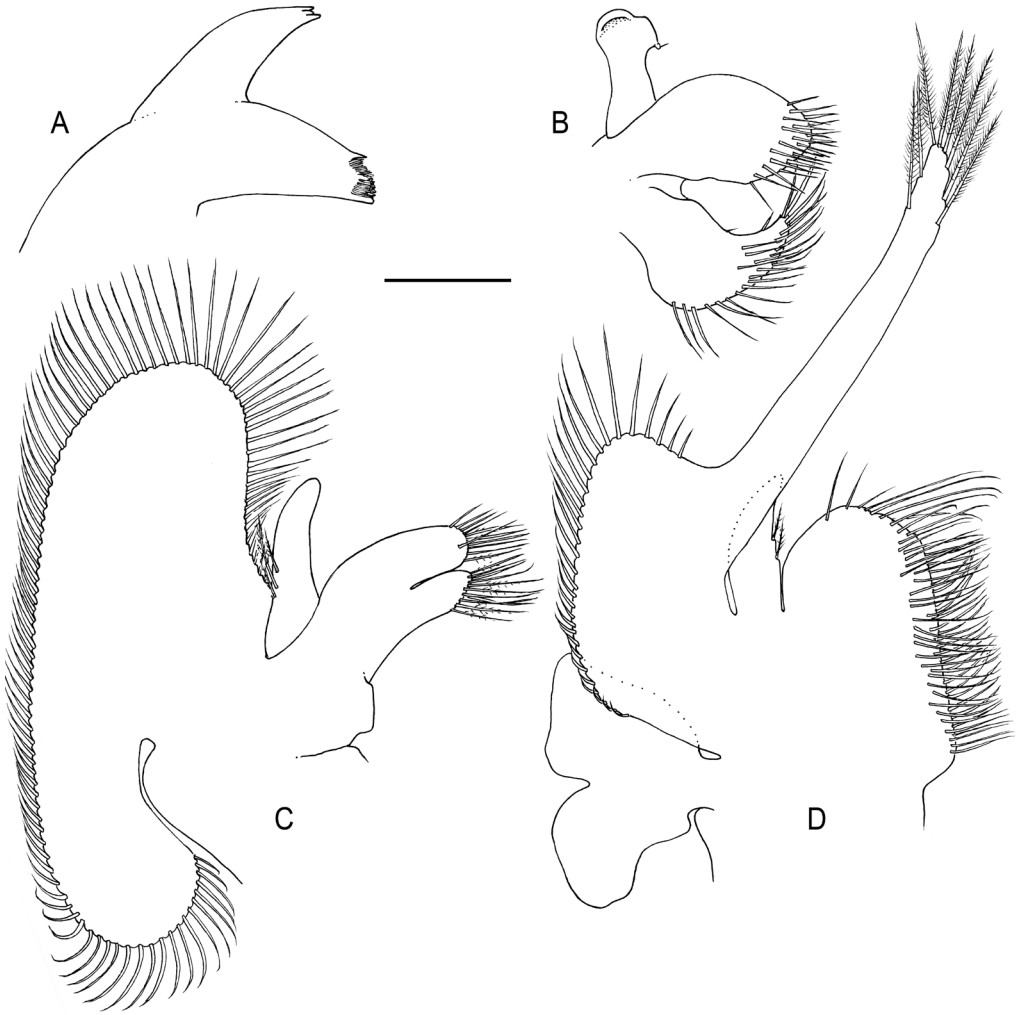
1967: 568; Bruce, 1972a: 219 (listed); Monod, 1972: 24, figs. 65-86; Hipeau-Jacquotte, 1972b: 9; Bruce, 1975a: 162; Bruce, 1977a: 47-50, figs. 2, 3; Bruce, 1983c: 200 (listed); Bruce, 1990: 15, 18 (listed); Bruce, 1991a: 260, 275, fig. 23; Müller, 1993: 9 (listed); Fransen, 1994a: 107, 111, fig. 2a; Li, 2000: 10, fig. 10 (listed); Hayashi, 2002: 225; De Grave & Fransen, 2011: 340 (listed).

not *Anchistus gravieri* – McNeill, 1953: 89 (= *Polkamenes nobilii* (Holthuis, 1952)).



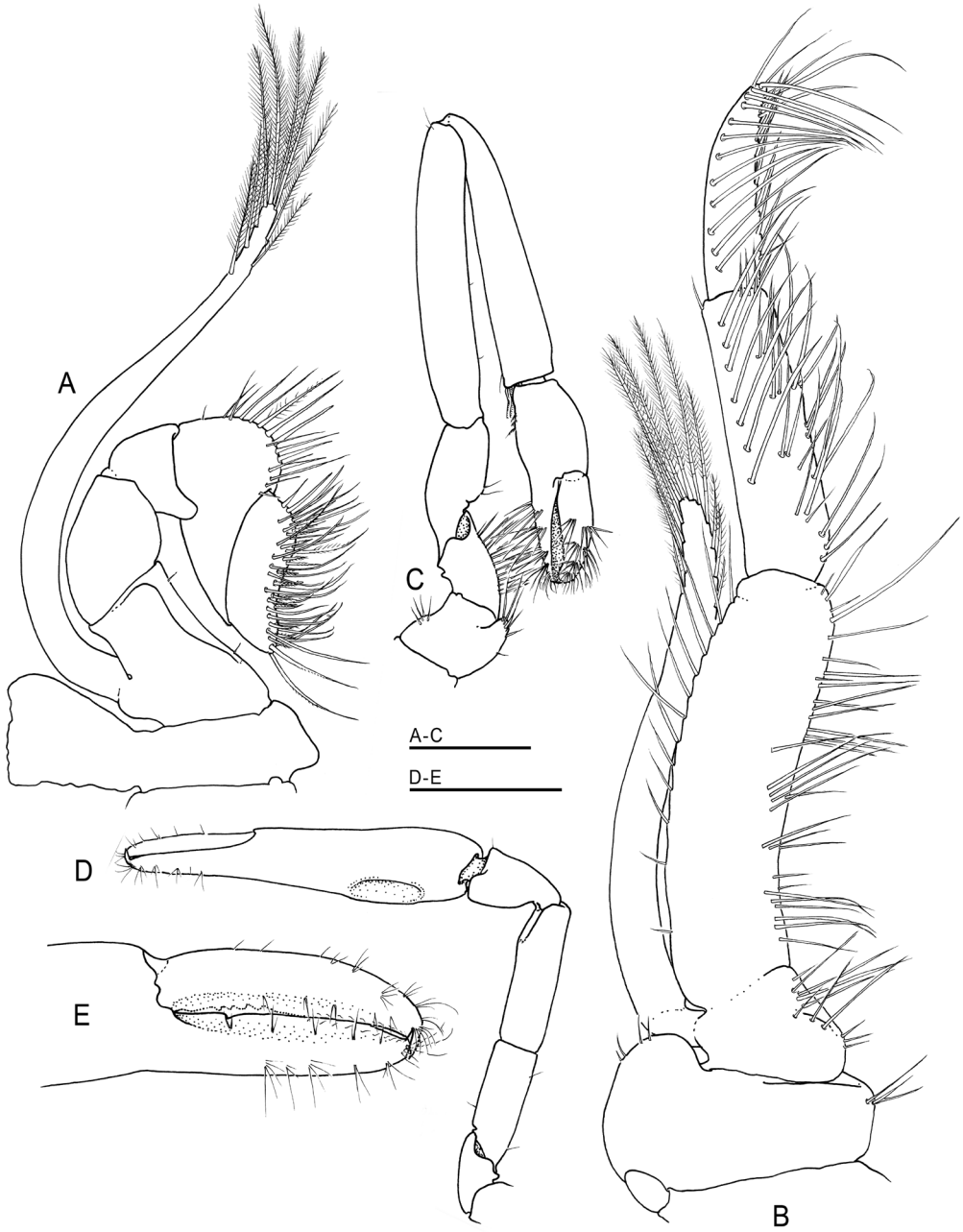
5.11 *Anchistus gravieri* Kemp, 1922, RMNH.CRUS.D.58052, Vanuatu (stn FR15). A–C, E) female, pocl. 4.2 mm; D, F, G) male, pocl. 3.9 mm. A) habitus, lateral view; B) anterior appendages, lateral view; C, D) idem, dorsal view; E, F) rostrum, lateral view; G) tail fan, dorsal view. Scale A = 4 mm; B–D = 2 mm; E–G = 1 mm. Artwork: Charles Fransen.

Material examined. VIETNAM: 1 ovigerous female pocl. 6.9 mm (RMNH.CRUS.D.58051), Nathrang, entree 1948, st. 870 E. 413, in *Tridacna* sp., coll. R. Serène. **VANUATU**: 1 male pocl. 3.9 mm, 1 female pocl. 4.2 mm (RMNH.CRUS.D.58052), stn fr15, S Aoré Island, Bruat Channel, 15°21'56"S 167°5'44"E, 16.ix.2006, depth 3 m, hand picked, in *Hippopus hippopus*, coll. C.H.J.M. Fransen, fcn. cf57, photo T.-Y. Chan.

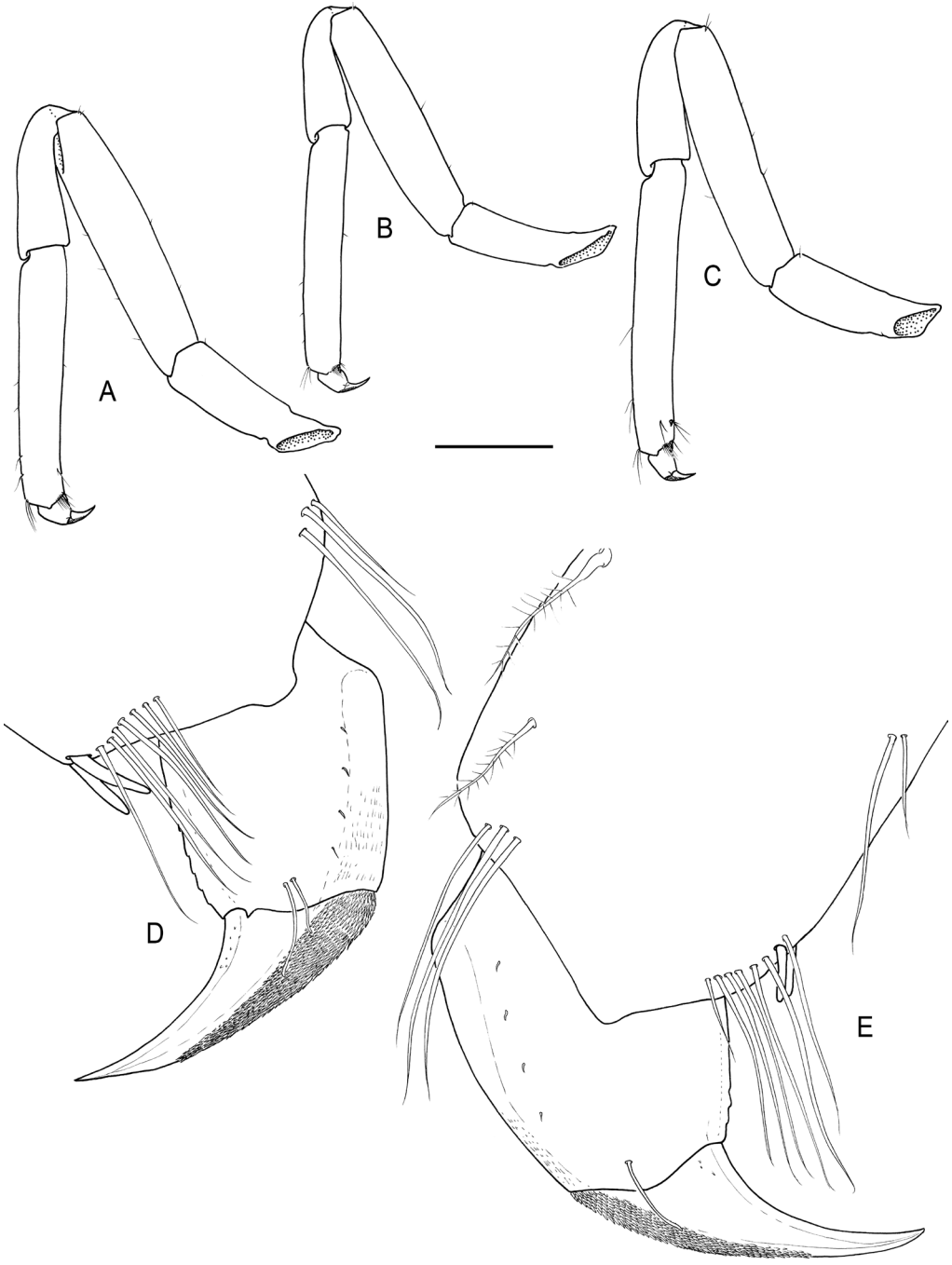


5.12 *Anchistus gravieri* Kemp, 1922, RMNH.CRUS.D.58052, Vanuatu (stn FR15), female, pocl. 4.2 mm. A) left mandible, dorsal view; B) left maxillula, dorsal view; C) left maxilla, dorsal view; D) left first maxilliped, dorsal view. Scale = 0.5 mm. Artwork: Charles Fransen.

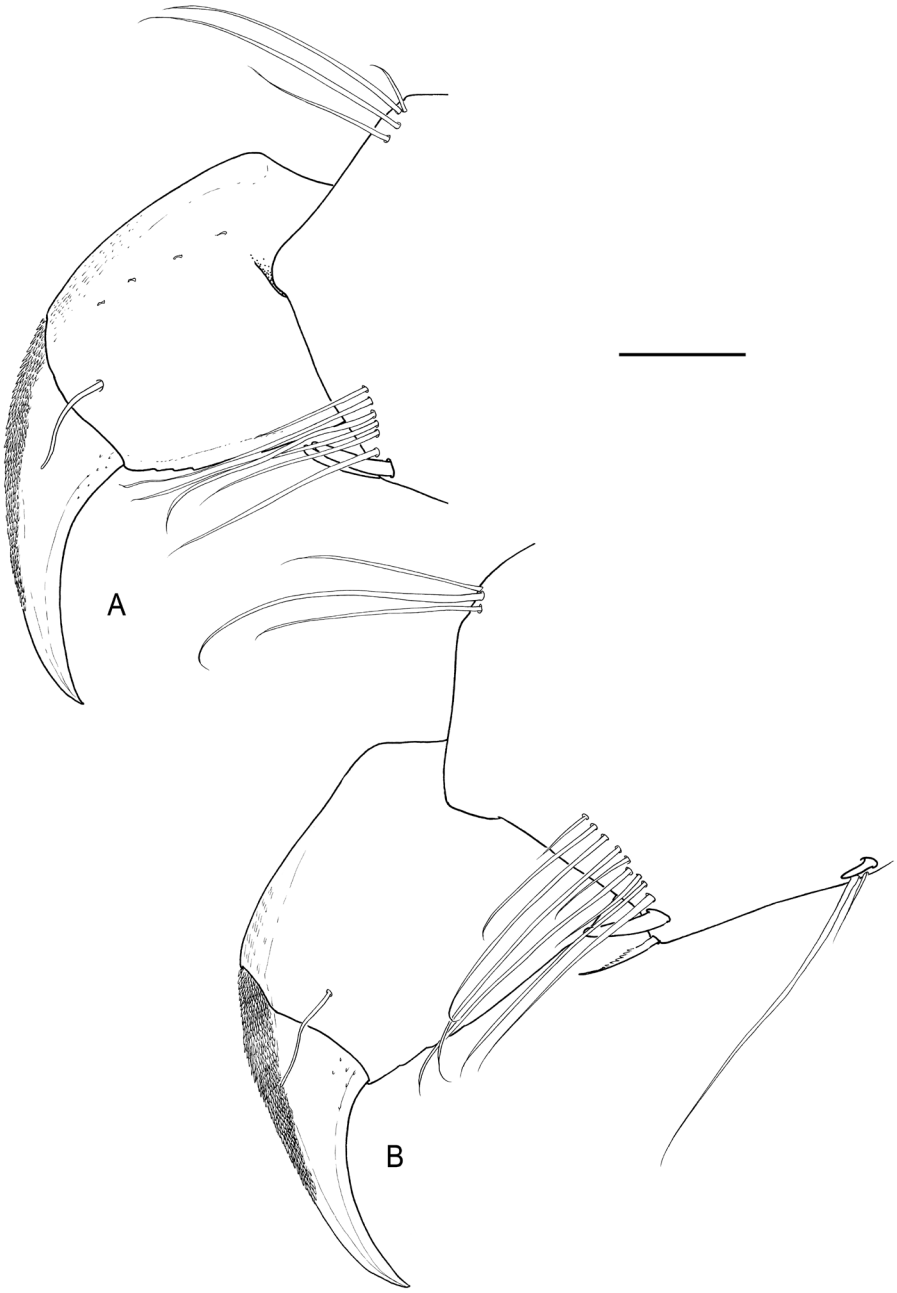
Diagnosis. Rostrum reaching end of penultimate segment of antennular peduncle, bent downward, with 2–4 dorsal distal teeth and one or none ventral tooth. Antennal spine well developed. Ventral orbital angle produced. Antero-lateral angle of carapace rounded. Anterior margin of carapace between ventral orbital angle and antero-lateral angle straight. Sixth abdominal segment with posterior ventral angle rounded. Telson with small dorsal spines at 0.61 and 0.79 of telson length, with lateral pair of terminal spines small, situated next to intermediate spines. Basal segment of antennular peduncle with or without distolateral tooth. Stylocerite acute. Scaphocerite with straight or slightly convex lateral margin, slit between distolateral tooth and lamina of moderate depth. First pereiopods with chela spatulate, not cannulate, with lateral cutting edges



5.13 *Anchistus gravieri* Kemp, 1922, RMNH.CRUS.D.58052, Vanuatu (stn FR15), female, pocl. 4.2 mm. A) left second maxilliped, dorsal view; B) left third maxilliped, dorsal view; C) left first pereiopod, lateral view; D) right second pereiopod, ventral view; E) idem, chela, medial view. Scale A, B = 0.5 mm; C = 1.25 mm; D = 2 mm; E = 1 mm. Artwork: Charles Fransen.



5.14 *Anchistus gravieri* Kemp, 1922, RMNH.CRUS.D.58052, Vanuatu (stn FR15), female, pocl. 4.2 mm. A) right third pereiopod, lateral view; B) right fourth pereiopod, lateral view; C) left fifth pereiopod, medial view; D) right third pereiopod dactylus, medial view; E) idem, lateral view. Scale A–C = 1.25 mm; D, E = 0.125 mm. Artwork: Charles Fransen.



5.15 *Anchistus gravieri* Kemp, 1922, RMNH.CRUS.D.58052, Vanuatu (stn FR15), female, pocl. 4.2 mm. A) right fourth pereiopod dactylus, lateral view; B) left fifth pereiopod dactylus, medial view. Scale = 0.125 mm. Artwork: Charles Fransen.

minutely pectinate in distal 2/3, medially entire. Second pereiopods unequal or equal, proximal half of palm with oval tympanal organ medio-ventrally in proximal part; dactylus with one, sometimes two, moderate triangular teeth in proximal half; fixed finger with 6–8 small teeth in proximal half. Ambulatory pereiopods with simple dactyli, not biunguiculate, dorsal flattened surface unguis with fine scale-like spinules. Protopod of uropod acutely produced.

Remarks. The specimens studied agree well with former descriptions of the species (Kemp, 1922; Monod, 1972; Bruce, 1977a, 1991a).

Colour (fig. 5.4E). Large dark blue dots on carapace, abdomen, pereiopods, scaphocerite, antennal peduncles and caudal fan.

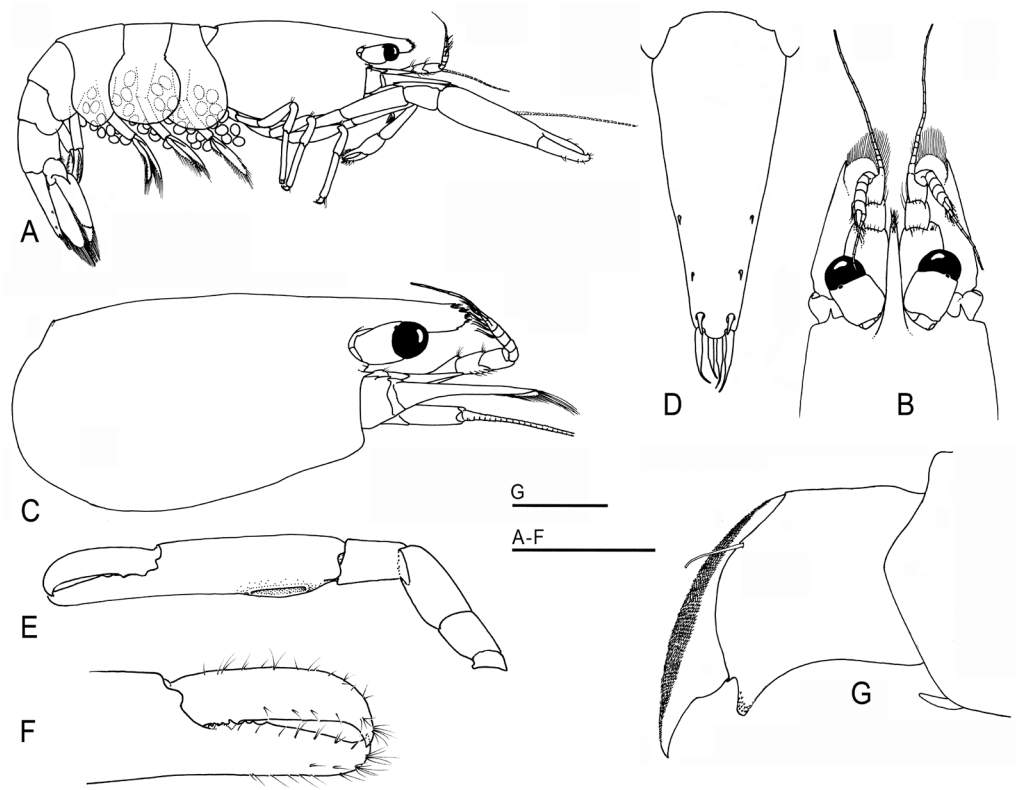
Hosts. Recorded from Cardiidae, Tridacninae: *Hippopus hippopus* (L., 1758) (cf. Bruce, 1977a, 1991a); newly recorded from an unidentified *Tridacna*.

Distribution. Vanikoro Island, Santa Cruz Islands, Polynesia (Kemp, 1922); New Caledonia (Monod, 1972; Bruce, 1991a); Herald Island, Australia (Bruce, 1977a); Nathrang, Vietnam (Fransen, 1994a). Now recorded for the first time from Vanuatu.

***Anchistus miersi* (De Man, 1888)**

(figs. 5.4F–G, 5.16, 5.39B, D)

Harpilius Miersi De Man, 1888: 274, pl. 17 figs. 6–10 [type locality: Elphinstone Island];? Whitelegge, 1897: 148.
Coralliocaris nudirostris – Nobili, 1899: 235.
non *Anchistus miersi* – Nobili, 1906a: 48; Pérez, 1920: 1027 (both *Polkamenes nobilii* (Holthuis, 1952)).
Anchistus miersi – Nobili, 1906b: 63; Nobili, 1907: 359; Holthuis, 1955: 62 (listed).
Marygrande mirabilis Pesta, 1911: 573, figs. 1–5 (partim) [type locality: Samoa]; De Grave, 2001: 129–134, figs. 4–5.
Anchistus miersi – Borradaile, 1898: 387; Borradaile, 1917: 324; Tattersall, 1921: 391; Seurat, 1934: 60; Kubo, 1940: 52–54, figs. 18-20 (pro parte); Holthuis, 1952: 110, 111 (full synonymy), fig. 45; Holthuis, 1953: 56; Johnson, 1961: 59, 62, 63, 76; Ooishi, 1964: 196 (listed); Johnson & Liang, 1966: 434; Miyake & Fujino, 1968: 414-415 (pro parte); Bruce, 1972a: 219, 222 photograph (listed); Miyake, 1972: 65, 210 (listed); Bruce, 1973: 136; Bruce, 1975b: 26 (listed); Fujino, 1975: 252-255, fig. 1, pl. 1; Monod, 1976b: 24,



5.16 *Anchistus miersi* (De Man, 1888). A–D) RMNH.CRUS.D.41390, ovigerous female pocl. 3.9 mm; E–G) RMNH.CRUS.D.41389, ovigerous female pocl. 4.7 mm. A) habitus, lateral view; B) anterior appendages, dorsal view; C) idem, lateral view; D) telson, dorsal view; E) left second pereiopod, ventral view; F) idem, chela, medial view. Scale A = 4 mm; B, C, E = 2 mm; D, F = 1 mm. Artwork: Charles Fransen.

figs. 29-36; Bruce, 1976b: 147; Bruce, 1976c: 22; Bruce, 1976d: 464-465; Bruce, 1976e: 448; Watabe & Tomida, 1977: 46; Bruce, 1977b: 174-175; Bruce, 1978a: 119; Bruce, 1978b: 279; Bruce, 1979a: 232-233; Johnson, 1979: 31; Bruce, 1982: 267 (listed); Miyake, 1982: 32, pl. 11, fig. 6 (listed); Bruce, 1983c: 200 (listed); Bruce & Svoboda, 1983: 36 (listed); Bruce, 1984: 147 (listed); Vine, 1986: 101 (listed); Devaney & Bruce, 1987 (listed); Bruce, 1990: 15, 18 (listed); Holthuis, 1993: 130, fig. 111; Müller, 1993: 9 (listed); Bruce, 1993: 57, fig. 3A; Chace & Bruce, 1993: 72; Britayev & Fachrutdinov, 1994: 129, fig. 3E-F; Fransen, 1994a: 107, 110, 111, fig. 2c; Fransen, 1994b: 89, fig. 1D; Poupin, 1998: 12 (listed); De Grave, 1999: 132, pl. 1 figs. d, e; Li, 2000: 11, fig. 11 (listed); De Grave, 2001: 129-134, figs. 4–5; Hayashi, 2002: 223, figs. 432i, j, 434g, h, 435, g, h; Bruce, 2003b: 211 (listed); Li, 2004: 67; Hayashi, 2006: 510, 512; Marin & Savinkin, 2007: table 2 (listed); Li et al., 2007: 178, fig. 75 (listed); De Grave & Ashelby, 2011: table 1 (listed); De Grave & Fransen, 2011: 340 (listed); Radhakrishnan et al., 2012: table 1

(listed); Fransen & Reijnen, 2012; Hosie et al., 2015: 283 (listed); Neo et al., 2015: 877-878, figs. 1, 2; Anker & De Grave, 2016: 407; Samuel et al., 2016: table 1 (listed).
Anchistus meirsi – Fankboner, 1972: 35–41, figs. 1-3 [erroneous spelling].
Auchistus miersi – Strack, 1993: 46 [erroneous spelling].
Ancistus miersi – Ng et al., 1995: 43, fig. [erroneous spelling].

Material examined. INDONESIA: 2 ovigerous females pocl. 4.29, 4.74 mm (RMNH.CRUS.D.41389), stn RBE.30, Suli, 29.xi.1990, depth 1–3 m, snorkeling, in 2 *Hippopus hippopus* (L.) (Ø 15–20 cm), coll. H.L. Strack, one with pinnotherid. – 1 ovigerous female pocl. 3.90 mm (photo by M.S.S. Lavaleye 3/4-8) (RMNH.CRUS.D.41390), stn RBE.11, Nusaniwe, 12.xi.1990, snorkeling, depth 2 m, in *Tridacna* sp., coll. C.H.J.M. Fransen and J.C. den Hartog. – 1 male pocl. 3.25 mm, 1 female pocl. 4.87 mm (RMNH.CRUS.D.41391), Karimuajawa, Rotenoa, 25.iii.1974, don. PPPO. – 1 male pocl. 3.90 mm, 1 female pocl. 4.94 mm (RMNH.CRUS.D.41392), Ambon Island, Ambon Bay, Tantoci, 3.vi.1975, coll. M.K. Moosa, Banner & Banner, Rumphius II Expedition, don. PPPO, fcn. cm 133. – 1 ovigerous female pocl. 7.47 mm (RMNH.CRUS.D.41393), Sunda Strait, Panaitan Island, 1983, don. PPPO. – 1 male pocl. 5.00 mm, 4 females pocl. 4.74, 4.81, 6.04, 6.30 mm (RMNH.CRUS.D.41394), Java, Seribu Archipelago, Tikus Island, Goba, 24.x.1974, coll. S. Sidabutan, don. PPPO. – 1 male pocl. 5.19 mm, 1 female pocl. 7.47 mm (RMNH.CRUS.D.41395), Java, Seribu Archipelago, Tikus Island, 26.v.1975, in *Tridacna* sp., don. PPPO. – 1 male pocl. 5.84 mm, 2 females pocl. 5.84, 5.91 mm (RMNH.CRUS.D.41396), Java, Seribu Archipelago, Tikus Island, 27.v.1975, in *Tridacna* sp., coll. V. Toro, don. PPPO. – 4 males pocl. 4.35, 4.74, 5.13, 5.39 mm, 3 females pocl. 2.47, 5.71 (with bopyrid under carapace), 7.34 mm (RMNH.CRUS.D.41397), Java, Seribu Archipelago, Tikus Island, 28.v.1975, in *Tridacna* sp., coll. V. Toro, don. PPPO. – 1 male pocl. 5.3 mm (RMNH.CRUS.D.58053), Java, Seribu Archipelago, Tikus Island, 25.i.1975, coll. S. Sidabutan, don. PPPO. – 2 females pocl. 4.48, 5.71 mm (RMNH.CRUS.D.41398), Ambon Island, Sowahtelu Archipelago, Morela, 03°32’00”S 128°12’30”E, 8.i.1973, coll. M.K. Moosa, don. PPPO, fcn. cm 324. – 1 ovigerous female pocl. 7.79 mm (RMNH.CRUS.D.41399), E coast of Ajer Island, 21.iv.1971, 11.00 hr., R.V. “Mutiara II”, don. PPPO. – 1 male pocl. 4.87 mm, 1 ovigerous female pocl. 7.14 mm (RMNH.CRUS.D.41400), Sulawesi, Ujung Pandang, Kayangan Island, 7.vi.1975, coll. M.K. Moosa, Banner & Banner, Rumphius II Expedition, don. PPPO, fcn. cm 260. – 1 male pocl. 2.9 mm, 1 ovigerous female pocl. 3.8 mm (RMNH.CRUS.D.46472), SW Sulawesi, Spermonde Archipelago, Pulau Badi, nnw side, 3.x.1994, depth 0.5 m, snorkeling, in *H. hippopus* (Ø 20 cm), coll. J.C. den Hartog. – 1 male pocl. 3.2 mm, 1 ovigerous female pocl. 4.4 mm (RMNH.CRUS.D.46473), SW

Sulawesi, Spermonde Archipelago, Barang Lompo, NW side, 23.ix.1994, depth 3–5 m, snorkeling, from *Tridacna squamosa* Lamarck, coll. J.C. den Hartog. – 1 male pocl. 2.1 mm, 1 ovigerous female pocl. 2.6 mm (RMNH.CRUS.D.46474), SW Sulawesi, Spermonde Archipelago, Gusung, harbour, NE side, 8.x.1994, depth 1–2 m, snorkeling, from *T. squamosa* (Ø 10 cm) in very turbid water, coll. J.C. den Hartog. – 1 ovigerous female pocl. 4.9 mm (RMNH.CRUS.D.46475), SW Sulawesi, Spermonde Archipelago, Kudingareng Keke, S side, 26.ix.1994, depth 3–5 m, snorkeling, in *T. squamosa* (Ø 15 cm), coll. J.C. den Hartog. – 1 male pocl. 3.4 mm, 1 ovigerous female pocl. 4.0 mm (RMNH.CRUS.D.46476), stn SUL.06, N Sulawesi, Selat Lembeh, Pulau Lembey, Pantai Parigi, 01°28'N 125°14'E, 15.x.1994, small fringing reef, gently sloping from beach to 10 m, depth 1-3 m, snorkeling, in *H. hippopus*, coll. J.C. den Hartog. – 1 male pocl. 3.3 mm (RMNH.CRUS.D.46477), stn SUL.08, N Sulawesi, Selat Lembeh, S of Tanjung Batuangus, channels between lava outflows, 01°30'N 125°15'E, 16.x.1994, fragile corals on slopes, down to muddy bottom, depth 1-3 m, snorkeling, in *T. squamosa* (Ø 25 cm), coll. I.J. Smit. – 1 ovigerous female pocl. 3.0 mm (RMNH.CRUS.D.46478), stn SUL.13, N Sulawesi, Selat Lembeh, Air Bajo near Kareko, 01°29'N 125°15'E, 21.x.1994, sandy bay between rocks, N-exposed, gently sloping bottom, sandy beach to 4 m, large boulders, depth 0-5 m, snorkeling, in *Tridacna maxima* (Röding), coll. I.J. Smit. – 1 male pocl. 3.0 mm, 1 non-ovigerous female pocl. 2.5 mm (RMNH.CRUS.D.46479), stn SUL.22, N Sulawesi, Selat Lembeh, Pulau Lembeh, near Batu Riri, 01°28'N 125°14'E, 31.x.1994, small enclosed bay, from sandy beach, seagrass belt, depth 2 m, scuba diving, in *H. hippopus* (Ø 20 cm), coll. C.H.J.M. Fransen. – 1 male pocl. 2.7 mm (RMNH.CRUS.D.46480), stn SUL.22, N Sulawesi, Selat Lembeh, Pulau Lembeh, near Batu Riri, 01°28'N 125°14'E, 31.x.1994, small enclosed bay, from sandy beach, seagrass belt, depth 2 m, scuba diving, in *H. hippopus* (Ø 15 cm), coll. C.H.J.M. Fransen. – 1 male pocl. 3.7 mm, 1 female pocl. 6.1 mm (RMNH.CRUS.D.47688), stn MAL.28, Moluccas, NW Seram, Kotania bay, SW of Pulau Marsegu, 03°01'S 128°03'E, 11.xi.1996, depth 20 m, scuba diving, in T. squamosa (Ø 40 cm) with *Xanthasia* sp. or *Tridacnatheres* sp., coll. C.H.J.M. Fransen. – 1 non ovigerous female pocl. 1.9 mm (RMNH.CRUS.D.53568; GenBank JX185706), stn TER.19, Halmahera, Tidore, SW of Tobala, 0°44'56.6"N 127°23'13.5"E, 4.xi.2009, depth 10 m, scuba diving, sloping rubble reef, in small *T.*? *squamosa*, coll. C.H.J.M. Fransen, fcn. CF-00075, photo TER.19.0101-108. – 1 female pocl. 6.49 mm (ZMA.CRUS.D.204368), Siboga Expedition stn 78, Borneo Bank, Lumu-lumu Shoal, 3°24'S 117°36'E, 10/11.vi.1899, shore exploration, in *Tridacna* sp., depth 34 m, bottom coral and coral sand (publ. Holthuis, 1952: 110). – 1 male pocl. 2.66 mm, 1 ovigerous female pocl. 3.90 mm (RMNH.CRUS.D.41487), West Papua, Kameri, Noemfoor, 2.iii.1955, from local people, in *Tridacna* sp., leg. L.B.

Holthuis no. 714. – 1 ovigerous female pocl. 6.4 mm (RMNH.CRUS.D.58030), stn SER.25, Java Sea, Kepulauan Seribu (Thousand Islands), off Jakarta, Kotok Besar Island, NW-side, 5°41'56"S 106°32'23"E, 16.ix.2005, depth 5 m, scuba diving and snorkelling, in *T. squamosa*, coll. C.H.J.M. Fransen. – 1 ovigerous female pocl. 3.8 mm (RMNH.CRUS.D.58032), stn SER.23, Java Sea, Kepulauan Seribu (Thousand Islands), off Jakarta, 15.ix.2005, depth 20 m, in *Hyotissa hyotis* (L.), coll. C.H.J.M. Fransen, film 9. – 1 ovigerous female, pocl. 3.6 mm (RMNH.CRUS.D.58054), stn BER.03, NE Kalimantan, Berau Islands, Derawan Island, S-side (jetty Derawan Dive Resort), 02°17'03.3"N 118°14'48.8"E, 13.x.2003, depth 0-2 m, snorkeling, in *H. hippopus*, coll. C.H.J.M. Fransen, film 10. **MALAYSIA**: few specimens (RMNH.CRUS.D.53798; GenBank JX185704); stn SEM.40, Semporna, Ribbon Reef, 04°36'10.0"N 118°45'53.6"E, 11.xii.2010, depth 5 m, scuba diving,? in *Tridacna* sp., collected by boatman. – 1 non ovigerous female pocl. 2.0 mm, 1 ovigerous female pocl. 3.2 mm (RMNH.CRUS.D.53806; GenBank JX185705), stn SEM.22, Semporna, Bumbun Island W (channel), 04°27'40.7"N 118°38'09.1"E, 5.xii.2010, depth 3 m, scuba diving, in *H. hippopus*, coll. C.H.J.M. Fransen. – 1 non ovigerous female pocl. 1.8 mm (RMNH.CRUS.D.53938; GenBank JX185707), stn SEM.14, Semporna, Ligitan Isl., Ligitan 2, 04°09'35.8"N 118°52'22.2"E, 3. xii.2010, depth 5 m, in *H. hippopus*, coll. B.T. Reijnen. – 1 male pocl. 2.73 mm, 1 female pocl. 4.61 mm, 1 ovigerous female pocl. 4.03 mm (RMNH. CRUS.D.41387), West Malaysia, Tioman Island, Telok Dalam, 22.i.1983, in *T. squamosa*, depth 10 feet, coll. P.K.L. Ng. – 1 male, pocl. 4.55 mm; 1 female, pocl. 3.64 mm (RMNH.CRUS.D.41388); West Malaysia, Tioman Island, Tekek Bay, 23.vi.1983, depth 20 feet, in *T. squamosa*, coll. P.K.L. Ng. **MALDIVES**: 2 ovigerous females pocl. 7.79, 8.70 mm (RMNH.CRUS.D.15162), Addu Atol, Gan Island, 26.xii.1957, coll. S. Gerlach, Xarifa Expedition. **RED SEA**: 1 ovigerous female pocl. 6.10 mm (RMNH.CRUS.D.41486), Landing Bay, Entedibir Island, 3.iv.1962, Israel Red Sea Expedition no. E62/3842. – 1 ovigerous female pocl. 7.27 mm (RMNH.CRUS.D.41488), Dahab, 10.x.1968, in *Tridacna* sp., coll. L. Fishelson, NS 4401. – 1 ovigerous female pocl. 6.04 mm (RMNH.CRUS.D.41489), Landing Bay, Entedibir Island, 6.iv.1962, Israel Red Sea Expedition no. E62/2391. – 1 ovigerous female pocl. 6.62 mm (RMNH. CRUS.D.41490), Ras-A-Tanrar, 2.vii.1969, coll. L. Fishelson, NS 5826. **SEYCHELLES**: 1 ovigerous female pocl. 5.3 mm (RMNH.CRUS.D.42793), niop-E, stn SEY.797, Platte Island atoll, 05°49'S 55°21'E, 7.i.1993, depth 12 m, lagoon of inner atoll, scuba diving, in *T.*? *maxima*, coll. C.H.J.M. Fransen. – 1 male pocl. 2.9 mm, 1 ovigerous female pocl. 4.0 mm (RMNH.CRUS.D.42790), niop-E stn SEY.723, Bird Island, off N coast 3°42'S 55°12'E, 21xiiI.1992, depth 7 m, coral reef, near drop-off, scuba diving, in *T. squamosa*, coll. C.H.J.M. Fransen. **PHILIPPINES**: 1 male pocl. 2.99 mm (RMNH.CRUS.D.41386),

Mactan Island near Cebu, Maribago, summer 1979, in *Tridacna* sp., coll. V. Storch. **CAROLINE ISLANDS**: 1 ovigerous female pocl. 3.38 mm (RMNH. CRUS.D.16511), Ifaluk, in front of Falarik, 26.ix.1953, depth 0.5–3 m, in *H. hippopus*, lagoon reef, coll. F.M. Bayer no. 402, don. usnm. **MARSHALL ISLANDS**: 1 ovigerous female pocl. 6.10 mm (RMNH.CRUS.D.16510), Eniwetok Atoll, N end of Island, 10.vii.1959, in *Tridacna* sp., coll. J.S. Garth, 59–3.

Diagnosis. Rostrum reaching just beyond basal segment of antennular peduncle, with 3–5 dorsal distal teeth, 0–2 ventral distal teeth. Antennal spine present. Ventral orbital angle slightly produced. Antero-lateral angle rounded. Anterior margin between ventral orbital angle and antero-lateral angle straight. Sixth abdominal segment with posterior ventral margin rounded. Telson with small dorsal pairs of spines at 0.63 and 0.79 of telson length, with lateral pair of terminal spines robust, placed just behind intermediate pair. Basal segment of antennular peduncle distally rounded, or with small distolateral tooth, not overreaching lamina. Scaphocerite with lateral margin straight, slit between distolateral tooth and lamina of moderate depth. Chela of first pereiopods spatulate, not cannulate, cutting edge laterally minutely pectinate in distal 2/3, medially entire. Dactylus of second chela with one large triangular tooth in proximal half, fixed finger with 5–10 small teeth in proximal half; palm with oval tympanal organ medio-ventrally in proximal part. Ambulatory dactyli biunguiculate; accessory tooth well developed, accessory tooth and flattened dorsal surface of unguis with fine scale-like spinules. Protopod of uropod acutely produced.

Remarks. The material corresponds closely to published descriptions. The distolateral tooth of the basal segment of the antennular peduncle is usually minute or absent. The position of the lateral pair of terminal spines of the telson just behind the intermediate pair is present in all investigated specimens.

The specimens investigated by Kubo (1940) appear not all belong to this species. The telson (fig. 5.20G) has the lateral terminal spines not in the characteristic position just behind the intermediate spines, but in the lateral position known of other *Anchistus* and *Polkamenes*. As Kubo (1940) had specimens from the bivalve *Pteria* sp., it is possible that his specimens belonged to *Polkamenes pycnodontae*.

The tympanal organ was first described and figured for this species by Monod (1976b: 24, fig. 33) as: “Le propode des chélipèdes porte d’un côté, et vers la base, une sorte d’aire déprimé ovalaire et cette fossette n’est pas un artefact

puisqu'elle existe sur les deux appendices. Je n'ai rien trouvé à ce sujet dans la littérature mai ce détail a certainement dû se voir déjà remarqué". This means that Monod had not encountered such a structure before and gave no suggestions for its function.

Colour (fig. 5.4F–H). Transparent with large blue or red-brown spots over body and appendages, ova green.

Hosts. Recorded from Cardiidae, Tridacninae: *Hippopus hippopus* (L., 1758) (Holthuis, 1953; Miyake & Fujino, 1968, Fankboner, 1972; Bruce, 1977b; Fransen, 1994a; Hayashi, 2002, 2006; Fransen & Reijnen, 2012); *Tridacna crocea* Lamarck, 1819 (Miyake & Fujino, 1968; Hayashi, 2002, 2006); *Tridacna gigas* (L., 1758) (Fankboner, 1972; Bruce, 1983b; Hayashi, 2006); *Tridacna elongata* Lamark, 1819 (= *Tridacna maxima* (Röding, 1798))(Ooishi, 1964; De Grave, 1999); *T. maxima* (Kubo, 1940; Bruce, 1976d, 1978a, 1993; Britayev & Fachrutdinov, 1994; Hayashi, 2002, 2006); *T.*? *maxima* (Fransen, 1994b); *Tridacna squamosa* Lamarck, 1819 (many records; Borradaile, 1917; Kubo, 1940; Miyake & Fujino, 1968; Bruce, 1976b, 1976c, 1976d, 1979a, 1993; Britayev & Fachrutdinov, 1994; Fransen, 1994b; Ng et al., 1995; Hayashi, 2002, 2006; Neo et al., 2015); *Tridacna*? *squamosa* (Fransen & Reijnen, 2012); *Tridacna* sp. (Holthuis, 1952; Johnson, 1961; Bruce, 1973, 1976e, 1978b, 1979a, 1993; Fujino, 1975; De Grave, 1999; Li, 2004; Fransen & Reijnen, 2012). Also recorded from Margaritidae: *Pinctada maxima* (Jameson, 1901) (Kubo, 1940; Miyake & Fujino, 1968; Hayashi, 2002, 2006); *Pinctada margaritifera* (L., 1758) (Seurat, 1934; Hayashi, 2006); *Pinctada fucata* (A. Gould, 1850) (Hayashi, 2006, as *Pinctada martensii* (Dunker, 1880)). From Pinnidae: *Pinna* sp. (Nobili, 1906b; Tattersall, 1921; Hayashi, 2002, 2006); *Streptopinna saccata* (L., 1758) (Hayashi, 2006). From Pteriidae: *Pteria penguin* (Röding, 1798) (Kubo, 1940, as *Pteria macroptera* (Lamarck, 1819); De Grave, 1999, as *Magnavicula penguin*/*Magnavicula bennetti* Iredale, 1939); *Pteria* sp. (Nobili, 1907 (with doubt); Hayashi, 2002, 2006).

Distribution. Widely distributed in the Indo-Pacific, from the Red Sea to Moçambique and Madagascar to the Gambier Archipelago in the east. Kenya, Tanganyika and Zanzibar, East Africa (Bruce, 1976c, 1976d); Red Sea (Nobili, 1899, 1906b; Tattersall, 1921; Vine, 1986); Persian Gulf (Nobili, 1906a); Comoro Islands (Bruce, 1978a); Seychelles (Borradaile, 1917; Bruce, 1976b); Egmont, Chagos Islands (Borradaile, 1917, see also Bruce, 1976b); Maldives (Borradaile, 1917; Bruce, 1976e, 1993); Madagascar (Bruce, 1978b); Perhentian Islands, Malaysia (Bruce, 1979a); Sabah, Malaysia (Fransen & Reijnen, 2012); Penang, Malaysia (Johnson, 1979); Lumulumu Shoal, Borneo Bank,

Indonesia (Holthuis, 1952); Anambas, Indonesia (Li, 2004); Ambon, Indonesia (Holthuis, 1993; Fransen, 1994a); Tidore, Indonesia (Fransen & Reijnen, 2012); Singapore (Johnson, 1961, 1979; Anker & De Grave, 2016; Neo et al., 2015); Tre Island, Vietnam (Bruce, 1993); Philippines (Chace & Bruce, 1993); Mergui Archipelago (De Man, 1888); British New Guinea (= Southern Papua New Guinea) (Borradaile, 1898); Queensland, Australia (Bruce, 1977b); Papua New Guinea (De Grave, 1999); Nouméa, New Caledonia (Monod, 1976b); Japan (Hayashi, 2002, 2006); Ryukyu Islands, Japan (Fujino, 1975); Marshall Islands (Holthuis, 1953); Palau (Kubo, 1940); French Polynesia (Nobili, 1907); Gambier Islands (Seurat, 1934); Funafuti Atoll (Whitelegge, 1897). Now for the first time recorded from the Caroline Islands, Micronesia; and from the following locations in Indonesia: Panaitan Island, Seribu, and the Thousand Islands, Sunda strait; NE and S Kalimantan; Numfor, West Papua; SW and N Sulawesi; and NW Seram, the Mollucas.

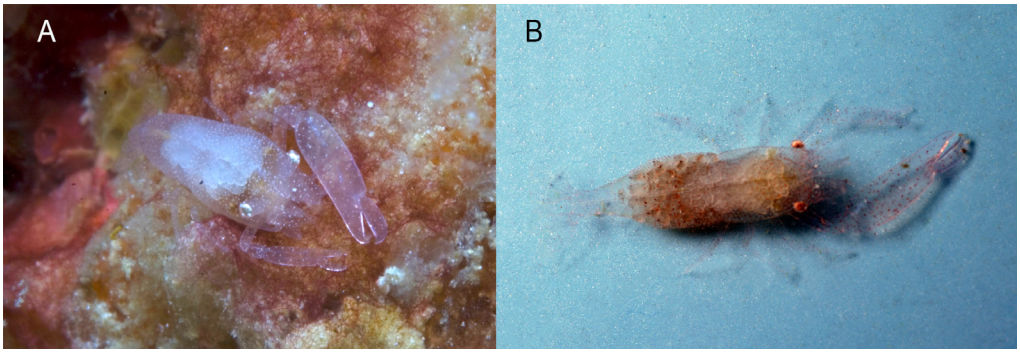
Genus *Dasella* Lebour, 1945

Dasia Lebour, 1938: 650. Type species, by monotypy: *Dasia herdmaniae* Lebour, 1938. Gender: feminine. Invalid junior homonym of *Dasia* Gray, 1839 (Reptilia), and *Dasia* van der Goot, 1918 [in B. Das, 1918] (Hemiptera).
Dasella Lebour, 1945: 279. Replacement name for *Dasia* Lebour, 1938. Type species therefore *Dasia herdmaniae* Lebour, 1938.

Diagnosis (emended from Bruce, 1995: 47). Small sized shrimps of subcylindrical body form. Rostrum well developed, compressed, dorsal margin without teeth, ventral margin with or without minute distal tooth, lateral carinae feebly developed. Carapace smooth, glabrous; inferior orbital angle produced, orbit feebly developed, antennal spine present, hepatic spine mobile, supraorbital and epigastric spines absent, anterolateral margin rounded, not produced. Abdomen smooth, glabrous, third segment not posterodorsally produced, pleura broadly rounded. Telson with two pairs of large dorsal spines, three pairs of posterior spines. Eye small, cornea globular. Antennula with basal segment of peduncle with distolateral tooth, flagella short, short free ramus reduced. Antenna with basicerite unarmed, scaphocerite well developed with small distolateral tooth. Epistome unarmed. Mandible normal, without palp, molar process robust, incisor process dentate; maxillula with feebly bilobed palp, laciniae moderately broad; maxilla with simple palp, basal endite broad, bilobed, coxal endite obsolete, scaphognathite broad; first maxilliped with slender palp, basal endite broad, fused with coxal endite, exopod well developed, caridean lobe broad, flagellum with numerous plumose setae distally, epipod rectangular or rounded, without podobranch; third maxilliped with endopod slender,

ischiomerus and basis fused, exopod with numerous plumose setae distally, coxa not medially produced, with large lateral plate, without arthrobranch. Fourth thoracic sternite without median process. First pereiopods moderately robust, chelae with fingers subspatulate, non-cannulate, cutting edges minutely pectinate. Second pereiopods well developed, similar, unequal, fingers simple, unarmed; palm without tympanal organ. Ambulatory pereiopods with dactyli strongly compressed, unguis distinct with scattered scale-like spinules, corpus with armed or unarmed distoventral process. Uropod with protopodite acutely produced, exopod of uropod with small distolateral tooth with larger mobile spine medially.

Three species are designated to this genus: *Dasella ansoni* Bruce, 1983, *Dasella brucei* Berggren, 1990, and *Dasella herdmaniae* (Lebour, 1938).



5.17 *Dasella herdmaniae* (Lebour, 1938) from *Herdmania momus* (Savigny, 1816). A) RMNH.CRUS.D.53924, stn SEM.52; B) RMNH.CRUS.D.53535, stn TER.10. Photo credit: Charles Fransen.

Key to the species of *Dasella*

1. - Propodal spines on pereiopod 3–5 long, sharp; minute ventral tooth on rostrum (sometimes absent); length of rostrum barely about as long as the antennular peduncle **2.**

- Propodal spines on pereiopod 3–5 long, club-shaped; no teeth on rostrum; length of rostrum much shorter than antennular peduncle *D. herdmaniae*

2. - Large blunt tooth on dactylus of pereiopod 3–4 with hooked process *D. ansoni*

- Large blunt tooth on dactylus of pereiopod 3–4 without hooked process *D. brucei*

Dasella ansoni Bruce, 1983

Dasella ansoni Bruce, 1983d: 22, figs. 1–5 [Type locality: Arafura Sea, 10°58'S 132°10'E, 27 m; see Bruce & Coombes, 1995]; Berggren, 1990: 558 (listed); Bruce, 1990: 10, 16, 18 (listed); Müller, 1993: 23 (listed); Holthuis, 1993: 140, fig. 123 (listed); Bruce & Coombes, 1995: 108 (listed); Li, 2000: 41, fig. 41 (listed); De Grave & Fransen, 2011: 348 (listed).

Diagnosis (based on Bruce, 1983d). Small-sized shrimp. Rostrum barely reaching distal border of distal segment of antennular peduncle, slightly depressed, acute, dorsally thickened, without distinct lateral carinae, without dorsal teeth, with one acute minute ventral tooth near tip. Antennal spine present, small. Hepatic spine well-developed, small, mobile, below level of than antennal spine. Inferior orbital angle distinct, produced. Antero-lateral angle of carapace broadly rounded, feebly produced. Anterior margin of carapace between ventral orbital angle and antero-lateral angle straight. Sixth abdominal segment with posterolateral angle acute, posteroventral angle feebly acute. Telson with large dorsal spines at 0.35 and 0.50 of telson length, lateral pair of terminal spines just behind intermediate pair, distal margin of telson rounded, without median process. Basal segment of antennular peduncle with one small distolateral tooth. Scaphocerite with lateral margin almost straight, slit between small distolateral tooth and lamina shallow. Chela of first pereiopods spatulate, with cutting edges minutely pectinate along entire rim. Second pereiopods unequal; almost straight dactylus barely exceeding fixed finger on both chelae, without teeth on cutting edges; palm without tympanal organ. Ambulatory dactyli with large broad distoventral process on flexor margin of corpus, accompanied by small hooked tooth on tip in third and fourth pereiopods; unguis simple, acute. Distoventral spines on ambulatory propodi long, acute.

Colour. Generally transparent; rostrum, antennular peduncles and chelae of second pereiopods feebly spotted with small white dots (Bruce, 1983d).

Hosts. Only known to be associated with one species of ascidian: *Phallusia depressiuscula* (Heller, 1878) (Ascidiidae) (Bruce, 1983d).

Distribution. Only known from its type locality: Arafura Sea (10°58'S 132°10'E), Australia (Bruce, 1983d; Bruce & Coombes, 1995).

***Dasella brucei* Berggren, 1990**

Dasella herdmaniae Bruce, 1981a: 50-56, figs. 1–2. (not *Dasella herdmaniae*

(Lebour, 1938)).
Dasella brucei Berggren, 1990: 558 [Type locality: Heron Island, Queensland, Australia, 15 m]; Bruce, 1990: 10, 16, 18 (listed); Müller, 1993: 23 (listed); Li, 2000: 42, fig. 42 (listed); Bruce, 2003a: 111, fig. 1; De Grave & Fransen, 2011: 348 (listed).

Diagnosis (based on Bruce, 1981a). Small-sized shrimp. Rostrum exceeding proximal segment of antennular peduncle, slightly depressed, acute, dorsally thickened, without distinct lateral carinae, without dorsal teeth, without or with one acute minute ventral tooth near tip. Antennal spine present, small. Hepatic spine well-developed, small, mobile, below level of antennal spine. Inferior orbital angle distinct, produced. Antero-lateral angle of carapace broadly rounded, feebly to strongly produced. Anterior margin of carapace between ventral orbital angle and antero-lateral angle somewhat straight. Sixth abdominal segment with posterolateral angle acute, posteroventral angle feebly acute. Telson with large dorsal spines at 0.40 and 0.70 of telson length, lateral pair of terminal spines just behind intermediate pair, distal margin of telson with median acute process. Basal segment of antennular peduncle with one small distolateral tooth. Scaphocerite with lateral margin almost straight, slit between small distolateral tooth and lamina shallow. Chela of first pereiopods spatulate, with cutting edges minutely pectinate along entire rim. Second pereiopods unequal, with dactylus barely exceeding fixed finger on both chelae, without teeth on cutting edges; palm without tympanal organ. Ambulatory dactyli biunguiculate, with large broad distoventral process on flexor margin of corpus; unguis simple, acute. Distoventral spines on ambulatory propodi long, acute.

Colour. Unknown.

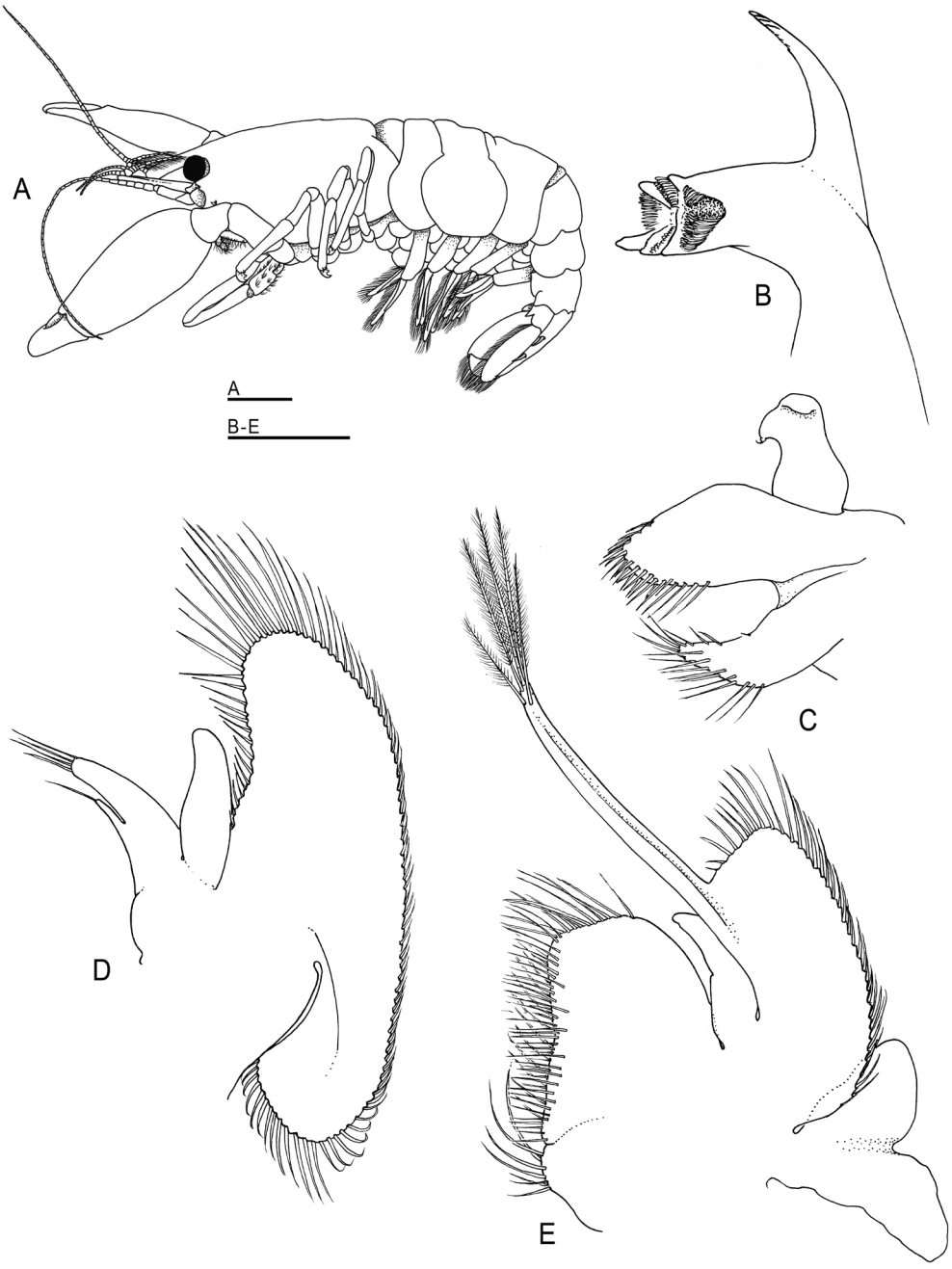
Hosts. Known from two species of ascidians: *Herdmania grandis* (Heller, 1878) (Bruce, 2003a) and *Herdmania momus* (Savigny, 1816) (Pyuridae) (Bruce, 1981a, 2003a).

Distribution. Three localities off Queensland, Australia: Heron Island, Murdock Point (Mooloolaba), and Moreton Bay (Berggren, 1990; Bruce, 2003a).

***Dasella herdmaniae* (Lebour, 1938)**

(figs. 5.17–5.19)

Dasia herdmaniae Lebour, 1938: 650, plate 1 [type locality: Tuticorin, Gulf of Manaar, Madras, India, associated with ascidian *Herdmania pallida* (Heller,

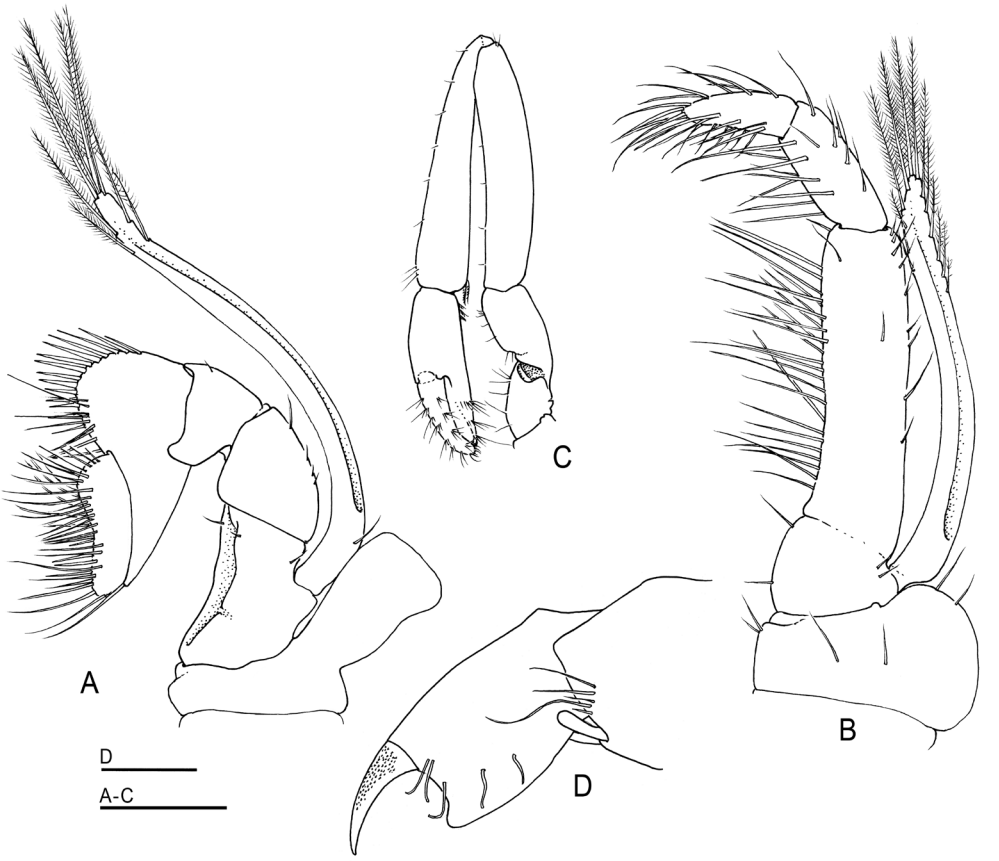


5.18 *Dasella herdmaniae* (Lebour, 1938). A) RMNH.CRUS.D.57964, ovigerous female, pocl. 3.8 mm; B–E) RMNH.CRUS.D.48249, 1 ovigerous female pocl. 3.4 mm. A) habitus, lateral view; B) left mandible, ventral view; C) left maxillula, ventral view; D) left maxilla, ventral view; E) left first maxilliped, ventral view. Scale A = 2 mm; B–E = 0.5 mm. Artwork: A) Werner de Gier and B–E) Charles Fransen.

1878) (= *Herdmania momus* (Savigny, 1816))].
Dasella herdmaniae – Lebour, 1945: 279; Holthuis, 1952: 17 (listed); Holthuis, 1955: 68, fig. 4lc, d (listed); Bruce, 1976a: 89; Bruce, 1981a: 50, figs. 1, 2; Bruce, 1981c: 5; Bruce, 1983c: 201 (listed); Berggren, 1990: 554, figs. 1–3; Chace & Bruce, 1993: 78–79; Müller, 1993: 23 (listed); Li, 2000: 42, fig. 43 (listed); Bruce, 2010a: 63 (listed); De Grave & Fransen, 2011: 348 (listed); Radhakrishnan et al., 2012: table 1 (listed); Horká et al., 2016: 6, figs. 2-4; Samuel et al., 2016: table 1 (listed); Chow et al., 2021: table A.1.

Material examined. INDONESIA: 1 male pocl. 2.6 mm, 1 ovigerous female pocl. 3.8 mm (RMNH.CRUS.D.49846; GenBank KU170689), stn BAL.14, Bali, NE-side Pulau Serangan, off lighthouse, 08°43'17"S 115°15'31"E, 5.iv.2001, 16 m depth, scuba diving, slowly declining reef slope, sandy base, in *Herdmania momus* (Savigny), translucent, coll. C.H.J.M. Fransen. – 1 male, pocl. 2.5 mm, 1 ovigerous female pocl. 2.5 mm, (RMNH.CRUS.D.58055), stn BAL.05, Sanur, Penjor Point, 08°41'36"S 115°16'20"E, 1.iv.2001, depth 15 m, scuba diving, slowly declining reef slope, sandy base, in *H. momus*, translucent with red chromatophores of body and appendages, coll. C.H.J.M. Fransen, photo C.H.J.M. Fransen & W. Renema. – 1 male pocl. 2.2 mm, 1 non-ovigerous female pocl. 2.2 mm, (RMNH.CRUS.D.58056), stn BAL.05, Sanur, Penjor Point, 08°41'36"S 115°16'20"E, 1.iv.2001, depth 15 m, scuba diving, slowly declining reef slope, sandy base, in *H. momus*, translucent with red chromatophores over body and appendages, coll. C.H.J.M. Fransen. – 1 male pocl. 2.7 mm, 1 non-ovigerous female pocl. 1.9, 2.8, 3.5, 3.6 (with bopyroid) mm, (RMNH.CRUS.D.58057), stn BAL.13, NE-side Pulau Serangan, Loloan Serangan, 08°43'39"S 115°15'13"E, 5.iv.2001, depth 15 m, scuba diving, slowly declining reef slope, sandy base, in *H. momus*, translucent with red chromatophores over body and appendages, coll. C.H.J.M. Fransen. – 1 specimen pocl. 1.1 mm (RMNH.CRUS.D.58058), stn BAL.12, E-side Nusa Dua, off Club Med Hotel, N of channel, 08°47'06"S 115°13'57"E, 4.iv.2001, depth 18 m, slowly declining reef slope, sandy base, in ascidian, coll. C.H.J.M. Fransen. – 1 ovigerous female pocl. 4.3 mm (RMNH.CRUS.D.48254), stn SUL.18, N Sulawesi, Selat Lembeh, near Tandarusa, Sarena Kecil West, 01°27'N 125°13'E, 24.x.1994, depth 2 m, snorkeling, sandy bank, from sandy shore to coral covered slope, in tunicate, coll. J.C. den Hartog. – 1 non ovigerous female pocl. 1.8 mm (RMNH.CRUS.D.48253), SW Sulawesi, Spermonde Archipelago, Barang Lompo, NW side, 23.ix.1994, depth 3–5 m, scuba diving, in *Polycarpa* sp. ascidian, coll. C.H.J.M. Fransen. – 1 ovigerous female pocl. 3.0 mm, 1 male pocl. 2.1 mm (RMNH.CRUS.D.48252), SW Sulawesi, Spermonde Archipelago, Kudingareng Keke, ssw side, 28.ix.1994, depth 3–5 m, scuba diving, in ascidians, coll. C.H.J.M. Fransen. – 1 ovigerous female

pocl. 4.6 mm, 1 male pocl. 3.1 mm, 1 non ovigerous female pocl. 3.5 mm, 1 juvenile pocl. 1.6 mm (RMNH.CRUS.D.48251), SW Sulawesi, Spermonde Archipelago, Pulau Badi, nnw side, 3.x.1994, depth 15 m, scuba diving, in large solitary milkey white transparent ascidians, coll. C. Massin. – 1 ovigerous female pocl. 4.1 mm (RMNH.CRUS.D.48250), stn SUL.08, N Sulawesi, Selat Lembeh, S of Tanjung Batuangus, channels between lava outflows, 01°30'N 125°15'E, 25.x.1994, depth 2–5 m, snorkeling, fragile corals on slopes, down to muddy bottom, coll. J.C. den Hartog. – 1 ovigerous female pocl. 3.4 mm (RMNH.CRUS.D.48249), stn RBE.38, Moluccas, Ambon, Hitu, E coast, N of Tengah tengah, 8.xii.1990, depth 10 m, scuba diving, in ascidian, coll. C.H.J.M. Fransen. – 1 ovigerous female pocl. 2.5 mm (RMNH.CRUS.D.48248), stn RBE.34, Moluccas, Ambon, Hitu, Ambon Bay, outer bay, Rumahtiga, 5.xii.1990, depth 3–5 m, scuba diving,? from ascidian, coll. C.H.J.M. Fransen, photo M.S.S. Lavaleye. – 1 male pocl. 2.9 mm (RMNH.CRUS.D.47589), stn MAL.24, Moluccas, Ambon, S coast, Seri bay, 03°45'S 128°09'E, 22.xi.1996, depth 15 m, scuba diving, in solitary ascidian, *H. momus*, coll. C.H.J.M. Fransen. – 1 male pocl. 2.6 mm, 1 ovigerous female pocl. 4.7 mm (RMNH.CRUS.D.47582), stn MAL.20, Moluccas, Ambon, Ambon bay, N coast W of Lilibooi, 03°45'S 128°01'E, 20.xi.1996, depth 30 m, scuba diving, from large solitary ascidian aff. *Phallusia* Savigny, coll. C.H.J.M. Fransen. – 1 male pocl. 2.8 mm, 1 female pocl. 4.2 mm (RMNH.CRUS.D.47580), stn MAL.24, Moluccas, Ambon, S coast, Seri bay, 03°45'S 128°09'E, 22.xi.1996, depth 15 m, scuba diving, in *H. momus*, coll. C.H.J.M. Fransen. – 1 non ovigerous female pocl. 2.0 mm, 1 male pocl. 2.5 mm (RMNH.CRUS.D.47579), stn MAL.32, Moluccas, Ambon, E coast, close to Cape Meriam, 03°38'S 128°21'E, 23.xi.1996, depth 10 m, scuba diving, in solitary ascidian aff. *Phallusia*, coll. C.H.J.M. Fransen. – 1 male pocl. 2.5 mm with pair of abdominal bopyroids, 1 non ovigerous female pocl. 1.8 mm (RMNH.CRUS.D.47577), stn MAL.24, Moluccas, Ambon, S coast, Seri bay, 03°45'S 128°09'E, 22.xi.1996, depth 15 m, scuba diving, in *H. momus*, leg. C.H.J.M. Fransen. – 1 ovigerous female pocl. 2.4 mm (RMNH.CRUS.D.53535; GenBank OQ603086 (COI)), stn TER.10, Halmahera, Maitara, Maitara NW, 0°44'32"N 127°21'50.9"E, 29.x.2009, depth 7 m, scuba diving, coral reef with sandy patches, corals and sea fans abundant, in *H.* cf. *momus* (Asc. 33), coll. C.H.J.M. Fransen, fcn. cf-00039, photo TER.10.0171-175. – 1 ovigerous female pocl. 3.0 mm, 1 male pocl. 2.6 mm (RMNH.CRUS.D.58059), stn TER.21, Tanjung Ratemu (S of river), Halmahera mainland, 0°54'24.7"N 127°29'17.7"E, 5. xi.2009, depth 8 m, in ascidian (Asc 91), coll. A. Gittenberger. – 3 males pocl. 1.6, 1.7 and 2.0 mm, 1 non ovigerous female pocl. 1.4 mm (RMNH. CRUS.D.57964; GenBank OQ603089 (COI) & OQ600397 (16S)), stn. lem.03, NE Sulawesi, Lembeh Strait, E Sarena Besar, 1°27'34.1634"N 125°14'1.896"E, 31.i.2012, depth 15 m, in *H. momus*, coll. C.H.J.M. Fransen. – 2 ovigerous



5.19 *Dasella herdmaniae* (Lebour, 1938). RMNH.CRUS.D.48249, 1 ovigerous female pocl. 3.4 mm. A) left second maxilliped, ventral view; B) left third maxilliped, ventral view; C) left first pereiopod, lateral view; D) right fifth pereiopod dactylus, medail view. Scale A, B = 0.5 mm; C = 1.25 mm; D = 0.1 mm. Artwork: Charles Fransen.

females pocl. 3.3 mm, 1 male pocl. 2.8 mm, with abdominal bopyroid), 1 non ovigerous female pocl. 2.8 mm (RMNH.CRUS.D.58060), stn lem.21, NE Sulawesi, Lembeh Strait, Tanjung Kungkungan, 1°27'58.392"E 125°14'2.2554"E, 10.ii.2012, depth 4 m, in *H. momus*, coll. C.H.J.M. Fransen. – 3 males pocl. 2.7 mm and 2.4 and 2.5 mm, 1 ovigerous females pocl. 4.1 mm (RMNH.CRUS.D.58061), stn lem.33, NE Sulawesi, Lembeh Strait, Tanjung Nanas I, 1°27'39.492"N 125°13'35.7954"E, 17.ii.2012, depth 15 m, each pair in *H. momus*, coll. C.H.J.M. Fransen. – few specimens (RMNH.CRUS.D.58062), stn SER.16, Java Sea, Kepulauan Seribu (Thousand Islands), off Jakarta, Sepa Island, NW-side, 5°34'32"S 106°34'48"E, 13.ix.2005, unknown depth, scuba diving, in ascidian, coll. C.H.J.M. Fransen. – 1 ovigerous female pocl. 4.4 mm (RMNH.CRUS.D.54134), stn RAJ.19, W Papua, Raja Ampat Islands, Maya's mimpi, 00°30.456'S 130°39.919'E, 25.xi.2007, depth 20 m, scuba diving, in *H. momus*, coll. J.D. Thomas. – 1 ovigerous female pocl. 4.0 mm (RMNH.

CRUS.D.58063), stn RAJ.26, W Papua, Raja Ampat Islands, S. Gam, E entrance Besir Bay, Bun Isl., 00°30.988'S 130°33.812'E, 27.xi.2007, depth 2–12 m, in ascidian? *Polycarpa* sp., coll. J.D. Thomas. **MALAYSIA**: 1 ovigerous female pocl. 3.3 mm (RMNH.CRUS.D.53860), stn SEM.37, Sabah, Semporna area, S Boheydulang Isl., outer reef, 04°35'00.3"N 118°46'39.1"E, 11.xii.2010, depth 7 m, scuba diving, in *H. momus*, coll. C.H.J.M. Fransen. – 1 male pocl. 2.3 mm (RMNH.CRUS.D.53870; GenBank OQ603087 (COI)), stn SEM.31, Sabah, Semporna area, N Kulapuan Isl., 04°32'09.6"N 118°50'18.6"E, 9. xii.2010, depth 20 m, scuba diving, in *H. momus*, coll. C.H.J.M. Fransen. – 1 non ovigerous female pocl. 4.4 mm, 1 ovigerous female pocl. 2.9 mm, 1 male pocl. 2.6 mm (RMNH.CRUS.D.53967; GenBank OQ603088 (COI)), stn SEM.57, Sabah, Semporna area, S Larapan Isl. 2, 04°32'51.1"N 118°36'31.3"E, 17. xii.2020, depth 12 m, scuba diving, in *H. momus*, coll. C.H.J.M. Fransen. – 1 non ovigerous female pocl. 2.4 mm (RMNH.CRUS.D.53924; GenBank KU064966), stn SEM.52, Sabah, Semporna area, Silumpat Isl., 04°45'58.7"N 118°23'25.6"E, 16.xii.2010, depth 25 m, scuba diving, in *H. momus*, coll. C.H.J.M. Fransen, photo 1263–1270. **MOZAMBIQUE**: 1 male pocl. 1.4 mm, 1 ovigerous female pocl. 2.3 mm (RMNH.CRUS.D.39850), Inhaca Island, 26°S 33°W, 19.i.1990, depth 5 m, scuba diving, associated with *Pyura stolonifera* (Heller), don. M. Berggren, Kristineberg Marine Biol. Stn., Sweden (published by Berggren, M., 1990. J. Crust. Biol 10(3): 554–558). **VANUATU**: 1 specimen (RMNH.CRUS.D.58064), stn FR06, NW Tutuba Island, 15°19'33"S 167°10'06"E, 13.ix.2006, depth unknown, scuba diving, hand picked, in *Herdmania* sp., fcn. CF35.

Diagnosis (based on Berggren, 1990). Small-sized shrimp. Rostrum falling short of the distal segment of the antennular peduncle, slightly depressed, acute, dorsally thickened, without distinct lateral carinae, without dorsal nor ventral teeth. Antennal spine present, small. Hepatic spine well-developed, small, mobile, far below level of antennal spine. Inferior orbital angle distinct, produced. Antero-lateral angle of carapace slightly rounded, slightly produced. Anterior margin of carapace between ventral orbital angle and antero-lateral angle straight. Sixth abdominal segment with posterolateral angle acute, posteroventral angle feebly acute. Telson with large dorsal spines at 0.35 and 0.50 of telson length, lateral pair of terminal spines just behind intermediate pair, distal margin of telson rounded, without median process. Basal segment of antennular peduncle with one small distolateral tooth. Scaphocerite with lateral margin almost straight, slit between small distolateral tooth and lamina shallow. Chela of first pereiopods spatulate, with cutting edges minutely pectinate along entire rim. Second pereiopods unequal, almost straight dactylus barely exceeding fixed finger on both chelae, without teeth on cutting edges, palm

without tympanal organ. Ambulatory dactyli biunguiculate, with large broad distoventral process on flexor margin of corpus; unguis simple, acute, minute microstructures present on dorsal margin. Distoventral spines on ambulatory propodi long, club-shaped.

Colour (fig. 5.17). Whitish/opaque with small red dots scattered over the carapace, abdomen, and chelae of the second pereiopods (Berggren, 1990).

Hosts. Known from various species of ascidians. Pyuridae: *Herdmania momus* (Savigny, 1816) (Horká et al., 2016); *Herdmania pallida* (Heller, 1878) (Lebour, 1938); *Pyura stolonifera* (Heller, 1878) (Berggren, 1990); unidentified ascidian (Bruce, 1981c). Now recorded for the first time from Styelidae: *Polycarpa* sp. and Ascidiidae: *Phallusia* sp.

Distribution. Known from various regions of the Indo-West Pacific and the Indian Ocean: Ponta Torres, Inhaca Island (approximately 26°S, 33°W), Mozambique (Berggren, 1990); Gulf of Mannar, India (Lebour, 1938); Heron Island, Australia (Bruce, 1981c); Indonesia (Chow et al., 2021); Bali, Indonesia (Horká et al., 2016); Sabah, Malaysia (Horká et al., 2016). Now recorded for the first time from Vanuatu (Tutuba Island), and the following new localities in Indonesia: N, SW, and NE Sulawesi; Ambon, the Mollucas; Halmahera; Seribu (Thousand Islands); and Raja Ampat, West Papua.

Genus *Ensiger* Borradaile, 1915

ZooBank accession link: urn:lsid:zoobank.org:act:012A13F1-C8A9-4C9F-B4E7-E8B5EE345560

Ensiger Borradaile, 1915: 207. Type species, being the first species subsequently placed in this subgenus, which was described without any nominal species: *Anchistia aurantiaca* Dana, 1852 (a junior subjective synonym of *Cancer custos* (Forskål, 1775); first placed in *Ensiger* by Borradaile, 1917. The genus is herein resurrected. Gender: masculine. Etymology: Borradaile (1915, 1917) did not provide information on the derivation of the name. From *Ensiger* (Latin) = sword-bearer; probably in reference of the sword-like rostrum of the type-species.

Both the type species, *Anchistus custoides* Bruce, 1977, and *Anchistus custos* (Forskål, 1775) are herein transferred to *Ensiger* Borradaile, 1915.

Diagnosis. Moderately sized shrimps of subcylindrical body form. Rostrum



5.20 A–C) *Ensiger custoides* (Bruce, 1977) in *Atrina vexillum* (Born, 1778). A) RMNH.CRUS.D.58065, stn RAJ.08; B) RMNH.CRUS.D.58066, stn SER.24; C) RMNH.CRUS.D.53610, stn TER.36; D) *Ensiger custos* (Forskål, 1775) from *Pinna bicolor* Gmelin, 1791, RMNH.CRUS.D.41446, stn RBE.01. Photo credit: Charles Fransen.

well developed, compressed, without teeth or with very shallow indistinct dorsal teeth, lateral carinae feebly developed. Carapace smooth, glabrous; orbit feebly developed, inferior orbital angle distinct, antennal spine present, supraorbital, hepatic and epigastric spines absent, anterolateral margin somewhat produced, rounded. Abdomen smooth, glabrous, third segment not posterodorsally produced, anterior pleura rounded, posterior blunt. Telson with two pairs of small dorsal spines, three pairs of posterior spines. Eye small, cornea globular. Antennula normal, with basal peduncular segment with distolateral tooth, upper flagellum biramous, short ramus reduced. Antenna with basicerite unarmed, scaphocerite well developed, with moderately developed distolateral tooth. Epistome unarmed. Mandible normal, without palp, molar process robust, incisor process dentate; maxillula with feebly bilobed palp, laciniae broad; maxilla with simple palp, basal endite broad, bilobed, coxal endite obsolete, scaphognathite broad; first maxilliped with slender palp, basal endite broad, fused with coxal endite, exopod well developed, caridean lobe broad, flagellum with numerous plumose setae distally, epipod subrectangular, without podobranch; third maxilliped, ischiomerus and basis fused broad, exopod with numerous plumose setae distally, coxa with oval lateral plate, with or without rudimentary arthrobranch. Fourth thoracic sternite without median

process. First pereiopods slender, chelae cannulate or sub-cannulate, with fingers subspatulate, cutting edges minutely pectinate. Second pereiopods well developed, similar, unequal, dactylus with large acute triangular tooth in proximal half, fixed finger with series of teeth in proximal part; propodus without tympanal organ. Ambulatory pereiopods with simple dactyli, without basal protuberance; unguis simple, without dorsal pad of fine scale-like spinules. Uropod with protopodite posterolaterally acute, exopod with small mobile distolateral spine.

Key to the species of *Ensiger*

1. - Chela first pereiopod sub-cannulate; rostrum with minute dorsal and distal shallow teeth; antennal spine well developed; dactylus of chela second pereiopod with one or two small teeth in proximal half *E. custoides*

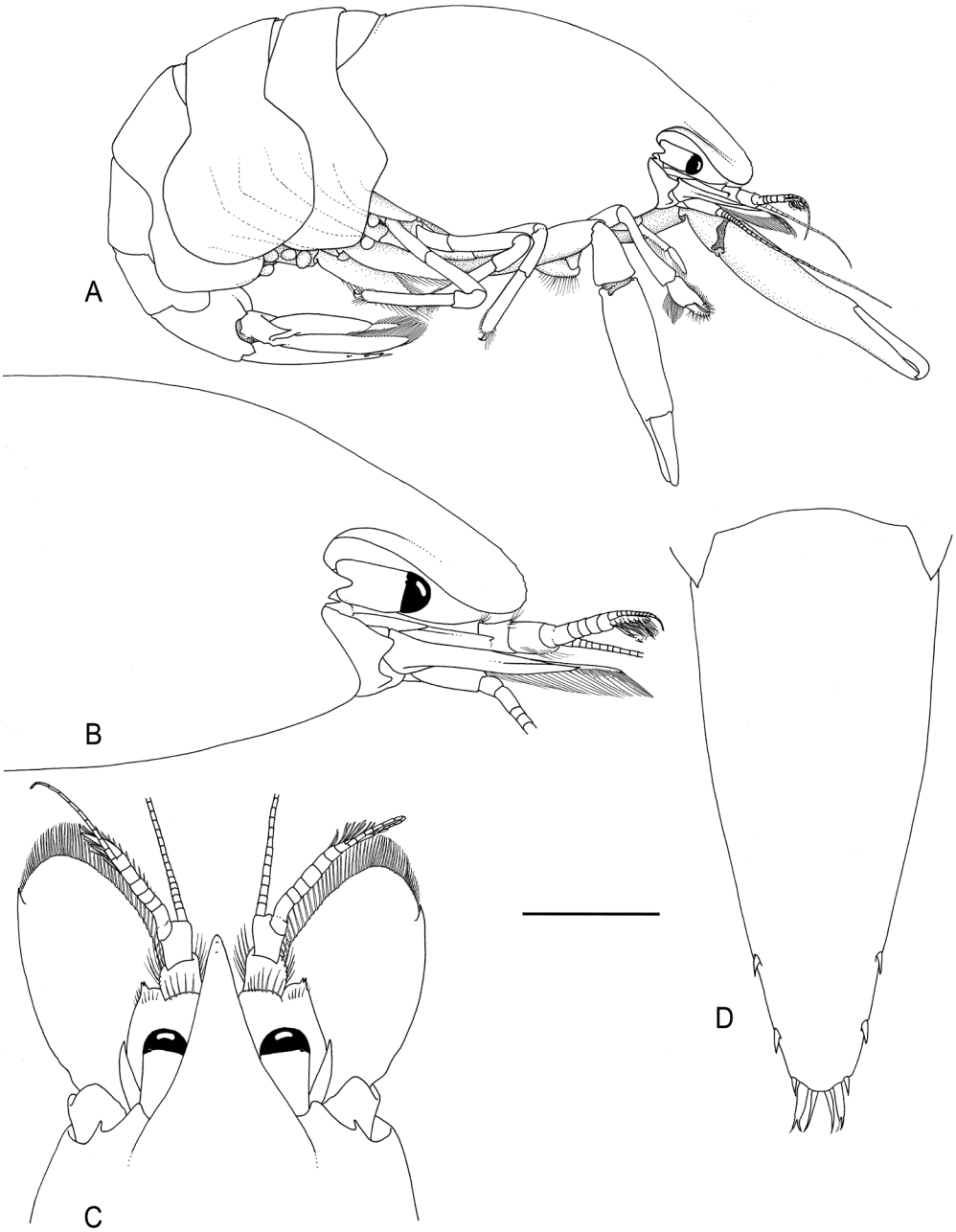
- Chela first pereiopod cannulate; rostrum devoid of dorsal and distal teeth; antennal spine minute; dactylus of chela second pereiopod with large triangular tooth in proximal half *E. custos*

***Ensiger custoides* (Bruce, 1977)**

(figs. 5.20A–C, 5.21–5.22)

Anchistus inermis – Kubo, 1940: 48–51, figs. 15–17.
Anchistus custos – Holthuis, 1952: 106 (pro parte); Miyake & Fujino, 1968: 415–417.
Anchistus custoides Bruce, 1977a: 47, figs. 4–6 [Type locality: north-west end, Gillett Cay, Great Barrier Reef, 21°43'S 152°25'E, in bivalve mollusk *Atrina vexillum* according to Springthorpe & Lowry, 1994]; Bruce, 1981c: 2-3; Bruce, 1983b: 892; Bruce, 1983c: 199 (listed); Bruce, 1990: 15, 18 (listed); Müller, 1993: 7 (listed); Bruce, 1993: 56; Chace & Bruce, 1993: 72 (listed); Fransen, 1994a: 108, 110, 111 (listed); Britayev & Fachrutdinov, 1994: 124, fig. 2D-F; De Grave, 1999: 128, fig. 2; Li, 2000: 7, fig.7 (listed); Hayashi, 2002: 222, figs. 432c–e, 434c, d, 435c, d; Bruce, 2003b: 210 (listed); Hayashi, 2006: 115; Marin & Savinkin, 2007: table 2, 3 (listed); Bruce, 2010a: 63 (listed); De Grave & Fransen, 2011: 340 (listed); Fransen & Reijnen, 2012; Gan et al., 2015: 3, fig. 1; Anker & De Grave: 2016: 406, fig. 72; Horká et al., 2016: 6, figs. 1m, 2–4; Chow et al., 2021: table A.1 (listed); Idris et al., 2022: 136–148, figs. 4–5.

Material examined. INDONESIA: 1 ovigerous female pocl. 9.09 mm (RMNH. CRUS.D.41440), Snellius II Expedition sta. 4.079, E of Komodo, Selat Linta,



5.21 *Ensiger custoides* (Bruce, 1977), RMNH.CRUS.D.41440, ovigerous female pocl. 9.1 mm. A) habitus, lateral view; B) anterior appendages, lateral view; C) idem, dorsal view; D) telson, dorsal view. Scale A= 4 mm; B, C, = 2 mm; D = 1 mm. Artwork: Charles Fransen.

8°35'S 119°34.2'E, 18.ix.1984, snorkeling, coastal reef flat, in large *Pinna* sp. (also collected). – 1 male pocl. 5.79 mm (RMNH.CRUS.D.41441), Java, off Jakarta, Seribu Archipelago, Kongsi Island, 26.vi.1973, sandy bottom, coll. Sapri

D, don. PPPO. – 1 ovigerous female pocl. 6.17 mm (RMNH.CRUS.D.41442), Java, off Jakarta, Seribu Archipelago, Pari Island, 27.i.1973, coll. Sukardi, don. PPPO. – 1 ovigerous female pocl. 8.47 mm (RMNH.CRUS.D.41443), Seram Island, Piru Archipelago, Piru Bay, 03°04'45"S 128°11'27"E, 10.vi.1975, coll. A.H. Banner & D.M. Banner, Rumphius II Expedition, don. PPPO, fcn. cm 334. – 1 ovigerous female pocl. 6.49 mm (RMNH.CRUS.D.41444), Ambon Island, Ambon Bay, Tantoci, 3.vi.1975, coll. M.K. Moosa, A.H. Banner & D.M. Banner, Rumphius II Expedition, don. PPPO, fcn. cm 195. – 1 ovigerous female pocl. 9.22 mm (RMNH.CRUS.D.8996), Kera Island near Timor, 11-13.xi.1929, Snellius I Expedition 1929-1930, published in Holthuis, 1952: 106 as *Anchistus custos*. – 1 ovigerous female pocl. 6.2 mm (RMNH.CRUS.D.46640), stn SUL.10, N Sulawesi, Selat Lembeh, Pulau Lembeh, W of Kelapadua, 01°26'N 125°13'E, 17.x.1994, large *Acropora* field, scuba diving to 20 m, in *Atrina vexillum* (Born), coll. J. Goud. – 1 ovigerous female pocl. 7.4 mm, 1 male pocl. 3.4 mm (RMNH. CRUS.D.46641), SW Sulawesi, Spermonde Archipelago, Pulau Badi, nnw side, depth 30 m, scuba diving, 3.x.1994, in *Atrina vexillum* (Born) (Ø 35 cm), coll. C.H.J.M. Fransen. – 1 ovigerous female pocl. 7.5 mm (RMNH.CRUS.D.46643), SW Sulawesi, Spermonde Archipelago, Barang Lompo, NW side, 23.ix.1994, depth 20 m, scuba diving, in *A. vexillum* (Ø 25 cm), coll. B.W. Hoeksema. – 1 ovigerous famel pocl. 5.6 mm, 1 male pocl. 2.6 mm (RMNH.CRUS.D.53610; GenBank OQ603090 (COI)), stn TER.36, off Halmahera mainland, Teluk Dodinga E, N of Pulau Jere, 0°50'47.8"N 127°37'48.7", 13.xi.2009, depth 2 m, scuba diving, muddy bay shallow area with coral cover and rubble, in *A. vexillum*, coll. A. Gittenberger, fcn. CF-00123, photo TER.36.0086-91. – 2 ovigerous females pocl. 5.7 and 7.0 mm, 1 male pocl. 3.9 mm (RMNH.CRUS.D.53615), stn TER.38, off Halmahera mainland, Teluk Dodinga, Karang Galiasa Besar E, 0°50'45.6"N 127°35'7.4"E, 14.xi.2009, depth 2 m, scuba diving, exposed shallow reefflat followed by a sloping reef with coral and rubble, in *A. vexillum*, coll. C.H.J.M. Fransen, fcn. cf-00128, photo TER.38.0048-57. – 1 ovigerous female pocl. 9.0 mm, 1 male pocl. 3.5 mm (RMNH.CRUS.D.49844; GenBank OQ600398 (16S)), stn BAL.32, Bali, N side of Nusa Penida, off Tukad Adegan, 08°40'32"S 115°31'18"E, 20.iv.2001, scuba diving to 30 m depth, deep reef slope with patches of sand, in large *Atrina* sp., coll. Angus, film 32. – 1 male pocl. 4.6 mm, 2 ovigerous females pocl. 5.9 and 8.2 mm (RMNH.CRUS.D.58029), stn lem.29, NE Sulawesi, Lembeh Strait, Teluk Walemetodo, 1°24'11.3394"N 125°10'20.3154"E, 15.ii.2012, depth 5 m, in each pair in an *A. vexillum*, coll. C.H.J.M. Fransen. – 1 ovigerous female pocl.8.4 mm, 1 male pocl. 4.5 mm (RMNH.CRUS.D.58065), stn RAJ.08, W Papua, Raja Ampat Islands, Mioskon Island, 00°29.808'S 130°43.623'E, 21.xi.2007, depth 3 m, scuba diving, in *A. vexillum*, coll. B.W. Hoeksema, fcn. CF025. – 1 ovigerous female pocl. 5.5 mm (RMNH.CRUS.D.58066), stn SER.24, Java Sea, Kepulauan Seribu (Thousand

Islands), off Jakarta, Kotok Kecil Island, NW-side, 5°41'20"S 106°32'01"E, 16.ix.2005, depth 15 m, scuba diving, in *A. vexillum*, coll. C.H.J.M. Fransen, photo film 11. – 1 ovigerous female pocl. 8.5 mm (RMNH.CRUS.D.58067), stn SER.30, Java Sea, Kepulauan Seribu (Thousand Islands), off Jakarta, Tikus Island, S-side, 5°51'55"S 106°34'55"E, 18.ix.2005, depth 20 m, scuba diving, in *A. vexillum*, coll. C.H.J.M. Fransen, photo film 16. – 1 male pocl. 4.0 mm (RMNH.CRUS.D.58068), stn SER.30, Java Sea, Kepulauan Seribu (Thousand Islands), off Jakarta, Tikus Island, S-side, 5°51'55"S 106°34'55"E, 18.ix.2005, depth 20 m, scuba diving, in *A. vexillum*, coll. C.H.J.M. Fransen, photo film 16. – 1 male pocl. 4.6 mm, 1 ovigerous female pocl. 7.0 mm (RMNH.CRUS.D.58069), stn BER.06, NE Kalimantan, Berau Islands, shoal between Lighthouse-2 reef and Derawan Island, 02°12'08.6"N 118°11'34.9"E, 5.x.2003, depth 10 m, scuba diving, in *A. vexillum*, coll. C.H.J.M. Fransen. **MALAYSIA**: 1 male pocl. 3.3 mm (RMNH.CRUS.D.53810; GenBank JX185712), stn SEM.24, Sabah, Semporna area, Bumbun Isl., 04°26'54.1"N 118°46'31.0"E, 7.xii.2010, depth 3 m, scuba diving, in *A. vexillum*, coll. B.T. Reijnen. – 1 male pocl. 3.6 mm (RMNH.CRUS.D.53795; GenBank JX185710), sta. SEM.22, Sabah, Semporna area, Mata Pahi Isl., 04°27'40.7"N 118°38'09.1"E, 5.xii.2010, depth 3 m, scuba diving, in *A. vexillum*, coll. C.H.J.M. Fransen. – 1 non ovigerous female pocl. 3.4 mm (RMNH.CRUS.D.53807; GenBank OQ603091 (COI)), stn SEM.56, Sabah, Semporna area, Silawa Isl., 04°34'29.8"N 118°33'59.6"E, 17.xii.2010, depth 4 m, scuba diving, in *A. vexillum*, coll. C.H.J.M. Fransen. **PHILIPPINES**: 1 male pocl. 3.2 mm, 1 ovigerous female pocl. 5.9 mm (RMNH.CRUS.D.48353), Stn CEB.12, Cebu Strait, W of Bohol, NE side of Sandingan Island, 9°51.53'N 123°48.81'E, 15.xi.1999, depth 3–5 m, scuba diving, sand with coral patches, in *Atrina pectinata* (L.), coll. C.H.J.M. Fransen. – 1 male pocl. 3.9 mm, 1 ovigerous female pocl. 5.3 mm (RMNH.CRUS.D.48352), Stn CEB.12, Cebu Strait, W of Bohol, NE side of Sandingan Island, 9°51.53'N 123°48.81'E, 15.xi.1999, depth 3–5 m, scuba diving, sand with coral patches, in *A. pectinata*, coll. C.H.J.M. Fransen. **VANUATU**: 2 males pocl. 4.7 and 4.9 mm, 3 females pocl. 7.8, 8.5 and 9.4 mm (RMNH.CRUS.D.58070), stn DR11, Aoré Island, off port Lautour, 15°21'58"S 167°06'02"E, 12.ix.2006, scuba diving, hand picked, 2 pairs and a single female in 3 *A. vexillum* – 1 ovigerous female pocl. 7.5 mm, 1 male pocl. 4.5 mm (RMNH.CRUS.D.58071), stn db13, no further data, 13.ix.2006, depth unknown, scuba diving, in *Atrina* sp., coll. C.H.J.M. Fransen, fcn. CF29, photo T.-Y. Chan. – 1 ovigerous female pocl. 7.6 mm (RMNH.CRUS.D.58072), stn FR12, S Aoré Island, W Ratua Island, 15°22'07"S 167°06'17"E, 15.ix.2006, depth 0–3 m, snorkeling, in *A. vexillum*, coll. C.H.J.M. Fransen, fcn. CF51. – 2 ovigerous females pocl. 6.5 and 7.7 mm (RMNH.CRUS.D.58073), stn H1, Vanuatu maritime College, 22.ix.2006, hand picked, intertidal, mangrove environment, in 2 separate bivalves. – 1 ovigerous female pocl. 9.0 mm, 1 male

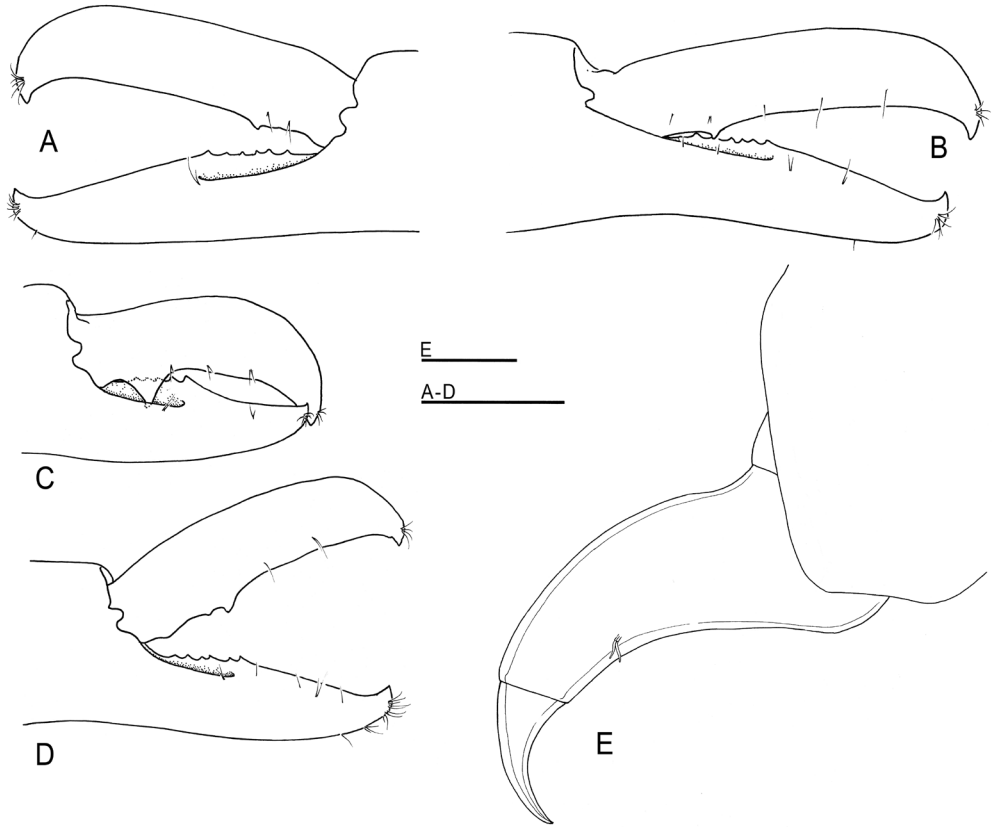
pocl. 4.7 mm (RMNH.CRUS.D.58074), stn DR68, no further data, 26.ix.2006.

Diagnosis. Rostrum broad proximally, compressed distally, almost triangular in dorsal view, strongly bent downwards (especially in females), reaching distal segment of antennular peduncle, with 4–8 obtuse dorsal distal teeth, ventral margin unarmed, distally rounded, lateral carina feebly developed. Inferior orbital angle produced. Antennal spine present. Antero-lateral angle of the carapace broadly rounded, produced. Anterior margin of carapace between ventral orbital angle and antero-lateral angle sinuate. Sixth abdominal segment with posterior ventral angle acute. Telson with dorsal spines at 0.75 and 0.88 of length. Basal segment of antennular peduncle with one or two distolateral teeth, lateral margin slightly convex. Scaphocerite with convex lateral margin; shallow slit between distolateral spine and lamina. Third maxilliped with robust penultimate segment and broad antepenultimate segment. Basis and ischium of first pereiopod with row of long slender setae along their ventral margins; chela sub-cannulate, slightly sub-spatulate, palm with row of long plumose setae on opposite sides; cutting edges minutely pectinate in distal half. Second pereiopods relatively small with slender fingers; dactylus with one or two small teeth in proximal half; fixed finger with 4–7 small teeth in proximal half; palm without tympanal organ. Ambulatory pereiopods with dactylus simple, not biunguiculate; reflected margin of corpus with transverse ridges; unguis sparsely provided with small triangular flattened scale-like spinules.

Remarks. The female specimen collected at sta. 4.079 of the Snellius II expedition (RMNH.CRUS.D.41440) shows two closely set distolateral teeth on the basal segment of the antennular peduncle (fig. 5.21C). The same specimen has bifurcated tips on the intermediate pair of terminal spines of the telson (fig. 5.21D) The specimen collected at Kongsi Island (RMNH.CRUS.D.41441) has two teeth on the left and one tooth on the right basal segment of the antennal peduncle, while the other specimens have one distolateral tooth on both sides as described for the type material (Bruce, 1977a: 47). The second pereiopods are usually small in relation to the carapace length and subequal.

Re-examination of Snellius I material described by Holthuis, 1952 yielded one ovigerous female (RMNH.CRUS.D.8996) identified as *A. custos* which belong to *A. custoides*.

Colour (fig. 5.20A–C). Specimens are translucent with numerous small red and white chromatophores all over the body, giving it an orange appearance. The cornea is strongly reflecting, colouring silver.



5.22 *Ensiger custoides* (Bruce, 1977). A, B, E) RMNH.CRUS.D.41440, ovigerous female pocl. 9.1 mm. C, D) RMNH.CRUS.D.41444, ovigerous female pocl. 6.5 mm. A) right major second pereiopod chela; B) left minor second pereiopod chela; C) left major second pereiopod, medial view; D) right minor second pereiopod, medial view; E) right third pereiopod dactylus, medial view, propodal setae omitted. Scale A, B, D = 1 mm; C = 2 mm; E = 0.1 mm. Artwork: Charles Fransen.

Hosts. Recorded from Pinnidae: *Atrina vexillum* (Born, 1778) (Miyake & Fujino, 1968; Bruce, 1977a, 1981c; De Grave, 1999; Hayashi, 2002, 2006; Fransen & Reijnen, 2012; Idris et al., 2022); *Pinna atropurpurea* G.B. Sowerby I, 1825 (Anker & De Grave, 2016); *Pinna bicolor* Gmelin, 1791 (Britayev & Fachrutdinov, 1994; De Grave, 1999; Hayashi, 2006; Idris et al., 2022); *Pinna deltodes* Menke, 1843 (Idris et al., 2022); *Pinna muricata* L., 1758 (Idris et al., 2022); *Pinna* sp. (Bruce, 1993). Also from Pteriidae: *Pteria* (*Avicula*) sp. (Bruce, 1983c). Now for the first time recorded from *Atrina pectinata* (L., 1767).

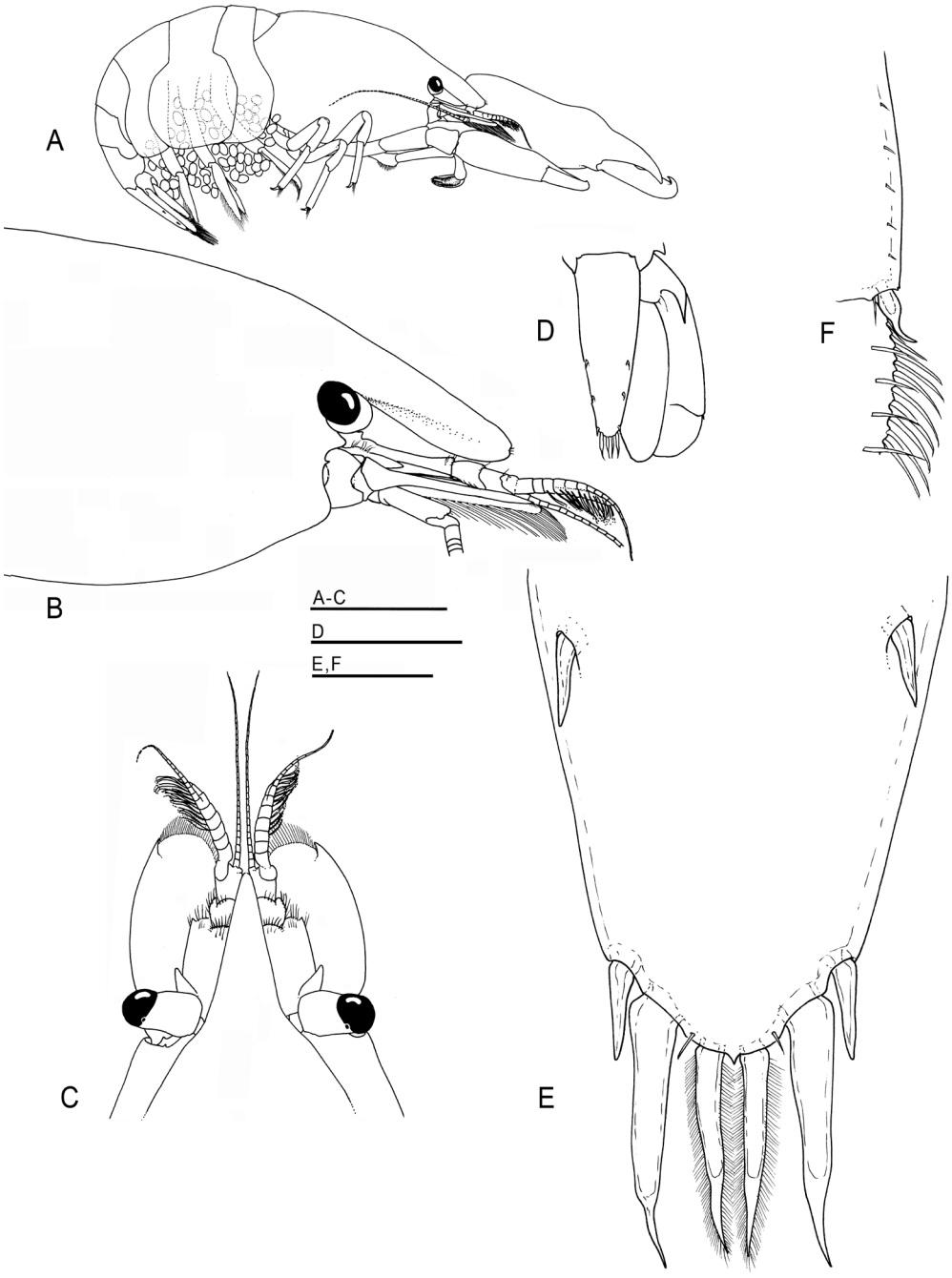
Distribution. Gillett Cay, Swains Reefs, One Tree Island, Capricorn Group, Australia (Bruce, 1977a); Heron Island, Australia (Bruce, 1981c); Caroline Islands, Palau (Kubo, 1940; Miyake & Fujino, 1968); Seram Island, Indonesia (Bruce, 1983c); Sabah, Malaysia (Fransen & Reijnen, 2012); Johor, W Malaysia

(Idris et al., 2022); Singapore (Anker & De Grave, 2016); Tre Island, Vietnam (Bruce, 1993); Hainan, China (Gan et al., 2015); Okinawa, Japan (Horká et al., 2016); Japan (Hayashi, 2002, 2006). Now recorded for the first time from various localities in Indonesia: Ambon Island, Kera Island near Timor, Selat Linta E of Komodo, the Seribu Archipelago off North Java, Bali, NE and SW Sulawesi, Teluk Dodinga (off mainland Halmahera), W Papua (Raja Ampat), and NE Kalimantan (Berau Islands). Additionally, now also newly recorded from Vanuatu and the Philippines (Cebu Strait).

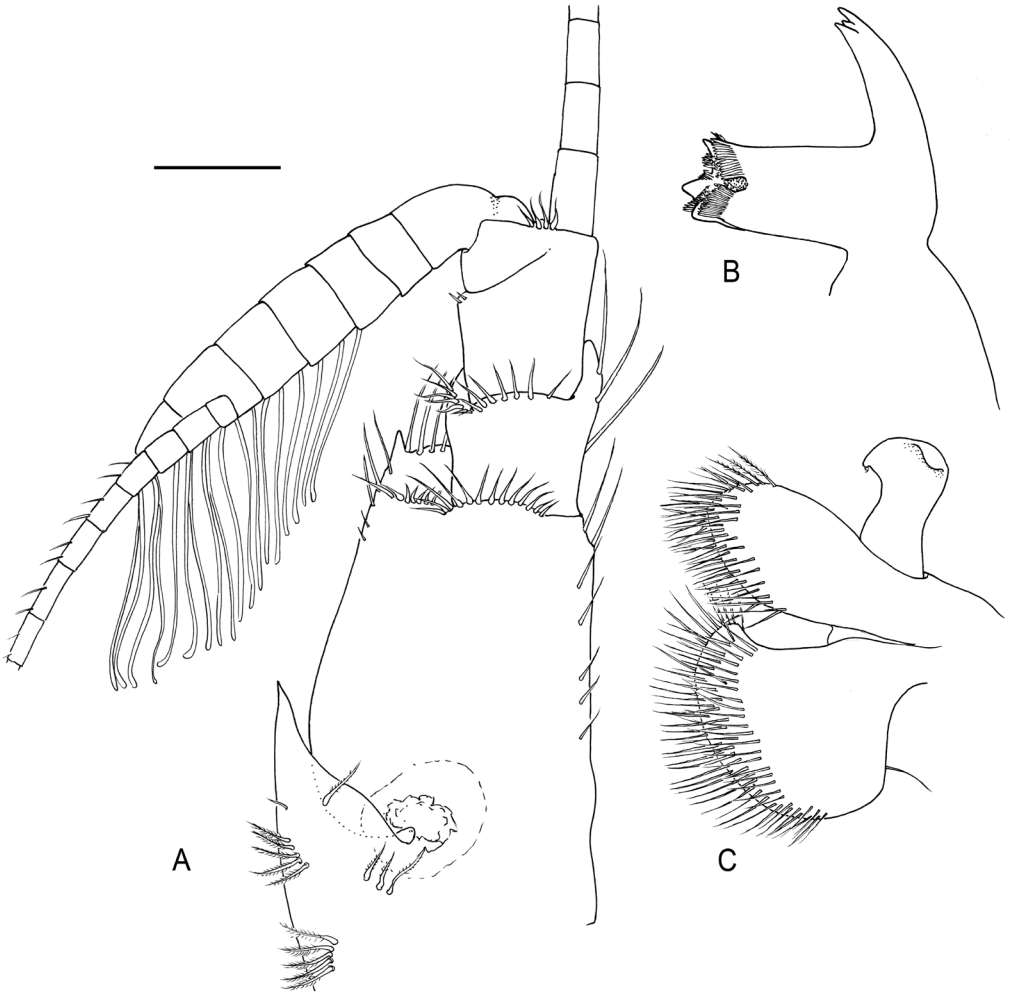
***Ensiger custos* (Forskål, 1775)**

(figs. 5.20D, 5.23–5.27)

Restricted synonymy:
Cancer custos Forskål, 1775: 94 [Type locality: Lohajæ, intra *Pinna nigras*].
Pontonia inflata H. Milne Edwards, 1840 [in H. Milne Edwards, 1834-1840]: 633 [Type locality: Ceylan et sur les côtes de Vanicoso].
Anchistia aurantiaca Dana, 1852a: 25 [Type locality: archipelago Viti].
Harpilius inermis Miers, 1884: 291, pl. 32 fig. B [Type locality: Port Molle, in the interior of a shell of a species of *Pinna*]; Müller, 1887: 471.
Pontonia pinnae Ortmann, 1894: 16, pl. 1 fig. 3; nec Lockington, 1878a [Type locality: Dar-es-Salaam, Chokirbank, in den Schalen einer *Pinna*-Art].
Anchistus inermis – Borradaile, 1898: 387 (listed); Lanchester, 1902: 565; Pearson, 1905: 77; Rathbun, 1914: 656; Borradaile, 1917: 388 (listed); Tattersall, 1921 (listed); Hale, 1927: 57, fig. 52.
Pontonia custos – Borradaile, 1898: 388 (listed).
Periclimenes aurantiacus – Borradaile, 1898: 382 (listed).
Marygrande mirabilis Pesta, 1911: 573, figs. 1–5 (partim) [Type locality: Samoa]; Pesta, 1913: 88, figs. 31, 32; De Grave, 2001: 129–134, figs. 1–3.
Periclimenes (*Ensiger*) *aurantiacus* – Borradaile, 1917: 376 (listed).
Pontonia spinax Dawydoff, 1952: 136. [nomen nudum]
Anchistus custos – Holthuis, 1955: 62, fig. 34 (listed); Holthuis, 1952: 105–109 (full synonymy), figs. 43, 44 (specimen from Kera Island excluded (= *Ensiger custoides* (Bruce, 1977)); Johnson, 1961: 59; Johnson & Liang, 1966: 433–455, figs. 1–10; Jacquotte, 1963: 59–62; Hipeau-Jacquotte, 1967: 153–166; McNeill, 1968; Hipeau-Jacquotte, 1971: 194; Bruce, 1972a: 219 (listed); Hipeau-Jacquotte, 1972a: 287–294, figs. 1, 2a1; Hipeau-Jacquotte, 1973a: 96–116, figs. 1a1–f1, 2, 3a1–b1, 4a1–d1,5, 6a, 7a1–f1, 8a1–d1; Hipeau-Jacquotte, 1973b: 63-70; Hipeau-Jacquotte, 1974a: 367-382; Hipeau-Jacquotte, 1974b: 383-402; Hipeau-Jacquotte, 1974c: 403–408; Hipeau-Jacquotte, 1974d: 359–386; Thomassin, 1974: 306; Bruce, 1975a: 150, 160–



5.23 *Ensiger custos* (Forskål, 1775). A–C) RMNH.CRUS.D.41446, ovigerous female pocl. 6.5 mm; D–F) RMNH.CRUS.D.41445, male pocl. 4.0 mm. A) habitus, ectina view; B) anterior appendages, lateral view; C) idem, dorsal view; D) tailfan, dorsal view; E) telson, tip, dorsal view; F) disolateral part uropodal exopod, ectin view. Scale A= 4 mm; B, C, = 2 mm; D = 2 mm; E, F = 0.25 mm. Artwork: Charles Fransen.



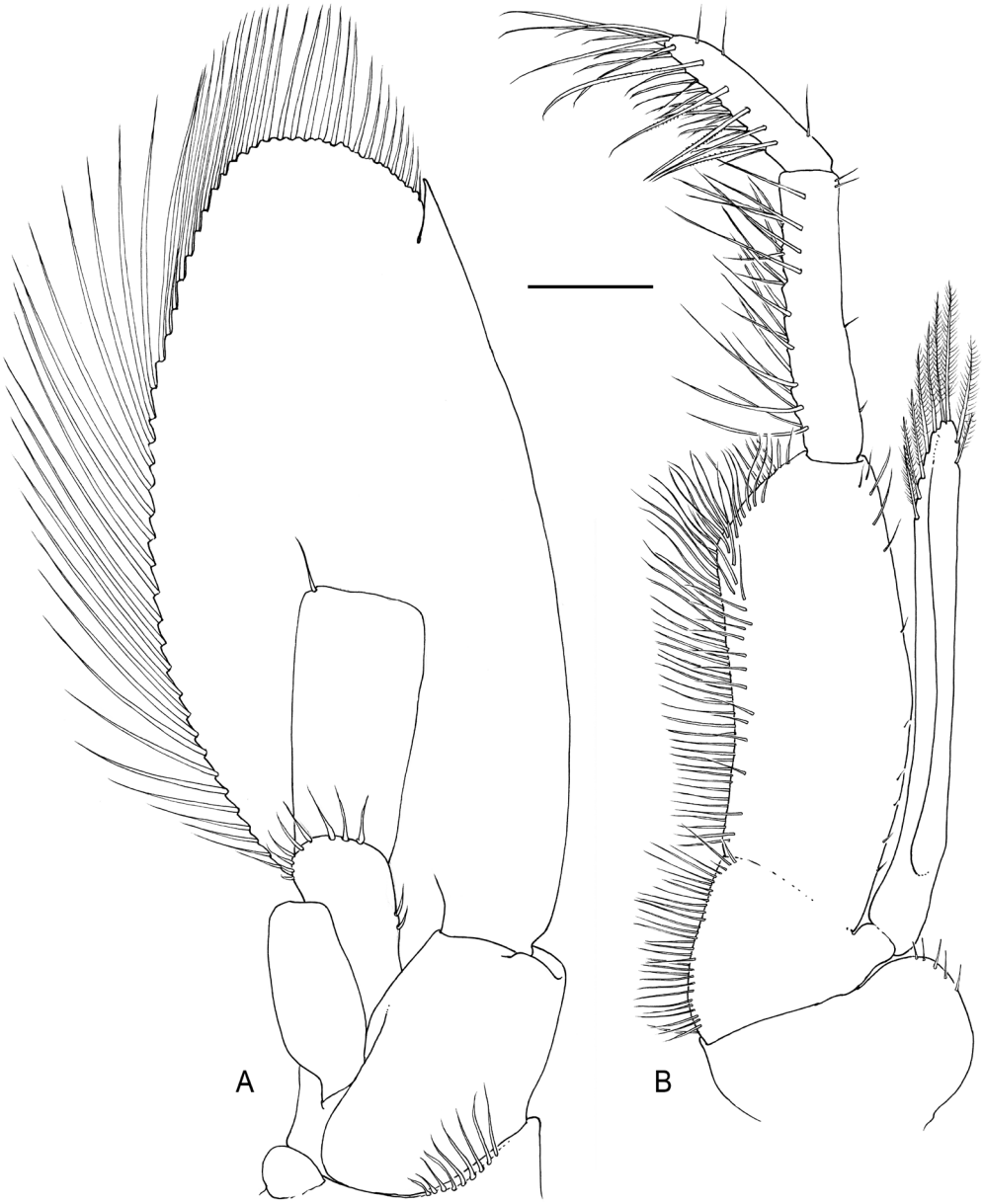
5.24 *Ensiger custos* (Forskål, 1775). RMNH.CRUS.D.41445, male pocl. 4.0 mm. A) antennula, dorsal view; B) left mandible, ventral view; C) left maxillula, ventral view. Scale = 0.5 mm. Artwork: Charles Fransen.

162, fig. 7; Bruce, 1976a: 93; Bruce, 1976d: 464; Bruce, 1976e: 447; Bruce, 1977a: 47; Bruce, 1977b: 174; Bruce, 1978b: 279; Bruce, 1979a: 231–232; Johnson, 1979: 31; Tirmizi & Kazmi, 1982: 115–117; Bruce, 1982: 264–265, fig. 19; Bruce, 1983a: 44; Bruce, 1983c: 199 (listed); Bruce & Svoboda, 1983: 36 (listed); Vine, 1986: 101 (listed); Bruce, 1989: 178; Bruce, 1990: 15, 18 (listed); Bruce, 1993: 56; Chace & Bruce, 1993: 72 (listed); Müller, 1993: 8 (listed); Fransen, 1994a: 106, 108, 111, fig. 4; Britayev & Fachrutdinov, 1994: 126, figs. 3A–C, 4–5; Bruce & Coombes, 1995: 106; Bruce, 1996: 205; Davie, 1998: 96, unnumbered colour photo; Davie, 2002: 305 (listed); De Grave, 1999: 129, fig. 3, pl. 1b–c; Li, 2000: 7, fig. 8 (listed); Li, 2001: 76; De Grave, 2001: 129–134, figs. 1-3; Hayashi, 2002: 21, fig. 433, 434e, f; Li & Liu, 2002:

271–272, fig. 1; Li et al., 2004: 528; Li & Bruce, 2006: 625; Bruce, 2006: table 1 (listed); Hayashi, 2006: 115; Marin & Savinkin, 2007: table 2 (listed); De Grave, 2007: 145 (listed); Li et al., 2007: 176, fig. 73 (listed); Li, 2008a: 235, fig. 2; De Grave & Fransen, 2011: 340 (listed); De Grave & Ashelby, 2011: table 1 (listed); Dobson et al., 2014: suppl. fig. 1, appendix 1; Kou et al., 2015: table 1, fig. 1; Gan et al., 2015: 3, fig. 1; Hosie et al., 2015: 283 (listed); Dobson et al., 2016: fig. 7, suppl. inf. I; Anker & De Grave, 2016: 406, fig. 73; Samuel et al., 2016: table 1; Ghory & Kazmi, 2018: 23–26; Al-Kandari et al., 2020, 263, fig. 8; Chow et al., 2021: table A.1.
not *Anchistus custos* – Miyake & Fujino, 1968: 415 (= *Ensiger custoides* (Bruce, 1977)).
not *Pontonia custos* – Sharp, 1893: 119 (= *Pontonia pinnophylax* (Otto, 1821)).

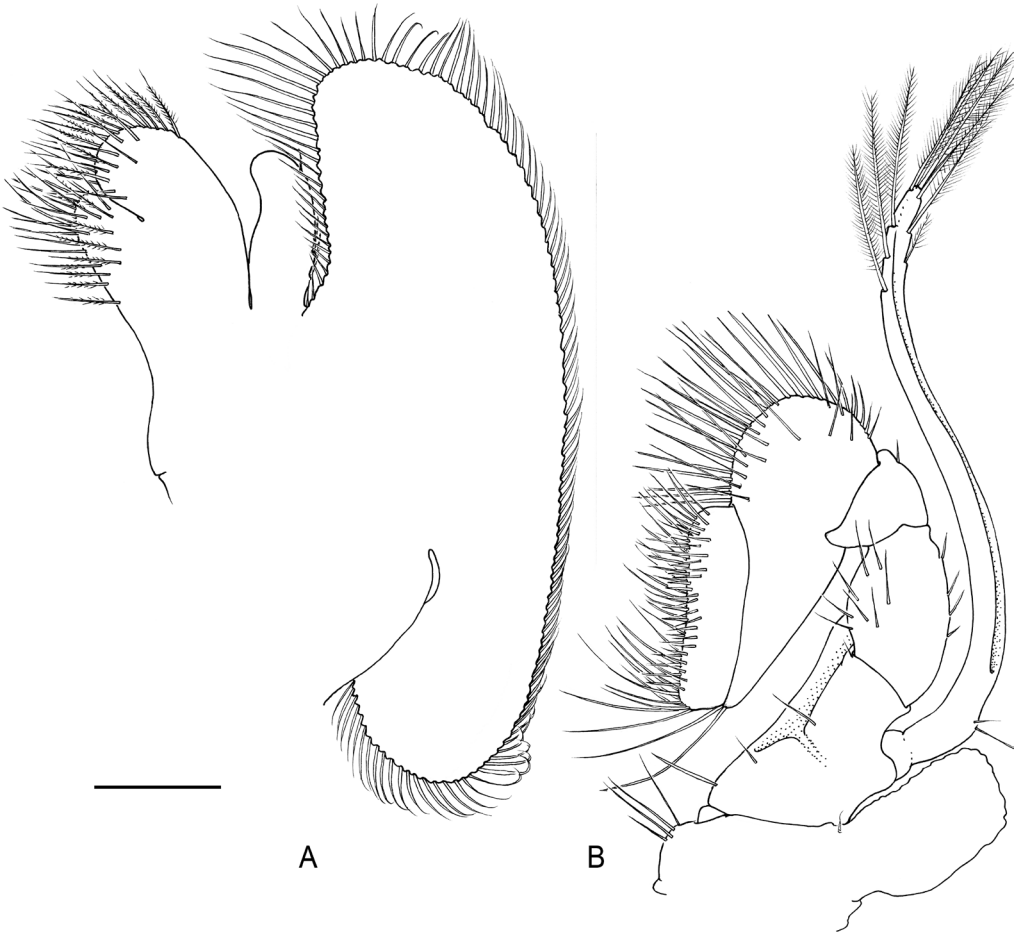
Material examined. INDONESIA: 1 male pocl. 4.03 mm (RMNH.CRUS.D.41445), stn RBE.01, Ambon Island, Ambon Bay, inner bay, Hunut, basecamp, 28.xi.1990, depth 1-3 m, snorkeling, in *Pinna* sp., coll. H.L. Strack. – 1 ovigerous female pocl. 6.49 mm (photo M.S.S. Lavaleye 51/23-27). – 1 male pocl. 5.65 mm (photo M.S.S. Lavaleye 51/25-29) (RMNH.CRUS.D.41446), stn RBE.01, Ambon Island, Ambon Bay, inner Bay, Hunut, basecamp, 11.xii.1990, depth 10 m, scuba diving, in large *Pinna bicolor* Gmelin, coll. C.H.J.M. Fransen. – 1 male pocl. 3.83 mm, 1 ovigerous female pocl. 6.10 mm (RMNH.CRUS.D.41447), stn RBE.35, Ambon Island, Ambon Bay, Martafons, 4.xii.1990, littoral collecting, from wreck, in *Pinna* sp., coll. H.L. Strack. – 1 ovigerous female pocl. 6.36 mm (RMNH.CRUS.D.41448), stn RBE.35, Ambon Island, Ambon Bay, inner bay, Martafons, 4.xii.1990, depth 2–4 m, snorkeling, from piles of lightbouy, in *Pinna* [or *Atrina*?], coll. J.C. den Hartog. – 1 male pocl. 3.18 mm (RMNH.CRUS.D.41449), sta. 35, Ambon Island, Ambon Bay, inner bay, Martafons, 5.xii.1990, depth 1–3 m, snorkeling, from piles of lightbouy, in oyster [or *Atrina*?], coll. C.H.J.M. Fransen and J.C. den Hartog. – 2 males pocl. 5.06, 5.26 mm (RMNH.CRUS.D.41450), Java, off Jakarta, Seribu Archipelago, Tikus Island, 23.vii.1978, depth 1-2 m, in *Pinna* sp., coll. Sikardi, don. PPPO. – 2 males pocl. 5.00, 6.29 mm, 3 ovigerous females pocl. 8.44, 8.70, 9.61 mm (RMNH.CRUS.D.41451), Pulau Seribu Archipelago, 29.xi.1984, in *P. bicolor*, coll. Sapri, Don. PPPO. – 1 male pocl. 5.32 mm, 1 ovigerous female (damaged) (RMNH.CRUS.D.41452), Java, off Jakarta, Seribu Archipelago, Kongsi Island, 25.v.1972, intertidal, dead coral and sand, coll. M.K. Moosa & Sitabatan, don. PPPO, fcn. CM 7. – 1 male pocl. 6.75 mm (RMNH.CRUS.D.41453), Java, off Jakarta, Seribu Archipelago, Pari Island, 27.i.1973, in *Pinna* sp., coll. Sukardi, don. PPPO. – 1 male pocl. 5.00 mm (RMNH.CRUS.D.41454) Ambon Island, Ambon Bay, Tantoci, 3.vi.1975, coll. M.K. Moosa, A.H. Banner & D.M. Banner,

don. PPPO, fcn. CM 195. – 4 males pocl. 5.58, 5.84, 6.10, 6.23 mm, 3 ovigerous females pocl. 7.27, 7.27, 7.33 mm (RMNH.CRUS.D.41455), near east coast of Tegal Island, near Sunda Island, in *Pinna* sp., 16.i.1983, coll. Indra & Sukardi, don. PPPO. – 1 ovigerous female pocl. 5.84 mm (ZMA.CRUS.D.101.612), Siboga Expedition stn 127, Sangihe Group, Siau Island, anchorage off Sawan, 18/19.vii.1899, depth 27 m (publ. Holthuis, 1952: 106). – 1 male pocl. 6.17 mm, 1 female pocl. 11.04 mm, 1 ovigerous female pocl. 7.14 mm (ZMA. CRUS.D.101.613), Lesser Sunda Islands, 18.xii.1909, leg. H.J.M. Laurense (publ. Holthuis, 1952: 106). – 1 non ovigerous female pocl. 3.5 mm (RMNH. CRUS.D.58075), stn SER.01, Java Sea, Kepulauan Seribu (Thousand Islands), off Jakarta, Onrust Island, NW-side, 6°02'01"S 106°43'58"E, 7.ix.2005, depth 1–2 m, scuba diving and snorkeling, in *P. bicolor*, coll. P.G. Rachello Dolmen. – 6 males pocl. 5.1, 5.3, 5.7, 5.9, 6.5 and 6.5 mm, 6 ovigerous females 7.0, 7.5, 7.7, 8.1, 8.7 and 8.8 mm (RMNH.CRUS.D.57963; GenBank OQ603092 (COI) & OQ600399 (16S)), stn SER.01, Java Sea, Kepulauan Seribu (Thousand Islands), 6°02'01"S 106°43'58"E, 7.ix.2005, depth 1–2 m, scuba diving and snorkeling, in in *Atrina pectinata* (L.), coll. C.H.J.M. Fransen. – 1 ovigerous female pocl. 6.4 mm (RMNH.CRUS.D.58076), stn SER.02, Java Sea, Kepulauan Seribu (Thousand Islands), off Jakarta, Damar Besar Island, NW-side, 5°57'17"S 106°50'27"E, 8.ix.2005, depth 5 m, scuba diving and snorkelling, in A. ectinate, coll. C.H.J.M. Fransen. – 1 male pocl. 5.1 mm (RMNH. CRUS.D.58077), stn SER.02, Java Sea, Kepulauan Seribu (Thousand Islands), off Jakarta, Damar Besar Island, NW-side, 5°57'17"S 106°50'27"E, 8.ix.2005, depth 7 m, scuba diving and snorkelling, in A. ectinate, coll. C.H.J.M. Fransen. – 1 ovigerous female pocl. 5.3 mm, 1 male pocl. 3.7 mm (RMNH.CRUS.D.58078), stn SER.02B, Java Sea, Kepulauan Seribu (Thousand Islands), off Jakarta, Damar Besar Island, NW-side, 5°57'17"S 106°50'27"E, 8.ix.2005, depth 2 m, scuba diving and snorkelling, in *P. bicolor*, coll. J. Goud. – 1 ovigerous female pocl. 4.3 mm (RMNH.CRUS.D.58079), stn SER.03, Java Sea, Kepulauan Seribu (Thousand Islands), off Jakarta, Damar Kecil Island, NW-side, 5°59'00"S 106°50'43"E, 8.ix.2005, depth unknown, scuba diving and snorkelling, in *P. bicolor*, coll. J. Goud. – 3 ovigerous females pocl. 5.0, 5.7 and 6.4 mm, 3 males pocl. 4.5, 4.7 and 5.4 mm (RMNH.CRUS.D.58080), stn SER.07, Java Sea, Kepulauan Seribu (Thousand Islands), off Jakarta, Bidadari Island, NW-side, 6°01'55"S 106°44'47"E, 9.ix.2005, depth 2–3 m, scuba diving and snorkelling, in *P. bicolor*, coll. C.H.J.M. Fransen. – 1 ovigerous female pocl. 6.8 mm, 1 male pocl. 4.4 mm (RMNH.CRUS.D.58081), stn SER.08, Java Sea, Kepulauan Seribu (Thousand Islands), off Jakarta, Ayer Besar Island, NW-side, 6°00'06"S 106°46'48"E, 10.ix.2005, depth 3 m, scuba diving and snorkelling, in *P. bicolor*, coll. C.H.J.M. Fransen. – 1 ovigerous female pocl. 6.2 mm, 1 male pocl. 5.2 mm (RMNH.CRUS.D.58082), stn SER.10, Java Sea, Kepulauan Seribu (Thousand



5.25 *Ensiger custos* (Forskål, 1775). RMNH.CRUS.D.41445, male pocl. 4.0 mm. A) left antenna, ventral view; B) left third maxilliped, ventral view. Scale = 0.5 mm. Artwork: Charles Fransen.

Islands), off Jakarta, Kelor Island, NW-side, 6°01'31"S 106°44'39"E, 20.ix.2005, depth 2–3 m, scuba diving and snorkelling, in *P. bicolor*, coll. C.H.J.M. Fransen. – 1 ovigerous female pocl. 6.9 mm, 1 male pocl. 5.1 mm (RMNH.CRUS.D.58083), stn SER.10, Java Sea, Kepulauan Seribu (Thousand Islands), off Jakarta,



5.26 *Ensiger custos* (Forskål, 1775). RMNH.CRUS.D.41445, male pocl. 4.0 mm. A) left first maxilliped, ventral view; B) left second maxilliped, ventral view. Scale = 0.5 mm. Artwork: Charles Fransen.

Kelor Island, NW-side, 6°01'31"S 106°44'39"E, 20.ix.2005, depth 2–3 m, scuba diving and snorkelling, in *P. bicolor*, coll. B.W. Hoeksema. – 1 ovigerous female pocl. 6.8 mm, 1 male pocl. 4.7 mm (RMNH.CRUS.D.58084), stn SER.10, Java Sea, Kepulauan Seribu (Thousand Islands), off Jakarta, Kelor Island, NW-side, 6°01'31"S 106°44'39"E, 20.ix.2005, depth 2–3 m, scuba diving and snorkelling, in *P. bicolor*, coll. P.G. Rachello Dolmen. – 1 ovigerous female pocl. 7.7 mm, 1 male pocl. 5.8 mm (RMNH.CRUS.D.58085), stn SER.10, Java Sea, Kepulauan Seribu (Thousand Islands), off Jakarta, Kelor Island, NW-side, 6°01'31"S 106°44'39"E, 20.ix.2005, depth 1-2 m, scuba diving and snorkelling, in *P. bicolor*, coll. R. Moolenbeek. – 1 ovigerous female pocl. 8.5 mm, 1 male pocl. 5.5 mm (RMNH.CRUS.D.52028), stn SER.13, Pulau Seribu Archipelago, Lancang, 5°55'37"S 106°35'29"E, 12.ix.2005, depth 12 m, in *P. bicolor*, Pulau Seribu. – 1 ovigerous female pocl. 3.9 mm, 1 male pocl. 3.6 mm (RMNH.

CRUS.D.58086), stn SER.14, Java Sea, Kepulauan Seribu (Thousand Islands), off Jakarta, Bokor Island, NW-side, 5°56'35"S 106°37'38"E, 12.ix.2005, depth 10 m, scuba diving and snorkelling, in *P. bicolor*, coll. P.G. Rachello Dolmen. – few specimens (RMNH.CRUS.D.58087), stn SER.20, Java Sea, Kepulauan Seribu (Thousand Islands), off Jakarta, Sepa Island, W-side, 5°34'39"S 106°34'48"E, 14.ix.2005, unknown depth, scuba diving from jetty (night), in *P. bicolor*, coll. C.H.J.M. Fransen. – few specimens (RMNH.CRUS.D.58058), stn SER.20, Java Sea, Kepulauan Seribu (Thousand Islands), off Jakarta, Sepa Island, W-side, 5°34'39"S 106°34'48"E, 14.ix.2005, unknown depth, scuba diving from jetty (night), in *P. bicolor* coll. C.H.J.M. Fransen. – 1 male pocl. 6.5 mm, 1 ovigerous female pocl. 8.6 mm (RMNH.CRUS.D.46642), SW Sulawesi, Spermonde Archipelago, Gusung, NW side, 7.x.1994, depth 8 m, scuba diving, in *Atrina vexillum* (Born) (Ø 35 cm), coll. B.W. Hoeksema. – 1 male, pocl. 5.6 mm, 1 female pocl. 6.9 mm (RMNH.CRUS.D.47464), stn MAL.01, Moluccas, Ambon, Inner bay, N coast near laboratory Poka, 03°38'S 128°11'E, 1-4.xi.1996, depth 1–2 m, snorkeling, muddy sublittoral near rocky shore, in *P. bicolor*, coll. C.H.J.M. Fransen. – 3 ovigerous females pocl. 7.9, 7.8 and 7.3 mm, 2 males pocl. 6.0 and 7.0 mm (RMNH.CRUS.D.42649), Padando Gr., Konori, Mios Woendi, New Guinea, in *Pinna* sp., ex. Natural Science Foundation, no. 464 and no. 00112. – 1 specimen, total length 24 mm (ZMA. CRUS.D.101612), Anchorage off Sawan, Siau Islands, 18.vii.1899, Siboga Expedition. – 1 ovigerous female total length 28 mm, 1 specimen, total length 27 mm (ZMA.CRUS.D.101613), Tual Anchorage, Kei Islands, 12.xii.1899, Siboga Expedition. **MALAYSIA**: 2 males pocl. 4.74, 5.00 mm, 1 female pocl. 5.00 mm, 1 ovigerous female pocl. 5.32 mm (RMNH.CRUS.D.41456), Lankawi, Pantai [= beach] Kok, 22.ix.1991, littoral collecting, depth 0.1–0.2 m, each pair in *Pinna* sp., in one *Pinna* together with pinnotherid crab, coll. H.L. Strack. **SINGAPORE**: 1 female pocl. 8.44 mm (RMNH.CRUS.D.41288), near Sentosa Island, -vi.1983, tidal zone, in *Pinna* sp. (Ø 15cm), coll. P.K.L. Ng. **TAIWAN**: 1 ovigerous female pocl. 9.09 mm (RMNH.CRUS.D.4162), S Taiwan, Takao, 15.ix.1907, in *Pinna* sp., coll. H. Sauter. **JAPAN**: 1 male pocl. 4.1 mm, 1 female pocl. 5.4 mm (RMNH.CRUS.D.56147), Ryukyu Islands, Okinawa, Yagai Island, 19.ix.2013, intertidal, in *Pinna* sp., coll. Yoshida, Ryuta. **PERSIAN GULF**: 2 males pocl. 6.10, 6.23 mm, 2 ovigerous females pocl. 8.05, 8.83 mm (RMNH. CRUS.D.16550), off Zeel Island, near stn 2, 26.ix.1956, coll. C.E. Dawson. – 1 male pocl. 5.4 mm (RMNH.CRUS.D.48723), Oman, Masicoh, C2, no more data, don. MNHN, Paris through A. Anker, 2000. **MADAGASCAR**: 1 male pocl. 4.87 mm (RMNH.CRUS.D.16546), Esimipoika Bay, on gills of *Pinna* sp., coll. G. Petit, don. Zool. Staatssammlung München. – 1 male pocl. 4.74 mm (RMNH. CRUS.D.16547), Normandia, don. Zool. Staatssammlung München. – 1 male pocl. 6.88 mm, 1 female pocl. 7.79 mm (RMNH.CRUS.D.16548), province of

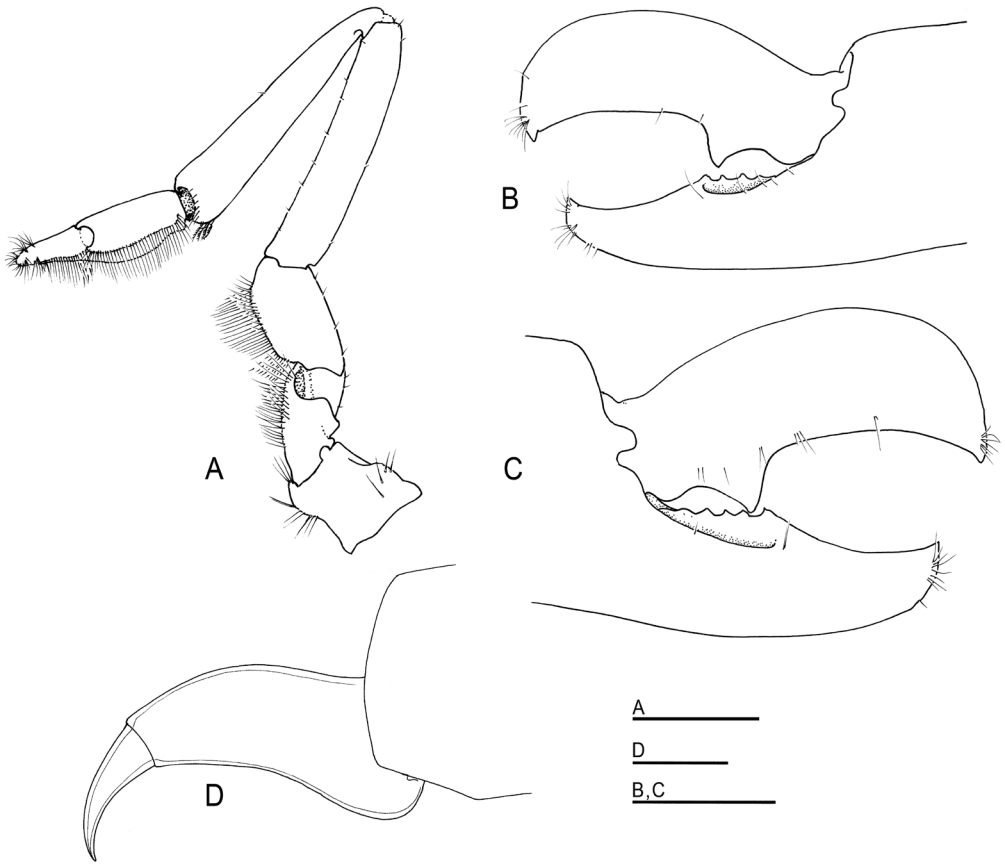
Tuléar, Ankalibé, 1906, coll. F. Geay, don. Zool. Staatssammlung München. – 1 ovigerous female pocl. 9.09 mm (RMNH.CRUS.D.16549), province of Tuléar, 1921, coll. G. Petit, don. Zool. Staatssammlung München. **UNKNOWN LOCALITY**: 1 ovigerous female pocl. 8.05 mm (RMNH.CRUS.D.4163), in *Pinna* sp., coll. M.J. Landauer (publ. Holthuis, 1952: 106).

Diagnosis. Rostrum broad proximally, compressed distally, oblong triangular in dorsal view, distally rounded, bent downwards, reaching distal end of ultimate segment of antennular peduncle, without teeth. Small antennal spine present. Ventral orbital angle produced. Antero-lateral angel of carapace rounded, slightly produced. Sixth abdominal segment posterior ventral angle rounded. Telson with minute dorsal spines at 0.60 and 0.82 of telson length. Basal segment of antennular peduncle with one distolateral tooth. Scaphocerite with convex lateral margin; shallow slit between distolateral tooth and lamina. Stylocerite blunt. Third maxilliped with narrow penultimate segment and very broad antepenultimate segment. Basis and ischium of first pereiopod with row of long slender setae on ventral margins; chela slightly sub-spatulate, cannulate, palm with rows of long slender plumose setae on opposite margins; cutting edges minutely pectinate along distal 2/3. Second pereiopods well developed; chela robust; dactylus with concave outer margin and large triangular tooth in proximal half of cutting edge; fixed finger with 3–6 small teeth in proximal half; palm without tympanal organ. Ambulatory dactyli simple, sparsely provided with flattened scale-like spinules on unguis.

Remarks. The specimens of this well-known species show no aberrant features.

Colour (fig. 5.20D). Specimens are translucent with numerous small red and white chromatophores all over the body, giving it an orange appearance. The cornea is strongly reflecting, colouring silver.

Hosts. Recorded from Pinnidae: *Atrina vexillum* (Born, 1778) (Forskål, 1775 (as *Pinna* nigras); Kemp, 1922 (as *Pinna vexillum* Born, 1778 and *Pinna nigra* Chemnitz, 1785), 1925 (as *P. nigra*); Jacquotte, 1963; Hipeau-Jacquotte, 1967, 1973a, 1973b, 1974b; Britayev & Fachrutdinov, 1994; Hayashi, 2006 (also as *P. nigra*); Li, 2008a); *Pinna atropurpurea* G. B. Sowerby I, 1825 (Johnson & Liang, 1966; Bruce, 1979a; Hayashi, 2002, 2006; Anker & De Grave, 2016); *Pinna bicolor* Gmelin, 1791 (Kemp, 1922; Jacquotte, 1963; Hipeau-Jacquotte, 1967, 1973b, 1974b; Bruce, 1976b, 1976c; 1976d, 1976e, 1978b, 1993; Britayev & Fachrutdinov, 1994; Bruce & Coombes, 1995; De Grave, 1999; Hayashi, 2002, 2006; Li, 2008a; Al-Kandari et al., 2020); *Pinna deltodes* Menke, 1843 (Bruce & Coombes, 1995; Hayashi, 2006 as '*Pinna deltoides*'); *Pinna dolabrata* Lamarck,



5.27 *Ensiger custos* (Forskål, 1775). A–C) RMNH.CRUS.D.41445, male pocl. 4.0 mm. A) left first pereiopod, lateral view; B) right second pereiopod chela, medial view; C) left second pereiopod chela, medial view; D, RMNH.CRUS.D.41448, ovigerous female pocl. 6.4 mm, right third pereiopod dactylus, medial view, propodal setae omitted. Scale A = 1 mm; B, C = 1 mm; D = 0.1 mm. Artwork: Charles Fransen.

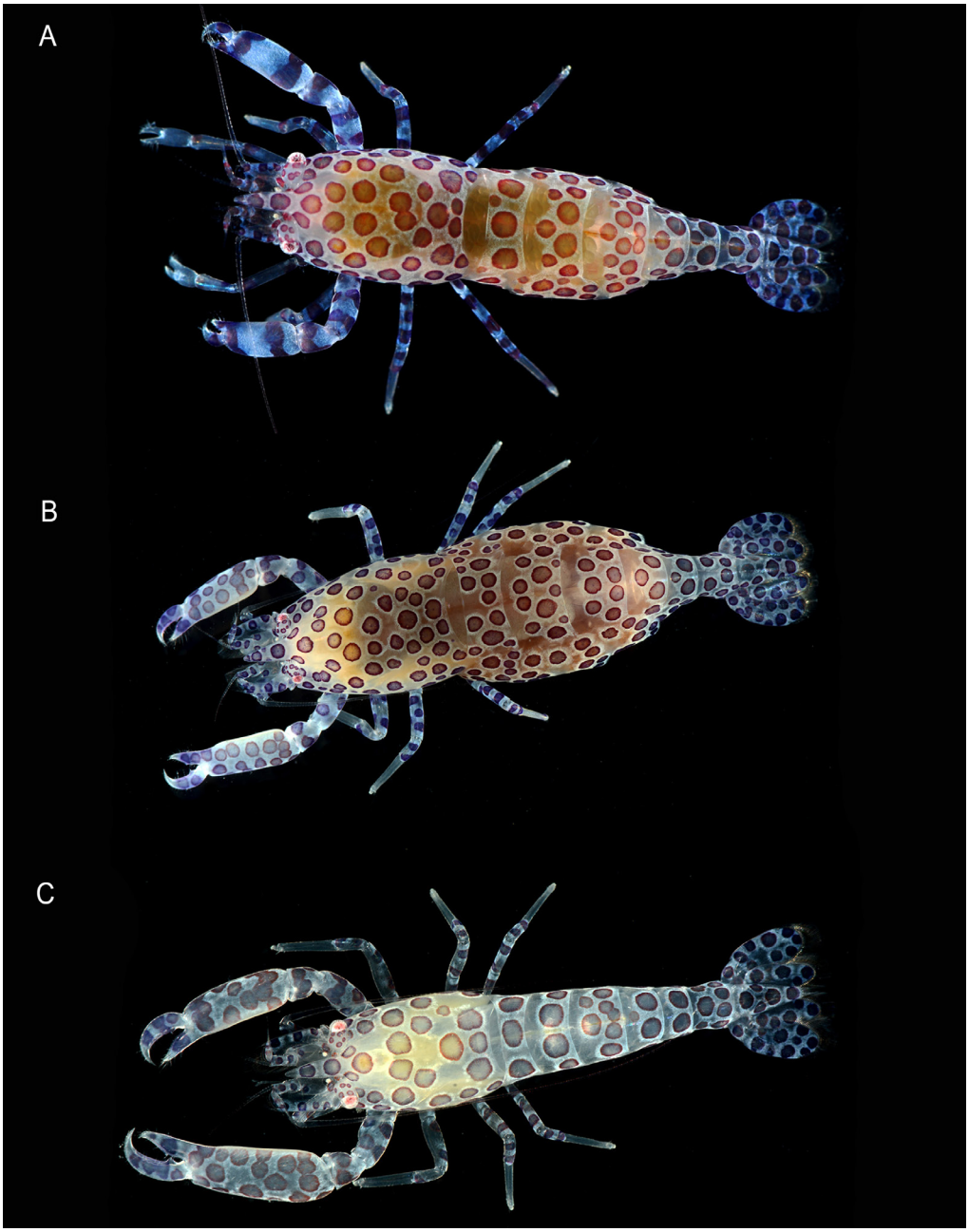
1819 (Hale, 1927; Hayashi, 2006); *Pinna madida* Reeve, 1858 (Hedley, 1924; Hayashi, 2006); *Pinna muricata* L., 1758 (Jacquotte, 1963; Hipeau-Jacquotte, 1967, 1974b; Bruce, 1976d, 1976e, 1978b, 1978c; Hayashi, 2006); *Pinna* sp. (Miers, 1884; Ortmann, 1894; Rathbun, 1914; Holthuis, 1952; Johnson, 1961; McNeill, 1968; Bruce, 1989, 1993; Bruce & Coombes, 1995; Li, 2001; Li & Liu, 2002; Li et al., 2004); *Streptopinna saccata* (L., 1758) (Forskål, 1775, as *Pinna saccata*; Forskål, 1775; Hayashi, 2002, 2006). Also recorded from Turbinellidae: *Vasum* sp. (Bruce, 1996; Hayashi, 2006); Cardiidae (Tridacninae): *Tridacna gigas* (?) (L., 1758) (Pesta, 1911, 1913; Hayashi, 2006); and Isognomonidae: *Isognomon isognomum* (L., 1758) (Hayashi, 2006). In addition, one record from an 'infra-branchial chamber of a large gastropod' (Lanchester, 1902). Now newly recorded from *Atrina pectinata* (L., 1767).

Distribution. Widely distributed in the Indo-Pacific, from the Red Sea south to Moçambique and Madagascar to Japan, the Philippines, eastward to Fiji. Lohajæ (= Al-Luhayya, Yemen), Red Sea (Forskål, 1775; Tattersall, 1921; Vine, 1986); Dar es Salaam, Tanzania (Ortmann, 1894); Madagascar (Jacquotte, 1963; Hipeau-Jacquotte, 1967, 1973b, 1974b, 1974c, 1974d, 1975b; Thomassin, 1974; Bruce, 1978b; Gan et al., 2015); Gulf of Mannar (Pearson, 1905; Bruce, 1976e); Sri Lanka (Müller, 1887); Kenya and Zanziber (Bruce, 1976d); Philippines (Bruce, 1989); Hong-Kong (Bruce, 1979a; Chow et al., 2021); Singapore (Johnson, 1961, 1979; Johnson & Liang, 1966; Anker & De Grave, 2016); Hainan Island, South China Sea (Li, 2001; Li & Liu, 2002; Li et al., 2004); Ambon, Indonesia (Fransen, 1994a); Bay of Jakarta, Indonesia (Holthuis, 1952); Kera, near Timor, Indonesia (Holthuis, 1952); Kai Island, Indonesia (Holtuis, 1952); Siau Island, Indonesia (Holthuis, 1952); Pulau Bidan, Penang, Malaysia (Lanchester, 1902); Australia (Bruce, 1977a); Queensland, Australia (Miers, 1884; McNeill, 1968; Bruce, 1977b); Moreton Bay, Australia (Li, 2008a; Kou et al., 2015); Monte Bello Islands, Australia (Rathbun, 1914); Tre Island, Vietnam (Bruce, 1993); South Vietnam (Britayev & Fachrutdinov, 1994); Papua New Guinea (De Grave, 1999); New Caledonia (Bruce, 1996); Fiji (Dana, 1852, as Viti); Takao, South Taiwan (Holthuis, 1952); Samoa (Pesta, 1911, 1913). Now for the first time recorded from these localities in Indonesia: Seribu Archipelago (Thousand Islands), Sunda Islands, SW Sulawesi, and New Guinea. Additionally, now also recorded from Okinawa, Japan; Masicoh, Oman; and Zeel Island (presumably Halat Za'al, Saudi Arabia).

Genus *Neoanchistus* Bruce, 1975

Neoanchistus Bruce, 1975a: 149. Type species, by original designation and monotypy: *Neoanchistus cardiodytes* Bruce, 1975. Gender: masculine.

Diagnosis (after Bruce, 1995: 83). Small sized shrimps of stout, depressed body form. Rostrum well developed, thickened, blunt, unarmed, lateral carinae indistinct. Carapace smooth, glabrous, inferior orbital angle and orbit obsolete, supraorbital, epigastric, hepatic and antennal spines absent. Anterolateral angel of carapace broadly rounded. Abdomen smooth, glabrous, pleura with posterior margins rounded. Telson narrow, with two pairs of minute dorsal spines and three pairs of posterior spines. Eye normal, with globular cornea. Antennula with basal segment of peduncle without distolateral tooth. Antenna with basicerite unarmed. Scaphocerite well developed with moderately sized distolateral tooth; slit between distolateral tooth and lamina shallow. Epistome unarmed. Mandible without palp, molar process robust, incisor process feeble; maxillula with feebly bilobed palp, laciniae normal; maxilla with simple palp,



5.28 A) *Neoanchistus cardiodytes* Bruce, 1975, from an unknown species of *Vasticardium* Iredale, 1927 (det. J. Goud), Madang Lagoon, Papua New Guinea (MNHN-IU-2013-10029 stn. PR107); B, C) *Neoanchistus nasalis* Holthuis, 1986, from *Mimachlamys townsendi* (G.B. Sowerby III, 1895), Oman (FLMNH UF). B) ovigerous female; C) male. Photo credit: Arthur Anker.

basal endite bilobed, scaphognathite moderately broad; first maxilliped with setose palp, basal endite broadly rounded, coxal endite fused to basal endite, exopod well developed, caridean lobe broad, exopod with plumose terminal setae, epipod bilobed; second maxilliped normal, dactylar segment narrow, exopod with terminal plumose setae, epipod subrectangular, without podobranch; third maxilliped with endopod slender, ischiomerus and basis fused, exopod flagellum with plumose terminal setae, coxa with oval lateral plate, arthrobranch rudimentary. Fourth thoracic sternite unarmed. First pereiopods slender, subspatulate, with cutting edges minutely pectinate. Second pereiopods small, subequal, similar, stout, dactylus hamate. Ambulatory pereiopods robust, dactyli hooked, unguis simple, large, with dorsal margin with minute scale-like spinules. Uropod with protopodite posterolaterally acutely produced, exopod with small mobile posterolateral spine.

Two species are recognised: *Neoanchistus cardiodytes* Bruce, 1975, and *Neoanchistus nasalis* Holthuis, 1986. Both species occur in bivalve hosts. The species can be distinguished as follows:

Key to the species of *Neoanchistus*

1. - Rostrum reaching end of antennular peduncle; outer antennular flagellum with two basal segments fused; dactyli of ambulatory pereiopods slender *N. cardiodytes*
- Rostrum reaching halfway second segment antennular peduncle; outer antennular flagellum with seven basal segments fused; dactyli of ambulatory pereiopods stout *N. nasalis*

***Neoanchistus cardiodytes* Bruce, 1975**

(fig. 5.28A)

Neoanchistus cardiodytes Bruce, 1975a: 149–165, figs. 1–6 [type locality: Nosy Bé, Madagascar]; Bruce, 1978b: 287; Müller, 1993: 39 (listed); Holthuis, 1993: 153, fig. 142 (listed); Fransen, 1994a: 111 (listed); Li, 2000: 81, fig. 81 (listed); Britayev & Marin, 2011: 1–3, fig. 1; De Grave & Fransen, 2011: 353 (listed).

Diagnosis. Rostrum stout, depressed, extending slightly beyond antennular peduncle, without teeth not setae, broadened in dorsal view by thick lateral expansions, distally truncated, proximally slightly constricted. Carapace without spines. Inferior orbital angle rounded, slightly produced. Anterolateral

angle of carapace rounded, not produced. Abdomen smooth, glabrous, pleura with posterior margins rounded. Telson narrow, with two pairs of small dorsal spines at 0.6 and 0.8 of telson length and three pairs of posterior spines. Basal segment of antennular peduncle distolaterally rounded; outer flagellum fused in basal two segments, shorter free ramus two-segmented, longer ramus four-segmented. Scaphocerite with straight lateral margins; slit between distolateral tooth and lamina shallow. First pereiopods with chela sub-spatulate; lateral cutting edges proximally gaping, distally minutely pectinate; merus with peg-like spinules on inner margin of proximal part. Dactylus of second chela strongly hooked, overreaching fixed finger, with one small blunt tooth at about one-third of its length; fixed finger with a small tooth halfway the cutting edge. Ambulatory pereiopods without distoventral spines in propodus of third pereiopod, with simple slender dactyli; unguis with minute scale-like spinules dorsally.

Remarks. The tympanal organ in the proximal medio-ventral surface of the palm in the second pereiopods is not reported in the type-description. This feature however could have been overlooked.

Colour (fig. 5.28A). Bruce (1975a: 158): “Body covered dorsally with round or slightly irregular reddish markings, with margins darker than centres. Appendages with similar red stripes (A.G. Humes, in litt.).” Britayev & Marin (2011) published a colour photograph of the species.

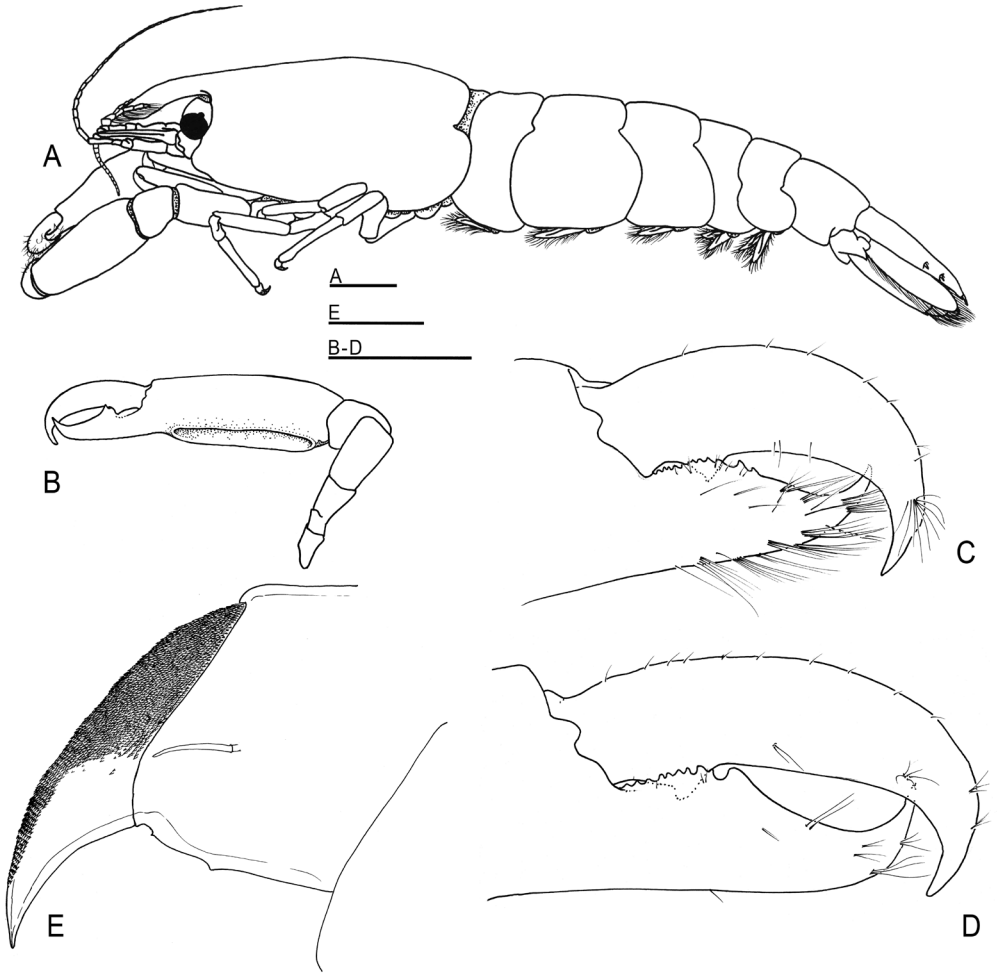
Hosts. Recorded from Cardiidae, Trachicardiinae: *Vasticardium pectiniforme* (Born, 1780) (Bruce, 1975a (as *Trachicardium*/*Cardium pectiniforme* Born, 1780)). Also from Mactridae: *Mactra grandis* Gmelin, 1791 (Britayev & Marin, 2011). Now recorded from an unidentified *Vasticardium* Iredale, 1927.

Distribution. Nosi Bé, Madagascar (Bruce, 1975a); Vietnam (Britayev & Marin, 2011). Now for the first time recorded from Madang Lagoon (stn. PR107; 05°10.2’S – 145°50.4’E), Papua New Guinea (Anker, pers. comm.; see fig. 5.28A).

***Neoanchistus nasalis* Holthuis, 1986**

(fig. 5.28B, C, 5.29)

Neoanchistus nasalis Holthuis, 1986: 264-269, figs. 1-2 [type locality: artificial reef near Raysut Harbour, Dhofar, southern Oman, 16°57’45”N 54°00’E, in *Chlamys townsendi* (= *Mimachlamys townsendi* (G. B. Sowerby III, 1895))]; Hogarth, 1989: 111; Müller, 1993: 39 (listed); Fransen, 1994a: 107, 111, fig.



5.29 *Neoanchistus nasalis* Holthuis, 1981. A) RMNH.CRUS.D.48724, non ovigerous female pocl. 4.0 mm; B–E) RMNH.CRUS.D.36608, ovigerus female, pocl. 14.3 mm. A) habitus, lateral view; B) left second pereiopod, ventral view; C) idem, chela, ovigerous female, pocl. 12.4 mm; D) idem, ovigerous female, pocl. 14.3 mm; D) left fifth pereiopod dactylus, lateral view. Scale A = 2 mm; B = 4 mm; C, D = 1 mm; E = 0.1 mm. Artwork: A) Werner de Gier; B–D) Charles Fransen.

2f; Li, 2000: 81, fig. 82 (listed); Bruce, 2006: table 1 (listed); De Grave & Fransen, 2011: 353 (listed); Dobson et al., 2014: 9, appendix 1; Dobson et al., 2016: fig. 7, suppl. inf. I.
Neanchistus nasalis – Hogarth, 1989: 111 [erroneous spelling].

Material examined. INDIAN OCEAN: 1 male pocl. 9.8 mm, 2 ovigerous females pocl. 12.4 14.3 mm (RMNH.CRUS.D.36608), Southern Oman, Dhofar, artificial reef near Raysut Harbour, 16°57'45"N 54°00'E, 24.ii.1983, in *Chlamys townsendi* (G.B. Sowerby), coll. P.J. Hogarth, no. 41, site no. IJI. – 1 specimen, pocl. 10 mm (RMNH.CRUS.D.36608), Southern Oman, Raysut, 9.x.1983,

depth 10 m, in *C. townsendi*, coll. P.J. Hogarth, no. 335. 1 non ovigerous female pocl. 4.0 mm (RMNH.CRUS.D.48724), Oman, Masicoh, C1, no more data, don. MNHN, Paris through A. Anker, 2000.

Diagnosis. Rostrum stout, depressed, extending halfway second segment antennular peduncle, without teeth nor setae, broadened in dorsal view by thick lateral expansions, distally rounded, proximally not constricted. Carapace without spines. Inferior orbital angle strongly produced. Anterolateral angle of carapace broadly rounded, produced. Basal segment of antennular peduncle distolaterally rounded; outer flagellum fused in basal seven segments, shorter free ramus with about four segments. Scaphocerite with almost straight lateral margins; slit between distolateral tooth and lamina shallow. First pereiopods with sub-spatulate, densely setose chela; lateral cutting edges proximally gaping, distally minutely pectinate; merus without peg-like spinules on inner margin of proximal part. Palm of second pereiopods with oblong oval tympanal organ in proximal two-third to three-fourth of medio-ventral surface; dactylus of second chela strongly hooked, overreaching fixed finger, one large triangular tooth at about one-third of its length; fixed finger with 10–12 small teeth in the proximal half of which the distalmost largest. Ambulatory pereiopods with propodi with distoventral spines, with simple stout dactyli, with one, seldom two, minute accessory teeth; unguis with minute scale-like spinulation dorsally.

Remarks. Dactyli of ambulatory pereiopods are very similar to those of *Tympanicheles pectinis*, both with a minute accessory tooth, dorsal transverse rims on the corpus, and dorsally minutely spinulate unguis. Chela of second pereiopods show differences in setation of the fixed finger, in two specimens it is provided with a dense cover of long setae on the ventral margin (fig. 5.29C), in the largest chela the number of setae is much smaller.

Colour (fig. 5.28B, C). Similar pattern as the other species in the genus. The colouration patterns on the appendages is, however, different from *N. cardiodytes*. In *N. cardiodytes*, the spotted pattern forms connected, continuous ‘bands’, most visible in the major chelipeds and walking legs. In *N. nasalis*, the spots are distinguishable and separated from each other.

Host. Pectinidae: *Mimachlamys townsendi* (G.B. Sowerby III, 1895) (Holthuis, 1986; Hogarth, 1989; Fransen, 1994a).

Distribution. Type-locality: Raysut, Southern Oman (cf. Holthuis, 1986; Hogarth, 1989; Fransen, 1994a). Now recorded for the second time from Oman (fig. 5.28B, C).

Genus *Paranchistus* Holthuis, 1952

Paranchistus Holthuis, 1952: 5, 13, 91. Type species, by original designation: *Anchistus biunguiculatus* Borradaile, 1898 (a junior subjective synonym of *Pontonia armata* H. Milne Edwards, 1837). Gender: masculine.

Diagnosis. Large sized shrimps of subcylindrical body form. Rostrum well developed, compressed, with or without indistinct shallow dorsal and distal teeth, lateral carinae feebly developed. Carapace smooth, glabrous; orbit feebly developed, inferior orbital angle distinct, slightly produced, antennal spine well developed, basally broad; hepatic, spine, if present, small, articulating; supraorbital and epigastric spines absent; anterolateral margin rounded, slightly produced. Abdomen smooth, glabrous, third segment not posterodorsally produced, anterior pleura rounded, posterior blunt. Telson with two pairs of dorsal spines in distal half, three pairs of posterior spines. Eye small, cornea globular. Antennula normal, upper flagellum biramous, short ramus reduced. Antenna with basicerite unarmed, scaphocerite well developed with distolateral tooth. Epistome unarmed. Mandible normal, without palp, molar process robust, incisor process dentate; maxillula with feebly bilobed palp; maxilla with simple palp, basal endite broad, bilobed, proximal lobe much smaller than distal lobe, coxal endite obsolete, scaphognathite broad; first maxilliped with palp, basally broad, distally slender, basal endite broad, well separated from coxal endite by suture, exopod well developed, caridean lobe broad, flagellum with numerous plumose setae distally, epipod bilobed; second maxilliped with normal endopod, dactylar segment slender, concave, exopod well developed, broad, coxa medially slightly produced, epipod rectangular, without podobranch; third maxilliped with endopod slender, ischiomerus and basis partly fused, exopod with numerous plumose setae distally, coxa with oval lateral plate, with rudimentary arthrobranch. Fourth thoracic sternite without median process. First pereiopods slender, chelae with fingers spatulate, non-cannulate, lateral cutting edges minutely pectinate. Second pereiopods well developed, similar, subequal, dactylus strongly hooked, overreaching fixed finger, with three broad teeth in proximal half, fixed finger with series of teeth in proximal part; propodus without ventral oval tympanal organ proximally. Ambulatory pereiopods with biunguiculate dactyli, with large triangular accessory tooth, with without basal protuberance; unguis compressed, curved, with few scattered minute scale-like spinules; propodi with strong distoventral spines. Uropod with protopodite posterolaterally acutely produced, exopod with small mobile distolateral spine, without distolateral tooth.

The genus is monospecific with *Pontonia armata* H. Milne Edwards, 1837 (=

Paranchistus armatus (H. Mile Edwards, 1837)) as its type species. *Paranchistus liui* Li, Bruce & Manning, 2004, *Paranchistus nobilii* Holthuis, 1952, *Paranchistus pycnodontae* Bruce, 1978, and *Paranchistus spondylis* Suzuki, 1971 are herby transfered to *Polkamenes*. *Paranchistus ornatus* Holthuis, 1952 is transfered to *Tympanicheles*.

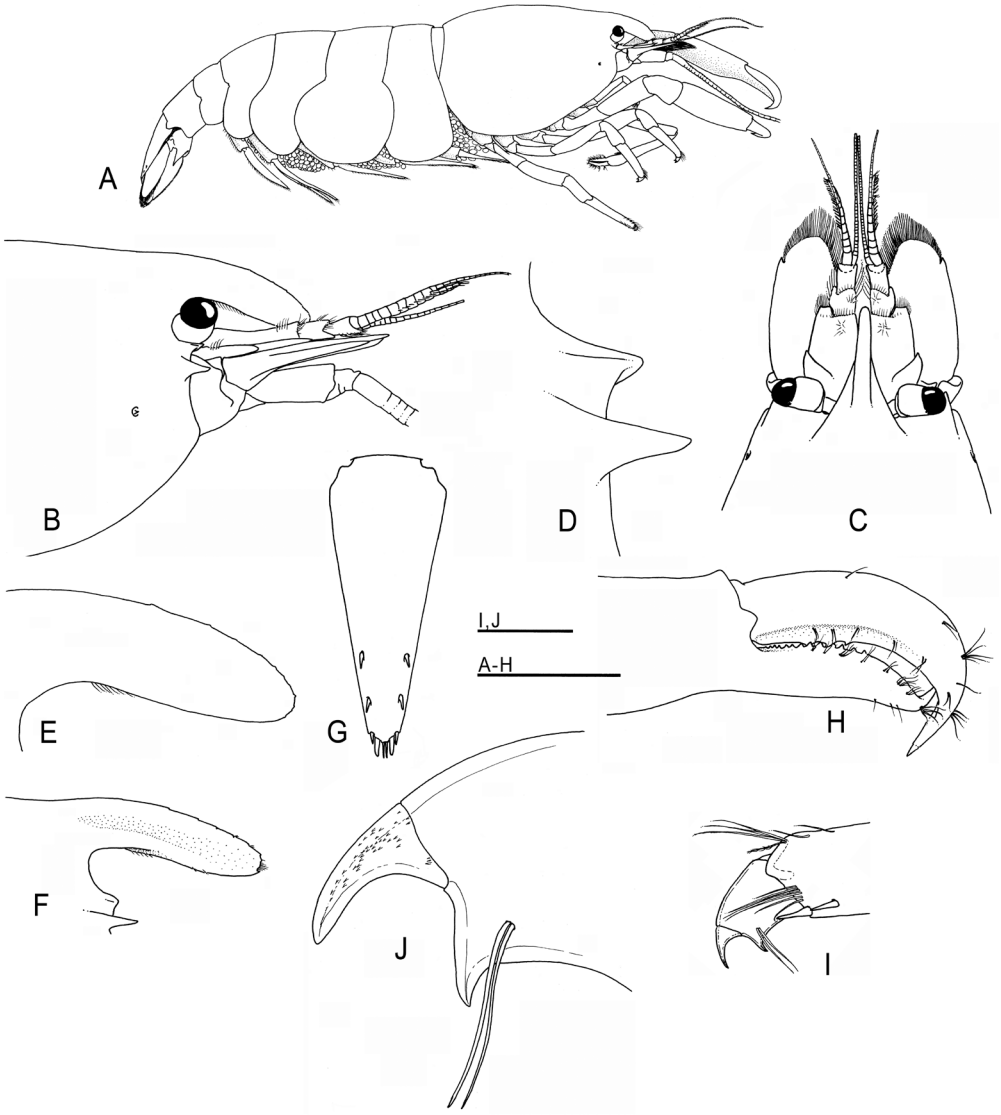
***Paranchistus armatus* (H. Milne Edwards, 1837)**

(figs. 5.30–5.32)

Pontonia armata H. Milne Edwards, 1837: 359 [type locality: près de côtes de la Nouvelle-Irlande]; Broderip, 1841: 421 (listed); Lucas, 1851: 183; Sharp, 1893: 119 (listed).
Anchistus biunguiculatus Borradaile, 1898: 387; Borradaile, 1900: 408, pl. 36 fig. 5; Borradaile, 1917: 388; Hayashi, 2005: 515.
Anchistus? *armatus* – Borradaile, 1898: 387 (listed); Borradaile, 1917: 389 (listed).
Tridacnocaris biunguiculata – Nobili, 1899: 235 [type locality: British New Guinea].
Anchistus oshimai Kubo, 1949: 26, figs. 1, 2 [type locality: Helen Atoll].
Paranchistus biunguiculatus – Holthuis, 1952: 13, 93, figs. 36–38; Holthuis, 1953: 56; Holthuis, 1955: 62, fig. 32b (listed); Rosewater, 1965: 370; McNeill, 1968: 21; Miyake & Fujino, 1968: 417; Suzuki, 1971: 110; Bruce, 1972a: 221 (listed); Bruce, 1975a: 162 (listed); Bruce, 1975b: 27; Bruce, 1976f: 59 (listed); Devaney & Bruce, 1987: 230 (listed).
Anchistus armatus – Bruce, 1967: 564; Hipeau-Jacquotte, 1972b: 9.
Paranchistus armatus – Bruce, 1975c: 49-54, figs. 1-3; Bruce, 1979a: 226–227; Bruce, 1983c: 204; Bruce, 1986: 165; Devaney & Bruce, 1987: 230 (listed); Bruce, 1990: 16, 18 (listed); Holthuis, 1993; 160, fig. 151; Müller, 1993: 10 (listed); Chace & Bruce, 1993: 90-91 (listed); Fransen, 1994a: 111 (listed); De Grave, 1999: 134, pl.2 fig. a; Bruce, 2000: 91-96, figs. 1-2; Li, 2000: 110, fig. 119 (listed); De Grave & Fransen, 2011: 357 (listed).

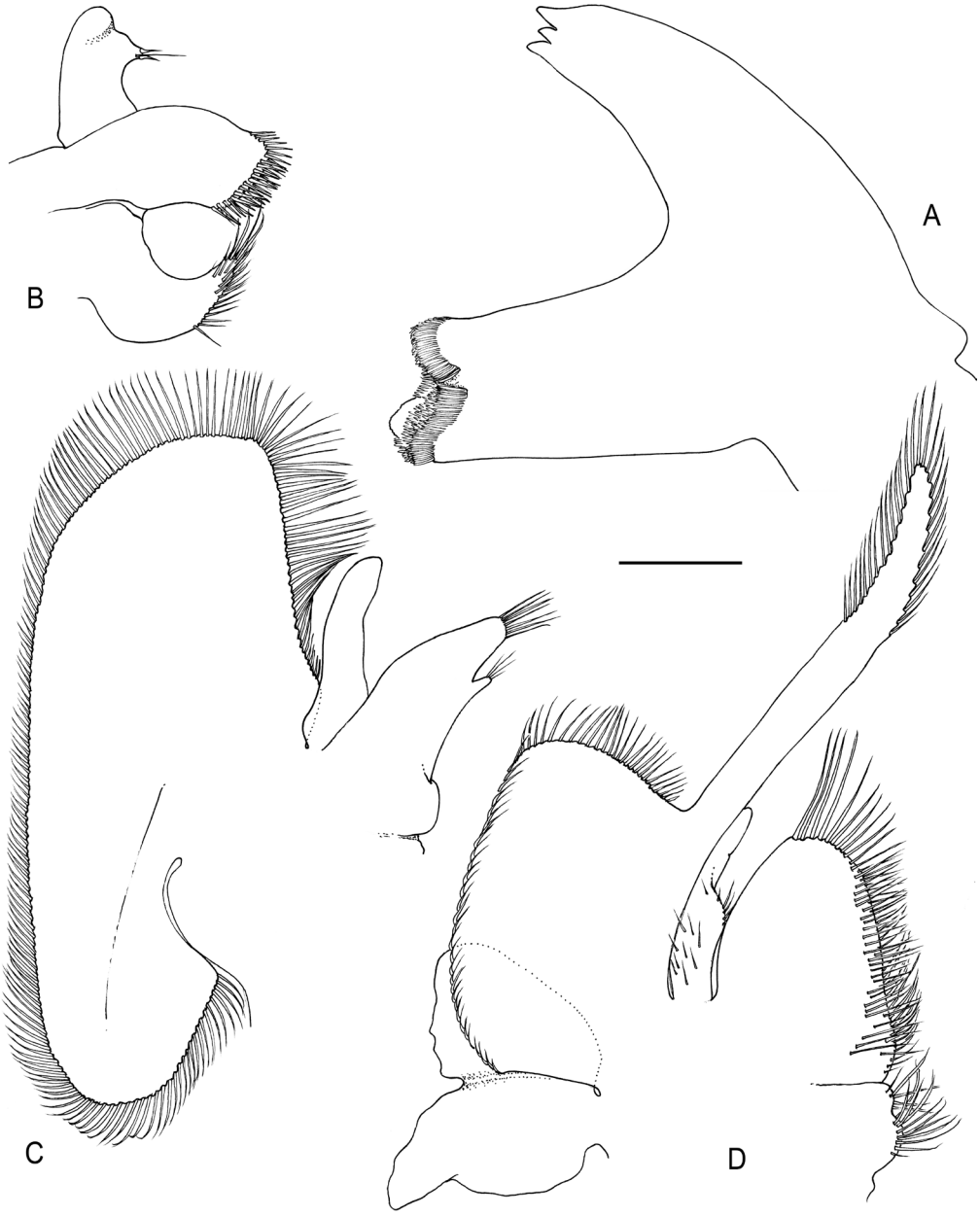
Material examined. INDONESIA: 1 male pocl. 8.70 mm, 1 ovigerous female pocl. 13.77 mm (RMNH.CRUS.D.46092; GenBank OQ600400 (16S)), Kei Islands, Tanimbar, 16.xi.1990, depth 8–10 m, scuba diving, in large *Tridacna gigas* (L.) (Ø 50cm), coll. Zainal. – 1 male pocl. 5.80 mm (RMNH.CRUS.D.8991), Moluccas, Obi Latu, shore and reef, 23-27.iv.1930, Siboga Expedition, published by Holthuis, 1952: 93–97, figs. 36–38, as *Paranchistus biunguiculatus*.

Diagnosis. See diagnosis of genus.



5.30 *Paranchistus armatus* (H. Milne Edwards, 1837), RMNH.CRUS.D.46092. A–E, H) ovigerous female, pocl. 13.8 mm; F, G, I, J) male, pocl. 8.7 mm. A) habitus, lateral view; B) anterior appendages, lateral view; C) idem, dorsal view; D), antennal spine and infraorbital angle; E, F) rostrum, lateral view; G) telson, dorsal view; H) left second pereiopod chela; I) right fifth pereiopod dactylus, medial view; J) idem, detail unguis. Scale A = 8 mm; B, C = 4 mm; E–G = 2 mm; D = 1 mm; I = 0.4; J = 0.1 mm. Artwork: Charles Fransen.

Remarks. In smaller specimens the dorsal teeth on the rostrum are more prominent with setae in between, in larger specimens the teeth become indistinct (Bruce, 1975c: 5). This indicates that the dorsal rostral teeth become less prominent after each moult this in contrast to *Anchistus australis* and *Tympanicheles ornatus* where they are placed more distally after each moult.



5.31 *Paranchistus armatus* (H. Milne Edwards, 1837), RMNH.CRUS.D.46092, ovigerous female, pocl. 13.8 mm. A) left mandible, ventral view; B) right maxillula, ventral view; C) right maxilla, ventral view; D) right first maxilliped, ventral view. Scale A = 0.5 mm; B–D = 1.25 mm. Artwork: Charles Fransen.

The hepatic spine is normally developed in small specimens and reduced or absent in larger specimens.

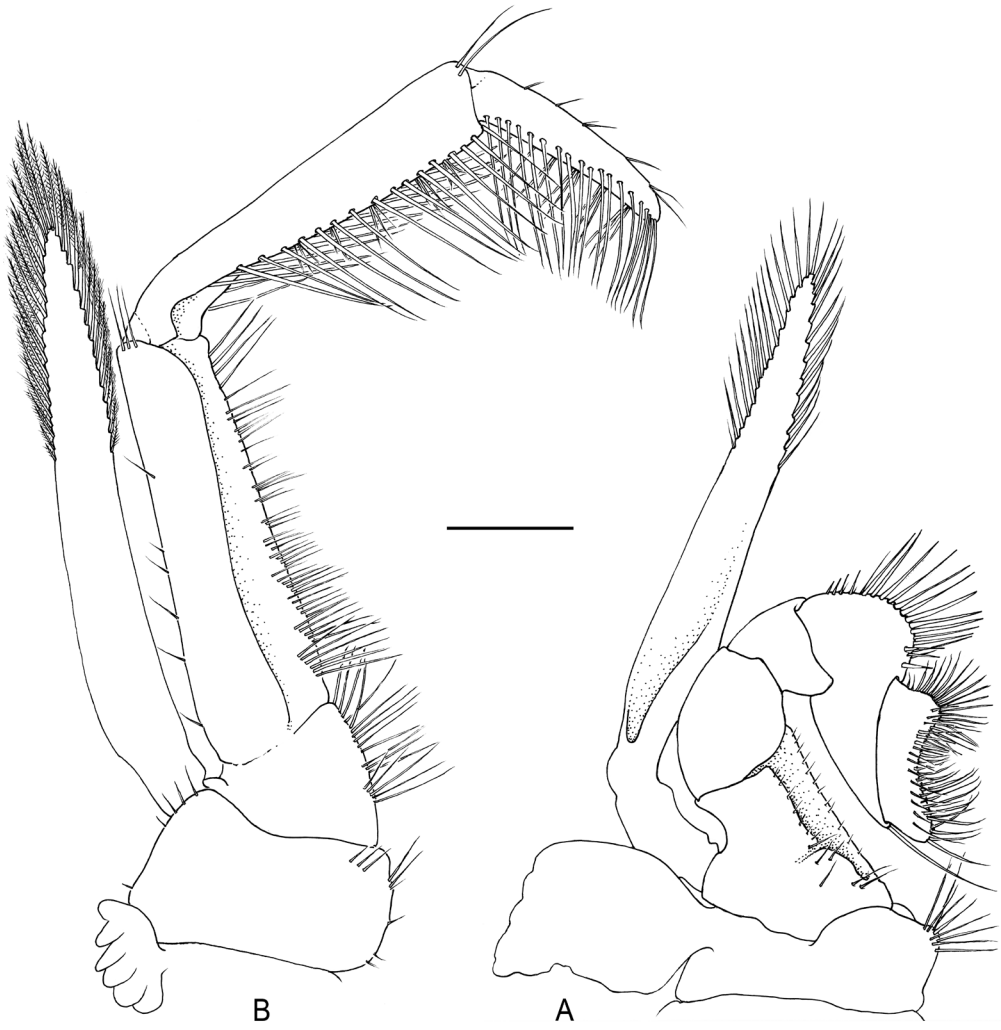
The dactylus of the ambulatory pereiopods is very different from those of other

species in the '*Anchistus* group'. With *E. custos* and *E. custoides* it has in common the sparcely distributed scale-like spinules on the unguis, whereas in other species the unguis is densely covered with spinules on the dorsal surface. The broad robust accessory tooth is very different from the slender simple or minutely spinulate accessory tooth in *A. miersi*, *Polkamenes nobilii*, *P. pycnodontae*, *P. spondylis*, or the minute accessory tooth in *A. demani*, *A. australis*, *N. nasalis*, *Tympanicheles ornatus*, and *T. pectinis*.

Colour. Various colour descriptions are known. "Great Barrier Reef specimens were noted to be transparent or whitish. Freshly preserved specimens showed a dense covering of minute red chromatophores all over the body, with similar but more sparse dots over de second pereipods." (cf. Bruce, 1975c: 51). "The ovigerous female in life is transparent or light blue with dark blue spots except for the carapace, in which they are enclosed with white rings. The cornea is bitter orange, and the stalk is light blue. The antennal flagellum is reddish purple. The colour of the male is unknown." (cf. Miyake & Fujino, 1968: 417). "Body generally translucent whitish in colour, generally and evenly with numerous small white and inconspicuous chromatophores. Ovary a deep olive green. Antennal peduncles and eyestalks whitish. Cornea white. Pereiopods whitish, finely spotted with red. Caudal fan whitish, more heavily speckled with red." (cf. Bruce, 1979b: 227). From the present specimens was noted that the carapace was more or less translucent and the antennae were blue.

Hosts. Only known from giant clams (Cardiidae: Tridacninae): *Tridacna gigas* (L., 1758) (Kubo, 1949; Rosewater, 1965; McNeill, 1968; Bruce, 1975c, 1979a, 1983b; De Grave, 1999). Several authors recorded the species from *Tridacna* sp. (Borradaile, 1917; Holthuis, 1952; Bruce, 1975c, 1979b).

Distribution. Same presumed distribution as its host: *Tridacna gigas* (L., 1758), e.g., Moluccas, Indonesia to the Marshall Islands. NE Australia (cf. McNeill, 1968; Bruce, 1975c); Marshall Islands (Holthuis, 1953; Rosewater, 1965; Bruce, 1975c, 1979b); Eniwetok Atoll (Rosewater, 1965); Obi latu, Moluccas, Indonesia (Holthuis, 1952); Aru Islands, Indonesia (Holthuis, 1993); Batanta and Numfoor Island, New Guinea, Indonesia (Nobili, 1899); Papua New Guinea (De Grave, 1999); New Ireland, Papua New Guinea (cf. H. Milne Edwards, 1837; Bruce, 1975c); Tubetube, Engineer Islands, Papua New Guinea (Borradaile, 1898); 'British New Guinea' (southeastern part of Papua New Guinea) (Borradaile, 1898); Helen Atoll, Ngaianges Island and Ngadarak Reef, Palau Islands (Kubo, 1940, 1949; Miyake & Fujino, 1968). Now newly reported from a second locality in the Mollucas: Kei Islands, Tanimbar.



5.32 *Paranchistus armatus* (H. Milne Edwards, 1837), RMNH.CRUS.D.46092, ovigerous female, pocl. 13.8 mm. A) right second maxilliped, ventral view; B) right third maxilliped, ventral view. Scale = 0.5 mm. Artwork: Charles Fransen.

Genus *Polkamenes* gen. nov.

ZooBank accession link: urn:lsid:zoobank.org:act:7AE1F106-EED4-499E-8D49-9BAFB5235D9A

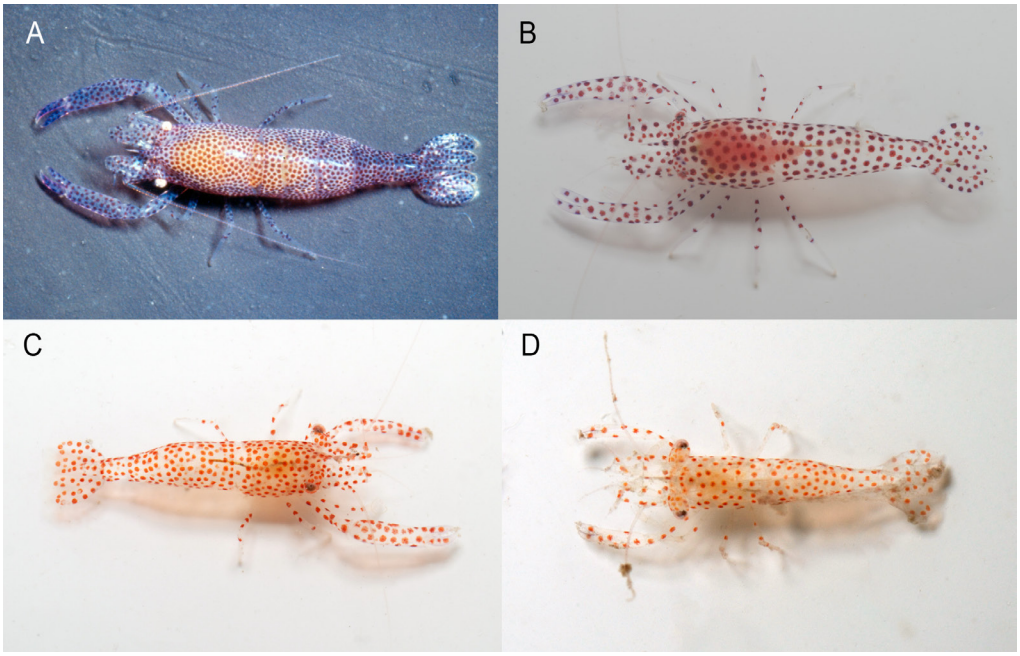
Diagnosis. Small to moderately sized shrimps of subcylindrical body form. Rostrum well developed, compressed, with teeth in distal part, lateral carinae feebly developed. Carapace smooth, glabrous; orbit feebly developed, inferior orbital angle distinct, antennal spine present, supraorbital and epigastric spines absent, hepatic spine mobile, anterolateral margin not or feebly produced.

Abdomen smooth, glabrous, third segment not posterodorsally produced, anterior pleura rounded, posterior blunt. Telson with two pairs of dorsal spines, three pairs of posterior spines. Eye small, cornea globular. Antennula normal, proximal segment of peduncle with distolateral tooth, upper flagellum biramous, flagella short, short ramus reduced. Antenna with basicerite unarmed, scaphocerite well developed. Epistome unarmed. Mandible normal, without palp, molar process robust, incisor process dentate; maxillula with feebly bilobed palp, laciniae normal or broad; maxilla with simple palp, basal endite broad, bilobed, coxal endite obsolete, scaphognathite broad; first maxilliped with slender palp, basal endite broad, fused to coxal endite, exopod well developed, caridean lobe broad, flagellum with numerous plumose setae distally, epipod bilobed; second maxilliped with normal endopod, flagellum with numerous plumose setae distally, epipod subrectangular, without podobranch; third maxilliped with endopod slender, ischiomerus distinct from basis, exopod with numerous plumose setae distally, coxa with oval lateral plate, with rudimentary arthrobranch. Fourth thoracic sternite without median process. First pereiopods chelae with fingers sub-spatulate, cutting edges entire or minutely pectinate. Second pereiopods well developed, similar, unequal, dactylus with large acute triangular tooth in proximal half, fixed finger with series of teeth in proximal part; propodus without ventral oval tympanal organ proximally. Ambulatory pereiopods with simple or biunguiculate dactyli, without basal protuberance; unguis with dorsal pad of fine scale-like spinules. Uropod with protopodite posterolaterally acute, exopod with small mobile distolateral spine.

Etymology. '*Polkamenes*': named after the characteristic blue-, red-, and orange-dotted pattern seen on the species' carapace, abdomen, and appendages 'Polka dots' (English, in combination with the common palaemonid suffix '-*menes*'; see e.g., *Ancylomenes* Okuno & Bruce, 2010, *Laomenes* Clark, 1919, *Periclimenes*). Gender: masculine.

Type species by present designation: *Paranchistus pycnodontae* Bruce, 1978 (= *Polkamenes pycnodontae* (Bruce, 1978)).

We recognise four species: *Polkamenes liui* (Li, Bruce & Manning, 2004); *Polkamenes nobilii* (Holthuis, 1952); *Polkamenes pycnodontae* (Bruce, 1978); and *Polkamenes spondylis* (Suzuki, 1971). All have bivalve hosts. The latter three species, with the biunguiculate ambulatory dactyli, are very similar. *Polkamenes nobilii* and *P. spondylis* are only known from a few specimens and DNA sequences could only be obtained from *P. pycnodontae*. It thus remains unclear if the differences presently used to distinguish these nominal species are indeed species specific or mere intraspecific variation. If more material



5.33 *Polkamenes pycnodontae* (Bruce, 1978). A) RMNH.CRUS.D.48261, from *Hyotissa hyotis* (L., 1758); B) RMNH.CRUS.D.54136, in *Pteria penguin* (Röding, 1798); C) RMNH.CRUS.D.54139, in *P. penguin*; D) RMNH.CRUS.D.57918, stn RAJ.14, in *Lopha* sp. Photo credit: Charles Fransen.

becomes available, for both morphological and molecular phylogenetic analyses, it will be possible to determine with more certainty if these three nominal species are indeed separate biological species. The species can be distinguished as follows:

Key to the species of *Polkamenes*

1. - Ambulatory pereiopods with dactyli simple, not biunguiculate *P. liui*

- Ambulatory pereiopods with dactyli biunguiculate **2.**

2. - First pereiopod chela with cutting edges minutely pectinate **3.**

- First pereiopods with cutting edges entire, not minutely pectinate *P. spondylis*

3. - Anterior margin of basal segment of antennular peduncle rounded; accessory tooth of ambulatory dactyli not ventrally bifurcate *P. pycnodontae*

- Anterior margin of basal segment of antennular peduncle with tooth; accessory tooth of ambulatory dactyli usually ventrally bifurcate *P. nobilii*

***Polkamenes liui* (Li, Bruce & Manning, 2004)**

Paranchistus liui Li, Bruce & Manning, 2004: 535, figs. 21–23 [type locality: [21°N 108°E, 30 m]; Li et al., 2007: 247, fig. 119 (listed); De Grave & Fransen, 2011: 357 (listed).

Diagnosis (based on Li, Bruce & Manning, 2004). Rostrum slightly ventral directed, reaching distal margin of basal segment of antennular peduncle, dorsal and ventral margins subparallel, apex blunt in lateral view, with one distodorsal tooth and one apical tooth which situated at the below middle of the apex. Carapace with small antennal spine; with small articulating hepatic spine situated in depression somewhat posteroventrally of antennal spine; inferior orbital angle produced; anterolateral angle rounded, not produced. Telson with two pairs of small dorsal spines at 0.60 and 0.76 of telson length. Basal segment of antennular peduncle with one distolateral tooth. Scaphocerite well developed with slightly convex lateral margin; distolateral tooth normal, almost reaching the distal margin of the lamina; slit between distolateral tooth and lamina shallow. First pereiopods slender, chela sub-spatulate, cutting edge of fingers entire. Second pereiopods equal in size and form; fingers of equal length; dactylus with one large tooth in proximal half; fixed finger with five small teeth in proximal half; palm flattened. Ambulatory pereiopods with dactyli compressed, unguis not flattened, with sparse fine tubercles on extensor surface, ventral margin simple, not biunguiculate, corpus with distal margin slightly deeper than base of unguis. Protopodite of uropods with strong acute lateral tooth; lateral margin of uropod exopod slightly convex, distolateral with mobile spine.

Colour. Unknown (Li et al., 2004).

Hosts. Unknown (Li et al., 2004).

Distribution. Only known from the type locality in the South China Sea, China (21°N 108°E).

Remarks. The cutting edge of the chela of the first pereiopods is without minute denticulation (Li et al., 2004). As all species in the '*Anchistus* clade' possess a minute denticulation on the cutting edges of the first pereiopod chelae, this would be a reversion to the ancestral state. As only one small specimen is known, more material is needed to check if this character is typical for the species.

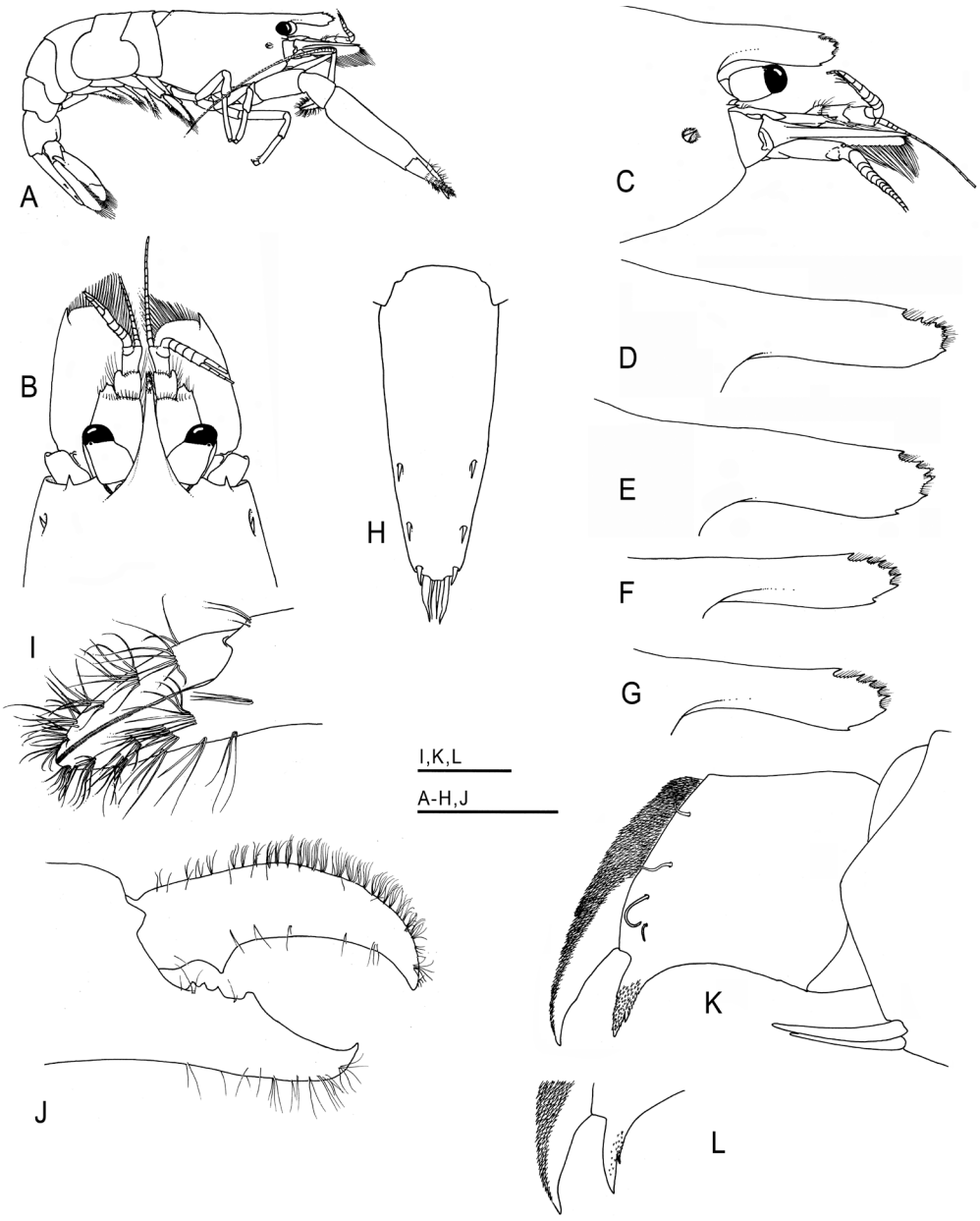
***Polkamenes nobilii* (Holthuis, 1952)**

(fig. 5.34)

Anchistus miersi – Nobili, 1906a: 48; Pérez, 1920: 1027. (Not *A. miersi* (De Man, 1888)).
Paranchistus nobilii Holthuis, 1952: 100–104, figs. 41, 42 [type locality: Arzana Island, Rakas Zakoum Bank, Persian Gulf, out of *Spondylus gaederopus*]; Suzuki, 1971: 110; Bruce, 1972a: 221 (listed); Bruce, 1975c: 162 (listed); Bruce, 1977a: 47; Bruce, 1983b: 890, figs. 6E, 8I, J; Müller, 1993: 51 (listed); Chace & Bruce, 1993: 91 (listed); Fransen, 1994a: 107, 111, text-fig. 2h; Li, 2000: 111, fig. 120 (listed); De Grave & Ashelby, 2011: table 1 (listed); De Grave & Fransen, 2011: 357 (listed).
Anchistus gravieri – McNeill, 1953: 89. (Not *A. gravieri* Kemp, 1922).

Material examined. PERSIAN GULF: 2 males pocl. 3.96 mm, 2 ovigerous females pocl. 5.78 mm, cotypes (ZMA.CRUS.D.102828), Rakas Zakoum Bank, Arzana Island, 1901, in *Spondylus gaederopus* L., coll. C. Pérez, collection J.G. den Man.

Diagnosis. Rostrum well developed, laterally compressed in distal part, straight, almost reaching end of second segment of antennular peduncle, with 5–7 dorsal distal teeth and 0-1 ventral distal tooth; row of setae between dorsal teeth. Carapace with antennal spine well developed; articulating hepatic spine present, situated in depression somewhat posteroventrally of antennal spine; inferior orbital angle slightly produced; anterolateral angle rounded, slightly produced. Telson with two pairs of small dorsal spines at 0.62 and 0.82 of telson length. Basal segment of antennular peduncle with two distal teeth in males, one distolateral tooth and strongly convex anterior margin in females; outer flagellum with basal five segments fused, free shorter ramus of two or three segments. Scaphocerite well developed with almost straight lateral margin; distolateral tooth normal, almost reaching distal margin of lamina, sometimes just overreaching distal margin of lamina; slit between distolateral tooth and lamina shallow. First pereiopod slender, chela sub-spatulate, minutely pectinate in distal two-thirds of lateral margin. Second pereiopods subequal in shape and size; dactylus overreaching fixed finger, with one large triangular tooth in proximal half, densely setose on lateral margin; fixed finger with 4–6 small teeth in proximal half. Ambulatory pereiopods with biunguiculate dactyli; slender accessory tooth usually ventrally bifurcate, more or less covered with minute scale-like spinules; unguis dorsally flattened, with dense cover of minute scale-like spinules. Uropods with protopodite with strong acute lateral tooth; lateral



5.34 *Polkamenes nobilii* (Holthuis, 1952). A–C, E, H–L) ZMA.CRUS.D.102.828, ovigerous female, pocl. 5.8 mm; D) idem, pocl. 5.8 mm; F) male, pocl. 4.0 mm; G) male, pocl. 4.0 mm. A) habitus, lateral view; B) anterior appendages, dorsal view; C) idem, lateral view; D–G) rostrum, lateral view; H) telson, dorsal view; I) left first pereiopod chela, lateral view; J) right second pereiopod chela, lateral view; K) left third pereiopod dactylus, lateral view; L) left fifth pereiopod distal part dactylus and unguis, lateral view. Scale A = 4 mm; B, C = 2 mm; D–H, J = 1 mm; I = 0.4 mm; K, L = 0.1 mm. Artwork: Charles Fransen.

margin of exopod convex with small mobile spine distolaterally.

Remarks. Reexamination of the type specimens yielded some additional morphological data. The first pereiopods have the cutting edges pectinate (fig. 5.34I) in the distal part, this in contrast with Holthuis (1952: 102) who stated that they were not pectinate. The accessory tooth on the ambulatory pereiopods is more or less provided with minute scale-like spinules and bifurcate ventrally. The basal segment of the antennular peduncle has two teeth distolaterally in the males; in the females one distolateral tooth is present while the distal margin is is strongly convex, overreaching the distolateral tooth. The lateral posterior spines of the telson are relatively long in the small males compared with the larger females, in which they are more of the size of Bruce's specimen (Bruce, 1983b: fig. 6E).Teeth on the rostrum are regarded dorsal when setae are in between them. The ventral side of the rostrum does not bear a row of setae.

Polkamenes spondylis (Suzuki, 1971) is only differing from the present species in having a stouter accessory tooth in the dactylus of the ambulatory pereiopods. According to the original description, the chela of the first pereiopods are not sub-spatulate and have entire cutting edges, but Suzuki's illustration (fig. 8a) shows the accessory tooth of the ambulatory pereiopods to be minutely spinulate. This should be checked with the holotype. The species may be conspecific with *Polkamenes nobilii* Holthuis, 1952.

Colour. The colouration of the type-specimens of *P. nobilii* is unknown.

Hosts. Recorded as *Paranchistus nobilii* from *Spondylus gaederopus* L., 1758 (Nobili, 1906a: Pérez, 1920; Holthuis, 1952; Fransen, 1994a); *Tridacna* sp. (Bruce, 1983b); *Pinna* sp. (McNeill, 1953).

Distribution. Rakas Zakoum Bank, Arzana Island, Persian Gulf (Nobili, 1906a; Pérez, 1920; Holthuis, 1952; Fransen, 1994a); Gilbert Islands (McNeill, 1953); Seram Island, Mollucas, Indonesia (Bruce, 1983b).

***Polkamenes pycnodontae* (Bruce, 1978)**

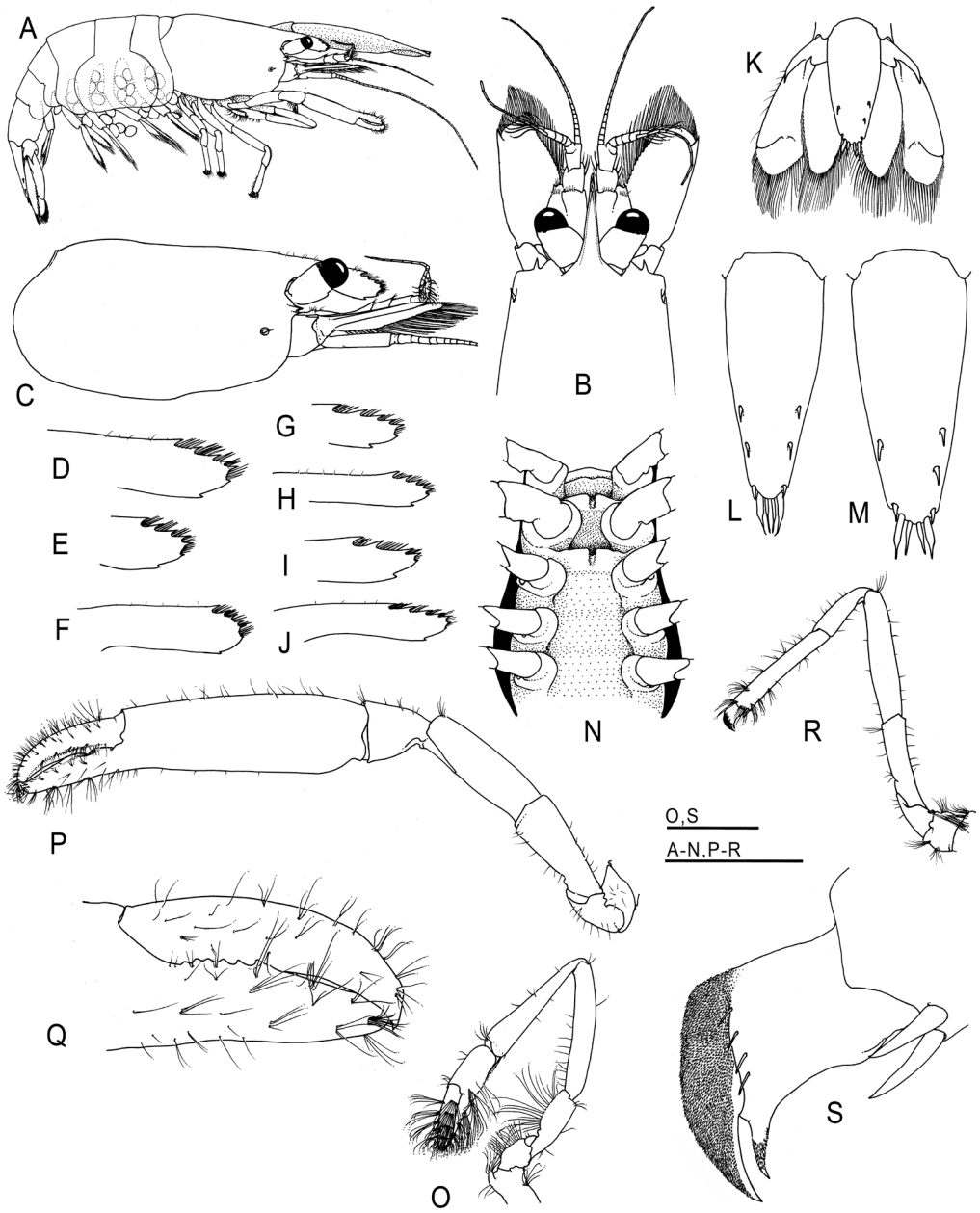
(figs. 5.33, 5.35)

Paranchistus pycnodontae Bruce, 1978c: 233–243, textfigs. 1–5, pl. 39 [type locality: Heron Island, Capricorn Group, Queensland, 3 m]; Bruce, 1980: 180, text-fig. 16, pl. 1 fig.; Bruce, 1981c: 9; Bruce, 1983c: 204 (listed); Bruce, 1990: 16, 18 (listed); Müller, 1993: 51 (listed); Fransen, 1994a: 111; De Grave, 1999:

138, fig. 6, pl. 2b–g; Li, 2000: 112, fig. 122; Li, 2008b: 218; Bruce, 2010a: 64 (listed); Poupin & Juncker, 2010: 276, fig. 276b; De Grave & Fransen, 2011: 357 (listed); Baeza et al., 2013: 172–181, figs. 1–4; Dobson et al., 2014: 8, suppl. fig. 1, appendix 1; Dobson et al., 2016: fig. 7, suppl. inf. I; Horká et al., 2016: 6, figs. 2–4; Chow et al., 2021: table A.1.
Paranchistus serenei Bruce, 1983b: 890, figs. 7H, 9 [type locality: Seleman Bay, Seram Island]; Bruce, 1990: 18 (listed); Chace & Bruce, 1993: 91 (listed); Müller, 1993: 52 (listed); Strack, 1993: 58; Fransen, 1994a: 107, fig. 2e, 112, pl. 2: fig. 4; Poupin, 1998: 15; Li, 2000: 112, fig. 123.

Material examined. INDONESIA: 1 female pocl. 3.87 mm (RMNH.CRUS.D.48261), stn RBE.01, Moluccas, Ambon, Hitu, Ambon Bay, inner bay, W-side of Hunut (= 2 km N of Poka), in front of basecamp, 30.xi.1990, depth 12 m, scuba diving, in large *Pteria* sp., coll. C.H.J.M. Fransen, photo C.H.J.M. Fransen & M.S.S. Lavaleye 36/1-4. – 3 males pocl. 1.93, 2.47, 2.47 mm, 3 ovigerous females pocl. 3.33, 3.40, 4.00 mm (RMNH.CRUS.D.48265), stn RBE.35, Moluccas, Ambon, Hitu, Ambon Bay, inner bay, Martafons, 4.xii.1990, depth 1–3 m, snorkeling, in oyster shells on piles, coll. C.H.J.M. Fransen & J.C. den Hartog, photo M.S.S. Lavaleye 41/34-35, 42/1. – 1 male pocl. 2.67 mm (RMNH.CRUS.D.48266), stn RBE.35, Moluccas, Ambon, Hitu, Ambon Bay, inner bay, Martafons, 4.xii.1990, depth 2–4 m, scuba diving, in the bivalve *Vulsella vulsella* (L.), coll. C.H.J.M. Fransen. – 2 males pocl. 2.13, 2.2 mm, 1 female pocl. 3.07 mm, 1 ovigerous female pocl. 3.25 mm (RMNH.CRUS.D.48262), stn RBE.35, Moluccas, Ambon, Hitu, Ambon Bay, inner bay, Martafons, 4.xii.1990, depth 4–6 m, snorkeling, in *Pteria* sp. shells on piles, coll. J.C. den Hartog. – 1 male pocl. 2.8 mm (RMNH.CRUS.D.48263), stn RBE.35, Moluccas, Ambon, Hitu, Ambon Bay, inner bay, Martafons, 4.xii.1990, depth 2-4 m, snorkeling, in *Pteria* sp. shells on piles, coll. J.C. den Hartog. – 2 males pocl. 1.8, 2.3 mm, 2 ovigerous females pocl. 3.3, 3.7 mm (RMNH.CRUS.D.48264), stn RBE.35, Moluccas, Ambon, Hitu, Ambon Bay, inner bay, Martafons, 4.xii.1990, depth 2–4 m, snorkeling, in oyster shells on piles, coll. J.C. den Hartog. – 1 ovigerous female pocl. 4.00 mm (RMNH.CRUS.D.48260), Snellius II stn 4.069, E of Komodo, Teluk Slawi, northern cape of entrance, 8°36'S 119°31.2'E, 17.ix.1984, rocky shore adjacent to sandy beach. – 1 male pocl. 4.2 mm, 1 ovigerous female pocl. 4.0 mm (RMNH.CRUS.D.49845; GenBank OQ600401 (16S)), stn BAL.26, Bali, N side Nusa Penida, off Desa Ped, 08°40'28"S 115°30'50"E, 21.iv.2001; depth 25 m, scuba diving, deep reef slope, in *Pteria* sp. (Ø 20cm) on *Gorgonaria*, coll. C.H.J.M. Fransen. – 1 male pocl. 2.9 mm (RMNH.CRUS.D.53612; GenBank OQ603093 (COI)), stn TER.37, off Halmahera Island, Teluk Dodinga E, NW of Pulau Jere, 0°50'26"N 127°37'54"E, 13.xi.2009, depth 18 m, scuba diving, in *Pteria penguin* (Röding) on rock, coll. C.H.J.M. Fransen, fcn. CF-00125, photo

TER.37.0072-75. – 1 ovigerous female pocl. 2.9 mm (RMNH.CRUS.D.58089), stn. lem.27, NE Sulawesi, Lembeh Strait, S N Tanjung Pandean, 1°24'21.7074"N 125°10'4.5114"E, 14.ii.2012, depth 22 m, in *P. penguin*, coll. B.T. Reijnen. – 1 ovigerous female pocl. 2.2 mm (RMNH.CRUS.D.58090), stn lem.35, NE Sulawesi, Lembeh Strait, Batu Kapal, 1°32'56.832"N 125°17'31.848"E, 18.ii.2012, depth 22 m, in *P. penguin* (Ø 20 cm), coll. C.H.J.M. Fransen. – 1 ovigerous female pocl. 3.7 mm, 1 male pocl. 3.1 mm (RMNH.CRUS.D.58091), stn SER.06, Java Sea, Kepulauan Seribu (Thousand Islands), off Jakarta, Dapur Island, NW-side, 5°56'44"S 106°43'27"E, 9.ix.2005, unknown depth, scuba diving and snorkelling, in *Hyotissa hyotis* (L.), coll. C.H.J.M. Fransen, photo film 4. – 1 male pocl. 3.0 mm with abdominal bopyroids, 1 ovigerous female, pocl. 3.1 mm, (RMNH.CRUS.D.58092), stn BER.06, NE Kalimantan, Berau Islands, shoal between Lighthouse-2 reef and Derawan Island, 02°12'08.6"N 118°11'34.9"E, 5.x.2003, depth 10 m, scuba diving, in *P. penguin* (Ø 20 cm), coll. J. Goud. – 1 male pocl. 3.8 mm, 1 ovigerous female, pocl. 4.2 mm (RMNH.CRUS.D.58093), BER.05, NE Kalimantan, Berau Islands, Berau delta, Lighthouse-2 reef, 02°09'33.9"N 118°10'11.4"E, 5.x.2003, depth 5-8 m, scuba diving, in *P. penguin* (Ø 20 cm), coll. C.H.J.M. Fransen. – 1 male pocl. 1.3 mm (RMNH.CRUS.D.57918), stn RAJ.14, W Papua, Raja Ampat Islands, Akber Reef, 00°34.253'S 130°39.561'E, 23.xi.2007, depth 6 m, scuba diving, in *Lopha* sp., together with a pair of fish of the genus *Onuxodon* Smith (Carapidae), coll. J. van Egmond, fcn. CF054, photo. – 1 specimen (pleopods missing) pocl. 1.9 mm (RMNH.CRUS.D.54139), stn RAJ.14, W Papua, Raja Ampat Islands, Akber Reef, 00°34.253'S 130°39.561'E, 23.xi.2007, depth unknown, scuba diving, in *P. penguin*, coll. B.W. Hoeksema, fcn. CF055, photo. – 1 male pocl. 2.7 mm (RMNH.CRUS.D.54136), stn RAJ.15, W Papua, Raja Ampat Islands, SW Kri, Kuburan, 00°33.713'S 130°39.673'E, 23.xi.2007, depth unknown, scuba diving, in *P. penguin*, coll. B.W. Hoeksema, fcn. CF062, photo. – 1 male pocl. 2.9 mm (RMNH.CRUS.D.57920), stn RAJ.33, W Papua, Raja Ampat Islands, S Gam, E Besir Bay, 00°29.306'S 130°35.510'E, 29.xi.2007, depth 15 m, scuba diving, in *P. penguin*, coll. C.H.J.M. Fransen, fcn. CF125. **MALAYSIA**: 1 male pocl. 2.4 mm (RMNH.CRUS.D.53797; GenBank OQ603095 (COI)), stn SEM.20, Sabah, Semporna area, Creach Reef, 04°18'58.8"N 118°36'17.3"E, 5.xii.2010, depth 5 m, scuba diving, in *P. penguin* on Antipathes, coll. C.H.J.M. Fransen. – 1 non ovigerous female pocl. 2.7 mm (RMNH.CRUS.D.53867), stn SEM.15, Sabah, Semporna area, Ligitan Isl., Ligitan 3, 04°12'43.0"N 118°54'36.6"E, 3.xii.2010, depth 15 m, scuba diving, in *P. penguin* (Ø 20 cm), coll. C.H.J.M. Fransen. – 1 juvenile pocl. 1.1 mm (RMNH.CRUS.D.53872), stn SEM.15, Sabah, Semporna area, Ligitan Isl., Ligitan 3, 04°12'43.0"N 118°54'36.6"E, 3.xii.2010, depth 15 m, scuba diving, in *P. penguin* (Ø 6 cm), coll. C.H.J.M. Fransen. – 1 male pocl. 3.0 mm (red dots) (RMNH.CRUS.D.53787; GenBank OQ603094 (COI) &



5.35 *Polkamenes pycnodontae* (Bruce, 1978). A–C, E, K) RMNH.CRUS.D.48262, ovigerous female, pocl. 3.3 mm; L) idem, non-ovigerous female, pocl. 3.1 mm; M) idem, non-ovigerous female, pocl. 3.3 mm; G, N–Q) idem, male, pocl. 2.1 mm; I) male, pocl. 2.2 mm; D, R, S) RMNH.CRUS.D.48261, ovigerous female, pocl. 3.9 mm; I) RMNH.CRUS.D.48263, male pocl. 2.8 mm; J) RMNH.CRUS.D.48266, male, pocl. 2.7 mm; H) idem, male, pocl. 2.5 mm; F) idem ovigerous female, pocl. 3.4 mm. A) habitus, lateral view; B) anterior appendages, dorsal view; C) idem, lateral view; D–J) rostrum, lateral view; K) tail fan, dorsal view; L, M) telson, dorsal view; N) thorax, ventral view; O) left first pereiopod, lateral view; P) left second pereiopod, ventral view;

5.35 (*Cont.*) Q) idem, chela, medial view; R) left third pereiopod, lateral view; S) idem, dactylus. Scale A = 4 mm; B, C, K = 2 mm; D–J, L, M, N = 1 mm; O, P, R = 1 mm; Q = 0.4 mm; S = 0.1 mm. Artwork: Charles Fransen.

OQ600402 (16S)), stn SEM.36, Sabah, Semporna area, N Gaya Island (back), 04°37'57.6"N 118°45'32.3"E, 10.xii.2010, depth 10 m, in ? *Spondylus* sp. with even valves, not attached, rim brown-white, gills orange, coll. C.H.J.M. Fransen. – 1 male pocl. 1.8 mm (RMNH.CRUS.D.53926; GenBank OQ603096 (COI)), stn SEM.51, Sabah, Semporna area, Tabawan Isl., 04°47'15.6N" 118°25'00.8"E, 16.xii.2010, depth 18 m, scuba diving, in *P. penguin* on *Antipathes*, coll. C.H.J.M. Fransen. **PHILIPPINES**: 1 male pocl. 3.0 mm, 1 ovigerous female pocl. 4.1 mm (RMNH.CRUS.D.48386), stn CEB.21, Cebu Strait, SW of Bohol, S of Panglao, NE side of Balicasag Island, 'black forest', 9°30.97'N 123°41.34'E, 20.xi.1999, depth 30 m, scuba diving, large octocorals on sand, in *Pteria* sp. (Ø 20 cm) on Antipatharia, coll. C.H.J.M. Fransen. – 1 ovigerous female pocl. 4.6 mm (RMNH.CRUS.D.48385), stn CEB.21, Cebu Strait, SW of Bohol, S of Panglao, NE side of Balicasag Island, 'black forest', 9°30.97'N 123°41.34'E, 20.xi.1999, depth 30 m, scuba. diving, large octocorals on sand, in *Pteria* sp. on Antipatharia, coll. C.H.J.M. Fransen. – 1 male pocl. 2.7 mm, 1 ovigerous female pocl. 3.6 mm (RMNH.CRUS.D.48384), stn CEB.09, Cebu Strait, W of Bohol, N side of Cabilao Island, NE of Looc, 9°53.59'N 123°46.92'E, 12.xi.1999, depth 20 m, scuba divng, overhanging wall with caves, in *P. penguin* on Antipatharia, coll. J. Goud. – 1 male pocl. 1.8 mm (RMNH.CRUS.D.48383), stn CEB.08, Cebu Strait, W of Bohol, S side of Cabilao Island, Cabacungan Point, 9°51.55'N 123°45.95'E, 11.xi.1999, depth 25 m, overhanging wall with caves, in *Lopha cristagalli* (L.) on Antipatharia, coll. C.H.J.M. Fransen. – 1 male pocl. 2.9 mm (RMNH.CRUS.D.48382), stn CEB.05, Cebu Strait, W of Bohol, W side of Cabilao Island, S side fish sanctuary, 9°52.60'N 123°45.61'E, 8.xi.1999, depth 25 m, scuba diving, vertical wall with caves, in large *Pteria* sp. on Antipatharia, coll. C.H.J.M. Fransen.

Diagnosis. Rostrum well developed, laterally compressed in distal part, straight, almost reaching end of second segment of antennular peduncle, with 5–7 dorsal distal teeth and 0-1 ventral distal tooth; row of setae between dorsal teeth. Carapace with antennal spine well developed; articulating hepatic spine present, situated in depression somewhat posteroventrally of antennal spine; inferior orbital angle slightly produced; anterolaterlal angle rounded, slightly produced. Telson with two pairs of small dorsal spines at about 0.6 and 0.8 of telson length. Basal segment of antennular peduncle with distolateral tooth; outer flagellum with basal four segments fused, free shorter ramus of two or three segments. Scaphocerite well developed with almost straight lateral margin; distolateral tooth normal, reaching distal margin of lamina, sometimes

just overreaching distal margin of lamina; slit between distolateral tooth and lamina shallow. First pereiopod slender, chela sub-spatulate, minutely pectinate in distal two-thirds of lateral margin. Second pereiopods subequal in shape and size; dactylus just overreaching fixed finger, with one large triangular tooth in proximal half, densely setose on lateral margin; fixed finger with 4–7 small teeth in proximal half. Ambulatory pereiopods with biunguiculate dactyli; slender accessory tooth, covered with minute scale-like spinules; unguis dorsally flattened, with dense cover of minute scale-like spinules. Uropods with protopodite with strong acute lateral tooth; lateral margin of exopod convex with small mobile spine distolaterally.

As *Polkamenes nobilii* except for the accessory tooth on the dactyli of the ambulatory pereiopods, which is without minute spinulation.

Remarks. Since *Polkamenes nobilii* appears to have the chela of the first pereiopods with pectinate cutting edges, the differences between *P. pycnodontae* and *P. nobilii* are smaller than previously thought. Both species have many setae on the dactylus of the second pereiopods. The only remaining difference is the absence of a minute spinulation in the accessory tooth of the ambulatory pereiopods of *P. pycnodontae* while in *P. nobilii* this is usually present although sometimes indistinct. As no other differences have been found, *P. pycnodontae* may prove to be a synonym of *P. nobilii*.

Differences with *Polkamenes spondylis* are minor. In *P. spondylis* the accessory tooth on the dactylus of the ambulatory pereiopods seems stouter than in the type-species of *P. pycnodontae*. Although not mentioned in the description, fig. 8a of Suzuki (1971) shows a minute spinulation on the accessory tooth of the ambulatory dactyli. Of both species, only the holotypes are known. Additional material could provide information on their intraspecific variation, elucidating their relationship. The holotype of *P. spondylis* should be checked for lateral cutting edges of the chela of the first pereiopods being pectinate and sub-spatulate or not. As in *P. nobilii*, this character could be overlooked in *P. spondylis*.

Colour. “Semi-transparent with numerous small round evenly distributed red chromatophores, all over body, ambulatory pereiopods, antennae and caudal fan. Similar but slightly larger chromatophores over second pereiopods. Chromatophores absent from antennal flagella and propods of ambulatory pereiopods.” (Bruce, 1978c: 238).

Hosts. Recorded from Grypheidae: *Hyotissa hyotis* (L., 1758) (Bruce, 1978c,

1980 as *Pycnodonta hyotis*; De Grave, 1999); *Hyotissa* sp. together with a species of the fish genus *Onuxodon* Smith, 1955 (Carapidae) (Bruce, 1981c; Bruce, 1983b). From Pteriidae: *Pteria penguin* (Röding, 1798) (De Grave, 1999, as 'Magnavicula penguin'). From Margaritidae: *Pinctada margaritifera* (L., 1758) (Poupin, 1998; Li, 2008b). Now newly recorded from: *Lopha cristagalli* (L., 1758) (Ostreidae), *Vulsella vulsella* (L., 1758) (Vulsellidae), and an unknown species of *Spondylus* L., 1758 (Spondylidae).

Distribution. Heron Island, Queensland, Australia (cf. Bruce, 1978c, 1980, 1981c); Lizard Island, Australia (Horká et al., 2016); Taiwan (Chow et al., 2021); Ambon, Indonesia (Fransen, 1994a); Tuamotu Islands, French Polynesia (Poupin, 1998; Li, 2008b). Now for the first time recorded from the following localities in Indonesia: E of Komodo, Bali, Teluk Dodinga (off Halmahera mainland), NE Sulawesi, Seribu (Thousand Islands), NE Kalimantan, West Papua. Also newly recorded from Sabah, Malaysia; and Cebu, Philippines.

***Polkamenes spondylis* (Suzuki, 1971)**

Paranchistus spondylis Suzuki, 1971: 15–19, figs. 8, 9 [type locality: Shiraiso, near the Manazuru M.B.L.]; Bruce, 1972a: 221 (listed); Bruce, 1975a: 162 (listed); Müller, 1993: 52 (listed); Fransen, 1994a: 111 (listed); Li, 2000: 113, fig. 124 (listed); Bruce, 2003b: 223 (listed); Li et al., 2004: 538, fig. 24; Hayashi, 2005: 516, figs. 511, 512; Hayashi, 2006: 510; Li et al., 2007: 247, fig. 120 (listed); De Grave & Fransen, 2011: 357 (listed).
Paranchistus spondylus – Müller, 1993: 52 [erroneous spelling].

Diagnosis. Rostrum well developed, laterally compressed in distal part, straight, almost reaching end of second segment of antennular peduncle, with four dorsal distal teeth and 0–1 ventral distal tooth; row of setae between dorsal teeth. Carapace with antennal spine well developed; articulating hepatic spine present, situated in depression somewhat posteroventrally of antennal spine; inferior orbital angle slightly produced; anterolateral angle rounded, slightly produced. Telson with two pairs of small dorsal spines at about 0.6 and 0.8 of telson length. Basal segment of antennular peduncle with distolateral tooth; outer flagellum with basal three segments fused, free shorter ramus of four segments. Scaphocerite well developed with slightly convex lateral margin; distolateral tooth normal, reaching distal margin of lamina, slit between distolateral tooth and lamina shallow. First pereiopod slender, chela sub-spatulate, cutting edges entire. Second pereiopods subequal in shape and size; dactylus as long as fixed finger, with one large triangular tooth in proximal half, densely setose on lateral margin; fixed finger with 4–6 small teeth in proximal half. Ambulatory

pereiopods with biunguiculate dactyli; slender accessory tooth, with minute scale-like spinules; unguis dorsally not flattened, with dense cover of minute scale-like spinules. Uropods with protopodite with strong acute lateral tooth; lateral margin of exopod convex with small mobile spine distolaterally.

Remarks. Like in *Polkamenes liui*, the cutting edge of the chela of the first pereiopods is without minute denticulation according the author (Suzuki, 1971). As the other species in the '*Anchistus* clade' do possess the minute denticulation on the cutting edges of the first pereiopod chelae, this is a reversion to the plesiomorphic state. As only the holotype and two juveniles (Li et al., 2004) are known, more material will be needed to check if this character really is typical for the species. Although not reported in the description of the species, Suzuki (1971, fig. 8a) figures the accessory tooth of the ambulatory dactyli with small spinules.

Colour. "The body is translucent. Many small reddish spots can be seen on the body and thoracic appendages as shown in text-fig. 8 in dotting." (Suzuki, 1971: 18). The specimen from Semporna also had red dots.

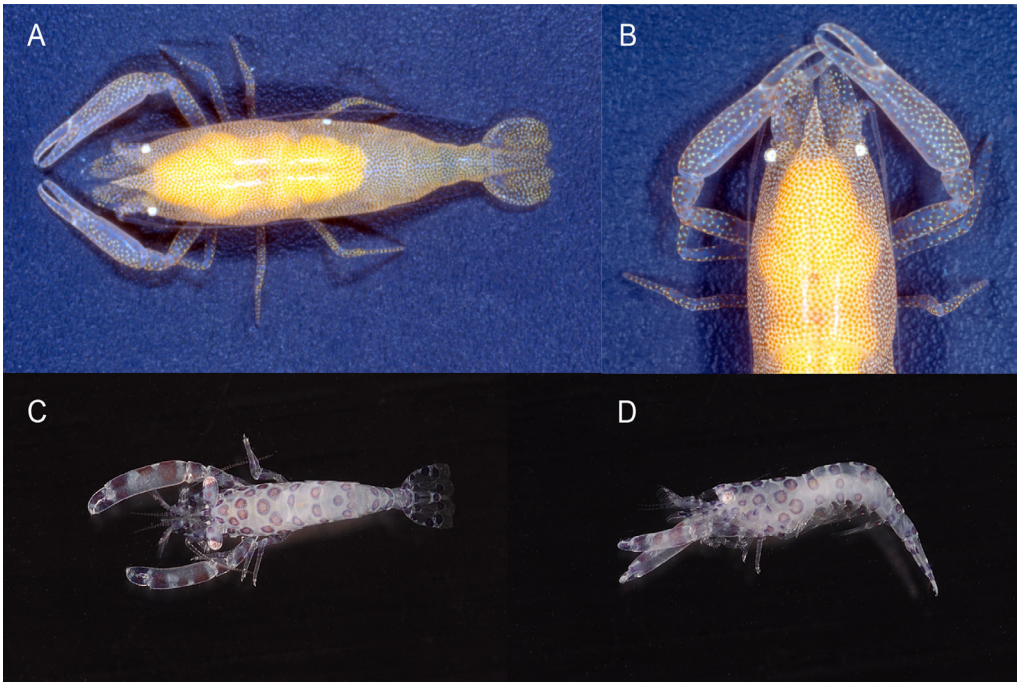
Hosts. Recorded from Spondylidae: *Spondylus squamosus* Schreibers, 1793 (Suzuki, 1971; Hayashi, 2005, 2006; as *Spondylus barbatus* Reeve, 1856). Also from Vulsellidae: *Vulsella vulsella* (L., 1758) (Li et al., 2004; Hayashi, 2006).

Distribution. Shiraiso, Manazura-Machi, Kanagawa Prefecture, Japan, (cf. Suzuki, 1971, Hayashi, 2005, 2006); South China Sea (Li et al., 2004).

Genus *Tympanicheles* gen. nov.

ZooBank accession link: urn:lsid:zoobank.org:act:5020D378-966A-4801-BD63-C26BA625E675

Diagnosis. Small to moderately sized shrimps of subcylindrical body form. Rostrum well developed, compressed, with teeth in distal part, lateral carinae feebly developed. Carapace smooth, glabrous; orbit feebly developed, inferior orbital angle distinct, antennal spine present, supraorbital and epigastric spines absent, hepatic spine absent or present, if present mobile, anterolateral margin not or feebly produced. Abdomen smooth, glabrous, third segment not posterodorsally produced, anterior pleura rounded, posterior blunt. Telson with two pairs of dorsal spines in distal half, three pairs of posterior spines. Eye small, cornea globular. Antennula normal, proximal segment of peduncle with distolateral tooth, upper flagellum biramous, flagella short, short ramus reduced.



5.36 A, B) *Tympanicheles ornatus* (Holthuis, 1952), RMNH.CRUS.D.42794, from *Atrina vexillum* (Born, 1778); C, D) *Tympanicheles pectinis* (Kemp, 1925), RMNH.CRUS.D.58094, Vanuatu, stn LD25, host unknown. Photo credits: A, B) Charles Fransen; C, D) Tin-Yam Chan.

Antenna with basicerite unarmed, scaphocerite well developed. Epistome unarmed. Mandible normal, without palp, molar process robust, incisor process dentate; maxillula with feebly bilobed palp, laciniae broad; maxilla with simple palp, basal endite broad, bilobed, coxal endite obsolete, scaphognathite broad; first maxilliped with slender palp, basal endite broad, fused with coxal endite, exopod well developed, caridean lobe broad, flagellum with numerous plumose setae distally, epipod bilobed; second maxilliped with normal endopod, flagellum with numerous plumose setae distally, epipod subrectangular, without podobranch; third maxilliped with endopod slender, ischiomerus distinct from basis, exopod with numerous plumose setae distally, coxa with oval lateral plate, with rudimentary arthrobranch. Fourth thoracic sternite without median process. First pereiopods chelae with fingers sub-spatulate, cutting edges or minutely pectinate. Second pereiopods well developed, similar, unequal, dactylus with large acute triangular tooth in proximal half, fixed finger with series of teeth in proximal part; palm with oblong ventral oval tympanal organ over entire length. Ambulatory pereiopods with dactyli simple, corpus without distinct accessory tooth, with carina with small denticle(s) on flexor margin, with dorsodistal transverse wrinckles; without basal protuberance; unguis not flattened, with fine scale-like spinules dorsally. Uropod with protopodite posterolaterally acute, exopod with small mobile distolateral spine.

Etymology. '*Tympanicheles*': named after the 'drum-like' veil on the species' major chela. Built from '*Tympani-*', a suffix for drum in Latin ('Tympanum'), and '*-cheles*' for claws in Latin. Gender: masculine.

Type species by present designation: *Paranchistus ornatus* Holthuis, 1952 (= *Tympanicheles ornatus* (Holthuis, 1952)).

We tentatively recognise two species: *Tympanicheles ornatus* (Holthuis, 1952), and *Tympanicheles pectinis* (Kemp, 1925).

Key to the species of *Tympanicheles*

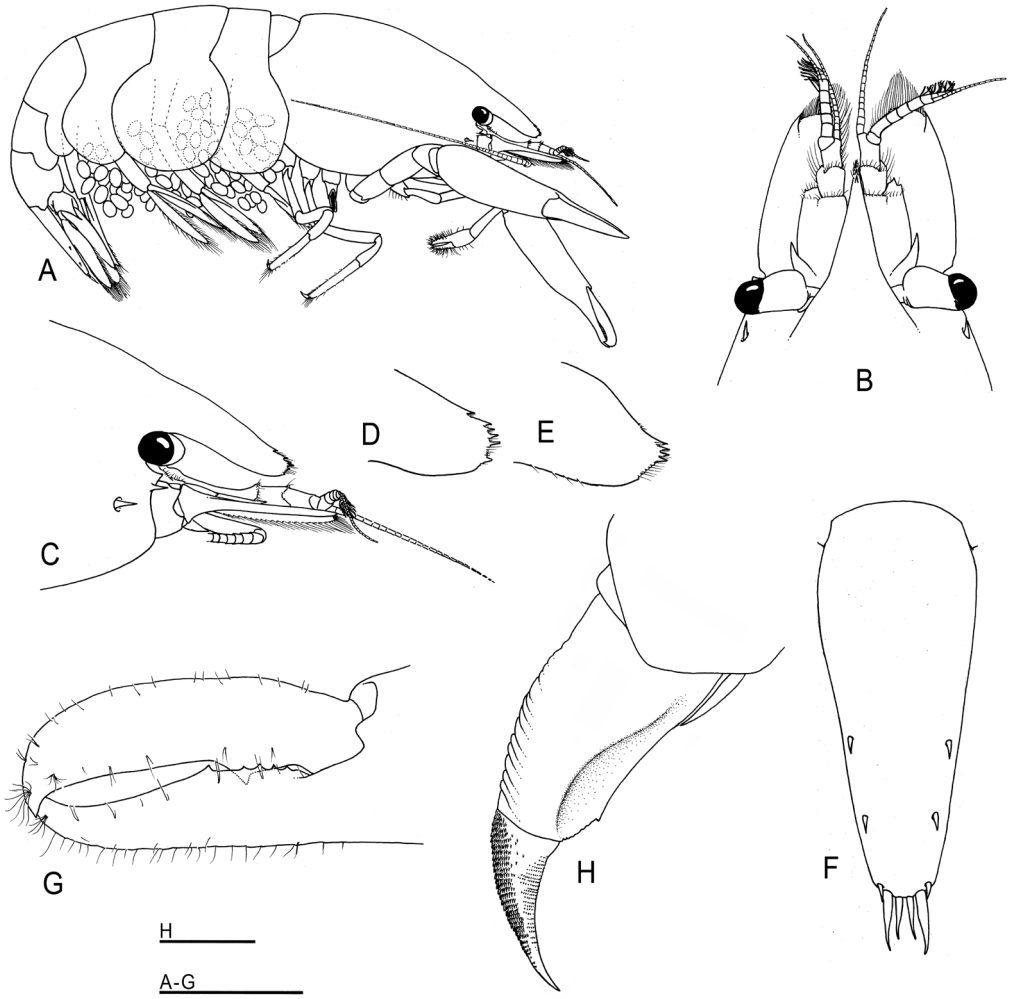
1. - Movable hepatic spine present; rostrum with series of 4–6 subdistal and distal teeth; ambulatory pereiopod dactyli oblong, with unguis slightly curved *T. ornatus*
- Hepatic spine absent; rostrum with 3 distal teeth; ambulatory pereiopod dactyli short, compact, with unguis strongly hooked *T. pectinis*

***Tympanicheles ornatus* (Holthuis, 1952)**

(figs. 5.36A–B, 5.37, 5.39A, C)

Paranchistus ornatus Holthuis, 1952: 97–100, figs. 39, 40 [type locality: Mozambique]; Barnard, 1955 (listed): 5; Barnard, 1958 (listed): 12; Rosewater, 1961: 195; Jacquotte, 1963: 61; Hipeau-Jacquotte, 1967: 153–166; Hipeau-Jacquotte, 1971: 194, figs. 4, 5 9; Bruce, 1972a: 219–221, colour figures (listed); Hipeau-Jacquotte, 1972a: 288, fig. 2a2; Hipeau-Jacquotte, 1972b: 10, 14, 17, figs. 1a2–e2, 3a2, 3b2, 4a2–d2, 6b, 7a2–f2, 8a2–d2; Thomassin, 1972: 311; Hipeau-Jacquotte, 1973a; 96–116, fig. 1a2–e2, 3a2–b2, 4a2–d2, 5, 6b, 7a2–f2, 8a2–d2; Hipaeu-Jacquotte, 1973b: 63–70; Hipeau-Jacquotte, 1974a: 367–382; Hipeau-Jacquotte, 1974b: 383–402; Hipeau-Jacquotte, 1974c: 403–407; Hipeau-Jacquotte, 1974d: 359–386; Thomassin, 1974: 306; Bruce, 1975a: 162 (listed); Bruce, 1976a: 93; Bruce, 1976d: 471: Monod, 1976a: 839 (listed); Bruce, 1978a: 119; Bruce, 1978b: 279; Bruce, 1980: 179, textfig. 1a, pl. 1 figs. a–c; Müller, 1993: 51 (listed); Fransen, 1994a: 107, 111, 112, fig. 2g, pl. 2 fig. 3; Fransen, 1994b: 117, fig. 2g, pl. 2D; Li, 2000: 111, fig. 121 (listed); De Grave & Fransen, 2011: 357 (listed); Chow et al., 2021: table A.1 (listed).

Material examined. RED SEA: 3 males pocl. 3.90, 4.22, 4.74 mm, 7



5.37 *Tympanicheles ornatus* (Holthuis, 1952). A–C, F–H) RMNH.CRUS.D.41497, ovigerous female, pocl. 6.4 mm; D) RMNH.CRUS.D.41491, ovigerous female, pocl. 8.7 mm; E) idem, pocl. 8.8 mm. A) habitus, lateral view; B) anterior appendages, dorsal view; C) anterior appendages, lateral view; D, E) rostrum, lateral view; F) telson, dorsal view; G) right second pereiopod chela, medial view; H) right third pereiopod dactylus, medial view. Scale A = 4 mm; B, C = 2 mm; D, E, G = 1 mm; H = 0.1 mm. Artwork: Charles Fransen.

ovigerous females pocl. 7.40, 7.92, 8.18, 8.18, 8.31, 8.70, 8.83 mm (RMNH.CRUS.D.41491), Nocra Island, 18.iii.1962, Israel Red Sea Expedition no. E62/2028. – 1 ovigerous female pocl. 6.10 mm (RMNH.CRUS.D.41492), Loe Salina, 1965, in *Atrina* sp., Israel Red Sea Expedition no. E65/1868. – 3 males pocl. 3.64, 3.90, 4.42 mm, 3 ovigerous females pocl. 7.53, 7.66, 8.70 mm (RMNH.CRUS.D.41493), off Nocra Island, 17.iii.1962, in *Atrina* sp., Israel Red Sea Expedition no. E62/1350. – 1 male pocl. 5.32 mm, 1 ovigerous female pocl. 9.09 mm (RMNH.CRUS.D.41494), NE of Nocra Island, 18.ii.1962, depth 2-3

m, in *Atrina* sp. (10049), Israel Red Sea Expedition no. E62/1354. – 3 males pocl. 4.35, 4.87, 4.94 mm (RMNH.CRUS.D.41495), Nocra Island, 18.iii.1962, Israel Red Sea Expedition no. E62/2059. – 1 male pocl. 4.48 mm (RMNH.CRUS.D.41496), Umm Aabak, 4.v.1962, Israel Red Sea Expedition no. 1452. – 2 males pocl. 3.05, 3.57 mm, 1 female pocl. 5.52 mm, 1 ovigerous female pocl. 6.36 mm (RMNH.CRUS.D.41497), SW of Umm Aabak, near Nocra Island, 22.iii.1962, depth 0-3 m, in *Atrina* sp., Israel Red Sea Expedition no. E62/1379. **MOZAMBIQUE**: 1 male holotype pocl. 5.97 mm (ZMA.CRUS.D.102827), published by Holthuis, 1952: 97–100, figs. 39, 40. **SEYCHELLES**: 1 ovigerous female pocl. 7.7 mm (RMNH.CRUS.D.42794), niop-E stn SEY.748, E of Mahé, E coast of Sainte Anne Island, 4°37'S 55°31'E, 25.xii.1992, depth 10 m; scuba diving, in *Atrina vexillum* (Born), coll. C.H.J.M. Fransen.

Diagnosis. Rostrum well developed, laterally compressed towards distal part, bent downwards, reaching end of TE second segment of antennular peduncle, with 3–5 dorsal distal teeth, without ventral distal teeth; row of setae between dorsal teeth. Carapace with well-developed antenna tooth, with small articulating hepatic spine, situated somewhat posteroventrally of antennal spine; inferior orbital angle acute, slightly produced; anterolateral angle broadly rounded, slightly produced. Sixth abdominal segment with posterior ventral margin rounded. Telson with two pairs of small dorsal spines at 0.58 and 0.81 of telson length; lateral pair of terminal spines placed just behind intermediate pair. Basal segment of antennular peduncle with one (seldom two) distal teeth, anterior margin almost straight; outer flagellum with basal 4–6 segments fused, free shorter ramus of two or three segments. Scaphocerite well developed with slightly convex lateral margin; distolateral tooth normal, almost reaching the distal margin of the lamina; slit between distolateral tooth and lamina shallow. First pereiopod slender, chela sub-spatulate, minutely pectinate in distal two-third of lateral margin. Second pereiopods usually equal in size and shape in females, subequal in size in males; dactylus with one large tooth in proximal half; fixed finger with 5–6 small teeth in proximal half; palm with oblong ventral oval tympanal organ over entire length. Ambulatory pereiopods with slender long slightly curved non-biunguiculate dactyli; serrated carinae on distoventral margin of corpus; unguis not flattened, dorsally and ventrally with transverse rows of scale-lake spinules. Uropod with protopodite posterolaterally acute, exopod with small mobile distolateral spine.

Remarks. Smaller specimens have the dorsal rostral teeth in the distal fourth of the rostrum while larger specimens have them all at the tip. This indicates that the dorsal rostral teeth are placed more distally after each moult as has been observed in *Anchistus australis*.

Colour (fig. 5.36A,B). “Uniformly covered all over body and appendages with small red chromatophores, with minute yellow and white chromatophores interspersed.” (Bruce, 1978a: 119; see colour figures Bruce, 1972a).

Hosts. Reported from Pinnidae: *Atrina vexillum* (Born, 1778) (Hipeau-Jacquotte, 1967, 1973b, 1974b; Bruce, 1976e, 1978a, 1978b, 1980; Fransen, 1994b); *Pinna bicolor* Gmelin, 1791 (Rosewater, 1961; Hipeau-Jacquotte, 1967).

Distribution. Distributed along the East African coast: Mocambique (Holthuis, 1952); Zanzibar and Kenya (cf. Rosewater, 1961; Bruce, 1976d, 1980), Madagascar (cf. Hipeau-Jacquotte, 1967, 1971, 1972a, 1972b, 1973a, 1973b, 1974a, 1974b, 1974c, 1974d; Thomassin, 1972, 1974; Bruce, 1978b), Comoro Islands (cf. Bruce, 1978a); Seychelles (Fransen, 1994b); Nocra Island, Red Sea (Fransen, 1994a).

***Tympanicheles pectinis* (Kemp, 1925)**

(figs. 5.36C, D, 5.38)

Anchistus pectinis Kemp, 1925: 327–330, figs. 19, 20 [Type locality: Octavia Bay, Nancowry Harbor, Nicobars]; Holthuis, 1952: 13 (listed); Bruce, 1967: 568; Fujino & Miyake, 1967: 293-296, figs. 1–3; Suzuki, 1971: 101–106, figs. 5–7, pl. 2; Bruce, 1972a: 219 (listed); Bruce, 1975a: 150, 162 (listed); Bruce, 1976d: 465; Bruce, 1977b: 175–176, fig. 6e; Bruce, 1983c: 200 (listed); Bruce, 1990: 15, 18 (listed); Bruce, 1991a: 261, 275, fig. 24; Bruce, 1991b: 378–381, figs. 56–57, 71 d-f; Müller, 1993: 11 (listed); Fransen, 1994a: 107, 111, fig. 2b; Bruce, 1996: 205, figs. 2, 3; Li, 2000: 12, fig. 12 (listed); Yamaguchi & Holthuis, 2001: 107, 109, 135, fig.; Hayashi, 2002: 224, figs. 432k, l, 434i, j, 435i, j; Hayashi, 2006: 510, 512; De Grave & Fransen, 2011: 340 (listed); Radhakrishnan et al., 2012: table 1 (listed); Samuel et al., 2016: table 1 (listed).
Anchistus misakensis Yokoya, 1936: 136, fig. 5 [Type locality: Octavia Bay, Nancowry Harbor, Nicobars]; Holthuis, 1952: 13; Bruce, 1967: 568 (key).

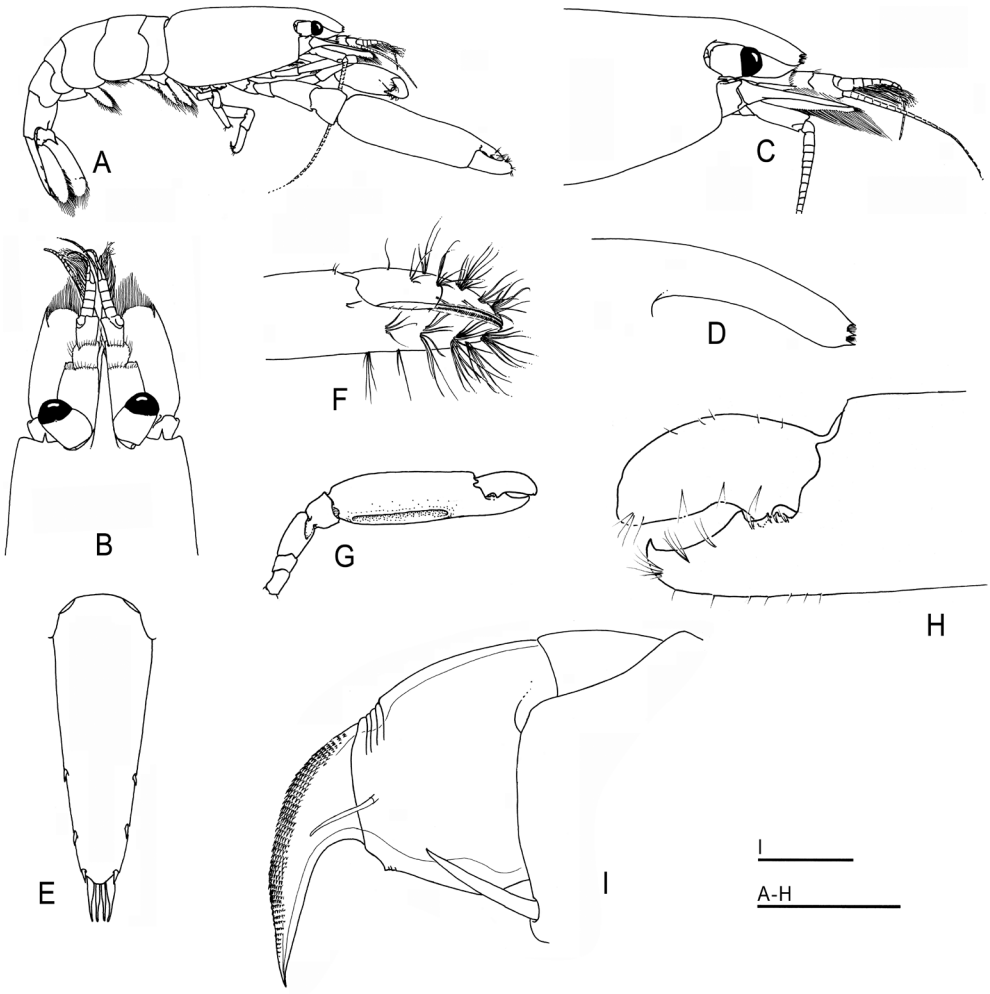
Material examined. JAPAN: 1 ovigerous female pocl. 3.51 mm, 1 male pocl. 3.90 mm (RMNH.CRUS.D.26972), Kanagawa prefecture, Sagami Bay near Manazuru, S of Odawara, 25.viii.1968, coll. H. Suzuki. **INDONESIA**: 1 non ovigerous female pocl. 1.8 mm (RMNH.CRUS.D.47465), stn MAL.21, Moluccas, Ambon, Ambon bay, N coast Cape Hatupero, E of Lilibooi, 03°44’S 128°02’E, 20.xi.1996, depth 10 m, scuba diving, in pectinid shell, coll. C.H.J.M. Fransen. **VANUATU**: 1 non ovigerous female pocl. 1.6 mm (RMNH.CRUS.D.58094),

stn LD25, N Aoré, 15°19'29"S 167°06'19"E, 7.x.2006, scuba diving, depth unknown.

Diagnosis. Rostrum well developed, laterally compressed towards distal part, bent downwards; reaching just beyond basal segment of antennular peduncle, with 3, seldom 2 distal teeth with setae in between. Antennal spine present, hepatic spine absent; inferior orbital angle slightly produced; anterolateral angle rounded, not produced. Sixth abdominal segment with posterior ventral margin rounded. Telson with dorsal pairs of spines at 0.61 and 0.79 of telson length; lateral pair of terminal spines placed just behind intermediate pair. Basal segment of antennular peduncle with one small distolateral tooth. Scaphocerite with lateral margin almost straight, slit between distolateral tooth and lamina of moderate depth. Chela of first pereiopods spatulate, with minutely pectinate cutting edges distolaterally. Second pereiopods unequal; dactylus exceeding fixed finger, distally strongly hooked, with one large triangular tooth in proximal half, fixed finger with 4-6 small teeth in proximal half of which distalmost largest; palm with oblong oval tympanal organ on entire medio-ventral surface. Ambulatory dactyli simple; corpus with carinated ventral margin with small triangular subdistal denticle, distodorsally transversely wrinckled; unguis strongly hooked, not flattened, with transverse rows of minute scale-like spinules dorsally. Uropod with protopodite posterolaterally acute, exopod with small mobile distolateral spine.

Remarks. The variability in the development of the accessory tooth on the dactylus of the ambulatory pereiopods ranging from its absence in Kemp's material to well developed in the type of *Anchistus* misakiensis Yokoya, 1936 and the illustrated specimen from New Caledonia (indicated as Lagon, stn. CC 147) of Bruce (1991b) attributes to the opinion that *A. misakiensis* is conspecific with *Tympanicheles pectinis*. In the two specimens studied the accessory tooth was absent or minute (fig. 5.38I). Differences in the male first endopod as noted by Bruce (1991b: 381) could be due to different developmental stages involved. In the present male (pocl. 3.90 mm) 13 spinules are present on the proximal median margin. In the male specimen (pocl. 2.7 mm) from New Caledonia (Bruce, 1991b: fig. 56k) only six spinules are present while in a large male specimen (pocl. between 3.65 and 5.88 mm) from Japan (Suzuki, 1971: fig. 7.4) at least 10 spinules are present.

Colour (fig. 5.36C, D). "The body is translucent. A number of reddish circular marking, which are encircled with purplish colouration, are found on the body and appendages; these marks are variable in size and number. The distal portion of the antennular and antennal peduncles, the scaphocerite, the second



5.38 *Tympanicheles pectinis* (Kemp, 1925), RMNH.CRUS.D.26972, male, pocl. 3.9 mm. A) habitus, lateral view; B) anterior appendages, dorsal view; C) idem, lateral view; D) rostrum, lateral view; E) telson, dorsal view; F) left first pereiopod chela, lateral view; G) right second pereiopod, ventral view; H) idem, chela, medial view; I) right third pereiopod dactylus, lateral view. Scale A, G = 4 mm; B, C = 2 mm; D–F, H = 1 mm; I = 0.1 mm. Artwork: Charles Fransen.

chela, the telson and the uropod is purplish in colour." (Suzuki, 1971: 13, textfig. 5, pl. 2).

Hosts. Reported from Pectinidae: *Bractechlamys vexillum* (Reeve, 1853) (Bruce, 1996, as *Semipallium vexillum* (Reeve, 1853)); *Mimachlamys crassicostata* (G. B. Sowerby II, 1842) (Hayashi, 2002, 2006, as *Mimachlamys nobilis* (Reeve, 1852); *Pecten albicans* (Schröter, 1802) (Fujino & Miyake, 1967; Suzuki, 1971; Hayashi, 2002, 2006, as *P.* (*Notovola*) *albicans*); *Pecten* sp. (Kemp, 1925); *Yilistrum balloti* (Bernardi, 1861) (Yokoya, 1936; Suzuki, 1971; Bruce, 1977b,

1983b, 1991a, 1991b, 1996; Hayashi, 2002, 2006, as *Amusium* '*japonicum*' *balloti* (Bernardi, 1861)); Pectinid bivalve (Bruce, 1976d). Also one record from an unidentified bivalve (Bruce, 1977b).

Distribution. Nicobar Islands (Kemp, 1925); Zanzibar (Bruce, 1976d); Japan (Yokoya, 1936; Fujino & Miyake, 1967; Suzuki, 1971; Hayashi, 2002, 2006); Townsville, Australia (Bruce, 1977b), Keppel Bay and Magnetic Island, Australia (Bruce, 1983b); New Caledonia (Bruce, 1991a, 1991b, 1996). Now newly recorded from Ambon, Indonesia, and Vanuatu.

Key to the genera of the '*Anchistus*' clade

1. - Unguis of ambulatory pereiopods with few scattered minute scales dorsally **2.**
- Unguis of ambulatory pereiopods with dense cover of minute scales dorsally **4.**

2. - Ambulatory dactyli without obtuse compressed ventral process **3.**
- Ambulatory dactyli with obtuse compressed ventral process *Dasella*

3. - Ambulatory pereiopods biunguiculate; mobile hepatic spine present *Paranchistus*
- Ambulatory pereiopods simple; hepatic spine absent *Ensiger*

4. - Rostrum laterally compressed, distally acute, with teeth in distal part **5.**
- Rostrum broad, swollen, distally truncate, without teeth *Neoanchistus*

5. - Palm of second chelipeds with tympanal organ **6.**
- Palm of second chelipeds without ventral tympanal organ; mobile hepatic spine present *Polkamenes* gen. nov.

6. - Tympanal organ on palm of second cheliped oblong; dorso-distal part of corpus of ambulatory dactyli wrinkled *Tympanicheles* gen. nov.
- Tympanal organ on palm of second cheliped restricted to proximal part of palm; dorso-distal part of corpus of ambulatory dactyli glabrous *Anchistus*

Discussion

Outgroup placements

All outgroup species, except *Cuapetes tenuipes* and *Palaemonella rotumana*, are part of 'Clade 5' recovered by Horká et al. (2016), which has a topology that only partly matches the current results. The coral-associated *Actinimenes inornatus*, *A. ornatus*, and *Periclimenes kempi* split off as a separate clade in the phylogenies resulting from the concatenated and TE alignment (figs. 5.1, 5.2). This seems to be congruent with the results of Horká et al. (2016). Here, the present ingroup (consisting of three species) is a sister of a clade comprised of *Lipkemenes lanipes*, *Periclimenes colemani*, and *Zenopontonia rex*. In our analyses (TE and concatenated datasets), these outgroups seem to be placed elsewhere: *L. lanipes* is placed basal to a clade including both *P. colemani*, *Z. rex*, as well as the ingroup (figs. 5.1, 5.2); and *Z. rex* and *P. colemani* seem to be clustering together in all analyses. In the phylogeny reconstruction resulting from the concatenated dataset, *Z. soror* clusters together with *Z. rex* and *P. colemani*, while in the TE-analysis *Z. soror* is placed basally to these species and the ingroup (figs. 5.1, 5.2). Horká et al. (2016) found *Z. soror* to be basal to a clade containing all three before-mentioned outgroups, as well as the ingroup. In Chow et al. (2021), the placement of these species is also different, placing *Z. soror* basal to the current ingroup, and *L. lanipes* basal to *Z. rex* and *P. colemani* (similar to Horká et al., 2016). One has to keep in mind that the present phylogeny reconstructions are based on two molecular markers, compared to four and eight markers used by Horká et al. (2016) and Chow et al. (2021), which partially use the same sequences.

In short, the systematic position of the selected outgroups remains elusive. In order to explore the systematic position of all members of our outgroup genera, a large-scale systematic review, combining molecular and morphological data, is needed, especially involving *Zenopontonia* and related echinoderm-associated genera (e.g., *Sandimenes* Li, 2009, various members of *Periclimenes*, and *Allopontonia* Bruce, 1972, which appear to be somewhat related (Chow et al., 2021)).

Uncertain placements and polytomies

Although most ingroup clades seem to be recovered in a similar way across the various analysis methods, there are a few species with aberrant placements. *Paranchistus armatus*, the type species of *Paranchistus*, was recovered based on the concatenated alignment as a part of a large polytomy, including various outgroups (see above) and the rest of the ingroup, suggesting a basal placement of the species. In the tree based on the TE-approach, the species is placed next to a clade including *Ensiger custos* and *E. custoides*, with *Dasella* being placed

basal to this group. The morphological features placed *P. armatus* with the two species of *Ensiger*. Although this difference in placement is not interfering with the taxonomic decisions made in this study, a more basal placement of this species in the TE-approach would probably directly influence the low ML-value of the character state reconstruction of the common ancestor (now P = 0.508; fig. 5.3) to be higher in favour of a bivalve host.

The genera *Anchistus* and *Paranchistus* were in need of reappraisal after being recovered as polyphyletic in the phylogeny reconstruction (figs. 5.1, 5.2). Two species, however, were also represented by more than one lineage. This is only the case in the phylogenetic tree resulting from the concatenated molecular dataset (fig. 1). First, *Anchistus demani* is represented by three specimens, two of which cluster together (KP759379/KP725496 and JX185707) next to *A. miersi*. The other specimen, making the placement of the species paraphyletic, is only represented by a newly acquired 16S barcode (RMNH.CRUS.D.48354), and is placed basal to a clade comprising the other specimens of *A. demani* and *A. miersi*. This deviating placement is probably caused by the limited phylogenetic information of 16S, when used on its own (supplementary fig. S1). In a similar light, the specimens representing *Ensiger custos* are grouping together, but in two lineages. Two specimens group together (KF738360 and KJ584120), while one other specimen is placed basal to this clade (RMNH. CRUS.D.57963), in both the tree resulting from the TE-approach (fig. 5.2), as well as the tree resulting from the concatenated molecular alignment (fig. 5.1). Again, this could be the result from the first two specimens only being represented by 16S (supplementary fig. S1), while the latter is represented by both molecular markers.

Horká et al. (2016) included only three of the present ingroup species: *Dasella herdmaniae* was recovered in a basal position to *Ensiger custoides* and *Polkamenes pycnodontae* (sensu Horká et al., 2016), which complies to the current results. Similarly, Chow et al. (2021) recovered a clade including *E. custoides* and *E. custos* as a sister group to a clade including *P. pycnodontae* and an unidentified species of *Paranchistus* (probably part of *Polkamenes*). This clade was, however, recovered as a sister to a branch containing *D. herdmaniae* and *Tympanicheles ornatus* (Chow et al., 2021). In our results, *Dasella* is always placed basally to the other mentioned species and *T. ornatus* is grouped together with the more derived species of the ingroup (figs. 5.1, 5.2). The 16S barcode of *T. ornatus* featured in Chow et al. (2021) is the same as the one used in the current study, only here it is accompanied by a COI-barcode provided by Aznar-Cormano et al. (2015). Based on this, we believe that a more derived placement of *T. ornatus* is correct.

The phylogenetic trees that are solely based on molecular data (fig. 5.1, supplementary figs. S1–S3) do not pose problems regarding polytomies. The tree resulting from combined molecular and morphological data, however, shows one major polytomous split (fig. 5.2). Polytomies are common in trees based on limited morphological data, and can only be resolved by increasing the number of morphological characters (Neumann et al., 2021). The present polytomous branch splits into three monophyletic clades: *Anchistus*, *Neoanchistus*, and *Tympanicheles*. Considering the molecular trees (fig. 5.1, supplementary figs. S1–S3), the placement of the three *Anchistus* species and *T. ornatus* seems to be fixed. The placement of *T. pectinis* inside *Tympanicheles*, solely based on morphology, is tentative but convincing. What remains is the placement of *Neoanchistus* within this group; both species' placement is based solely on morphological features, possibly causing the polytomy. Whether *Neoanchistus* should be placed more basal to *Tympanicheles*, or closer to *Anchistus*, remains unclear. Acquiring DNA extractions from fresh specimens of *Neoanchistus* may solve this polytomy in a future study (Anker, pers. comm.).

Host switching

An ancestral character state reconstruction of the host affiliations resulted in ML probabilities for all nodes in the tree based on the TE-approach (fig. 5.3, supplementary fig. S4, supplementary table S3). Most interesting are nodes where host switching might have happened, and the host affiliation of the common ancestor of the entire ingroup. In this case, a single host switch seemed to have happened in the split from the common ancestor of the entire ingroup. In contrast to the other bivalve- and ascidian-associated palaemonid clade (de Gier et al., 2022), which appeared to have an ascidian-associated ancestor, the common ancestor of the present ingroup was recovered as a bivalve-associated species, albeit with a low probability (fig. 5.3, supplementary fig. S4, supplementary table S3). Consequently, there seems to have been one host switch from a bivalve to an ascidian in the branch including *Dasella*. Horká et al. (2016) were not able to recover an ancestral host affiliation from the currently studied clade, and Chow et al. (2021) recovered the ancestral node of the entire clade as a bivalve-associated species. This node also included various species of the echinoderm-associated genera *Zenopontonia*, *Sandimenes*, *Lipkemenes* Bruce & Okuno, 2010, etc., and one free-living species, *Periclimenes* josephi Li, 2008.

Phylogenetical significance of morphological features

Comparing phylogenetic trees resulting from the concatenated molecular

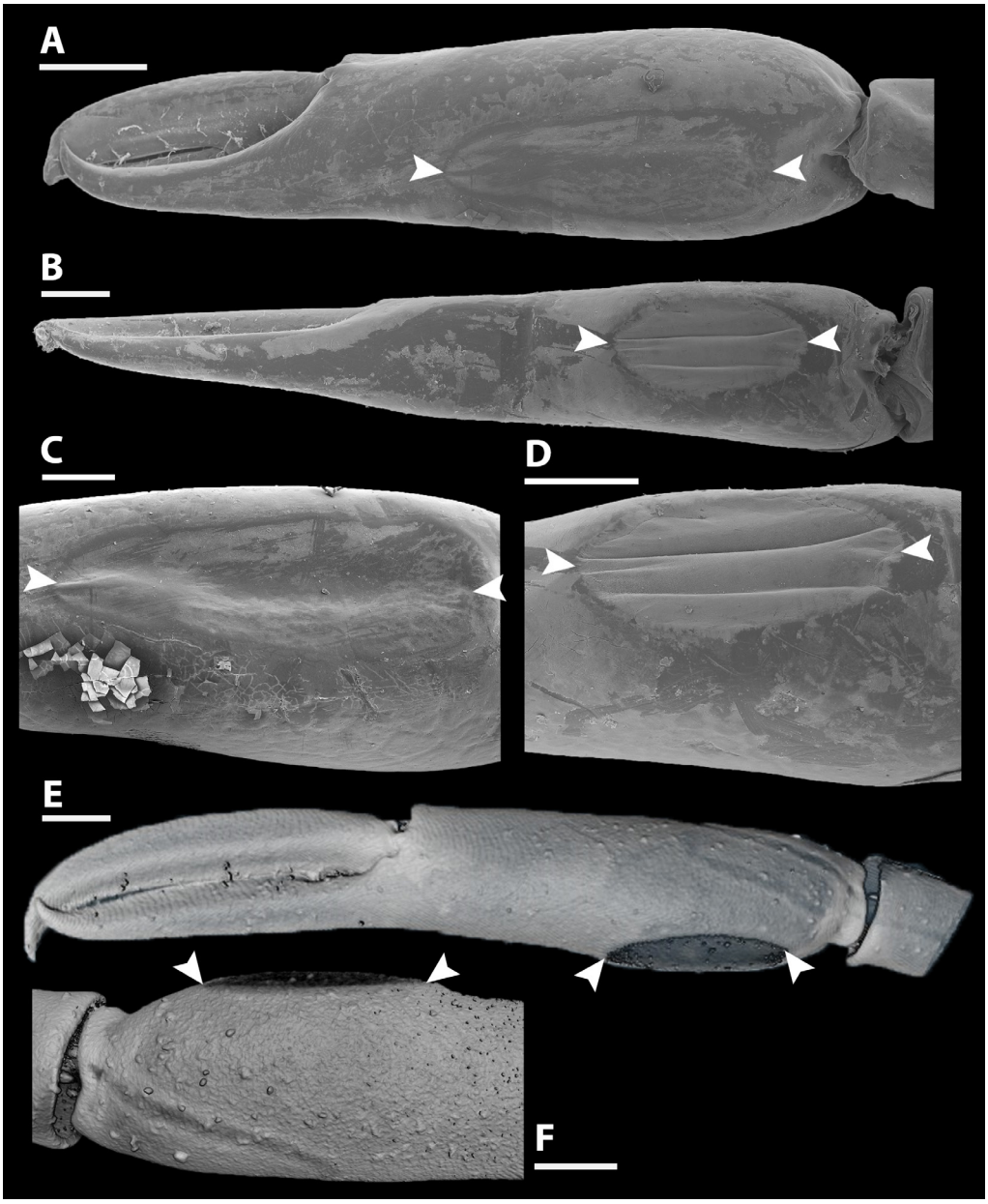
alignment and the morphological character state dataset could give us an insight in which morphological characters are more phylogenetically significant than others. Previous authors have made some remarks on the subject, which will be discussed below, together with new findings.

Bruce (1975b, 1976a) mentioned that the "irrelevant" colour patterns of *Anchistus* (s.l.) have little specific relevance due to the shrimps endosymbiotic lifestyle and their monogamous mating system (Neo et al., 2015). This implies that the conspicuous colourations have no obvious advantages or disadvantages (Bruce, 1976a). Consequently, there would be no strong selection pressure for this character. The whole ingroup clade can be seen exhibiting a spotted (or polka-dotted) colour pattern (figs. 5.4, 5.17, 5.20, 5.33, 5.36; Bruce, 1972a), with the exception of *Neoanchistus* (fig. 5.28; Anker, pers. comm.), and to some extent *A. pectinis*. The colour pattern of *Neoanchistus* and *A. pectinis* somewhat resembles that of other endosymbiotic shrimps, such as those of the genus *Platypontonia* Bruce, 1968 (see de Gier et al., 2022). Why these species exhibit a distinct pattern of larger red spots is unknown, but this may be concordant with the species' aberrant host choice, consisting of pectinid and cardiid bivalves. All other ingroup species appear to have a similar spotted pattern, although two groups can be distinguished by their spots and colouration (Bruce, 1972a; supplementary appendix S1, supplementary table S2). Minute white, red or yellow spots can be found in *Dasella*, *Tympanicheles ornatus* and both species of *Ensiger*. In addition, minute spots can also be found in the more closely related outgroups, like *Zenopontonia*, *Lipkemenes* and *Periclimenes kempi*, confirming the basal placement of some of the before-mentioned species. Larger spots, usually a combination of both blue and red (or orange) chromatophores, can be found in all other ingroup species: *Anchistus australis*, *A. demani*, *A. miersi*, *Polkamenes pycnodontae*, *P. spondylis*, and additionally to some extent in *Tympanicheles ornatus*. The colour pattern of *T. ornatus* resembles the minute spots of *E. custoides* (figs. 5.20, 5.36), although closer inspection (e.g., MNHN-iu-2010-4971) reveals that the yellow spots are larger and that additional orange spots are present. Projection of the colour patterns on the molecular trees show that larger spots are derived characters and that they are only found in symbionts of larger bivalves.

The presence of minutely pectinate cutting edges of spatulate to subspatulate first chelipeds is considered to be a definitive character for the studied ingroup genera (e.g., Bruce, 1995; Fransen & Van der Meij, 2010), but was also found in the related outgroup species *Lipkemenes*, *Zenopontonia*, *Actinimenes* and both *Periclimenes colemani* and *P. kempi* (supplementary appendix S1, supplementary table S2). A quick investigation also proves that *Sandimenes*

and various members of *Periclimenes* and *Allopontonia* (which were proven to be somewhat related to the currently studied ingroup by Chow et al., 2021) also have subspatulate first chelipeds, although the associated minutely pectinate cutting edges cannot be seen in the published illustrations (Bruce, 1971, 1972b, 1995, 2010b; Li, 2009; Rauch et al., 2019), except for the illustrations of *P. josephi*, which show "a distal row of pectinate short setae" (Li, 2008b). Closer examination of collection material is needed in order to see if minutely pectinate cutting edges are also present in related ectosymbiotic species. The potential function of the (sub)spatulate cheliped shape is currently unknown, although it may be linked to the dietary habits of the shrimp. Species could be using the minor chelipeds to harvest and digest their hosts' mucus and 'steal' food particles, such as in *E. custos* (Johnson & Liang, 1966), although the cheliped morphology of *E. custos* and *E. custoides* is quite aberrant (e.g., fig. 5.27A). Interestingly, De Grave et al. (2021) concluded that representatives of the currently studied clade, *Actinimenes ornatus*, *Zenopontonia soror*, and *Anchistus miersi*, were facultative or obligate parasites, feeding from the hosts' tissue. Comparatively, the limited information on the bivalve-associated species from the other endosymbiotic palaemonid clade (de Gier et al., 2022) were found to feed from host mucus strands and/or pseudofeces (Kennedy et al., 2001; Ashelby et al., 2015). In addition, *Pontonia pinnophylax* (Otto, 1821) was found to be feeding from similar carbon sources as its host (De Grave et al., 2021), making it a 'true' commensal symbiont.

The flat oval-shaped 'tympanal' organ found on the underside of the second chelipeds was first described and figured by Monod (1976a) for *Anchistus miersi*. This structure can be found in various members of the ingroup: *Anchistus australis*, *A. demani*, *A. miersi*, and *A. gravieri* bear major claws with small, distinct tympanal organs (fig. 5.39B, D–F), while both species of *Tympanicheles*, *Neoanchistus nasalis*, and possibly *N. cardiodytes*, bear major claws with larger, less-pronounced tympanal organs (fig. 5.39A, C). In *A. australis*, the 'veil' is much thinner than the surrounding rim and the rest of the claw (fig. 5.39E, F). Although the systematic position of *T. pectinis*, *A. gravieri*, and *Neoanchistus* cannot be confirmed with the current molecular data, *T. ornatus* has a more basal placement than *A. australis*, *A. demani*, and *A. miersi* (fig. 5.1). Thus, it seems that a dinstinctly outlined smaller organ evolved from a less clearly delimited larger organ. More morphological and histological work is needed in order to see if the surface of the tympanal organ is homologous to the outer surface of the chelae of other species in the ingroup. The potential function of the organ is unknown, and a potential link to the host choice could also not be found (with *T. ornatus* occupying the same hosts as *Ensiger*). Similar drum-looking organs are used for interspecies communication, host detection,



5.39 Tympanal organ on the second chelipeds of various ingroup members, and the veil figured in detail for every claw. SEM captures: A (C detail)) *Tympanicheles ornatus* (Holthuis, 1952) (RMNH.CRUS.D.41491); B (D detail)) *Anchistus miersi* (De Man, 1888) (RMNH. CRUS.D.58030). µCT model: E (F detail; rotated)), *Anchistus australis* Bruce, 1977 (RMNH. CRUS.D.53540). White arrows indicate proximal and distal border of tympanal organ. Scale A = 1 mm; B–F = 500 µm. Photo credits: A–D) Pepijn Helleman, Jurriaan van den Oever; E–F) Werner de Gier.

or predator avoidance in various groups of insects (described as a 'tympanum': Yack, 2004; Strauß & Lakes-Harlan, 2014) and as a 'directional air motion sensor' in the spider genus Hortipes Bosselaers & Ledoux, 1998 (Ramírez, 2014). Perhaps the functionality of the drum-like organ for shrimps can be found in the field of bioacoustics (e.g., Lovell et al., 2005). In crustaceans, similarly looking structures called 'gas windows' or also 'tympana' can be seen in the last walking leg pairs and sometimes thoracic sternites of semi-terrestrial sand bubbler crabs (Brachyura: Dotillidae), which they use for osmoregulation (Matsumasa et al., 2001). In various other semi-terrestrial crabs, small 'window-like' organs called 'Barth's myochordotonal organs' can be found, which play a role in intraspecific communication, being able to respond to vibrations transmitted through the exoskeleton from the substrate and the air (Davie et al., 2015). In male Australian freshwater crayfish *Cherax quadricarinatus* (von Martens, 1868), sexually dimorphic 'red patches' on the propodus of the major claws' can be found. The function of this uncalcified structure was reviewed by Karplus et al. (2003) and hypothesised to be a sensory organ, possibly used in intraspecific social interactions. Various species of amphipods within the Bogidiellidae, Niphargidae, and Ingolfiellidae exhibit 'windows' in the cuticle of the pereiopods, pleon, and pereion, called 'lentiform organs' (Pretus & Stock, 1990). As for now, the function of these structures remains unknown (Vonk & Schram, 2003). In addition, a semi-transparent structure can be found on the dorsal sides of the heads of 'mountain shrimp' (Anaspidacea) in the genus *Allanaspides* Swain, Wilson, Hickman & Ong, 1970, which resembles the structures found in amphipods, mentioned above (Vonk & Schram, 2003).

The walking legs' (third to fifth pair of pereiopods') dactyli bear many of the characteristic features used for species and genera identifications within the present shrimp clade (Fransen, 2002). In most cases, the dactyli are well-illustrated in taxonomic literature, revealing the wide range of morphological ornamentations and setation patterns on the corpus and unguis. Although homology and sequential evolution in the structures are sometimes hard to dinstinguish (supplementary appendix S1; supplementary table S2), some characters give the impression to be unique to bivalve- and ascidian-associated endosymbiotic shrimp (Fransen, 1994a, 2002; de Gier & Fransen, 2018; de Gier et al., 2022). However, Chow et al. (2021) showed that the overall shape of a 'biunguiculate' dactylus has evolved many times in both endo- and ectosymbionts, and bears no phylogenetic signal on a higher taxonomic level. Similarly, in the present clade, traces of convergent evolution can be found in the accessory teeth on the flexor margin of the dactylar corpus: all species of *Dasella* show a blunt, distoventral process (fig. 5.19D), while *Paranchistus armatus* has a somewhat sharper tooth (fig. 5.30J; almost resembling species of *Pontonia*

and *Odontonia* (Fransen, 2002; de Gier & Fransen, 2018). In addition, both species of *Ensiger* have no accessory teeth whatsoever (figs. 5.22E, 5.27D). Higher up in the tree (see fig. 5.1), *Polkamenes pycnodontae* (as well as *P. spondylis* and *P. nobilii*) exhibit a strong accessory tooth (figs. 5.34K, L, 5.35S), while *Tympanicheles ornatus* has just one minor denticle (fig. 5.37H). Similar to *T. ornatus*, *T. pectinis*, *A. gravieri*, as well as both species of *Neoanchistus* (although the structures on the illustrations of *N. cardiodytes* of Bruce (1975a) are difficult to identify) bear none or one small denticle on the flexor margin (fig. 5.29E). The elusive *P. liui* also has no accessory teeth (Li et al., 2004). Interestingly, the species in the more derived clade of *Anchistus*, comprised of *A. australis*, *A. demani*, and *A. miersi*, show a presence of a larger accessory tooth, most apparent in *A. miersi* (figs. 5.5L, M, 5.6J, 5.16G). In contrast, the microscopic spinules which can be found on the dorsal surface of the unguis, can exclusively be found in the more derived lineages, and is not found in *Ensiger* and *Dasella*. *Paranchistus armatus* exhibits a few microspinules on the unguis, although preliminary SEM-images prove that these structures are not homologous (de Gier et al., unpublished observation).

Sexual dimorphic and ontogenetic characters

To keep the dataset uniform, mostly female ovigerous specimens were used to score almost all of the character states (with the exception of the exclusively male features), both regarding data from the literature as from collected material (supplementary appendix S1, supplementary table S2). Since the species in the ingroup are considered to exhibit reverse sexual dimorphism (Baeza et al., 2013), the morphological characters of the bigger female specimens are easier to score. The differences between male and female specimens can mostly be found in the abdominal region (male and female first and second pleopods, size of abdominal segments), which has an inclusion that is limited in the morphological analysis. The bigger size of females may result in a stronger selection for ecomorphological characters, due to the limited size of their hosts' internal cavities. This link has not been studied in palaemonid shrimp as for now, although there is strong evidence for sex-related selection pressure in other crustaceans with more extreme sexual dimorphisms (e.g., where males visit sedentary females in gall and pea crabs (Brachyura: Cryptochiridae, Pinnotheridae); Van der Meij, 2014; Vehof et al., 2016; de Gier & Becker, 2020). Sexual dimorphism in the colouration of the chromatophores (dichromatism) was recently found in *Anchistus miersi*, with the male showing red spots, and the female blue spots (Neo et al., 2015). This causes no problems in our dataset (supplementary appendix S1, supplementary table S2).

All chosen characters were picked based on their visibility in adult specimens,

since juvenile anatomy may differ a lot from their adult form (Fransen, 1994a). In most cases, these juvenile characters are easily distinguishable, such as a shorter rostrum and relatively larger eyes (Fransen, 2002, 2006). In extreme cases, juvenile characters may be completely reduced in the adult stages, and can provide erroneous character states. Examples can be seen in the (articulate) hepatic spine of *Paranchistus armatus*, which is much larger in juveniles than in adults (Bruce, 1975c). More extreme examples can be found in the 'biunguiculate' juvenile dactyli of the walking legs of *Tympanicheles pectinis*, which grow away in later stages (Bruce, 1996), and in the juvenile rostrum morphology of *Anchistus australis*, which grows from an acute to a more rounded shape (Fransen, 1994a). Some juvenile characters result in unknown species-identifications, as is the case for a single specimen of a juvenile shrimp found in a boring mussel (*Spengleria mytiloides* (Lamarck, 1818) belonging to the Gastrochaenidae; Fransen & Van der Meij, 2010). The specimen is thought to be related to the species in the currently studied ingroup, and was described as an unknown species of *Anchistus* (Fransen & Van der Meij, 2010), although its juvenile characters could not be included in the morphological analysis.

Conclusions

New distributions, hosts, and classification

In this study, numerous new records of distribution ranges and host affiliations are presented, summarised in table 5.2. In addition, this study establishes three new genera, and resulted in some additional taxonomic changes (table 5.2).

Future perspectives

Table 5.2 Summary of new distribution and host records, with information about the taxonomic changes. Species without new records and taxonomic changes are omitted.

Species name	Taxonomic changes	New locality records	New host records
Anchistus			
A. australis	-	Indonesia: West Papua: Raja Ampat; NE Kalimantan; Nanaka Is., Sulawesi. Malaysia: Tioman Is.	
A. demani	-	Indonesia: N Sulawesi; NE Kalimantan. Red Sea; Palau; Philippines: Cebu strait.	*Hippopus hippopus*; *Tridacna deresa*; *Tridacna crocea* (confirmed after Bruce, 1979a)
A. gravieri	-	Vanuatu	*Tridacna* sp.

Table 5.2 *Cont.*

A. miersi	-	Micronesia: Caroline Islands. Indonesia: NW Java: Thousand Islands; NE and S Kalimantan; West Papua: Numfor; SW and N Sulawesi; Mollucas: NW Seram.	-
Dasella			
D. herdmaniae	-	Vanuatu: Tutuba Is.; Indonesia: N, SW, and NE Sulawesi; Mollucas: Ambon; Halmahera; NW Java: Thousand Islands; West Papua: Raja Ampat.	*Polycarpa* sp.; *Phallusia* sp.
Ensiger			
E. custoides	Transferred from *Anchistus*	Indonesia: Ambon, Kera Is. near Timor, Komodo, NW Java: Thousand Islands, Bali, NE and SW Sulawesi, Halmahera; W Papua: Raja Ampat; NE Kalimantan: Berau Islands. Vanuatu. Philippines: Cebu Strait.	*Atrina pectinata*
E. custos	Transferred from *Anchistus*	Indonesia: NW Java: Thousand Islands; Sunda Islands, SW Sulawesi; West Papua. Japan. Oman: Masicoh (?); Saudi Arabia.	*Atrina pectinata*
Paranchistus			
P. armatus	-	Indonesia: Moluccas, Kei Islands, Tanimbar.	-
Polkamenes gen. nov.			
P. liui	Transferred from *Paranchistus*	-	-
P. nobilii	Transferred from *Paranchistus*	-	-
P. pycnodontae	Transferred from *Paranchistus*	Indonesia: E of Komodo; Bali; Halmahera; NE Sulawesi, NW Java: Thousand Islands; NE Kalimantan; West Papua. Malaysia: Sabah. Philippines: Cebu.	*Lopha cristagalli*; *Vulsella vulsella*; *Spondylus* sp. (possibly *S. sinensis*)
P. spondylis	Transferred from *Paranchistus*	-	-
Tympanicheles gen. nov.			
T. ornatus	Transferred from *Paranchistus*	-	-
T. pectinis	Transferred from *Anchistus*	Indonesia: Ambon. Vanuatu	-

The analyses revealed two genera to be polyphyletic and that three genera needed to be (re)erected to make a naturally sound classification. Various species of the present study did not have any molecular data, causing various branches to have low support values and their exact evolutionary placement to remain uncertain. Similar to an earlier study (de Gier et al. 2022), additional molecular data are needed for this clade to obtain better supported and resolved phylogeny reconstructions regarding host associations and ancestral state reconstructions, which would require more field observations and studies addressing the feeding habits, host choices, and distribution ranges.

Some authors have suggested that the mollusc- and ascidian-associated shrimp clades display various host-specific (eco)morphological adaptations (Bruce, 1972a, 1976a; Fransen, 1994a), although the statistical evidence for this is limited (Dobson et al., 2014, 2016; Chow et al., 2021). Ecomorphological analyses using new imaging methods (µCT-scanning, SEM), combined with ecological and molecular data may help us to understand how exactly the currently studied species have adapted to their endosymbiotic way of life.

Supplementary information

Supplementary material is available online at: https://doi.org/10.6084/m9.figshare.22310059.

Acknowledgements

The authors would like to thank Prof. Dr. Bert W. Hoeksema (Naturalis Biodiversity Center, Rijksuniversiteit Groningen), Dr. Ronald Vonk (Naturalis Biodiversity Center), and Dr. Jeroen Hubert (Universiteit Leiden) for their helpful comments and feedback during the writing phase of this project. The lab-technicians Frank Stokvis, Roland Butôt, and Marcel Eurlings (all Naturalis Biodiversity Center) are thanked for their assistance generating the DNA-barcodes which were used during this study. Collection managers Jeroen Goud, Bram van der Bijl, and Esther Dondorp (all Naturalis Biodiversity Center) are thanked for their support during the examinations of the physical specimens. Bertie-Joan van Heuven and Dirk van der Marel (both Naturalis Biodiversity Center) are thanked for their assistance manning the SEM and µCT scanner in the Naturalis imaging facilities. Research interns Jurriaan van den Oever en Pepijn Helleman are thanked for their help in the SEM lab, acquiring images of the dactyli and claws of various species studied in this paper. Dr. Arthur Anker (King Abdullah University of Science and Technology,

Saudi Arabia) is thanked for his contributing colour pictures of *Neoanchistus cardiodytes* and *N. nasalis* (fig. 5.28), and Jeroen Goud (Naturalis Biodiversity Center) for the identification of the *Vasticardium* host of *N. cardiodytes*. In addition, two anonymous reviewers are thanked for their helpful comments and suggestions, which helped to improve the manuscript. Mr. H.L Strack organised the 'Rumphius Biohistorical Expedition' (1990) to Ambon, in cooperation with the Nationaal Natuurhistorisch Museum (NNM; now Naturalis Biodiversity Center), Leiden, the Netherlands; Oceanological Institute, Indonesian Institute of Sciences (LIPI, now RCO-BRIN); and Universitas Pattimura, both from Ambon, Indonesia. The R.V. 'Tyro' 'Oceanic Reefs Expedition' to the Seychelles (1992–1993) was organised by the NNM, and the Netherlands Indian Ocean Program. Dr. J. van der Land (NNM) was chief-scientist at these cruises. The 'Sulawesi expedition' (1994) was organised was organised by the NNM in cooperation with the Hasanuddin University at Makassar (UNHAS) and the field station of the Oceanological Institute of LIPI at Bitung with chief-scientist Dr. J. van der Land. The 'Maluku Expedition' (1996) was organised by the NNM in cooperation with the Oceanological Institute of LIPI at Ambon with chief-scientist Dr. J. van der Land and counterpart Dr. Dwi Listyo Rahayu (LIPI). The 'Cebu Strait' Expedition (2000) by the NNM and the University of San Carlos (USC), Cebu, with chief-scientists Dr. Bert W. Hoeksema, Dr. Thomas Heeger (USC) and Dr. Filipina B. Sotto (USC). The 'Lombok Strait' Expedition (2001) by the NNM, the Center of Oceanological Research and Development (PPPO) of LIPI in Jakarta, and the World Wildlife Foundation (WWF) Wallacea project at Denpasar, with chief-scientists Dr. B.W. Hoeksema and Ir. M.I. Yosephine Tuti (PPPO-LIPI). The 2003 'Berau 2003' expedition around the Berau Islands, East Kalimantan was organised by the NNM (chief scientist Dr. B.W. Hoeksema) and the RCO-LIPI (chief scientist Dr. Suharsono), as part of the NWO-LIPI East Kalimantan Programme. The 'Thousand Islands Expedition, September 2005', organised by the RCO-LIPI and the NNM. The 'santo 2006 Global Biodiversity Survey' was initiated by the Muséum national d'Histoire naturelle, Paris (MNHN), Institut de Recherche pour le Développement (IRD) and ProNatura International. Dr. Philippe Bouchet acted as expedition leader for the marine theme. The '2007 Raja Ampat expedition' was organised by the NNM (at that time NCB Naturalis) and RCO-LIPI, under the umbrella of Ekspedisi Widya Nusantara (E-Win) of LIPI. We are grateful to LIPI for granting research permits and to Dr. B.W. Hoeksema for his efforts in organizing the expedition. The 'Ternate expedition' in the northern Moluccas was organised by the RCO-LIPI and the NNM (at that time Naturalis Biodiversity Center). This expedition was part of the Ekspedisi Widya Nusantara project (E-Win expeditions), with chief-scientists Dr. B.W. Hoeksema and Ir. M.I.Y. Tuti (PPPO-LIPI). The 'Semporna Marine Ecological Expedition' (2010) was jointly organised by WWF-Malaysia, Universiti Malaysia

Sabah's Borneo Marine Research Institute, Naturalis Biodiversity Center and Universiti Malaya's Institute of Biological Sciences. Research permission was granted by Economic Planning Unit, Prime Minister's Department, Economic Planning Unit Sabah, Sabah Parks and Department of Fisheries Sabah. Dr. B.W. Hoeksema and Ms. Zarinah Waheed acted as expedition leaders for the biodiversity theme. The 'Marine Biodiversity Workshop at Lembeh Strait' (2012) was organised by Universitas Sam Ratulangi (UNSRAT in Manado) and the Bitung field station of the Research Centre of Oceanography (RCO-LIPI) in cooperation with Naturalis Biodiversity Center. Prof. Dr. Suharsono, former director of the Research Centre for Oceanography (PPO-LIPI), is acknowledged for his support. Prof. Dr. Markus T. Lasut (Universitas SamRatulangi), Dr. B.W. Hoeksema, and Ir. Y. Tuti (PPO-LIPI) acted as workshop coordinators. Dr. Francesca Benzoni organised the 'Marine Biodiversity Workshop' (2014) at the Marine Research and Higher Education Centre University of Milano-Bicocca, Magoodhoo Island, Faafu Atoll, Republic of Maldives. Prof. Mike Berumen (Red Sea Research Center, King Abdullah University of Science and Technology, KAUST), hosted a Naturalis field team under leadership of Dr. B.W. Hoeksema in November 2014 off the Red Sea coast of Thuwal, Saudi Arabia.

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6

Phylomorphometrics reveal ecomorphological convergence in pea crab carapace shapes (Brachyura: Pinnotheridae)

Werner de Gier

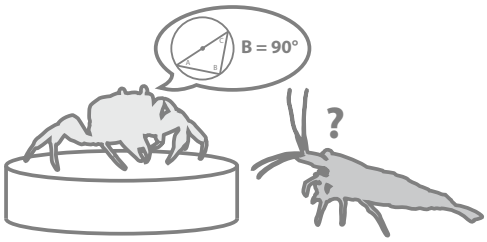
This chapter is published as:

de Gier, W. (2023) Phylomorphometrics reveal ecomorphological convergence in pea crab carapace shapes (Brachyura, Pinnotheridae). Ecology and Evolution, 13(1): e9744.

Abstract

Most members of the speciose pea crab family (Decapoda: Brachyura: Pinnotheridae) are characterised by their symbioses with marine invertebrates in various host phyla. The ecology of pea crabs is, however, understudied, and the degree of host dependency of most species is still unclear. With the exception of one lineage of ectosymbiotic echinoid-associated crabs, species within the subfamily Pinnotherinae are endosymbionts, living within the body cavities of mollusks, ascidians, echinoderms, and brachiopods. By contrast, most members of the two other subfamilies are considered to have an ectosymbiotic lifestyle, sharing burrows and tubes with various types of worms and burrowing crustaceans (inquilism). The body shapes within the family are extremely variable, mainly in the width and length of the carapace. The variation of carapace shapes in the family, focusing on pinnotherines, is mapped using landmark-based morphometrics. Mean carapace shapes of species groups (based on their host preference) are statistically compared. In addition, a phylomorphometric approach is used to study three different convergence events (across subfamilies; between three genera; and within one genus), and link these events with the associated hosts.

Keywords: Ecomorphology; morphometry; parasitism; phylomorphospace; symbiosis



Introduction

Most pea crab species (Crustacea: Decapoda: Brachyura: Pinnotheridae) are characterised by their symbiotic lifestyle, often thought to be obligate parasites or commensals (Baeza, 2015). The degree of host dependency is, however, still unclear for most species. The species within the subfamily Pinnotherinae are known to be endo- or ectosymbiotic inhabitants of bivalves, polyplacophorans, gastropods, holothurians, echinoids, brachiopods, and ascidians (de Gier & Becker, 2020). Although some species have a broad host spectrum spanning across various phyla, most species of Pinnotherinae are considered to be specialists. This means that these species are only inhabiting specific genera or orders within a host phylum. Several species of the two smaller subfamilies (Pinnixinae and Pinnixulalinae) are also found in bivalve, or in/on holothurian hosts in their adult stages, but most are considered to be free-living or tube/burrow-dwelling, sharing this habitat with a worm, ghost shrimp, or mud lobster as a host (inquilism; see Palacios Theil et al., 2016; Palacios Theil & Felder, 2020a; Schmitt et al., 1973; Zmarzly, 1992). It is worth noting that also in these subfamilies, the host specificity is understudied and some species now considered to be free-living might have unknown host associations (McDermott, 2009).

Pinnotherids have evolved a wide range of ecomorphological adaptations that could be linked to their presumed host choice (described for the subfamily Pinnotherinae in de Gier & Becker, 2020). These include: (A) several types of setae on the walking legs and claws used for feeding, swimming, or camouflaging; (B) asymmetry and widening of the walking legs' segments for feeding purposes and/or grip within the host (or host tube/burrow); and (C) various ornamentations, setaetion and colouration patterns, shape differences, and a variation of thickness of the carapaces in order to fit inside their hosts or to blend with their hosts' colouration (de Gier & Becker, 2020, and references therein for examples of adapted species). In addition, in sexual dimorphic species, females have evolved an enlarged pleon to carry eggs, making them almost immobile and very vulnerable to predation if they ever leave their host (Baeza, 2015). This is likely a consequence of living hidden within a host (de Gier & Becker, 2020). Although the variation in the above-mentioned characters is most diverse in the speciose pea crab family, similar adaptations to endo- or ectosymbiotic lifestyles can be found in other brachyuran ("true" crab) taxa (Castro, 2015; Serène, 1961). For example, coral-inhabiting parasitic gall crabs (Cryptochiridae) share a strong sexual dimorphism and enlarged pleons in females (Vehof et al., 2016). Adaptive walking-leg morphology and host-specific camouflage can be found in the echinoderm-associated Eumedoninae

subfamily (Pilumnidae) and the coral-associated Domeciidae, Tetraliidae, and Trapeziidae (Castro et al., 2004; Chia & Ng, 2000; Ng & Jeng, 1999; Števčić et al., 1988). In addition, symbiotic members of the families Portunidae (e.g., the cnidarian- and echinoderm-associated members of *Lissocarcinus* Adams & White, 1849 and Caphyra Guérin, 1832) and Varunidae (the burrow-dwelling *Sestrostoma* Davie & Ng, 2007) have also evolved adapted walking-leg dactyli, carapace shapes, and cryptic camouflage (Davie & Ng, 2007; Evans, 2018). Two clades of endosymbiotic shrimp (Caridea: Palaemonidae), which share a similar lifestyle with ascidian- and bivalve-associated pea crabs, evolved similar morphological features, mainly in the round, swollen shape of their abdomen and smooth carapace (de Gier et al., 2022; Fransen, 1994).

Most of the ecomorphological adaptations mentioned above have only briefly been described in taxonomic and phylogenetic literature (Campos, 1996a, 2016; Manning, 1993; Palacios Theil & Felder, 2020b), and were not directly studied with respect to the crabs' host choice. In a large-scale study, Laughlin (1981) mentioned that various pinnixine pea crabs (mainly including species then attributed to *Pinnixa* White, 1846) have a much wider carapace than the studied pinnotherines (in this case, species of *Pinnotheres* Bosc, 1801), and linked this character to their biology. More recently, Hultgren et al. (2022) analyzed the relationship between the host choice of a wide range of pea crabs and their carapace size ratios, considering also their phylogenetic positions. In this way, convergence in carapace shapes could be studied. They did this by testing the aspect ratios of 149 species, 59 of which had known phylogenetic positions (see Palacios Theil et al., 2016).

The present study elaborates on the analyses by Hultgren et al. (2022), by using additional morphometrics to investigate the relationship between the adult female carapace shape, and the host choice, focusing on all currently included pinnotherine members. A phylomorphospace approach will be used, including both symbiotic, as well as free-living outgroup species from the two other subfamilies. This projection of the phylogeny should reveal clusters and convergence patterns in the data (Stayton, 2015), indicating that the colonization of similar host phyla has led to analogous carapace shapes in the evolution of pea crabs.

Materials and methods

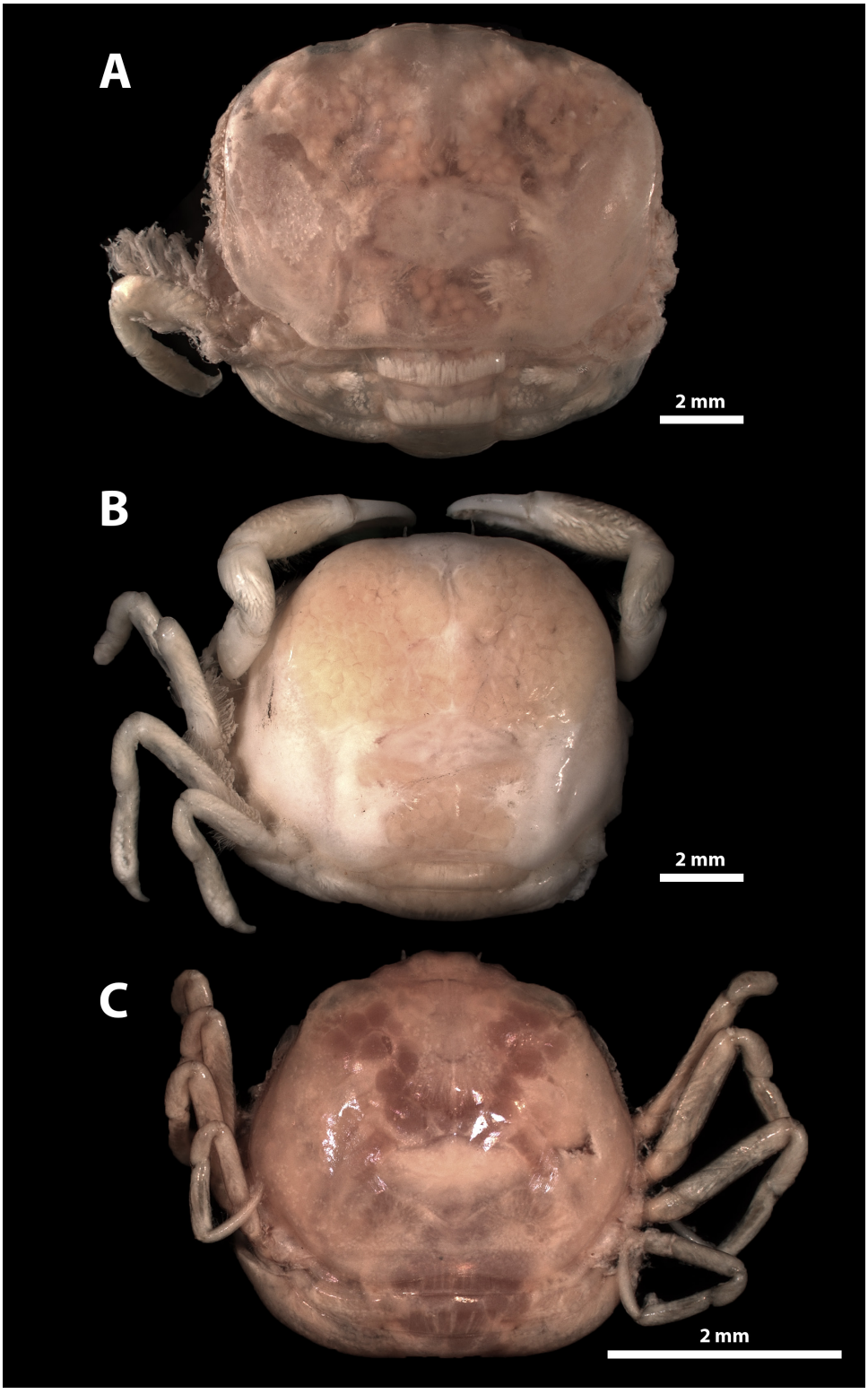
Selection of illustrations

Similarly to the methods described by Hultgren et al. (2022), published

illustrations of 181 pea crab species (in particular Pinnotherinae; see below) were collected through an extensive literature search. Available dorsal views of adult female carapaces (independently of the number of pereiopods depicted in the illustration) were selected for the analyses due to their often obligatory symbiosis, being restricted to remain inside their host. By contrast, males often leave their host, and juveniles have been found to switch hosts multiple times before reaching their adult stages (de Gier & Becker, 2020). In addition, one rarely figured species, and two species without previously published illustrations were photographed using a Leica M165c stereo microscope with a Leica DFC420 microscope-mounted camera, using Leica LAS 4.4 software and Helicon Focus stacking software. These species are *Mesotheres serrei* (Rathbun, 1909) (SMF 9435) and *Pinnotheres pectunculi* Hesse, 1872 (SMF 34002) from the Senckenberg Forschungsinstitut und Naturmuseum collection (Frankfurt, Germany), and *Arcotheres quadratus* (Rathbun, 1909) (ZMA. CRUS.D.242266) from the collection of Naturalis Biodiversity Center (fig. 6.1).

From all the currently recognised species of Pinnotherinae (excluding two genera discussed below), 168 species could be included in the study. Thirty-five species were excluded due to the absence of illustrations of the dorsal view of adult female crabs (Appendix S1). To cover the morphological variation of the non-pinnotherine pea crabs, outgroup illustrations were selected of ten species from the other pea crab subfamilies. Nine were selected from Pinnixinae and one from Pinnixulalinae. A representative of both the genera *Sakaina* Serène, 1964 and *Parapinnixa* Holmes, 1895 were also added but considered as outgroups, although they are still within the subfamily Pinnotherinae (WoRMS, 2022), as the phylogeny of Palacios Theil et al. (2016) suggests that this placement is rather questionable. The subfamily classification of pea crabs seems to be unstable and in need of further research, and therefore the subfamily status for these species will be annotated as “Pinnotherinae?”. In addition, one species with a tentative placement basal to the three subfamilies is included as an outgroup: *Tetrias fischerii* (A. Milne-Edwards, 1867). This species has no subfamily status (WoRMS, 2022). Five of the outgroup species were chosen based on their recorded, or presumed endosymbiotic lifestyle: *Tetrias fischerii* is thought to be associated with bivalves (Milne-Edwards, 1873), the pinnixines *Pinnixa barnharti* Rathbun, 1918 and *Pinnixa tumida* Stimpson, 1858 are thought to be internal symbionts of holothurians (Dai & Yang, 1991; Zmarzly, 1992), and adults of the pinnixines *Scleroplax faba* (Dana, 1851) and *Scleroplax littoralis*

6.1 Three newly made photographs of pinnotherine species, which were used in the current analyses; A) *Mesotheres serrei* (Rathbun, 1909) (SMF 9435) (all walking legs but one, and both claws were severed); B) *Pinnotheres pectunculi* Hesse, 1872 (SMF 34002) (right side walking legs were severed); C) *Arcotheres quadratus* (Rathbun, 1909) (ZMA.CRUS.D.242266).


A
2 mm
B
2 mm
C
2 mm

(Holmes, 1895) are commonly found in bivalve hosts (Zmarzly, 1992). The latter two are suggested to be morphotypes of the same species (Palacios Theil & Felder, 2020a; Zmarzly, 1992) but are treated as separate species in the analyses. *Scleroplax faba* was reported in various other host types (gastropods, ascidians, and holothurians) in juvenile specimens (Zmarzly, 1992).

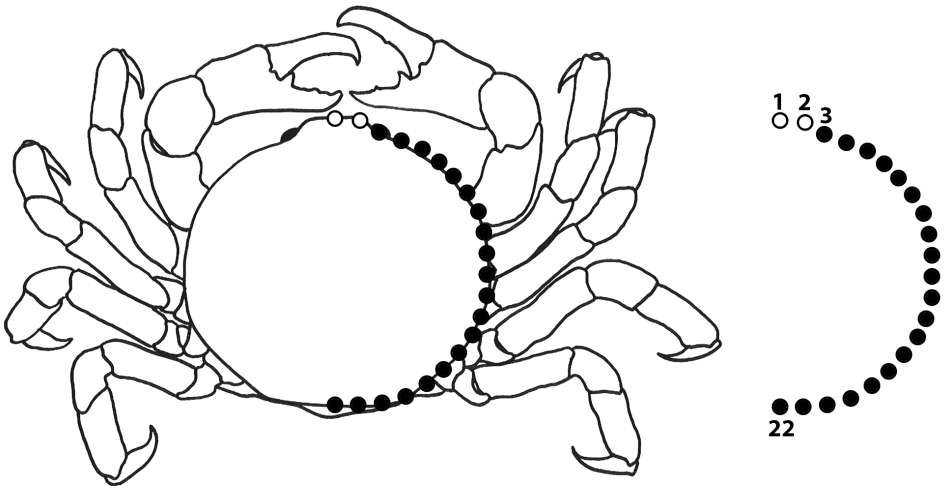
Sources of illustrations used are presented as supplementary data (Appendix S2). Taxonomy follows WoRMS (2022), taking the taxonomic decisions made after de Gier and Becker (2020) into account, mainly within *Arcotheres* Manning, 1993 (e.g., Ng & Ahyong, 2022).

Landmark selection and morphometrics

Collector bias is a common problem in morphometric studies when selecting landmark data (e.g., Percival et al., 2019), as is the use of nonhomologous and inconsistent datapoints (e.g., nonuniform orientations of specimens; Collins & Gazley, 2017). These problems could be evaded due to the uniformity in the orientations of illustrations used in taxonomic pea crab publications: only uniform (dorsal) orientations with visible ocular carapace ridges (cavities for the eyes) were used (with the exception of anteriorly ornamented species). Landmark (LM) selection was done to digitize the right half of the pea crabs' carapace shape, with the inclusion of three landmarks (LM 1, 3, 22), one semi-landmark (LM 2), and 18 sliding semi-landmarks (curve) (LM 4 to 21) along the lateral and caudal margin of the carapace (see fig. 6.2). Because of the lack of homologous anatomical features on the lateral curvature of pea crab carapaces, sliders were used to capture the shape variation.

1. Central tip of the rostrum
2. Halfway between LM 1 and 3
3. Central point of the ocular cavity (if the eye is not visible, the part of the carapace directly above the eye is marked) — beginning of curve
4 to 21. Curve of 18 sliding semi-landmarks with fixed distance along the anterior, lateral, and caudal margin of the carapace
22. Most caudal tip of the carapace — end of curve

Landmark data were gathered in tpsDig2 (v. 2.31) (Rohlf, 2017) and analyzed using R v. 4.2.1 and Rstudio v. 2022.07.0 (R Core Team, 2022; RStudio Team, 2022), using the packages geomorph v. 4.0.4 and ggplot2 v. 3.3.6 (Adams et al., 2022; Baken et al., 2021; Wickham, 2016). A generalized Procrustes analysis was performed to scale, transform and rotate all images, or a subset of the images for morphospace analyses. In this way, the scale was set to "uniform",



6.2 *Ostracotheres tridacnae* (Rüppell, 1830) after Ahyong (2018), with the 22 landmarks plotted on the crab's carapace. Darkened circles are part of the sliding semi-landmark curve, whereas landmarks 3 and 22 are stationary. Setae in illustration omitted.

also to take into account potential uniform swelling due to preservation in ethanol. A Procrustes pairwise (M)ANOVA with a residual randomization permutation procedure (1000 permutations, RRPP; Collyer & Adams, 2021) was performed to find significant differences in the mean shape data based on the host associations of the specimens. The Procrustes pairwise ANOVA test compares the mean shapes of two specified groups by checking the relative distance between these two shapes (Goodall, 1991). In this way, it explains if the differences between the groups are large enough (e.g., significant) in comparison to the variation within the groups. The full dataset, including outgroup species, as well as a subset only including the "true" Pinnotherinae (i.e., excluding the members of *Parapinnixa* and *Sakaina*), was tested and compared. All species were labelled considering their host association: bivalve-, gastropod-, holothurian-, ascidian-, echinoid-, and brachiopod-associated, and tube/burrow-dwelling. The echinoderm associates were separated based on the external or internal nature of their symbiosis (ecto- or endosymbiotic). In addition, two outgroup species are labelled as "free-living", although they could have been dislodged from their host (see McDermott, 2009; Appendix S2). Five ingroup species with a generalist multi-phylum host range are labelled separately (de Gier & Becker, 2020). These species are: *Afropinnotheres dofleini* (Lenz in Lenz & Strunck, 1914), *Austrotheres holothuriensis* (Baker, 1907), *Nepinnotheres pinnotheres* (L., 1758), *Opisthopus transversus* Rathbun, 1894, and *Pinnaxodes major* Ortmann, 1894 (Appendix S2). Ingroup species with an unknown host association are labelled as "unknown" in the dataset. These 21

species are often rarely caught, poorly described, or have a very questionable host association. Two of these species (*Hospitotheres powelli* Manning, 1993 and *Pinnotheres taichungae* Sakai, 2000) were previously recorded as burrow-dwelling or free-living. It has been argued that they may have been dislodged from their host or that their host was destroyed during collection (de Gier & Becker, 2020; McDermott, 2009). Despite this, the choice was made to include them in the dataset, in order to test whether there is a predictive value in the analysis (see Discussion).

Phylomorphospace analyses

In order to include the available phylogenetic information of 33 species and to link this to the morphospace, a pruned (trimmed-down) ultrametric version of the phylogeny reconstruction of Palacios Theil et al. (2016) and Hultgren et al. (2022) was used. This phylomorphospace analysis was also performed in R, using the packages phytools v. 1.0-3, ape v. 5.6-2, and geiger v. 2.0.10 (Paradis & Schliep, 2019; Pennell et al., 2014; Revell, 2012). Three convergence events were highlighted in the phylomorphospace using ggplot2. The phylogeny reconstruction is treated as an overlay on the presented morphospace. The branches were also used to statistically test three potential convergence events in the phylomorphospace plot. Similarity-based measures (C_1 to C_4, and corresponding P-values) were calculated using the R package convevol v. 1.3 (Stayton, 2018) as described by Stayton (2015). Examples of their uses are presented by Serb et al. (2017), Zelditch et al. (2017), Stange et al. (2018), and Grossnickle et al. (2020). A custom R-script (Zelditch et al., 2017; Zelditch, pers. comm.) was used to run 1000 replicates to check the results from the convevol package. For the calculations of the C-values, PC-values were used from PC1 to PC3 (84.5% of the explained data).

A phylogenetically informed ANOVA (Phylogenetic Generalized Least Squares; PGLS) was performed to investigate the impact of host choice on the shape variation in the data while controlling for the independence of the residuals from the phylogeny (Adams & Collyer, 2018; Mundry, 2014). This was done for 33 species, using the procD.pgls() command in geomorph, with Pagel's lambda (λ) (Pagel, 1999) set at 1.0 (a high phylogenetic signal—Brownian motion model). For comparison, a regular Procrustes ANOVA/regression, without the implementation of a phylogenetic framework, was performed for the 33 species (similar to the pairwise test explained above). In both analyses, a similar RRPP approach was used as mentioned above (1000 permutations).

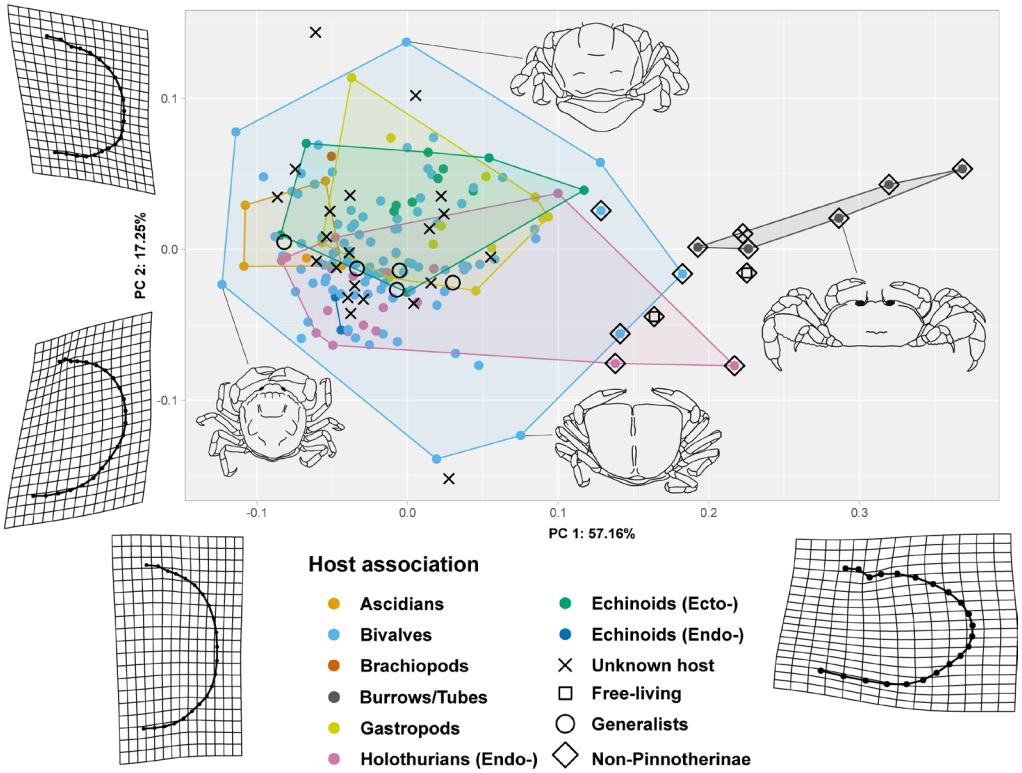
Results

Morphospaces and mean shapes

The morphometric analyses of the scored landmarks revealed the overall variation in carapace shapes (for a morphospace plot with numbers indicating the species, see Appendix S2 (list) and S3 (figure)). When including the outgroup (non-pinnotherinae) species, the first two of 43 principal components (PCs) explain 74.4% of the variation in the data (fig. 6.3). PC1 to PC11 together explain 99% of the data, meaning that the rest of the 32 PCs explain less than 1% of the data. Along these first two axes, the mean shapes change mainly in width and length: along PC1, the carapace changes from an elongated and rounder shape ($PC1_{min}$) to a widened, angular shape, with more defined ocular cavities in dorsal view ($PC1_{max}$). Along PC2, the widest point of the carapace seems to slightly shift from the anterolateral side ($PC2_{min}$) to the posterolateral side ($PC2_{max}$), meaning that the shape in the middle would have the widest point in the middle of the carapace. In addition, the rostrum seems to be much wider and more defined in specimens from the upper side of the plot ($PC2_{max}$) (fig. 6.3). This also means that a perfectly round species would approximately be found in the center of this plot (0,0).

There is a clear separation between the ingroups and the outgroups, except for one outgroup species: *Tetrias fischerii*. Outgroup species with a similar host association to ingroup members (namely endosymbionts of holothurians or bivalves; see Appendix S2, S3) are closer to the ingroup than to the rest of the outgroup species, which are burrow- and tube-dwelling (fig. 6.3). The significant difference between the point cloud of this host type and the rest of the host categories was confirmed by the pairwise Procrustes ANOVA ($p < .01$; table 6.1).

All species of the ingroup are covered by a vast cloud of bivalve-associated points (fig. 6.3; Appendix S2, S3). Although overlapping, significant results were found in the data by the pairwise ANOVA, taking all 43 PCs into account (table 6.1). The five ascidian-associated species group on the left side of the plot. Ascidian-associated species were found to be shaped significantly different from gastropod-associated ($P = 0.010$) and externally echinoid-associated species ($P = 0.027$). In addition, a nearly significant difference was found between the mean shape of the ascidian-associated species and the bivalve ($P = 0.056$), and internal holothurian associates ($P = 0.069$). Bivalve-associated species were significantly different from gastropod associates ($P = 0.044$), internal associates of holothurians ($P = 0.047$), and external echinoid associates ($P = 0.007$). Between these last two groups, a significant result was found (P

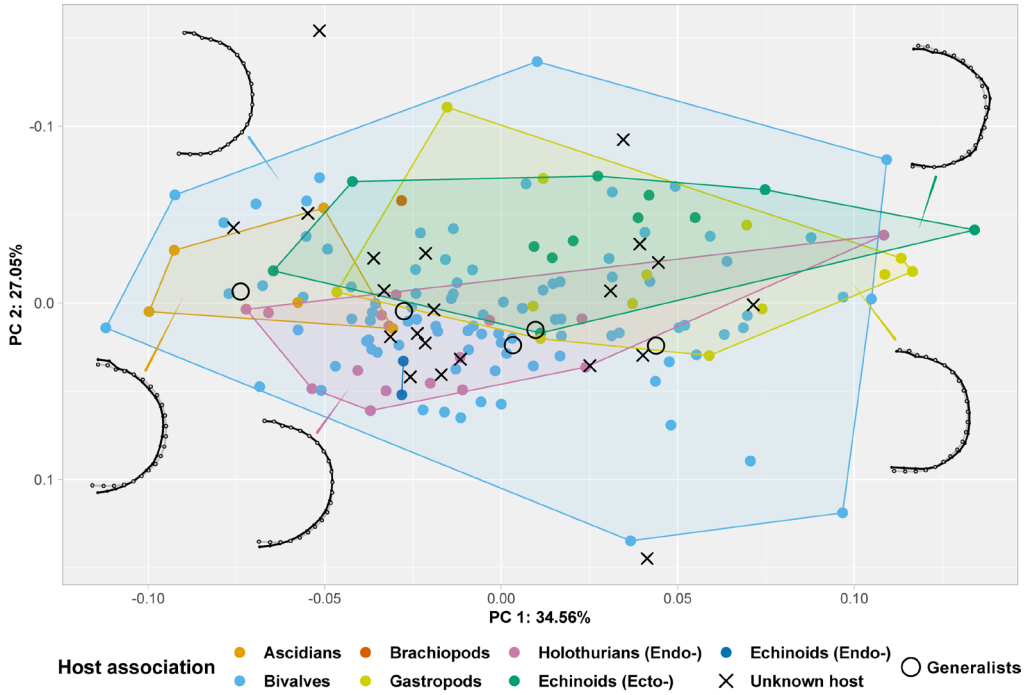


6.3 Morphospace plot showing the total variation of dorsal carapace shapes of both the in- and outgroups. Warps show extreme shape variation along the first and second PCs. Colours of points and convex hulls correspond to host association type, and shapes give an indication if the species have an unknown, free-living, or generalist symbiotic lifestyle. Diamonds show the non-pinnotherine outgroups. Illustrated species correspond to linked datapoints in morphospace: top, *Fabia tellinae* Cobb, 1973, after Campos (1996b); right, *Glassella floridana* (Rathbun, 1918), after Felder & Palacios Theil (2020a); bottom, *Durckheimia lochi* Ahyong & Brown, 2003, after Ahyong & Brown (2003); left, *Austrotheres pregenzeri* Ahyong, 2018, after Ahyong (2018) (setae in illustrations omitted; crabs not to scale).

= 0.006). Lastly, internal holothurian associates were significantly differently shaped than gastropod associates (P =0.015). The actual morphological differences between the carapace shapes are explained in detail below.

When excluding the outgroup from the analyses, the bivalve-associated convex hull overlaps all but five specimens with a known host association (fig. 6.4). These species (the external echinoid associate *Dissodactylus latus* Griffith, 1987, the holothurian-associated *Holothuriophilus trapeziformis* Nauck, 1880, and the three gastropod-associated *Mesotheres unguifalcula* (Glassel, 1936), *Orthotheres bayou* Ng & Ho, 2016, and *Orthotheres turboe* Sakai, 1969) have a broader body shape than the rest of the ingroup (i.e., a higher AR carapace

aspect ratio; Hultgren et al., 2022). Running the analysis with only pinnotherine members influences the P-values of the Procrustes pairwise ANOVA to be lower in all significant values from the analysis with the outgroup species included (table 6.1), and the first two PCs to explain less of the calculated variation (61.6%) (in addition, the first 12 of 43 PCs explain 99% of the data). In addition, four differences between the mean shapes of the carapaces, which were in the previous analysis labelled as insignificant, were calculated and found to be significant between the following groups: ascidian and bivalve associates ($P = 0.011$); gastropod and external echinoid associates ($P = 0.021$); gastropod and internal echinoid associates (of which there are only two) ($P = 0.045$); and between external and internal associates of echinoids ($P = 0.036$). When sorted based on host association, the mean shapes of the carapaces can be calculated and projected on the mean shape of the entire dataset (fig. 6.4). The mean of the bivalve-associated crab carapace shapes is almost identical to the mean shape of the entire ingroup. External echinoid-associated species have more angular lateral sides of the carapace, while the internal symbionts are rounded. Additionally, a somewhat wider body shape can be seen in the



6.4 Morphospace plot showing the total variation of dorsal carapace shapes of the ingroup. Mean shapes of major host-associated groups are plotted (black) and compared with the mean shape of the entire ingroup (gray/white). Colours of points and convex hulls correspond to host association type, and shapes give an indication if the species have an unknown or generalist symbiotic lifestyle. Note that the y-axis (PC2) is flipped compared with figure 6.3, and the aspect ratio is reduced to 0.5 for comparison with other plots.

Table 6.1 Output of pairwise Procrustes ANOVA of both datasets (with and without outgroup species included).

	With outgroup			Without outgroup		
Host type combination	***d***	**UCL (-95%)**	**Pr > *d***	***d***	**UCL (-95%)**	**Pr > *d***
Asc:Biv	0,078	0,081	0,056	0,075	0,059	0.011*
Asc:Bra	0,084	0,213	0,531	0,083	0,146	0,445
Asc:Bur/Tub	0,356	0,108	0.001*			
Asc:Ech_Ecto	0,108	0,096	0.027*	0,109	0,069	0.001*
Asc:Ech_Endo	0,08	0,147	0,397	0,081	0,11	0,219
Asc:Free	0,277	0,15	0.001*			
Asc:Gas	0,117	0,092	0.010*	0,117	0,067	0.001*
Asc:Gen	0,065	0,111	0,33	0,066	0,077	0,148
Asc:Hol_Endo	0,084	0,09	0,069	0,063	0,065	0,061
Biv:Bra	0,081	0,22	0,439	0,078	0,136	0,416
Biv:Bur/Tub	0,294	0,077	0.001*			
Biv:Ech_Ecto	0,072	0,051	0.007*	0,077	0,039	0.001*
Biv:Ech_Endo	0,063	0,121	0,422	0,061	0,096	0,31
Biv:Free	0,217	0,132	0.003*			
Biv:Gas	0,05	0,049	0.044*	0,054	0,039	0.002*
Biv:Gen	0,038	0,076	0,558	0,039	0,058	0,363
Biv:Hol_Endo	0,044	0,043	0.047*	0,044	0,034	0.005*
Bra:Bur/Tub	0,335	0,222	0.011*			
Bra:Ech_Ecto	0,086	0,214	0,446	0,084	0,137	0,361
Bra:Ech_Endo	0,114	0,235	0,369	0,112	0,159	0,261
Bra:Free	0,272	0,236	0.028*			
Bra:Gas	0,09	0,211	0,402	0,09	0,137	0,295
Bra:Gen	0,096	0,209	0,398	0,095	0,143	0,291
Bra:Hol_Ecto	0,113	0,212	0,215	0,099	0,14	0,197
Bur/Tub:Ech_Ecto	0,276	0,091	0.001*			
Bur/Tub:Ech_Endo	0,334	0,139	0.001*			
Bur/Tub:Free	0,109	0,145	0,182			
Bur/Tub:Gas	0,256	0,09	0.001*			
Bur/Tub:Gen	0,301	0,111	0.001*			
Bur/Tub:Hol_Endo	0,29	0,088	0.001*			
Ech_Ecto:Ech_Endo	0,104	0,13	0,119	0,106	0,099	0.036*
FreeL:Ech_Ecto	0,209	0,134	0.004*			
FreeL:Ech_Endo	0,249	0,175	0.007*			
FreeL:Gas	0,188	0,131	0.010*			
FreeL:Gen	0,218	0,149	0.005*			
FreeL:Hol_Endo	0,204	0,136	0.002*			
Gas:Ech_Ecto	0,054	0,066	0,141	0,058	0,052	0.021*
Gas:Ech_Endo	0,103	0,128	0,126	0,103	0,1	0.045*
Gas:Gen	0,073	0,09	0,122	0,073	0,067	0.030*

Table 6.1 *Cont.*

Gas:Hol_Endo	0,075	0,061	0.015*	0,084	0,048	0.001*
Gen:Ech_Ecto	0,079	0,091	0,098	0,083	0,068	0,009
Gen:Ech_Endo	0,058	0,144	0,665	0,06	0,112	0,528
Gen:Hol_Ecto	0,025	0,087	0,908	0,018	0,064	0,973
Hol_Endo:Ech_Ecto	0,086	0,061	0.006*	0,091	0,048	0.001*
Hol_Endo:Ech_Endo	0,059	0,13	0,537	0,05	0,102	0,587

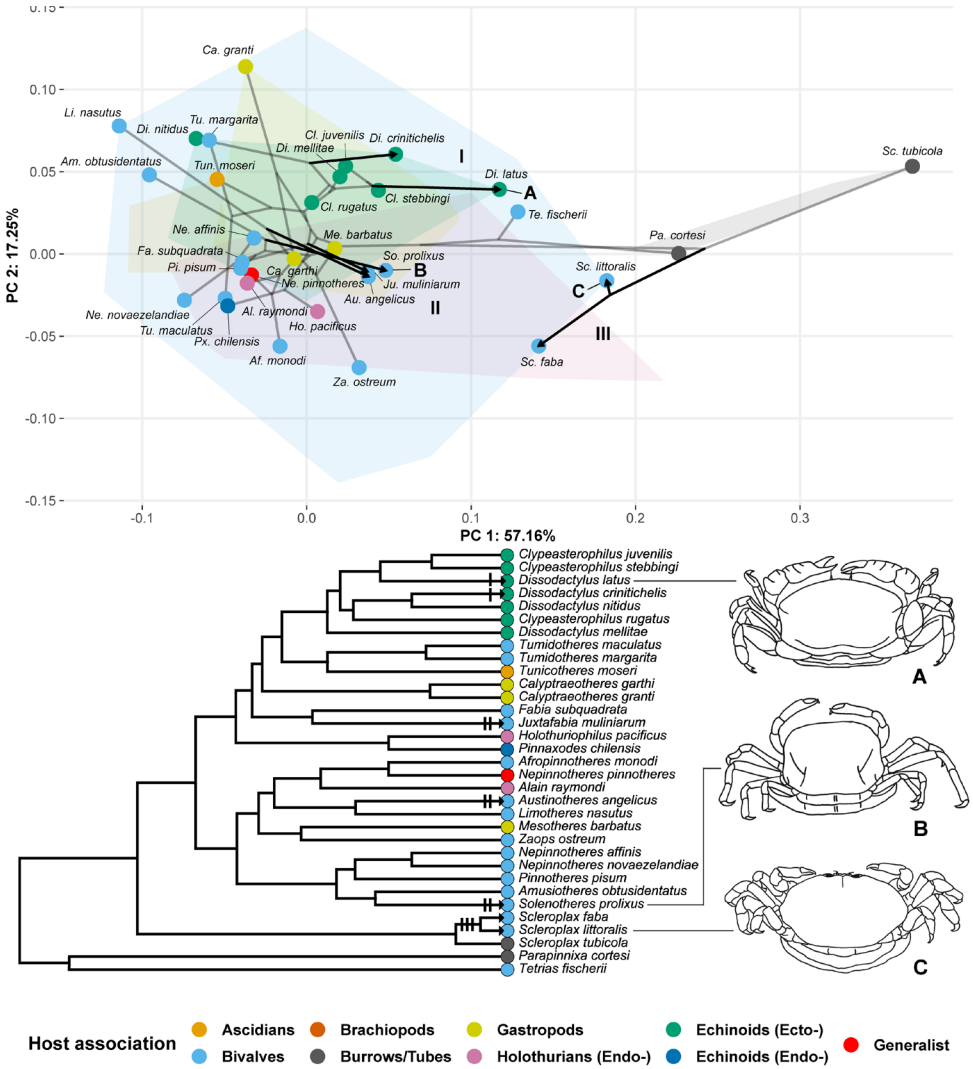
Notes: Host associations are abbreviated: Asc) Ascidians; Biv) Bivalves; Bra) Brachiopods; Bur/Tub) Burrows/Tubes; Gas) Gastropods; Hol_Endo) Holothurians (Endosymbiotic); Ech_Ecto) Echinoids (Ectosymbiotic); Ech_Endo) Echinoids (Endosymbiotic); Gen) Generalists; Free) Freeliving. *d* = distance between mean shapes, UCL (95%) = 95% upper confidence level, Pr > *d* = P-value associated with the mean shape distances (d). Significant values ($p < 0.05$) are indicated by an asterisk (*).

gastropod-associated species. The ascidian- and holothurian-associated species show a more pointed posterior side of their carapace, and the widest point of the carapace is found on the anterolateral side. The bivalve- and gastropod-associated species have a straight posterior side of their carapace. Lastly, the rostrum of ascidian-associated species is slightly broader and more pronounced.

Phylomorphospace approach

A pruned phylogeny tree was projected on the morphospace (fig. 6.3), resulting in a so-called phylomorphospace (fig. 6.5). The tree “starts” in the center and to the right side of the plot with the branches containing *Tetrias fischerii* and *Parapinnixa cortesi* Thoma, Heard & Vargas, 2005, respectively. These two “ancestral” branches come together in the center of the plot, after which the tree splits into two branches, directed into opposite directions of the plot. To the right, representing Pinnixinae, the three species of *Scleroplax* Rathbun, 1894 (of which two are endosymbiotic and somewhat more round, and one is tube-dwelling and extremely wide), and to the left the phylogeny projection stays on one side of the plot, overlapping while branching off into all the ingroup species (Pinnotherinae).

Convergence in morphology can be observed when branches split off in separate instances, but point in the same direction. Instances of presumed convergence can be observed in the plot above (fig. 6.5). Three examples of presumed convergence are highlighted here: within a genus (I), between three different genera (II), and between subfamilies (III). Morphological convergence within a genus is suggested here within *Dissodactylus* Smith, 1870, where *Dissodactylus latus* (fig. 6.5: crab A) and *Dissodactylus crinitichelis* Moreira, 1901 share a wider carapace shape than the rest of the genus. A similar shift in



6.5 Phylomorphospace plot and the projected ultrametric phylogeny reconstruction. Specimens not included in the phylogeny are omitted in the morphospace for better readability. Colors of points and convex hulls correspond to the species' host association. PCs and corresponding shape changes along these axes same as in figure 6.3. Three convergence events are highlighted with arrows in both the phylomorphospace (I to III) and the tree, of which three species (A to C) are illustrated next to the tree: A) *Dissodactylus latus* Griffith, 1987, after Griffith (1987); B) *Solenotheres prolixus* Ng & Ngo, 2010, after Ng & Ngo (2010); C) *Scleroplax littoralis* (Holmes, 1895), after Palacios Theil & Felder (2020a) (setae omitted in illustrations; crabs not to scale).

morphology can be found in the branches including *Solenotheres prolixus* Ng & Ngo, 2010 (fig. 6.5: crab B), *Juxtafabia muliniarum* (Rathbun, 1918), and *Austinotheres angelicus* (Lockington, 1877), where the evolution of three different clades resulted in almost the same wide carapace morphology.

Interestingly, the two sister species of *S. prolixus* and *A. angelicus* appear to also be morphologically similar: both *Amusiotheres obtusidentatus* (Dai, 1980) and *Limotheres nasutus* Holthuis, 1975 have a relatively more angular, elongated shape with a defined rostrum. A presumed morphological convergence to a rounder carapace in the outgroup species can be observed for the two bivalve-associated species *Scleroplax faba*, and *S. littoralis* (fig. 6.5: crab C) when they are together compared with the ingroup species with the shortest morphological distance to the outgroup, *Zaops ostreum* (Say, 1817). For morphological comparison to the burrow/tube-dwelling outgroup species *Glassella floridana* (Rathbun, 1918), see above (fig. 6.3).

The three above-mentioned convergence events were statistically tested for their significance (table 6.2), indicated by their similarity-based measures (C-values) and significance

Table 6.2 Similarity-based measures of converge for three presumed convergence events in pea crab species combinations (1000 replicates, PC1 to PC3; 84.5% of the data explained).

Species combinations		C_1	C_2	C_3	C_4	P-Value for C_1
(I)	*Dissodactylus crinitichelis*, *Dissodactylus latus*	0,816	0,299	0,525	0,952	**0,004**
(II)	*Solenotheres prolixus*, *Juxtafabia muliniarum*, *Austinotheres angelicus*	0,845	0,139	0,5	0,031	**<.001**
(III)	*Scleroplax faba*, *Scleroplax littoralis*, *Zaops ostreum*	0,657	0,221	0,391	0,049	**<.001**

Notes: Due to *Zaops ostreum* (Say, 1817) being the ingroup species with the shortest overall distance to the two Scleroplax species, this species was chosen to represent the ingroup in this (III) calculation. P-values indicating the probability that the degree of convergence exceeds what would be expected from a randomly evolving lineage are in **bold** if significant ($P < 0.05$).

A PGLS analysis was performed to test the dependency of the morphometric data, taking the phylogenetic history of the included species into account. The PGLS resulted in an insignificant P-value ($P = 0.823$; $R^2 = 0.151$). Thus, the landmark (shape) data and the placement within the morphospace of the 33 included species are not associated with a host group, once phylogenetic nonindependence is taken into consideration (fig. 6.5). The comparative Procrustes ANOVA test, excluding the phylogenetic framework, resulted in a lower but also insignificant P-value ($P = 0.285$; $R^2 = 0.247$).

Discussion and Conclusions

Shape differences

Although the pairwise Procrustes ANOVA shows significant differences between the mean carapace shapes of the host-associated groups (table 6.1), the differences appear to be very inconspicuous (fig. 6.4). The mean shape of all bivalve-associated pinnotherine species included in this study was very similar to that of the entire set of the analyzed ingroup species. Therefore, assigning a particular pea crab carapace shape to a specific host group seems impossible at this point. However, hypotheses can be made to explain the observed shape variation of the data, based on possible physical limitations of the habitat. For instance: ascidian-associated species might need a more elongated shape (fig. 6.4) to fit in the basket-like structure inside the body of the host, and the limited space of the mantle tissue of gastropods might result in wider (and perhaps flatter) shaped associates (fig. 6.4) to keep the fragile appendages covered. The angular shape of echinoid ectosymbionts appears to be very typical for this host association, but all species in this group are closely related and part of the "*Dissodactylus* complex." Members of this complex are known to have evolved a wide range of morphological adaptations on their appendages and have a flattened body shape, which might be beneficial for living an ectosymbiotic lifestyle (see de Gier & Becker, 2020). Perhaps the angularly shaped carapace margins might be linked to this dorsal flattening of the body.

Different P-values are observed for the same Procrustes pairwise ANOVA tests, depending on whether the outgroup is included or not in the analyses. Although these differences might be unexpected due to most of the ingroup data being uniformly placed in both analyses (e.g., when comparing the gastropod and ectosymbiotic echinoid associates; table 6.1), they can be explained by the way the P-values are calculated. The P-values are based on the permutation of residuals, meaning that if the morphospace is expanded or condensed, the available data for running permutations would also change (Adams & Collyer, 2018; Kaliontzopoulou, pers. comm.). This also means that choosing appropriate outgroups is extremely important in morphometric analyses.

Convergence events and host specificity

Hultgren et al. (2022) already suggest that two bivalve-associated species (*Scleroplax faba* and *S. littoralis*; both included in the present analyses as outgroup) have evolved from having a wide carapace shape (as can be seen in the tube- and burrow-dwelling outgroup species) to having a relatively round carapace. The mean AR (carapace aspect ratio) of these two species was found

to be significantly higher than the pinnotherine bivalve associates, but the green-coloured branches of figure 2 in Hultgren et al. (2022) clearly show the species to be rounder than their pinnixine relatives. Here, this proposed convergence between members of two subfamilies was shown in the phylomorphospace of the first two PCs (fig. 6.5, III), where the ancestral lineage of these two species shifts into the direction of the ingroup, with *S. faba* being more closely placed to the ingroup than *S. littoralis*. This difference in placement might be the result of an independent evolution of *S. faba*, evolving to be rounder, with the widest part of the carapace being on the lower side. The similarity-based measures for convergence (C_1 to C_4) indicate that these taxa (*S. faba* and *S. littoralis*, compared with a member of the ingroup, *Zaops ostreum*) show an average of 65.7% convergence (table 6.2; following Stayton, 2015). Although this is a rather low value due to the distance between the three species, under a Brownian motion model, this result is significant ($p < .001$), indicative of "true" convergence between these members of the in- and outgroup. Similarly, the two holothurian-associated outgroup species (*Pinnixa barnharti* Rathbun, 1918 and *Pinnixa tumida* Stimpson, 1858) seem to have undergone a similar presumed convergence event, shifting away from the rest of the outgroups towards the ingroup (fig. 6.3). This possible convergence might be related to the endosymbiotic host choice of these two species, as is also discussed for *S. faba* and *S. littoralis* by de Gier and Becker (2020) and Hultgren et al. (2022). However, whether these two *Pinnixa* species are phylogenetically related is unknown, although *P. barnharti* is found in Californian waters (Zmarzly, 1992), whereas *P. tumida* is known from Japan and China (Dai & Yang, 1991), which might suggest they are not very closely related. DNA analyses are needed to investigate their relatedness to the other species of *Pinnixa*, and consequently if their carapace shapes are the result of a single or of two separate convergence events associated with their host choice.

Another trend seen in the data is the convergence from different lineages in the ingroup, where branches move along the morphospace towards a wider body shape (fig. 6.5, II). In contrast to the outgroups, the three convergence events in the branches (including the bivalve-associated *Austinotheres angelicus*, *Juxtafabia muliniarum*, and *Solenotheres prolixus*) cannot easily be attributed to their host choice on a phylum level. While *A. angelicus* and *J. muliniarum* are relative generalists, inhabiting various families of bivalves, *S. prolixus* has been found so far exclusively in the elongated bivalve *Solen corneus* Lamarck, 1818. *Juxtafabia muliniarum* can also be found in one species of elongated bivalve, *Tagelus affinis* (Adams, 1852) (see de Gier & Becker, 2020). A potential correlation between the shape of the host bivalve and the carapace shape of the symbiont cannot be tested with the current datasets, but more symbionts

of elongated bivalves can be found among the pinnotherine (some of which take wide and/or otherwise aberrant shapes: e.g., *Raytheres* (Campos, 2004), *Serenotheres* Ahyong & Ng, 2005, and *Visayeres* Ahyong & Ng, 2007(Ahyong & Ng, 2007; Campos, 2002; Ng & Meyer, 2016)). The C_1 value of this event shows an average of 84.5% convergence, with a highly significant probability ($p < .001$; table 6.2).

There are several other species with a wide carapace, of which no phylogenetic information was available (see fig. 6.4). For this reason, no conclusions on the origins of their wide body shape and potential convergence events can be drawn. Most notably are the holothurian-associated *Holothuriophilus trapeziformis*, the giant-clam-associated *Xanthasia murigera* White, 1846, and the gastropod-associated *Mesotheres unguifalcula* and the three species of *Orthotheres* Sakai, 1969.

An example of a convergence event where the host choice seems to have played at least a partial role can be found within the *Dissodactylus* complex (fig. 6.5, I). *Dissodactylus* and *Clypeasterophilus* Campos & Griffith, 1990 are two closely related paraphyletic genera, known for their ectosymbiotic association with echinoids (de Gier & Becker, 2020; Griffith, 1987; Palacios Theil et al., 2016). Two species of *Dissodactylus* were found to move in the phylomorphospace along the first PC axis in a similar manner, after splitting from their theoretical ancestor, away from the rest of the species complex: *D. crinitichelis* and *D. latus*. These species appear to have a relatively wide carapace shape, compared with the rest of the clade (fig. 6.5, I; see crab A). When running a similarity-based convergence measure analysis for these two species, a C_1 value of 81.6% was found, with a significant P-value of 0.004 (table 6.2). This relatively higher P-value could be an indication of incomplete convergence or parallel shifts in the morphology (see Grossnickle et al., 2020). Both species occur in different, but overlapping regions in the tropical West Atlantic, living on various species of flattened echinoids (*D. crinitichelis* being more generalistic in its host choice; see de Gier & Becker, 2020). The current plots suggest that these species both could have occupied a similar niche in the same distribution range, and therefore evolved towards a similar shape. The sister species of *D. crinitichelis*, *Dissodactylus nitidus* Smith, 1870, has a more rounded carapace, and might be expected to live on different host groups. However, *D. nitidus* can be found on similar flattened sand dollars, albeit on different species from the East Pacific (de Gier & Becker, 2020). A detailed study focusing on the historical biogeography and morphological ancestral character states of the species in the *Dissodactylus* complex needs to be done in order to disentangle the two genera, resolve their phylogenetic positions, and

discern if certain body shapes are favorable for living on specific host species. Host switching within one life history, as was shown by Jossart et al. (2013) in *Dissodactylus primitivus* Bouvier, 1917, might also play an important role in the morphological plasticity of the genera. In addition, 3D morphometrics might be applied to also acquire information about their flattened body shapes, which might be linked to the widened carapace shapes and their host choices, also suggested for other genera by Hultgren et al. (2022).

The PGLS analysis, evaluating whether the host associations explain the variation of shape, given the phylogenetic framework of the 33 included species, resulted in an insignificant P-value. This means that the shape differences are not associated with the host groups, once phylogenetic nonindependence is taken into account. The linear model, excluding the phylogenetic framework, also resulted in an insignificant P-value. While this means that the shape differences between these 33 included species are not explained by the predefined host associations, performing the analysis on the full dataset (181 specimens) results in contrasting, significant values ($P = 0.001$, $R^2 = 0.347$). The low sample size of the previous tests (18.8% of the original data) might be the case of the deviating results of the analyses (see Mundry, 2014). Increasing the sample size by including molecular data of more pea crab species might prove if the phylogenetic framework plays a significant role in the diversification of the entire (sub)family.

Ancestral reconstructions and ecomorphological trends

Besides analyzing convergence events, the phylomorphospace approach allows for a close examination of the evolution of shape in the deeper branches of the phylogeny (e.g., Ford et al., 2016). The currently presented phylogeny reconstruction “starts” with a somewhat widened outgroup species, *Tetrias fischerii*, and the much-more widened species *Parapinnixa cortesi* (fig. 6.5). This first bivalve-associated species is plotted between the large cloud of ingroup species, and the main tube- and burrow-dwelling outgroups (including its currently designated sister species *P. cortesi*).

In the phylogeny reconstructions of Palacios Theil et al. (2016), the placement of *Parapinnixa* and *Tetrias* Rathbun, 1898, as well as *Sakaina* and *Pseudopinnixa* Ortmann, 1894, appears to be unstable due to low support values. In the phylogeny reconstruction resulting from a concatenated alignment, Palacios Theil et al. (2016; fig. 5.7) found a clade combining *Tetrias* and *Pseudopinnixa* to have the most basal placement, after which a clade including *Sakaina* and *Parapinnixa* branch off. In another study, these clades switch places, with high support values for the basal placement of *Parapinnixa* and *Sakaina* (Palacios

Theil & Felder, 2020a; fig. 6.2). In addition, the placement of the Pinnixulalinae appears to be switching from a placement next to the Pinnotherinae (Palacios Theil et al., 2016), to a placement basal to the other two subfamilies (Palacios Theil & Felder, 2020a). Taking the wide body shapes of the Pinnixulalinae into account, it is likely that the typical round body shapes of symbiotic pinnotherine pea crabs have here evolved once, and that the carapace of the ancestor of the entire clade including the three subfamilies was broadly shaped. In addition, the somewhat round body shape of *Tetrias* could have evolved independently to live within a host. Future phylogenetic studies, including more species, will probably result in different tree topologies and new lineages, and will consequently be able to test hypotheses about the ancestral morphology of the most recent ancestor of the entire family (including *Tetrias*, *Parapinnixa*, and other related pea crab genera without a stable subfamily status).

Future perspectives

A problem posed by the currently presented data was a large number of species with unknown host, presumed free-living, or with generalist host associations. Not all of these species will truly be free-living and might have been dislodged or wandering from their host organism when sampled (McDermott, 2009). Using the present morphospace plots, the association type (endo- or ectosymbiotic) might be speculated by looking at the data clouds (e.g., the outgroup species *Pinnixulala heardi* Felder & Palacios Theil, 2020b, whose carapace shape is perfectly in line with the other tube- and burrow-dwelling outgroups, as was already speculated by Felder and Palacios Theil (2020b); fig. 6.3). Within the ingroup, “predicting” a specific host type for species without a known association may be more difficult, and unexpected host associations might influence the shape of the convex hulls in the morphospaces and consequently influence the P-values of the pairwise (M)ANOVA (table 6.1). Similarly, landmarking extremes in the morphological variation of certain (often synonymized) species (e.g., within *Arcotheres*; Ng & Ahyong, 2022) might result in unexpected shifts in the datapoint clouds. In future studies the number of specimens per species should be increased, to account for this variation. In addition, efforts should be made to make the dataset as complete as possible by adding host records of species that currently have an unknown host association, or have been regarded as free-living as for now. Moreover, a large number of species that could not be included in this study due to the lack of images might be added in the future. In the case of convergence analyses, additional molecular data are needed to include more species in the phylogeny reconstruction, and therefore in the phylomorphospace. This way, the statistical strength of the methods used in this study can be improved considerably. These more elaborate analyses can only be done if future works in the field of pea crab systematics, phylogenetics,

and ecology allow for it.

Using (phylo)morphometrics allows users to visualize morphological evolutionary pathways and to see shape variation on a scale that might not be perceivable by using measurements only (e.g., “traditional morphometrics”; Zelditch et al., 2012). The field of ecomorphology (sensu Feilich & López-Fernández, 2019) has been using the currently presented methods for years, studying the evolution of shape as a result of ecological factors, mainly in vertebrates (Claverie & Wainwright, 2014; Curth et al., 2017; Dugo-Cota et al., 2019; Kulemeyer et al., 2009; Sherratt et al., 2019), and less so in invertebrates (Bush et al., 2006; Malcicka et al., 2017). This is due to the sampling limitation of selecting homologous 2D, or 3D landmarks in all samples (Zelditch et al., 2012). Firm homologous structures like skeletons seem to be easier to compare, but soft-bodied invertebrate taxa pose a problem in this respect. Invertebrates like mollusk bivalves or gastropods have, however, been studied thanks to their hard (partial) exoskeleton (Burridge et al., 2015; Potkamp et al., 2017; Sherratt et al., 2016; Verhaegen et al., 2018), but for unknown reasons, crustaceans seem to be underrepresented in the field of eco-, and phylomorphometrics (with the exception of Marochi & Masunari, 2016; Trontelj et al., 2012; Vermeiren et al., 2021).

In the current study, the phylomorphospace approach shows already multiple presumed convergent evolutionary pathways in a limited phylogenetic framework. The presented data suggest that host-switching events could have had an important role in the evolution of carapace shapes of non-pinnotherinae pea crabs, moving from a tube/burrow-dwelling biology to a strictly endosymbiotic lifestyle within bivalves, in adult crabs. Within the pinnotherinae, however, host switches between phyla seem to have had almost no effect on the evolution of carapace shape. This suggests that a shift in lifestyle from ecto- to endosymbiotic could be the driver for carapace (and overall body) shape diversification, rather than between-phyla host switches. This might also be the case in other symbiotic crustacean taxa with similar evolutionary switches in their lifestyles. For example, various palaemonid shrimp lineages have evolved from having a free-living lifestyle to a life in symbiosis with an invertebrate host (e.g., Frolová et al., 2022). In addition, some lineages have had multiple between-phyla host switches, some resulting in a shift from ecto- to endosymbiosis (Chow et al., 2021; Horká et al., 2016). These evolutionary pathways resulted in a wide range of morphological adaptations, ranging from changes in the morphology of the walking legs, eyes, and overall carapace shape (Dobson et al., 2014; Fransen, 1994, 2002). Although not studied in detail, symbiotic amphipods from the family Leucothoidae (inhabiting coral rubble,

but also bivalve, ascidian, and sponge hosts) might also have diversified in a similar matter (e.g., White, 2011). Lastly, the extremely specious copepod order Harpacticoida has had multiple shifts from a free-living life to a commensal or parasitic ecto- or endosymbiosis. A wide range of vertebrate and invertebrate hosts are utilised by these copepods, which is possibly the driver for their body shape diversification (e.g., Huys, 2016).

Supplementary information

Supplementary material is available online at: https://doi.org/10.1002/ece3.9744 [follow link to: Supporting Information – Supinfo: ece39744-sup-0001-Supinfo.docx].

Acknowledgements

The author would like to thank Charles Fransen, Bert Hoeksema, and Ronald Vonk (all Naturalis Biodiversity Center, the Netherlands) for their insightful comments on the manuscript, in all stages of writing. Bianca Trautwein and Angelika Brandt are thanked for the loan on the material from the Senckenberg Forschungsinstitut und Naturmuseum collection (Frankfurt, Germany). The author would like to thank Kristin Hultgren, Christine Foxx (both Seattle University, USA), and Emma Palacios Theil (University of Łódź, Poland) for sharing the phylogeny reconstruction of their paper (Hultgren et al., 2022). In addition, the author would like to thank Max Willems (Leiden University, Universiteit van Amsterdam; the Netherlands) for her help in making CT scans of the species in the *Dissodactylus* complex; Ton de Winter (Naturalis Biodiversity Center) for his help in making three of the photographs of the collection materials (fig. 6.1); and Jeroen Hubert (Leiden University), Brigida Gallone, and Vicky Beckers (both Naturalis Biodiversity Center) for their help and feedback building the script in R. Miriam Zelditch (University of Michigan, USA) is thanked for her extensive feedback and support in adding statistical analyses in this study, therefore improving it considerably. Antigoni Kaliontzopoulou (University of Barcelona, Spain), Mike Collyer (University of Sussex, UK), and Dean Adams (Iowa State University, USA) from the geomorph forum are thanked for their quick, elaborate answers. Lastly, the author would like to thank three anonymous reviewers for their insightful comments on the manuscript after submission.

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7

Ecomorphological convergence in the evolution of two clades of ascidian- and mollusc-associated shrimps (Decapoda: Caridea: Palaemonidae)

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A part of this chapter is published as:

de Gier, W., Helleman, P., Van den Oever, J. & Fransen, C. H. J. M. (2023) Ecomorphological convergence in the walking leg dactyli of two clades of ascidian- and mollusc-associated shrimps (Decapoda: Caridea: Palaemonidae). Ecology and Evolution, 13(12): e10768.

Abstract

Symbiotic species, living within or on the surface of host organisms, may evolve a wide range of adaptations as a result of various selection pressures, host specificity of the symbiont, and the nature of the symbiosis (obligatory or facultative commensalism, mutualism, or parasitism). In tropical marine coral reef ecosystems, palaemonid shrimps (Crustacea: Decapoda: Caridea) live in association with at least five different invertebrate phyla. Host switches between (distantly) related host groups, and the thereby associated selection pressures, were found to play a major role in the diversification of these shrimp lineages, giving rise to various host-specific adaptations. Two lineages of palaemonid shrimp, which have switched from an ectosymbiotic association towards endosymbiosis, are studied for their morphological diversification and possible convergence. Special attention is given to the rapid between-phyla host switches involving ascidian- and bivalve hosts, which are characteristic for these lineages. Using Comparative Phylogenetic Methods, landmark-based (phylo)morphometrics, and Scanning Electron Microscopy, three morphological character traits are studied, based on their presumed heavy selection pressures: body length, overall walking leg dactylus shape, and walking leg dactylus microstructures. No specific bivalve- or ascidian-associated morphotypes were found, but morphological convergence in both body lengths, as well as dactylus morphology was found in various species with similar hosts. In addition, presumed higher morphological diversification rates were found in lineages of bivalve-associated species, with intermediate morphotypes in lineages close to near host-switching events.

Keywords: Adaptive radiation; morphometrics; phylomorphospace; SEM-photography; symbiosis



Introduction

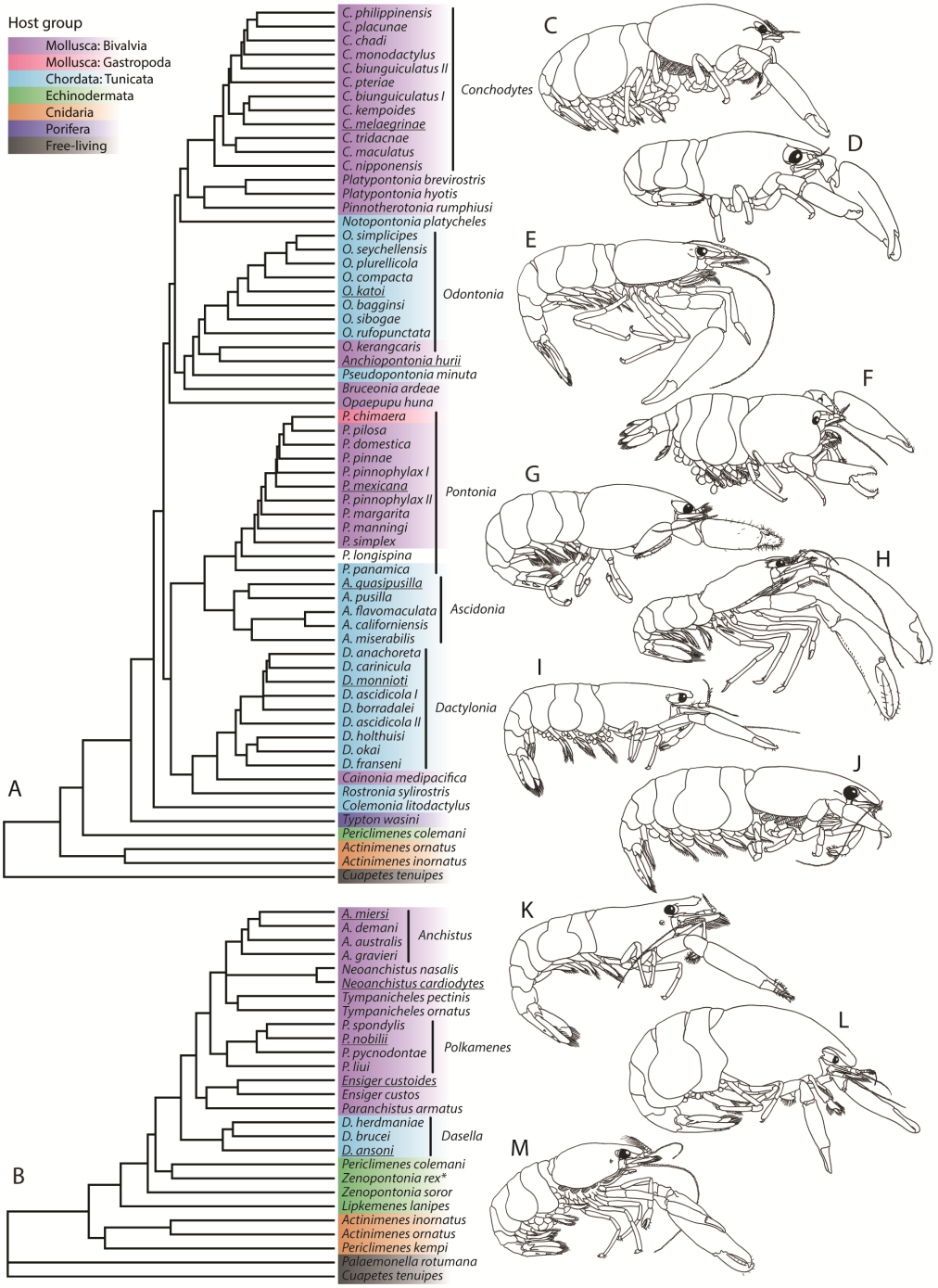
Biodiversity, and its variation in morphology, is shaped by various types of selection pressures (e.g., new dietary habits, predators, or environmental factors; Losos & Ricklefs, 2009; Burin et al., 2016; Edelaar et al., 2017), which result in evolutionary processes like speciation and extinction (e.g., Jablonski, 2008). In most classical examples, these selection pressures are the result of a species colonizing a newly available habitat and filling up a niche (e.g., adaptive radiation; Schluter, 2000). Although schoolbook examples mostly focus on macroevolutionary processes, such as island biogeography, habitat shifts might also occur on a much smaller, sympatric scale. For instance, in symbiotic relationships, a shift of a symbiont from one host species to another might also result in new selection pressures for the symbiont. Depending on the strength of the selection pressures, the host specificity of the symbiont, and the nature of the symbiosis (obligatory or facultative commensalism, mutualism, or parasitism), new species might rapidly arise from these host switches (Doña et al., 2018). Moreover, depending on the variation in host types due to host-switching events between distantly related hosts, a wide range of morphological adaptations might evolve in the symbiont species, resulting in a relatively high degree of morphological variation (Sapp, 1994; Munday et al., 2004; Ghalambor et al., 2007; Joy, 2013; Kise et al., 2023). Convergent evolution may also play a role, if similar selection pressures are at play in different clades of symbionts, resulting in the same, or similar adaptations (Pérez-Losada et al., 2009; Goto et al., 2012; Poulin & Randhawa, 2015; Horká et al., 2018; Li et al., 2018; Kise et al., 2023).

A group of animals where this rapid evolution of adaptive features seems to have occurred, is a clade of marine symbiotic shrimp species in the family Palaemonidae (Crustacea: Decapoda: Caridea). It is estimated that about 70% of the marine species in this family have established some form of symbiotic interaction (De Grave, 2001). Most species of symbiotic palaemonid shrimps are involved in protective symbioses (Chow et al., 2021), meaning that they live inside the body cavity of their host (endosymbiotic, as used in Baeza, 2015) or on the surface of their host (ectosymbiotic), both supplying a shrimp with dietary needs or a certain level of protection through either providing shelter or camouflaging possibilities. Most of these associations have evolved with fellow coral reef inhabitants, including species of the phyla Porifera, Cnidaria, Mollusca, Echinodermata, and Chordata (Bruce, 1995; Horka et al., 2016; Chow et al., 2021). In addition, so-called ‘inquilinistic forms’ of palaemonid shrimps have evolved to share a burrow with pistol shrimps (Caridea: Alpheidae), opisthognath fish, and echiurids (Frolová et al., 2022). This above-

mentioned host range is presumably an incomplete record, as many other symbiotic interactions have not been identified yet, and more associations are being discovered (de Gier & Fransen, 2018; Rauch et al., 2019; Anker & De Grave, 2021; Fransen et al., 2021, 2022; Komai et al., 2023). Several studies have demonstrated interphylum host-switching to be very important for the past diversification of the family (Kou et al., 2013, 2015; Gan et al., 2015; Horká et al., 2016, 2018; Davis et al., 2018). Host-switching events, together with coadaptation and cospeciation, have been argued to be the main drivers of diversification within this group (Horká et al., 2016).

Host switching events seem to have occurred a few times in ectosymbiotic clades within the Palaemonidae, mainly between echinoderm and cnidarian hosts (Horká et al., 2016). In endosymbiotic lineages, like clade 5 and 6 of Horká et al. (2016), these events are hypothesised to have happened much more frequent (de Gier et al., 2022). The endosymbiotic mollusk- and ascidian-associated palaemonid species are generally grouped in two clades (fig. 7.1; de Gier et al., 2022; de Gier & Fransen, 2023), from hereon called the '*Conchodytes* clade' and the '*Anchistus* clade'. The first clade includes the larger genera *Ascidonia* Fransen, 2002, *Conchodytes* Peters, 1852, *Dactylonia* Fransen, 2002, *Odontonia* Fransen, 2002, and *Pontonia* Latreille, 1829, in addition to some minor, often monotypic genera (de Gier et al. 2022). The second clade includes the genera *Anchistus* Borradaile, 1898; *Dasella* Labour, 1945; *Ensiger* Borradaile, 1915; *Neoanchistus* Bruce, 1975; *Paranchistus* Holthuis 1952; *Polkamenes* de Gier & Fransen, 2023; and *Tympanicheles* de Gier & Fransen, 2023 (de Gier & Fransen, 2023).

The endosymbiotic lifestyle of these clades seems to be mirrored by their morphological variation: lots of species exhibit morphological adaptations that seem to be linked to an endosymbiotic lifestyle inside a soft-bodied host. These adaptations often include: (A) adaptive colouration, thereby enhancing camouflage; (B) a cylindrical-shaped body to fit in the internal cavity of the host (fig. 7.1); (C) a reduction in the size and number of frontal, dorsal and lateral protrusions from the body, thereby aiding movement inside the host's cavity (fig. 7.1); (D) altered eye morphology; and (E) presence of microstructures on the ambulatory dactyli (such as denticles, hooked teeth and microsetae) (Bruce, 1972, 1976, 1980, 1995; Fransen, 1994; Dobson et al., 2014, 2016; de Gier et al., 2022). In contrast, most free-living species of palaemonid shrimp carry more spiny appendages on the rostrum and carapace (Fransen, 1994), and have less complex dactyli (Chow et al., 2021). The body sizes of symbiotic shrimps, and the observed (limited) sexual dimorphism in size, might also be linked to their endosymbiotic lifestyle, and therefore their (presumed)



7.1 Phylogenetic and morphological variation of the two studied clades. A, B) Ultrametric phylogeny reconstructions based on TE-analyses from de Gier et al. (2022) (A) and de Gier & Fransen (2023) (B); C–M) Lateral views of a number of representatives from the two clades. Representatives are underlined in the phylogeny trees: C) *Conchodytes meleagrinae* Peters,

(7.1 *Cont.*) 1852, after Fransen & Reijen (2013); D) *Odontonia katoi* (Kubo, 1940), after Hayashi (2003); E) *Anchiopontonia hurii* (Holthuis, 1981), after Bruce (1992); F) *Pontonia mexicana* Guérin-Méneville, 1855, after an illustration by Alberto Guerra, for BioDiversidad Marina de Yucatán (BDMY); G) *Ascidonia quasipusilla* (Chace, 1972), new illustration of RMNH. CRUS.D.51678; H) *Dactylonia monnioti* (Bruce, 1990), after Bruce (1990); I) *Anchistus miersi* (De Man, 1888), after de Gier & Fransen (2023); J) *Neoanchistus cardiodytes* Bruce, 1975, after Bruce (1975); K) *Polkamenes nobilii* (Holthuis, 1952), after de Gier & Fransen (2023); L) *Ensiger custoides* (Bruce, 1977), after de Gier & Fransen (2023); M) *Dasella ansoni* Bruce, 1983, after Bruce (1983). Shrimp dimensions not to scale. Colours indicate host groups (see legend), with *Zenopontonia rex* (Kemp, 1922) being ectosymbiotic with holothurians and nudibranch gastropods (indicated by an asterisk (*)).

mating systems. Baeza and Thiel (2007) reviewed and modelled the mating systems of various taxa of symbiotic crustaceans, and found that most strictly monogamous symbionts have limited to no degree of sexual dimorphism in both size and morphology (Baeza & Thiel, 2007). The models also suggest that males of endosymbiotic monogamous species might evolve smaller sizes, due to the limited food sources within the host's body cavity. Dwarf males can also be found in a more complex mating system that involves a pure-search polygynandry of sedentary females (as in most pea and gall crabs, and some shrimp species (Baeza & Thiel, 2007; Van der Meij, 2014; Van der Meij et al., 2023)). A number of adaptations mentioned above (B, C, E) are also found in other groups of endosymbiotic crustaceans, such as pea and gall crabs (Decapoda: Brachyura: resp. Pinnotheridae and Cryptochiridae) (Vehof et al., 2016; de Gier & Becker, 2020), and commensal or parasitic copepods (Gotto, 2004).

The above-mentioned adaptations may be linked to an endosymbiotic lifestyle, but most information is based on short remarks on a few selected species in taxonomic and ecological literature (Bruce, 1976, 1995; Fransen, 1994, 2002). We now have the means (an extensive voucher collection, as well as various new figuration and analysis methods) to properly study these morphological adaptations in an evolutionary context. As of today, there is no information on the host specificity of these adaptations; although hosts from different phyla may share a similar protective microhabitat (Horká et al., 2016), we suspect some morphological adaptations to be ascidian- or mollusc- specific in these shrimp clades. Additionally, some morphological adaptations might be present in species which are restricted to one family of bivalve or ascidian hosts. We also expect convergent evolution to play a major role in the morphological variation within these clades when switching back to a similar host, and will study these patterns in detail. This study combines phylogenetic information from previous literature (de Gier et al., 2022; de Gier & Fransen, 2023) with various phylogenetic comparative methods (PCMs), 2D morphometric analyses, and

microscopic imaging methods (SEM), and attempts to elucidate the plasticity in shrimp morphology in relation to the various known host-switches in the evolutionary history of the selected clades.

Materials and methods

Species selection and data acquisition

The '*Anchistus* clade' and '*Conchodytes* clade', consisting of respectively 18 and 55 currently known species (de Gier et al., 2022; de Gier & Fransen, 2023), were used as ingroups. We included a wide range of endo- and ectosymbiotic shrimps that are related to the studied clades, as well as two species with a free-living lifestyle, as outgroups. The outgroup selection is the same as in the studies by de Gier et al. (2022) and de Gier & Fransen (2023). Of these species, three types of data were acquired through the use of imaging techniques and literature. Published information of currently known host associations, distribution data, and maximum body measures were collected. In addition, dactylus shape data was acquired from taxonomic literature. For the newly acquired SEM images, collection material was used that was obtained during historical and recent expeditions and has been deposited in the decapod collection of Naturalis Biodiversity Center (previously Rijksmuseum van Natuurlijke Historie; RMNH.CRUS.D). These samples were collected using standard collection procedures in the field (mainly SCUBA diving) and stored in 70% ethanol. The materials used for the SEM study, as well as the references used during the literature search, are listed in table S1.

Continuous character mapping

Continuous trait evolution could be mapped on the trees by including information of the maximum post-orbital carapace length (pocl), measured from the posterior margin of the orbit to the posterior margin of the carapace, as a proxy for the body size in palaemonid shrimp species, for both female and male specimens, found in taxonomic literature (table S1). Missing measurements for one of the two sexes resulted in an exclusion from the analysis. Ancestral character values for all internal nodes were calculated by using the built-in R function fastAnc() as a Maximum likelihood (ML)-method. Character maps were constructed in R using the packages phytools v. 1.0-3, ape v. 5.6-2, and geiger v. 2.0.10 (Revell, 2012; Pennell et al., 2014; Paradis & Schliep, 2019) and the contMap() command. Hosts were coded by colours overlapping the names and a continuous scale from 'hot' to 'cold' was used to indicate maximum pocl measurements. Similar colours would indicate convergence towards the same pocl, and therefore the same body size, in the selected species. In addition,

boxplots were made to show pocl variation for every clade, per host type (only focusing on bivalve- and ascidian hosts. A GLM-test was performed on the '*Conchodytes* clade' to see if the differences were significant ($p < 0.05$). This could not be done for the '*Anchistus* clade' due to the low sample size ($n = 3$ for ascidian associates).

To measure phylogenetic signaling for the pocl value as a character, two tests were performed on the continuous data and the used phylogeny reconstructions: The Blomberg et al.'s (2003) K statistic and Pagel's (1999) lambda (λ) (Revell, et al., 2008). The command phylosig() in phytools was used, running with 1000 replicates. K-, λ-, and corresponding P-values are given (Revell & Harmon, 2022). The most recent phylogeny reconstructions were used to map the data for the '*Anchistus* clade' (de Gier & Fransen, 2023) and for the '*Conchodytes* clade' (de Gier et al., 2022). This could only be done after excluding any degree of dependency of the Total Evidence (TE) tree topologies on body-size related character states.

2D morphometrics

A total of 18 landmarks (7 stationary landmarks, 11 semi-landmarks) were placed on 2D lateral images of the third walking leg dactylus of all studied species (table S1). Homologous landmarks were chosen based on the full dataset; where comparable anatomical features could not be landmarked due to their absence, the landmarks fall on the same anatomical point (Gómez-Robles et al., 2011). The landmarks are listed below and also plotted in a schematic drawing of a shrimp (fig. 7.2).

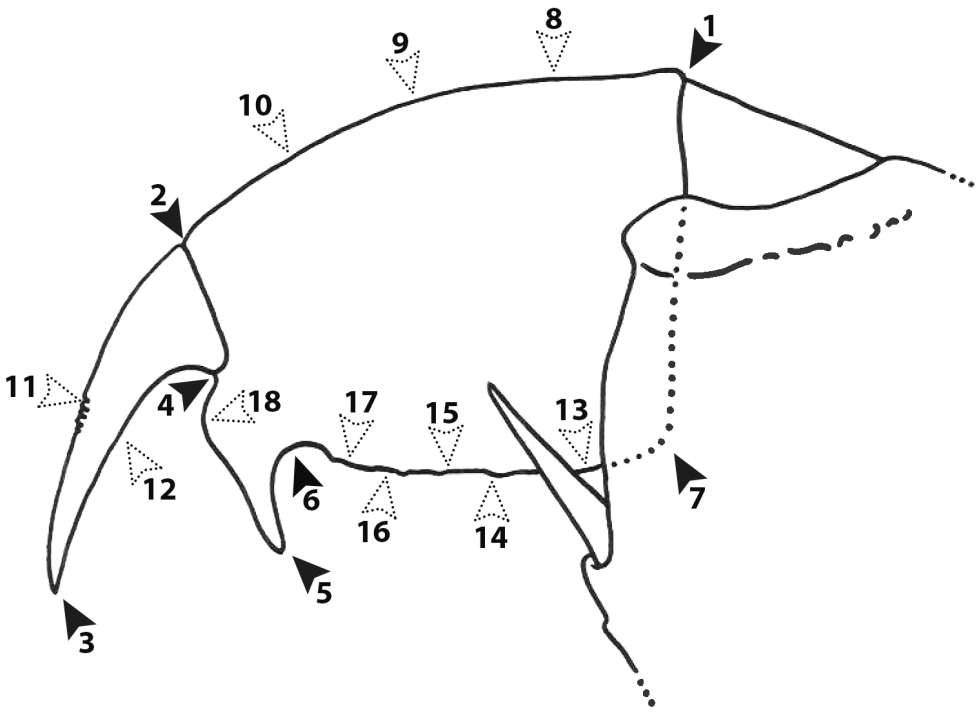
Landmarks:

1. The dorsoproximal end of the corpus, adjacent to the intersegmental membrane (approximation).
2. Dorsal border of the unguis with the corpus.
3. Terminal point of unguis.
4. Ventral border of the unguis with the corpus.
5. Terminal end of accessory tooth (in species without accessory tooth, same as landmark 4).
6. Proximal base of the accessory tooth (in species without accessory tooth, same as landmark 4).
7. The ventroproximal end of the corpus, adjacent to the intersegmental membrane (approximation).

Semi-landmarks:

8. Dorsal side of the corpus, quarter of the length to the unguis.
9. Dorsal side of the corpus, half of the length to the unguis.

10. Dorsal side of the corpus, three-quarter of the length to the unguis.
11. Dorsal side of the unguis, half of the length.
12. Ventral side of the unguis, half of the length.
13. Ventral side of the corpus, quarter of the length to the accessory tooth.
14. Ventral side of the corpus, half of the length to the accessory tooth.
15. Ventral side of the corpus, 5/8th of the length to the accessory tooth.
16. Ventral side of the corpus, three-quarter of the length to the accessory tooth.
17. Ventral side of the corpus, 7/8th of the length to the accessory tooth.
18. Distal base of accessory tooth (in species without accessory tooth, same as landmark 4).



7.2 Landmark selection for the morphometric analyses on the third pair of walking legs dactyli. Dactylus of *Pontonia manningi* Fransen, 2000 is shown with seven landmarks (filled with arrows) and 11 semi-landmarks (white totted arrows). Illustration altered after Fransen (2002).

Landmark data was gathered in tpsDig2 (v. 2.31) (Rohlf, 2017) and analysed using R v. 4.2.1 and Rstudio v. 2022.07.0 (R Core Team, 2022; RStudio Team, 2022), using the packages geomorph v. 4.0.4 and ggplot2 v. 3.3.6 (Wickham, 2016; Baken et al., 2021; Adams et al., 2022). A generalised Procrustes analysis (GPA) was performed to scale, transform and rotate all images for

morphospace analyses. In this way, scale was set to “uniform” and centroid size was not taken into account. Species were coloured in the morphospaces based on their host association (similar to de Gier, 2023). Morphospaces with all species annotated in the plot can be found in the supplementary datafile (Appendix 1: figs. A1, A2; numbers in table S1). A Procrustes (M)ANOVA with a residual randomization permutation procedure (1000 permutations, RRPP) (Collyer & Adams, 2021) was performed to detect significant effects of the host associations on the placement of the species in the morphospaces, and a pairwise test was performed to find significant differences in the mean shape data in relation to host specificity. Distance between the mean shapes (d), Upper Confidence level (UCL (95%)) and P-values associated with the mean shape distances (Pr > d) are given (de Gier, 2023).

Phylomorphospace analyses for convergence

Previously published phylogeny reconstructions of the two clades were used to project the evolutionary history of the species on the morphospaces, resulting in so-called phylomorphospaces (Stayton, 2015). The latest published Total Evidence tree topologies were used to project the phylogeny reconstruction on the data (de Gier et al., 2022; de Gier & Fransen, 2023). Since the TE trees are partially based on morphological characters found in the walking leg dactyli, the phylogenies were trimmed to include only specimens of which molecular data was available. This excludes any dependency of the tree topologies on the morphological character state analysis. Only one specimen of those species was included for each branch, with the exception of species with a polyphyletic placement (de Gier et al., 2022). Phylomorphospaces were built in R using the packages phytools, geiger, and ape (see above). The similarity-based measures C_1 to C_4 with corresponding P-values and 1000 replicates (Stayton, 2015; de Gier, 2023) were calculated using the R package convevol v. 1.3 (Stayton, 2018). The convergence measures and their calculations are described by Stayton (2015). For the calculations of the C-values, PC-values were used from PC1 to PC3 (81% and 80% of the explained data for the ‘*Conchodytes*-’ and ‘*Anchistus* clade’, respectively).

Two phylogenetically informed ANOVAs (Phylogenetic Generalized Least Squares; PGLS) were performed in a similar way as the one described by de Gier (2023). This was done to investigate the impact of host choice on the shape variation in the 2D data of the two studied clades, while controlling for independence of the residuals from the phylogeny (Mundry, 2014; Adams & Collyer, 2018). This was done for 37 species in the ‘*Conchodytes* clade’, including five outgroup species and two polyphyletic species, and 18 species in the ‘*Anchistus* clade’ (including nine outgroup species), by using the procD.pgls()

command in geomorph, with Pagel's lambda (λ) set at 1.0 (a high phylogenetic signal – Brownian motion model). For comparison, regular Procrustes ANOVA regression was performed on these subsets of the data. This was done to see if exclusion of the phylogenetic framework had any effect. In all analyses a RRPP-approach was used with 1000 permutations.

SEM study

A total of 49 specimens were sampled for a Scanning Electron Microscope (SEM) study; 41 ingroup species (including 1 duplicate - *Polkamenes pycnodontae* (Bruce, 1978) from an aberrant host (de Gier & Fransen, 2023) and eight outgroup species (table S1). Specimens are studied and prepared under a dissecting microscope (Zeiss Discovery v.8). From every specimen, the right third pereiopod was subsampled using dissecting tweezers, preferably from an ovigerous female specimen. The dactyli were cleaned with a fine brush under the dissecting microscope to remove any host tissue and sediment material. The pereiopods were then dehydrated in an ethanol series: 2×15 min in 80% ethanol, followed by 2×15 min in 96% ethanol, followed by 2×30 min in 100% acetone. Afterwards, the samples were dried using Critical Point Drying-methods (CPD) with a Leica EM CPD300 (Naturalis Biodiversity Center, Imaging facilities), according to the protocol used by de Gier & Fransen (2018). Each pereiopod was placed on a single stub using double-sided tape and prepared for the SEM by coating the samples with a 20 nm Pt/Pd coating using a Quorum Q150T S.

The dactyli were photographed using a JEOL JSM 6480LV Scanning Electron Microscope (Naturalis Biodiversity Center, Imaging facilities). The SEM gives the possibility to take very detailed photographs of microstructures on the dactyli, which were scored as four different morphological character states. The following character states were used:

- Unguis dorsal surface: coverage smooth (0); with randomly placed small teeth (1); with teeth placed in rows (2); with a striated pattern (3); and with a patch of teeth at the distal end only (4 – only for *Odontonia rufopunctata* Fransen, 2002).
- Unguis ventral surface: coverage smooth (0); with shallow grooves (1); with randomly placed small teeth (2); and with teeth placed in rows (3).
- Unguis tip morphology: simple (0); with one to three scale-like structures (1); with dorsal teeth also covering the unguis tip (2); with a scoop-like distal end, covered in teeth (3); and with a patch of teeth at the distal end only (4 – only for *O. rufopunctata*).
- Accessory tooth: absent, or barely visible (0); present, but without

coverage (1); covered with minute teeth (2); covered with shallow grooves and teeth on the proximal base (3); with patches of minute teeth on the distal side (4).

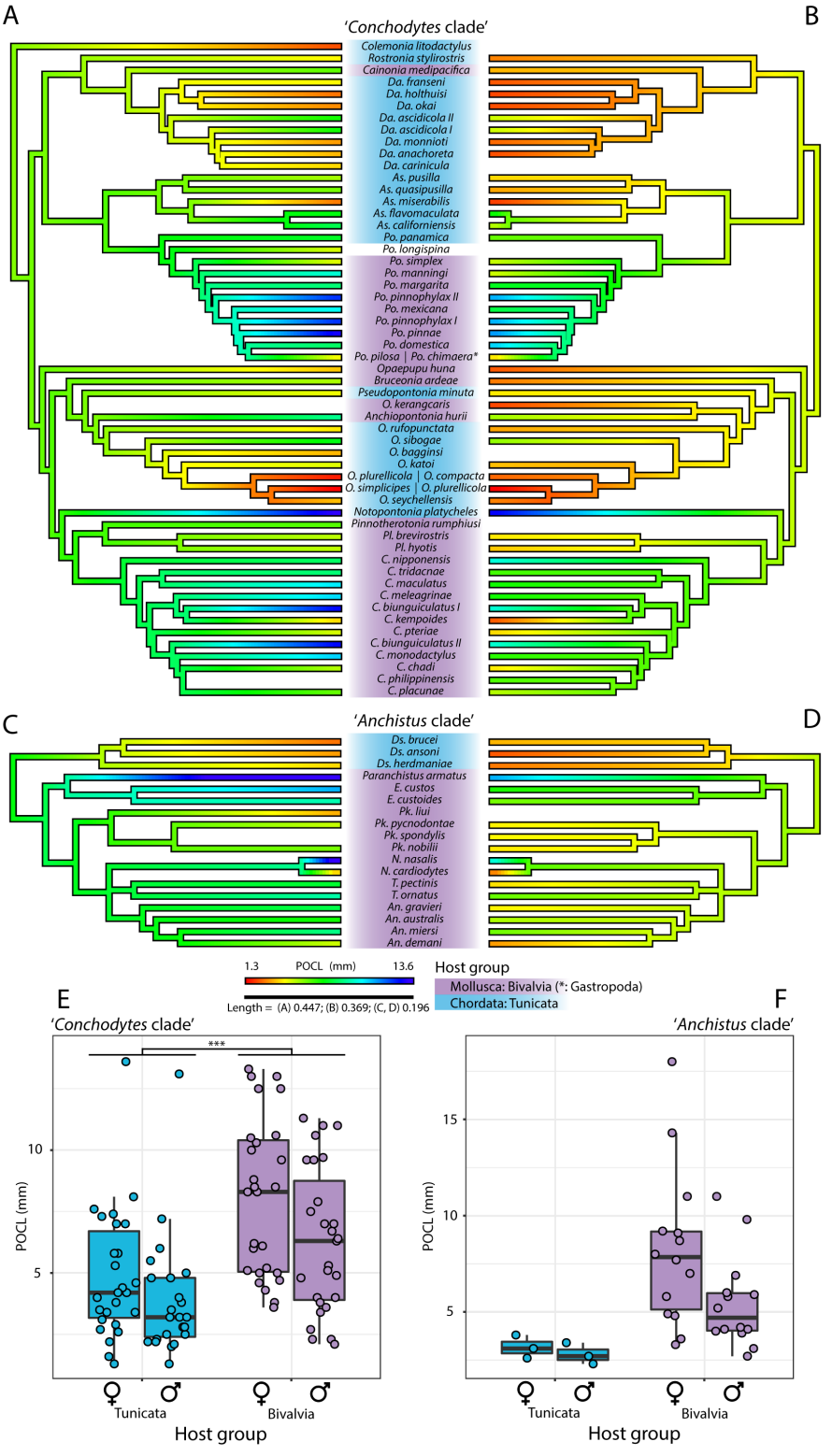
Species of which the microstructures were tentatively placed in a character state, were annotated with an asterisk in the results (e.g., the small, but still visible accessory tooth of the species in *Zenopontonia* Bruce, 1975 are annotated as 0* in the fourth character list). The entire dactylus, the unguis and the accessory spine (if present) were photographed for every sample. Additionally, minute microstructures were photographed in more detail if present. Plates were edited, and backgrounds were deleted in Adobe Photoshop. All SEM-captures, with insets of relevant details, can be found in Appendix 1 (figs. A1–A7).

Results

POCL measurements

Continuous character mapping of the maximum pocl-values resulted in four phylogeny reconstructions, with a colour gradient indicating the shortest (1.3 mm) to the longest (13.6 mm) measurements found in published and unpublished literature (fig. 7.3A–D). For species of which measurements of both sexes could be found, the colouration of the branches of the phylogeny seems to generally match for both clades (between female (fig. 7.3A, C) and male (fig. 7.3B, D) measurements), with males being in most cases smaller than the female specimens (fig. 7.3E, F). The only exceptions to this are the species *Conchodytes nipponensis* (De Haan, 1844), *Dasella brucei* Berggren, 1990, and *Pontonia simplex* Holthuis, 1951, in which the male measurements were found to be somewhat bigger (9.7 vs. 8.3 mm; 3.4 vs. 2.6 mm; and 4.9 vs. 4.6 mm, respectively).

The ancestral character state analysis, using a ML-approach, gave an indication on how much the body sizes changed over time. In the '*Conchodytes* clade', a relatively small body size evolved during several different occasions: in *Dactylonia* (once at least); in *Ascidonia miserabilis* (Holthuis, 1951); in *Odontonia* (most apparent in the clade containing *Odontonia plurellicola* de Gier & Fransen, 2018, *Odontonia simplicipes* (Bruce, 1996), *Odontonia compacta* (Bruce, 1996), and *Odontonia seychellensis* Fransen, 2002); and once in *Conchodytes* (in *Conchodytes kempoides* Bruce, 2013). Basally a small body size can also be observed in *Colemonia litodactylus* Bruce, 2005 (discussed below). In the male specimens, comparatively small body sizes were also found in *Opaepupu huna* Anker & De Grave, 2021, *Bruceonia ardeae* (Bruce, 1981), and *Odontonia kerangcaris* Fransen, Groenhof & de Gier, 2021. In contrast, a

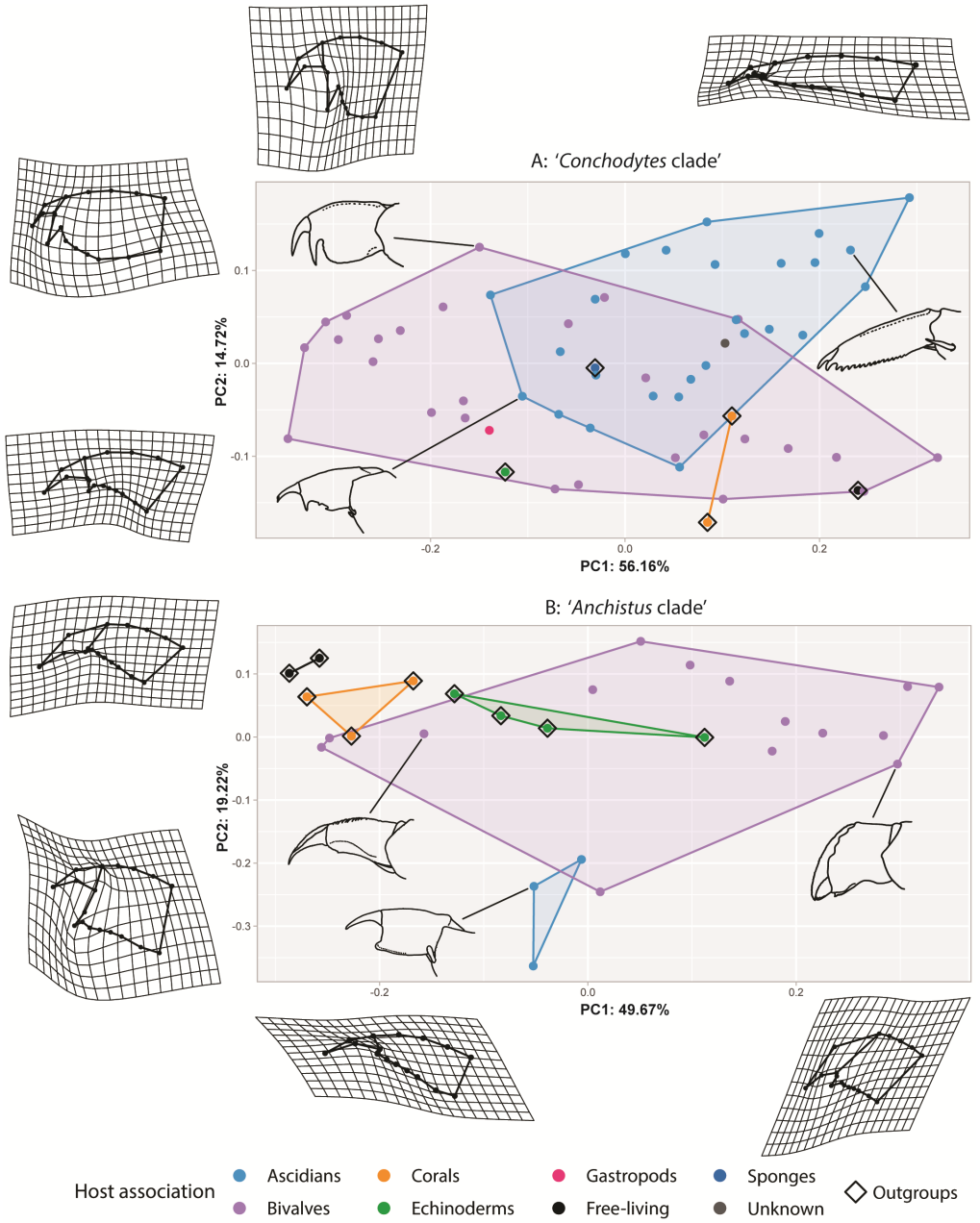

A
'Conchodytes clade'
B
Colemonia litodactylus
Rostronia stylirostris
Cainonia medipacifica
Da. franseni
Da. holthuisi
Da. okai
Da. ascidicola II
Da. ascidicola I
Da. monnioti
Da. anachoreta
Da. carinicula
As. pusilla
As. quasipusilla
As. miserabilis
As. flavomaculata
As. californiensis
Po. panamica
Po. longispina
Po. simplex
Po. manningi
Po. margarita
Po. pinnophylax II
Po. mexicana
Po. pinnophylax I
Po. pinnae
Po. domestica
Po. pilosa | Po. chimaera*
Opaepupu huna
Bruceonia ardeae
Pseudopontonia minuta
O. kerangcaris
Anchiopontonia hurii
O. rufopunctata
O. sibogae
O. bagginsi
O. katoi
O. plurellicola | O. compacta
O. simplicipes | O. plurellicola
O. seychellensis
Notopontonia platycheles
Pinnotherotonia rumphiusi
Pl. brevirostris
Pl. hyotis
C. nipponensis
C. tridacnae
C. maculatus
C. meleagrinae
C. biunguiculatus I
C. kempoides
C. pteriae
C. biunguiculatus II
C. monodactylus
C. chadi
C. philippinensis
C. placunae
C
'Anchistus clade'
D
Ds. brucei
Ds. ansoni
Ds. herdmaniae
Paranchistus armatus
E. custos
E. custoides
Pk. liui
Pk. pycnodontae
Pk. spondylis
Pk. nobilii
N. nasalis
N. cardiodytes
T. pectinis
T. ornatus
An. gravieri
An. australis
An. miersi
An. demani
1.3
POCL (mm)
13.6
Length = (A) 0.447; (B) 0.369; (C, D) 0.196
Host group
Mollusca: Bivalvia (*: Gastropoda)
Chordata: Tunicata
E
'Conchodytes clade'
***
POCL (mm)
10
5
♀
♂
♀
♂
Tunicata
Bivalvia
Host group
F
'Anchistus clade'
15
10
5
POCL (mm)
♀
♂
♀
♂
Tunicata
Bivalvia
Host group

7.3 Continuous character mapping and corresponding boxplots of the maximum pocl measurements in the studied clades, for both sexes. A, B, E) '*Conchodytes* clade', female (A), and male (B) specimens, with boxplot (E); C, D, F) '*Anchistus* clade', female (C), and male (D) specimens, with boxplot (F). Statistical tests were performed for boxplot E, but not for boxplot F due to a too low sample size (n = 3 for ascidian associates). The 'hot' to 'cold' colour gradient of branches indicate a short (min. 1.3 mm) or a long (max. 13.6 mm) pocl measurement. Colours behind the species names indicate host choice (with *Pontonia longispina* Holthuis, 1951 having no confirmed host record, and *Pontonia chimaera* Holthuis, 1952 living in gastropods, indicated by an asterisk (*)). Branch lengths are given for all phylogeny trees, and members of non-monotypic genera are abbreviated: (from top to bottom:) A, B) *Da.* = *Dactylonia*; *As.* = *Ascidonia*; *Po.* = *Pontonia*; *O.* = *Odontonia*; *Pl.* = *Platypontonia*; *C.* = *Conchodytes*; C, D) *Ds.* = *Dasella*; *E.* = *Ensiger*; *Pk.* = *Polkamenes*; *N.* = *Neoanchistus*; *T.* = *Tympanicheles*; *An.* = *Anchistus*. Species of which no male of female measurements were available, are excluded in the phylogeny reconstruction.

relatively large body size evolved in several species of *Pontonia*, *Conchodytes*, and the monotypic genus *Notopontonia* Bruce, 1991. Compared to the rest of the genus, *Dactylonia ascidicola* (Borradaile, 1898) has also evolved a larger body size. In the '*Anchistus* clade', a small body size can be found to have evolved in the branch including *Dasella*, but also in *Polkamenes liui* (Li, Bruce & Manning, 2004), *Neoanchistus cardiodytes* Bruce, 1975, and to some degree in *Anchistus demani* Kemp, 1922. A relatively large body size can be found in the basal branches of *Paranchistus armatus* (H. Milne Edwards, 1837), and the genus *Ensiger*. Interestingly, *Neoanchistus nasalis* Holthuis, 1986, was found to have a much larger body size than its congener mentioned above (14.3 and 9.8 vs. 3.6 and 2.7 mm).

Looking at the boxplots (fig. 7.3E, F), the pocl measurements can be compared between the two major host groups (bivalve vs. ascidian hosts). The bivalve-associated species from the '*Conchodytes* clade' have significantly larger POCL measurements than ascidian associates (intercept: 4.88, bivalves: 3.17, $p < 0.01$; fig. 7.3E). Visual observation of pocl measurements of the '*Anchistus* clade' reveals a similar pattern as for the '*Conchodytes* clade' (fig. 7.3E, F), however, we did not perform a statistical test for this clade because of a shortage of ascidian associates in our dataset.

Statistical tests for phylogenetic signaling resulted in a significant value for both tests (Blomberg et al.'s K-statistic and Pagel's λ) for both sexes of the '*Conchodytes* clade' (table 7.1). This means that there is a significant phylogenetic signal in the pocl data. Thus, the pocl variance among clades is higher than the variance within clades. For the '*Anchistus* clade', this is different: both values for both sexes were found to be lower than the ones found in the '*Conchodytes* clade', with non-significant P-values, meaning that there seems



7.4 Morphospaces of the third walking leg dactylus shape variation, resulting from the Generalized Procrustes Analysis, including warps of the maximum and minimum shape differences possible along the first two PC axes, relative to the mean shape of the morphospace. A) '*Conchodytes* clade' (including five outgoups); B) '*Anchistus* clade' (including nine outgroups). Colours of convex hulls and points indicate host associations, and three representatives are illustrated for each clade: A) *Conchodytes meleagrinae* Peters, 1852 (top left) (after Fransen & Reijnen, 2013), *Odontonia compacta* (Bruce, 1996) (bottom left), *Dactylonia franseni* Bruce,

(7.4 *Cont.*) 2003 (middle right) (both after Fransen, 2002); B) *Tympanicheles ornatus* (Holthuis, 1952) (top left), *Dasella herdmaniae* (Lebour, 1938) (bottom left), *Anchistus demani* Kemp, 1922 (middle right); all after de Gier & Fransen (2023). Diamonds indicate outgroup species. Annotations for each species are given in fig. S1.

to be no phylogenetic signal in the pocl data (figs. 7.3A, B, D, E). Blomberg et al.'s K-statistics and Pagel's λ values, with their corresponding P-values for both clades (per sex) are found above (table 7.1).

Table 7.1 Phylogenetic signalling statistics, Blomberg et al.'s K statistic, Pagel's lambda (λ), and corresponding P-values are given. Significant P-values are indicated with an asterisk (*).

Clade	Sex	K	P_K	λ	$P_λ$
'*Conchodytes*'	females	0,911	0.002*	0,687	0.002*
	males	0,764	0.006*	0,649	0.001*
'*Anchistus*'	females	0,442	0,897	<0.001	1
	males	0,362	0,928	<0.001	1

Landmark analyses and morphospaces

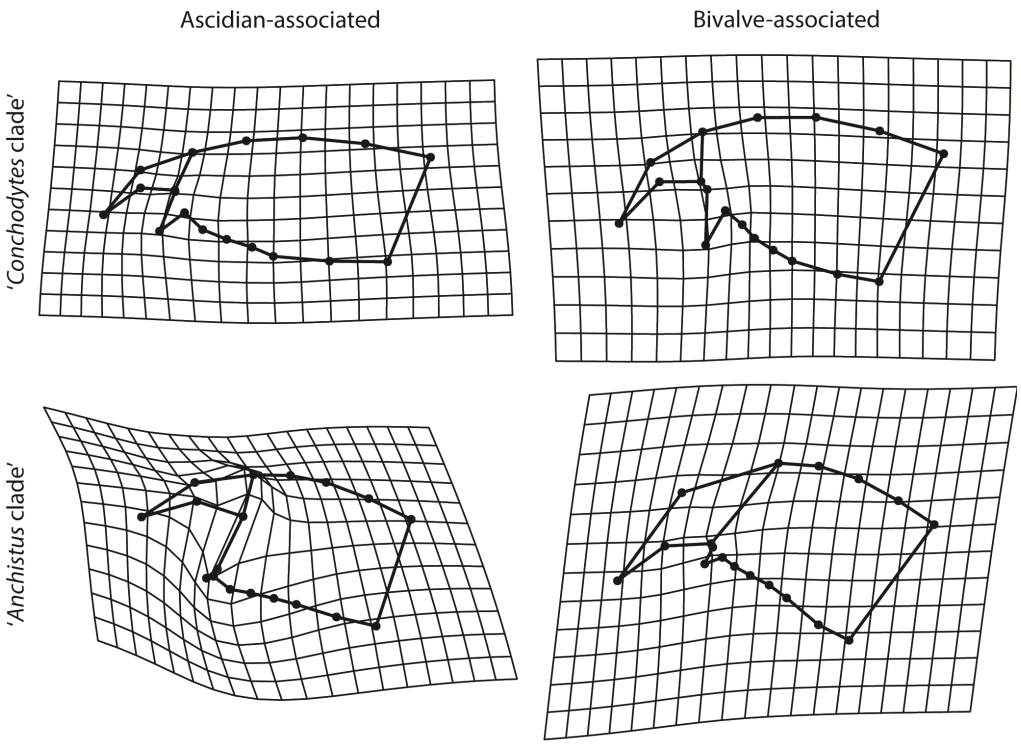
In the morphospace of the '*Conchodytes* clade', the first two PC axes explain 72.88%, with 99% being explained in the first 14 PCs, out of 32 dimensions (fig. 7.4A). The first two PC axes of the '*Anchistus* clade' explain 68.89% of the data, with 99% being explained in the first 10 PCs, out of 26 dimensions (fig. 7.4B).

The variation of the dactyli shapes in the '*Conchodytes* clade' show almost no differences in the unguis morphology, while the shape of the corpus, including the accessory tooth, varies the most (fig. 7.4A). This can be seen in the first axis of the morphospace, ranging from an almost round corpus with a long accessory tooth ($PC1_{min}$) to an elongated corpus shape with almost no accessory tooth ($PC1_{max}$; see *Dactylonia franseni* Bruce, 2003; fig. 7.4A). The second axis shows a variation in the curvature of the ventral side of the corpus, ranging from a curved corpus with a short accessory tooth ($PC2_{min}$), to an inflated corpus with a long tooth ($PC2_{max}$; see *Conchodytes meleagrinae*; fig. 7.4A, top left). Although the outgroups are scattered within the two major convex hulls of the bivalve- and ascidian-associated species (fig. 7.4A), these two latter groups seem to be only partially overlapping. Six bivalve-associated species (*Anchiopontonia hurii*; *Cainonia medipacifica* (Edmondson, 1935); *Conchodytes monodactylus* Holthuis, 1952; *Pontonia mexicana*; *P. pinnae* Lockington, 1878; and *P. pinnophylax* (Otto, 1821)) and one species with an unknown host (*P. longispina*) overlap with the ascidian-associated convex hull.

The species within the '*Anchistus* clade' also show some variation in the shape of the corpus, but in contrast with the species above, the unguis also

displays a large degree of variation (fig. 7.4B). The first displayed axis shows a morphological gradient, ranging from an elongated corpus, with a minute accessory tooth and a sharp, thin unguis ($PC1_{min}$) to a more rounded corpus with a large and often broad (de Gier & Fransen, 2023) unguis ($PC1_{max}$). The unguis might even be rounded at the apex, as is seen in *Anchistus demani*.

(fig. 7.4B). On the second axis, the morphological variation ranges from a wide, almost square corpus, with a broad outgrowth between the ventral border of the unguis and the accessory tooth ($PC2_{min}$; see *Dasella* in fig. 7.4B), to a dactylus with an elongated corpus and unguis, with the accessory tooth being absent ($PC2_{max}$). This last shape, seen in three bivalve-associated species (both species of *Ensiger*, and *Tympanicheles ornatus*; fig. 7.4B) is shared with the free-living and coral-associated outgroups. The chosen outgroups keep to one side of the plot, except for the echinoderm-associated species, partially overlapping with the bivalve-associated species. Only the ascidian-associated *Dasella brucei* overlaps with the convex hull of the bivalve-associated species (fig. 7.4B).



7.5 Mean dactylus shape warps of both clades: mean shapes of host associated groups (landmarks and lines) are compared with the mean shape of the entire clade (deformation grid).

Procrustes (M)ANOVA tests were performed (in all PC dimensions) to test for the effect of host-association on the placement of the species in the morphospaces. A significant effect was found for both clades ('*Conchodytes* clade': $R^2 = 0.248$, P = 0.001; '*Anchistus* clade': $R^2 = 0.411$, P = 0.001), thus the host associations seem to be influencing the shape variation. The pairwise tests resulted in significant shape differences between the bivalve- and ascidian-associated species, as well for both clades (fig. 7.5): '*Conchodytes* clade': d = 0.177, UCL (95%) = 0.097, Pr>d = 0.001; '*Anchistus* clade': d = 0.337, UCL (95%) = 0.273, Pr>d = 0.013). As was mentioned above, the number of ascidian-associated species in the '*Anchistus* clade' is relatively low and the power of these statistical tests is therefore limited. Visual inspection, however, indicates a strong difference in the dactylus morphology of these species compared with the bivalve-associated species in the clade (figs. 7.4B, 7.5). These morphological differences can be found in the shape of the unguis and the distance between the accessory tooth and the ventral unguis border. For the '*Conchodytes* clade' the differences are less conspicuous: only the shape of the corpus influences the deformation grid to expand in height (fig. 7.5).

Phylomorphometrics

Several suspected convergence events have been annotated in the phylomorphospace plots (roman numerals; I to V, and I to III, fig. 7.6). Stayton's similarity-based measures for convergence (C_1 to C_4, and a P-value for C_1) can be found below (table 7.2).

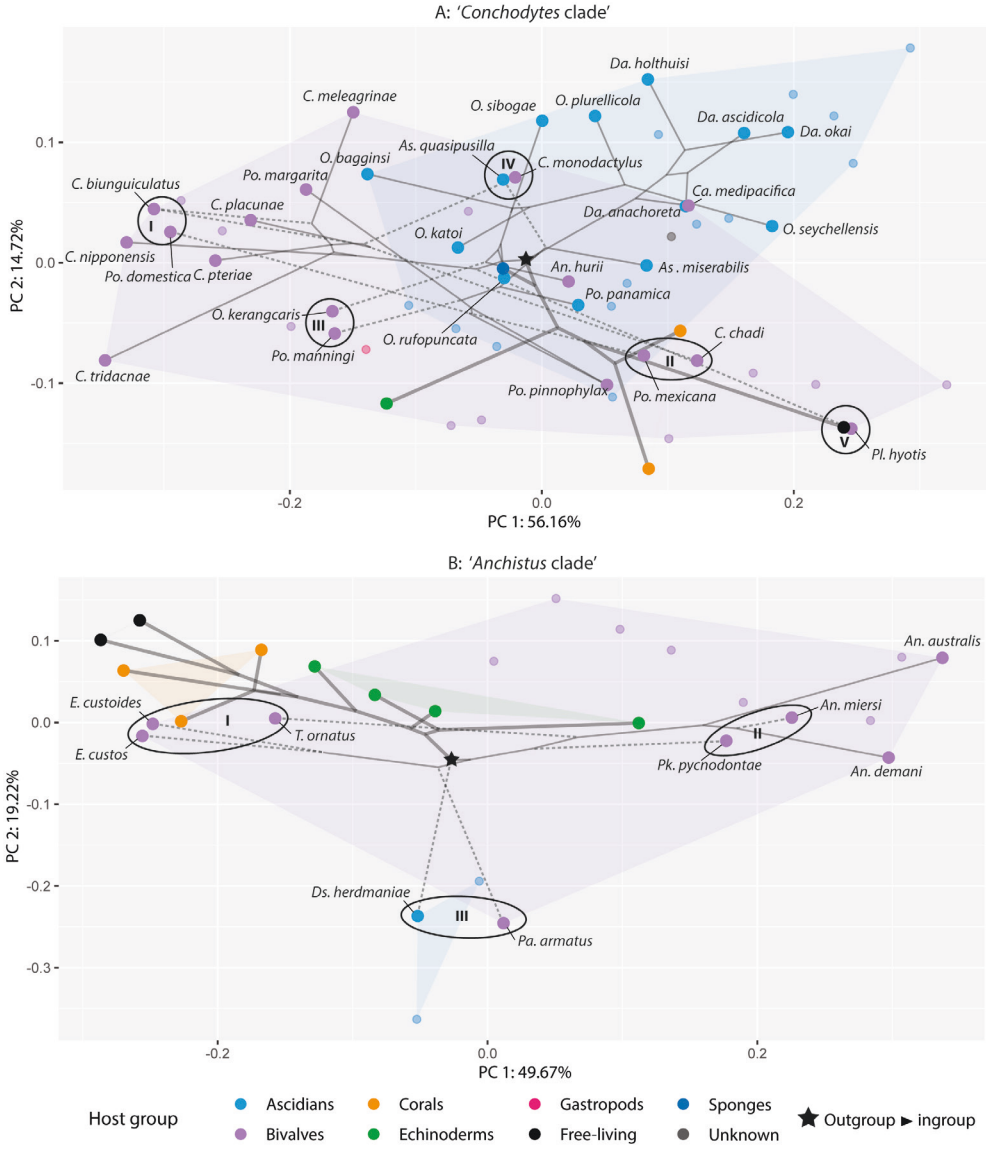
In the '*Conchodytes* clade', the outgroups are placed in the bottom and right part of the plot, but the ingroup clade 'starts' in the middle of the plot (fig. 7.6A). The tree splits into two clades: one consisting of *Ascidonia*, *Dactylonia*, and *Pontonia*, and the other of *Conchodytes* and *Odontonia* (also including one species each of *Anchiopontonia* Bruce, 1992 and *Platypontonia* Bruce, 1968) (fig. 7.6A). Within this first clade, the ascidian-associated *Dactylonia* and *Ascidonia* cluster together, diverging within the ascidian-associated convex hull. *Pontonia* also diverges within this convex hull, first with the ascidian associate *Pontonia panamica* Marin & Anker, 2008. Later, the branches split off in both directions, with *P. mexicana* and *P. pinnophylax* diverging towards a more elongated dactylus shape, and *Pontonia domestica* Gibbes, 1850, *Pontonia manningi*, and *Pontonia margarita* Smith in Verrill, 1869 towards a more round dactylus shape (fig. 7.4). The other major branch splits near the starting point of the phylogeny in the morphospace, into two clades: one including *Conchodytes* and basally *Platypontonia*, and the other *Odontonia* with basally *Anchiopontonia*. *Odontonia* and *Anchiopontonia* cluster somewhat together in the center and top right part of the morphospace, with species of *Odontonia* making up the

7.2 Similarity-based measures of convergence for eight presumed convergence events in the two studied clades (1000 replicates, PC1 to PC3; 81% and 80% of the data explained, for both clades, respectively). [1]) Two values are given for both the species combinations with *Conchodytes biunguiculatus* (Paulson, 1875) I and II; [2]) *Cuapetes tenuipes* (Borradaile, 1898) is part of the outgroup. Significant P-values are indicated with an asterisk (*).

Species combinations	C_1	C_2	C_3	C_4	P-value for C_1
'*Conchodytes* clade'					
(I) *Conchodytes biunguiculatus* (I/II)[1] & *Pontonia domestica*	0.703/ 0.733	0.215/ 0.250	0.272/ 0.281	0.046/ 0.053	0.019*/ 0.014*
(II) *Conchodytes chadi* &*Pontonia mexicana*	0,765	0,254	0,335	0,054	0.011*
(III) *Odontonia kerangcaris* & *Pontonia manningi*	0,813	0,219	0,354	0,047	0.002*
(IV) *Ascidonia quasipusilla* & *Conchodytes monodactylus*	0,563	0,115	0,202	0,025	0.037*
(V) *Cuapetes tenuipes*[2] & *Platypontonia hyotis*	0,828	0,243	0,539	0,044	0.003*
'*Anchistus* clade'					
(I) *Ensiger custoides* & *Tympanicheles ornatus*	0,751	0,312	0,376	0,19	0.003*
(II) *Anchistus miersi* & *Polkamenes pycnodontae*	0,648	0,237	0,33	0,313	0.016*
(III) *Dasella herdmaniae* & *Paranchistus armatus*	0,698	0,195	0,452	0,105	0.010*

outer edges of the ascidian-associated convex hull. The only exception to this is the bivalve-associated *Odontonia kerangcaris*, diverging towards various species of *Conchodytes* and *Pontonia*. The branch including *Conchodytes* and *Platypontonia* splits near the middle of the morphospace, towards the right side of the plot with *Platypontonia* (with an elongated dactylus), and towards the left side with the species of *Conchodytes*. The divergence of *Conchodytes* from there resembles *Pontonia* (see above), with *Conchodytes biunguiculatus* (Paulson, 1875), *C. nipponensis*, *C. placunae* (D.S. Johnson, 1967), *C. pteriae* Fransen, 1994, and *C. tridacnae* Peters, 1852 clustering together in the left side of the plot, and *C. chadi* (Marin, 2011) and to some extent, *C. monodactylus*, diverging to the right side.

In the phylomorphospace plot of the '*Anchistus* clade', the phylomorphospace is less tangled (fig. 7.6B). After the somewhat clustered outgroup, the first clade within the ingroup including *Dasella* splits off towards the lower side of the plot. Here, *Dasella* is only represented by one species, *Dasella herdmaniae*. After *Dasella*, the tree splits in two: one branch including both species of *Ensiger* and *Paranchistus armatus*, and one branch including *Anchistus*, *Polkamenes* and *Tympanicheles* (of which the latter two are only represented by one species).



7.6 Phylomorphospaces of the third walking leg dactylus shape variation, for both studied clades. A) '*Conchodytes* clade' (including five outgoups), B) '*Anchistus* clade' (including nine outgroups). Trimmed phylogeny trees from de Gier et al. (2022) and de Gier and Fransen (2023) are projected on the morphospaces; large datapoints are included in the phylogeny reconstructions, smaller datapoints make up the convex hulls of figure 7.4. Colors indicate host association, and the black star indicate the split between in- (thin lines), and outgroups (wide lines). Possible convergence events are indicated with roman numerals, and the concerned species are encircled. Terminal branches of the (possibly) converging species are indicated with dotted lines. Genera names are abbreviated: A) *An.* = *Anchiopontonia*; *As.* = *Ascidonia*; *C.* = *Conchodytes*; *Ca.* = *Cainonia*; *Da.* = *Dactylonia*; *O.* = *Odontonia*; *Pl.* = *Platypontonia*; *Po.* = *Pontonia*, B) *An.* = *Anchistus*; *Ds.* = *Dasella*; *E.* = *Ensiger*; *Pa.* = *Paranchistus*; *Pk.* = *Polkamenes*; *T.* = *Tympanicheles*.

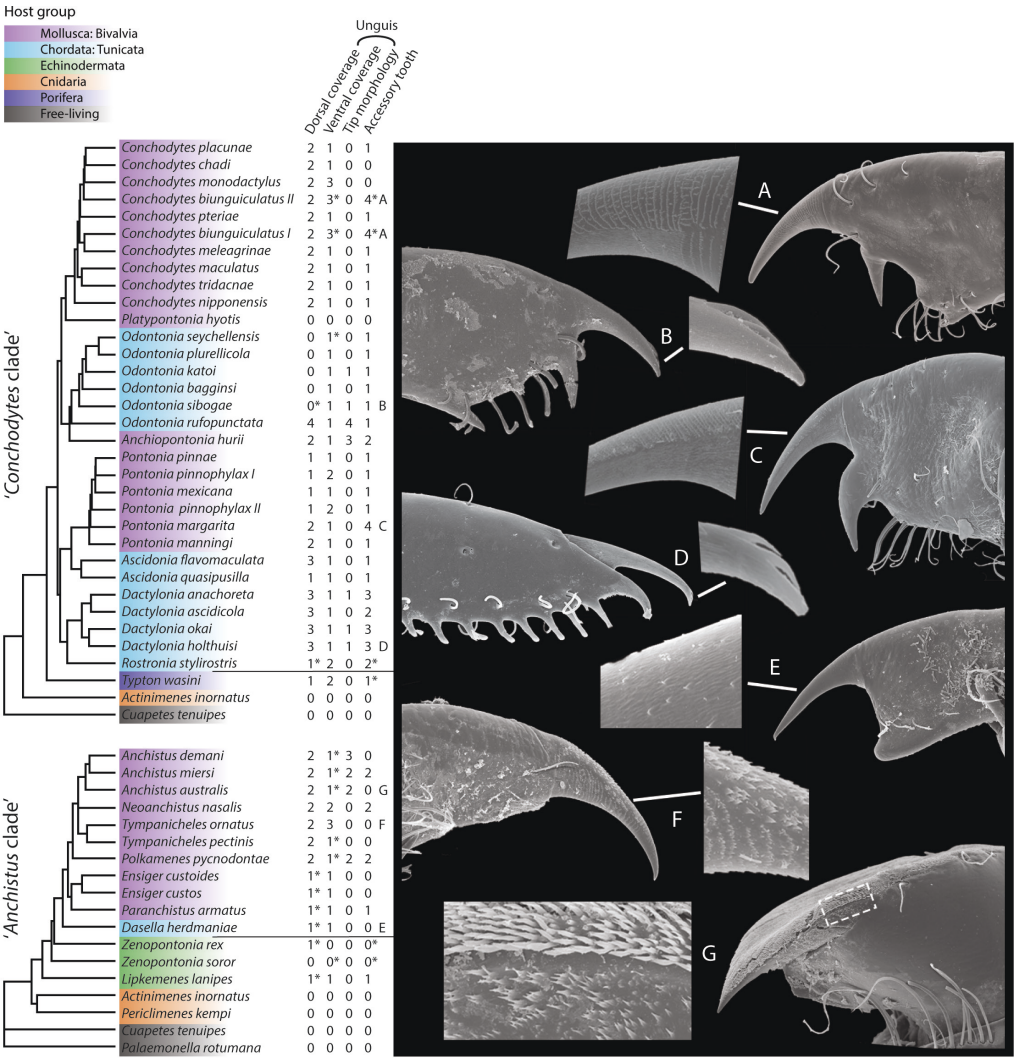
Ensiger diverges from the center of the plot towards the left side, near the cnidarian-associated outgroups, indicating that they have an elongated dactylus, without accessory tooth. *Paranchistus armatus* clusters together with *Dasella*, due to the large ventral outgrowth of the corpus of this species. The other major branch first splits off into *Polkamenes pycnodontae*, of which the dactylus resembles the more derived species of the ingroup, such as *Anchistus miersi*. Afterwards, *Tympanicheles ornatus* splits of in the direction of *Ensiger* and the cnidarian-associated outgroups. The rest of the species of the branch, *A. miersi*, *Anchistus australis* Bruce, 1977, and *A. demani*, cluster together on the right side of the plot, characterised by a broad unguis.

The PGLS analyses resulted in insignificant values for both the '*Conchodytes* clade' ($P = 0.749$; $R^2 = 0.102$), and the '*Anchistus* clade' ($P = 0.346$; $R^2 = 0.263$). This means that the landmark (shape) data and thus the placement of the selected species within the morphospace is not associated with a host group, once phylogenetic non-independence is taken into account. The P-values of the regular ANOVA regressions came back as significant for both clades ('*Conchodytes* clade': $P = 0.001$; $R^2 = 0.312$; '*Anchistus* clade': $P = 0.024$; $R^2 = 0.402$). This gives the impression that, for this subset of the original data, the phylogenetic framework is influencing the separation of our landmark (shape) data, with respect to host choice.

Dactylar microstructures

The hidden diversity of microstructures on the third walking leg dactyli of the studied species was presented by the SEM-captures (fig. 7.7; Appendix 1: figs. A1–A7). The dorsal coverage of the unguis could range from no microstructures in most outgroups, as well as in most species of *Odontonia*. In two outgroup species, sparsely placed minute teeth could be seen, which was a character shared by the genera *Dasella* (fig. 7.7E; on the proximal dorsal surface of the unguis), *Paranchistus*, *Ensiger*, but also in the other clade, in *Rostronia* Fransen, 2002. More, but irregular, coverage could be seen in most species of *Pontonia*, and in *Ascidonia quasipusilla*. A conspicuous patterning in the dorsal coverage was found in most species of *Conchodytes* (fig. 7.7A), but also in *Anchiopontonia hurii*, *Pontonia manningi* and *P. margarita* (fig. 7.7C). A comparable coverage was found in the rest of the species in the '*Anchistus* clade', covering the entire dorsal surface of the unguis in the included species of *Anchistus* (fig. 7.7G) and *Polkamenes*.

The ventral coverage of the unguis follows a similar pattern in the phylogeny as the dorsal coverage: no coverage was found in most outgroups, while a dense coverage of shallow grooves was found in most ingroup species (Appendix 1:



7.7 Character states of microstructures on the third walking leg dactyli of the species in the studied clades, with SEM-captures of examples of microstructure morphologies. Trimmed TE phylogeny trees from de Gier et al. (2022) and de Gier and Fransen (2023) are used. Four character states are scored, for the character states, and asterisks were added for character states which were tentatively placed in that category (see Material and Methods section). Letters A to G correspond to the SEM-captures on the right (see also Appendix 1: figs. A1–A7). Sem-captures not to scale. Colors indicate host association of the species, with *Zenopontonia rex* also being associated with nudibranch gastropods. The dactylus of *Odontonia sibogae* (Bruce, 1973) (B) after de Gier & Fransen (2018).

figs. A1–A7). Randomly placed teeth were found in *Pontonia pinnophylax* and *Neoanchistus nasalis* (Appendix 1: figs. A3E, A6C). In addition, patterning of the teeth on the ventral side of the unguis was found in *Tympanicheles ornatus* (fig. 7.7F) and *Conchodytes biunguiculatus* (fig. 7.7A). In the latter, the patterning

stops halfway the unguis (fig. 7.7A).

The tip of the unguis can be pointed, as in most studied species (Appendix 1: figs. A1–A7), but also flattened and shaped like a scoop-like structure. This character state was found in *Anchiopontonia hurii*, as well as in *Anchistus demani* (Appendix 1: figs. A2A, A6F). The unguis is in these cases fully covered with minute teeth. A low number of large 'scales' on the unguis tip was found in species of *Odontonia*, such as *Odontonia sibogae* (fig. 7.7B), and in various species of *Dactylonia*, such as *D. holthuisi* Fransen, 2002 (fig. 7.7F).

The accessory tooth also shows some variation in microstructure coverage. A morphotype with an accessory tooth covered with shallow grooves and teeth on the proximal base was found: three species of *Dactylonia* share this character state (see *D. holthuisi*, fig. 7.7D). An accessory tooth covered with minute teeth was found in *Anchiopontonia hurii*, *Dactylonia ascidicola*, and in the other clade in *Anchistus miersi*, *Neoanchistus nasalis*, and *Polkamenes pycnodontae* (Appendix 1: figs. A5F, A6C, E). The accessory tooth of *Rostronia stylirostris* (Holthuis, 1952) shows a coverage with large teeth, a unique feature (see Appendix 1: fig. A4A). The species *Conchodytes biunguiculatus* and *Pontonia margarita* both show a patterning of minute teeth only on the distal side of the accessory tooth, placed in patches (Appendix 1: figs. A1F, A3D).

Discussion and Conclusions

Linking sex related body sizes with mating systems

One of the major patterns found in the continuous character trait analysis of the pocl values is that female shrimp specimens appear to be larger in size than the male specimens of the same species, with the exception of three species (fig. 7.3A–D). The (limited) degree of sexual dimorphism in our studied taxa might suggest a fully monogamous mating system, or a mating system in which males move between hosts to reproduce with as many females as possible. Indeed, previous studies showed that the ascidian-associated *Odontonia katoi* exhibits a promiscuous mating system with males, and possibly females, moving among (commonly available) host individuals in search of receptive sexual partners (Baeza et al., 2015; Levitt & Shenkar, 2017). Here, male specimens are smaller than their female counterparts, but the relative size of their claws is larger than those of the female specimens. This is likely the result of intraspecific competition between males over females (Baeza & Thiel, 2007). Similarly, the ascidian-associated species *Ascidonia flavomaculata* (Heller, 1864) shows evidence for the same mating system (Baeza & Díaz-Valdés, 2011). Here the females seem to be sedentary in their hosts, while males move around and

compete with each other during the mating season. In contrast, studies on *Pontonia domestica*, *P. margarita*, *P. mexicana*, and an unidentified species of *Pontonia* showed these species to be socially monogamous (Baeza et al., 2011). The duration of the male-female pairing might, however, vary between species: male specimens from *P. domestica*, *P. margarita*, and an unidentified species of *Pontonia* were found to have long lasting bonds with their female partner (Courtney & Couch, 1981; Baeza, 2008; Aucoin & Himmelman, 2010). In the case of *P. margarita*, the male major claws did not display positive allometry, suggesting limited intraspecific competition (Baeza, 2008). In *P. mexicana*, a high degree of sexual dimorphism in the size of the major claws was found, suggesting the social monogamous system to be less strict in this species (Baeza et al., 2011). This appears to be the same for *P. pinnophylax* (Fransen, 1994; Richardson et al., 1997). In addition, while all studied *Pontonia* species were mostly found in pairs (as is suggested by theory for monogamous species), male *P. mexicana* were found with females that were close to molt and became sexually receptive more frequently than expected by chance alone, suggesting that the male specimens switch among host individuals (Baeza et al., 2011). There is not much known about the mating systems of the other species in the currently studied ingroups, except for evidence for long-lasting monogamous pairing in *Polkamenes pycnodontae* (in Baeza et al., 2012), and possible strict protandry in *Paranchistus armatus* (in Bruce, 2000a).

As was found in the above-mentioned studies, competition between male conspecifics, available host sizes, host abundance, and risk of predation all might influence the evolution of mating systems, and therefore the maximum body sizes in our ingroups (Baeza & Thiel, 2007; Baeza et al., 2015). As can be seen in the generated boxplots, species from ascidian hosts are generally smaller than bivalve symbionts, in both clades (fig. 7.3E, F). Infested bivalve host species might be able to grow much bigger (e.g., pen shells, giant clams), compared to ascidian hosts (e.g. Humann & DeLoach, 2010), resulting in a different degree of variation in the body sizes of these associated species.

One remarkable exception to this 'rule' is *Notopontonia platycheles* Bruce, 1991, being much larger than the other ascidian associates and even most bivalve-associated species (see fig. 7.3E, F). The only member of *Notopontonia* shares the same host (*Herdmania momus* (Savigny, 1816)) as other species in the currently studied group (e.g., members of *Dasella*, *Ascidonia*, and *Odontonia*), and one might think that this species has a different mating strategy than its relatives. However, Berggren (1999) mentioned the species to be living solitary in its host, similar to *Ascidonia flavomaculata* (mentioned above), indicating host-switching behaviour in this species. Why *Notopontonia* exhibits such large

body sizes remains unknown, but their supposed phylogenetic placement might give some insights (see below).

The species in the '*Conchodytes* clade' appear to have a higher pocl variance among clades than the variance within clades, suggesting that there is a high phylogenetic signal in this character. This was proven by Blomberg et al.'s K-statistics and Pagel's λ values (see table 7.1). However, it can be deduced that a large pocl has evolved in multiple instances, mainly once in *Pontonia*, *Notopontonia*, and (possibly) multiple times in *Conchodytes*. It might be possible that the common ancestor of the clade including *Conchodytes*, *Notopontonia*, *Platypontonia*, and *Pinnotherotonia* Marin & Paulay, 2010 has been a larger-sized shrimp, explaining the size of *Notopontonia*. Exceptionally small-sized shrimp have also evolved multiple times: mainly in *Odontonia*, *Ascidonia*, and *Dactylonia*. Interestingly, the bivalve-associated and basally-placed *Colemonia litodactylus* is also small in size, suggesting a small-sized ancestral character state for the entire clade. Within the '*Anchistus* clade', no phylogenetic signal for pocl was found. This is probably the result of the low sample size of this clade, definitely when considering that most outgroup species, as well as the first branch (including *Dasella*) are all small-sized. The species in the branch including *Ensiger* and *Paranchistus* appear to be much larger. This again might be the result of a host switch from an ascidian to a bivalve host, but this could not be proven in the present study. The female size of *Paranchistus armatus* specimens does not only exceed the maximum pocl value for all other species in the presently studied taxa, but also most other symbiotic palaemonid shrimp (Bruce, 2000a). This species lives in the largest species of giant clam, *Tridacna gigas* (L., 1758), possibly influencing the maximum body size of the symbiont (Bruce, 1972).

Convergence in dactylus shapes and host switching in a morphospace

Although the pairwise Procrustes (M)ANOVA resulted in significant values when comparing the mean shapes of the bivalve- and ascidian-associated species in both clades, there still seems to be an overlap in convex hulls of the first two PCs (fig. 7.4). One of these overlaps is also translated in the mean shapes to be almost the same in the '*Conchodytes* clade', with the bivalve-associated species having a somewhat more inflated corpus (fig. 7.5). The question arises if there are features in the walking leg dactyli that are typical for a certain host-association, and how much of the variation is explained by the phylogenetic placement of the species. In the '*Conchodytes* clade', both the ascidian- and bivalve-associated groups feature species with a large accessory tooth (most species of *Conchodytes* and *Pontonia panamica*), a proportionally elongated

unguis (most species of *Conchodytes*, of *Pontonia*, and *Pseudopontonia minuta* (Baker, 1907)), or a very flattened, elongated corpus (e.g., *Conchodytes chadi* and *Odontonia seychellensis*) (fig. 7.4A). The outgroup species do not cluster together in these first two PCs, and are scattered within the point clouds of both major host-associated groups (fig. 7.4A). In the '*Anchistus* clade', the same variation from an elongated dactylus shape (e.g., in *Ensiger*) to a more robust, inflated shape (e.g., in *Anchistus* and *Polkamenes*) can be found in the more numerous bivalve-associated species (fig. 7.4B). Less variation can be found in the ascidian-associated species: *Dasella* shows low intrageneric variation for the three known species (fig. 7.4B). In the '*Anchistus* clade', the mean shapes for the host-associated groups are easier to distinguish (fig. 7.5), and the outgroup species group somewhat together, although the overlap with the bivalve-associated species point cloud is evident in the echinoderm-associated species (fig. 7.4B).

The variation in shapes of both the ascidian- and bivalve-associated species, as well as the placement of the outgroups in the presented morphospaces, gives us the impression that, in general, there is no evidence for a typical 'bivalve- or ascidian-associated morphotype'. This was already suggested by a previous study from Chow et al. (2021), wherein character mapping was performed for a whole range of symbiotic shrimp dactylus shapes, grouped into nine categories (Chow et al., 2021; fig. 6). Most bivalve- and ascidian-associated species studied here (within clade IIIC and IIIH, sensu Chow et al., 2021) were grouped into category I (having no or a reduced accessory tooth) or II (the typical 'biunguiculate' dactylus shape), which they shared with numerous coral-, echinoderm-, and sponge-associated species. In addition, a lot of free-living species also share these general morphotypes. Four other species from the '*Conchodytes* clade' were found in the monotypic category IV for the bivalve-associated *Anchiopontonia hurii* (having a scoop-like unguis), and in category V for the species with a serrated ventral corpus. This was the case for the bivalve-associated *Cainonia medipacifica*, and the ascidian-associated *Dactylonia ascidicola* and *Odontonia sibogae*. This last category was shared with numerous sponge-, and echinoderm-associated species. *Dasella herdmaniae* was placed together with the included species of the coral-associated *Jocaste* Holthuis, 1952 and *Coralliocaris* Stimpson, 1860 in category VIII, due to the distoventral dilatation of the corpus. As can be seen, the morphotypes of the included bivalve- and ascidian-associated species are shared by a wide range of symbionts from related, and unrelated, clades. However, if the similarities between these morphologically similar species and our studied ingroup species are homologous, and if they share the same functional morphology, needs to be studied in more detail. It is also worth noting that other endosymbiotic

shrimp lineages show a lot of intra-generic variation in dactylus shape (as can be seen in the related, species-rich, and ascidian- and sponge-endosymbiotic genera *Periclimenaeus* Borradaile, 1915 and *Typton* Costa, 1844; see Bruce, 2013, 2000b, figs. 5E & 8H; Neves, 2020, fig. 3C; Anker et al., 2021, fig. 8). In the analysis by Chow et al. (2021), the morphological variation of these genera seems to be conserved, possibly due to the sampling size (as is the case of our ingroup species).

When following the phylogeny projections on our phylomorphospace plots (fig. 7.6), it can be deduced that there have been major morphological radiations in the evolution of the studied branches, even with the limited number of included species. Several potential instances of convergent evolution have been highlighted and statistically tested (fig. 7.6, table 7.2). These convergences, and the link with the host-switching events described in previous studies (de Gier et al., 2022; de Gier & Fransen, 2023), are discussed below.

Starting with the '*Conchodytes* clade' (fig. 7.6A), the genera *Conchodytes*, *Odontonia*, and *Pontonia* seem to be spreading out the most in the phylomorphospace. Various convergence events between species of *Conchodytes* and *Pontonia* can be discerned: *P. domestica* and *P. margarita* occur on the 'typical' *Conchodytes*-side of the plot, at $PC1_{min}$ (fig. 7.6A: event I); and *C. chadi*, *P. mexicana*, and *P. pinnophylax* all share a more elongated dactylus, on the positive side of PC1 (fig. 7.6A: event II). Moreover, *P. manningi* can also be found on the negative side of PC1, together with the only bivalve-associated *Odontonia* species, *O. kerangcaris* (see fig. 7.6A: event III). Closer examination of the dactylus of *O. kerangcaris* (see Fransen et al., 2021) shows a very '*Conchodytes*-like' dactylus for this species. The aberrant host-association and the basal placement of the bivalve-associated *O. kerangcaris* might be the cause of its placement close to various species of *Conchodytes* and *Pontonia*, instead of closer to its ascidian-associated congeners. The convergence between the two groups of *Conchodytes* and *Pontonia* cannot be explained by their host choice; they do not share the same host genera. Possible convergence between two species from different genera, and with different host associations could be found between *Ascidonia quasipusilla* and *Conchodytes monodactylus* (fig. 7.6A: event IV). No apparent reason was found for this very apparent-looking convergence event; the host of *C. monodactylus* is shared with more species within the genus (from *Pinna* and *Pteria*). The shapes of the dactyli do not match at first sight, it seems the shape of the unguis placed *C. monodactylus* between the ascidian-associated species in the first two principal components. When plotting the second and third principal component, the datapoints are placed more separately (Appendix 1: fig. S2).

The last convergence event in this plot can be observed to have happened between *Platyponontonia hyotis* Hipeau-Jacquotte, 1971, and the included free-living outgroup species *Cuapetes tenuipes* (Borradaile, 1898) (fig. 7.6A: event V). This indicates an evolutionary pathway from a more complex dactylus shape, to a simple, elongated shape. This has not only happened in this first clade, but also twice in the '*Anchistus* clade', with both species of *Ensiger* and *Tympanicheles ornatus* moving towards the direction of the cnidarian-associated and free-living outgroups ($PC1_{min}$) (fig. 7.6B: event I). It is worth noting that *T. ornatus* does, however, show more complex microstructures than the other species with an elongated dactylus. Another convergence event was found between the seemingly complex dactylus shapes of *Anchistus miersi* (and the other species of *Anchistus*) and *Polkamenes pycnodontae*, towards the right side of the plot ($PC1_{max}$) (fig. 7.6B: event II). In addition, two different genera with different host associations (*Dasella* and *Paranchistus*) have evolved similar dactylus shapes, according to the plot (fig. 7.6B: event III). This might be the result of the basal placement of the species, which will be discussed below.

When mapping the host-switching events from previous studies (de Gier et al., 2022; de Gier & Fransen, 2023) on the (limited) phylomorphospace data, it can be noted that the switches from an ascidian to a bivalve association result in a comparably higher degree of diversification. This is the case for the initial switch from an ascidian-associated ancestor for the branch including *Conchodytes*, *Odontonia* and related genera, towards a bivalve association in *Conchodytes*, *Anchiopontonia hurii*, and *O. kerangcaris* (de Gier et al., 2022, fig. 7.6, event V). After this initial switch, the branch including the other *Odontonia* species remains somewhat conservative in its morphological disparity (fig. 7.6A). The same happens in the branch including *Ascidonia*, *Dactylonia*, and *Pontonia*, which branches out in the top right and middle part of the phylomorphospace, until the switch from ascidian-association towards bivalve-association takes place after *P. panamica* (fig. 7.6A). The other *Pontonia* species, like *Conchodytes*, spread out towards the morphological limits, seen in PC1. Within the '*Anchistus* clade', this cannot be seen due to the ancestral state being recovered as bivalve-associated (de Gier & Fransen, 2023). However, the bivalve-associated species have diversified much after the initial switch from ectosymbiosis towards endosymbiosis, covering a wide range of the morphospace (fig. 7.6B). Host-switches have been proven to be driving force behind diversification in symbiotic shrimp (Horká et al., 2016; Chow et al., 2021), which can now also be quantified on a morphological scale in our phylomorphospace plots.

Evolution and diversification of microstructures

The character matrix of the microstructure morphotypes shows some

evolutionary patterning of microstructures on the walking leg dactyli of the included species (fig. 7.7). Convergence in microstructure coverage was found within, and to some extent, between the clades. First up, convergence towards a complex, regular patterning of scales or teeth on the dorsal and ventral side of the unguis was found in various bivalve-associated lineages (fig. 7.7A, C, F, G). Most striking is the convergence between various species of *Conchodytes* (e.g., *C. biunguiculatus*; fig. 7.7A) and *Pontonia* (*P. margarita*; fig. 7.7C and *P. manningi*; Appendix 1: fig. A3C). This convergence of microstructures among these species was also seen in the overall dactylus shape (fig. 7.6A, also for *P. domestica*). No similarity between the host taxa of the species with this particular microstructure morphotype was found, including shrimp species of which no SEM-images were taken, but are known to have similar microstructures (e.g., *Pontonia panamica*, *P. simplex*, and *P. pilosa* Fransen, 2002 (Fransen, 2002; Marin & Anker, 2008). This morphotype, although in this case possibly not homologous, was also found in the more derived branches of the '*Anchistus* clade'. In *Tympanicheles* and *Neoanchistus*, these scales still somewhat resemble the ones in *Conchodytes* and *Pontonia*, while *Anchistus* and *Polkamenes* the scales formed a regular coverage on the full dorsal surface of the unguis, accompanied by the lateral expansion of the unguis and corpus (Appendix 1: figs. A1, A3, A5, A6). This second type of coverage was already discussed and compared by Fujino (1975) and Bruce (1980), accompanied by SEM captures.

The selected outgroup species might give an indication of the evolutionary pathway the species in our clades have undergone to show such elaborate microstructures. The free-living and cnidarian-associated species appear to have no microstructures on their unguis, while the sponge symbiont *Typton wasini* Bruce, 1977 (Appendix 1: fig. A7A) displays very fine scales on the dorsal and ventral base. The echinoderm-associated *Lipkemenes lanipes* (Kemp, 1922) and *Zenopontonia rex* (also associated with nudibranches) both exhibit very disperse scales on the dorsal side of the unguis, a character shared with some basal species of the '*Anchistus* clade' (*Dasella herdmaniae*, both species of *Ensiger*, and *Paranchistus armatus*). This is in contrast with the unguis morphology of the other two species of *Zenopontonia*: *Z. soror* (Nobili, 1904) (Appendix 1: fig. A7C), and *Z. noverca* (Kemp, 1922) (pers. obs.), which have no dorsal microstructures. *Z. soror* only has proximally placed microstructures on the ventral side of the unguis (Appendix 1: fig. A7C); *Zenopontonia* already appears to be polyphyletic in published phylogeny reconstructions (Horka et al., 2016; Chow et al., 2021), for which the SEM-photographs might warrant taxonomic reappraisal. The presence of microstructures in somewhat related outgroups already show that these features are not unique to our ingroup species.

Moreover, inspection of SEM images from dactyli of species in the unrelated, also endosymbiotic, clade of *Periclimenaeus* show elaborate microstructure patterning in the ascidian-associated *Periclimenaeus storchi* Bruce, 1989, but not the sponge-associated *Periclimenaeus bidentatus* Bruce, 1970 (pers. obs.). If the elaborate microstructures are strictly found in endosymbiotic lineages of the palaemonid family tree, remains unclear.

Five ascidian-associated species from different lineages within the '*Conchodytes* clade' exhibit the same morphotype for the unguis tip (fig. 7.7B, D). This convergence towards an unguis tip with only a small number of large scales has happened at least once in both *Odontonia* and *Dactylonia* (de Gier & Fransen, 2018; Appendix 1: figs. A2, A4). This morphotype could tentatively be linked to the host choice on a lower taxonomic level. The species *Ascidonia quasipusilla* and all species of *Odontonia* (excluding the bivalve-associated *O. kerangcaris* and phlebobranch-associate *O. plurellicola*) reside inside of ascidians from the order Stolidobranchia, and appear to have no or limited microstructures on the surface of their unguis (de Gier & Fransen, 2018, see also fig. 7.7). This character state is also shared with the stolidobranch-associated genus *Dasella* (fig. 7.7E). In contrast, three species within *Dactylonia* and *Ascidonia flavomaculata* can be found in ascidians from the order Phlebobranchia. These species share an intricate pattern of rows on the dorsal surface of the unguis (Appendix 1: figs. A3A, A4). This feature is, however, shared with *Dactylonia anachoreta* (Kemp, 1922) (Appendix 1: fig. A4E), which lives in stolidobranch ascidians. In addition, the phlebobranch-associated *O. plurellicola* (Appendix 1: fig. A2G) does not share this morphotype. Excluding these two exceptions, it might be proposed that the internal anatomical differences between the two ascidian orders might influence the selection pressures for the symbionts (e.g., for easy movement in the host).

Similar to the overall dactylus shape evolution discussed above, plotting the host-switching events on the tree might help to understand when microstructure diversification has occurred. In the '*Conchodytes* clade', the variation in microstructures appears to be more apparent in the bivalve-associated species, with the exception of *Platypontonia* showing no complex structures whatsoever (Appendix 1: fig. A1A). Similar to the evolution of the dactylus shapes, a host switching event at the base of the clade containing *Conchodytes*, *Odontonia*, and the smaller related genera appears to have jumpstarted a wide range of microstructure differences within *Conchodytes* (and *Anchiopontonia*). After this initial switch towards a bivalve host, the clade of *Odontonia* switched back to an ascidian host, and with it losing most microstructures. This switch seems to have happened somewhere between *Anchiopontonia* and *O. rufopunctata*,

with the latter having an 'intermediate' microstructure morphology (Appendix 1: fig. A2B). *Odontonia kerangcaris* (which is basal to *O. rufopunctata*) also shows regularly placed teeth on the dorsal surface of the unguis (Fransen et al., 2021). Moreover, the switch between an ascidian host towards a bivalve host in the branch containing *Ascidonia*, *Dactylonia* and *Pontonia*, seems to have caused some diversification of microstructures in *Pontonia*. Hypothetically, this switch should have happened in the branch after the ascidian-associated *P. panamica*, but the original description of the species shows regular microstructures at the dorsal base of the unguis (Marin & Anker, 2008, fig. 7.6A). New observations using a light microscope prove this (pers. obs. on the paratypes: RMNH.CRUS.D.51824). This means that the evolution of complex microstructures possibly has happened before *P. panamica* splits off in the phylogeny reconstruction, possibly proven by *Ascidonia quasipusilla* having basic, but conspicuously placed teeth on the dorsal unguis surface (Appendix 1: fig. A3B). As mentioned above, the first bivalve-associated branch in the '*Anchistus* clade', including *Ensiger* and *Paranchistus*, shows no elaborate microstructures. Theories about host-switching cannot prove why this clade did not evolve similar microstructures as in the other clade, including *Anchistus*, *Neoanchistus*, *Polkamenes*, and *Tympanicheles*. It is worth noting that members of *Ensiger* and *Paranchistus* are usually larger in size than their relatives from the other genera, which might play a role in the grip within the host.

Functional morphology and its implications

As mentioned before, host switching can result in convergent evolution in both the dactylar morphology, as well as the body sizes of the studied shrimp species. Also, between the studied clades parallels can be drawn. For instance, the two clades of endosymbionts show a high variation in pocl values, both ranging from species being a bit larger than a few millimeters, to species reaching a pocl of 13 mm (fig. 7.3). Comparing the high values with species from ectosymbiotic clades gives us the impression that selection pressures for sedentary endosymbionts are very different from ectosymbiotic conditions. For instance, the females often grow large abdominal segments, capable of holding a relatively high number of eggs. This adaptation to endosymbiotic life, which might be linked to the high overall maximum body size, is possibly caused by a low degree of predation. This is also observed in various other endosymbiotic shrimp (e.g., the coral endosymbionts *Paratypton* Balss, 1914 and *Anapontonia* Bruce, 1966, the sponge- and ascidian-associated *Periclimenaeus*, and various other genera of sponge symbionts, such as *Typton* and *Onycocaris* Nobili, 1904 (Bruce, 1995), but also in other endosymbiotic crustaceans, such as gall crabs and pea crabs (Vehof et al., 2016; de Gier & Becker, 2020). While the carapace

is often less inflated compared to the abdomen, various species in our ingroup have evolved a flattened (e.g., *Conchodytes placunae*) or compressed (e.g., *Dasella*, *Odontonia*) carapace shape, with a reduction in the number of teeth present on the rostrum and frontal margin (fig. 7.1; Bruce, 1995). The only exception are some species within the '*Anchistus* clade' (genera *Anchistus* and *Polkamenes*) showing some dentition on the tip of the rostrum (de Gier & Fransen, 2023). It is thought that these teeth gradually migrate to the frontal margin of the rostrum after shedding, as can be seen in juvenile specimens (de Gier & Fransen, 2023). The reduction in teeth is thought to be an adaptation to live inside soft-bodied hosts (Bruce, 1972; Fransen, 1994), making it harder for the host to 'feel' and consequently remove the symbiont, or to not get stuck within the body structures of the host. This reduction in armature can also be found in other endosymbiotic shrimp clades (Bruce, 1995), most pea crabs (de Gier & Becker, 2020; Chow et al., 2023; de Gier, 2023), some echinoderm-endosymbiotic crabs (e.g., Pilumnidae: Eumedoninae: genus *Echinoecus* (Chia et al., 1999)), and parasitic amphipods, isopods, and copepods (Baeza, 2015).

The huge variation of dactylus shapes and microstructures, is thought to be the result of different selection pressures in the different host groups (Chow et al., 2021). Although no true 'bivalve- or ascidian-specific morphotype' could be identified, the shape of the dactyli might serve similar purposes in both endo- as well as ectosymbionts. For instance, the typical '*Conchodytes*-like', biunguiculate, dactylus shape is shared between various bivalve associates (e.g., in *Conchodytes* and *Pontonia*, but also in *Odontonia kerangcaris*; Appendix 1: figs. A1B–H, J, A3C, D; Fransen et al., 2021), but also in various species of the ascidian- and sponge-associated *Periclimenaeus* (e.g., *Periclimenaeus djiboutensis* Bruce, 1970, *Periclimenaeus matherae* Bruce, 2005, and *Periclimenaeus pachydentatus* Bruce, 1969 (Marin, 2007; Bruce, 2013). As shown by Chow et al. (2021), dactyli with a large accessory tooth are also present in various ectosymbiotic clades. It is safe to assume that the accessory tooth is used to grasp host tissue for better grip where strong currents are at play. Interestingly, the convergence towards more 'simple' elongated dactylus shapes seen in the studied ingroups (e.g., in *Conchodytes chadi*, *Ensiger*, *Platypontonia*, *Odontonia seychellensis*, and various species of *Pontonia*; Appendix 1: figs. A1A, I, A2F, A3E, F, A5C, D) can also be observed in *Periclimenaeus* (see Bruce, 2013) and various other clades (Chow et al., 2021). The same goes for dactyli of which the ventral side is serrated, as can be seen in *Dactylonia*, *Odontonia*, and *Rostronia* (Appendix 1: figs. A2B–G, A4A–E; for comparison to *Periclimenaeus*, see Bruce, 2013). The microstructures might also play a role in the grip within the host specimens: it seems like endosymbiotic species (also including the third endosymbiotic

lineage sensu Chow et al., 2021) display more microstructures on the accessory teeth and unguis than their ectosymbiotic relatives (Appendix 1: fig. A7; for *Periclimenaeus*, see Bruce, 2013). The mucus-covered body walls of ascidian and bivalve hosts is possibly selecting for symbionts with dactyli covered in elaborate micro-ornamentations. This does however not explain the lack of elaborate microstructures in *Ensiger*, *Paranchistus* (Appendix 1: fig. A5B–D), and various other species in our ingroups. A study focusing on the selection pressures within these microhabitats, comparing bivalves, ascidians, but also other hosts for endosymbionts (in sponges, see above), might give insights to why some types of microstructures have evolved in these clades of symbionts.

The morphology of the other appendages may reflect the host choice of the symbionts as well. Chow et al. (2021) demonstrated that the variation mandible shape, although expected, did not show any link with host choice, or type of symbiosis (endo- versus ectosymbiotic). The methods of food transport, different dietary compositions, and weaker selection pressures might have influenced the mandibular diversity of the studied clades of palaemonid shrimp. The type of symbiosis might also be reflected by the dietary habits, and perhaps the morphology of the mouthparts (Ashelby et al., 2015; De Grave et al., 2021). For comparison, Chow et al. (2023) showed some links between the host choice and the mouthpart morphology of a large group of pea crabs (Pinnotheroidea), which were either filter-feeding, or feeding from the host tissue and/or mucus. In contrast to the study by Chow et al. (2021), Dobson et al. (2014; 2016) suggest endosymbionts to have smaller and simpler eyes, compared to their free-living and ectosymbiotic relatives. It is thought that this is the result of the protective symbiosis, where species do not need good eyesight to survive (Dobson et al., 2014). A study should be done to investigate the effect of the mating system (e.g., if the males or even females leave their host to seek for new mates) on the eye morphology of the currently studied species. For comparison, males of host-hopping pea crab species have relatively larger eyes than the females (de Gier & Becker, 2020).

Limitations and future perspectives

The present study describes the variation in body size, walking leg dactylus shape and unguis microstructure in two clades of palaemonid shrimp species. While the supposed convergence events uncovered in this study are studied extensively, it is inevitable that major patterns were missed due to a lack of known morphological and ecological data. First, incomplete sampling of some of the rare species resulted in a limited dataset. For example, sometimes only one specimen of a species was caught (e.g., *Conchodytes philippinensis* Bruce, 1996; see Bruce, 1996), or no host records are known (*Pontonia longispina*; see

Fransen, 2002). In addition, some species are also thought to be synonyms of well-known species (*Polkamenes spondylis* (Suzuki, 1971) and *Polkamenes nobilii* (Holthuis, 1952) being variations of *Polkamenes pycnodontae*; see de Gier & Fransen, 2023), or are 'cryptic species' and part of so-called species complexes, in which multiple species might be waiting to be discovered (polyphyletic species in the phylogeny reconstruction in de Gier et al., 2022). This all leads to noise in our usable dataset, causing us to exclude species from the analyses, and consequently to miss possible instances of host-switching, body-size evolution, and general convergence patterning in morphological structures (figs. 3, 6, 7).

Incomplete sampling also causes us to have insufficient molecular data for phylogeny reconstructions. This was, however, bypassed by implementing Total Evidence analyses in de Gier et al. (2022) and de Gier & Fransen (2023) to build phylogenetic trees using molecular and morphological information, which were used here in the pocl- and phylomorphospace analyses. Species of which only morphological data was available, were excluded from the analyses in the phylomorphospace. Although 'trimming' the tree disables us to calculate convergence events with these excluded species, some hypotheses can be drawn using the dactylar (phylo)morphospaces (figs. 7.4, 7.6, S1, S2) and TE-topologies from de Gier et al. (2022) and de Gier & Fransen (2023). For instance, in the '*Conchodytes* clade', we highlighted only two convergence events towards a simple elongated dactylus shape (fig. 7.6A: convergence events II and V), but this can also be seen in at least two other lineages within the clade (excluding *Platypontonia brevirostris* (Miers, 1884) which possibly is closely related to *P. hyotis*). Two other *Pontonia* species (*P. simplex*, and *P. pilosa*), and to some extent *Opaepupu huna*, all exhibit elongated dactyli similar to the ones seen in the free-living outgroups (see Fransen, 2002; Anker & De Grave, 2021), indicating two to three new possible convergence events. In the '*Anchistus* clade' similar events can also be observed in *Neoanchistus nasalis*, grouping together with *Anchistus australis*, and in *N. cardiodytes*, which groups together in the top middle part of the plot with two species of *Polkamenes*, and *Anchistus gravieri* Kemp, 1922. These placements also indicate possible convergence events between three genera within the clade.

More sampling efforts might help us in building better and naturally sound phylogeny reconstructions, placing rarely caught species in existing trees (Van der Meij et al., 2023). As can be seen in this study, using these phylogeny reconstructions in combination with morphological data, gathered through historical as well as recent museum collections, helps us in understanding when and how convergent evolution of adaptive features occurred in symbiotic

clades. Palaemonid shrimps have already been suggested to be preferred model organisms to study mating systems and speciation under sympatric conditions (Baeza et al., 2015; Chow et al., 2021), but this study proves that this speciose and morphologically variable clade can also be used to study convergent evolution on a small scale. In addition, this knowledge should be supplemented with studies on the microhabitats of the symbionts, as they might give us insights in why symbionts have evolved in such intricate ways. The same can be mentioned about some groups of parasitic molluscs that live in association with selected taxa of corals and also occupy specific places in or on their hosts (Gittenberger & Gittenberger, 2005, 2011; Gittenberger & Hoeksema, 2013; Owada & Hoeksema, 2013; Potkamp et al., 2017).

Supplementary information

Supplementary material of this chapter was submitted seperately to a Zenodo page: https://doi.org/10.5281/zenodo.10473768. The supplementary datafile from the published article is available online at: https://doi.org/doi/10.1002/ece3.10768 [follow link to: Supporting Information – Supinfo: ece310768-sup-0001-Supinfo.docx].

Acknowledgements

The authors would like to thank Bert Hoeksema and Ronald Vonk (both Naturalis Biodiversity Center, Leiden) for their helpful comments and feedback during all phases of this study. Liam Revell (University of Massachusetts Boston) is thanked for his help regarding streamlining the general phylomorphospace analysis in R. The authors are grateful to Bertie Joan van Heuven (Naturalis Biodiversity Center) for her help in the SEM lab of Naturalis, assisting with drying, coating, and photographing the dactyli. Bastiaan Broeders is thanked for his pilot study on the '*Anchistus* clade' in 2017, using the SEM to study the walking leg morphology of a number of species. Max Willems (UvA, Amsterdam), Vicky Beckers (Naturalis Biodiversity Center), and Jeroen Hubert (Leiden University) are thanked for their feedback on the layout and figures.

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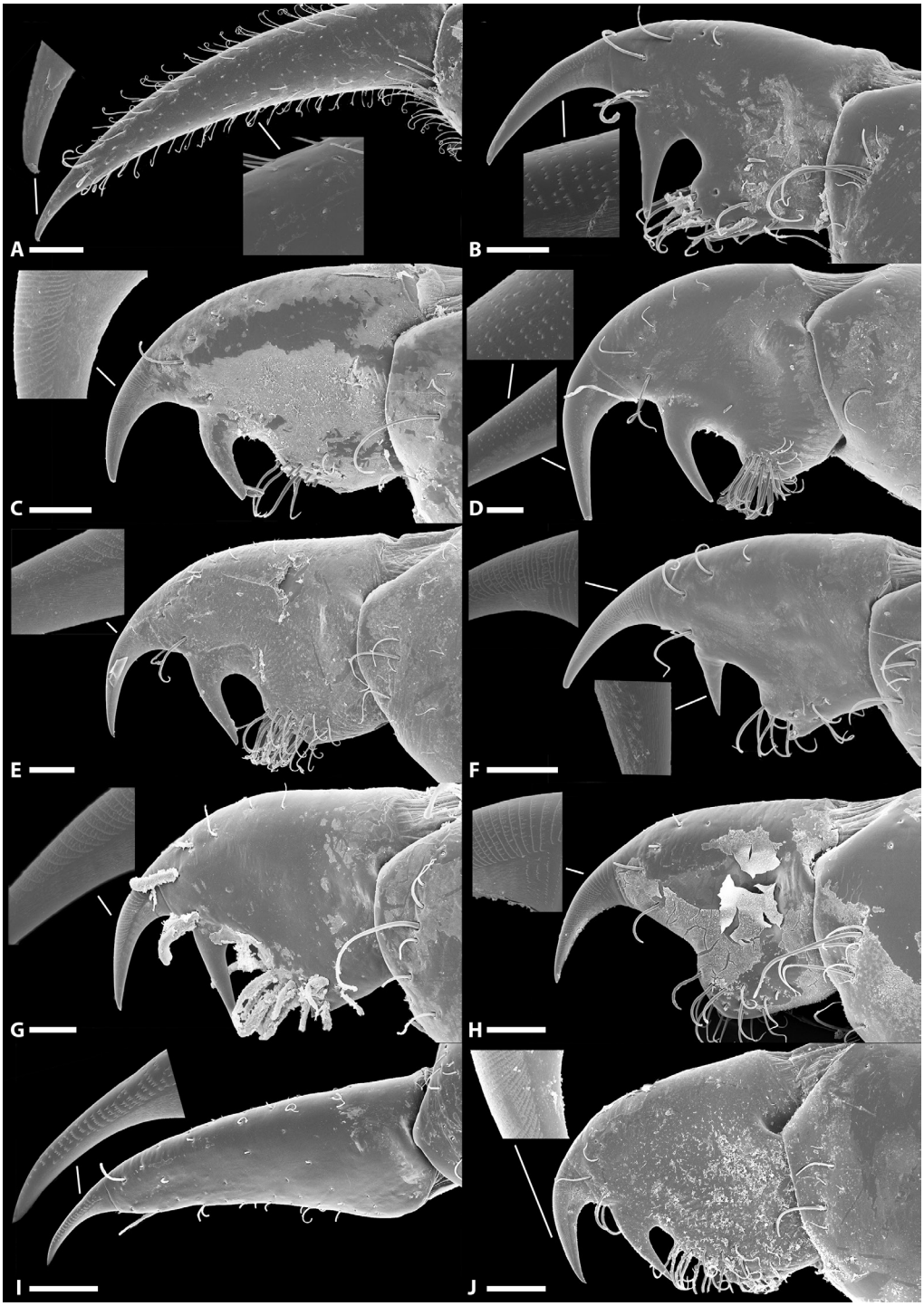
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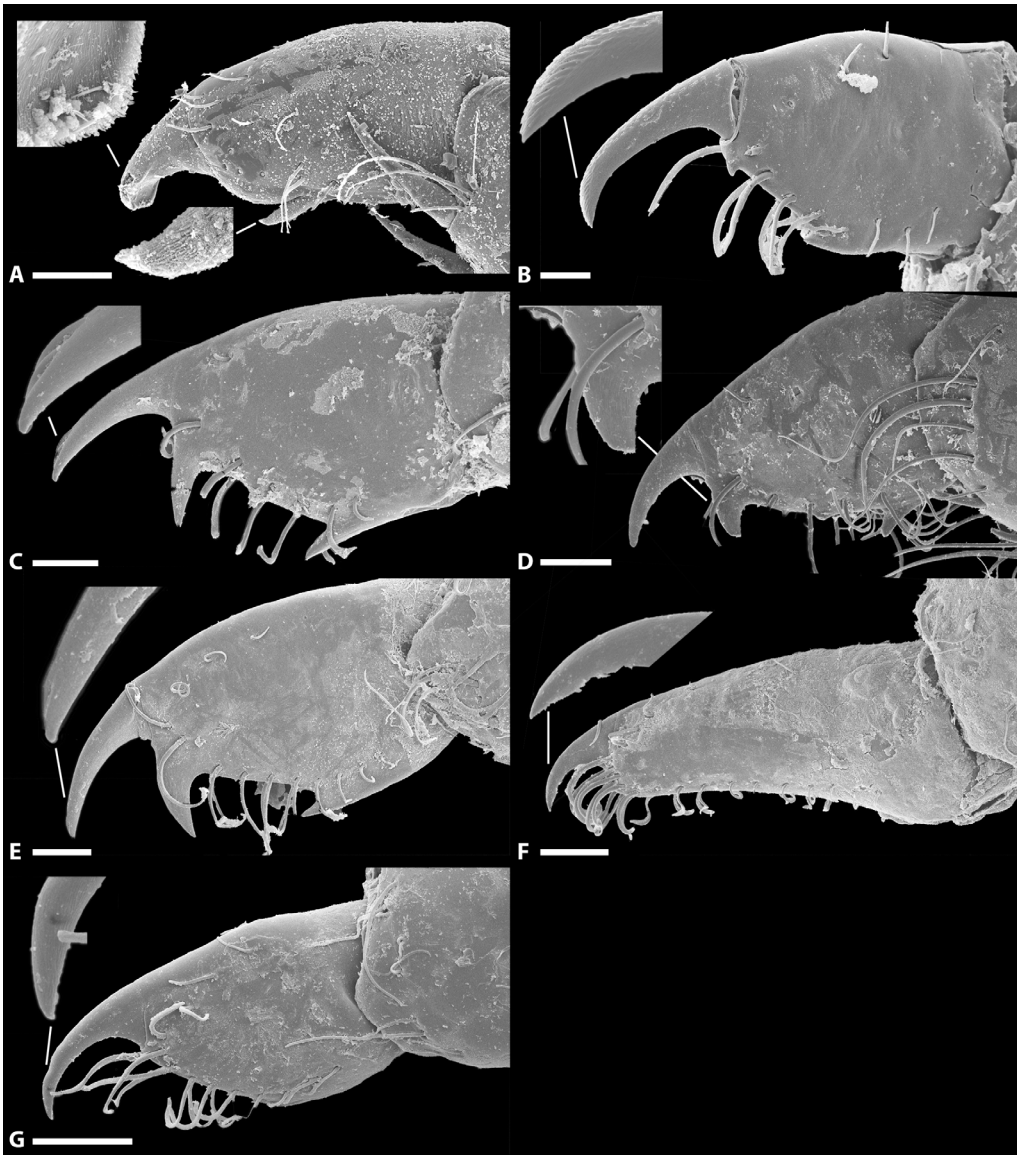
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Appendices

The following figures (on pages 356 to 362) are provided in the supplementary datafile in the published article, but are reffered to in this chapter as Appendix 1: fig. A1 to A7.

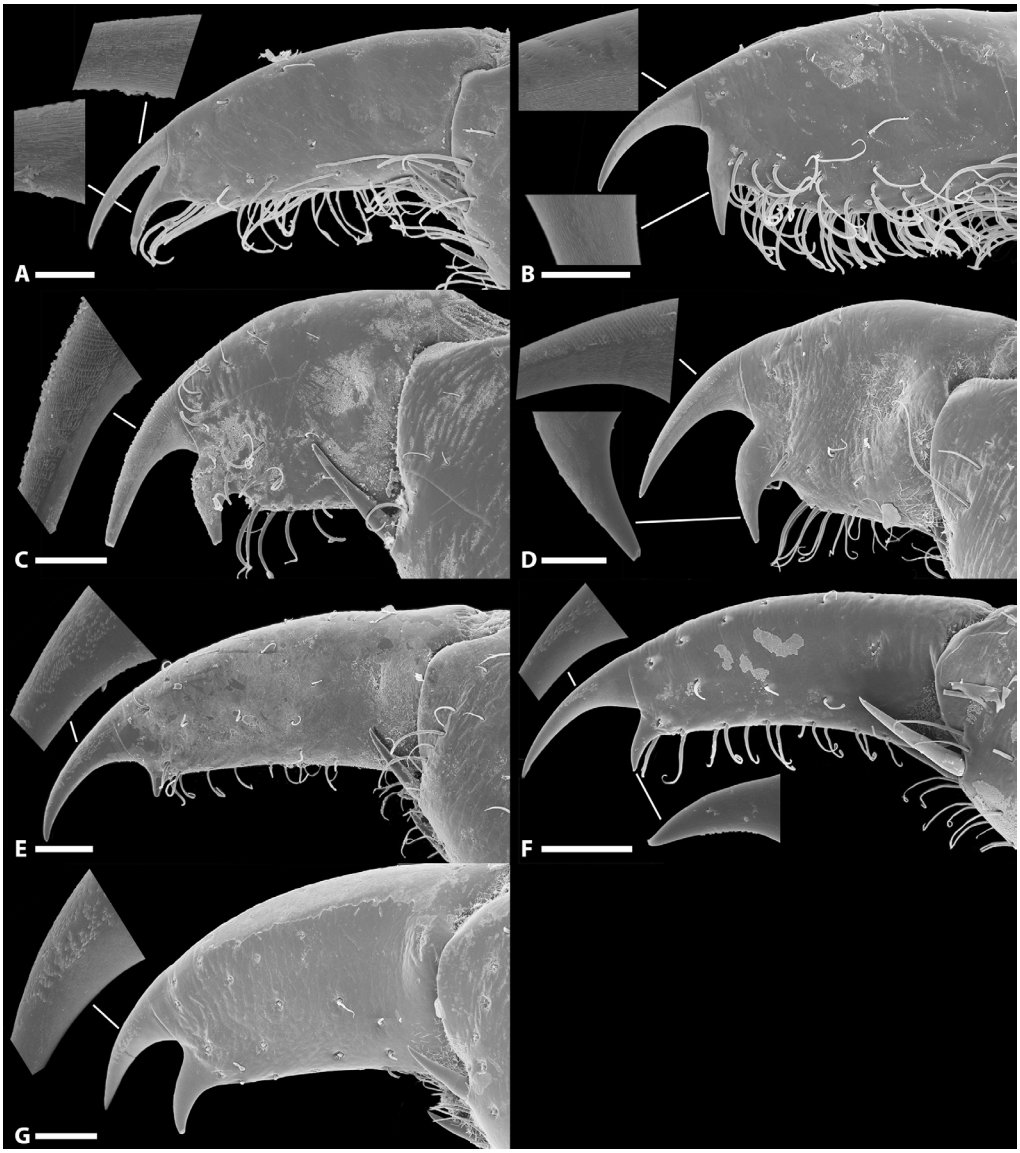


A1 SEM captures of third pair of walking leg dactyli of species within the '*Conchodytes* clade' (1/4) with detailed insets: genera *Conchodytes* and *Platypontonia*. A) *Platypontonia hyotis* Hipeau- 35 Jacquotte, 1971; B) *Conchodytes nipponensis* (De Haan, 1844); C) *C. maculatus*

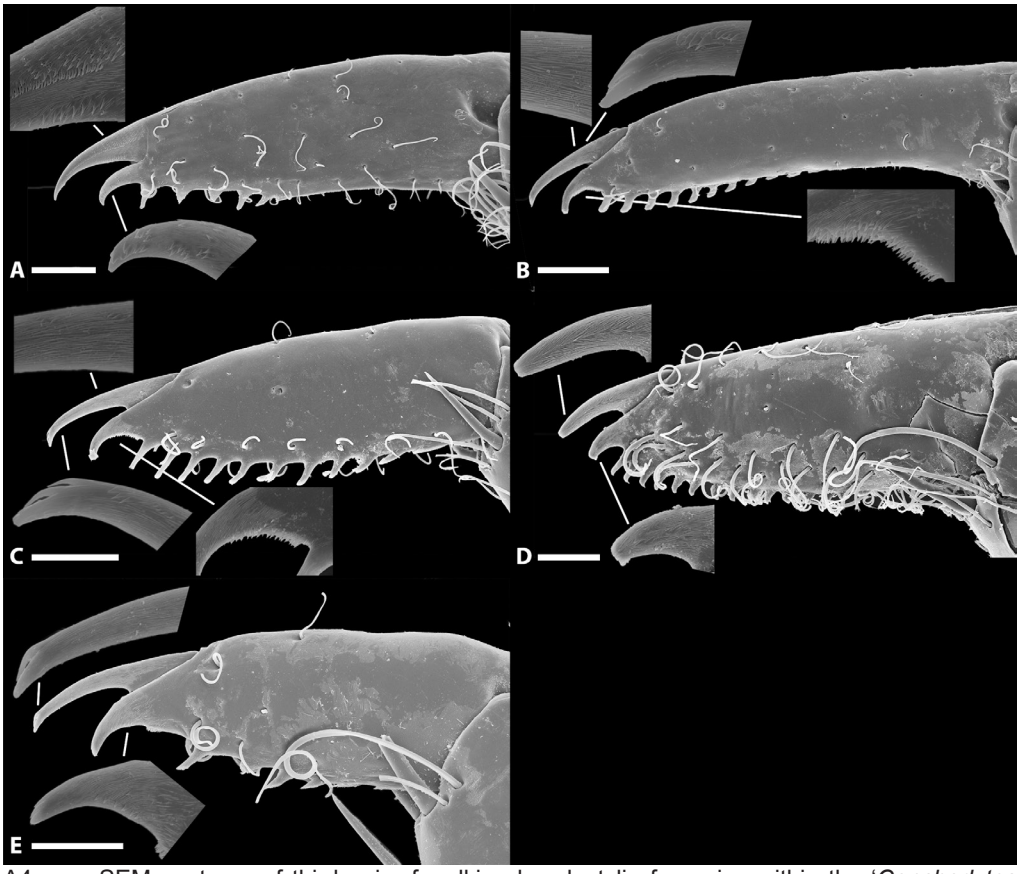


A2 SEM captures of third pair of walking leg dactyli of species within the '*Conchodytes* clade' (2/4) with detailed insets: genera *Anchiopontonia* and *Odontonia*. A) *Anchiopontonia hurii* (Holthuis, 1981), with part of the underside of the unguis showing in the inset; B) *Odontonia rufopunctata* Fransen, 2002; C) *O. sibogae* (Bruce, 1973); D) *O. bagginsi* de Gier & Fransen, 2018; E) *O. katoi* (Kubo, 1940); F) *O. seychellensis* Fransen, 2002; G) *O. plurellicola* de Gier & Fransen, 2018. Scale A = 100 µm; B = 20 µm; C–G = 50 µm. Figures of *Odontonia* after de Gier & Fransen (2018).

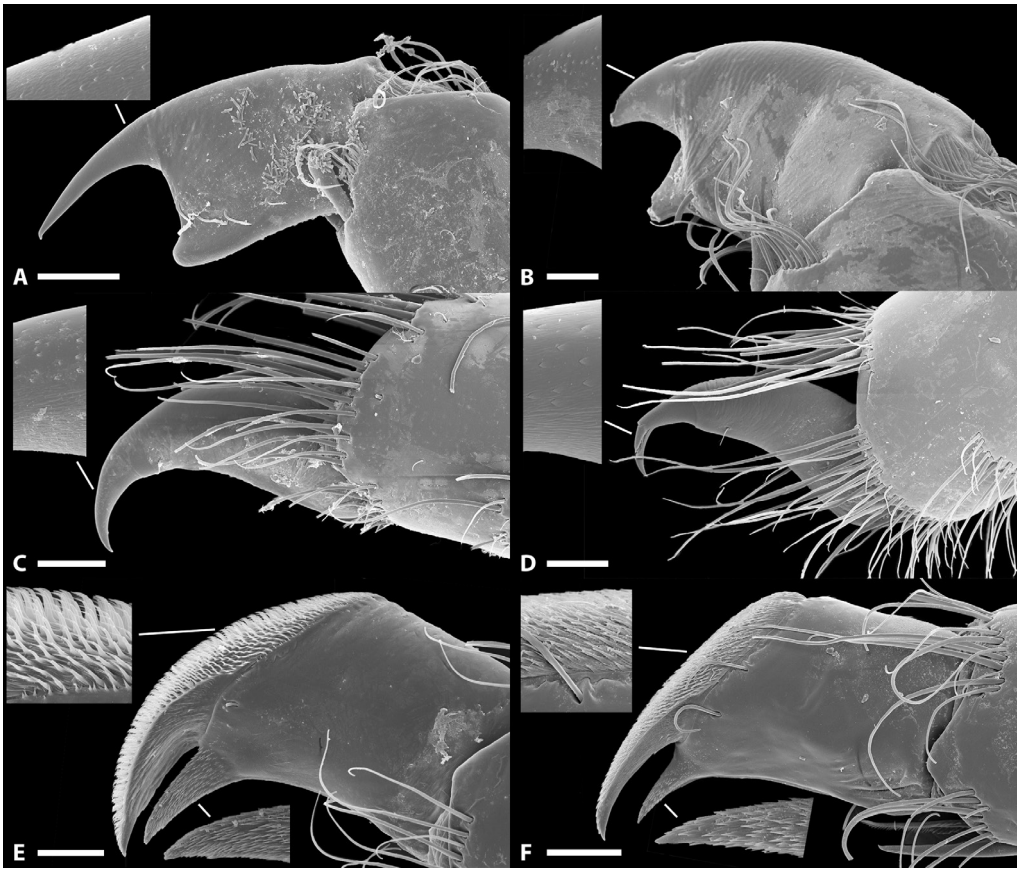
(A1 *Cont.*) Bruce, 1989; D) *C. tridacnae* Peters, 1852; E) *C. meleagrinae* Peters, 1852; F) *C. biunguiculatus* (Paulson, 1875); G) *C. pteriae* Fransen, 1994; H) *C. monodactylus* Holthuis, 1952; I) *C. chadi* (Marin, 2011); J) *C. placunae* (D. S. Johnson, 1967). Scale A = 200 µm; B–F, H–J = 100 µm; G = 50 µm.



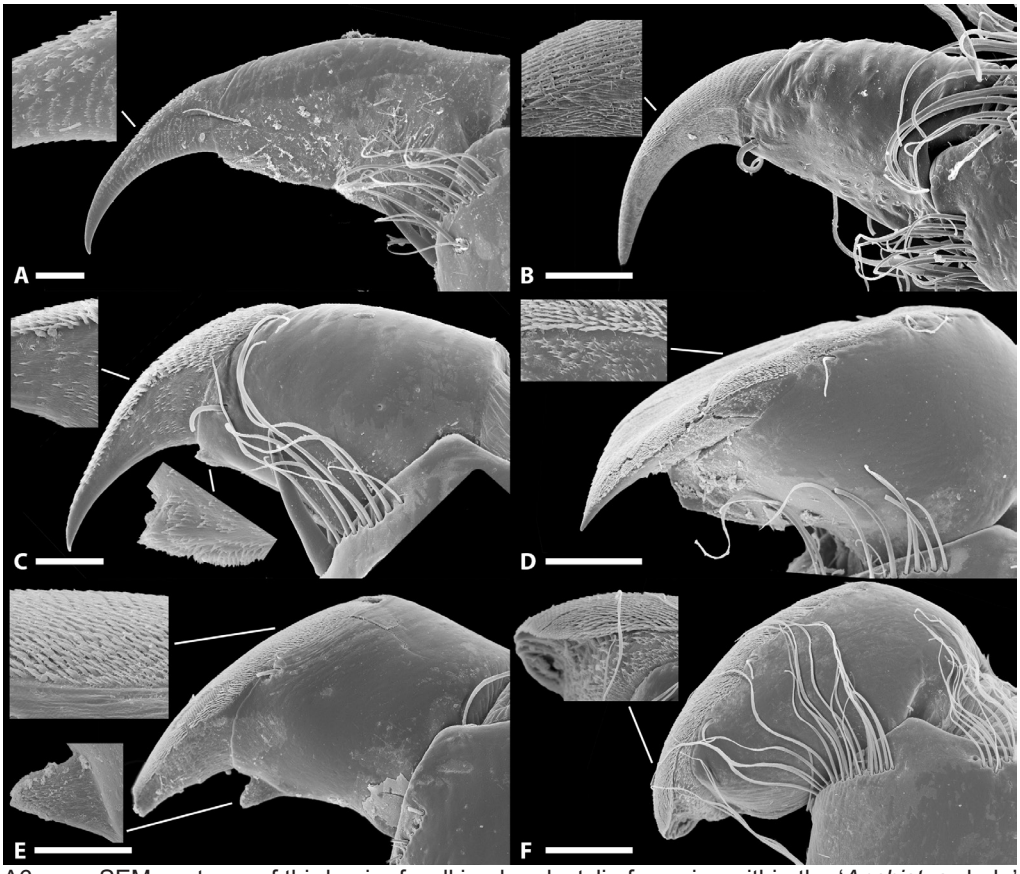
A3 SEM captures of third pair of walking leg dactyli of species within the '*Conchodytes* clade' (3/4) with detailed insets: genera *Ascidonia* and *Pontonia*. A) *Ascidonia flavomaculata* (Heller, 1864); B) *Ascidonia quasipusilla* (Chace, 1972); C) *Pontonia manningi* Fransen, 2000; D) *P. margarita* Smith in Verrill, 1869; E) *P. pinnophylax* (Otto, 1821); F) *P. mexicana* Guérin-Méneville, 1855; G) *P. pinnae* Lockington, 1878. Scale A = 50 µm; B–G = 100 µm.



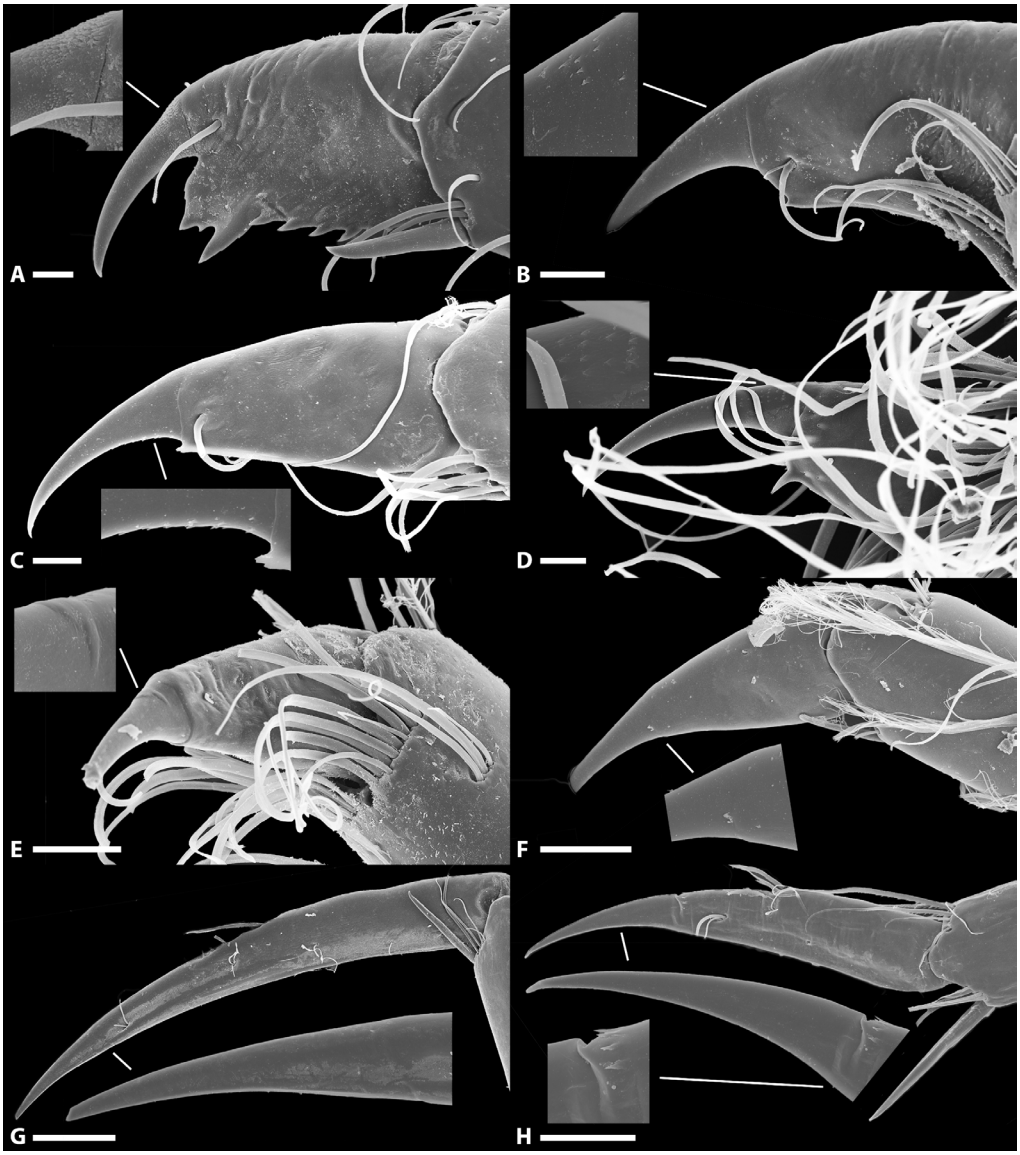
A4 SEM captures of third pair of walking leg dactyli of species within the '*Conchodytes* clade' (4/4) with detailed insets: genera *Dactylonia* and *Rostronia*. A) *Rostronia stylirostris* (Holthuis, 1952); B) *Dactylonia okai* (Kemp, 1922); C) *D. holthuisi* Fransen, 2002; D) *D. ascidicola* (Borradaile, 1898); E) *D. anachoreta* (Kemp, 1922). Scale A–C, E = 50 µm; D = 100 µm.



A5 SEM captures of third pair of walking leg dactyli of species within the '*Anchistus* clade' (1/2) with detailed insets: genera *Dasella*, *Ensiger*, *Paranchistus*, and *Polkamenes*. A) *Dasella herdmaniae* (Lebour, 1938); B) *Paranchistus armatus* (H. Milne Edwards, 1837); C) *Ensiger custos* (Forskål, 1775); D) *E. custoides* (Bruce, 1977); E) *Polkamenes pycnodontae* (Bruce, 1978) (hosted by *Hyotissa hyotis* (L., 1758)); F) *P. pycnodontae* (hosted by *Spondylus* sp.). Scale A–D = 100 µm; E, F = 50 µm.



A6 SEM captures of third pair of walking leg dactyli of species within the '*Anchistus* clade' (2/2) with detailed insets: genera *Anchistus*, *Neoanchistus*, and *Tympanicheles*. A) *Tympanicheles ornatus* (Holthuis, 1952); B) *T. pectinis* (Kemp, 1925); C) *Neoanchistus nasalis* Holthuis, 1986; D) *Anchistus australis* Bruce, 1977; E) *A. miersi* (De Man, 1888); F) *A. demani* Kemp, 1922. Scale A–C, E, F = 50 µm; D = 100 µm.



A7 SEM captures of third pair of walking leg dactyli of species in the studied outgroup, with detailed insets: A) *Typton wasini* Bruce, 1977; B) *Zenopontonia rex* (Kemp, 1922); C) *Z. soror* (Nobili, 1904); D) *Lipkemenes lanipes* (Kemp, 1922); E) *Actinimenes inornatus* (Kemp, 1922); F) *Periclimenes kempi* Bruce, 1969; G) *Cuapetes tenuipes* (Borradaile, 1898); H) *Palaemonella rotumana* (Borradaile, 1898). Scale A, C–D = 20 μm; B, E–F = 50 μm; G = 200 μm; H = 100 μm.

8

Thesis summary & General discussion



Taxonomy, focusing on the systematic and phylogenetic placement of organisms in the 'Tree of Life', is the first and arguably the most important step in studying life on Earth. This type of 'pre-work' is relevant, if not necessary for all subsequent studies on the evolution, ecology, behaviour, and conservation of the studied groups (Thomas et al., 2018). Even outside of these taxonomic groups, the analytical and visualization methods used in phylogenetic and 'integrative taxonomic' studies might help in understanding the evolution and morphological diversification of completely different clades of organisms (Santos et al., 2019). In studies focusing on symbiotic species, this link between morphology, ecology, and the evolutionary history of the studied symbionts and hosts, helps us to fundamentally understand how these often well-hidden species of 'cryptofauna' adapt to new surroundings, and how they take up new niches in sympatric conditions (Meca et al., 2019). In particular, species with complex evolutionary histories, in which host-switching events are known to be common occurrences, might give us insights on how they might rapidly adapt to newly-available hosts in an ever-changing world (Brooks & Hoberg, 2007).

The collection of papers in this thesis can be seen as a case study for a proposed workflow for ecomorphological research on symbiotic species. This is done by using: data collection focusing on ecological and morphological observations from previous literature, making it possible to pinpoint potentially host-specific morphological features for future studies (Chapter 2); integrative taxonomic and phylogenetic studies performed on understudied clades, which are needed to understand the evolution of morphological features (Chapter 3 to 5); and phylomorphometrics and phylogenetic comparative methods (PCMs) to study the ecomorphological convergence and diversification of the studied clades (Chapter 6 & 7). Below, the main findings of all chapters are summarised and discussed. Consequently, the three studied clades (pinnotherine pea crabs and two clades of endosymbiotic palaemonid shrimps) are compared and suggestions for future studies are presented.

New insights in the ecomorphology of pea crabs

The literature review presented in the first chapter focused on the ecology, life histories, and morphology of pinnotherine pea crabs. Here, we did not only collect, summarise, and discuss previous literature, but also showed the 'knowledge gap' in ecomorphological research on pea crabs (Chapter 2). Questions arose like: "are all pea crabs parasitic?", "how do they find their hosts?", and "are there truly two separate mating strategies (with sedentary or host-hopping females)?"

The present update of the list of host associations already indicated the variation

in host groups and the host specificity of all included species (Chapter 2: table 2.1). This raised some questions about the host-switching events which have taken place in this subfamily: multiple genera have presumably switched from the most common association with bivalves towards a symbiosis with gastropods, various groups of echinoderms (both ecto- and endosymbiotic), ascidians, and even chitons and brachiopods (Chapter 2). Using the phylogeny reconstructions by Palacios Theil et al. (2016) and Chow et al. (2023), host-switching patterns can be plotted on the included lineages. Unfortunately, these tree topologies do not include members of all genera due to incomplete sampling. The available trees do, however, give us some indications on at least a portion of the host-switching events in the Pinnotherinae: in the phylogenetic model presented by Palacios Theil et al. (2016), at least two lineages have switches towards a symbiosis with holothurians (in *Holothuriophilus* and *Alain*); two have switched towards a gastropod host (in *Calyptraeotheres* and *Orthotheres*); and at least two switches have happened towards a full or partial association with ascidians (in the generalist species *Nepinnotheres* pinnotheres, and in the specialistic genus *Tunicotheres*). Two instances of switches towards an echinoid association are recorded in this tree: one as endosymbiont in *Pinnaxodes*, and one lineage ectosymbionts in the species in the '*Dissodactylus* complex'. In the phylogeny reconstruction by Chow et al. (2023), more host-switches were found: one more towards a symbiosis with ascidians (in *Austrotheres pregenzeri*); two switches towards a life within holothurians (in *Holotheres* and *Buergeres*); and one partial switch towards a life within echinoids (in *Pinnaxodes major*).

Some morphological features of pea crabs are considered be adaptively evolved, related to their host choices (Chapter 2). For instance, the broad body shapes and strong third walking-leg-pairs of members of the Pinnixinae are perfectly adapted towards a co-inhabitance within the tubes and burrows of various invertebrate groups, while endosymbiotic Pinnotherinae have soft, bulbous bodies, often associated with long slender legs. These walking legs might be evolved to be asymmetrical, thought to be linked to the host-collecting feeding habits of some species. Furthermore, some species of (partial) ectosymbionts have adaptive (cryptic) colouration as camouflage to blend in with that of their host. On a smaller scale, some species have been studied for their third maxilliped shape, a feature that is often used in taxonomic literature, and the scales and hairs on their claws. The morphology of the feeding apparatus, including the third maxillipeds and these claw-ornamentations, seems to be linked to the dietary habits, and therefore host-specificity, of the symbionts. While the walking leg dactylus shapes of symbiotic shrimps have been studied in detail (Fransen, 2002; Chow et al., 2021), pea crab walking legs are often only used in the identification of species (Ahyong & Ng, 2007; Ng & Ahyong,

2022). However, some species show adaptive evolution in the overall shape and microstructures of these anatomical features. For example: the species within the '*Dissodactylus*-complex' show 'bifurcate' or 'forked' dactylus shapes, thought to aid in the movement between the spines of their host sand dollars and sea urchins, while most species of *Arcotheres* have minute scales on their last walking leg dactyli, thought to help in holding themselves in place within their slimy bivalve hosts (Griffith, 1987; Ng & Ahyong, 2022).

Some very conspicuous, morphological features have not been studied in detail before, and the potential functions of these features are far from known. For instance, the furry appearance of some holothurian endosymbionts is proposed to be some sort of 'structural camouflage' so the host does not repel the symbiont. In addition, the functions of the seemingly complex carapace ornamentations of *Durckheimia*, *Xanthasia*, *Serenotheres*, and *Visayeres* are still unknown (Chapter 2). Using modern visualization methods, like µCT-scanning, can assist us to track down anatomical 'clues' to find out why these species have evolved like this.

Some of the highlighted features can also be found in the studied endosymbiotic shrimp clades, indicating convergent patterns spanning across entire lineages. For example, most palaemonid shrimps also evolved compressed body shapes (with an inflated abdomen) when entering into an endosymbiotic association with bivalves and ascidians (Fransen, 1994). In addition, specific setae on the claws of pea crabs can also be found on a few of the presently studied shrimp species, such as those of the genus *Ensiger* (Chapter 5). While the more unique carapace ornamentations of some above-mentioned pea crab species cannot be seen in the endosymbiotic palaemonids, the 'furry' appearance of some pea crabs is also shown in some bivalve-associated shrimps, such as the genera *Bruceonia* and *Pinnotherotonia*, and the species *Pontonia pilosa* (Fransen, 2002; Marin & Paulay, 2010). While the microstructures on the pea crabs' walking legs have not been studied in detail, the scales on the last pair of walking legs of Arcotheres seem to resemble the microstructures of some endosymbiotic shrimp species (Chapter 7), which possibly bear the same function.

Discovering a 'missing link'

New species are not only found in the field, but also in museum collections, either unidentified or misidentified. Morphological re-examination of collection material used for DNA-barcoding resulted in the discovery of the minute shrimp species *Odontonia kerangcaris* (Chapter 3). This species appears to have features that are characteristic for species of the genus *Odontonia*, but some

of the walking leg characters match more with those of a species of the related genus *Conchodytes*. The preferred host of this species, which is thought to be a spiny oyster (*Chama lazarus*), is also aberrant for the ascidian-associated genus (de Gier & Fransen, 2018).

The basal placement of the species within the genus, which was found during the phylogeny reconstruction of the larger '*Conchodytes* clade' (Chapter 4), indicated that this species might be a 'missing link' in the host switch from an ancestral bivalve-associated state (found in the thought-to-be related genera *Anchiopontonia* and *Bruceonia*) towards an endosymbiosis with ascidians found in the rest of the species of *Odontonia*. This intrageneric host switch is apparently paired with a switch in dactylus morphology (Chapter 3). The dactylus of *O. kerangcaris* features a long unguis with apparent microstructures, an inflated corpus, and a long accessory tooth. Based on morphological analyses performed after the discovery of the new species (Chapter 7), this shape resembles two species from the '*Conchodytes* clade' the most: *Pontonia manningi*, and *Conchodytes kempoides*. The latter was not included in the tree topology due to limited molecular data. In addition, the analyses also show that the shape of the dactylus has evolved separately from the rest of the *Conchodytes* clade, representing a perfect example of convergent evolution. This convergence is probably the result of similar selection pressures related to overlapping host choices.

Other intrageneric host switches in the studied shrimp clades are mostly found in the (mainly) bivalve-associated genus *Pontonia*. The most basally placed species, *Pontonia panamica*, is hosted by an ascidian host, while one of the most derived species, *P. chimaera*, is found in gastropods (Fransen, 2002). Linking these host-switching events to the dactylar morphology of the species results in less conspicuous results: the walking leg dactyli of *P. panamica* resemble those of various other bivalve-associated members of the genus (e.g., *P. mexicana*). The applies to *P. chimaera*, which resembles the bivalve-associated *P. pinnae* (Fransen, 2002). This teaches us that the switch from bivalve association towards an ascidian association may have more extreme effects on the symbionts' morphology than the other way around (Chapter 7).

Host switching and biogeography in the evolutionary history of the '*Conchodytes* clade'

The Total-Evidence (TE) approach used to phylogenetically study the placement of all members of the mainly bivalve- and ascidian-associated '*Conchodytes* clade' resulted in a more complete hypothetical evolutionary history of the clade (Chapter 4). The method works by using a combination of morphological and

molecular data, so that even de rarest of species could be added to the tree of life. These are mainly obscure species only known from historical descriptions with limited records or somewhat well-known species known from museum collections, but not from molecular data. Some researchers have also dated trees using this method, by incorporating morphological features of species from the fossil record (Ronquist et al., 2012), although this was impossible to do in the present study, due to the lack of fossils.

The TE tree topology, as well as the molecular- and morphology-only tree topologies, includes five outgroup species chosen from both distantly related lineages, as well as members found in the same branch as the '*Conchodytes* clade' of Horká et al. (2016). The outgroup choice showed us that the studied clade was monophyletic instead of a poly- or paraphyletic range of species. However, this is hypothetical and should be used with caution owing to the unstable branch-support values and because of the fact that inclusion of various species is only based on morphological data. The aberrant morphology of *Colemonia litodactylus* lead it to be placed basally to the entire ingroup, after which two main lineages emerged: a clade including *Conchodytes*, *Platypontonia*, *Odontonia*, and some minor (often monotypic) genera; and a clade including *Dactylonia*, *Pontonia*, and *Ascidonia* (and some minor genera). Two ancestral character state analyses were used to find out the historical, native range of the species of the latter lineage, and to shed some light on the presumed complex host-switching events that have taken place in the entire clade.

The ancestral biogeographic range reconstruction focused mainly on the species in *Pontonia* and *Ascidonia*, which have an Atlantic (Atl.) and East Pacific (EP) range. The other species in the clade, also including the more closely related *Dactylonia*, *Cainonia*, and *Rostronia*, have a native Indo-West Pacific (IWP) range. The ancestral range of the most common ancestor of the branches including *Pontonia*, Ascidionia, and the three genera mentioned before, was found to be native to the Pacific, which was limited to an East Pacific range in the branch of only *Pontonia* and *Ascidonia*. This reflects the combination of two main historical events that transpired around 12-18 Myr ago (the TTE; Terminal Tethyan Event) and the past 65 Myr (EPB; the East Pacific Barrier). These two events prove to be a hard and soft barrier (resp.) that separated the IWP and EP populations, giving rise to new species lineages. The internal topology of the clade of *Pontonia* and *Ascidonia* (including West and East Atlantic, as well as East Pacific members) is reflected by the subsequent closure of the Panama Isthmus (around 3.1 Myr ago), which separated the species on the East (Pacific) and West (East Atl.; Caribbean) side of the Americas, and the opening of the

Atlantic Ocean (in effect throughout the past ca. 60 Myr), separating many West and East Atlantic species. The complex biogeography of the area resulted into (sister-) species pairs that can be found on either side of the Panama Isthmus (like *Pontonia manningi* and *P. margarita*, and *Ascidonia pusilla* and *A. quasipusilla*), or on either side of the Atlantic Ocean (like the closely resembling *P. pinnophylax* and *P. mexicana*). The ancestral native ranges of various pistol shrimps (Alpheidae) have been studied in detail before (Anker & Baeza, 2012), revealing similar patterns. This is the first group of palaemonid shrimp to be studied this way, giving rise to perform these biogeographical analyses on other groups of symbiotic shrimps.

The ancestral state reconstruction focusing on ancestral host associations resulted in a full overview of the host-switching events that presumably have happened in the evolutionary history of the clade. Horká et al. (2016) could not resolve the ancestral state of the clade, and Chow et al. (2021) found the ancestral state to be in favour of bivalve association. By raising the number of species in the '*Conchodytes* clade', the analyses presented in Chapter 4 showed the ancestral character state to be in favor of an ascidian host. These analyses also showed that the clade had shifted to a bivalve host in at least four instances: once in *Pontonia*, once in the monotypic genus *Cainonia*, once in the monotypic *Opaepupu*, and once at the base of the clade including *Odontonia*, *Conchodytes* and related smaller genera. Interestingly, some of the branches which have switches from an ascidian to a bivalve host, have switched back to an association with an ascidian host. This is the case for the branch containing the monotypic genus *Pseudopontonia*, and possibly twice in *Odontonia* (in *O. rufopunctata*, and in the other species of *Odontonia*, excluding *O. kerangcaris*). In *Pontonia*, a host switch towards a gastropod host has also occurred, in the branch of *P. chimaera*. This evolutionary history, featuring multiple back-and-forth inter-phylum host switching events, is unique for this clade (Chapter 5). Looking at the hosts' morphology, it seems that the similar internal microenvironment of the bivalve- and ascidian hosts could explain the host-switching events in this clade. Host switching between phyla without these proposed matching internal microenvironments seems to have occurred less frequently (Horká et al., 2016; Chow et al., 2021).

The ancestral range and host-association analyses in Chapter 4 show only overlap in one species. The (ancestrally) ascidian-associated *Pontonia panamica* switches towards a bivalve-association in (most of) the other species in *Pontonia*, just when the lineage switches from an East Pacific to West Atlantic distribution. The link between host switches and biogeography has been studied in the past (Hoberg & Brooks, 2008; Hoeksema et al., 2018), but needs to be

further addressed in the evolution of these commensal shrimps.

Revising the '*Anchistus* clade'

Similar to how the species in the '*Conchodytes* clade' were studied, a Total-Evidence approach was used to study the phylogeny of the species in the '*Anchistus* clade'. This endosymbiotic lineage was previously defined by Horká et al. (2016) and Chow et al. (2021) to be separately evolved from the species in the '*Conchodytes* clade' and the endosymbiotic species in (mainly) *Typton* and Periclimenaeus. However, in contrast to the species of the '*Conchodytes* clade', members of the genera *Anchistus* and *Paranchistus* (both s.l.) were already found to be polyphyletic by Horká et al. (2016) and Chow et al. (2021). This polyphyly was also proved by the phylogenetic framework constructed in Chapter 5, resulting in a new taxonomic classification of the clade. Three (of which two new) genera were (re-)established: *Ensiger* was re-established for *Anchistus custos* and *A. custoides*; *Polkamenes* was newly erected for four species of *Paranchistus* (leaving *Paranchistus* to be monotypic); and *Tympanicheles* was erected for two species, i.e., one from *Anchistus* and one from *Paranchistus*. A full taxonomic revision, including new illustrations and a key to the genera and species, can be found in Chapter 5.

An ancestral character state analysis focusing on the host associations of the species in the '*Anchistus* clade' was be performed. The clade was found to have a common ancestor in slight favour of a bivalve-associated host specificity, which is in contrast to the '*Conchodytes* clade's' ascidian-associated ancestral state (Chapter 4). Regardless if the common ancestor of the clade had a bivalve- or an ascidian-associated lifestyle, one host switch seems to have occurred when the basally placed genus of *Dasella* separated from the bivalve-associated lineage comprised of the other genera in the clade.

Comparing the phylogeny reconstructions based on molecular, morphological and combined data sparked some interest in the phylogenetic significance of the studied morphological features, and the possible convergence of these characters. First off, the colour patterns of the endosymbionts are a paradox on their own: why would a species with a protective endosymbiosis evolve intricate colour patterns that do not match with their host's? Bruce (1976) mentions that the conspicuous colour patterns have no obvious advantages or disadvantages, and might be regarded as "irrelevant". Projecting the 'polka-dotted' patterns on the phylogeny reconstruction, including the outgroup species, seems to suggest something else. Larger spots on the dorsal side of the carapace, and all extremities seem to be more phylogenetically derived traits. We were, however, not able to find the same evolutionary trend in the

'*Conchodytes* clade'. In addition, considering both endosymbiotic clades, some types of colouration patterns could be linked to the species' host choices. For instance, the larger, brightly-coloured, blotches in *Neoanchistus* (and to some extent *Tympanicheles pectinis*) is shared with *Platypontonia* (part of the '*Conchodytes* clade'), which all share similar pectinid and cardiid bivalves as hosts (Chapter 4: fig. 4.1J; Chapter 5: figs. 5.28, 5.36).

The minor and major claws of the clade members bear features which should be studied more in the future for their phylogenetic and functional-mechanic properties. The (sub)spatulate minor claws with pectinate rows on the cutting edges can be found in almost all members of the ingroup, as well as a selection of related outgroup species (*Zenopontonia*, *Lipkemenes*, *Actinimenes*, and various members of *Periclimenes*). This feature has to have something to do with the feeding habits of the species, possibly used for scraping off mucus from their host. Interestingly, this feature cannot be found in members of the '*Conchodytes* clade', making it less host-specific. Based on a pilot study on the dietary habits of the shrimps (De Grave et al., 2021), it can be deduced that the species (and the outgroups) of the '*Anchistus* clade' can be classified as facultative or obligate parasites, while the sole representative of the '*Conchodytes* clade', *Pontonia pinnophylax*, appears to be a 'true' commensal. It is possible that the pectinate minor claws found in the species of the '*Anchistus* clade' and the related outgroups are used in (klepto-)parasitic feeding of host tissue and mucus.

The major claw of some of the shrimps in the '*Anchistus* clade' features an interesting 'organ', which was re-discovered in the present study. The drum-like veil on the underside of the major claws in species of *Anchistus*, *Neoanchistus*, and *Tympanicheles*, was found using SEM-photography and µCT-scanning (Chapter 5: fig. 5.39). The structure seems to be most developed in species of *Anchistus* associated with giant clams (*Tridacna*), and is possibly used to feel the vibrations of the host to sense danger. Interestingly, *Tridacna*-associated species of the '*Conchodytes* clade', as well as pea crabs with the same host, do not display this organ. In other arthropods, veil-like drum organs (so-called tympanums) are used for sound-perception and intraspecific communication, or even as osmoregulators (references in Chapter 5).

Lastly, some species in the '*Anchistus* clade' bear intricate microstructures on the walking-leg dactyli, almost resembling some sort of 'Velcro'. Interestingly, this patterning of microsetae was found in two separate lineages of the clade: within *Anchistus*, and within *Polkamenes* (mostly visible in *P. pycnodontae*). The species that were placed between these lineages (within *Neoanchistus*

and *Tympanicheles*) showcase some microstructures, but not to the same extend. This leads us to believe that *Anchistus* and *Polkamenes* have evolved these characters independently from each other, making it a good example of convergent evolution. Convergence between these two lineages and some species in the '*Conchodytes* clade' was also found for this character. This morphological convergence is possibly due to the same host choices and therefore selection pressures found in these groups, and is studied in detail in Chapter 7 and below.

Phylomorphospace analysis of pea crab carapace shapes

In order to study the carapace shape variation of all (previously illustrated) species of pinnotherine pea crabs, a morphometric analysis was performed using 2D landmarks. The information on host associations gathered in the first chapter (Chapter 2) was used to make a full ecological dataset of pinnotherine pea crabs, which was used to label the specimens in the so-called morphospace plots, resulting from the morphometric analyses (Chapter 6). The shape variation in the first two principal components (or morphospace dimensions) was mostly found in the general appearance of the carapace: most outgroup species, which were from the other subfamilies (Pinnixinae, Pinnixulalinae), were found to be wider in shape, and the Pinnotherinae (in general) rounder in shape. In addition, on the second axis, the shape variation was less conspicuous: some species had a flattened, wide frontal margin (e.g. in *Durckheimia*), while other species display more pointed frontal margin (e.g., in *Fabia* and *Limotheres*). Although the pairwise Procrustes ANOVA tests resulted in significant differences between the mean carapace shapes, based on their host associations, the differences between the groups were inconspicuous. Only the ectosymbiotic clade of the '*Dissodactylus* complex' could easily be distinguished from their endosymbiotic relatives, based on the angular carapace margins. In addition, outgroup species with an inquiline lifestyle were found to be much wider built than their endo- and ectosymbiotic counterparts in the ingroup (Chapter 6).

Combining these morphometric analyses with the phylogeny reconstruction from Hultgren et al. (2022), resulted in a so-called 'phylomorphospace' analysis (Chapter 6). This projection of the phylogeny reconstruction on top of the specimens in the morphospace results in a 2D-network that shows how the lineages changed their carapace morphology during their evolutionary history. Using this technique, multiple convergent pathways to similar morphologies were found: between members of the same genus, between three species from different genera, and between subfamilies. Within the ingroup, morphological convergence within one genus, and between different genera, was found in

species with a wider carapace shape: between the ectosymbiotic *Dissodactylus crinitichelis* and *D. latus*; and between species of *Austinotheres*, *Juxtafabia*, and *Solenotheres*, which are bivalve endosymbionts. The host association of the species from the different genera could not be used to explain the convergence towards a wider body shape: both *Austinotheres* and *Juxtafabia* are generalist genera, and the elongated bivalve hosts of *Solenotheres* is shared with many other species of pea crabs (e.g., *Raytheres*, *Serenotheres*, and *Visayeres*). The morphological convergence within *Dissodactylus*, could however be explained by their host choice, as well as their distributions. Both species appear to have colonised the Caribbean waters independently, and have evolved to utilise the same sand dollar taxa as hosts.

Morphological convergence of the carapace shape between two species of the outgroup (*Scleroplax faba* and *S. littoralis*) and the species in the ingroup was already found by Hultgren et al. (2022) and Chow et al. (2023). These two outgroup species are part of a lineage that seems to have independently evolved an endosymbiosis with bivalves, resulting in a more Pinnotherinae-looking, round, carapace shapes for these species. Moreover, while the third maxilliped shapes of these species were found to look similar to other pinnixine crabs, the ambulatory walking legs also appear to be convergently evolved towards a more 'endosymbiotic form' (Chow et al., 2023). Similarly, two other species (*Pinnixa barnharti* and *P. tumida*) which have evolved an endosymbiosis with holothurians, can also be found to the left side of the outgroup in the morphospace in Chapter 6, indicating a similar switch towards a rounder carapace shape in these species. Due to the incomplete molecular dataset, the phylogenetic position of these species could not be deduced. Therefore, questions about the number of host switches leading up to these endosymbioses with bivalves and holothurians, and the way the species have converged with the ingroup, remain.

Due to some vague host records and limited figurations featured in old pea crab species descriptions, a complete dataset could not be obtained. This, in combination with limited molecular data, although recently improved for the Indo-West Pacific pea crab species (Chow et al., 2023), resulted in an incomplete overview of the morphological convergence of endosymbiotic pea crabs in general. However, the geometric morphometric approach, using landmarks as a basis to translate 2D morphology into data, is a massive improvement from other analyses which either use some type of categorization for the different (author-defined) morphotypes (Chow et al., 2023), or ratios and measurements, the so-called classical morphometrics (Zelditch et al., 2004). Although these methods work to showcase larger patterns of convergence,

geometric morphometrics help us in capturing minute details, and let us statistically compare shapes. It is foreseen that in the future, 3D morphometrics can be used to take the overall roundness of the species into account, which might help in answering questions about the ecomorphological aspects in the body shapes found in the '*Dissodactylus* complex'.

Morphological diversification and convergence in endosymbiotic shrimps

Using the host records, illustrations, and additional data from published taxonomic papers and field reports, an almost-complete morphological and ecological dataset could be produced for the two studied bivalve- and ascidian-associated endosymbiotic shrimp clades. In this dataset, a special interest was taken in the dactylar morphology of the third pair of walking legs, and the so-called 'post orbital carapace length' (or 'pocl') measurements, for their potential host-specific adaptability. As was mentioned above, the dactylus shapes are mostly used to identify species, but they might show host-specific shape variation due to their functionality in maintaining grip within the host (Chapter 7). Similarly, host-specific selection pressures could also be influencing the maximum body sizes of the shrimps (Baeza & Thiel, 2007). In addition, collection materials for the available species were subsampled for a large-scale Scanning Electron Microscopy study, to investigate the micro-ornamentations on the surface of the dactyli, which might also play a role in the host-specific biology of these shrimp species.

The maximum pocl values, functioning as a proxy for the maximum body sizes, for both male and female specimens were mapped on the TE phylogeny reconstructions from the phylogenetic studies on the clades (Chapters 4 and 5), showing also the (continuous) ancestral character states for the internodes. We found that, in general, female specimens reach larger body sizes than their male conspecifics, and that bivalve-associated species usually grow larger than ascidian-associated species. Convergence between and within the clades was therefore found between genera and species with the same host phyla (e.g., low pocl values were found in the ascidian-associated genera *Odontonia*, *Dactylonia*, and *Dasella*; and high values were mainly found in some bivalve-associated members of *Pontonia*, *Conchodytes*, *Ensiger*, and *Paranchistus*). There were, however, some exceptions such as the highest pocl value in the '*Conchodytes* clade', which was recorded for the ascidian-associated *Notopontonia platycheles* (Chapter 7).

Similar to the study discussed above (Chapter 6), landmarks were placed on the gathered illustrations, producing an overview ('morphospaces') of the

dactylus shape variation in both clades (Chapter 7). Similar to the study on pea crab carapace shapes (Chapter 6), the species with an association with bivalves take up the largest proportion of the morphospaces. In this case, the bivalve-associated dactyli ranged from an elongated shape, without any accessory teeth (e.g., in *Platypontonia*, and *Ensiger*) towards an inflated shape, in some species armed with excessive accessory teeth, such as in the genera *Conchodytes* and *Anchistus*. In contrast, the ascidian-associated species were found to be grouped more together, and may have intricate ornamentations of the flexor margin, such as in most species of the '*Conchodytes* clade' (e.g., in *Dactylonia* and *Odontonia*). In the '*Anchistus* clade', all species of the ascidian-associated *Dasella* were found to share a similar shape of the dactylus, with the bivalve symbiont *Paranchistus armatus* resembling them the most. Interestingly, averaging the shape differences for the bivalve- and ascidian-associated species resulted in almost the same shape for both host groups, with the bivalve-associated having a somewhat more inflated corpus. This does not suggest a phylum-specific dactylus shape for the '*Conchodytes* clade', as suggested for the whole family by Chow et al. (2021). For the '*Anchistus* clade', no conclusions could be drawn due to the low sample size of ascidian-associated shrimps.

Consequently, using the same TE phylogeny topologies phylomorphospaces could be constructed and the convergent pathways to similar dactylus shapes studied. A total of respectively five and three possible convergence events from the '*Conchodytes* and *Anchistus* clade' were studied in detail, similar to the study in Chapter 6. Most convergence events were found between species with the same host phylum, but convergence between species with different host groups was also found (Chapter 7). In the '*Conchodytes* clade', convergences towards the '*Conchodytes*-like' dactylus shape were most prevalent, characterised by an inflated corpus and a long accessory tooth. This morphotype was also found in *Odontonia kerangcaris* (Chapter 3), and several species of *Pontonia*. Interestingly, some species of *Pontonia* and *Conchodytes* also shifted towards a less complex dactylus shape, as could be seen in *C. chadi*, *P. mexicana*, and *P. pinnophylax*. This convergence towards a more elongated dactylus was also seen in other bivalve-associated genera like *Platypontonia*, and the ascidian-associated *Odontonia seychellensis*. In the '*Anchistus* clade' convergence towards more inflated and complex dactylus shapes were found between the species of *Anchistus*, and *Polkamenes* (Chapter 5). In addition, convergence towards a more 'outgroup-looking' elongated shape was found in species of *Ensiger*, and in *Tympanicheles ornatus*. The resemblance of the dactyli of the bivalve-associated *Paranchistus armatus* to those of the ascidian-associated *Dasella*, also appears to be the result of convergent evolution (Chapter 7).

Projection of the characters studied by SEM on top of the TE phylogeny topologies revealed some patterning in the dactylar microstructure diversity in the two clades. Some species shared the lack of microstructures with the outgroup species (e.g., *Ensiger*, *Dasella*, *Odontonia*). In contrast, a variety of elaborate scales, teeth, and microsetae were found on the tip, as well as the dorsal and ventral side of the unguis, and on the accessory tooth. Morphological convergence was found in a number of characters: an unguis covered in a few large scales was found in a few members of ascidian-associated *Odontonia* and *Dactylonia*; (sometimes well-spaced) rows of microstructures were found on the dorsal side of the unguis in both *Conchodytes* and *Pontonia*, and to some extend also in all bivalve-associated genera in the '*Anchistus* clade', excluding *Paranchistus*; and microstructures on the ventral side of the unguis were found in a few members of *Conchodytes*, as well as *Tympanicheles ornatus*. Interestingly, the species of *Conchodytes* and *Pontonia* which share a similar dactylus morphology, also share similar microstructures, only reinforcing the possibility of morphological convergence in these clades. Although the SEM images could not quantitively be tested like the other datatypes mentioned above, it is expected that these results will mirror the results from the dactylus-shape analysis. In addition, it is unknown if these possible instances of convergences resulted in homologous morphological features, which can only be studied using juvenile specimens.

When comparing the body sizes, dactylus shapes, and dactylar microstructures of the species in both clades, no 'bivalve- or ascidian-specific associated morphotypes' could be identified. However, the presumed evolutionary convergence of all three studied features suggest that similar selection pressures are at play for these clades. Moreover, bivalve-associated species appear to be showcasing the largest variation in all three features, in both clades, indicating a higher morphological diversification rate in this group. In contrast, ascidian-associated species appear to be more conserved in their morphology, even when taking the lower sample size of this group into account. This is most apparent in the dactylar morphospaces, also in the context of the known host-switching events (Chapter 7). For example: a host switch from a bivalve towards an ascidian association results in a lower disparity in the clade of *Odontonia*, compared to the related genus *Conchodytes*. The other way around, a host switch from a more morphologically conserved ascidian-associated group towards a more morphologically variable group with a bivalve association can be found in *Pontonia*. Here, the related ascidian-associated species (the genera *Ascidonia*, *Dactylonia*, and *P. panamica* as the most basally placed species of *Pontonia*) also group together. Host-switches have already been proven to be driving force behind diversification in symbiotic shrimps

(Horká et al., 2016; Chow et al., 2021), which can now also be quantified on a morphological scale in the phylomorphospace plots (Chapter 7).

Linking it all together – future studies on the ecomorphology of symbiotic crustaceans

When comparing the results of the ecomorphological and phylogenetic studies of this thesis, it can be concluded that the more data is available, the more accurate and interpretable the results seem to get. This is most evident in the studies on the pinnotherine pea crabs, in which mainly the molecular data coverage is worth improving. This was recently done for the Indo-West Pacific genera (Chow et al., 2023), but many others are still under-represented (Chapter 2). Outside of Pinnotherinae, massive advances have been made to make the pinnixine and pinnothereliine Tree of Life as complete as possible (Felder & Palacios Theil, 2020; Palacios Theil & Felder, 2020). Certain 'key species' would, however, be interesting to add in the future, like the holothurian endosymbionts mentioned above (Chapter 6). For the studies on Palaemonidae, the coverage of the species in the clades is better (Chapters 4 and 5), but could also be improved by including more molecular data of poorly known species. Barcodes from these obscure crab and shrimp species could be used to study their phylogenetic placement, convergent evolution, host switching, and biogeographical ranges. Ideally, a better coverage of molecular markers could assist in dating the phylogeny trees without the use of fossils, by linking molecular clocks with biogeographical events (as done by Frolová, 2022). For this to be working, the mutation rates of mitogondrial markers in the Palaemonidae and Pinnotheridae need to be studied in detail. As for now these rates are not yet known, leaving no alternative but to use general mutation rates known from other crustacean groups (as is used in Frolová, 2022; for more information, see Schubart et al., 1998).

With regard to the molecular analyses of endosymbiotic-shrimp clades, many ingroup taxa could not be included due to limited availability of molecular data. They could, however, still be included in phylogeny reconstructions by use of the TE approach (Chapters 4 and 5). Most of these species are rare members of well-represented genera, such as *Conchodytes kempoides* and *Pontonia longispina*, or species of obscure monotypic genera (e.g., *Bruceonia*, *Colemonia*). In the present phylogeny reconstructions, only close resemblance of morphological characters resulted in their phylogenetic placement next to other ingroup members. In theory, species that are only represented by morphological data could also be separate lineages, as was suggested for *Opaepupu huna* (Anker & De Grave, 2021). The morphological similarity of the species between our ingroup and these 'rogue' species can, of course, be

the result of similar selection pressures being at play due to the microhabitat provided by the hosts being similar, suggesting convergent evolution between these potentially different lineages. For now, we have to trust phylogeny reconstructions based on molecular data and the a priori assumptions from taxonomic papers, suggesting relatedness between the genera included in these studies. The latter was the case for *Notopontonia* in Chapter 4, which was based on a remark by Bruce (1991). A species that should perhaps be added in future phylogenetic studies came to light more recently. The elusive gall-inducing *Paratypton siebenrocki*, hosted by scleractinian corals, was found to be related to two species from the '*Conchodytes* clade' (Van der Meij et al., 2023). Re-running the analyses from Chapter 4 by including *Paratypton* might shed some light on how this species is related to the genera in the '*Conchodytes* clade', and how host-switching could have happened between an ascidian- or bivalve and a scleractinian host. The morphological adaptations of *Paratypton* should also be studied more in detail, also for their resemblance with the species in the '*Conchodytes* clade'.

Using the phylogeny topologies from the TE methods, ecomorphological features of both crabs and shrimps were studied in an evolutionary context. These features could be adaptive to specific selection pressures because of their interspecific variation. In pea crabs, only carapaces were studied because of their variation in overall shape, size, and ornamentation (Chapter 6), but this selection in adaptive features might be expanded by including the eyes, mouthparts, claws, and walking legs (Chapter 2). There might even be more morphological features, which cannot be observed using the currently available species descriptions. Examples of these are microstructures that can only be seen using SEM or similar methods, such as the scales on the last walking leg pairs in members of *Arcotheres* (Chapter 2). More recently, Chow et al. (2023) made the next step by also studying the third maxillipeds and walking legs. They also found patterning of convergence, although this was not statistically tested using Stayton's C-values (Stayton, 2015). In the two shrimp clades, we studied the dactylus shapes, dactylar microstructures, and a proxy for maximum body sizes (Chapter 7). Some early studies focused on adaptions to host associations in the evolution of other morphological features, such as the eyes (Dobson et al., 2014, 2016) and mouthparts (Ashelby et al., 2015; Chow et al., 2021). This selection might be expanded by also including the carapace shapes, as in Chapter 6. The plotting of three-dimensional landmarks on shrimp carapaces can perhaps help in finding possible body-shape differences between ascidian- and bivalve-associated representatives.

Evidence for the evolutionary adaptability of morphological features can be

provided by studying the endosymbionts when they are alive inside their hosts (Wainwright, 1991). For instance, the drum-organ ('tympanum') seen in some shrimp species in the '*Anchistus* clade' was morphologically studied in Chapter 5. It would be interesting to see how the shrimps use the drum in intra- or interspecific communication, or perhaps in symbiont-host interactions. Another way to get more information on the symbiotic lifestyle of these symbionts (in this case, the trophic positioning) is to use meta-barcoding techniques on the symbionts' stomach contents or stable isotope analyses. The latter was done in a pilot study by De Grave et al. (2021). As was mentioned before, a clear difference was found between the ecological notes on *Pontonia pinnophylax* (thought to be a true commensal), and the isotopic composition of *Anchistus miersi*, which was found to be an obligate or facultative parasite. This demonstrated that the host association by itself does not predict the lifestyle and feeding habits of the symbiont. Moreover, this method also raises questions about the potential phylogenetic relevance of the trophic characterization of the shrimps. In other words, are all species in the '*Anchistus* clade' parasites, and are all species in the '*Conchodytes* clade' true commensals? Only observational data and improving the coverage of the species in our ingroups can help in finding an answer in these types of studies.

While we studied the pinnotherine crabs in a wider context by also taking the other subfamilies into account, the palaemonid shrimps were only studied in the context of the two endosymbiotic clades. This could be expanded by including other endosymbiotic clades, such as the clade including *Periclimenaeus*, Onycocaris, and *Typton*, symbionts of colonial ascidians and sponges, and the clade including *Apopontonia*, *Climeniperaeus*, *Paraclimenaeus*, and *Unesconia*, mainly living in sponges (Horká et al., 2016; Odijk & Fransen, 2017). Convergence between these four endosymbiotic clades has not yet been studied before, and it may be interesting to look for host-specific adaptations in the overall morphology and linked microstructures. In addition, the selection of included species may also be expanded family-wide (Chow et al., 2021). Here, no direct evidence of host-specific morphological convergence of the dactyli and mouthparts was found. There are, however, a few cases of morphological convergence that are based on a similar host choice. For example, among lookalikes of Caribbean/Indo-West Pacific species, such as *Periclimenes perryae* vs. *Lipkemenes lanipes*, *Periclimenes commensalis* vs. *Laomenes* spp., and *Pontonia* spp. vs. *Isopontonia platycheles*, the counterparts resemble each other in cryptic colouration and body size, but are not directly related (Horká et al., 2016). By including more types of figuration and analytic methods (like SEM and geometric morphometrics), a more detailed view of the morphological variation of these clades can be studied. In this case, it would be interesting to

find more host-specific micro- or macro-adaptations in these species.

A study of convergence can be done on a much broader scale by including symbiotic crustacean clades from other orders, such as true crab lineages, symbiotic porcelain crabs, and squat lobsters. In this way, the origin of symbiosis could be studied in more detail. In addition, a study of morphological adaptations in parasitic amphipods, copepods, fish lice, isopods, and cirripeds might assist us in finding host-specific adaptations, and distinguish them from general lifestyle adaptations. For example, the round body shape of endosymbiotic pea crabs appears to be an adaptation to life within a host in general, and not for a certain host group (Chapter 6). The evolutionary history of these groups might also feature multiple host-switching events, as seen in the shrimps and crabs studied in this thesis.

Concluding words

This thesis is a first step in clarifying how three clades of endosymbiotic decapods have evolved to live in their own unique microhabitats. As is the case for most taxonomy-based studies, the work never appears to be finished. New symbiotic species of palaemonid shrimps and pinnotherid crabs continue to be discovered, sometimes found in remote places, in elusive host organisms, or in a museum collection (Chapter 3; Fransen et al., 2022, 2023; Neves, 2022; Komai et al., 2023). Hopefully, the phylogenetic and morphometric methods from this thesis will be used to include additional species, or they can be applied in different biological fields. For example, many specimens of symbiotic decapods in natural history museums that were dredged from the deep sea have no host record. New advances in the field of remote-controlled deep-sea expeditions may prove that relatives of these species are associated with a wide range of invertebrate hosts (Komai et al., 2022, 2023). The ecomorphological methods presented in this thesis, together with ecological data on species that phylogenetically are closely related to the present species, may help authors to go back to older specimens and ‘predict’ their host associations based on the species’ morphology, following the ‘Darwin Hawkmoth principle’ (Kritsky, 1991).

Carlson et al. (2017) state that a large portion of parasite biodiversity already faces extinction in the upcoming 50 years due to climate-change alone. This is clearly the case in coral-associated fauna (Stella et al., 2011; Montano, 2020, 2022). These threatened parasites, as well as symbionts as a whole, were found to require a specialised conservation approach, tailored to their unique life histories, tremendous diversity, and the complex ecosystem services they provide. Therefore, I also hope I can assist subsequent authors in filling in the puzzle-pieces to answer questions about the lifestyle and evolution of symbiotic

organisms in general, and therefore also help in the future conservation of these symbiotic species.

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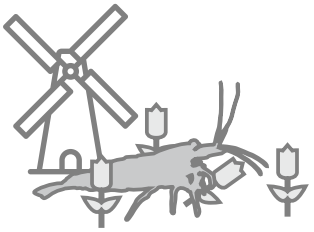
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Nederlandse samenvatting



Het samenleven van twee (of meer) verschillende soorten organismen noemt men ‘symbiose’. Hierbij biedt een gastheer een thuis voor een doorgaans kleinere ‘symbiont’ of gast. Er bestaan verschillende soorten symbioses, in te delen op basis van de voor- en nadelen voor beide partijen, en hoe strikt de samenwerking is. De meest voorkomende symbioses zijn mutualisme, parasitisme, en commensalisme. Bij mutualisme is de samenwerking gunstig voor beide partijen; bij parasitisme profiteert de symbiont (in dit geval een parasiet), waardoor de gastheer erop achteruitgaat; en bij commensalisme profiteert de symbiont zonder dat de gastheer een positief of negatief effect ervaart. Als de partners ook onafhankelijk van elkaar kunnen leven, spreekt men van een facultatieve symbiose, terwijl een noodzakelijke samenwerking een obligate symbiose wordt genoemd. Symbioses zijn ook in te delen aan de hand van de positie van de symbiont ten opzichte van de gastheer: ectosymbionten leven aan de buitenzijde van de gastheer (zoals bijvoorbeeld een vlo of een teek), en endosymbionten leven binnenin lichaamsholtes van de gastheer (zoals bijvoorbeeld een lintworm). Het begrijpen van deze variatie in samenlevingsvormen is noodzakelijk om meer te weten te komen over hoe huidige soorten leven en hoe ze zijn geëvolueerd.

De groep van de kreeftachtigen (Crustacea) is deel van de Geleedpotigen stam, waarin ook miljoen- en duizendpoten, insecten, spinnen en schorpioenen te vinden zijn. De kreeftachtigen omvatten zo’n zesenzeventigduizend beschreven soorten, waarbij vertegenwoordigers van de tienpotigen (Decapoda) het meest herkenbaar zijn. Veel soorten van deze groep hebben economische waarde en worden door de vishandel te koop aangeboden als krabben, kreeften, en garnalen. Bijna alle kreeftachtige hoofdgroepen bevatten soorten die als symbiont beschouwd kunnen worden en in een zoutwatermilieu voorkomen. Hier leven ze in, op, of met een grote diversiteit aan gastheren. De grootste fylogenetische variatie in gastheren is te vinden in de symbioses van twee families tienpotigen: de symbiotische palaemonide garnalen (ook wel de ‘commensale garnalen’) en de pinnotheride krabben (ook wel ‘erwtenkrabbetjes’).

Symbiotische palaemonide garnalen zijn te vinden tussen de tentakels van zeeanemonen, takken van zachte en harde koralen, armen van zeesterren en zeelelies, maar ook tussen stekels van zee-egels, bovenop zeekomkommers en naaktslakken, en in sponzen. Ook zijn er symbiotische garnalen die samen met wormen en vissen leven, in de gangen die hun gastheer uitgraaft. De twee groepen palaemonide garnalen die in dit proefschrift behandeld worden, leven als endosymbionten in de mantelholte (in het zachte lichaam tussen de schelpen) van tweekleppigen (zoals mossels en oesters), en in de zogenaamde kieuwkorf van zakpijpen (een primitieve groep chordadieren), die worden

gebruikt om plankton te filteren. De meeste garnalen in deze familie worden gezien als commensaal, hoewel recent onderzoek aan sommige soorten aanwijzingen geeft van een (klepto-)parasitaire levenswijze. De krabben die in dit proefschrift worden behandeld, de groep van de erwtenkrabbetjes, staan bekend echter bekend als parasitair, hoewel dit nog niet bij alle soorten is bewezen. In Nederland leven een paar soorten erwtenkrabbetjes, die mede te vinden zijn in commercieel verkrijgbare oesters en mosselen. Een groot deel van de endosymbiotische erwtenkrabbetjes zijn te vinden in de subfamilie Pinnotherinae, waarvan de meeste soorten in tweekleppigen leven. Daarentegen zijn er ook nog pinnotherine soorten die leven in zeekomkommers, (kever-)slakken, zakpijpen, en op en in zee-egels. Met uitzondering van een paar soorten, leven de vertegenwoordigers van de andere twee subfamilies in tunnels die zijn uitgegraven door wormen, of sommige kreeftachtigen (zoals molkreeften).

De extreme variatie in gastheerkeuze resulteerde gedurende de evolutie van palaemonide garnalen met een nog grotere variatie in uiterlijk, die met name tot uitdrukking komt in verschillende camouflagepatronen. Vooral ectosymbiotische garnalen lijken geëvolueerd te zijn om qua kleur overeen te komen met die van de gastheer, waarschijnlijk om niet opgemerkt te worden door roofdieren. Voor de erwtenkrabbetjes geldt hetzelfde, de minder vaak voorkomende ectosymbionten zijn wit van kleur om niet op te vallen tussen het sediment op de bovenkanten van hun gastheer zee-egels. De endosymbiotische vertegenwoordigers van de onderzochte krabben en garnalen vertonen daarentegen vaak regelmatige stippenpatronen, die niet overeenkomen met het interieur van hun gastheren. De endosymbiotische levenswijze van de in dit proefschrift bestudeerde groepen heeft niet alleen maar selectiedruk uitgeoefend op de kleur van de symbionten, maar ook op hun lichaamsbouw, zowel op macro- als microniveau. Deze adaptaties, verkregen door de selectiedruk van de leefomgeving, worden ook wel ‘ecomorfologische aanpassingen’ genoemd.

De ecomorfologie van erwtenkrabbetjes werd bestudeerd en bediscussieerd in Hoofdstuk 2 door een literatuuroverzicht te maken van zo veel mogelijk gepubliceerde wetenschappelijke artikelen en notities. Hierbij lag de focus op morfologische kenmerken die gastheer-specifiek, of kenmerkend voor de levensstijl lijken te zijn, zodat deze later door onderzoekers kunnen worden behandeld in morfologische analyses (zoals in hoofdstuk 6). Ook werd de lijst met gastheer-associaties van alle soorten binnenin de pinnotherine subfamilie geactualiseerd. Volgens Hoofdstuk 2 wordt de ecomorfologie van endosymbiotische erwtenkrabbetjes bepaald door de aanwezigheid van een zacht, rond rugschild (de carapax); een extreem opgezwollen achterlijf voor

het beschermen van de relatief grote eieren; en het bezit van lange, soms asymmetrische, dunne poten en scharen. Op een nog kleiner niveau is te zien, dat voornamelijk de mannetjes van sommige soorten erwtenkrabbetjes haren hebben ontwikkeld om beter tussen gastheren te kunnen zwemmen, hoewel deze structuren ook bij sommige vrouwtjes te vinden zijn. De aanwezigheid van deze zwemharen lijkt gekoppeld te zijn aan de levenswijze van de soorten: bij sommige soorten erwtenkrabbetjes wordt de gastheer zowel door de mannetjes als de vrouwtjes verlaten om te kunnen paren. Verder lijken de vrouwtjes van sommige soorten aanpassingen te hebben ontwikkeld op de poten en scharen, in de vorm van kleine haren of schubben. Waarschijnlijk gebruiken ze deze structuren om zich te kunnen voeden met slijm dat door de gastheer wordt uitgescheiden. Sommige soorten blijken vergrote en sterk behaarde monddelen te hebben die gebruikt kunnen worden voor het vangen van plankton.

Bij de endosymbiotische garnalen waren de meeste (potentieel) ecomorfologische aanpassingen al bekend in de literatuur: de meeste hiervan lijken overeen te komen met die van de erwtenkrabbetjes. Bij veel soorten is er een afgeronde carapax met een aangepast achterlijf te zien. Verder is ook door voorgaand onderzoek aangetoond dat de ogen van endosymbiotische garnalen een andere bouw hebben dan hun ectosymbiotische verwanten. Op de pootjes en scharen van de garnalen zijn daarentegen ook klittenband-achtige structuren te vinden die de grip binnenin de gastheer lijken te bevorderen. Deze structuren, in combinatie met de vorm van de pootjes, worden ook gebruikt in soortherkenning, en bij het indelen van soorten in genera. In Hoofdstuk 3 is een nieuwe soort beschreven, waarbij de vorm van de pootjes niet overeenkomt met die van de rest van het geslacht. De nieuwe soort *Odontonia kerangcaris* werd opgemerkt tijdens de moleculaire analyses die in Hoofdstuk 4 werden uitgewerkt, waarin deze soort een basale fylogenetische positie heeft ten opzichte van de andere soorten binnen het geslacht *Odontonia*. De nieuwe soort was gevonden in een tweekleppige gastheer, wat erop duidt dat er een gastheerwisseling heeft plaatsgevonden binnen het geslacht, omdat de andere *Odontonia* soorten in zakpijpen leven. De plaatsing in de stamboom en de afwijkende gastheer worden weerspiegeld door de vorm van de pootjes, die meer overeenkomen met die van andere geslachten die in tweekleppigen voorkomen, zoals *Pontonia* en *Conchodytes*.

Wanneer de gastheerkeuze van een groep krabbetjes of garnalen wordt uitgezet tegenover hun fylogenetische stamboom, kan worden nagegaan hoe de evolutie van de groep is verlopen en wanneer elk gastheertype werd gekoloniseerd. Hierin zijn zogenaamde ‘gastheer-switches’ te vinden, waarbij een groep symbionten de overstap heeft gemaakt tussen twee onverwante

gastheergroepen. Voor de palaemonide garnalen is een stamboomoverzicht gemaakt waaruit blijkt dat bijvoorbeeld de overstap tussen een koraalgastheer en een stekelhuidige (zeesterren, -egels, -komkommers, en -lelies) vaak onafhankelijk heeft plaatgevonden. Het overzicht geeft ook een inkijkje in de twee endosymbiotische aftakkingen van de boom, waarbij niet duidelijk is of de zakpijp- of tweekleppige-associatie het meest oorspronkelijk was. Om dit te onderzoeken zijn er in Hoofdstukken 4 en 5 nieuwe fylogenetische stambomen gepresenteerd voor zowel de '*Conchodytes* tak' als de '*Anchistus* tak'. De '*Conchodytes* tak' bevat een groot aantal geslachten, waarvan de tweekleppige-geassocieerde geslachten *Conchodytes* en *Pontonia*, en de zakpijp-geassocieerde geslachten *Odontonia* en *Dactylonia* de grootste zijn. Gebaseerd op een combinatie van moleculaire en morfologische gegevens bleek deze tak monofyletisch te zijn, wat betekent dat ze allemaal een gedeelde gemeenschappelijke voorouder hebben. Deze vooroudersoort bleek een zakpijp-geassocieerde soort te zijn, in tegenstelling tot bevindingen van eerder onderzoek. Met behulp van zogenaamde 'ancestral character state analyses' zijn de gastheer-switches in kaart gebracht: binnen de aftakkingen van de stamboom wordt zeker zes keer van gastheer gewisseld, waarbij in drie gevallen zelfs de initiële switch (van zakpijp naar tweekleppige) wordt opgevolgd door een secundaire switch, terug naar de oorspronkelijke staat. In het geval van de genera *Ascidonia* en *Pontonia* konden de aangetoonde gastheer switches voor een deel gekoppeld worden aan de kolonisatie van nieuwe geografische gebieden in de Atlantische Oceaan.

Met behulp van morfologische en moleculaire data werd er ook van de '*Anchistus* tak' een stamboom gemaakt, en werd er gekeken naar de gastheer switches in deze groep. De statistische testen wezen op een gemeenschappelijke voorouder met een tweekleppige-associatie, wat haaks stond op de '*Conchodytes* tak'. In tegenstelling tot de '*Conchodytes* tak' waarbij de geslachten geen taxonomische aanpassingen nodig hadden, werden drie geslachten van de '*Anchistus* tak' als polyfyletisch bevonden. Dit betekende dat, wederom op basis van nieuwe inzichten door een combinatie van moleculaire en morfologische gegevens, de soorten een nieuwe indeling nodig hadden. In dit geval waren er drie extra geslachten nodig om de soorten in te kunnen delen. Het ongeldige geslacht *Ensiger* kreeg opnieuw een geldige status voor twee soorten, het nieuwe geslacht *Polkamenes* werd beschreven voor vier soorten, en het nieuwe geslacht *Tympanicheles* werd beschreven voor twee soorten. De nieuwe naam *Polkamenes* is gebaseerd op de kleurpatronen van de soorten en *Tympanicheles* is vernoemd naar een 'trommel-achtig' orgaan dat voorkomt aan de onderzijde van de grote scharen bij enkele soorten. Dit orgaan werd afgebeeld door middel van SEM- en micro-CT-opnames en blijkt

een fylogenetisch relevant kenmerk te zijn. Het orgaan het meest duidelijk ontwikkeld te zijn in de soorten die recent zijn afgetakte. De functie van het orgaan is nog onduidelijk, maar gedragsobservaties in het veld geven de indruk dat het trommel-achtig vlies wordt gebruikt om de gastheer af te tasten.

Zoals hierboven beschreven, zijn er in de bouw van zowel de garnalen als de krabben dezelfde aanpassingen te vinden, wat een voorbeeld is van zogenaamde 'convergente evolutie', waarbij dezelfde milieuomstandigheden (in dit geval, dezelfde typen gastheren) tot eenzelfde type aanpassing heeft geleid. Convergente evolutie kan te zien zijn tussen soorten binnenin hetzelfde geslacht, tussen groepen binnen een familie, en in dit geval zelfs tussen twee ver verwante groepen kreeftachtigen. De laatste twee hoofdstukken presenteren voorbeelden van hoe convergente evolutie op basis van gastheerkeuze kan hebben plaatsgevonden, in zowel de groep van de endosymbiotische erwtenkrabbetjes (Hoofdstuk 6) en in de twee verschillende groepen endosymbiotische garnalen (Hoofdstuk 7).

In beide hoofdstukken wordt een methode gebruikt die we de 'phylomorphospace' methode noemen, die hierna wordt uitgelegd aan de hand van Hoofdstuk 6 over een ecomorfologische analyse van erwtenkrabbetjes carapaces. De 'phylomorphospace methode' start bij een geometrische morfometrische landmark-analyse, waarbij karakteristieke oriëntatiepunten (zogenaamde landmarks) worden geplaatst op een 2D of 3D afbeelding. In dit geval zijn tekeningen en foto's verzameld van 181 soorten erwtenkrabbetjes, waarbij de oriëntatiepunten op de rechterkant van de carapax werden gemarkeerd. De opeenvolgende 'Procrustes' analyse maakt de data uniform door alle variaties in rotatie, schaal, en plaatsing eruit te filteren. Met behulp van multivariate statistiek (PCA) is het dan mogelijk om de variatie in carapaxvorm te plotten, waarbij elke soort wordt vertegenwoordigd door een unieke set van datapunten. Op basis van het resulterende 'morphospace', kan men al zien welke soorten meer op elkaar lijken, en of er een ecologische factor bestaat (in dit geval de gastheerkeuze) die een rol speelt in het verklaren van de data. In het geval van de erwtenkrabbetjes bleek dat de gastheerkeuze een effect had op de vorm van de carapax. Zo hebben zakpijpgeassocieerde krabbetjes een langwerpigere carapax dan bijvoorbeeld slakgeassocieerde soorten, die wat breder in vorm zijn. Opvallend waren vooral de scheiding tussen de buis- en tunnelbewonende soorten enerzijds en de endosymbionten anderzijds, en de grote variatie in carapaxvormen binnen de groep van soorten die samenleven met tweekleppigen.

De resulterende 'morphospace' kan gecombineerd worden met evolutionaire

informatie, om op deze manier convergentie in vorm aan te kunnen tonen tussen soorten. Dit werd in dit geval gedaan door de meest recente stamboom van de erwtenkrabbetjes te projecteren op de 'morphospace', waarbij de takken gevolgd kunnen worden om de evolutionaire stappen naar een bepaalde lichaamsvorm te kunnen herkennen. Als de takken bij elkaar komen vanuit een andere oorsprong, dan is er een aanwijzing van convergente evolutie naar eenzelfde lichaamsvorm toe. Er is gekeken naar drie verschillende (potentiële) gevallen van convergentie in vorm: tussen twee soorten binnenin het ectosymbiotische zee-egel-geassocieerde '*Dissodactylus* complex'; tussen drie soorten tweekleppige-geassocieerde endosymbionten uit verschillende geslachten binnen de Pinnotherinae; en tussen drie soorten tweekleppige-geassocieerde endosymbionten, waarvan twee uit de subfamilie Pinnixinae komen. De drie gevallen werden statistisch getest en als convergent aangeduid, hoewel de reden voor hun convergentie niet werd gevonden. Voor de convergentie tussen de twee subfamilies kon er echter wel een verklaring worden gevonden: de afwijkende endosymbiotische gastheerkeuze van twee soorten Pinnixinae lijkt ervoor gezorgd te hebben dat de soorten weer tot meer ronde vormen zijn geëvolueerd. Voor nog twee andere soorten binnen de Pinnixinae, welke in de anale opening van zeekomkommers leven, lijkt dit ook het geval te zijn. Dit kon echter niet getest worden door een gebrek aan moleculaire data.

De 'phylomorphospace' methode werd ook bij de twee groepen endosymbiotische garnalen toegepast. In dit geval werd gekeken naar de vorm van het derde paar looppoten. Op deze manier is geprobeerd om inzicht te krijgen in hoe de pootjes van de garnalen zijn geëvolueerd en potentieel zijn geconvergeerd, en of de gastheerkeuze hierin een rol speelde. Net als bij de erwtenkrabbetjes werd de grootste variatie bij de tweekleppige-geassocieerde soorten waargenomen, in zowel de '*Conchodytes* tak' als in de '*Anchistus* tak'. Hoewel de groep zakpijp-geassocieerde soorten in de '*Anchistus* tak' te klein was om harde conclusies te trekken, bleek de soortenrijke groep in de '*Conchodytes* clade' een kleiner gebied binnen de 'morphospace' in te nemen. Dit kan betekenen dat de selectiedruk op symbionten in zakpijpen een stuk sterker is dan die in tweekleppige weekdieren, waardoor de morfologische variatie een stuk lager uitvalt. Het meest opvallend was de graad van convergentie tussen tweekleppige-geassocieerde soorten: in beide groepen garnalen kwamen zowel langwerpige, simpelgebouwde pootjes voor, als brede, complex geornamenteerde pootjes.

Naast de convergentie in de vorm van de pootjes in de 'phylomorphospace', is er ook gekeken naar de convergentie in lichaamsgrootte, en in de microstructuren op de looppootjes. De lichaamsgrootte is geplot op stambomen uit Hoofdstukken 4 en 5, waarbij voor zowel de mannetjes als de vrouwtjes de maximaal bekende

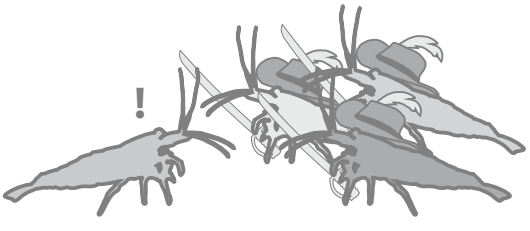
groottes werden gebruikt. Bij bijna alle soorten blijken de vrouwtjes groter te zijn dan de mannetjes. Deze seksuele dimorfie heeft waarschijnlijk te maken met hun rol in de productie en het uitbroeden van de eieren. Er was ook een duidelijk verschil in lichaamsgrootte op basis van gastheerkeuze. Op één soort na, worden zakpijpbewonende garnalen kleiner dan de verwante symbionten in tweekleppigen. Dit heeft waarschijnlijk te maken met de maximale grootte van de beschikbare ruimte in de gastheer: de gevouwen kieuwkorf van zakpijpen biedt waarschijnlijk minder ruimte dan de mantelholte van tweekleppigen. De levenswijze van de garnalen en hun voortplantingsstrategieën kunnen ook hebben bijgedragen aan het verschil in lichaamsgroottes. Soorten die voor het paren moeten wisselen tussen gastheren zullen waarschijnlijk kleiner blijven dan soorten die in hun gastheer blijven en monogame koppels vormen.

Ten slotte is er convergentie gevonden tussen de microstructuren die te zien zijn op de looppootjes van de garnalen. Met behulp van SEM-opnames met hoge resolutie kon worden gekeken naar morfologische variatie van schubben, haartjes, en klittenband-achtige structuren, waarbij werd genoteerd welke soorten vergelijkbare microstructuur-dekking hadden. De meer primitieve soorten bleken doorgaans een gebrek aan microstructuren te hebben en de recent afgetakte soorten toonden juist complexe microstructuren. Ook was er een connectie tussen de convergentie binnen de ‘phylomorphospace’ van pootjes tussen verschillende soorten *Pontonia* en *Conchodytes* en verschillen in hun microstructuurdekking. In andere woorden: soorten die convergent dezelfde pootvormen hadden geëvolueerd, hadden vaak ook dezelfde type microstructuren. Dit betekent dat de pootvormen en de microstructuren waarschijnlijk samen geëvolueerd zijn en er dus relatief vaak selectiedruk uitgeoefend wordt op deze anatomische kenmerken.

De volgorde van de besproken hoofdstukken fungeert als voorbeeld voor een protocol voor het bestuderen van de ecomorfologie van symbiotische soorten in een evolutionaire context. Net als de meeste andere onderzoeken met een taxonomische basis, laat ook dit proefschrift zien dat dit werk nooit af is. Het is wenselijk dat met de ontdekking van nieuwe ecologische, evolutionaire, en taxonomische inzichten, de analyses van dit proefschrift gebruikt kunnen worden in toekomstig onderzoek aan symbiotische krabben- en garnalen. Daarnaast kunnen de hoofdstukken ook gebruikt worden als voorbeelden voor analyses die focussen op andere groepen symbiotische ongewervelden. Eerder onderzoek heeft uitgewezen dat ook voor het beschermen van symbiotische soorten een gespecialiseerde aanpak nodig is, waarin rekening wordt gehouden met de unieke levenswijzen, diversiteit, en ecosysteemdiensten die worden geleverd door symbiotische soorten. Veel groepen symbiotische soorten

hebben mogelijk bescherming nodig zonder dat er fundamentele kennis over hen bestaat. Dit is zeker het geval voor de ‘cryptofauna’ van koraalriffen die over het algemeen niet in één oogopslag te zien zijn. Met name voor deze diergroepen dient dit proefschrift bij te dragen aan het oplossen van de grote biodiversiteitspuzzel en daarmee aan kennis ten behoeve van de bescherming van onzichtbare maar onmisbare soorten.

Acknowledgements



I have to admit that this chapter was the most difficult to write. Unlike the scientific chapters from this thesis, I'm afraid the information listed here is far from complete. It's simply impossible to list all the people who helped me during this project, not only with practical, but also emotional support. In addition, my gratitude is impossible to describe with words only, so please come have a cup of tea or a beer with me – you all know I like to chat. So, to all the people listed here, but especially the ones I missed, I would like to thank you from the bottom of my heart.

First off, I would like to thank my supervisors Bert, Charles, and Ronald for the wonderful time I had during my PhD, but also before and after. Your knowledge of fieldwork, taxonomy, phylogenetics, and marine biology in general led to fruitful discussions about the past, present and future, combined with lots of laughs, and the occasional collaboration. I learned how to do research, to write, and to publish, even before the start of my contract. I'd like to believe this helped me to create a similar inspiring working environment for the students we co-supervised. Bert, thank you for teaching me how to write less (in your words) "fuzzy" – I hope my payment in cookies makes up for the number of times I made the same grammar mistakes over and over again. Charles, thank you so much for everything you taught me about shrimp anatomy, biology, and evolution – but also sorry for using you as my personal shrimp fact library, I hope that one day I can return the favour. Ronald, I know for certain that without your feedback my thesis would not have been the same. In addition to learning all the ins and outs on publishing, I love reciting your fieldwork stories from the ZMA-days. Heren, Musketiers, bedankt voor alles.

I'm really happy I got to know so many people at Naturalis, despite the two 'scientist migrations' from one office building to another, and a seemingly endless pandemic. First up, a big thank you to all my peers: other institutes can only dream of having a PhD/Postdoc community like the one we have at Naturalis. In no particular order, I would like to thank the following PhD's and Postdocs: Kasper, Kevin, Mátyás, Roderick and Eka, James, Elsa (and Fritjof), Aleksandre, William, Auke-Florian, Sander, Mehrdad, Ajaree, Bob, Richa, Irene, Merijn, Aafke, Marcel, Dewi, Andrès, Lizzie, Jimmy, Hector, Panos, Manon, Leon, Deyi, Aleid, Nicolaas, Giovanni, Niels (and Charlotte), Maartje, Sjoerd, Brigida, Mariya, Izai, Jordy (and Floor), Pasha, Yanell, Tim, Nemi, Eva, Margret, Caroline, Benoît, Eduard, Annabelle, and Isaak. Thank you all for the fun times at the museum, and in the city of Leiden during the PhD/Postdoc events. In addition, I also want to thank a few peers and indirect colleagues from the Sylvius' behavioural biology department: thanks for the amazing advice and good times.

At Naturalis, I was part of three research groups, the first two (Taxonomy and Systematics, and Taxonomy, Systematics and Geodiversity) consisted of a handful of researchers with a shared interest in fundamental research. A major thanks goes out to Erik, not only for being our group leader, but also for nudging me in the right direction to chase my dreams at Naturalis. I would also like to thank all our former group members: Leo, Jan, Hanco, Herman, Pim, Isolde, Esther v. d. E., and Isabel. Special thanks go out to my former supervisors Steve and Ton: thank you for all the discussions we had during our daily coffee moments. I hope you're both proud of this thesis, for I could not have written it without your help.

After Erik left Naturalis, the research group of Katja kindly adopted us as fellow marine biologists. Katja, thank you so much for your help in the last few months of my PhD. Trying to maintain a healthy work-life balance is almost impossible as an interested scientist, but I believe that you're a perfect example of someone who achieved this. In the same light, I would like to thank our other Plankton Diversity / Marine Evolution and Ecology group members: Lisa, Kaveh, Roel, Harry, Ronald S., Lisette, Mainah, Stephanie, Giada, Martien, Annelies, Illiana, Pane, Paula, and Le Qin.

In addition to direct colleagues, I also spent quite some time with many faces of the other research groups: Hans t. S. and Sylvia, Leni, Nicole, Marco R., Michael, Renske, Luis, Jeremy, Lars, Frederic, Koos, Martin, Jorinde, Frank W., Menno, Iva, Gab, Eelco, Anthonie, Rutger, Tinde, Barbara, Alexandra, Daan, Guido, Berry, Peter v. W., Peter H. (†) and Gerda, Willem (†) and Brigitta, Pieter, Hans A., Fritz A., Hans K., Hiltje and Paul, Ben, Aglaia, Erik v. N., Peter v. H., Esmée, Harold, and Corné. Thank you all for the nice conversations we had: even as a "beestjes-man" I almost felt like a proper botanist when joining the herbarium's morning coffees.

During my PhD, I was lucky enough to use the wonderful lab- and imaging-facilities at Naturalis. A group of technicians, true experts in their own field, helped me to get the best images from the CT-scanners and the SEM, and great-quality DNA data from our museum specimens. In this light, I would like to thank the following people: Bertie-Joan, Rob, Frank S., Oscar, Dirk, Elza, Roland, and Marcel. From the collection-tower, I would like to thank Hannco, Jeroen G., Bram, Wendy, Esther D., and of course, the people from the Alcohol-team, for their help in maintaining the 200-year-old spirit collection. From the Research Coordination Office, I would like to thank Dominique, Thirza, Johan, Ate, Caroline, Michiel, and especially Connie for always being there for me – from minute problems to huge career-changing decisions, I could always ask

for your advice.

Thanks to my science communication and educational tasks, and of course my sometimes a bit too extrovert personality, I also came into contact with the non-scientific staff. A big thank you goes out to the following people: my reception ladies Anneke and Angela, Krista, Maartje (†), Alice, Hansjorg, Bart, Amy, Marc, and Daphne. I would also like to thank Edwin and Maaike for leading the institute, and for providing me with the chance to work in this environment.

In 2019, I had the privilege to join a scientific expedition to Bonaire. Here, with the “Magnificent 8” led by our “fearless leader” Bert, I was able to work with the following people, who I owe my full gratitude for the amazing time on the island: Luna, Lukas, Jaaziel, Slava, James, Floris, Godfried, Simone, Marco F., and Marianne.

Even if you’re writing a “single author paper”, all scientists work together – collectively. Therefore, I want to thank the following people who helped me in conceptualising, writing, and publishing the chapters from this thesis: our student team, consisting of Max, Sherin, Mike G., Pepijn, and Jurriaan; my co-author Carola, and the team of carcinologists and collaborators, consisting of Shane, David, Sammy, Zdeněk, Peter N., Kristin, Quentin, Justin, Hossein, and Arthur. I also want to thank my NZ friends for the fun time in Wellington: Henry, Sienna, Anchita, and Victoria.

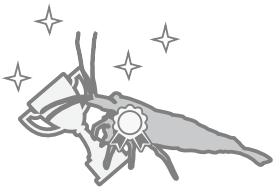
Bijna bij het eind van deze lijst aangekomen, wil ik nog mijn ouders Diny en Gert-Jan bedanken, en natuurlijk mijn zus Vivian. Ik hoop dat de hoeveelheid Waku Waku feitjes goed werd gemaakt met mijn leuke verhalen vanuit het museum, de artikelen die ik de wereld in hielp, en de media optredens die ik mocht doen tijdens mijn PhD. Ik weet dat jullie luisteren en kijken naar alle interviews, en ik merk dat jullie trots zijn als iemand die jullie kennen mij weer op TV gezien heeft. Mam, Pap, bedankt voor jullie steun.

Al sinds 2013 ben ik deel van een ontzettend hechte vriendengroep, met daarin (gelukkig) een ongezonde hoeveelheid biologen die elke wandeling weer op de beste manier kunnen verpesten. In willekeurige volgorde: Carola en Lucaß, Tobias en Marijke, Ihab, Lucas, Nathan en Michelle, Jerry en Nienke, en Willem en Larissa. Jongens, bedankt voor alle steun, zowel op professioneel als persoonlijk vlak – bedankt dat ik mezelf kan zijn bij jullie. Ook wil ik Dimmy en Roos, Caitlin en Bas, en Jamal en Tahnee bedanken voor de support de laatste vier jaar – ik weet dat ik met alles bij jullie terecht kan.

Two of my fellow PhDs really stood out since the moment they joined me at Naturalis: with Ryan and Mike H. our fine gentlemen's club really took shape, and luckily Vicky was there to save us every time we had stupid ideas. Thank you guys, Ryan and Mike H.: my para-paranymphs. Talking about Vicky – Vicky, zonder jou had ik dit niet eens kunnen uittikken en ik het is me een eer dat je me wil bijstaan bij de verdediging. Onze eindeloze koffiegesprekken over de meest heftige dingen, of juist over de meest nutteloze zooi zullen me altijd bijblijven. Ik weet zeker dat we elkaar blijven zien, en ik kan niet wachten om ook jouw paranimf te mogen zijn – hoewel ik na 4 jaar nog steeds niets weet over hout. Het zal een stuk stiller worden in Naturalis als onze giechels weg zijn, maar je weet nooit wat de toekomst brengt.

Dan rest mij nog maar één iemand te bedanken: Jeroen, bedankt voor alles de laatste jaren. Ik ben gekomen van niets naar een huis, een baan, en een vriend waarmee ik alles kan delen. Wat begon met praten over ons werk in bus 400, samen "harrie 5" kijken en een stuk fietsen door de Zoetermeerse polders, is gegaan naar samen de wereld verkennen, onze passies volgen, en zelfs samen publiceren. Men zegt altijd dat je een partner moet zoeken die iets totaal anders doet dan jijzelf, maar ik kan me niet voorstellen waar ik zou zijn geweest zonder je. Bij wie anders kan ik terecht als ik trots ben op een publicatie, en bij wie moet ik klagen over de tweede reviewer, of vragen stellen over mijn R-script? Van alle mensen in de wereld ben jij de enige die mij snapt, en voor dat ben ik je voor altijd dankbaar. Hou van je hun!

Curriculum Vitae



Werner de Gier was born on March 3rd, 1995, in Zoetermeer, the Netherlands. In 2013, Werner started studying Biology at Leiden University. During his Bachelor's degree, he was inspired by the scientific-illustration assignments during courses on botany and invertebrate zoology. This led him to seek out an internship in a field of invertebrate taxonomy, where classic illustration methods are still used for scientific publications. This search resulted in his first research project at Naturalis Biodiversity Center, under supervision of carcinologist Dr. Charles Fransen. Werner performed a phylogenetic analysis on a genus of tiny ascidian-dwelling shrimps, and illustrated two new species: *Odontonia plurellicola*, and *Odontonia bagginsi*. Due to its hairy legs, the latter species was named after the family name of the main character from the book 'The Hobbit'. The paper came out in 2018, paired with an international media storm.

During his Master degree in Ecology, Evolution and Conservation at Leiden University, Werner made a short switch to one of his many other loves: malacology. He started a research project with Dr. Ton de Winter, revising a species complex of West African predatory 'hunter snails', using both classical methods in conchology, as well as a new µCT-scanning methodology. Scanning snail shells was surprisingly easy, so Werner scanned as many species of the genus *Ptychotrema* as possible, and made a new classification based on the internal and external shell characters. Parts of this study were published in 2019: one paper on the identity of a *Mirellia* species, and one describing a new snail species from Nigeria: *Ennea nigeriensis*. Werner returned to carcinology during his second Master's research project, for which he sequenced the stomach contents of a Caribbean representative of the shame-faced crab family (Calappidae). These crabs were thought to only feed on right-handed snail shells, but were now also observed eating other prey items. During his fieldwork in Curaçao, Werner observed that the crabs were 'stalked' by various types of fish. This observation led to a paper in collaboration with a local diver, who was able to photograph the same behaviour in Bonaire.

After his Master, Werner started a short contract at Naturalis as a research assistant, helping with various DNA-barcoding and µCT-scanning projects for the research group of Prof. dr. Erik Smets. In addition, Werner also joined Prof. dr. Menno Schilthuizen during one of his field trips to the jungle of Brunei Darussalam, where Werner collected snails and land crabs for the local university's natural history collection.

In 2019, Werner started his PhD-project with Dr. Charles Fransen and Dr. Ronald Vonk, and his promotor Prof. dr. Bert Hoeksema. The thesis you are currently reading focusses on three taxonomic groups of symbiotic crustaceans:

a subfamily of pea crabs, and two clades of endosymbiotic shrimp species. Using geometric morphometrics – a way to translate shapes into quantitative data – and combining these with the evolutionary history of the studied clades, Werner was able to construct so-called 'phylomorphospaces'. In these plots, convergent evolution of morphological adaptations could be visualized and statistically proven, with special interest in 'host switching events' in the evolution of these crustaceans.

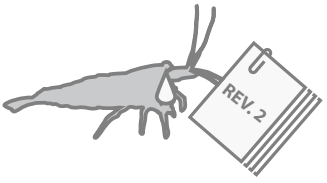
During his PhD project, Werner has been part of various committees and councils, representing the PhD community where needed. One of his biggest accomplishments was being able to organize a Pride-event at Naturalis, showing museum visitors that the animal kingdom is more sexually diverse than people would think, and that nothing is 'unnatural'.

Although Werner's heart lies with crustaceans, his interests are broader. He has been teaching in various courses at Leiden University, both in the lecture room and in the field. Werner also enjoys writing and presenting for a wider audience, being a common guest speaker in Naturalis, and writing press-releases for all his projects. While often appearing in Dutch radio shows, newspapers, and television interviews, Werner fills his last few free hours with another biology-based hobby: macro-photography of the Dutch fauna.

After Werner's PhD, he took some time to write both a NWO 'Rubicon' and a 'Humboldt post-doc' grant application, both for a two-year project to work with Drs. Kristina and Thomas von Rintelen at the Museum für Naturkunde, in Berlin. Here, Werner wants to expand his scientific toolkit by combining high-resolution molecular data with 3D-morphometrics, to unravel the evolution of a clade of freshwater *Caridina* shrimps from the Malili lake system (Sulawesi, Indonesia). The lakes are a natural lab for evolutionary processes, and showcase a great example of 'adaptive radiation'. In addition to studying the shrimps' evolution, a part of the post-doc project will focus on the aquarium trade in Western Europe: the shrimps are widely priced for their charismatic colouration and species-richness. Werner plans to use the results from his scientific works to aid the aquarists in species-identification, and therefore indirectly the protection of the species.

Although a scientific career is never a straightforward path, Werner aims to continue using his infectious enthusiasm to introduce people to the wonderful world of invertebrate animals by making these invisible animals more visible.

List of publications



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