Evolutionary diversification of coral-dwelling gall crabs (Cryptochiridae)

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Evolutionary diversification of coral-dwelling gall crabs (Cryptochiridae)

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Dr. F. Benzoni (University of Milano-Bicocca)

Aan mijn ouders

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> *"There is no task more fascinating to the naturalist than breaking up a block of some branching coral, such as* Pocillopora *or* Madrepora*, and dislodging from among its boughs the various animals that shelter there; nor of all these latter is there any more interesting than the crab* Hapalocarcinus*, which gives rise to the well-known galls that Semper [1881] described in his Animal Life."* (Borradaile, 1921)

Gall crabs (family Cryptochiridae) have fascinated scientists since the discovery of the first species described by William Stimpson in 1859. These crabs settle on stony corals (Scleractinia) as megalopa larvae and force the corals to form galls or pits as protective shelters around them. Their obligate association with scleractinian corals, hidden lifestyle, diminutive size and modified body shape (to fit their host) makes them stand out from all other crab species.

Cryptochiridae are amongst the smallest of crabs, ranging in carapace size from a few millimetres to about a centimetre at the most. There is conspicuous sexual dimorphism, with males being much smaller than females. Males mostly inhabit a dwelling on the same coral as a female, but may also be free-living on corals. Females of most species are permanently confined to their host, relying on it for food and shelter (Kropp, 1986). Their huge brood pouches, when ovigerous, prevent the females from being mobile. The males are mobile and visit the females for mating; hence their mating system is dubbed the 'visiting' mating system (Baeza and Thiel, 2007; Asakura, 2009).

For many years scientists have wondered where to place the family among the other crabs. Gall crabs have been linked to a wide range of families, and many researchers considered them to be closely related to pea crabs (Pinnotheridae) because of their similar size and host dependency. Although their placement within the overall crab phylogeny remained somewhat equivocal, they are placed in their own family and superfamily (Kropp and Manning, 1985). Over the years, many gall crab species have been described by Fize and Serène (1957), Takeda and Tamura (e.g. 1980, 1981, 1983) and Kropp (e.g. 1989, 1990a). The family Cryptochiridae currently consists of 21 genera and 51 species (Davie, 2014). Ongoing studies based on morphological, molecular and host specificity data revealed many cryptic species new to science (van der Meij, 2015, unpublished).

Gall crabs are recorded from shallow and deeper waters (over 500 m), but the majority of the species live in reef corals in the photic zone (Kropp and Manning, 1987; Kropp, 1990a; see also Van der Meij *et al*., 2015). Although gall crabs occur in almost all of the world's tropical oceans, they have their highest level of species and generic diversification in the Indo-West Pacific, where the coral diversity is highest (Fize and Serène, 1957; Kropp, 1990a; Hoeksema, 2007).

Many crabs have been recorded to live in some kind of association with stony corals (Stella *et al*., 2011), especially in branching corals belonging to the Acroporidae and Pocilloporidae. Cryptochiridae inhabit a wide range of branching and non-branching coral families, with Acroporidae, Euphylliidae and Poritidae as the most notable exceptions (Kropp, 1990a; van der Meij, unpublished). Gall crabs are very speciose as associates of Merulinidae and Agariciidae, in which many (closely related) coral species are inhabited by a host-specific gall crab species.

The interspecific interactions between gall crabs and their host corals trigger many research questions. Their unusual mode of life gives rise to questions about their phylogenetic position, which can be studied from molecular and morphological perspectives. Their close association with

their host corals allows for studies on (cryptic) speciation and possible coevolution - or more specifically i) coadaption or cospeciation, or ii) sequential evolution. Proven cospeciation or sequential evolution could make it possible to use gall crabs as phylogenetic indicators in scleractinian evolution. Studies on coevolutionary events in the marine realm are scarce – especially those focusing on invertebrates – and could hence provide novel insights in underlying mechanisms triggering diversification when compared to studies on terrestrial organisms. In a biogeographical context species diversity patterns of gall crabs can be compared with those of their scleractinian hosts, which are one of the most extensively investigated taxa in Indo-West Pacific biogeography. The symbiotic nature of the association allows for other comparisons, e.g. their distribution patterns at more local scale with regard to their abundance along environmental gradients, which is still a vastly unexplored field of study. Because gall crab dwellings can be observed and counted on reefs by experienced observers they can be used in studies on the distribution gall crabs over reefs and host species.

Thesis outline

After Fize (1956), Kropp (1988a) and Zayasu (2014), this is the fourth PhD thesis dealing with Cryptochiridae. It succeeds the work of a number of dedicated gall crab workers, in particular Potts, Utinomi [= Hiro], Fize and Serène, Takeda and Tamura, and Kropp. This thesis is divided into five sections, each containing one or more chapters, dealing with different aspects of cryptochirid evolution.

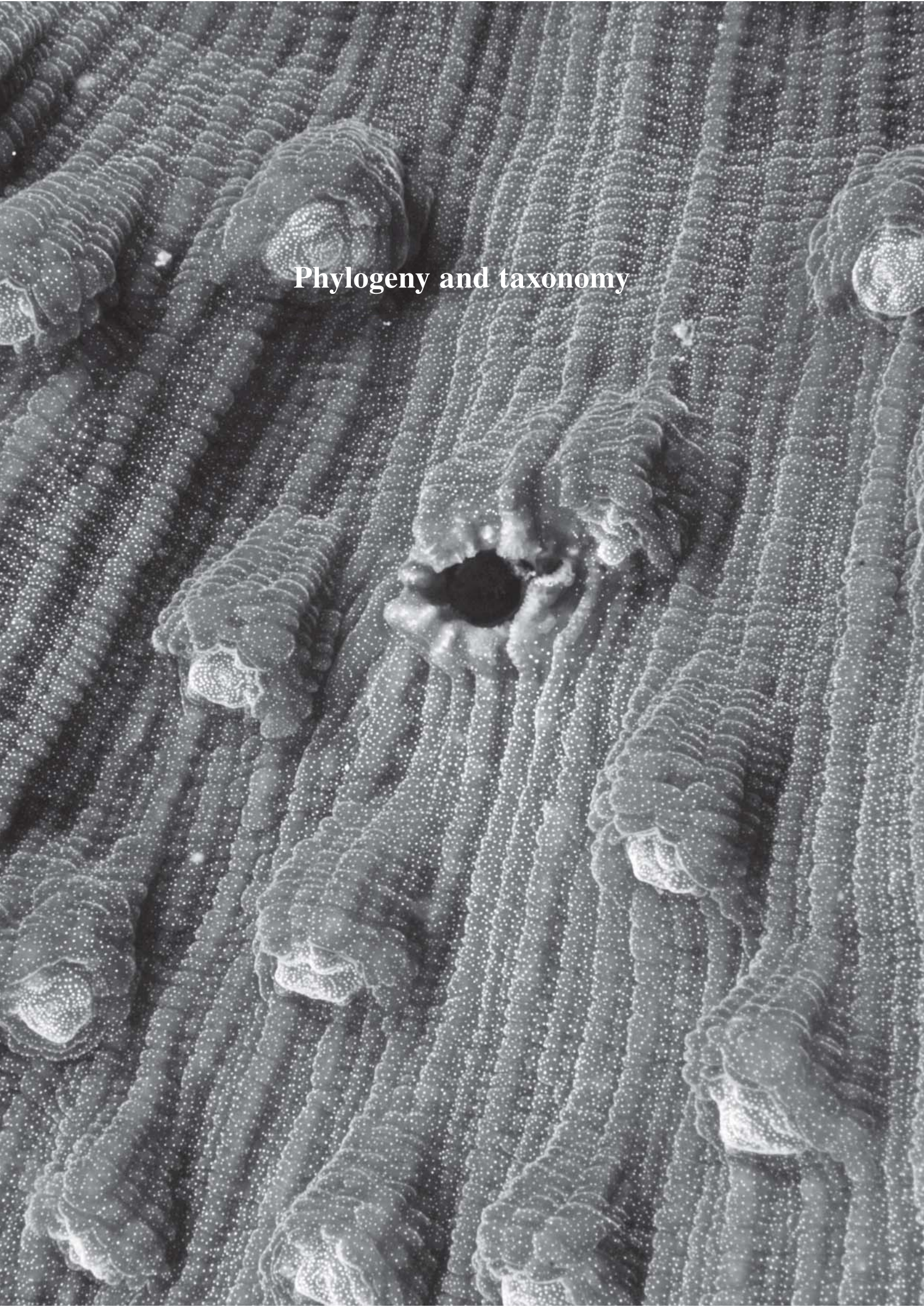
The section **Phylogeny and taxonomy** deals with the monophyly of the Cryptochiridae and their classification within the Thoracotremata (**chapter 1**). In the following chapters four new species are described: a new species from Indonesia and Malaysia, which is associated with the closely related coral species *Pavona bipartita* and *P. clavus* (Agariciidae) (**chapter 2**), a new species endemic to the Red Sea and Oman where it inhabits Lobophylliidae (**chapter 3**), a new species from Indonesia and Malaysia associated with the free-living coral *Trachyphyllia geoffroyi* (**chapter 4**) and a new and cryptic Fungiidae-associated species with a widespread distribution in the Indo-Pacific discovered by its host specificity in combination with molecular and morphological data (**chapter 5**). In the final chapter of this section, a molecular clock approach was used to estimate the time of origin of the Cryptochiridae and the diversification within the family based on nucleotide substitution rates (**chapter 6**).

The section **Host specificity and coevolution** is introduced by a study on the host specificity of Atlantic gall crabs, including new host coral records, extensions of known distribution ranges and an observation of their mating system (**chapter 7**). Host preferences and colour patterns for the Indo-Pacific species *Pseudocryptochirus viridis* are described in **chapter 8**, including new distribution records. In **chapter 9** the evolution of the association between gall crabs and mushroom corals is studied, which includes a test for possible coevolution between the Fungiidae and four Cryptochiridae species. Finally, coral and gall crab phylogeny reconstructions are compared, looking for congruence, by a test for coevolutionary events (**chapter 10**).

The section **Biogeography** starts with a study on the biogeographic patterns of *Neotroglocarcinus dawydoffi*, which occurs in the Red Sea, Indonesia and New Caledonia (**chapter 11**). Diversity patterns in the Red Sea – as a well-recognized biogeographic region of endemism – are studied by using gall crabs and their distribution in the Indo-Malayan region as a model (**chapter 12**).

The section **Distributions over reefs and shelves** deals with the cross-shelf distribution of mushroom coral-associated gall crabs across the Spermonde shelf in Indonesia (**chapter 13**). It also deals with the distribution of gall crabs in Fungiidae on reefs in the Semporna area, eastern Sabah, Malaysia (**chapter 14**).

The last section is on **Reproductive morphology**. The female reproductive morphology of three gall crab species is described using histological methods, and compared with that of other thoracotreme crabs (**chapter 15**).



Phylogeny and taxonomy

Chapter 1

Monophyly and phylogenetic origin of the gall crab family Cryptochiridae (Decapoda: Brachyura)

Sancia E.T. van der Meij & Christoph D. Schubart

Abstract

The enigmatic gall crab family Cryptochiridae has been proposed to be phylogenetically derived from within the Grapsidae (subsection Thoracotremata), based on the analysis of 16S mtDNA of one cryptochirid, *Hapalocarcinus marsupialis*, among a wide array of thoracotremes, including 12 species of the family Grapsidae. Here, we test the monophyly and phylogenetic position of Cryptochiridae using the same gene, but with an extended representation of cryptochirids spanning nine species in eight of 21 genera, in addition to further thoracotreme representatives. The results show that gall crabs form a highly supported monophyletic clade within the Thoracotremata, which evolved independently of grapsid crabs. Therefore, the Cryptochiridae should not be considered as highly modified Grapsidae, but as an independent lineage of Thoracotremata, deserving its current family rank. Further molecular and morphological studies are needed to elucidate the precise placement of the cryptochirids within the Eubrachyura.

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Introduction

Gall crabs (Cryptochiridae) are obligate symbionts of living scleractinian corals, residing in galls, tunnels or pits in the coral skeleton. The family consists of 21 genera and 49 species (Ng *et al.,* 2008; Davie, 2014) and is recorded from both shallow and deeper waters down to 512 m (Kropp and Manning, 1987; Kropp, 1990a). The first known gall crab species was described by Stimpson (1859), who named the species *Hapalocarcinus marsupialis* and referred to it as 'a remarkable new form of Brachyurous Crustacean'. Stimpson did not assign *H. marsupialis* to a crab family, but remarked that – in the series – it would probably fit between *Pinnotheres* and *Hymenosoma*, which belong to the Pinnotheridae De Haan, 1833 and the Hymenosomatidae MacLeay, 1838, respectively. Heller (1861) described a second gall crab species, *Cryptochirus coralliodytes*, and commented on its similarities with Ranina and Notopus (Raninidae De Haan, 1839). A. Milne-Edwards (1862) described yet another species, *Lithoscaptus paradoxus*, mentioning that this new species did not fit in any of the known crab families. Paul'son (1875) subsequently erected the subfamily Cryptochirinae within the Pinnotheridae to accommodate the gall crabs, which Richters (1880) elevated to family level. A more complete overview of the history of the family Cryptochiridae Paul'son, 1875, can be found in Kropp and Manning (1985).

Close phylogenetic affinities between the Cryptochiridae and Grapsidae s. str. (cf. Schubart *et al.,* 2002) were proposed by Wetzer *et al.* (2009). The authors recommended dropping the superfamily Cryptochiroidea (see Ng *et al.,* 2008) and suggested considering Cryptochiridae as just one of many separate 'grapsoid' families. The zoeal features of Cryptochiridae present numerous traits that are unique within the Brachyura (Tudge *et al.,* 2014 and references therein). Based on the larval development, a close relationship between grapsids and cryptochirids had been proposed by Fize (1956), who regarded cryptochirids as a transitional group between Grapsidae s.l. and Calappidae. Fize and Serène (1957) deviated from this placement and argued that Cryptochiridae has closest affinities with Pinnotheridae, based on the morphology of the female abdomen. When considering the larval morphology (based on *Troglocarcinus corallicola* Verrill, 1908), cryptochirids also appear closely related to Pinnotheridae, with close affinities to Hymenosomatidae and Leucosiidae (Scotto and Gore, 1981). Utinomi (1944) had previously considered the zoea of *Hapalocarcinus* and *Cryptochirus* to belong to the so-called Grapsizoea (including genera of the Cancridae, Grapsidae, Xanthidae and some Oxyrhyncha) and dismissed suggestions of a close affinity of Cryptochiridae with Pinnotheridae. Affinities with several other crab families (Hymenosomatidae, Leucosiidae, Pinnotheridae, Palicidae and Retroplumidae) were discussed by Kropp (1988a), who suggested monophyly of the Cryptochiridae based on a series of unique morphological characters (gastric mill, lateral lobe of the antennule, lack of mandibular palp). Guinot *et al.* (2013), based on several morphological structures, also concluded that the cryptochirids form a monophyletic group. The spermatozoa of *C. coralliodytes* and *H. marsupialis* were studied by Jamieson and Tudge (2000) and share a striking synapomorphy that is unique for the family Cryptochiridae (Tudge *et al.,* 2014). Tudge *et al.* (2014) also compared the sperm ultrastructure and operculum of Cryptochiridae to those of species belonging to the Majoidea and the Hymenosomatidae. The sperm ultrastructure proves to be somewhat equivocal with regard to placement of the cryptochirids in Thoracotremata or Heterotremata. The morphology of the female reproductive system was studied by Vehof *et al.* (in press) who showed that the Cryptochiridae share characteristics with the thoracotreme families Varunidae, Ocypodidae and Pinnotheridae. The cryptochirid reproductive system is nevertheless remarkable in having ovaries that are expanded into the abdomen (= pleon), which is exceptional among Brachyura and has only been known from pinnotherids so far (Becker *et al.,* 2011).

Fig. 1. The cryptochirid taxa used in this study: **A**, *Hapalocarcinus marsupialis*; **B**, *Utinomiella dimorpha*; **C**, *Opecarcinus lobifrons*; **D**, *Fungicola utinomi*; **E**, *Dacryomaia* sp.; **F**, *Fungicola fagei*; **G**, *Fizesereneia* sp.; **H**, *Lithoscaptus tri*; **I**, *Pseudocryptochirus viridis*. No picture is available for *Cryptochirus coralliodytes*. Not to scale.

Table 1. GenBank sequences used in molecular analyses (taxonomic authorities based on Ng *et al.,* 2008). * = sequences used in this study, but not included in Wetzer *et al.* (2009)

Family	Species	GenBank No.
Camptandriidae	*Baruna trigranulum* (Dai and Song, 1986)	AB002129
	Paracleistostoma depressum De Man, 1895	AB002128
Crossotonotidae	*Crossotonotus spinipes* (De Man, 1888)	AJ130807
Cryptochiridae	**Cryptochirus coralliodytes* Heller, 1861	KM114587
	**Dacryomaia* sp.	KM114582
	**Fizesereneia* sp.	KM114581
	**Fungicola fagei* (Fize and Serène, 1956)	KJ923707
	**Fungicola utinomi* (Fize and Serène, 1956)	KM114583
	Hapalocarcinus marsupialis Stimpson, 1859	EU743929
	Hapalocarcinus marsupialis Stimpson, 1859	EU743930
	**Hapalocarcinus marsupialis* Stimpson, 1859	KM114586
	**Lithoscaptus tri* (Fize and Serène, 1956)	KM114584
	**Opecarcinus lobifrons* Kropp, 1989	KJ923730
	**Pseudocryptochirus* viridis Hiro, 1938	KJ923710
	**Utinomiella dimorpha* (Henderson, 1906)	KM114585
Dotillidae	*Dotilla wichmanni* De Man, 1892	AB002126
	Ilyoplax deschampsi (Rathbun, 1913)	AB002117
	**Scopimera bitympana* Shen, 1930	AB002125
	Tmethypocoelis ceratophora (Koelbel, 1897)	AB002127
Gecarcinidae	*Cardisoma carnifex* (Herbst, 1796)	AM180687
	**Discoplax hirtipes* (Dana, 1852)	FM863830
	Gecarcinus lateralis (Fréminville, 1835)	AJ130804
	Gecarcoidae lalandii H. Milne Edwards, 1837	AM180684
Gecarcinucidae	**Holthuisana biroi* (Nobili, 1905)	FM180132
	**Lepidothelphusa cognetti* (Nobili, 1903)	FM180134
	Sartoriana spinigera (Wood-Mason, 1871)	AM234649
Glyptograpsidae	*Glyptograpsus impressus* Smith, 1870	AJ250646
	Platychirograpsus spectabilis De Man, 1896	AJ250645
Grapsidae	*Geograpsus lividus* (H. Milne Edwards, 1837)	AJ250651
	Goniopsis cruentata (Latreille, 1803)	AJ250652
	Grapsus grapsus (Linnaeus, 1758)	AJ250650
	Leptograpsus variegatus (Fabricius, 1793)	AJ250654
	Metopograpsus latifrons (White, 1847)	AJ784028
	Metopograpsus quadridentatus Stimpson, 1858	DQ062732
	Metopograpsus thukuhar (Owen, 1839)	AJ784027
	Pachygrapsus crassipes Randall, 1840	AB197814
	**Pachygrapsus fakaravensis* Rathbun, 1907	FR871306
	**Pachygrapsus gracilis* (Saussure, 1858)	FR871303
	Pachygrapsus marmoratus (Fabricius, 1787)	DQ079728
	Pachygrapsus minutus A. Milne-Edwards, 1873	AB057808
	**Pachygrapsus plicatus* (H. Milne Edwards, 1837)	FR871310
	Pachygrapsus transversus (Gibbes, 1850)	AJ250641
	Planes minutus (Linnaeus, 1758)	AJ250653
Heloeciidae	**Heloecius cordiformis* (H. Milne Edwards, 1837)	AM180695
Macrophthalmidae	**Macrophthalmus crinitus* Rathbun, 1913	AB537376
	**Hemiplax hirtipes* (Jacquinot, in Hombron and Jacquinot, 1846)	AB440189
Mictyridae	*Mictyris brevidactylus* Stimpson, 1858	AB002133
	**Mictyris guinotae* Davie, Shih and Chan, 2010	AB513632

Table 1. (continued)

Family	Species	GenBank No.
Ocypodidae	**Ocypode quadrata* (Fabricius, 1787)	FN539018
	**Uca borealis* Crane, 1975	AB535403
	**Uca tetragonon* (Herbst, 1790)	AB535405
	**Ucides cordatus* (Linneaus, 1763)	FN539019
Palicidae	*Palicus caronii* (Roux, 1828)	AM180692
Percnidae	*Percnon gibbesi* (H. Milne Edwards, 1853)	AJ130803
	**Percnon guinotae* Crosnier, 1965	FN539015
Pinnotheridae	*Austinixa aidae* (Righi, 1967)	AF503185
	Austinixa patagoniensis (Rathbun, 1918)	AF503186
	Pinnotheres pisum (Linnaeus, 1767)	AM180694
Plagusiidae	*Euchirograpsus americanus* A. Milne-Edwards, 1880	AJ250648
	**Plagusia depressa* (Fabricius, 1775)	AJ250649
	Plagusia squamosa (Herbst, 1790)	AJ311796
Potamidae	*Geothelphusa pingtung* Tan and Liu, 1998	AB266168
	**Potamon potamios* (Olivier, 1804)	AB428515
Potamonautidae	**Potamonautes perlatus* (H. Milne Edwards, 1837)	AM234647
Pseudothelpusidae	*Epilobocera sinuatifrons* (A. Milne-Edwards, 1866)	FM208778
Sesarmidae	*Armases elegans* (Herklots, 1851)	AJ784011
	**Chiromantes haematocheir* (De Haan, 1833)	AJ308414
	Sarmatium striaticarpus Davie, 1992	AM180680
	Sesarma meridies Schubart and Koller, 2005	AJ621819
	**Sesarma reticulatum* (Say, 1817)	AJ225867
Varunidae	*Austrohelice crassa* (Dana, 1851)	AJ308416
	Brachynotus atlanticus Forest, 1957	AJ278831
	Cyrtograpsus affinis Dana, 1851	AJ130801
	Eriocheir sinensis H. Milne Edwards, 1853	AJ250642
	Helograpsus haswellianus (Whitelegge, 1899)	AJ308417
	Hemigrapsus sanguineus (De Haan, 1835)	AJ493053
	Paragrapsus laevis (Dana, 1851)	AJ308418
	Pseudogaetice americanus (Rathbun, 1923)	AJ250643
	Varuna litterata (Fabricius, 1798)	AJ308419
Xenograpsidae	**Xenograpsus ngatama* McLay, 2007	FM863828
	**Xenograpsus testudinatus* Ng, Huang and Ho, 2000	FM863827
Xenophthalmidae	**Xenophthalmus pinnotheroides* White, 1846	EU934951

In the most recent treatments of the Brachyura (Ng *et al.,* 2008; De Grave *et al.,* 2009; Ahyong *et al.,* 2011; Tsang *et al.,* 2014), the Cryptochiridae is classified in the superfamily Cryptochiroidea, and placed in the subsection Thoracotremata. The main argument to place Cryptochiridae in the Thoracotremata is the sternal location of male gonopores (Guinot, 1977). This is in agreement with Scotto and Gore (1981), who regarded adults of the Atlantic species *Troglocarcinus corallicola* as exhibiting an advanced thoracotreme state. The Cryptochiridae have alternatively also been considered Heterotremata (e.g. Guinot and Richer de Forges, 1997; Guinot and Bouchard, 1998), advanced Heterotremata (Martin and Davis, 2001) or a 'basal heterotreme eubrachyuran superfamily' (Guinot *et al.,* 2013). Indeed, in the first paper employing molecular data to clarify the position of the gall crabs within other brachyurans, its placement in the subsection Thoracotremata was confirmed (Wetzer *et al.,* 2009).

The monophyly and phylogeny of the Cryptochiridae among the Thoracotremata were re-evaluated by using 16S mtDNA data for 10 gall crab species belonging to nine genera. We reused almost the entire dataset from Wetzer *et al.* (2009), but expanded it by adding 10 gall crab sequences, and 24 additional sequences from thoracotreme crab species and families not included in the previous study. We used this enlarged dataset for analysis of the position of the Cryptochiridae within the Thoracotremata and to test Wetzer *et al.*'s result that *Hapalocarcinus marsupialis* evolved from within the family Grapsidae.

Materials and methods

Wetzer *et al.* (2009) used two 16S mtDNA sequences of *Hapalocarcinus marsupialis*, combined with 49 GenBank sequences of thoracotreme species and four heterotreme species as outgroup to evaluate the relationships between Cryptochiridae and other Brachyura. To re-evaluate the position of the Cryptochiridae, we added nine additional species belonging to eight cryptochirid genera (see Fig. 1). We based our identifications on Fize and Serène (1957), Kropp (1989, 1990a) and van der Meij (2012). We included one additional sequence of *H. marsupialis* for comparison with the material of Wetzer *et al.* (2009).

An enlarged dataset encompassing a minimum of two species of all known thoracotreme families was used as a more complete dataset for research on the phylogenetic position of the gall crabs. Type genera and species were included whenever the corresponding data were available in GenBank. The full list of GenBank sequences and species authorities can be found in Table 1. The following changes and additions were made in comparison to the dataset of Wetzer *et al.* (2009):

(1) The Old World freshwater crabs used by Wetzer *et al.* (2009), *Sartoriana spinigera* (Gecarcinucidae) and *Geothelphusa pingtung* (Potamidae), were moved to the ingroup together with additional freshwater crabs from other continents, while *Crossotonotus spinipes* (Crossotonotidae) and *Palicus caronii* (Palicidae) were kept as outgroups. This was done in consequence to the newest brachyuran phylogeny by Tsang *et al.* (2014), which shows that Old World freshwater crabs of the superfamily Potamoidea (see Klaus *et al.,* 2009) are placed at the base of the Heterotremata which in turn are the sister group to all Thoracotremata. This implies that the Potamoidea are phylogenetically closer to Thoracotremata than most other Heterotremata are to Thoracotremata. Furthermore we wanted to root the tree in a comparable way to previous phylogenies of the Thoracotremata (Schubart *et al.,* 2000, 2002, 2006).
(2) *Sesarma windsor* (Sesarmidae) was deleted from the dataset as it is a close sister species of *S. meridies* (see Schubart and Koller, 2005) and does not contribute to the phylogenetic diversity, whereas *Sesarmoides longipes* (Sesarmidae) was removed, as it is a very basal sesarmid that often clusters weakly (see Schubart *et al.,* 2002) and will be dealt with separately. Instead, the type species of the family, *Sesarma reticulatum*, was added, as well as the Asian sesarmid representative *Chiromantes haematocheir.*
(3) *Hemigrapsus oregonensis* (Varunidae) was removed from the dataset, as it is not a typical representative of the genus, and will probably be placed in a separate genus after revision.

In addition to these changes, we noticed that GenBank no. AB002125 (Wetzer *et al.,* 2009: table 2) does not correspond to *Scopimera globosa* (De Haan, 1835), but to *S. bitympana* (Dotillidae). We used the latter in our analyses. Taxon selection for the enlarged dataset was also tested with species belonging to heterotreme families, but in all preliminary analyses the crypto-

chirids consistently nested in the Thoracotremata, similar to the results of Wetzer *et al.* (2009). Furthermore, several potential outgroups were tested.

Collecting

The gall crabs, with the exception of *Cryptochirus coralliodytes*, were collected in Indonesia (Raja Ampat, Papua; Ternate, Halmahera) and Malaysia (Semporna, E Sabah) by the first author from 2007 to 2010. Corals were searched for galls and pits, and subsequently split with hammer and chisel. The gall crabs were preserved in 80% ethanol, after being photographed with a digital SLR camera equipped with a 50 mm macrolens. The material is deposited in the collections of Naturalis in Leiden, The Netherlands (formerly Rijksmuseum van Natuurlijke Historie, collection coded as RMNH.Crus.D). The specimen of *C. coralliodytes* (made available by Dr Danièle Guinot) was collected in New Caledonia, more material of the same series is in the collections of the Muséum national d'Histoire naturelle (Paris).

Analyses

DNA was isolated from muscle tissue of the fifth pereiopod, using the Qiagen DNeasy® Kit according to the manufacturer's protocol for animal tissue. Maceration took place overnight for ~18 h. The final elution step was performed with 100 mL elution buffer. PCR was carried out with standard conditions (2.5 mL PCR buffer, 0.5 mL DNTPs, 1.0 mL of primers 16L2 and 16H10 (Schubart, 2009), 0.3 mL Taq, 18.7 mL MilliQ and 1.0 mL DNA template). Thermal cycling was performed as follows: initial denaturation at 95ºC for five minutes, followed by 39 cycles of 95ºC for five seconds, 47ºC for one minute, and 72ºC for one minute and finalised by 10 min at 72ºC. Sequences were assembled and edited in Sequencer 4.10.1.

The alignment was constructed with Clustal X (Larkin *et al.,* 2007) and minimally modified by hand. It includes 82 sequences consisting of 589 basepairs, of which 374 are variable and 319 are parsimony informative. A model selection analysis was carried out to select the best-fit model based on the Akaike Information Criterion (AIC) in jModelTest 2.1.1 (Darriba *et al.,* 2012), which rendered TrN+I+G as the best model. A Bayesian phylogeny was estimated with MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003) using the next most complex GTR+I+G model. Four Markov-Monte-Carlo chains were run for 3 000 000 generations with a sample tree saved every 1000 generations (outgroup *Palicus caronii*). The split frequency of the likelihood scores was 0.01042. The burnin was set to discard the first 25% of the sampled trees. The consensus tree, constructed using the 'sumt' option in MrBayes, was visualised using FigTree 1.3.1. (Rambaut, 2009).

Results

The topology of Fig. 2 is derived from the Bayesian inference 50% majority rule consensus of the trees remaining after the burnin, with high support values in the basal part as well as in the distal phylogenetic branches. The outgroup is separated by a long branch, whereas the freshwater crabs from four families form a sister clade to the highly supported monophyletic Thoracotremata. Within the Thoracotremata, four major clades can be distinguished. The cryptochirid taxa included in the analyses form a monophyletic clade with a long branch length compared to the other clades. Within this highly supported clade, *Utinomiella dimorpha*, *Pseudocryptochirus viridis* and *Opecarcinus lobifrons* hold a basal position with respect to the remaining gall crabs. Our specimen of *H. marsupialis* differs from the specimens used in Wetzer *et al.* (2009) by 15-17 basepairs (bp) out of 533 bp. Nevertheless, *Hapalocarcinus marsupialis* is for

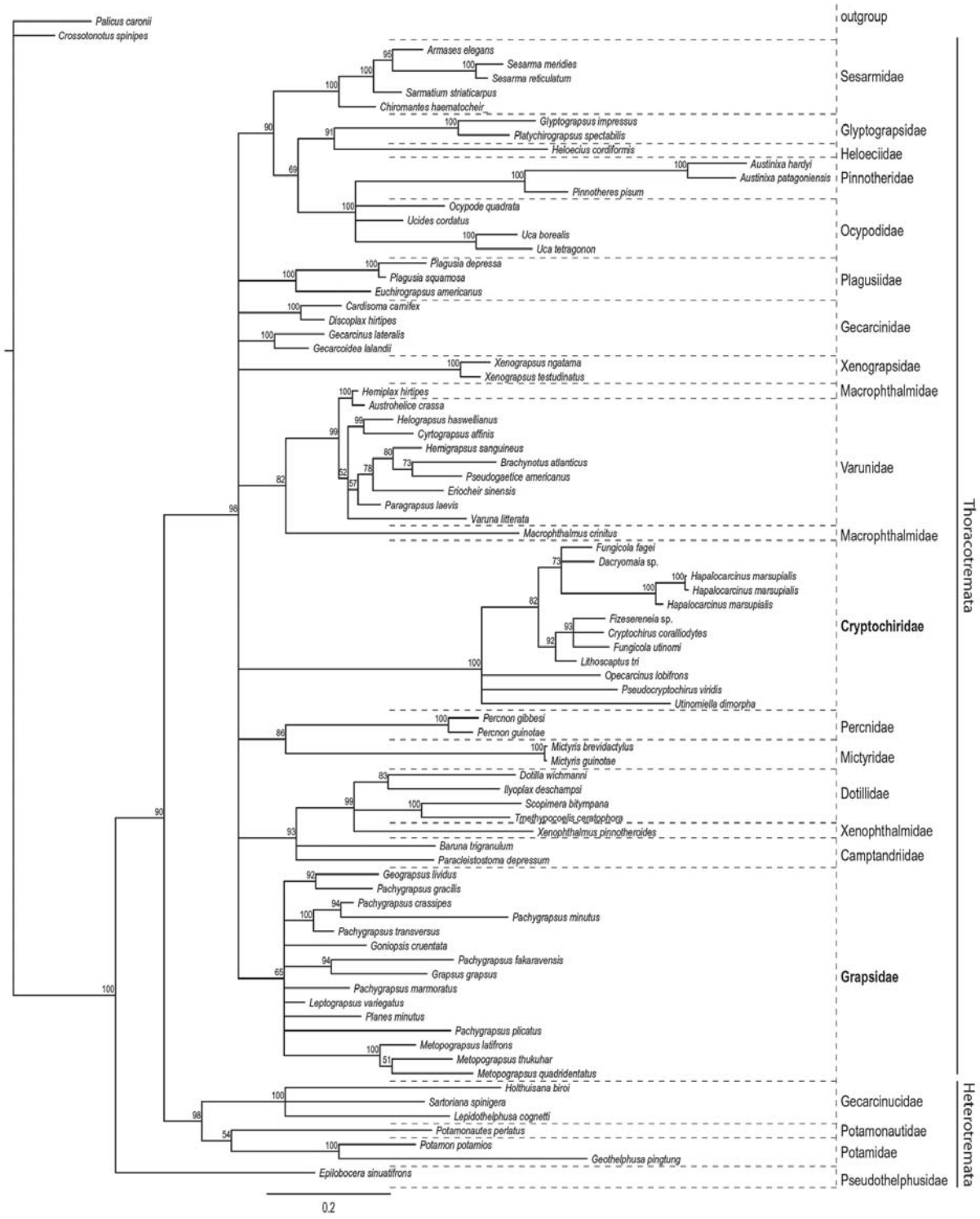

Fig. 2. Phylogenetic placement of the Cryptochiridae within the Thoracotremata, based on 16S mtDNA sequences of 82 taxa (including outgroups). This tree is rooted with *Palicus caronii*. Topology derived from Bayesian inference 50% majority rule, significance values are posterior probabilities.

now regarded a single species, but may well be a complex of species (see also Castro, 2011).

A second clade contains Glyptograpsidae, Heloeciidae, Pinnotheridae, Ocypodidae and Sesarmidae. Ocypodidae and Pinnotheridae together form a paraphyletic clade. The single representative of the Heloeciidae appears as a sister group of the Glyptograpsidae. All Sesarmidae taxa

form a monophyletic clade. A third clade is formed by the Macrophthalmidae and Varunidae. The Macrophthalmidae are polyphyletic, while the Varunidae are paraphyletic because of non-reciprocal monophyly (overlapping taxa) between these two families. Lastly, Grapsidae form the fourth monophyletic clade. The genus *Pachygrapsus* is paraphyletic, and the genus *Metopograpsus* clusters basally compared to the other grapsids. In addition to these major clades, several monophyletic families can be discerned based on our taxon sampling: the Mictyridae, Percnidae, Plagusiidae and Xenograpsidae. The Xenophthalmidae (represented by only one species) are included in the Dotillidae, which is a sister group of the Camptandriidae. The Gecarcinidae do not cluster together.

Discussion

The present molecular phylogeny, including 16S mtDNA of ten cryptochirid species belonging to nine genera, showed that Cryptochiridae form a highly supported monophyletic clade within the Thoracotremata with an unquestionable posterior probability of 100%. Within the Cryptochiridae, representatives of *Utinomiella*, *Pseudocryptochirus* and *Opecarcinus* cluster basally to the other included genera. These remaining genera form one clade, with three possible subclades. *Hapalocarcinus* clusters weakly with *Fungicola fagei* and *Dacryomaia* sp., but with a long branch. Our results are largely in agreement with Van der Meij and Reijnen (2014), who, based on 16S and COI mtDNA, retrieved *Utinomiella* as the basal genus to all other cryptochirids. They also found *Pseudocryptochirus* forming a well supported clade with *Neotroglocarcinus*, and *Opecarcinus* forming a highly supported clade with *Pseudohapalocarcinus*. In their study, the remaining six genera (seven species) formed a fourth clade, with *Hapalocarcinus* weakly clustering as a sister clade. The position of *Hapalocarcinus* within the Cryptochiridae therefore remains unclear to some degree.

According to our phylogeny, gall crabs should not be considered 'highly modified Grapsidae' (see Wetzer *et al.,* 2009), but an independent lineage deserving its current family rank. The conclusion that gall crabs are highly modified grapsids was based on low bootstrap (53%) and posterior probability (58%) values supporting the inclusion of *H. marsupialis* in the Grapsidae. Here we show that the conclusions of Wetzer *et al.* (2009) would have been different if there was better cryptochirid sampling. This may also be the case in the recent study by Tsang *et al.* (2014), where again only one cryptochirid taxon was used for a multi-gene phylogenetic analysis. In this case, *Dacryomaia* sp. is found in an unsupported sister taxon relationship with the family Xenograpsidae. It shows that conclusions on the phylogenetic position of (non-monotypic) families or other higher taxa, may be premature if based on a single species, especially when representatives are chosen that are not the type species of a genus, and when no information is available on the monophyly of the respective taxa.

Our results, and the ones by Tsang *et al.* (2014), do confirm the conclusion by Wetzer *et al.* (2009) that the Cryptochiridae belong to the Thoracotremata. In our analysis cryptochirids are consistently nested with thoracotreme crabs, when different heterotreme species were added to the dataset or used as outgroups. Yet, no clear affinities with a particular thoracotreme family could be identified. Thoracotreme crabs inhabit a wide diversity of habitats. Paulay and Starmer (2011) postulated that Thoracotremata evolved in 'safe places', such as intertidal, non-marine, deep water and endo-symbiotic habitats. Several thoracotreme families consist mainly of intertidal or shore crabs (e.g. Grapsidae, Sesarmidae, some Varunidae) occurring in different habitats, with some of them being specialised mangrove and mudflat dwellers (Camptandriidae, most Sesarmidae and Ocypodidae, with the exception of *Ocypode*, which specialises on sandy shores)

or freshwater-dependent crabs (Glyptograpsidae and some Varunidae) (Schubart *et al.,* 2002). Xenograpsidae with the genus *Xenograpsus* are specialised on hydrothermal vents (Ng *et al.*, 2007) and many Sesarmidae and Gecarcinidae have invaded repeatedly terrestrial and/or freshwater habitats (Schubart *et al.,* 2000). Only the Pinnotheridae have a similar lifestyle to the Cryptochiridae, by living in a permanent symbiosis with bivalves and ascidians (Becker *et al.,* 2011). Survival and diversification of thoracotreme crabs might therefore be related to their adaptability to new environments (Paulay and Starmer, 2011).

The branch support at the family/genus level is high for most clades. One of the largest clades is formed by the Glyptograpsidae, Heloeciidae, Ocypodidae, Pinnotheridae and Sesarmidae. A possible phylogenetic relationship between the Glyptograpsidae and Sesarmidae (see Schubart *et al.,* 2000; Wetzer *et al.,* 2009) or Glyptograpsidae and Ocypodidae (see Schubart and Cuesta, 2010) had previously been proposed based on the same gene (in addition to histone H3 in Schubart and Cuesta, 2010). However, a close affinity between these families was not confirmed by the study of Palacios-Theil *et al.* (2009). There is ongoing debate about the phylogenetic affinities of the genus *Ucides* (e.g. Ng *et al.,* 2008; Schubart and Cuesta, 2010). In our analyses, the relationship of *U. cordatus* with regards to the ocypodid genera *Ocypode* and *Uca* and the Pinnotheridae is not resolved. A study on the morphology of the female reproductive system shows that the overall anatomy of *U. cordatus* is similar to other ocypodids (Castilho-Westphal *et al.,* 2013). For now, we therefore continue to recognise *Ucides* as a genus within the Ocypodidae (see also Schubart and Cuesta, 2010) and not in its own family as suggested by Ng *et al.* (2008).

The Grapsidae form a monophyletic family. The separate clustering of the genus *Metopograpsus* within the Grapsidae has been shown before (e.g. Kitaura *et al.,* 2002; Wetzer *et al.,* 2009). In Schubart *et al.* (2006) and Schubart (2011), *Metopograpsus* holds a basal position within the Grapsidae in analyses carried out with the same molecular marker. The genus *Pachygrapsus* appears to be polyphyletic in this study, confirming results from Schubart (2011).

Kitaura *et al.* (2002) and Schubart *et al.* (2006) proposed that the Macrophthalmidae and Varunidae are sister groups, but with low confidence values. Our phylogeny shows a closer relationship between selected Macrophthalmidae and Varunidae, with high support levels. The species *Hemiplax hirtipes* clusters with the Varunidae (see also Kitaura *et al.,* 2010; McLay *et al.*, 2010). If *H. hirtipes* would be included in the Varunidae, then this family could again be considered monophyletic (see previous work by Schubart *et al.,* 2002), based on the included taxa. The Mictyridae appears related to the Percnidae (but with very long branches), which is a new and unexpected hypothesis considering the large phylogenetic distance between these two families in the trees of Schubart *et al.* (2006) and Wetzer *et al.* (2009). In their study on the Plagusiidae and Percnidae, Schubart and Cuesta (2010) did not include species belonging to the Mictyridae; there the genus *Percnon* holds a basal position to other thoracotreme families. In our tree, the Thoracotremata form a polytomy and thus no basal lineage can be postulated.

In Wetzer *et al.* (2009), the Camptandriidae are polyphyletic: *Paracleistostoma depressum* clusters as a sister group to the Mictyridae and the Pinnotheridae, whereas *Baruna triganulum* clusters with the Dotillidae. In our results both species form a clade with the Dotillidae. The species *Xenophthalmus pinnotheroides* stands together with the Dotillidae. Based on molecular data and larval morphology, Palacios-Theil *et al.* (2009) also suggest a close relationship of *Xenophthalmus pinnotheroides* with the family Dotillidae. Ng *et al.* (2008) already discussed the strange position of the Xenophthalmidae and found that it resembles the Dotillidae, but some characters argue against lumping them into the family. Hence they followed Serène and Umali (1972), and treated it as a good family. As the Xenophthalmidae and the Heloeciidae are represented by single

species in this study, no overall conclusions about their position in the Thoracotremata should be drawn.

Overall, several phylogenetic relationships (Heloeciidae–Glyptograpsidae, Varunidae–Macrophthalmidae, Pinnotheridae–Ocypodidae) argue against the classical and current (Ng *et al.,* 2008) superfamily concept within the Thoracotremata. Therefore, Schubart *et al.* (2006) suggested to refrain from this superfamily concept and treat the constituent families separately until a clearer picture of phylogenetic relationships within the Thoracotremata has been reached. The unsuitability of the current superfamilies has been re-confirmed by Schubart and Cuesta (2010) and Tsang *et al.* (2014). Here again we argue against it and would hence propose to refrain from using the superfamily Cryptochiroidea (see Ng *et al.*, 2008), until the evolutionary origin of Cryptochiridae (and taxonomic classification reflecting it) is better understood. In summary, the Cryptochiridae is a highly enigmatic family, for which the closest relatives so far remain unknown. The present study is based on a single gene fragment, and additional support needs to be obtained from independent molecular markers. Further studies on the evolution of Cryptochiridae within the Thoracotremata should for that reason be based on multiple markers, to obtain more insight in their unusual biology and life history.

Acknowledgements

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Chapter 2

A new species of *Opecarcinus* Kropp and Manning, 1987 (Crustacea: Brachyura: Cryptochiridae) associated with the stony corals *Pavona clavus* (Dana, 1846) and *P. bipartita* Nemenzo, 1980 (Scleractinia: Agariciidae)

Sancia E.T. van der Meij

Abstract

A new species of *Opecarcinus* Kropp and Manning, 1987, is described from Indonesia and Malaysia. Opecarcinus cathyae sp. nov. is associated with the scleractinian corals *Pavona clavus* (Dana, 1846) and *P. bipartita* Nemenzo, 1980, inhabiting crescent-shaped cavities or tunnels on the coral surface. The new species is the ninth assigned to the genus. It can be separated from congeners by the anterolateral orientation of the cornea, the carapace with shallow transverse depressions, lacking longitudinal depressions, and the smooth dorsal margin of the fifth female pereiopod carpus. The distinctive colour pattern can be used as a diagnostic character in live specimens.

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Introduction

Colonies of the scleractinian coral *Pavona clavus* (Dana, 1846), belonging to the Agariciidae, can occur in huge monospecific stands, covering large areas of reef flats and slopes (Veron and Pichon, 1980). Gall crabs belonging to the genus *Opecarcinus* Kropp and Manning, 1987, have been found to inhabit these large colonies in high densities (Hoeksema and van der Meij, 2013) and eight species are now recognised in the genus (cf. Ng *et al.*, 2008). *Opecarcinus* was established by Kropp and Manning (1987) to accommodate the Atlantic *Pseudocryptochirus hypostegus* Shaw and Hopkins, 1977, and *Cryptochirus crescentus* Edmonson, 1925, from the Pacific. An additional five species of *Opecarcinus* were described by Kropp (1989), who also removed *O. granulatus* from the synonymy of *O. crescentus*.

The Indo-Pacific species of *Opecarcinus* occur from the Red Sea to the Pacific coast of Central America (Kropp, 1989; pers. obs.), and have been recorded from corals belonging to several genera of the scleractinian family Agariciidae (Kropp, 1989). In the western Atlantic, Scott (1985, 1987) and Johnsson *et al.* (2006) recorded *O. hypostegus* from the genera *Agaricia* (family Agariciidae) and *Siderastrea* (family Siderastreidae), in contrast to Kropp and Manning (1987) and Van der Meij (2014a) who recorded *O. hypostegus* only from *Agaricia*.

Based on the observations by Hoeksema and van der Meij (2013), gall crabs collected from the Indo-Pacific agariciid *P. clavus* were studied in more detail, resulting in the identification of the present new species. This species, described herein, is the ninth assigned to the genus.

Material and methods

Gall crabs were collected in eastern Indonesia (Lembeh Strait, northern Sulawesi; Gura Ici, Halmahera) and Malaysian Borneo (Kudat, north Sabah; Semporna, east Sabah) from 2009 to 2012. Corals were searched for galls, cavities and pits, photographed, and subsequently split with hammer and chisel. Crab specimens were preserved in 80% ethanol after being photographed with a digital SLR camera equipped with a 50 mm macro-lens. All material is deposited in the collections of Naturalis Biodiversity Center in Leiden (formerly Rijksmuseum van Natuurlijke Historie, collection coded as RMNH.Crus.D). The identification of host corals was based on Veron and Pichon (1980) and Veron (2000). Drawings were made with a stereo microscope with camera lucida. Carapace lengths and widths were measured to the nearest 0.1 mm using an eyepiece micrometre, with the crabs positioned on a level surface.

Abbreviations used: CL, carapace length; CW, carapace width (at widest point); MXP, maxilliped; ovig., ovigerous; P, pereiopod; G1, male gonopod 1; G2, male gonopod 2. Carapace measurements are given as CL × CW, in mm.

Taxonomy

Family Cryptochiridae Paul'son, 1875
Opecarcinus Kropp and Manning, 1987

Opecarcinus cathyae sp. nov.
Figs 1-5

Type locality. Creach Reef, Semporna district, Sabah, Malaysia (04°18'58.8"N, 118°36'17.3"E).

Type material. Holotype (female) and allotype (male). RMNH.Crus.D.53648a, 10-14 m, host

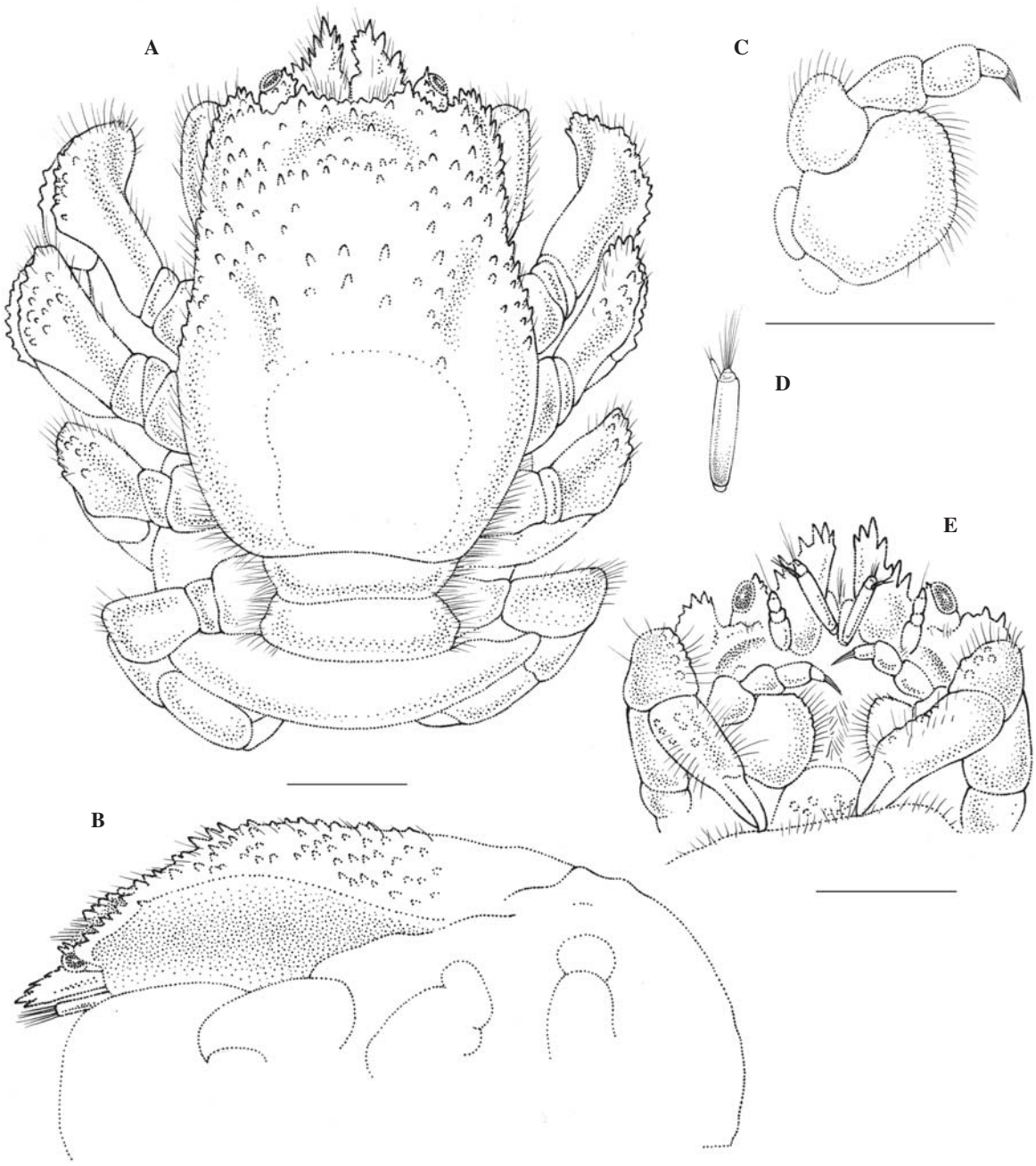

Fig. 1. A-E. Holotype of *Opecarcinus cathyae* sp. nov. (RMNH.Crus.D.53648a). **A**, habitus, dorsal view; **B**, carapace, lateral view; **C**, MXP3 (exopod hardly visible); **D**, close-up of antennules; **E**, anterolateral margin of carapace, ventral view. Scale bars = 1.0 mm.

Pavona clavus (Dana, 1846), 05.xii.2010, ovig. female (5.5 × 3.8), male (3.3 × 2.6), leg. Z Waheed. Paratypes. RMNH.Crus.D.53648b, from the same lot as holotype and allotype, 1 ovig. female (3.7 × 3.0), 1 juvenile male (1.6 × 1.1). A damaged male from this lot was used for DNA barcoding.

DNA barcoding. A COI sequence (partially, Folmer *et al.,* 1994) of one of the paratypes (damaged male) has been deposited in GenBank under accession number KM396420.

Additional material. ***Indonesia***. RMNH.Crus.D.53923, S Lela, Gura Ici, Halmahera (00°01'51.2"S 127°15'03.1"E), 10.xi.2009, 3 males, one with epicaridean parasite (*Carcinione platypleura* Bourdon, 1983) under carapace, host *Pavona clavus*, leg. SET van der Meij. RMNH. Crus.D.53916, 3 ovig. females, 1 male, host *Pavona clavus*, leg. SET van der Meij (same lot as

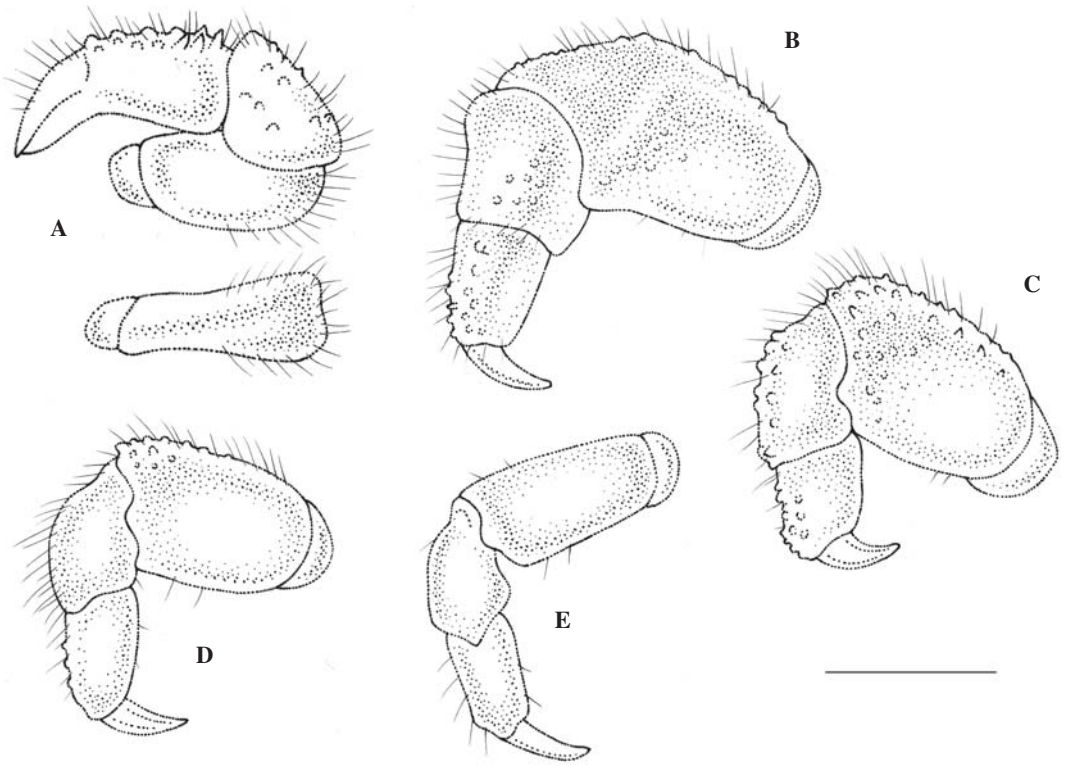

Fig. 2. A-E. Holotype of *Opecarcinus cathyae* sp. nov. (RMNH.Crus.D.53648a). **A**, right P1 (cheliped), merus drawn twice because of angle distortion; **B**, right P2; **C**, right P3; **D**, right P4; **E**, right P5. Scale bar = 1.0 mm.

RMNH.Crus.D.53923); RMNH.Crus.D.54202, Baturiri, Lembeh Strait (01°27'34.7"N 125°14' 23.1"E), 10 m, 6.ii.2012, 1 male, host *Pavona bipartita*, leg. SET van der Meij. RMNH.Crus. D.54214, Teluk Walemetodo, Lembeh Strait (01°24'11.3"N 125°10'20.3"E), 6 m, 15.ii.2012, 1 ovig. female, 1 male, host *Pavona bipartita*, leg. SET van der Meij. ***Malaysia (Borneo)***. RMNH. Crus.D.53656, Mataking I., Semporna district (04°34'57.6"N 118°56'46.5"E), 8.xii.2010, 1 ovig. female, 1 non-ovig. female, host *Pavona clavus*, leg. BW Hoeksema. RMNH.Crus.D.53768, Hanging Gardens, Sipadan I., Semporna district (04°06'45.3"N 118°37'29.3"E), 18.xii.2010, 2 ovig. females, host *Pavona clavus*, leg. Z Waheed. RMNH.Crus.D.54297, SW Mangsee Great Reef, Kudat (07°27'24.8"N 117°13'21.6"E), 9 m, 22.ix.2012, 1 ovig. female, 1 male, host *Pavona clavus*, leg. SET van der Meij. RMNH.Crus.D. 54275, Paliuk, Kudat (07°03'17.4"N 117°22' 32.6"E), 10.ix.2012, 2 ovig. females, 2 non-ovig. females, 2 males, host *Pavona clavus*, leg. SET van der Meij.

Description female holotype. Carapace vase-shaped, CL 1.4 CW; widest posterior to mid-length; anterior third of carapace deflected by about 40°, not sharply set off from posterior carapace, with shallow transverse depression across protogastric region; dorsal surface convex in lateral view, median third concave with scattered small conical tubercles. Mesogastric region slightly inflated with tubercles, cardiointestinal region outlined. Carapace surface ornamented with rounded, conical tubercles; posterior carapace smooth, tubercles most numerous at anterior, lateral carapace; anterolateral margins of carapace granular; anterolateral angle without prominent tubercle; margin inner orbital angle with tubercle. Front slightly concave with small tubercles, width about half of carapace at anterolateral angle. Orbit broadly V-shaped. Pterygostomial region fused to carapace (Fig. 1A-B). Brood pouch swollen (ovigerous), many short setae on distal margin (ventral view) (Fig. 1E). Posterior carapace, brood pouch margins fringed with many setae (Fig. 1A, E).

Antennular peduncle dorsal surface with small tubercles, slightly inflated distally, scarcely

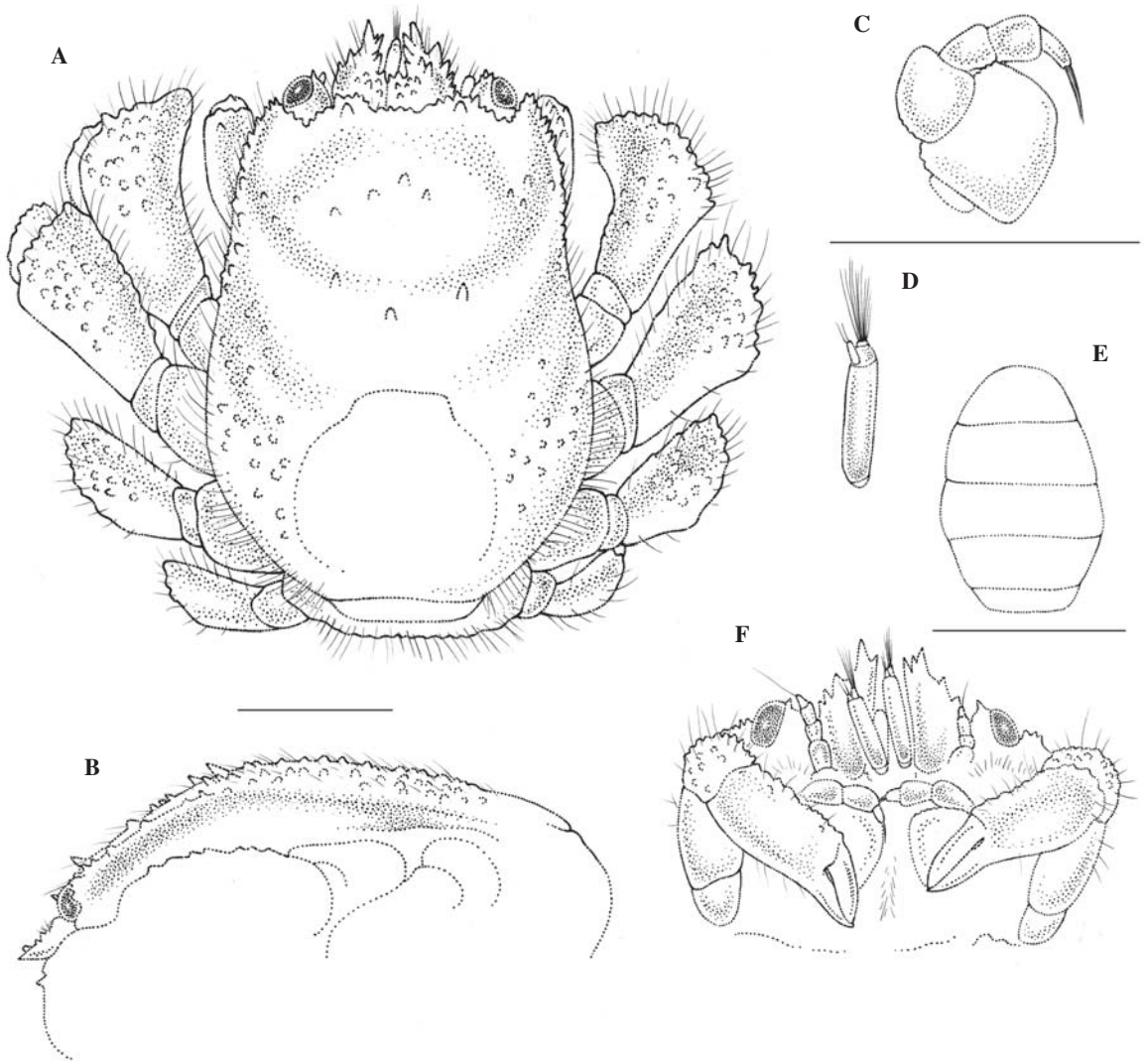

Fig. 3. A-F. Allotype of *Opecarcinus cathyae* sp. nov. (RMNH.Crus.D.53648a). **A**, habitus, dorsal view; **B**, carapace, lateral view; **C**, MXP3 (exopod hardly visible); **D**, close-up of antennules; **E**, abdomen; **F**, anterolateral margin of carapace, ventral view. Scale bars = 1.0 mm.

inflated mesially; apex of distal projection slightly extending beyond tip of eyestalk; spines on distal margin larger than those on mesial margin. Basal segment strongly tapering anteriorly in ventral view, length 1.5 times width; ventral surface relatively smooth (Fig. 1E).

Eyestalk partly exposed dorsally, slightly granular. Cornea anterolateral. Lateral margin of stalk not extending beyond anterolateral angle; distal margin with small spines (Fig. 1A, E). Distal segment of antennules with protruding segment, visible from ventral side (Fig. 1D-E).

MXP3 with exopod; mesial margin of ischium slightly crenulated; merus with distolateral projection, carpus to dactylus decreasing in size, latter with bundle of setae (Fig. 1C).

P1 (chelipeds) slender; merus length 2.8 times height; carpus granular on dorsal margin; propodus with stronger granulation on dorsal margin than carpus; cutting edge fingers entire, tips of fingers slightly crossing when closed (Fig. 2A).

P2 stout; merus length 1.8 times height, dorsal margin evenly convex, entire length crenulated, ventral margin straight, smooth; carpus, propodus of similar length with rows of conical tubercles; dactylus smooth, sharp, curved ventrally (Fig. 2B).

P3 stout; merus length 1.6 times height, dorsal margin slightly convex, entire length with

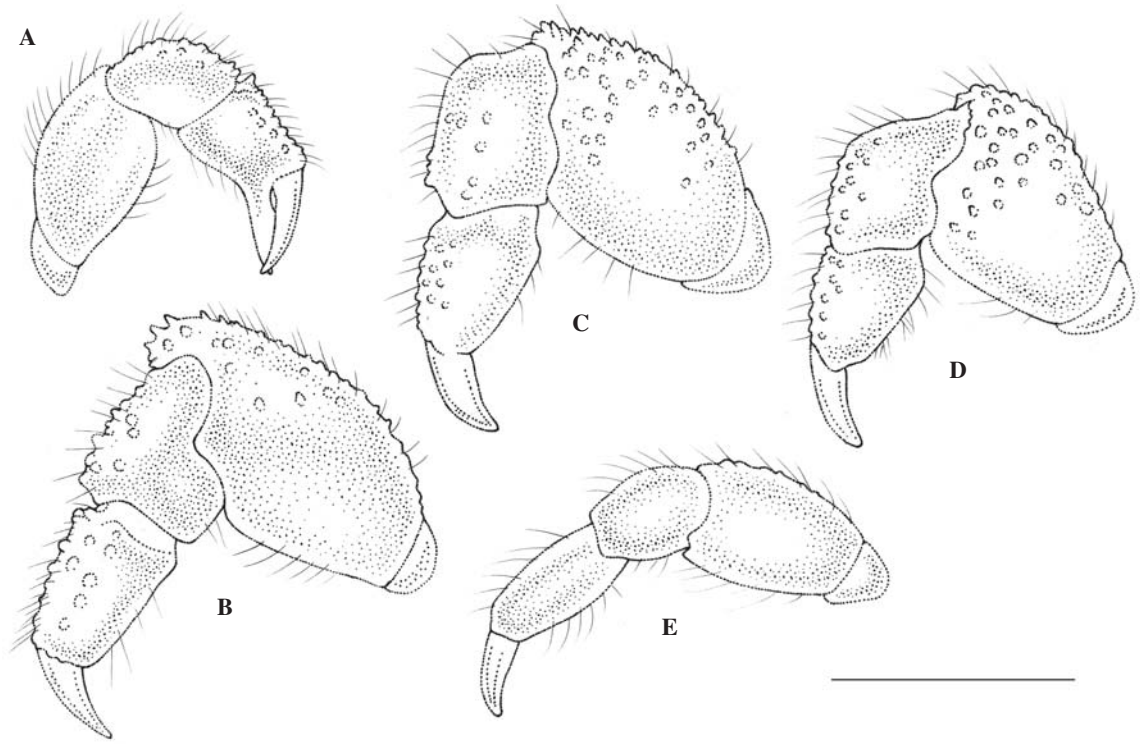

Fig. 4. A-E. Allotype of *Opecarcinus cathyae* sp. nov. (RMNH.Crus.D.53648a). **A**, right P1 (cheliped) - drawn from ventral side; **B**, right P2; **C**, right P3; **D**, right P4; **E**, right P5. Scale bar = 1.0 mm.

scattered conical tubercles, ventral margin straight, smooth; carpus, propodus of similar length with conical tubercles on dorsal margin; carpus with small anterior lobe; dactylus smooth, sharp, curved ventrally (Fig. 2C).

P4 relatively slender; merus length 1.4 times height, entire length dorsal margin with scattered conical tubercles, ventral margin straight, smooth; carpus, propodus of similar length; carpus with slight anterior lobe; propodus with conical tubercles on dorsal margin; dactylus smooth, sharp, curved ventrally (Fig. 2D).

P5 slender; merus length 2.0 times height, straight, smooth margins; carpus, propodus of similar length, margins smooth; dactylus smooth, sharp, curved ventrally (Fig. 2E).

Thoracic sternum 1-3 with transverse row of rounded tubercles at midlength, thoracic sternum 4 with fewer tubercles (Fig. 5B).

Gonopore (vulva); elliptical, lateral margin with small vulvar cover (examined in paratype).

Description male allotype. Generally similar to holotype, differences outlined hereafter. Carapace vase-shaped, CL 1.3 longer than CW; median third concave with few scattered small conical tubercles. Carapace surface ornamented with few rounded to conical tubercles, fewer than holotype, most numerous at lateral margins; anterolateral margins of carapace with row of small conical tubercles; anterolateral angle without prominent tubercle; inner orbital angle marked with tubercle. Orbit broadly V-shaped, margin somewhat crenulated. (Fig. 3A-B). Posterior carapace margins fringed with numerous setae (Fig. 3A).

Antennular peduncle dorsal surface with numerous spiny tubercles, slightly inflated distally, scarcely inflated mesially. Basal segment tapering anteriorly in ventral view, length 2.3-2.4 times width; surface relatively smooth (Fig. 3F).

Eyestalk partly exposed dorsally. Cornea anterolateral. Lateral margin of stalk not extending beyond anterolateral angle; distal margin with two small spines (Fig. 3F). Distal segment of

Fig. 5. A-D. Dorsal and ventral view of *Opecarcinus cathyae* sp. nov. **A**, **B**, RMNH.Crus.D.53916, female with regular colour pattern; **C**, **D**, RMNH.Crus.D.54297, male with pale colour pattern.

antennules with small protruding segment, visible from ventral side (Fig. 3D).

MXP3 with exopod; mesial distal margin of ischium very slightly crenulated; merus with distolateral projection; carpus, propodus dactylus of similar length, dactylus with tuft of setae (Fig. 3C).

P1 (chelipeds) somewhat stout; merus length 1.4 times height; carpus granular on dorsal margin; propodus with stronger granulation on dorsal margin than carpus; cutting edge fingers entire, tips of fingers crossing (Fig. 4A).

P2 stout; merus length 1.8 times height, dorsal margin slightly convex, entire length with tubercles, slightly larger distally, ventral margin straight, smooth (Fig. 4B).

P3 stout; merus length 1.5 times height, dorsal margin evenly convex, entire length with scattered conical tubercles, ventral margin rounded smooth; carpus with anterior lobe (Fig. 4C).

P4 stout; merus length 1.1 times height, dorsal margin slightly convex, entire length with scattered conical tubercles, ventral margin straight, smooth; carpus, propodus of similar length with conical tubercles on dorsal margin; carpus with anterior lobe (Fig. 4D).

P5 slender; merus length 1.3 times height, margins crenulated, ventral margin relatively

straight; carpus slightly shorter than propodus, margins smooth (Fig. 4E).

Thoracic sternum 1-3 with transverse row of rounded tubercles at midlength, thoracic sternum 4 with fewer, somewhat scattered tubercles (Fig. 5D). Abdomen widest at somite 3, somite 6 not visible in ventral view because of curvature; telson rounded (Fig. 3E).

Gonopods; G1: slightly curved laterally, slightly cinched in the middle, apex blunt, distal margin with 6-7 simple, long setae; G2: almost straight, slightly cinched in the middle, apex blunt with two large non-plumose setae at distal margin of the same length as G2.

Variation. The tubercle on the margin of the inner orbital margin is prominent in some individuals only. The setae along the carapace margins are more numerous in large individuals, especially in females.

Colour. Carapace bright orange-red to rust, darker rust on the lateral sides. Cardio-intestinal region outlined by a lighter colouration, off-white in some specimens. Anterolateral region off-white, sometimes with tubercles of contrasting (dark) colour. MXP ischium, merus off-with with orange hue, carpus, propodus, dactylus rust-coloured. P1 to P5 opaque with fine orange network of lines, giving an orange hue. Cornea bright rust colour (Fig. 5B, C). Some specimens are quite pale, and lack the intense orange-red colouration. These specimens do have the cardio-intestinal region outlined by a lighter colouration and have black chromatophores visible on the carapace, predominantly on the lateral margins (Fig. 5C).

Remarks. The orientation of the cornea on the eyestalk was used by Kropp (1989) to separate the species of *Opecarcinus* into two groups. *Opecarcinus cathyae* sp. nov. has anterolaterally oriented corneas, which places it in the same group as *O. hypostegus*, *O. granulatus* (Shen, 1936) and *O. pholeter* Kropp, 1989. The five remaining species of *Opecarcinus* have terminally oriented corneas. In *Opecarcinus hypostegus*, an Atlantic species, and *O. granulatus* the anterior third of the carapace is sharply set off from the posterior carapace and the transverse depression confined to the protogastric region. In *O. cathyae* sp. nov. and *O. pholeter* the anterior third is not sharply set off from the posterior carapace and the transverse depression is shallow. The new species can, furthermore, be separated from *O. granulatus* by the smooth dorsal margin of the P5 carpus in females, and from *O. pholeter* by the smooth surface of MXP3 and the lack of depressions on the carapace. *Opecarcinus cathyae* sp. nov. can also be separated from its Indo-West Pacific congeners in this species group by its colour pattern: *O. granulatus* is opaque with black chromatophores and *O. pholeter* has nine amber- coloured bands (Kropp, 1989), whereas *O. cathyae* sp. nov. is orange-red (rust) overall, with an off-white anterolateral region.

Coral hosts. The new species appears to be strictly associated with the *Pavona clavus* and *P. bipartita*, sister species that form a rather distinct lineage within the Agariciidae (F. Benzoni, pers. comm.). In his overview of the Pacific *Opecarcinus* species, Kropp (1989) does not mention *P. clavus* and *P. bipartita* as hosts, hence *O. cathyae* sp. nov. is the first species described in association with these corals. A figure of the dwelling of *O. cathyae* sp. nov. in *P. clavus* was provided by Hoeksema and van der Meij (2013: Fig. 1b, c). In *P. bipartita* the new species lives in tunnels on the coral surface. According to Kropp (1989) host specificity has been observed for *O. aurantius* Kropp, 1989 (host *Pavona minuta* Wells, 1954), *O. peliops* Kropp, 1989 (host *P. duerdeni* Vaughan, 1907), and *O. lobifrons* Kropp, 1989 (host *Gardineroseris planulata* (Dana, 1846)). *Opecarcinus cathyae* sp. nov. also seems to be host-specific by inhabiting two closely related species: *P. clavus* and *P. bipartita*.

Ecology. The carapace and pereiopods are fringed with numerous setae (Fig. 1A, E; Fig. 2A-E), which, in case covered with trapped sediment, can give the crab a mucky appearance.

Distribution. So far known from Indonesia and Malaysian Borneo. The holotype of *P. clavus*, illustrated by Veron and Pichon (1980), appears to have a dwelling of a cryptochirid. This

coral species was described by Dana (1846) from Fiji, which is therefore a possible distribution record for *O. cathyae* sp. nov. *Pavona clavus* is widespread, occurring from the Red Sea and East Africa to the eastern Pacific (Veron and Pichon, 1980; Veron, 2000). *Pavona bipartita* also shows a wide range, occurring from the Red Sea and East Africa to the Central Pacific (Veron, 2000). It is thus possible that *O. cathyae* has a wider distribution based on the distribution ranges of its host corals. *Opecarcinus cathyae* sp. nov. can be very abundant locally, with estimated densities up to 200 per m^{-2} because its coral host can form large monospecific stands (Veron and Pichon, 1980; Hoeksema and van der Meij, 2013).

Etymology. This species is named after Cathy [Catherine] DeGeorge to celebrate 15 years of Trans-Atlantic friendship.

Acknowledgements

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Chapter 3

A new species of *Fizesereneia* Takeda and Tamura, 1980 (Crustacea: Brachyura: Cryptochiridae) from the Red Sea and Oman

Sancia E.T. van der Meij, Michael L. Berumen & Gustav Paulay

Abstract

A new species of cryptochirid crab, *Fizesereneia panda* van der Meij, is described and illustrated based on specimens collected from the scleractinian corals *Lobophyllia* cf. *hemprichii* and *L.* cf. *corymbosa* from the Farasan Banks, Farasan Islands, and the reefs off Thuwal in the Saudi Arabian Red Sea, and from *Symphyllia recta* from reefs in the Gulf of Oman. This is the second cryptochirid species with the Red Sea as type locality. It can be separated from its congeners by the subrectangular carapace, raised midline and the complete division of the carapace depressions, and reddish black colour pattern of these concavities in live specimens. This new species is the seventh assigned to *Fizesereneia*. A DNA barcode for the new species has been deposited in GenBank.

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Introduction

Gall crabs (Cryptochiridae) occur on coral reefs worldwide. Cryptochirids are mostly found in tropical reef corals, but several species have been described from deep water corals (e.g. Kropp and Manning, 1996). Most gall crabs have been described from rather few areas where gall crab specialists worked (Guam, Japan, Vietnam), although they have been reported from most regions in the world, including the Pacific coast of Mexico (Hernández *et al.*, 2013), Saint Helena in the Atlantic Ocean (den Hartog, 1989), and northern Borneo (van der Meij and Hoeksema, 2013). Yet, most reefs have not been sampled for gall crabs, resulting in patchy known distribution ranges for most species (Kropp, 1990a).

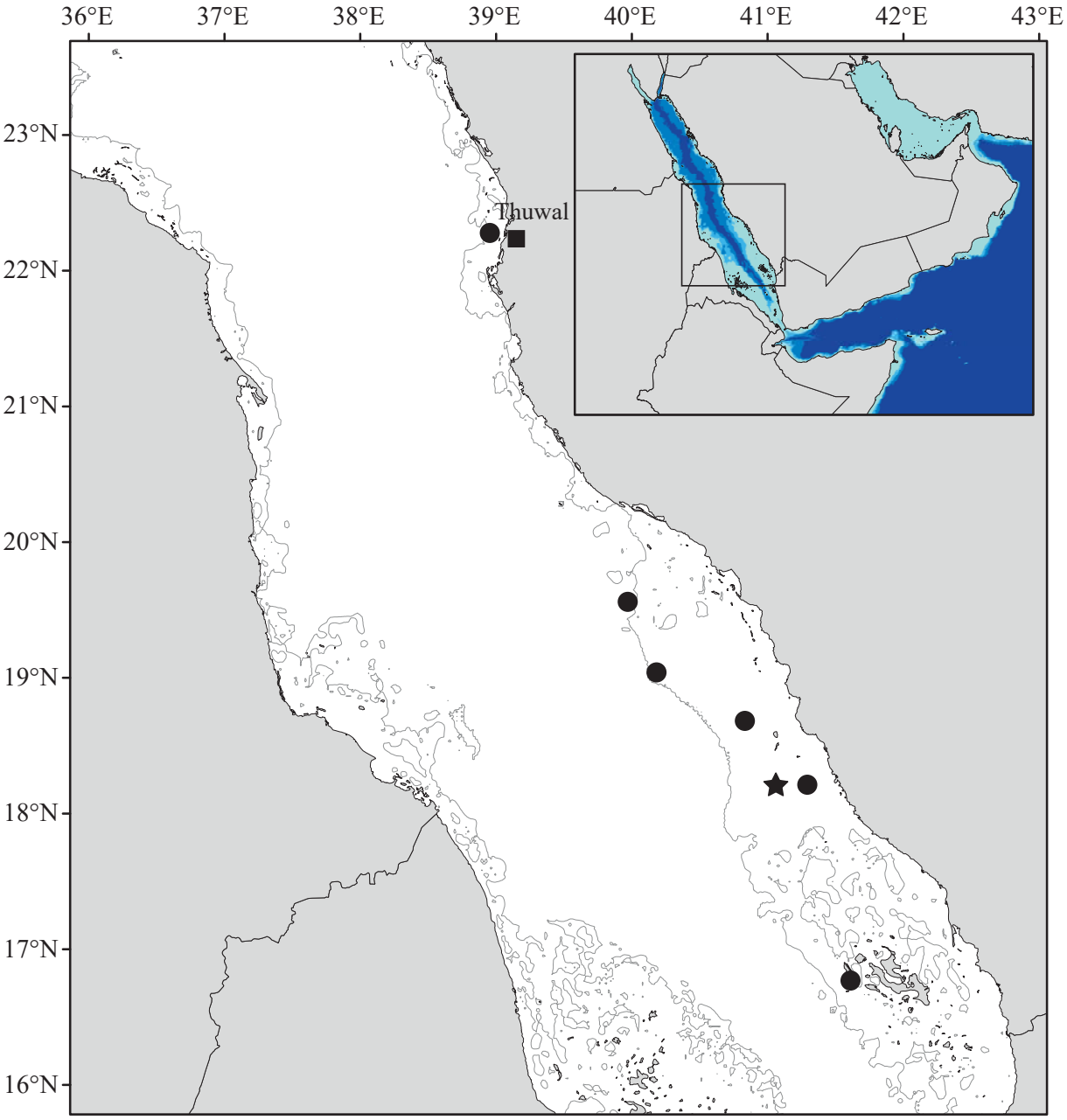

Fig. 1. Map of the collection sites in the Saudi Arabian Red Sea. The star indicates the type locality of *Fizesereneia panda* sp. nov., dots indicate the other Red Sea localities where *F. panda* sp. nov. was collected. One sample was collected in the Gulf of Oman (not on map).

To date, only one gall crab species has been described from the Red Sea: *Cryptochirus coralliodytes* Heller, 1861. Simon-Blecher and Achituv (1997) reported *C. coralliodytes* from the Gulf of Eilat inhabiting the former faviid genera *Favia* Milne Edwards, 1857 [= *Dipsastrea* Blainville, 1830], *Favites* Link, 1807, *Goniastrea* Milne Edwards and Haime, 1848, and *Platygyra* Ehrenberg, 1834. Based on the host specificity of gall crabs, however, it is likely that some of these host corals were inhabited by other gall crab species (Kropp 1990a; van der Meij, unpublished data). Two additional cryptochirid species have been recorded from the Gulf of Eilat: *Hapalocarcinus marsupialis* Stimpson, 1859, from *Stylophora pistillata* Esper, 1797 (Abelson *et al.*, 1991) and *Fungicola fagei* (Fize and Serène, 1956), from *Pleuractis granulosa* (Klunzinger, 1879) (Kramarsky-Winter *et al.*, 1995). The latter record, based on the host coral, should possibly be attributed to *F. syzygia* van der Meij, 2015. The only two species recorded to date from Saudi Arabia are *H. marsupialis*, which was recorded from Lidth [= Al Lith] and Djedda [= Jeddah] (Balls, 1924), and *Neotroglocarcinus dawydoffi* (Fize and Serène, 1956b) (van der Meij and Reijnen, 2014). Outside of the Gulf of Eilat, the Red Sea is a relatively understudied coral reef ecosystem, and non-coral invertebrates are particularly underrepresented in recent coral reef literature from the Red Sea (Berumen *et al.*, 2013).

During a biodiversity research cruise in the Saudi Arabian part of the Red Sea, gall crabs were collected from a wide range of coral hosts. An undescribed species of the genus *Fizesereneia* Takeda and Tamura, 1980 was collected from the scleractinian genus *Lobophyllia* de Blainville, 1830, and described below as *Fizesereneia panda* van der Meij sp. nov. The new species is the seventh assigned to the genus.

Material and methods

Gall crabs were collected in the southern Saudi Arabian Red Sea from Al Lith to Jizan in March 2013, with some additional sampling conducted in Oman in May 2008 and offshore of Thuwal, in the central Saudi Arabian Red Sea, in March 2013 and November 2014 (Fig. 1). Scleractinians corals were searched for galls and pits, photographed, and subsequently split with hammer and chisel. Gall crab specimens were preserved in 80% ethanol after being photographed with a digital SLR camera equipped with macro lens. The material (including holotype) is deposited in the collections of Naturalis Biodiversity Center in Leiden, the Netherlands (formerly Rijksmuseum van Natuurlijke Historie, collection coded as RMNH.Crus.D), paratypes are deposited in the collections of the King Abdullah University of Science and Technology (Thuwal, Saudi Arabia, collection coded as SAI)) and in the Florida Museum of Natural History, University of Florida (Gainesville, USA, collection coded as UF Arthropoda). Host corals were identified following Scheer and Pillai (1983) and Sheppard and Sheppard (1991). Drawings were made with a stereomicroscope with camera lucida. The chelipeds were drawn with the outer surface of the manus parallel to the plane of the paper, which somewhat distorts the other segments. The terms for carapace shape follow Zayasu *et al.* (2013). Carapace lengths (CL) and widths (CW) were measured using an eyepiece micrometre. All descriptions of colour patterns are based on pictures of live specimens.

Abbreviations used: CL, carapace length; CW, carapace width (at widest point); MXP, maxilliped; ovig., ovigerous; P, pereiopod; G1, male gonopod 1. Carapace measurements are given as CL × CW, in mm.

Taxonomy

Family Cryptochiridae Paul'son, 1875
Genus *Fizesereneia* Takeda and Tamura, 1980

Fizesereneia Takeda and Tamura, 1980: 137
Fizeserenia.— Kropp and Manning, 1987: 2 [erroneous spelling]

Type species. *Troglocarcinus heimi* Fize and Serène, 1956, subsequent designation by Kropp (1990b) Type locality. Nha Trang, Vietnam.

Remarks. The genus *Fizesereneia* presently includes six species: *Fizesereneia heimi* (Fize and Serène, 1956), *F. stimpsoni* (Fize and Serène, 1956), *F. ishikawai* Takeda and Tamura, 1980, *F. latisella* Kropp, 1994, *F. tholia* Kropp, 1994, and the recently described *F. daidai* Zayasu, 2013. The location of the holotypes of *Troglocarcinus heimi* and *T. stimpsoni* are unknown according to Kropp (1990a). The holotypes of the other *Fizesereneia* species are available in the collections of the National Museum of Nature and Science, Tokyo (*F. ishikawai*, *F. daidai*), the National Museum of Natural History, Smithsonian Institution, Washington D.C. (*F. latisella*), and the Muséum national d'Histoire naturelle, Paris (*F. tholia*).

Fizesereneia panda van der Meij sp. nov.
Figs 2-6

Type locality. Atlantis Shoal, Farasan Banks, Saudi Arabia (18.1917 N, 41.1138 E)

Coral host of holotype. *Lobophyllia* cf. *hemprichii* (Ehrenberg, 1834)

DNA barcoding. A sequence of the Folmer region of COI of the holotype (partially, Folmer *et al.*, 1994) has been deposited in GenBank under accession number KM491175.

Type material. Holotype: RMNH.Crus.D.54425, 1 ovig. female (4.2 × 3.6) on *Lobophyllia* cf. *hemprichii*, 7.iii.2013, ca. 10 m, leg. SET van der Meij; allotype: RMNH.Crus.D.54424, 1 male (4.2 × 3.2) on *Lobophyllia* cf. *hemprichii*, 7.iii.2013, ca. 10 m, leg. SET van der Meij. Paratypes: King Abdullah University of Science and Technology: SAI-001, Al-Fahal S, off Thuwal (22.2465 N 38.9592 E), 2 m, 9.xi.2014, 1 ovig. female on *Lobophyllia corymbosa* (coll. nr. SA1916), leg. SET van der Meij; UF Arthropoda 40384 (ex RMNH.Crus.D.54465), Marca I, Farasan Banks (18.2206 N 41.3244 E), ca. 10 m, 6.iii.2013, 1 non-ovig. female (4.3 × 3.4) on *Lobophyllia hemprichii*, leg. SET van der Meij. Other material. Saudi Arabia. RMNH.Crus.D.54449, Pelican (Ablo) I., Farasan Banks (18.6595 N 40.8270 E), 5 m, 5.iii.2013, 1 non-ovig. female on *Lobophyllia corymbosa*, leg. SET van der Meij; RMNH.Crus.D.54386, Shi'b Ammar, Farasan Banks (19.5707 N 40.0088 E), 7 m, 3.iii.2013, 1 ovig. female on *Lobophyllia corymbosa*, leg. SET van der Meij; RMNH.Crus.D.54490, Dolphen Lagoon, offshore of Farasan Banks (19.0005 N 40.1481 E), 0-3 m, 4.iii.2013, 2 ovig. female, 1 non-ovig. female on *Lobophyllia corymbosa*, leg. SET van der Meij; RMNH.Crus.D.54390, Marca Isl. II, Farasan Banks (18.2089 N 41.3346 E), 5-10 m, 7.iii.2013, 1 non-ovig. female on *Lobophyllia* cf. *hemprichii*, leg. SET van der Meij; RMNH. Crus.D.54387, Naf Shuma, Farasan Is. (16.7527 N 41.6049 E), 9.iii.2013, 2 ovig. female (1 damaged) on *Lobophyllia* cf. *corymbosa*, leg. SET van der Meij; RMNH.Crus.D.56801, Abu Gishaa, off Thuwal (22.2552 N 39.0235 E), 15 m, 10.xi.2014, 2 ovig. females on *Lobophyllia corymbosa*, leg. SET van der Meij; RMNH.Crus.D.56802, Tahlah, off Thuwal (22.2739 N 39.0503 E), 13 m, 13.xi.2014, 1 ovig. female on *Lobophyllia* cf. *corymbosa*, leg. SET van der Meij. Oman. UF

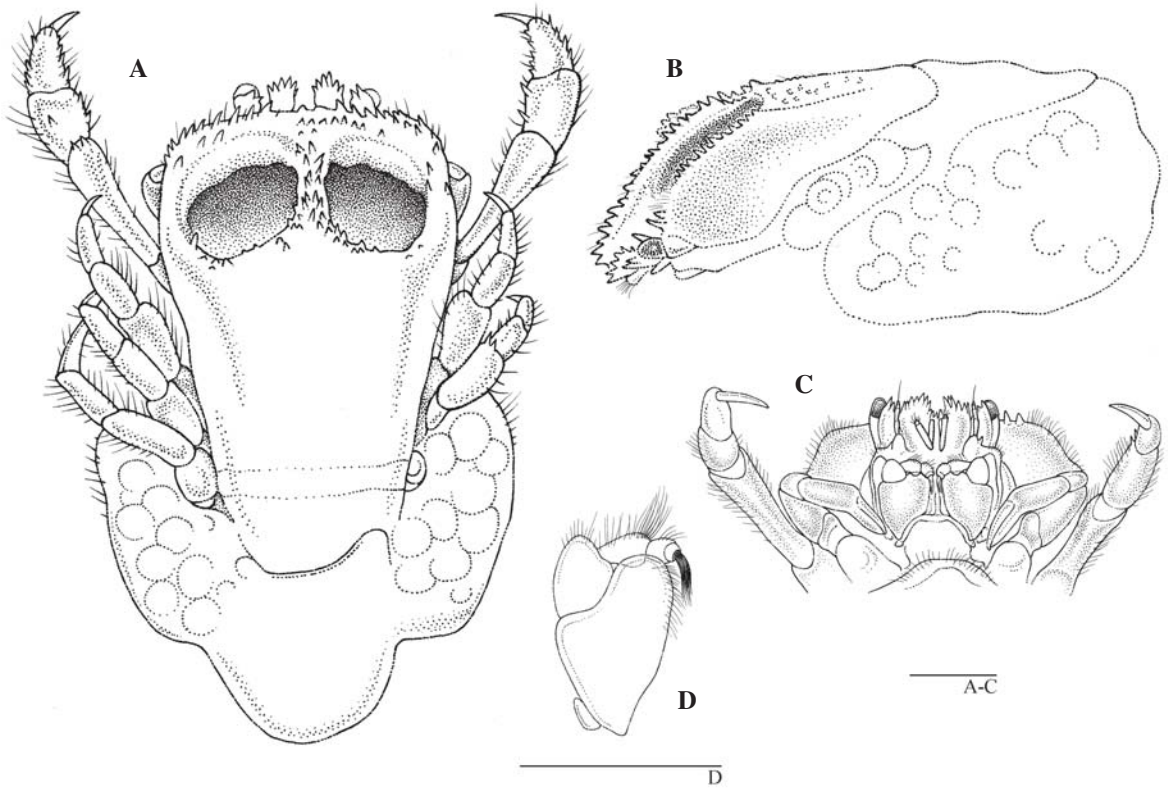

Fig. 2. A-D, Holotype *Fizesereneia panda* sp. nov. (RMNH.Crus.D.54425). **A**, habitus, dorsal view; **B**, carapace, lateral view; **C**, anterolateral margin of carapace, ventral view; **D**, MXP3. Scale bars 1 mm.

Arthropoda 20378, off Bandar Al-Khayran, Gulf of Oman (ca. 23.52 N 58.73 E), 6-9 m, 1 ovig. female (damaged), 1 male on *Symphyllia recta*, 23.v.2008, leg. M Malay.

Description female holotype. Carapace (Fig. 2A) subrectangular, longer than broad, CL 1.2 times longer than CW; greatest width of carapace where posterior margin of depression meets lateral carapace margin; dorsal surface convex in lateral view, deflected anteriorly (Fig. 2B). The anterior depressions divided completely into two concavities by median longitudinal ridge, armed with numerous spines crudely arranged in two rows; scattered spines on the margins of the depressions; carapace depressions smooth. Frontal margin armed with anteriorly directed spines. Frontal margin on ventral side features two substantial spines (Fig. 2C). Posterior half of dorsum smooth; cardio-intestinal region slightly outlined by shallow furrow; pterygostomial region is separated from the carapace by a membrane.

Ocular penduncles with two spines on distal margin, cornea elliptical, longer than broad; antennule same length as ocular penduncles; antennal segment two longer than broad, slightly extending beyond eyestalk, distal margin with several lateral spines.

MXP3 (Fig. 2D) exopod subrectangular, reaching approx. 1/3 length of ischium; ischium subtriangular, smooth, mesial and distal margin straight, anteromesial lobe with few setae; anterolateral margin of merus with few setae; distal portion of carpus with long setae; dactylus with bundle of long setae.

P1 (chelipeds, Fig. 3A) slender, smooth; ischium length ¾ height; merus length three times height, with few scattered short setae; carpus length twice height; propodus about same length as merus, fingers slender, mesial surfaces of fingers smooth, cutting edge entire, tips of fingers slightly crossing.

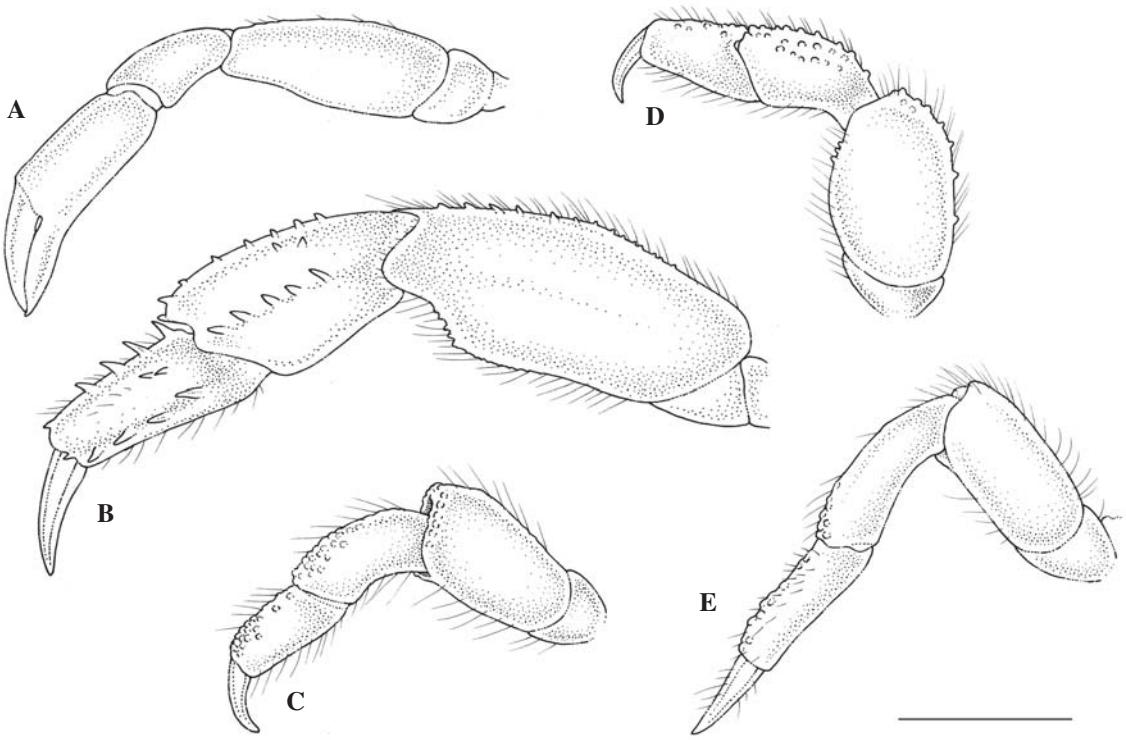

Fig. 3. A-E, Holotype *Fizesereneia panda* sp. nov. (RMNH.Crus.D.54425). **A**, left P1 (cheliped); **B**, left P2; **C**, left P3; **D**, left P4; **E**, left P5. Scale bar 1 mm.

P2 (Fig. 3B) longer, coarser than P1; ischium without setae; merus stout, slightly bent, few and small conical tubercles on distal half of dorsal surface, simple short setae on lateral and dorsal surface; joint between merus, carpus not extending more than at right angle; carpus 2/3 length of merus, surface smooth except for conical tubercles crudely arranged in two rows, no setae; propodus as long as carpus, surface smooth except for conical tubercles crudely arranged in two rows, fine setae on lateral and dorsal surface, dactylus half-length of propodus, smooth, sharp, slightly curved ventrally.

P3 (Fig. 3C) ischium with few setae; merus length twice height, rounded, tubercles and simple setae on dorsal surface, few small tubercles on distal half of lateral surface, simple setae along distal half of lateral surface; joint between merus, carpus not extending more than at right angle; carpus and propodus of equal length, rounded tubercles on dorsal surface, simple setae on lateral and dorsal surface; dactylus half-length of propodus, smooth, sharp, curved ventrally.

P4 (Fig. 3D) ischium with few setae; merus length twice height, small rounded tubercles close to joint with carpus, simple setae on dorsal and lateral surface; joint between merus, carpus not extending more than at right angle; carpus and propodus of equal length, rounded tubercles on dorsal surface, simple setae on lateral and dorsal surface; dactylus half-length of propodus, smooth, sharp, curved ventrally.

P5 (Fig. 3E) ischium with few setae; merus, carpus, propodus of equal length, all with simple setae; joint between merus, carpus not extending more than at right angle; carpus and propodus with rounded tubercles on dorsal surface; dactylus half-length of propodus, smooth, sharp, straight.

P3, P4 decreasing in size from P2. P5 right sampled for DNA analysis.

Pleon (= abdomen) enlarged, lateral margin fringed with setae.

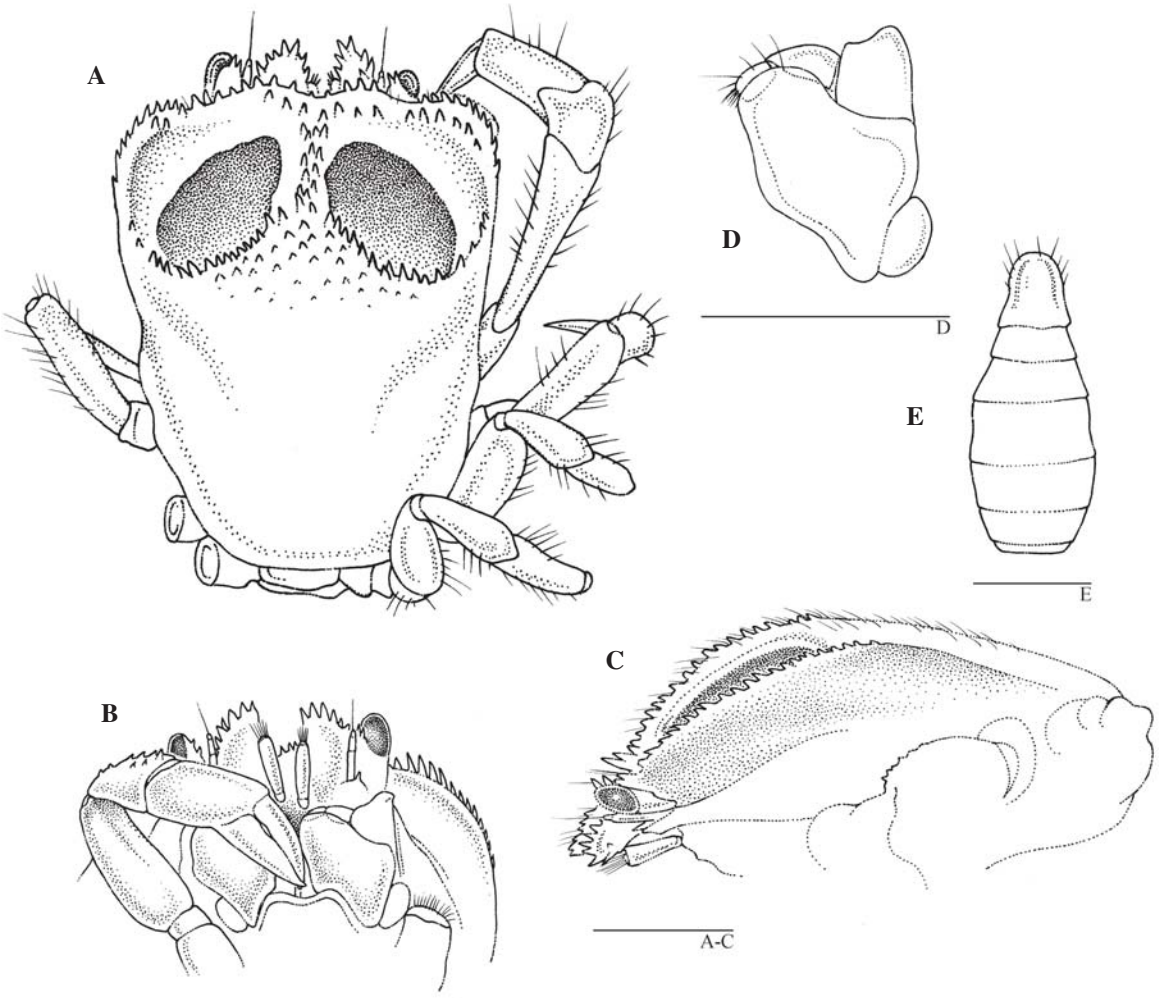

Fig. 4. A-E, Allotype *Fizesereneia panda* sp. nov. (RMNH.Crus.D.54424). **A**, habitus, dorsal view; **B**, anterolateral margin of carapace, ventral view; **C**, carapace, lateral view; **D**, MXP3; **E**, abdomen. Scale bars 1 mm.

Anterior margin thoracic sternites 1-3 almost straight (Fig. 6B).

Gonopore (vulva); reniform, size almost half the height of sternite 6 (examined in paratype UF Arthropoda 40384).

Description male allotype. Generally similar to holotype, differences as outlined below. Carapace (Fig. 4A) subrectangular, CL 1.3 times longer than CW (Fig. 4C). The anterior depressions divided completely into two concavities by median longitudinal; numerous spines on the margins of the depressions. Posterior half of dorsum smooth.

Ocular penduncles with small spines on distal margin, cornea elliptical, longer than broad; antennal segment extending beyond eyestalk (Fig. 4B).

MXP3 (Fig. 4D) exopod subrectangular, reaching approx. ½ length of ischium; ischium, smooth, mesial and distal margin slightly curved; anterolateral margin of merus with indentation; propodus with scattered setae; dactylus with bundle of short setae.

P1 (chelipeds, Fig. 5A) slender, smooth; merus length two times height; carpus with short spines on dorsal surface; propodus about same length as merus, fingers slender, mesial surfaces of dactyl with slight tooth.

P2 (Fig. 5B) longer, coarser than P1; ischium without setae; merus slender, simple short setae on lateral and dorsal surface; carpus ½ length of merus, slightly bent, few spiny tubercles on dorsal surface, few setae; propodus length twice height, surface smooth except for spiny tubercles

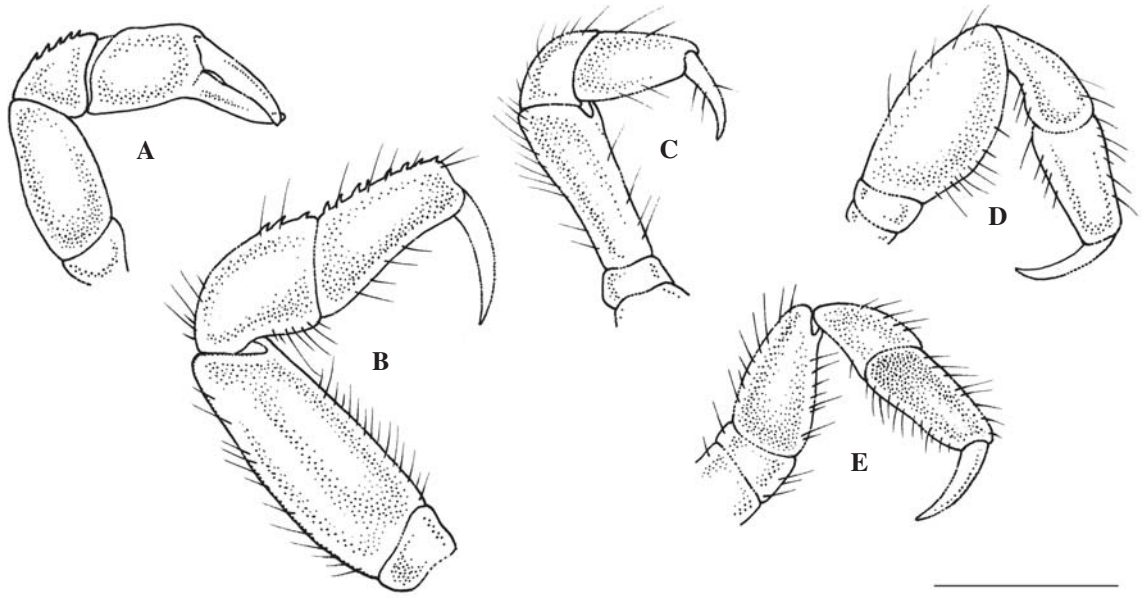

Fig. 5. A-E, Allotype *Fizesereneia panda* sp. nov. (RMNH.Crus.D.54424). **A**, right P1 (cheliped); **B**, right P2; **C**, right P3; **D**, right P4; **E**, right P5. Scale bar 1 mm.

on dorsal surface, fine setae on lateral and dorsal surface, dactylus smooth, sharp, slightly curved ventrally.

P3 (Fig. 5C) merus length three times height, simple setae on lateral and dorsal surface; carpus bent with few setae; propodus tapering towards dactyl, simple setae on lateral and dorsal surface; dactylus smooth, sharp, curved ventrally, few setae.

P4 (Fig. 5D) merus slightly rounded, simple setae on dorsal and lateral surface; carpus and propodus with simple setae on lateral and dorsal surface; dactylus smooth, sharp, curved ventrally.

P5 (Fig. 5E) ischium with few setae; merus length twice height, simple setae on dorsal and lateral surface; carpus 2/3 of propodus length, simple setae on lateral and dorsal surface; dactylus, smooth, sharp, curved. P3-5 roughly of equal size.

P1-2 left missing, P4-5 left sampled for DNA analyses.

Anterior margin of thoracic sternites 1-3 slightly concave (Fig. 6D). Abdomen bowling pin-shaped, longest and widest at 4th segment; telson rounded with few setae (Fig. 4E).

Gonopod; G1: slightly curved, tapering, apex pointed. Lateral margin with short, non-plumose simple setae, medial margin without setae.

Colour. Holotype (Fig. 6A-B): posterior 2/3 of the anterior depressions on the carapace have a black blotch with a reddish hue, whereas the remaining 1/3 is off-white. Several light blue spots are visible at the junction of the dark and off-white patterns. Remaining part of carapace translucent whitish-beige with a few scattered faint red spots on the posterior side of the carapace and brood pouch. All pereiopods translucent, P1 with many scattered brown spots and a few white spots, P2 more white than P3-5. Colour of MXP3 like P1. Antennules translucent with scattered white spots. Eyes reddish-brown with some white. Allotype (Fig. 6C-D) - differs from the holotype in the following - posterior 2/3 of the anterior depressions on the carapace have a deep red, almost black blotch, while the remaining 1/3 of the concavity is a soft yellow. Where the dark pattern meets the soft yellow a wine-red margin is visible. Remaining part of carapace translucent bluish-grey, with some scattered red spots, especially on the posterior side of the carapace

Fig. 6. A-D, *Fizesereneia panda* sp. nov., colouration in life. **A**, **B**, holotype RMNH.Crus.D.54425 (carapace 4.2 × 3.6), dorsal (A) and ventral (B) view. **C**, **D**, allotype RMNH.Crus.D.54424 (carapace 4.2 × 3.2), dorsal (A) and ventral (B) view. Photos by A. Anker and P.L. Norby.

and around the concavities. P1 with scattered white and brown spots, P2-5 with faint soft yellow bandings. Eyes red with some white.

Variation. *Fizesereneia panda* sp. nov. females show little morphological variation. There is some variation in colour pattern in live specimens. The size of the dark blotches in the carapace depressions varies but covers at least 2/3 of the concavities. Reddish hue of these blotches is more intense in some specimens. Several females lack the light blue spots of the holotype, whereas in other females the light part of the concavities appears more soft yellow. The male specimen of *F. panda* sp. nov. from Oman has mixed olive green and light blue spots on the overall reddish-black colour of the depressions.

Remarks. In *Fizesereneia heimi* and *F. stimpsoni* the anterior carapace depression is divided into two concavities by a median longitudinal ridge armed with spines, whereas it is incompletely divided in *F. latisella*, *F. ishikawai* and *F. tholia* (Fize and Serène, 1957, Takeda and Tamura, 1980, Kropp, 1994). The division of the depression in *F. daidai* is variable, but it is incomplete

Fig. 7. A-B, *Fizesereneia panda* sp. nov. (**A**, RMNH.Crus.D.54386; **B**, RMNH.Crus.D.54449) (circled) in *Lobophyllia corymbosa*. Photos by S.E.T. van der Meij, not to scale.

in most individuals (Zayasu *et al.*, 2013). The degree of division of the concavity is stronger in *F. panda* sp. nov. (females and males) than in any other *Fizesereneia* species, including *F. heimi* and *F. stimpsoni*. In addition, the median longitudinal ridge in *F. panda* sp. nov. is 'raised', whereas in the other two species the ridge is less pronounced. Based on the degree of division of the concavities, *Fizesereneia panda* sp. nov. is most similar to *F. heimi* and *F. stimpsoni*. The new species can be distinguished from these two species by its carapace shape and the colour pattern of the concavities. The carapace shape of *F. heimi* is roughly hexagonal (widest near the middle of the lateral margin), of *F. stimpsoni* subquadrangular (widest across the anterior margin, narrower posteriorly), whereas the carapace of *F. panda* sp. nov. is subrectangular (greatest width at the intersection of the posterior margin of the anterior depression with the lateral margin). The concavities of female *F. heimi* are predominantly brown-grey, and the concavities of male *F. heimi* are emerald green with some darker spots or lines. Female and male *F. panda* sp. nov. have dark reddish black blotches in the concavities. *Fizesereneia panda* sp. nov. can be distinguished from *F. stimpsoni* by the marbled pattern of the concavities in the latter (visible even in specimens in ethanol). Additionally, *F. stimpsoni* has only been recorded from the coral genus *Acanthastrea* (Fize and Serène, 1957; Zayasu *et al.*, 2013), whereas *F. panda* sp. nov. is associated with *Lobophyllia* and *Symphyllia*.

Coral hosts (Fig 6A-B). So far, *Fizesereneia* has only been found in association with Indo-Pacific coral species belonging to the Lobophylliidae Dai and Horng, 2009 (previously classified as Mussidae Ortmann, 1890, a family now restricted to the Atlantic (Budd *et al.*, 2012)). The coral hosts for this new gall crab species are identified as *Lobophyllia* cf. *corymbosa* (Forsskål, 1775) and *L.* cf. *hemprichii*, (Ehrenberg, 1834) based on Scheer and Pillai (1983) and Sheppard and Sheppard (1991), and as *Symphyllia recta* (Dana, 1846) based on Claereboudt (2006). Scheer and Pillai (1983) in their Red Sea study distinguished *Lobophyllia corymbosa* by its mostly monocentric corallites, from the mostly phacelomeandroid *L. hemprichii* but considered them potentially synonymous. They did not document the lobophyllid genus *Symphyllia* in the Red Sea. Sheppard and Sheppard (1991) discussed *Symphyllia erythraea* (Klunzinger, 1879), *S. radians* (Milne Edwards and Haime, 1849), and *Lobophyllia corymbosa* and L. *hemprichii* in the Red Sea. *Symphyllia erythraea* and *S. radians* are fully meandroid and not easy to confuse with *Lobophyllia*. Arrigoni *et al.* (2012) found *L. hemprichii*, *L. corymbosa* and *S. radians* to be genetically very closely related, while *S. erythraea* is distinct and basal to the *Symphyllia-Lobophyllia* clade.

Host specificity of *Fizesereneia* species appears to be less strict than that of species of some other gall crab genera, but this is possibly influenced by difficulties in host coral identification. So far, only *Fizesereneia daidai* and *F. stimpsoni* show strict host associations, respectively with the genera *Micromussa* and *Acanthastrea* (Fize and Serène, 1957; Zayasu *et al.*, 2013).

Distribution. Currently known from the Farasan Banks and Islands and the reefs off Thuwal in the Saudi Arabian part of the Red Sea (Fig. 1) and from off Bandar Al-Khayran in the Gulf of Oman. This is the first record of *Fizesereneia* from this area, a genus heretofore recorded from Vietnam, Indonesia, Japan, Australia, and Micronesia (Kropp, 1990a).

Etymology. This species is named *panda* owing to the dark colour pattern of its anterior carapace concavities, which resemble the dark spots around the eyes of the giant panda *Ailuropoda melanoleuca* (David, 1869) (Mammalia, Ursidae).

Acknowledgements

The photographs of *Fizesereneia panda* sp. nov. were taken by Arthur Anker and Patrick L. Norby, Erik-Jan Bosch made the scientific illustrations and Camrin Braun helped to create the Red Sea map. The COI sequences were produced as part of the Naturalis Barcoding project, with the help of Kevin Beentjes. Fieldwork in the Red Sea was supported by the King Abdullah University of Science and Technology under the Biodiversity in the Saudi Arabian Red Sea program, award number CRG-1-BER-002 to MLB. We thank Michel Claereboudt for organising and facilitating field work in Oman. We thank Francesca Benzoni for her comments on lobophylliid systematics and Roy Kropp for his constructive comments on the manuscript.

Chapter 4

A new gall crab species (Brachyura: Cryptochiridae) associated with the free-living coral *Trachyphyllia geoffroyi* (Scleractinia: Merulinidae)

Sancia E.T. van der Meij

Abstract

A new species of gall crab is described from the free-living stony coral *Trachyphyllia geoffroyi*. Specimens were collected during field work in Lembeh Strait (Indonesia) and off Kudat (Malaysian Borneo). This new species, here named *Lithoscaptus semperi* sp. n., is the ninth species assigned to the genus. It can be separated from its congeners by not having the internal orbital angle extending beyond the external orbital angle, and by the stout female P2 merus with prominent distomesial projection. In addition, the carapace surface appears smooth, despite having small tubercles on the anterior half, and is without noticeable spines, other than those on the frontal margin. The distinctive carapace pattern in life is a diagnostic character in male specimens.

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Introduction

During field work in Indonesia and Malaysia an undescribed gall crab species was encountered living in dwellings in free-living *Trachyphyllia geoffroyi* (Audouin, 1826) corals. This scleractinian species is usually found on soft substrate of reef bases near coral reefs, where it can occur in large numbers (Fisk, 1983; Best and Hoeksema, 1987). The polyps of *T. geoffroyi* are fleshy and a large mantle can extend beyond the perimeter of the skeleton.

Trachyphyllia geoffroyi was classified in its own family, Trachyphylliidae Verrill, 1901, but this taxon was recently synonymised with Merulinidae Verrill, 1865 (Huang *et al*., 2014). The sister genera of *Trachyphyllia* Milne Edwards and Haime, 1849 are *Coelastrea* Verrill, 1866 and *Dipsastraea* de Blainville, 1830, which include coral species that formerly belonged to *Goniastrea* Milne Edwards and Haime, 1848 and *Favia* Milne Edwards, 1857. Corals belonging to these genera are host to cryptochirids of the genus *Lithoscaptus* A. Milne-Edwards, 1862 (Fize and Serène, 1957; Kropp, 1990a).

Semper (1881) mentioned gall crabs associated with Indo-Pacific and Atlantic *"Trachyphyllia"*, but no formally described gall crab has been recorded living in association with *T. geoffroyi*. This new gall crab species, here named *Lithoscaptus semperi* sp. n., is the ninth assigned to the genus.

Material and methods

Gall crabs were collected in Indonesia (Lembeh Strait, N Sulawesi – February 2012) and Malaysia (off Kudat, N Borneo – September 2012). Corals were searched for gall crabs, taken to the field laboratory and subsequently split with hammer and chisel. The crabs were preserved in 80% ethanol, after being photographed with a digital SLR camera equipped with a macro lens to register colour patterns. All crab specimens are deposited in the Crustacea collection of Naturalis Biodiversity Center in Leiden, the Netherlands (formerly Rijksmuseum van Natuurlijke Historie, collection coded as RMNH.Crus.D). Drawings were made with a stereomicroscope with camera lucida. Carapace lengths and widths were measured to the nearest 0.1 mm using an eyepiece micrometre, with the crabs positioned on a level surface.

Abbreviations used: CL, carapace length; CW, carapace width (at widest point); MXP3, third maxilliped; ovig., ovigerous; P, pereiopod; G, male gonopod. Carapace measurements are given as CL × CW, in mm.

Taxonomy

Cryptochiridae Paul'son, 1875
Lithoscaptus A. Milne-Edwards, 1862

Lithoscaptus semperi sp. n.
Figs 1-3

Type locality. Tigabu Isl. (06°53'51"N, 117°27'36"E), Kudat, Sabah (N Borneo), Malaysia.

Coral host holotype. *Trachyphyllia geoffroyi* (Audouin, 1826).

Dna barcoding. A COI sequence (partially, Folmer *et al*., 1994) of paratype RMNH. Crus.D.54331 has been deposited in GenBank under accession number KP688583.

Type material. Holotype. RMNH.Crus.D.56962, ovig. female, 6.4 × 4.6. **Allotype** (with holo-

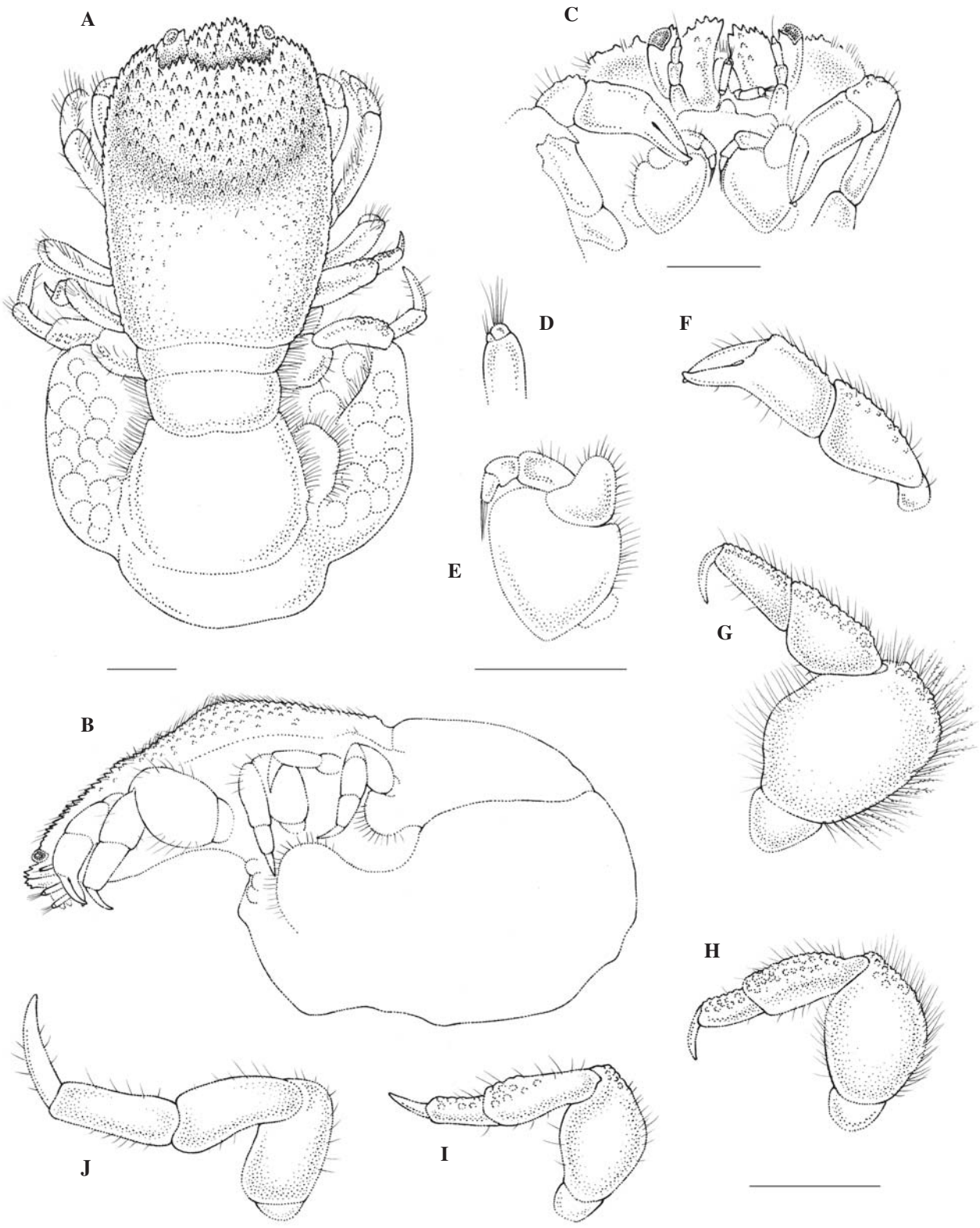

Fig. 1. Ovigerous female holotype (6.4 × 4.6) of *Lithoscaptus semperi* sp. n. (RMNH.Crus.D.56962). **A**, habitus, dorsal view; **B**, carapace, lateral view; **C**, anterior margin of carapace, ventral view; **D**, close-up of antennule; **E**, MXP3; **F**, left P1 (cheliped); **G**, left P2; **H**, left P3; **I**, left P4; **J**, left P5. Scale bars 1 mm; A-B, D-E, F-J share scale bars.

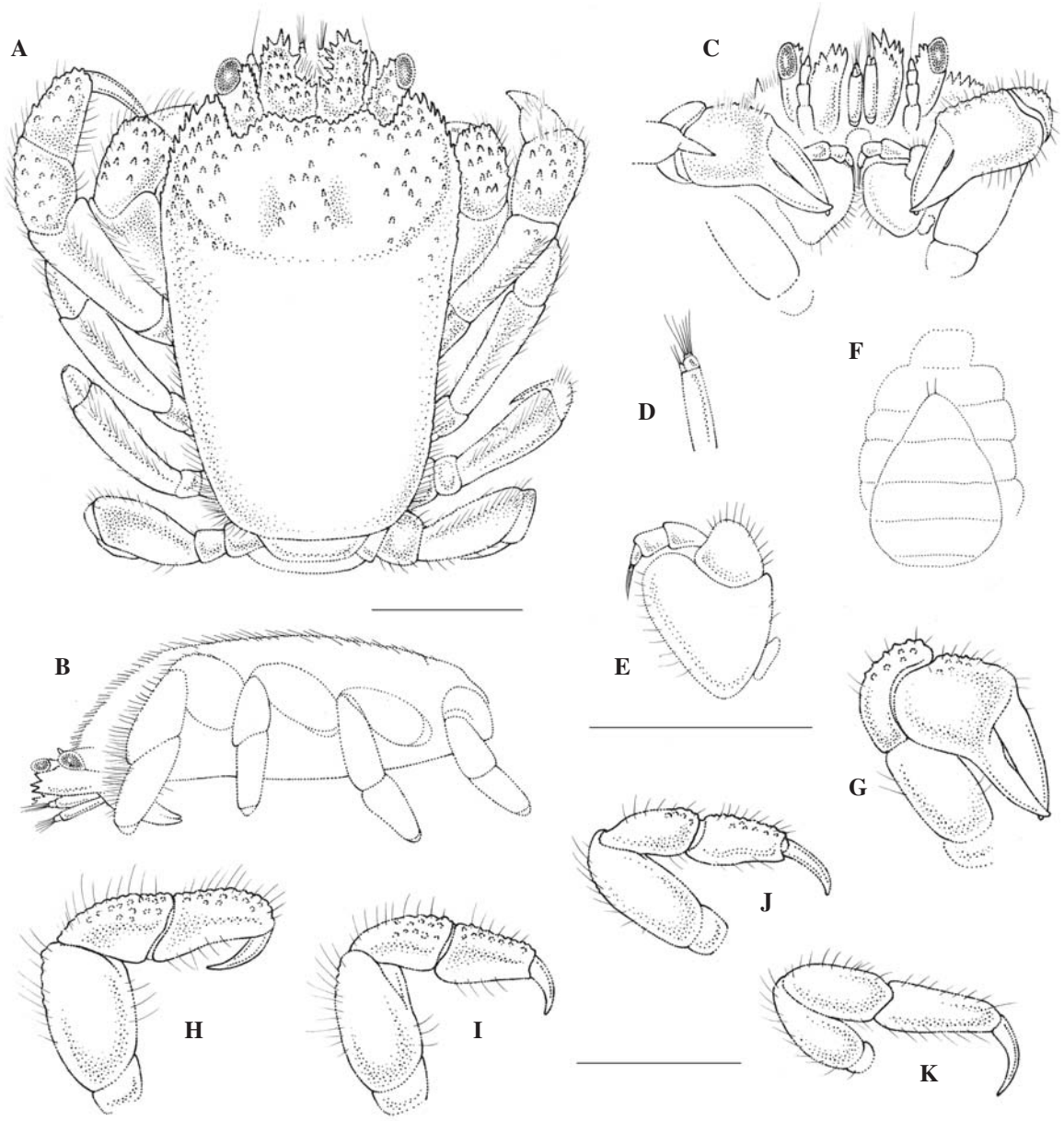

Fig. 2. Male allotype (3.6 × 2.5) of *Lithoscaptus semperi* sp. n. (RMNH.Crus.D.56962). **A**, habitus, dorsal view; **B**, carapace, lateral view; **C**, anterior margin of carapace, ventral view; **D**, close-up of antennule; **E**, MXP3; **F**, thoracic sternites; **G**, right P1 (cheliped); **H**, right P2; **I**, right P3; **J**, right P4; **K**, right P5. Scale bar 1 mm; A-C, D-E, F-K share scale bars.

type), male, 3.6 × 2.5. Collected by the author from 13 m depth on 8 September 2012. **Paratype.** RMNH.Crus.D.54331, Lubani Rock, Kudat, Sabah (N Borneo), Malaysia (06°53'45.0"N, 117°23'15.8"E), 10-15 m, 07.ix.2012, 1 ovig. female, 6.2 × 4.7, leg. SET van der Meij.

Material examined. Indonesia: RMNH.Crus.D.56957, Aer Perang, Lembeh Strait (01°28'25"N, 125°14'02"E), ca. 10 m, 02.ii.2012, 1 female, leg. BT Reijnen; RMNH.Crus.D.56958, Tanjung Labuhankompeni, Lembeh Strait (01°25'55"N, 125°11'10"E), 28 m, 04.ii.2012, 1 female, leg. BW Hoeksema; RMNH.Crus.D.56959, Kelapadua, Lembeh Strait (01°26'19"N, 125°12'49"E), 20 m, 09.ii.2012, 2 juvenile males, leg. BW Hoeksema; RMNH.Crus.D.54250, Tanjung Nanas I, Lembeh Strait (01°27'39"N, 125°13'35"E), 25-30 m, 17.ii.201, 1 ovig. female, 1 female, leg. BW Hoeksema; **Malaysia:** RMNH.Crus.D.54259, Lubani Rock, Kudat (06°53'45"N, 117°23'15"E),

10-15 m, 07.ix.2012, 1 ovig. female (slightly damaged), leg. BW Hoeksema; RMNH.Crus.D.54280, Lubani Rock, Kudat (06°53'45"N, 117°23'15"E), 10-15 m, 07.ix.2012, 1 ovig. female, 1 male, leg. BW Hoeksema; RMNH.Crus.D.56960, Lubani Rock, Kudat (06°53'45"N, 117°23'15"E), 10-15 m, 07.ix.2012, 1 male, leg. SET van der Meij; RMNH.Crus.D.56961, Lubani Rock, Kudat (06°53'45"N, 117°23'15"E), 10-15 m, 07.ix.2012, 1 ovig. female, leg. SET van der Meij; RMNH. Crus.D.54312, Tigabu Is., Kudat (06°53'51"N, 117°27'36"E), 9 m, 08.ix.2012, 1 ovig. female (damaged), 1 male, leg. SET van der Meij; RMNH.Crus.D.56963, Fairway Shoal, Kudat (07°07'06"N, 117°30'42"E), 12 m, 10.ix.2012, 1 male, leg. BT Reijnen; RMNH.Crus.D.56964, Belaruan, Kudat (07°01'50"N, 117°00'41"E), ca. 15m, 20.ix.2012, 1 male, leg. BW Hoeksema; RMNH.Crus.D.54258, Tajau, Kudat (06°59'36"N, 116°50'27"E), 21 m, 25.ix.2012, 1 female, 1 male, leg. BW Hoeksema. All material was collected from the scleractinian coral *Trachyphyllia geoffroyi*.

Description of female holotype. Carapace (Fig. 1A) rectangular, longer than broad, CL 1.4 times longer than CW; widest near midlength, dorsal surface in lateral view strongly convex in both directions, deflected anteriorly (Fig. 1B); anterior half of carapace with small, sharp tubercles, posterior half smooth with few, rounded granules, cardiointestinal region slightly inflated. Frontal margin armed with small anteriorly directed spines. Frontal margin on ventral side features few, small tubercles. Pterygostomial region fused to carapace.

Eyestalk exposed dorsally, slightly granular, small spines on mesial margin. Cornea anterolateral. Lateral margin of stalk at same level as anterolateral angle; distal margin with small spines (Fig. 1A, C). Distal segment of antennules with protruding article, visible from ventral side (Fig. 1C, D).

Antennular peduncle dorsal surface with small, sharp tubercles, slightly inflated distomesially; apex extending beyond tip of eyestalk; spines on mesial margin larger than those on distal margin. Ventral surface smooth, slightly tapering anteriorly in ventral view (Fig. 1C).

MXP3 (Fig. 1E) exopod rectangular; ischium subtriangular, smooth, mesial and distal margins straight, anteromesial lobe with few simple setae; merus with distolateralprojection, simple setae; distal portion of carpus with short, simple setae, dactylus with bundle of setae.

P1 (chelipeds, Fig. 1F) slender; carpus length twice height, scattered small tubercles on dorsal surface, simple setae; propodus length twice height, somewhat granulated, few, scattered setae, fingers slender, mesial surface of fingers smooth, cutting edge entire, tips of fingers crossing.

P2 (Fig. 1G) longer, coarser than P1; ischium without setae; merus stout, plump, smooth with few, small rounded tubercles on distal half of dorsal surface, simple setae on lateral surface, numerous plumose setae on dorsal surface; joint between merus, carpus not extending more than at right angle; carpus smooth with small rounded tubercles on dorsal surface, simple setae on dorsal surface; propodus slightly shorter than carpus, surface smooth with small rounded tubercles on dorsal surface, simple setae on lateral and dorsal surface; dactylus half-length of propodus, smooth, sharp, curved ventrally.

P3 (Fig. 1H) ischium without setae; merus length 1.5 times height, rounded, few rounded tubercles on distal half of dorsal surface, simple setae along dorsal, lateral surface; joint between merus, carpus not extending more than at right angle; carpus length 2.5 times height, rounded tubercles on dorsal surface, simple setae on lateral and dorsal surface; propodus length twice height, rounded tubercles on dorsal surface, scattered simple setae; dactylus similar length as propodus, smooth, sharp, slightly curved ventrally.

P4 (Fig. 1I) similar to P3, less coarse; ischium without setae; merus length 1.5 times height, small rounded tubercles close to joint with carpus, carpus length 2.5 times height, rounded tubercles on distal half of dorsal surface, scattered simple setae; propodus half-length carpus, rounded tubercles on distal half of dorsal surface, few scattered simple setae; dactylus similar length as

Fig. 3. Colour in life of *Lithoscaptus semperi* sp. n. **A-B**, non-ovigerous female (4.5 × 3.2; RMNH. Crus.D.54258) dorsal view and ventral view; **C-D**, male (2.5 × 1.9; RMNH.Crus.D.54258) dorsal view and ventral view; **E**, juvenile male (2.0 × 1.6; RMNH.Crus.D.56959) dorsal view; **F**, in-situ photograph of dwellings (left male, right female) of *L. semperi* sp. n. in *Trachyphyllia geoffroyi* on Lubani Rock reef, Kudat (Malaysia). Photos by BT Reijnen/SET van der Meij.

propodus, smooth, sharp, straight.

P5 (Fig. 1J) ischium without setae; merus, carpus, propodus, dactylus all of equal length, all with short simple setae; carpus, propodus slender compared to merus; dactylus smooth, sharp, slightly curved ventrally.

P3, P4 decreasing in size from P2.

Abdomen enlarged, lateral margins fringed with setae (Fig. 1A, B).

Gonopore (vulva); reniform, size half the height of sternite 6.

Description of male allotype. Carapace (Fig. 2A) subrectangular to trapezoid, CL 1.5 times longer than CW, widest near anterior half, convex in lateral view, deflected anteriorly, with broad W-shaped depression (Fig. 2A, B). Anterior half of carapace and carapace margins with small spines, posterior half of carapace smooth.

Ocular peduncles with small spines on distal margin, cornea elliptical, longer than broad; antennal article extending beyond eyestalk, with spines along margins (Fig. 2C). Antennule slender compared to holotype, distal segment of antennules with protruding article (Fig. 2D).

MXP3 (Fig. 2E) exopod rectangular; ischium smooth, triangular, few scattered simple setae on distal and lateral margins, merus with distolateral projection, simple setae; propodus, dactylus of similar length, latter with bundle of short setae.

P1 (chelipeds, Fig. 2G) stout; merus length twice height, smooth; carpus with rounded and conical tubercles, simple setae on dorsal surface; propodus stout, with conical tubercles, simple setae on dorsal surface; fingers slender, mesial surfaces of dactyl slightly gaping, tips of fingers crossing.

P2 (Fig. 2H) ischium without setae; merus relatively stout, smooth, length twice height, simple short setae on lateral and dorsal surface; carpus, propodus of similar length; carpus with few rounded tubercles and setae on dorsal surface; propodus smooth except for rounded tubercles on dorsal surface, few setae on lateral, dorsal surface, dactylus smooth, sharp, curved ventrally.

P3 and P4 (Fig. 2I, J) similar to P2, somewhat smaller; ischium without setae; merus smooth, simple short setae on lateral and dorsal surface; carpus, propodus ofsame length, few rounded tubercles and setae on dorsal surface; dactylus smooth, sharp, curved ventrally.

P5 (Fig. 2K) ischium with few setae; merus, carpus, propodus smooth, with simple short setae on dorsal and lateral surface; dactylus smooth, sharp, curved.

P3, P4 decreasing in size from P2.

Abdomen teardrop-shaped, widest at 4th somite; telson slightly pointed with few simple setae (Fig. 2F).

Gonopod 1 almost straight, tapering, apex sharply pointed. Distal margin with 2-3 non-plumose short simple setae, medial margin without setae (examined in RMNH. Crus.D.56964).

Colour. Female (Fig. 3A-B): Overall off-white. Pereiopods opaque, carpus, dactylus P1and P2 translucent violet, sometimes with a pale orange line. Eyes with wide longitudinal brownish-red lines. Male (Fig. 3C-D): Carapace opaque with an offwhite distinctive pattern over the whole carapace surface. Pereiopods opaque, P1 carpus, dactylus translucent violet, sometimes with a pale orange line. Eyes brown-red. In juvenile males (Fig. 3E), the carapace pattern is pale orange, pereiopods off-white.

Placement in genus. The placement of *Lithoscaptus semperi* sp. n. in the genus *Lithoscaptus* is somewhat tentative. The first (partial) molecular reconstruction of relationships within the Cryptochiridae shows that the genus *Lithoscaptus* is paraphyletic (van der Meij and Reijnen, 2014). However, following the diagnosis of *Lithoscaptus* by Kropp (1990a), the new species best fits the genus, except for the absence of a proximal tooth on the cutting edge of P1 dactylus and the presence of a distomesial projection of P2 merus in females. Kropp (1994) noted that his new

species, *L. prionotus*, had the pterygostomial region not fused to the carapace, unlike other species in the genus. It is likely that the characters defining the genus need to be redefined, or that certain species need to be moved to a new genus.

Comparisons. Eight species of *Lithoscaptus* are currently recognised (Ng *et al*., 2008: 212, Davie 2015). *Lithoscaptus semperi* sp. n. can be distinguished from *L. nami* (Fize and Serène, 1957), *L. tri* (Fize and Serène, 1956) and *L. pardalotus* Kropp, 1995 by not having the internal orbital angle extending beyond the external orbital angle. The new species can be separated from *L. grandis* (Takeda and Tamura, 1983), *L. paradoxus* A. Milne-Edwards, 1862 and *L. prionotus* Kropp, 1994 by the smooth appearance of surface of the carapace, despite the small tubercles on the anterior half of the carapace, and the lack of noticeable spines other than the small spines on the frontal carapace margin. *Lithoscaptus pacificus* (Edmonson, 1933) and *L. helleri* (Fize and Serène, 1957) lack the stout merus with prominent distomesial projection of P2 (female specimens). The off-white carapace colour and translucent violet colour on P1 and P2 in females, and the distinctive carapace pattern in males differs from patterns found on other *Lithoscaptus* species.

Distribution. The known distribution of *L. semperi* sp. n. includes northern Borneo and North Sulawesi. Specimens were collected at water depths between 9 and approximately 30 meters. Its host *Trachyphyllia geoffroyi* was described from the Gulf of Suez (Egypt), but this species has a wide distribution that includes the Red Sea, East Africa, Seychelles, Maldives, Nicobar Isls., 'East Indies', China Sea, Philippines, Japan, Australia and New Caledonia (Scheer and Pillai, 1983). Based on the widespread distribution of *T. geoffroyi*, a wider distribution range than the two presently recorded locations is expected for *L. semperi* sp. n.

Coral host. *Lithoscaptus semperi* sp. n. is so far strictly associated with *T. geoffroyi* (Fig. 3F). It is the first record of associated fauna for this coral host. Colonies of *T. geoffroyi* are free-living, have flabello-meandroid colony shapes and fleshy polyps. Cryptochirids have previously been recorded to inhabit free-living corals; crabs of the genus *Fungicola* are associated with free-living – and attached – mushroom corals (Fungiidae), whereas *Troglocarcinus corallicola* is associated with a wide range of Atlantic corals, including the free-living coral *Manicina areolata* (Mussidae) (Fize and Serène, 1957; van der Meij, 2014a, 2015a).

Remarks. Fize and Serène (1957: p. 163) report on *Cryptochirus coralliodytes* from *Trachyphyllia* based on a record of Semper (1881: p. 221) who writes: *"I found them* [C. coralliodytes] *in the Philippine Archipelago in cavities in* Goniastraea Bournoni [= Goniastrea retiformis (de Lamarck, 1816)], *in an undetermined true* Astræa, *which was unfortunately lost*, *also in an undescribed* Trachyphyllia*; finally I received a new form through A. Agassiz from the West Indian seas*, *which may perhaps form a distinct genus*, *though it is very nearly allied to the first. It also lives in a* Trachyphyllia." The coral genus *Trachyphyllia* is described from the Red Sea and has a widespread Indo-Pacific distribution, however it does not occur in the Atlantic Ocean. The most similar Atlantic species would be *Manicina areolata* (Linnaeus, 1758). Furthermore, on p. 453 (note 103 belonging to p. 221) Semper writes: *"This crab*, *living in* Trachyphyllia, *a West Indian coral*, *is extremely like* Cryptochirus, *and perhaps belongs to the same genus; this can only be determined by future and more exact examination. But the 'cave dwelling' of this West Indian crab is perfectly unlike that of the Eastern species*, *which is found from the Red Sea as far as the Pacific Ocean; it is not cylindrical*, *but has one side quite flat*, *so that its transverse section is almost exactly a half-circle; the underside of the crab rests against the flat side of the cavity.*" The gall crab *Troglocarcinus corallicola* Verrill, 1908 has been recorded from a wide range of hosts, including *M. areolata* (Kropp and Manning, 1987; van der Meij, 2014a). As mentioned by Semper (1881), the dwelling of *T. corallicola* in *M. areolata* is shaped like a half-circle (see e.g.

Van der Meij, 2014a: Fig. 1B), therefore it seems plausible that Semper was referring to the coral *M. areolata* when he discussed a West Indian *Trachyphyllia*. Alternatively, Semper could have been referring to the Atlantic genus *Colpophyllia* because Milne Edwards and Haime (1849), who established *Trachyphyllia*, compared their new genus with *Colpophyllia* (see Huang *et al*. [2014] for a discussion on the genus *Trachyphyllia*). Like *M. areolata*, *Colpophyllia natans* (Houttuyn, 1772) also hosts *T. corallicola* (see van der Meij, 2014a). It remains unclear whether Semper found gall crabs in Indo-Pacific corals currently recognized as *Trachyphyllia geoffroyi*. Semper is not known to have formally described any gall crab species (Ng *et al*., 2008).

Etymology. Named after the German naturalist Carl Gottfried Semper (1832-1893), who was the first to mention gall crabs occurring in *Trachyphyllia*.

Acknowledgements

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Chapter 5

Host relations and DNA reveal a cryptic gall crab species (Crustacea: Decapoda: Cryptochiridae) associated with mushroom corals (Scleractinia: Fungiidae)

Sancia E.T. van der Meij

Abstract

Mushroom corals of the Indo-West Pacific Fungiidae (Scleractinia) provide habitats for a rich associated fauna, including three species of gall crabs (Cryptochiridae). During the course of the present study gall crabs were sampled from many different fungiid hosts. Based on this 'reversed' approach - by studying coral symbionts from a host perspective - a previously unnoticed host specificity pattern was detected. The sampling of gall crab fauna per host coral combined with molecular analyses of H3 nDNA, 16S and COI mtDNA revealed a cryptic gall crab species closely related to *Fungicola fagei*. This new species, described hereafter as *Fungicola syzygia* sp. nov., is predominantly associated with the mushroom coral genera *Cycloseris* and *Pleuractis*, whereas its sibling species *F. fagei* is only known to be associated with the host genera *Podabacia* and *Sandalolitha*. Based on morphology *F. syzygia* sp. nov. is difficult to distinguish from *F. fagei*, but there are subtle differences in carapace shape, the lateral carapace margins, the border between the orbital angles and the merus of the third maxilliped, as well as in the carapace length/width ratio. The type material of *F. utinomi* and *F. fagei* is figured for comparison.

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Introduction

The mushroom coral family Fungiidae occurs in the Indo-West Pacific and tropical Eastern Pacific regions with a distribution ranging from the Red Sea and eastern Africa to the west coast of Central America (Hoeksema, 1989). Several species have been recorded in association with these fungiids (Hoeksema *et al*., 2012). Most of the associated fauna consists of crustaceans and molluscs, but fishes have also been recorded to live in symbiosis with fungiids (Bos, 2012; Hoeksema *et al*., 2012). Such heterospecific associations between a host and an associated organism can range from brief facultative encounters to lifelong obligate partnerships (Castro, 1988). Studies on the associated fauna of stony corals are often studied from a symbiont perspective. A 'reversed approach' aims to study associated fauna from the perspective of the host, by collecting specimens from as many host coral species as possible. This reversed approach has previously been applied to several endolithic and epibiotic mollusc taxa obligately associated with fungiids, resulting in the discovery of several cryptic, adaptive radiations (Hoeksema and Kleemann, 2001; Kleemann and Hoeksema, 2002; Gittenberger and Gittenberger, 2005, 2010).

The reversed approach in the examination of a complete inventory of associated fauna was also used in the present study on fungiid associated gall crabs (Cryptochiridae). Gall crabs are obligate symbionts of scleractinians and reside in dwellings in their host coral. They occur worldwide but are most diverse in the Indo-West Pacific region (Fize and Serène, 1957; Kropp, 1990a). Three gall crab species, *Fungicola fagei* (Fize and Serène, 1956), *F. utinomi* (Fize and Serène, 1956) and *Dacryomaia* sp., have so far been recorded from 32 mushroom corals (Fize and Serène, 1957; Takeda and Tamura, 1979; Kropp, 1990a; Hoeksema *et al*., 2012; van der Meij and Hoeksema, 2013). The reversed approach for studying gall crab associations already yielded new coral hosts (Hoeksema *et al*., 2012; van der Meij and Hoeksema, 2013; van der Meij, 2014a). Based on host relations and molecular analyses, a cryptic species closely related to *F. fagei* was discovered (van der Meij and Hoeksema, 2013) and described herein as *Fungicola syzygia* sp. nov. It is the third species assigned to the genus, and the fourth species recorded from mushroom corals.

Material and methods

Material was collected during fieldwork in Raja Ampat (Papua, Indonesia, Nov.-Dec. 2007), Bunaken National Marine Park (N Sulawesi, Indonesia, Dec. 2008), Ternate (Halmahera, Indonesia, Oct.-Nov. 2009), Semporna (E Sabah, Malaysia, Nov.-Dec. 2010), Lembeh Isl. (N Sulawesi, Indonesia, Jan.-Feb. 2012), and Kudat (N Sabah, Malaysia, Sep. 2012). A few additional samples were available from New Caledonia (2012), Payar Isl. (Malaysia, 2013), Loyalty Isl. (New Caledonia, 2014) and the Maldives (2014). Gall crabs were collected with their host coral and taken to the field station. After being photographed with a digital SLR camera with a 50 mm macro-lens, the crabs were preserved in 80% ethanol. The gall crabs are deposited in the collections of Naturalis Biodiversity Center in Leiden, the Netherlands (formerly Rijksmuseum van Natuurlijke Historie, collection coded as RMNH.Crus.D), and a paratype of *Fungicola syzygia* sp. nov. is deposited in the Lee Kong Chian Natural History Museum in Singapore (formerly Raffles Museum of Biodiversity Research, collection coded as ZRC). The mushroom coral identifications are based on revisions of the Fungiidae (Hoeksema, 1989; Gittenberger *et al*., 2011), all identifications were (confirmed) by Dr Bert W. Hoeksema. Species authorities for the Fungiidae are provided in the list of material examined.

Drawings were made with a stereo microscope with camera lucida. The chelipeds were drawn with the outer surface of the manus parallel to the plane of the paper, which somewhat distorts the other segments. Carapace lengths and widths were measured using an eyepiece micrometre.

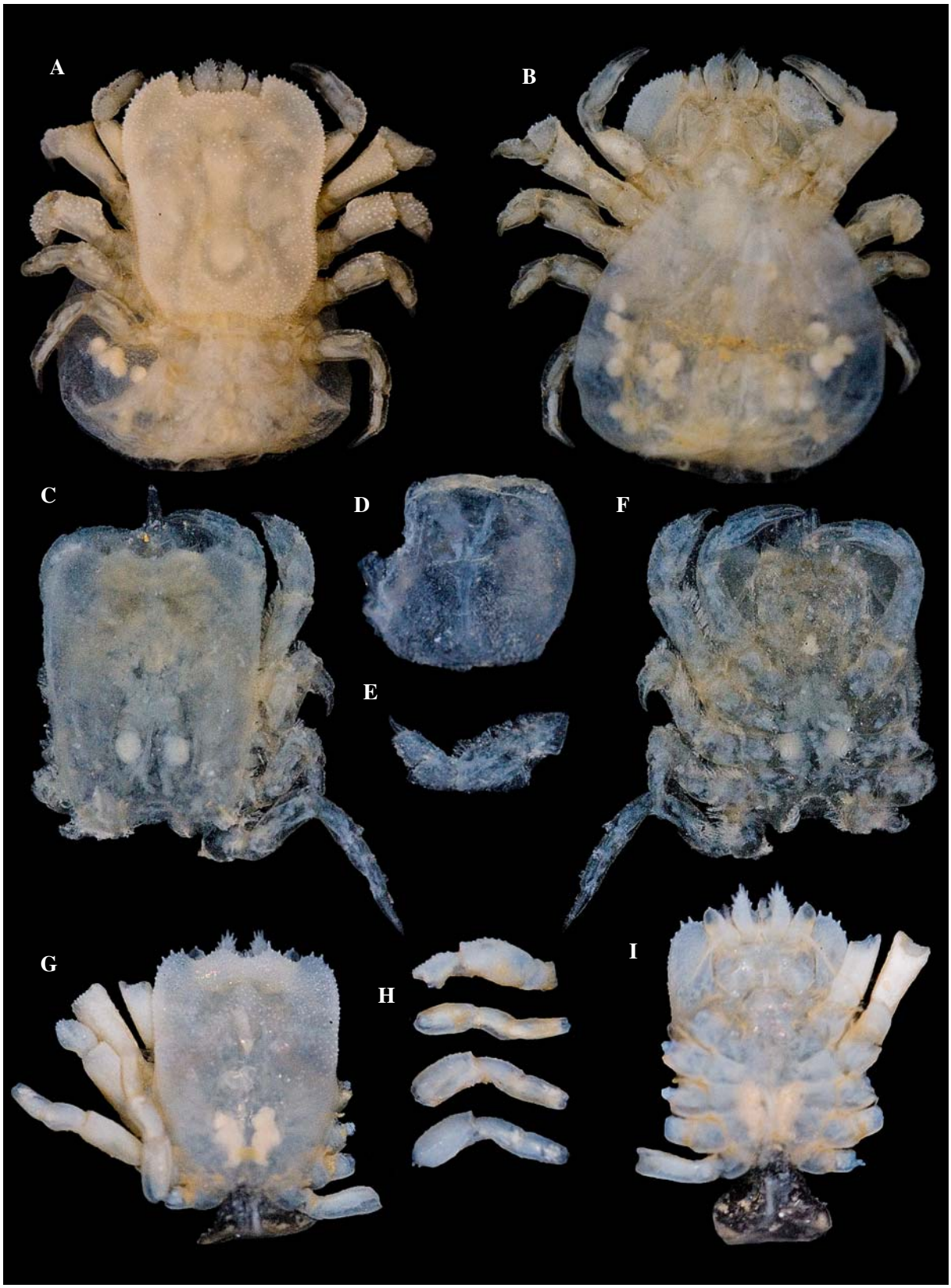


Fig. 1. Types in the Nha Trang collections; **A**, dorsal view and **B**, ventral view of the female holotype of *Troglocarcinus fagei* Fize and Serène, 1956 (E.38.444), carapace 4.5 × 4.0 mm; **C**, dorsal view, **D**, marsupium, **E**, pereiopod and **F**, ventral view of the female holotype of *T. utinomi* Fize and Serène, 1956 (E.37.277), carapace 5.0 × 4.0 mm; **G**, dorsal view, **H**, pereiopods and **I**, ventral view of the male allotype of *T. utinomi* Fize and Serène, 1956 (E.37.766), carapace 4.0 × 3.0 mm. Photos by SET van der Meij and BT Reijnen.

Abbreviations used: ovigerous, ovig.; MXP, maxilliped; P, pereiopod; PLP, pleopod; CL, carapace length at midpoint; CW, carapace width at widest point. Carapace measurements are given as CL × CW.

Nha Trang collections

Fize and Serène (1956a, b, 1957) described 16 new gall crab species in their monograph on the gall crabs of Vietnam and deposited the types in the museum of the Institute of Oceanography in Nha Trang (Vietnam). The Nha Trang collections were examined in October 2012 to search for the type material. Various specimens in the museum got displaced and subsequently lost during Vietnam's turbulent history, however, seven out of the 16 Fize and Serène's holotypes were located, including the types of *Troglocarcinus fagei* and *T. utinomi* (Fig. 1A-F). The allotype of *T. utinomi* was also located (Fig. 1G-I), but the vial that should have contained the allotype of *T. fagei* was empty, and this allotype is therefore considered lost. The lists of material examined includes all the *Fungicola* specimens that were located in the Nha Trang museum. Collection numbers were mentioned on labels on the outside of the collection jars and on labels inside the vials. In case of discrepancy, the information on the labels inside the vial was accepted as most likely the 'correct' information for the specimen(s) concerned. The locality data, 'Rte' (recolte) on the Vietnamese labels can be retrieved from Fize and Serène (1957).

Fize and Serène deposited duplicates of *Fungicola* specimens collected in Vietnam in the Muséum national d'Histoire naturelle (MNHN) in Paris, France. Additional specimens from Fize and Serène were unexpectedly encountered in the collections of the Natural History Museum (BMNH) in London, the United Kingdom. The details of this material are also included in the list of material examined.

Molecular analyses

For the molecular analyses specimens of *Fungicola fagei* and *F. utinomi* were included from many different host corals. *Utinomiella dimorpha* (Henderson, 1906) and *Pseudocryptochirus viridis* Hiro, 1938, associated with the coral genera *Pocillopora* and *Turbinaria*, respectively, were selected as outgroups because they belong to more distant clades within the monophyletic Cryptochiridae (van der Meij and Reijnen, 2014; van der Meij and Schubart, 2014).

Analyses of sequences from the mitochondrial cytochrome c oxidase subunit I gene (COI, partially, Folmer *et al*. (1994) and 16S mtDNA (16L2 and 16H10, Schubart (2009)) were used to infer phylogenetic relationships between the examined taxa. In addition, Histone H3 (H3) was used (Colgan *et al*., 2000). The reverse primer was optimized for gall crabs (H3_R_ SET: 5'-GCCGACMAGGTARGCCTCKG-'3).

DNA was isolated from the fifth pereiopod, using the QIAGEN DNeasy Kit according to the manufacturer's protocol for animal tissue. Maceration took place overnight for approximately 18 hrs. The final elution step was performed with 100 µl elution buffer. Polymerase chain reaction was carried out with standard PCR conditions (2.5 µl PCR buffer, 0.5 µl DNTP's, 1.0 µl primer FW, 1.0 µl primer RV, 0.3 µl Taq, 18.7 µl MilliQ and 1.0 µl DNA template). Thermal cycling for COI and 16S was as follows: initial denaturation at 95°C for five minutes, followed by 39 cycles of 95°C for five seconds, 47°C for one minute, and 72°C for one minute. This was followed by 10 minutes at 72°C. For H3 the standard PCR conditions were adjusted to 13.7 µl MilliQ and 5.0 µl Q-solution. Thermal cycling was as follows: initial denaturation at 95°C for three minutes, followed by 39 cycles of 95°C for ten seconds, 60°C for one minute, and 72°C for one minute. This was followed by 5 minutes at 72°C. Sequences were assembled and edited in Sequencer 4.10.1. A total of 1520 base pairs are included in the three marker dataset (n=50, including outgroups).

Sequences were aligned using ClustalW on the Guidance server (Penn *et al*., 2010), resulting in an alignment score of 0.991066. Prior to the model-based phylogenetic analysis, the best-fit model of nucleotide substitution was identified for each gene partition by means of the Akaike Information Criterion (AIC) calculated with jModeltest (Posada, 2008), resulting in GTR+I for 16S, GTR+G for COI and TIM2+G for H3. Because of the unavailability of TIM2 in MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003), I used the most complex GTR+G model of nucleotide substitution for H3. Bayesian inferences coupled with Markov chain Monte Carlo techniques (six chains) were run for 3,000,000 generations on the partitioned concatenated dataset, with a sample tree saved every 100 generations and the burnin set to 25%. Likelihood scores stabilized at a value of 0.005774. Consensus trees were visualized in FigTree v.1.3.1 (Rambaut, 2009). In addition, gene trees (H3 and concatenated 16S+COI) were constructed with the same parameters as for the concatenated three marker dataset (see online supplementary material Figs S1 and S2).

An analysis was also conducted in MEGA 6.06 (Tamura *et al*., 2013) to estimate the evolutionary divergence over sequence pairs between *a priori* determined groups (MCL model, 1000 bootstraps). Only specimens for which sequences for all three markers were obtained were included in the analysis (n=40).

Results

Molecular analyses

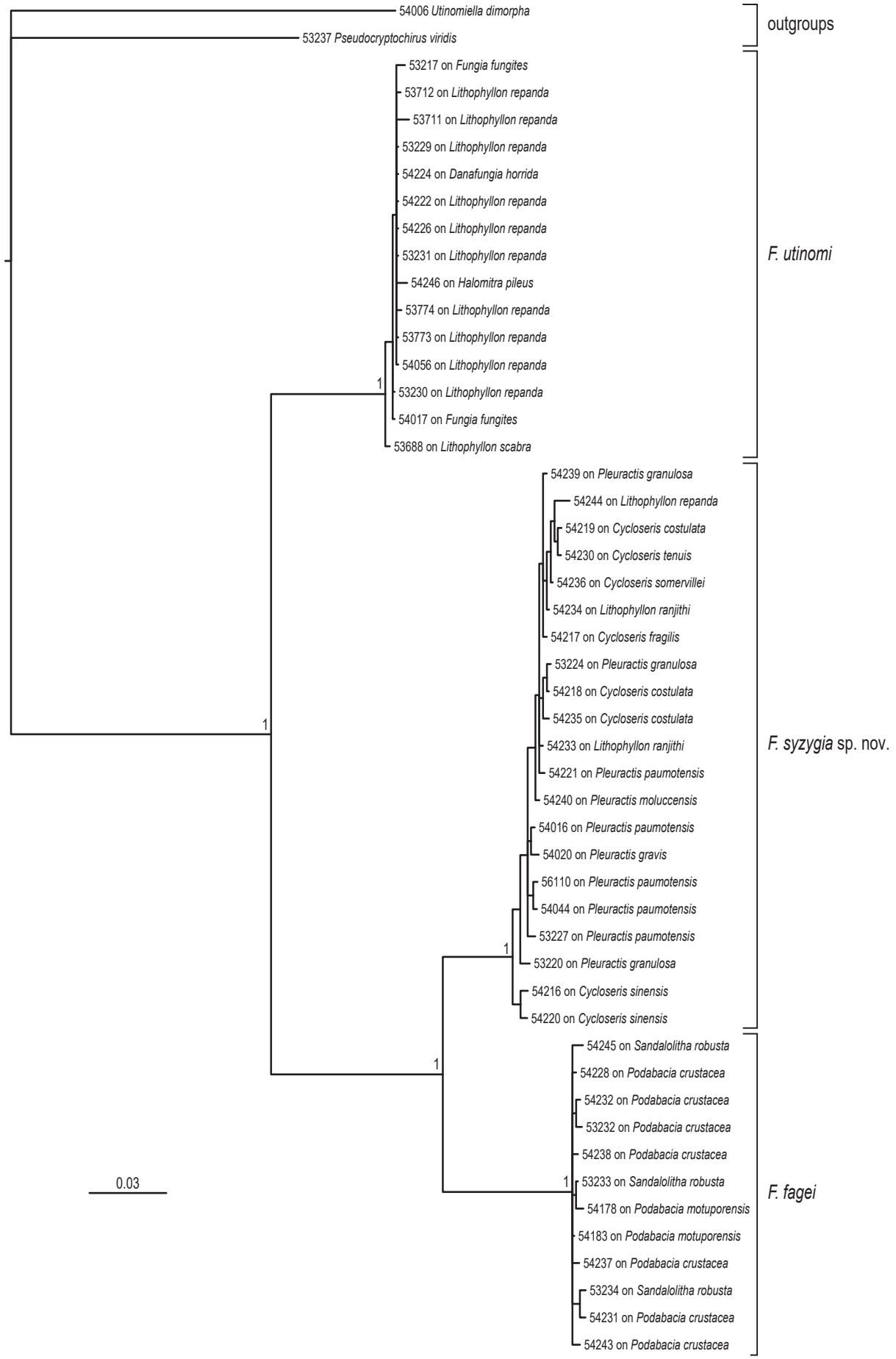
The analysis in MrBayes resulted in a tree with high posterior probabilities. Based on the results of this analysis and host specificity data a cryptic species of *Fungicola* was revealed, closely related to *Fungicola fagei* (Fig. 2). This new species is described as *F. syzygia* sp. nov. The concatenated 16S + COI mtDNA (Fig. S1) has the same topology as the three marker phylogeny reconstruction, the H3 gene tree (Fig. S2) recovers *F. utinomi* as monophyletic, but does not distinguish between *F. fagei* and *F. syzygia* sp. nov.

Table 1. Estimates of evolutionary divergence based on 16S, COI and H3. The number of base substitutions per site averaging over all sequence pairs **between** groups determined *a priori* is shown below the diagonal. Standard error (SE) estimates are shown above the diagonal.

	F. fagei	*F. syzygia* sp. nov.	*F. utinomi*
Fungicola fagei (n=10)		0.015	0.025
F. syzygia sp. nov. (n=21)	0.064		0.022
F. utinomi (n=9)	0.110	0.092	

Table 2. Estimates of evolutionary divergence based on 16S, COI and H3. The number of base substitutions per site averaging over all sequence pairs **within** groups determined *a priori* and the standard error (SE) estimates are shown.

	d.	SE
Fungicola fagei (n=10)	0.003	0.001
F. syzygia sp. nov. (n=21)	0.004	0.001
F. utinomi (n=9)	0.001	0.001


54006 Utinomiella dimorpha
53237 Pseudocryptochirus viridis
outgroups
53217 on Fungia fungites
53712 on Lithophyllon repanda
53711 on Lithophyllon repanda
53229 on Lithophyllon repanda
54224 on Danafungia horrida
54222 on Lithophyllon repanda
54226 on Lithophyllon repanda
53231 on Lithophyllon repanda
54246 on Halomitra pileus
53774 on Lithophyllon repanda
53773 on Lithophyllon repanda
54056 on Lithophyllon repanda
53230 on Lithophyllon repanda
54017 on Fungia fungites
53688 on Lithophyllon scabra
F. utinomi
54239 on Pleuractis granulosa
54244 on Lithophyllon repanda
54219 on Cycloseris costulata
54230 on Cycloseris tenuis
54236 on Cycloseris somervillei
54234 on Lithophyllon ranjithi
54217 on Cycloseris fragilis
53224 on Pleuractis granulosa
54218 on Cycloseris costulata
54235 on Cycloseris costulata
54233 on Lithophyllon ranjithi
54221 on Pleuractis paumotensis
54240 on Pleuractis moluccensis
54016 on Pleuractis paumotensis
54020 on Pleuractis gravis
56110 on Pleuractis paumotensis
54044 on Pleuractis paumotensis
53227 on Pleuractis paumotensis
53220 on Pleuractis granulosa
54216 on Cycloseris sinensis
54220 on Cycloseris sinensis
F. syzygia sp. nov.
54245 on Sandalolitha robusta
54228 on Podabacia crustacea
54232 on Podabacia crustacea
53232 on Podabacia crustacea
54238 on Podabacia crustacea
53233 on Sandalolitha robusta
54178 on Podabacia motuporensis
54183 on Podabacia motuporensis
54237 on Podabacia crustacea
53234 on Sandalolitha robusta
54231 on Podabacia crustacea
54243 on Podabacia crustacea
F. fagei
1
1
1
1
0.03

The evolutionary divergence over sequence pairs was estimated for *Fungicola fagei*, *F. syzygia* sp. nov. and *F. utinomi*. In this analysis, the groups were determined *a priori* based on Fig. 2. There is about a 6% difference between *F. fagei* and *F. syzygia* sp. nov. and an 11% difference between *F. fagei* and *F. utinomi*. *F. syzygia* sp. nov. and *F. utinomi* have a difference of 9.2% (Table 1). Table 2 shows the intraspecific difference within the three species, which ranges from 0.1 to 0.4%.

Host specificity

Different host occurrences for *Fungicola fagei* and its sibling species *F. syzygia* sp. nov. (Fig. 2, Table S3) were observed. *Fungicola fagei* occurs only in corals belonging to *Podabacia* or *Sandalolitha*. *Fungicola syzygia* sp. nov. is predominantly associated with *Cycloseris* and *Pleuractis*, with a few records from *Lithophyllon*. *Fungicola utinomi* is mostly found in association with species of *Danafungia*, *Fungia*, *Halomitra*, and *Lithophyllon*, but its most common host is *L. repanda*.

Systematic account

Family Cryptochiridae Paul'son, 1875

Genus *Fungicola* Serène, 1967

Fungicola.— Fize and Serène, 1957: 122 [name unavailable]
Fungicola Serène, 1967: 396
Fungicora.— Takeda and Tamura, 1986: 64 [erroneous spelling]

Type species. *Troglocarcinus utinomi* Fize and Serène, 1956: 377.

Diagnosis. Carapace rectangular to squaroid, longer than broad, widest anterior to midlength, flat in lateral view, not deflected anteriorly, surface covered with granules, mesogastric region slightly inflated, cardiointestinal region outlined; pterygostomial region fused to carapace; epistome with parallel lateral ridges; lateral lobe of antennule oval, extending slightly beyond eyestalk; antennal segment two longer than broad, distal margin lacking lateral spine; MXP3 with exopod, merus with small distolateral projection, mesial margin granulated, with setae; ventral thorax longer than broad; P1 cutting edges entire; P2 merus with distomesial projection; P3-4 coxae with well-developed anterior lobes; PLP3 of female uniramous; male abdomen triangle shaped (adapted from Kropp, 1990a).

Remarks. Fize and Serène (1957) described the subgenus *Fungicola* in the genus *Troglocarcinus* based on the following characteristics: 1) flat carapace on the dorso-ventral side, 2) flat dorsal surface in the shape of a spatula without much relief, 3) anterior-lateral angle shaped as a large round lobe, 4) rhomboid outline of the male abdomen, with the segments 3-5 enlarged. Fize and Serène unfortunately did not designate a type species. Serène (1967) elevated the subgenus to genus level and designated *T. utinomi* as the type species. Species in the genus *Fungicola* are only known to occur in association with fungiid corals.

◀ **Fig. 2.** Phylogeny reconstruction of the genus *Fungicola* based on a concatenated dataset of 16S and COI mtDNA and H3 nDNA of 50 taxa (including outgroups). Topology derived from Bayesian inference 50% majority rule, significance values are posterior probabilities. Numbers refer to collection numbers (RMNH. Crus.D).

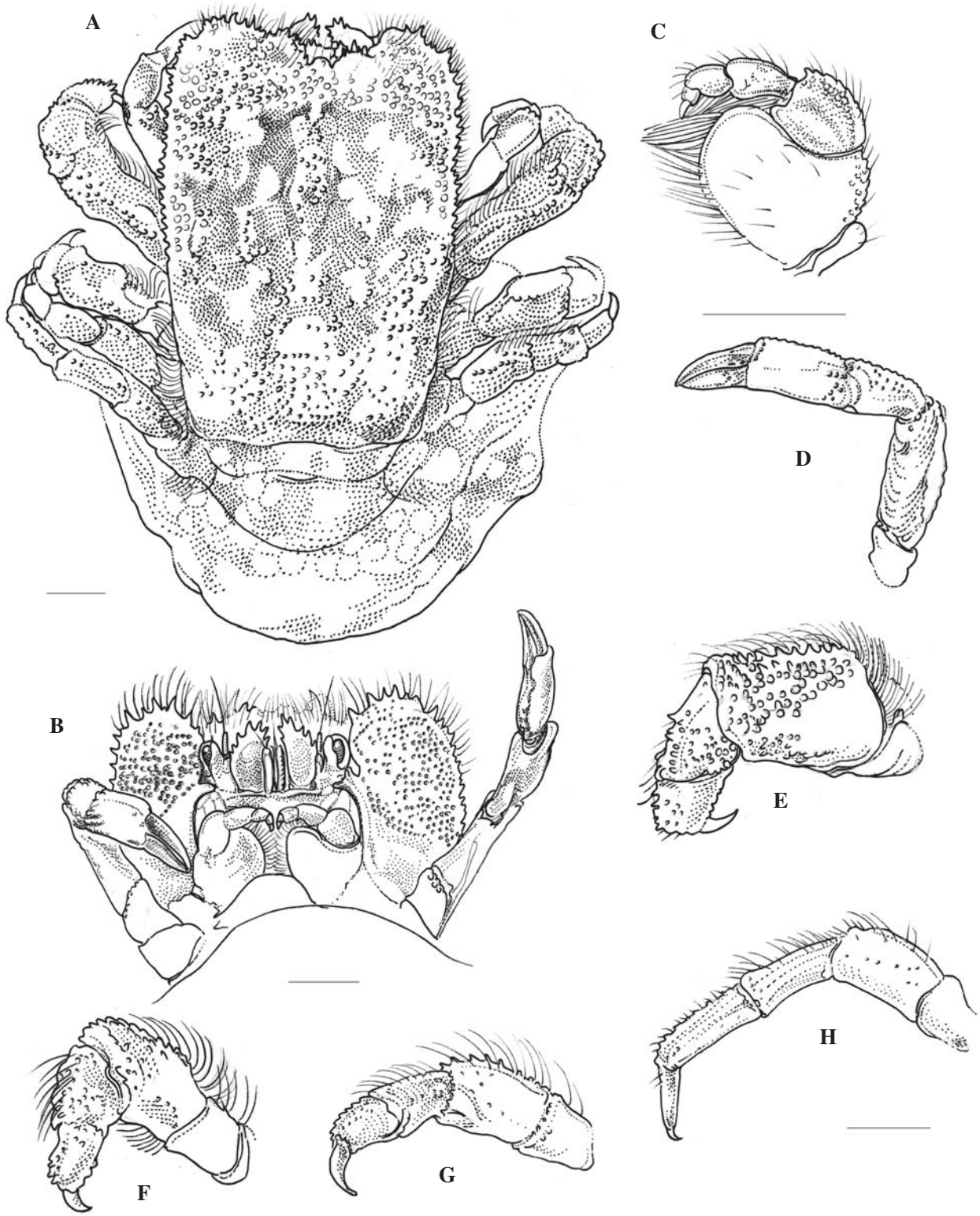


Fig. 3. *Fungicola utinomi* (RMNH.Crus.D.53229). **A**, habitus, dorsal view; **B**, anterolateral margin of carapace, ventral view; **C**, MXP3; **D**, left P1 (cheliped); **E**, left P2; **F**, left P3; **G**, left P4; **H**, left P5. Scale bars 1 mm, D-H share scale bar.

Fungicola utinomi (Fize and Serène, 1956)
Figs 1C-I, 3A-H

Troglocarcinus utinomi Fize and Serène, 1956: 377, fig. 2E
Troglocarcinus (*Fungicola*) *utinomi*.— Fize and Serène, 1957: 124
Fungicola utinomii.— Serène, 1967: 396 [unjustified emendation]
Pseudocryptochirus ishigakiensis Takeda and Tamura, 1979: 188
Hiroia ishigakiensis.— Takeda and Tamura, 1981: 20

Type locality. Nha Trang, Vietnam.

Holotype. E.37.277 ('Type' according to Fize and Serène, 1957) in the Institute of Oceanography in Nha Trang, Vietnam; allotype: E.37.766 (Fig. 2C-I), collected from *Fungia fungites* (Linnaeus, 1758).

Material examined. Institute of Oceanography (Nha Trang, **Vietnam)**: label E.37.277 (Rte 1560), in jar E.37.277, poor condition, **holotype**, 1 ovig. f; label E.37.183 (Rte 1555), in jar E.37.183 (Rte 1555) – E.37.776 (Rte 1569), good condition; label E.37.184 (Rte 1555), in jar E.37.183 (Rte 1555) – E.37.776 (1569), empty vial; label E.37.271 (Rte 1560), in jar E.37.898-38.276 (Rte 1589), residue; label E.37.272 (Rte 1560), in jar E.37.898- 38.276 (Rte 1589), empty vial; label E.37.275 (Rte 1560), in jar E.37.898-38.276 (Rte 1589), residue, on *Fungia* sp.; label E.37.276 (Rte 1560), in jar E.37.183 (Rte 1555) – E.37.776 (Rte 1569), reasonable condition; label E.37.283 (Rte 1560), in jar E.39.041 (1635) - E.39.217 (1641), good condition; label E.37.554 (Rte 1569), in jar E.37.183 (Rte 1555) – E.37.776 (Rte 1569), reasonable condition; label E.37.672 (Rte 1579), in jar E.39.041 (1635) - E.39.217 (1641), fair/reasonable condition; label E.37.673 (Rte 1579), in jar E.39.041 (1635) - E.39.217 (1641), good condition; label E.37.763 (Rte 1582), in jar E.39.041 (1635) - E.39.217 (1641), translucent but fair condition; label E.37.764 (Rte 1582), in jar E.39.041 (1635) - E.39.217 (1641), good condition; label E.37.765 (Rte 1582), in jar E.39.041 (1635) - E.39.217 (1641), fair condition; label E.37.766 (Rte 1569), in jar E.37.183 (Rte 1555) – E.37.776 (Rte 1569), fair condition, some pereiopods missing, **allotype**; label E.37.818 (Rte 1585), in jar E.39.041 (1635) - E.39.217 (1641), good condition; label E.37.898 (Rte 1589), in jar E.37.898-38.276 (Rte 1589), translucent and poor condition; label E.39.203 (Rte 1641), in jar E.37.183 (Rte 1555) – E.37.776 (1569), fair; label E.39.206 (Rte 1641), in jar E.39.041 (1635) - E.39.217 (1641), good condition; label E.39.207 (Rte 1641), in jar E.37.898- 38.276 (Rte 1589), residue, on *Fungia* sp.; label unreadable, in jar E.39.041 (1635) - E.39.217 (1641), good condition; jar E.39.041 (1635) - E.39.217 (1641) contains labelE.39.205 (Rte 1641) but no vial. **Muséum national d'Histoire naturelle (Paris**, **France)**: MNHNIU- 2014-10108 (was E.38.333 (Rte 1614)), good condition, 1 ovig. f, on *Fungia* [= *Lithophyllon*] *repanda*; MNHNIU- 2014-10107 (was E.38.425), good condition, 1 m, on *Fungia* [= *Lithophyllon*] *repanda*. **Natural History Museum (London**, **UK)**: BMNH.1958.1020.17-18 contains: E.39.336 (Rte 1644), good condition, 1 ovig. f, on *Fungia* sp.; E.39.337 (Rte 1644), good condition, 1 m, on *Fungia* sp. **Naturalis Biodiversity Center (Leiden**, **The Netherlands)**: see Table S3.

Diagnosis. Carapace rectangular, longer than broad, depressed, anterior half broader than posterior half, surface covered with small, low granules in posterior half, larger and rather spiniform in anterior; median gastric region moderately convex and its posterior part only indistinctly separated from cardio-intestinal region, branchial regions hardly separated from both, mid-gastric, cardio-intestinal regions by very shallow furrows; front moderately concave, armed with spines of different size; internal orbital nearly reaching level of external; antero-lateral borders armed

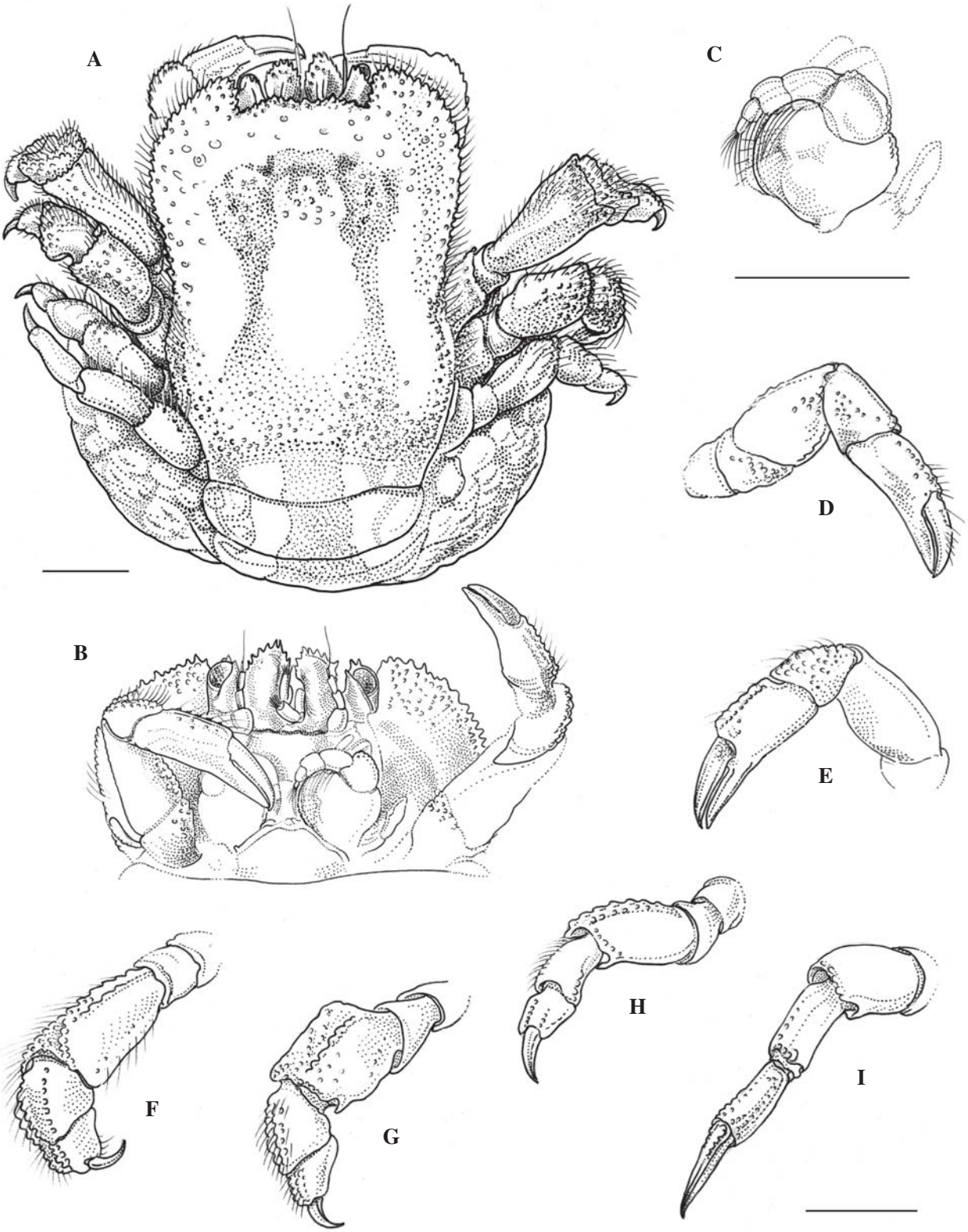


Fig. 4. *Fungicola fagei* (RMNH.Crus.D.53234). **A**, habitus, dorsal view; **B**, anterolateral margin of carapace, ventral view; **C**, MXP3; **D**, right P1 (cheliped); **E**, left P1 (cheliped); **F**, left P2; **G**, left P3; **H**, left P4; **I**, left P5. Scale bars 1 mm, B, D-I share a scale bar.

with spines and forming a convex lobe, lateral borders behind it straight or slightly converging. Cheliped slender; upper and external faces of merus, carpus and palm covered with fine granules. Upper face of coxae of pereiopods 3-4 with small forward protruding lobe. Carapace with striking brown, white patterns (visible in ethanol), pereiopods banded. For a full description see Fize and Serène (1957); for a colour figure of *F. utinomi* see Van der Meij and Schubart (2014: Fig. 1).

Host corals. *Fungicola utinomi* inhabits at least nine coral species belonging to eight genera, but is most often recorded from *Lithophyllon repanda* (Fig. 2, Table S3). In *L. repanda*, *F. utinomi* lives in an oval cavity with overhanging canopy, which can be raised above the coral surface (see Fize and Serène, 1957: Pl. XIII A-C; Takeda and Tamura, 1979: Pl. 1A-D, Pl. 2A-D).

Distribution range. *Fungicola utinomi* has so far been documented from Nha Trang, Vietnam (Fize and Serène, 1957); Maluku, Indonesia (Rumphius I expedition, Serène *et al*., 1974); Semporna, Malaysia (van der Meij and Hoeksema, 2013); Ishigaki-jima and Kuroshima, Okinawa, Japan (Takeda and Tamura, 1979); and Palau and Guam in Micronesia (Kropp, 1990a). New records from this study include Raja Ampat, Manado, Lembeh and Ternate in Indonesia, and Payar Isl. and Kudat in Malaysia.

Remarks. According to Kropp (1990a) the location of the holotype is unknown; however, the holotype and the allotype were located in the Institute of Oceanography in Nha Trang (Fig. 2C-F).

Fungicola fagei (Fize and Serène, 1956)
Figs 1A-B, 4A-I, 7F-G

Troglocarcinus fagei Fize and Serène, 1956: 378, fig. 2F
Troglocarcinus (*Fungicola*) *fagei*.— Fize and Serène 1957: 131

Type locality. Nha Trang, Vietnam
Holotype. E.38.444 ('Type' according to Fize and Serène, 1957) in the Institute of Oceanography in Nha Trang, Vietnam (Fig. 2A-B); allotype: E.37.727, collected from *Sandalolitha dentata* Quelch, 1884 (but see section on host corals).

Material examined. Institute of Oceanography (Nha Trang, Vietnam): E.38.444 (Rte 1614), no label on jar, good condition, **holotype**, 1 ovig. f; E.37.458 (Rte 1569), in jar E.37.458, residue; E.37479 (Rte 1569), in jar E.37479-39.215 (Rte 1641), empty vial; E.37.727 (Rte 1581), in jar E.37.479-39.215 (Rte 1641), empty vial, **allotype**; E.39.210 (Rte 1641), in jar E.37.458, poor condition; E.39.213 (1641), in jar E.37.479-39.215 (Rte 1641), empty vial, on *Parahalomitra* [= *Sandalolitha*] *robusta*; E.39.215 (Rte 1641), in jar E.37.479-39.215 (Rte 1641), empty vial, on *Parahalomitra* [= *Sandalolitha*] *robusta*; jar E.37.479-39.215 (Rte 1641) contains one label without vial, E.38.334 (Rte 1614). **Muséum national d'Histoire naturelle** (**Paris**, **France)**: MNHN-IU-2009-2245 (was E.37.729 (Rte 1581)), pereiopods present but not attached to carapace, 1 m, on *Parahalomitra* [= *Sandalolitha*] *robusta*; MNHN-IU-2009-2246 (was E.37.728 (Rte 1581), good condition, 1 ovig. f, on *Parahalomitra* [= *Sandalolitha*] *robusta*. **Naturalis Biodiversity Center** (**Leiden**, **The Netherlands)**: see Table S3.

Diagnosis. Carapace subrectangular, longer than broad, depressed, anterior half broader than posterior half, surface covered with fine granules; median gastric region feebly convex, its posterior part not separated from cardio-intestinal region, branchial regions separated from mid-gastric and cardio-intestinal regions by shallow furrows; front moderately concave; internal orbital angle falling much shorter than external; antero-lateral borders armed with spinules, moderately

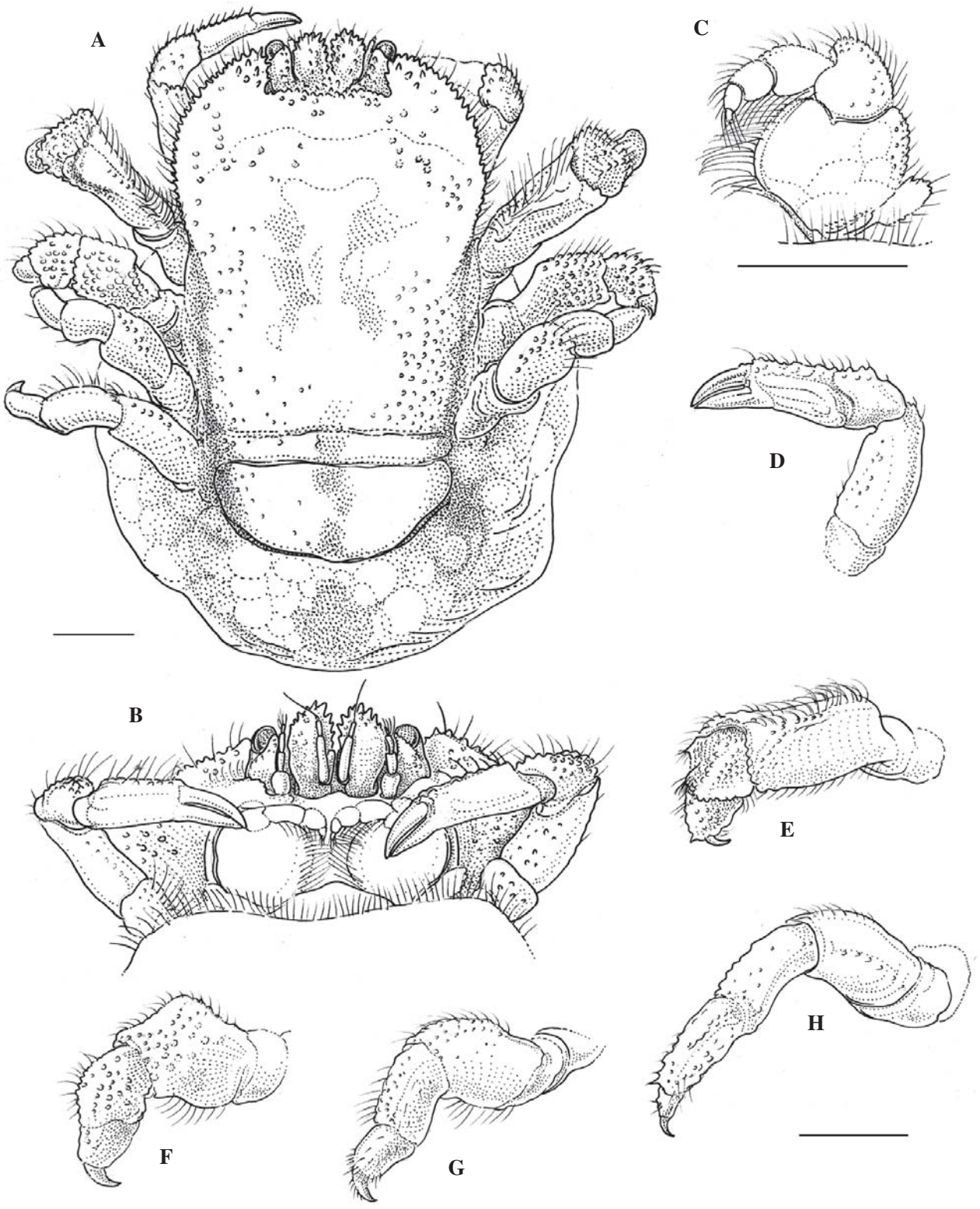


Fig. 5. Holotype *Fungicola syzygia* sp. nov. (RMNH.Crus.D.53220). **A**, habitus, dorsal view; **B**, anterolateral margin of carapace, ventral view; **C**, MXP3; **D**, left P1 (cheliped) **E**, left P2; **F**, left P3; **G**, left P4; **H**, left P5. Scale bars 1 mm, B, D-H share scale bar.

convex, lateral borders behind it slightly concave. Cheliped notstout; merus as well as upper borders of carpus, palm covered with fine granules. Upper face of coxae of third to fifth pereiopods with small forward protruding lobe. Carapace, pereiopods a dull beige-grey, semi-translucent. For a complete description of *F. fagei* see Fize and Serène (1957).

Host corals. *Fungicola fagei* can be found inhabiting flattened pits lodged between septae (see Fize and Serène, 1957: Pl. XIII D-F). According to Fize and Serène (1957) *Fungicola fagei* is associated with *Parahalomitra robusta* [= *Sandalolitha robusta*]. Based on Pl. XIII, fig. D-E in Fize and Serène (1957), however, this coral species should instead be identified as *Sandalolitha dentata* (B.W. Hoeksema, pers. comm.). The host genera of *F. fagei* are the attached *Podabacia* and the free-living *Sandalolitha*, two closely related genera. *Podabacia crustacea* is the most common host, and *P. sinai* is a new host for *F. fagei* (Table S3).

Distribution range. *Fungicola fagei* has is so far known from Nha Trang, Vietnam (Fize and Serène, 1957); Maluku, Indonesia (Serène *et al*., 1974); Semporna, Malaysia (van der Meij and Hoeksema, 2013); and Palau and Guam in Micronesia (Kropp, 1990). New records herein are New Caledonia and Raja Ampat and Ternate in Indonesia. A record of *F. fagei* from Japan by Takeda and Tamura (1979), collected from *Fungia* [= *Pleuractis*] *paumotensis*, most likely constitutes *F. syzygia* sp. nov. (see remarks in the species description of *F. syzygia* sp. nov).

Remarks. Kropp (1990a) stated that the location of the holotype was unknown; however, the type was located in the Institute of Oceanography in Nha Trang and is in relatively good condition. The allotype was not found and is considered lost. Out of the other material mentioned by Fize and Serène only sample E.39.210 was located, samples E.37.458, E.37.471, E.37.727, E.37.729, E.38.334 and E.39.211-215 appear to be lost.

Fungicola syzygia sp. nov.
Figs 5A-H, 6A-K, 7A-E

?Fungicola fagei.— Takeda and Tamura (1979: Fig. 2A-D)
?Fungicola sp.— Ng *et al*. (2008: Fig. 156)

Type locality. Sulamadaha Bay, Ternate, Indonesia (N 00°51’58”, E 127°19’53”)

Type material. Naturalis Biodiversity Center (Leiden, The Netherlands). **Holotype**: RMNH.Crus.D.53220, host *Pleuractis granulosa* (Klunzinger, 1879), 26.x.2009, 1 ovig. female (4.0 × 3.8), leg. SET van der Meij / BW Hoeksema (the holotype will be deposited in the **Museum Zoologicum Bogoriense, Bogor, Indonesia** with catalogue number MZB Cru 4130); **allotype**: RMNH.Crus.D.53224 (male), Tanjung Pasir Putih, Ternate, Indonesia (N 00°51’50”, E 127°20’ 36”), host *Pleuractis granulosa* (Klunzinger, 1 879), 0 2. xi.2009, 1 male (2.5 × 2.3), leg. SET van der Meij; **paratypes**: RMNH.Crus.D. 53225, Tanjung Ratemu (S of river), Ternate, Indonesia (N 00°54’24” E 127°29’17”), host *Pleuractis granulosa* (Klunzinger, 1879), 05. xi.2009, 1 non-ovig. female (4.3 × 4.3), leg. SET van der Meij; RMNH.Crus.D.53226, Pilongga N, Tidore, Indonesia (N 00°42’49” E 127°28’45”), host *Pleuractis granulosa* (Klunzinger, 1879), 12.xi.2009, 1 ovig. female (5.1 × 4.6), leg. SET van der Meij; **Lee Kong Chian Natural History Museum (Singapore)**. ZRC 2015.0006 (ex. RMNH.Crus.D.53219), Sulamadaha Bay, Ternate, Indonesia (N 00°51’58”, E 127°19’53”), host *Pleuractis granulosa* (Klunzinger, 1879), 26.x.2009, 1 ovig. female (5.3 × 5.3), leg. BW Hoeksema.

Material examined. Naturalis Biodiversity Center (Leiden, The Netherlands): see Table S3.

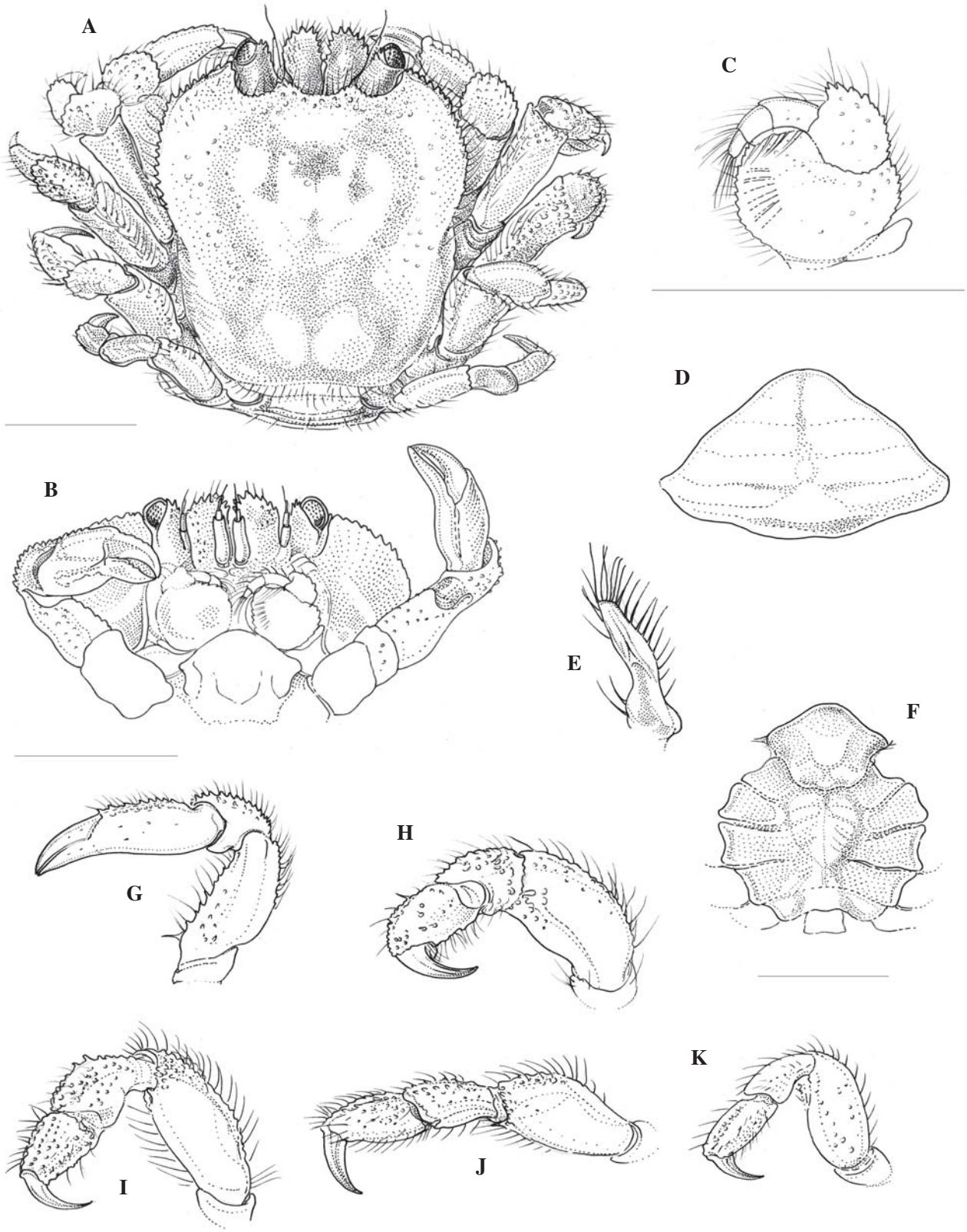


Fig. 6. Allotype *Fungicola syzygia* sp. nov. (RMNH.Crus.D.53224). **A**, habitus, dorsal view; **B**, anterolateral margin of carapace, ventral view; **C**, MXP3; **D**, abdomen; **E**, gonopod; **F**, thoracic sternites; **G**, left P1 (cheliped); **H**, left P2; **I**, left P3; **J**, left P4; **K**, left P5. Scale bars 1 mm, B, G-K as well as C-D and E-F share scale bar.

Description of holotype. Carapace (Fig. 5A) subrectangular to squaroid, longer than broad, anterior half rounded, broader than posterior half, surface covered with fine granules; flat in lateral view, not deflected anteriorly; cardio-intestinal region outlined; sharp internal orbital angle; frontal margin, anterolateral borders armed with spinules, few setae; anterolateral border moderately convex; lateral borders not clearly defined, somewhat concave. Pterygostomial region fused to carapace.

Ocular peduncles (Fig. 5B) granulated on distal margin, cornea round to oval, longer than broad; antennule same length as ocular peduncles; antennal segment 2.5 times longer than broad, extending beyond eyestalk, distal margin with several lateral spines.

MXP3 (Fig. 5C) exopod subrectangular, reaching approx. ½ length of ischium, with tubercles and setae; ischium subquadrangular, smooth, mesial and distal margin slightly rounded, anteromesial lobe with setae, distal margin with tubercles; merus with distolateral projection, anterolateral margin of merus with tubercles and setae; distal portion of carpus with tubercles and setae; dactylus with bundle of long setae.

P1 (chelipeds, Fig. 5D) slender; ischium length ½ height; merus length twice height with scattered small tubercles and few short setae; carpus with pronounced granules on distal margin, propodus with granules on distal margin, fingers slender, mesial surfaces of fingers smooth, cutting edge entire.

P2 (Fig. 5E) coarser than P1; ischium without setae; merus stout, two times longer than broad, few, small conical tubercles on distal half of dorsal surface, simple short setae on lateral, dorsal surface; carpus surface granulated, with setae; propodus about as long as carpus, surface granulated, fine scattered setae, dactylus smooth, sharp, curved ventrally.

P3 (Fig. 5F) ischium without setae; merus stout and rounded, prominent distomesial projection, surface with tubercles, simple setae; carpus not extending more than at right angle; carpus bend; carpus, propodus roughly of equal length, rounded tubercles on dorsal surface, simple setae on lateral, dorsal surface; dactylus halflength of propodus, smooth, sharp, curved ventrally.

P4 (Fig. 5G) ischium without setae; merus stout and rounded, small distomesial projection, surface with tubercles and simple setae; carpus not extending more than at right angle; carpus bend; carpus, propodus roughly of equal length, rounded tubercles on dorsal surface, simple setae on lateral, dorsal surface; dactylus half-length of propodus, smooth, sharp, curved ventrally, with setae.

P5 (Fig. 5H) slender; ischium without setae; merus, carpus, propodus of equal length; merus slightly bend, very few scattered tubercles and setae, carpus, propodus with sharp tubercles on dorsal surface; dactylus ⅓ length of propodus, smooth, sharp, straight, with few setae. P-5 right sampled for DNA analysis.

Abdomen (= pleon) enlarged, lateral margin fringed with setae (Fig. 5A-B).

Description allotype. Carapace (Fig. 6A) subrectangular to squaroid, slightly longer than broad, anterior half broader than posterior half, surface covered with fine granules; flat in lateral view, not deflected anteriorly; cardio-intestinal region outlined; internal orbital angle rounded; frontal margin and anterolateral borders armed with spinules, few setae; anterolateral border moderately convex; lateral borders not clearly defined, somewhat concave. Pterygostomial region fused to carapace.

Ocular peduncles (Fig. 6A-B) with small spines on distal margin, cornea round to oval, longer than broad; antennule shorter than ocular peduncles; antennal segment two longer than broad, slightly extending beyond eyestalk, distal margin with several lateral spines.

MXP3 (Fig. 6C) exopod subrectangular, reaching approx. ½ length of ischium, with tubercles, setae; ischium subquadrangular, smooth, mesial and distal margin slightly rounded, anteromesial

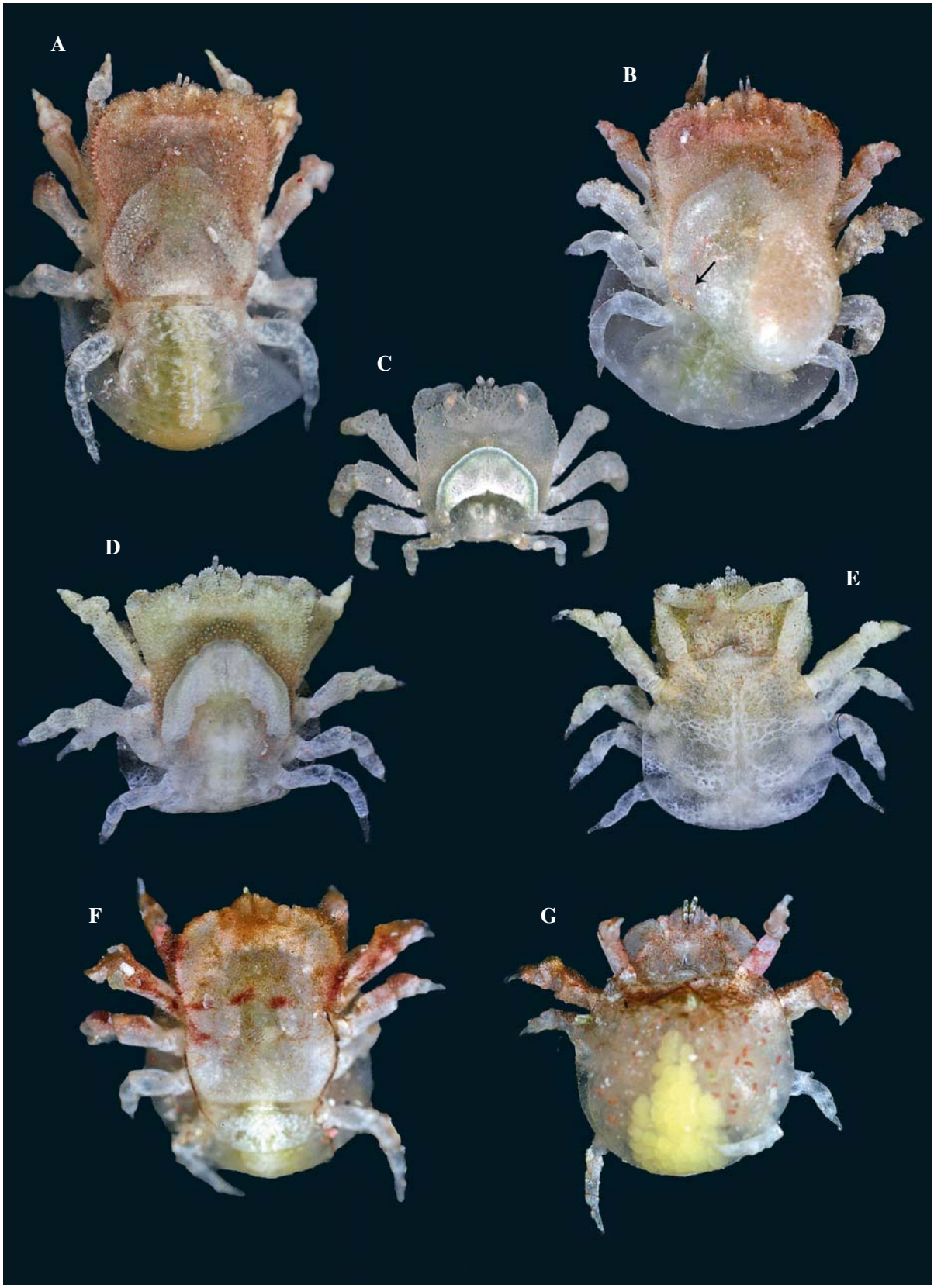


Fig. 7. *Fungicola syzygia* sp. nov., colouration in life. **A**, RMNH.Crus.D.53225, ovigerous female; **B**, RMNH.Crus.D.56481, non-ovigerous female parasitized by the isopod *Carcinione platypleura* Bourdon, 1983 (Isopoda: Bopyridae), male isopod indicated by arrow; **C**, RMNH.Crus.D.56479 male; **D-E**, RMNH. Crus.D.56480, non-ovigerous female. *Fungicola fagei*, colouration in life; **F-G**, RMNH.Crus.D.53234, ovigerous female.

lobe with setae, distal margin with tubercles; merus with distolateral projection, anterolateral margin of merus with tubercles, setae; distal portion of carpus with tubercles, setae; dactylus with bundle of long setae.

P1 (chelipeds, Fig. 6G) slender; ischium length ⅓ height; merus with scattered small tubercles, few simple setae; carpus, propodus with pronounced granules on distal margin, fingers slender, mesial surfaces of fingers smooth, cutting edge entire.

P2 (Fig. 6H) ischium without setae; merus stout, two times longer than broad, few, small conical tubercles on distal half of dorsal surface, simple short setae on lateral, dorsal surface; carpus surface granulated, with setae; propodus about as long as carpus, surface granulated, fine scattered setae, dactylus smooth, sharp, curved ventrally.

P3 (Fig. 6I) ischium without setae; merus surface with tubercles and setae; joint between merus, carpus not extending more than at right angle; carpus and propodus roughly of equal length, conical tubercles on spread over surface, with setae; dactylus smooth, sharp, curved ventrally.

P4 (Fig. 6J) similar to P3; ischium without setae; merus stout with distomesial projection, surface with tubercles and simple setae; joint between merus, carpus not extending more than at right angle; carpus and propodus roughly of equal length; conical tubercles on spread over surface, with setae; dactylus smooth, sharp, curved ventrally.

P5 (Fig. 6K) slender; ischium without setae; merus length twice height; carpus, propodus of equal length; merus, carpus, propodus with few scattered tubercles and setae; dactylus ½ length of propodus, smooth, sharp, curved ventrally.

Abdomen rhomboid, longest and widest at 5th segment; telson rounded (Fig. 6D).

Gonopod slightly curved laterally, broad shoulder, apex rounded, long setae. (Fig. 6E).

Colour. An overall pale beige-grey. On some specimens a horseshoe shaped pattern is visible on the carapace, more pronounced in males than in females (Fig. 7A-E). A similar pattern can occur in specimens of *F. fagei*, hence colour pattern is not a diagnostic character.

Host corals. *Fungicola syzygia* can be found inhabiting flattened pits lodged between septae (see Vehof *et al*., in press: Fig. 1A). Main host genera are *Cycloseris* and *Pleuractis*, but there are single records from other species (Fig. 2, Table S3). *Fungicola syzygia* sp. nov. (RMNH.Crus. D.54244) was found once in *Lithophyllon repanda*, the most common host of *F. utinomi* (Fig. 2). This is the fourth species recorded from mushroom corals.

Table 3. Characters distinguishing *Fungicola fagei* and *F. syzygia* sp. nov.

	F. fagei	*F. syzygia* sp. nov.
host genus	*Podabacia*, *Sandalolitha*	*Cycloseris*, *Pleuractis*
carapace shape	subrectangular	subrectangular to squaroid
CL / CW (all)	1.12 ± 0.10 (n=10)	1.02 ± 0.07 (n=20)
CL / CW (ovig. f)	1.13 ± 0.06 (n=7)	1.02 ± 0.08 (n=11)
CL / CW (m)	1.33 (n=1)	1.02 ± 0.03 (n=5)
border between orbital angles (f)	(mildly) undulating	straight to mildly undulating
lateral margin carapace	somewhat defined, less concave than *F. syzygia* sp. nov.	not well-defined, more concave than *F. fagei*
merus MXP3	modest distolateral projection	pronounced distolateral projection

Diagnostic characters. *Fungicola fagei* and *F. syzygia* sp. nov. can be separated based on their host specificity and DNA. For the latter, based on the results of this study, a positive identification can be achieved by analysis of a single mitochondrial marker. *F. fagei* and *F. syzygia* sp. nov. are more difficult to separate based on morphology. Both species show considerable intraspecific variation in the shape (angle) of the external orbital angle and the border between the orbital angles. Intraspecific variation is also observed on the lateral margins. In general, in *F. syzygia* sp. nov. the lateral margins are more concave then in *F. fagei*. Based on the carapace length / carapace width ration, it is clear that *F. fagei* is more rectangular than *F. syzygia* sp. nov., which is almost squaroid. The one available male of *F. fagei* is somewhat of an outlier, perhaps the results of sexual dimorphism. After removal of this single male from the CL / CW (all) category the mean ratio becomes 1.10 ± 0.08 (n=9) (Table 3).

Distribution range. *Fungicola syzygia* is so far known from various locations in Indonesia, Malaysia and the Maldives. The male specimens of Takeda and Tamura (1979) may belong to the new species *F. syzygia* sp. nov. (see remarks), which would add Japan to the known distribution range if the identification were confirmed. The figured specimen in Ng *et al*. (2008) was collected in Vanuatu (see remarks). The distribution of *F. syzygia* sp. nov. possibly overlaps the distribution of their host corals (for fungiid distributions see Hoeksema (1989)), but large areas of their host's distribution have not yet been examined for gall crabs.

Remarks. A sibling species is a cryptic sister species; two species that are the closest relative of each other and have not been distinguished from one another taxonomically (Bickford *et al*., 2007). *Fungicola syzygia* sp. nov. is therefore a sibling species of *F. fagei*. It is the third species assigned to the genus *Fungicola*. Previous workers most likely did not encounter this new species in their material. Fize and Serène (1957) collected *F. fagei* specimens from *S. dentata* (and possibly *S. robusta*; see host corals section of *F. fagei*), and did not collect gall crabs from *Cycloseris* or *Pleuractis*. Their material therefore most likely consists of solely *F. fagei*. Specimens of *F. fagei* were furthermore collected during the Rumphius I and II expeditions to Indonesia. Kropp (1994) re-examined the Rumphius material, but mentioned that the specimens of *F. fagei* were no longer available and that the host corals were not included. Takeda and Tamura (1979) did collect two male specimens, which, based on the host specificity data (*Fungia paumotensis* [= *Pleuractis paumotensis*]) and the illustrations, possibly could be identified as *Fungicola syzygia* (National Museum of Nature and Science Tokyo, NSMTCr. 5896/7). Ng *et al*. (2008: Fig. 156) illustrated a specimen of *Fungicola* sp., collected from *P. paumotensis* (B.W. Hoeksema, pers. comm.) in Santo, Vanuatu. *Pleuractis paumotensis* is only known to host *Fungicola syzygia*, therefore this material is tentatively included in the synonymy of this species.

Etymology. Syzygia in reference to the type locality Ternate, once the world's major producer of cloves (*Syzygium aromaticum* L. Merr. and Perry). The Latin syzygia is derived from the ancient Greek συζυγία [suzugia], for a pair of related things or union, referring to the obligate symbiosis between the gall crab and its host coral.

Discussion

Species having a small size or hidden habitat are often referred to as 'cryptic'. Although gall crabs are small and live a hidden life inside their corals hosts and hence 'cryptic', cryptic species is herein referred to as two or more species that were previously classified as a single one because of (at least superficial) morphological similarities (Bickford *et al*., 2007). This definition is applicable to *F. syzygia* sp. nov., which is difficult to separate from *F. fagei* based on morphology alone. DNA taxonomy is becoming an indispensable tool for unravelling cryptic speciation. This especially

holds true for endosymbionts which have prolonged relationships with their hosts that can lead to morphological stasis (e.g. Gittenberger and Gittenberger, 2010).

Fungicola syzygia sp. nov. and *F. fagei* are clearly separated based on DNA and host specificity (Fig. 2). *Fungicola syzygia* sp. nov. and *F. fagei* are more closely related to each other than to *F. utinomi*. The intraspecific variation is low (Tables 1-2). The mitochondrial markers 16S and COI showed high resolution at species level (Fig. S1), contrary to the nuclear marker H3 (Fig. S2). The latter marker recovered *F. utinomi* as a monophyletic clade, except for RMNH.Crus.D.54246, which clustered basally to all other specimens. H3 did not distinguish between *F. fagei* and *F. syzygia* sp. nov. A study by Dinapoli *et al*. (2007) showed that H3 is most informative on genus level in heterobranch Gastropoda, making this marker a possible candidate for further reconstructions of the deeper phylogenetic relationships within the Cryptochiridae.

Fungicola syzygia sp. nov. and *F. fagei* can also be separated based on their host occurrence. The usual pattern among host-specific organisms is a common or 'preferred' host, with a number of other hosts less frequently inhabited (Norton and Carpenter, 1998). This seems to be the case in fungiid-associated gall crabs as well. The present results showed that *Fungicola fagei* was strictly associated with the sister genera *Podabacia* and *Sandalolitha*, with *P. crustacea* as the most common host. *Fungicola syzygia* sp. nov. is mostly associated with the genera *Cycloseris* and *Pleuractis*, with *P. granulosa* and *P. paumotensis* as most common hosts. *Fungicola utinomi* is associated with a wider range of related genera but is most often encountered in *Lithophyllon repanda* (Fize and Serène, 1957; van der Meij and Hoeksema, 2013; Fig. 2, Table S3). Hoeksema *et al*. (2012) mentioned that gall crabs are not very host specific, but at the time of writing, the new sibling species of *Fungicola fagei* was not yet discovered. It turns out that *Fungicola* species are specific to at least genus level. The specimens referred to as 'cryptochirid sp.' in Hoeksema *et al*. (2012) belonged to one of the, now four, known species associated with Fungiidae. The identifications of these specimens was hampered by being juvenile stages or needed back-up from molecular work.

Several fungiid species have so far not been found occupied by a gall crab, possibly because their coral morphology makes for an unsuitable habitat for gall crabs (e.g. long tentacles in *Heliofungia actiniformis* or very thin plate-like morphology in *Halomitra clavator* and *Zoopilus echinatus*). Other species have distributions outside the range in which the fieldwork was carried out and might therefore not yet have been found in association with gall crabs.

Kropp and Manning (1987) removed the concept of host specificity as a generic character for gall crabs, which was previously believed to be a reliable character to distinguish genera (Fize and Serène, 1957). Many changes have been recently made and proposed in scleractinian phylogeny and taxonomy, largely based on new insights coming from molecular data and microstructures (e.g. Gittenberger *et al*., 2011; Benzoni *et al*., 2012b; Budd *et al*., 2012; Arrigoni *et al*., 2014a; Huang *et al*., 2014). The inconsistencies in the generic placement of the gall crabs based on host affinity, as observed by Kropp (1990a), are therefore likely related, at least in part, to past inconsistencies in scleractinian taxonomy.

Acknowledgements

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collected cryptochirids from Fungiidae over the last years, many of which were used in this study. Charles Fransen (Naturalis) helped to search for morphological characters distinguishing *Fungicola fagei* and *F. syzygia* sp. nov. Chris Boyko (Dowling College and American Museum of Natural History) helped to identify the epicaridean parasite. Bastian Reijnen (Naturalis) is thanked for all his help during our visit to the Institute of Oceanography in Nha Trang, as well as help with photography and lab work. The beautiful line drawings in this manuscript were made by Inge van Noortwijk (Naturalis). The COI sequences were produced as part of the Naturalis Barcoding project. The fieldwork in Indonesia was organized by Naturalis and the Indonesian Institute of Sciences (LIPI), under the umbrella of Ekspedisi Widya Nusantara (E-Win). Fieldwork in Lembeh Strait in 2012 took place during a Marine Biodiversity Workshop based at the Bitung Field Station (LIPI), co-organized by Universitas Sam Ratulangi in Manado, N Sulawesi (Indonesia). I am grateful to LIPI and RISTEK for granting research permits. Bert Hoeksema (Naturalis) and Yosephine Tuti Hermanlimianto (RCO-LIPI) are acknowledged for all their efforts in organizing the various expeditions in Indonesia. The 2010 Semporna Marine Ecological Expedition was jointly organized by WWF-Malaysia, Universiti Malaysia Sabah's Borneo Marine Research Institute, Universiti Malaya's Institute of Biological Sciences and Naturalis, and was funded through WWF-Malaysia. The research permits for Malaysia were granted by the Economic Planning Unit, Prime Minister's Department, Sabah Parks and Department of Fisheries Sabah. The Tun Mustapha Park Expedition (TMPE) 2012 was jointly organized by WWF-Malaysia, Universiti Malaysia Sabah (UMS), Sabah Parks and Naturalis. TMPE was funded by the Ministry of Science, Technology and Innovation (MOSTI) and USAID Coral Triangle Support Partnership (CTSP). The research permits were granted by the Economic Planning Unit, Prime Minister's Department and Sabah Biodiversity Centre. Permits to sample from Payar Isl. were granted to Zarinah Waheed (Naturalis & Universiti Malaysia Sabah) by the Economic Planning Unit, Prime Minister's Department Malaysia, and the Department of Marine Park Malaysia. Collecting in New Caledonia (2012) was done during the mission CORALCAL 4. Provinces Sud and Nord of New Caledonia provided sampling permits. Loyalty Island samples were collected during the BIBELOT campaign in 2014 onboard RV Alis of IRD at Nouméa. Sampling permits were granted by the Loyalty Islands Province, New Caledonia. For the samples from the Maldives the help of the University of Milano - Bicocca Marine Research and High Education Centre in Magoodhoo, the Ministry of Fisheries and Agriculture, Republic of Maldives and the community of Maghoodhoo, Faafu Atoll is gratefully acknowledged. Funding for the several fieldwork trips was provided by the AM Buitendijkfonds, LB Holthuisfonds, JJ ter Pelkwijkfonds (all Naturalis), Schure-Beijerinck-Poppingfonds (KNAW), Stichting Fonds Dr C van Tussenbroek (N Ongerboerfonds), LUF International Study Fund (Leiden University) and the Van Tienhoven Foundation for International Nature Protection. Travel to the Smithsonian Institution was funded by a 2009 EDIT Women in Science Fellowship. I am grateful to Peter Castro and Roy Kropp for their constructive comments on an earlier version of this manuscript.

Appendices S1-3 are available in the digital academic repository of Naturalis Biodiversity Center.

Chapter 6

Origin and diversification of coral-dwelling gall crabs (Decapoda: Cryptochiridae)

Sancia E.T. van der Meij & Sebastian Klaus

Abstract

Coral-dwelling gall crabs (Cryptochiridae) belong to the subsection Thoracotremata, which is estimated to have originated around 108 [± 11] Mya. The age of their most recent common ancestor is, however, unknown. A selection of 38 shallow-water gall crab species belonging to 17 of the 21 currently recognised genera, including type species for all genera and representatives from the Atlantic and Indo-Pacific oceans, was therefore used in this study to estimate their origin. Divergence time estimation was performed using a Bayesian relaxed molecular clock approach in BEAST with external brachyuran substitution rates. The analysis gave total support for the monophyly of the Cryptochiridae. The age of the most recent common ancestor was estimated at 50-23 Mya (early Eocene – early Miocene). Within the Cryptochiridae three large clades could be identified, which is in congruence with the phylogeny reconstruction of their scleractinian hosts. The short branches leading to these clades suggest an accelerated radiation during the last 10-2 Mya. The estimated origin and diversification of the Cryptochiridae corresponds with a general Cenozoic diversification of reef-associated taxa in the Tethys Ocean, with representatives in the Atlantic – Indo-Pacific divergence within the genus *Opecarcinus* most likely corresponding with the Pliocene closure of the Isthmus of Panama.

Manuscript in preparation

Introduction

Cryptochiridae, commonly known as gall crabs, are obligate symbionts of stony corals (Scleractinia). They live in dwellings (galls, pits or depressions) within corals and are fully dependent on their hosts for food and protection (Potts, 1915; Kropp, 1986). The relationship between the corals and crabs is tight with a high degree of host specificity (e.g. Fize and Serène, 1957; Kropp, 1990A; van der Meij, 2015a, b). There is a striking congruence between the phylogenetic reconstructions of Scleractinia (Fukami *et al*., 2008; Kitahara *et al*., 2010) and Cryptochiridae (van der Meij and Reijnen, 2014; van der Meij, chapter 10). This association even appears to be so tight that gall crabs can be used as phylogenetic indicators of scleractinian evolution (van der Meij, chapter 10).

The family Cryptochiridae is considered to be monophyletic, but their position within the brachyuran subsection Thoracotremata remains unclear (Guinot *et al*., 2013; van der Meij and Schubart, 2014). Within the Thoracotremata a wide variety of habitats occurs, as for example is observed among: 1) intertidal or shore crabs (e.g. Grapsidae, Sesarmidae), 2) specialised mangrove and mudflat dwellers (Camptandriidae, most Ocypodidae), 3) freshwater-dependent crabs (Glyptograpsidae, certain Varunidae), 4) hydrothermal vent specialists (Xenograpsidae), and 5) permanently symbiotic crabs (Cryptochiridae, Pinnotheridae). The superficial resemblance between the latter two families (small size, large brood pouches) and their host dependency lead previous authors to believe that they are closely related (e.g. Fize and Serene, 1957), however, based on molecular analyses this appears not to be correct (Tsang *et al*., 2014; van der Meij and Schubart, 2014).

In a multi-marker paper by Tsang *et al.* (2014) the age of the most recent common ancestor (tMRCA) of Thoracotremata is estimated at 108 [± 11] Mya, and the divergence of the Cryptochiridae from the Xenograpsidae is placed into the Cretaceous (83 [± 11] Mya). As their clade has no statistical support, the exact position of the Cryptochiridae within the Thoracotremata still remains enigmatic. The question of the origin of the gall crabs is interesting in the light of their obligate relationship with corals. In this study we aim to estimate the age of the MRCA of the Cryptochiridae and that of the clades within the Cryptochiridae, based on a dataset containing species from the Atlantic and Indo-Pacific ocean. This will allow a comparison of the diversification within the Cryptochiridae with the diversification times of their host corals. The placement of Atlantic and Indo-Pacific species will shed light on biogeographical patterns seen in the gall crabs and their host taxa.

Material and methods

Species selection

In this study, the same species selection was used as in the study of Van der Meij (chapter 10) on cospeciation, namely 38 shallow-water species belonging to 17 genera. The type species of each genus was included. The dataset includes three species from the West Atlantic, one endemic of waters surrounding the Arabian peninsula, and various species that are widespread in the Indo-Pacific. The Atlantic *Opecarcinus hypostegus* (Shaw & Hopkins, 1977) belongs to a genus that is otherwise exclusively Indo-Pacific. Unfortunately, deep sea gall crabs of, e.g., the genera *Cecidocarcinus* and *Zibrovia*, were not available. *Hemigrapsus pennicilatus* (de Haan, 1835) (Varunidae) was selected as the outgroup (van der Meij and Schubart, 2014).

Gall crabs were sequenced for three markers (mtDNA: 585 bp 16S rRNA gene, 643 bp COI; nDNA: 286 bp Histone H3). DNA extraction was performed following the protocols specified in Van der Meij (2015a). The total alignment length was 1514 bp.

Divergence time analyses

Divergence time estimation was performed using a Bayesian approach in BEAST 1.7.5 (Drummond *et al.,* 2012). One chain was ran for 50×10^6 iterations sampling every 10,000 iterations. Convergence of sampled parameters and potential autocorrelation (effective sampling size for all parameters >100) was investigated in Tracer 1.6 (Rambaut *et al.,* 2014). The first 500 trees were discarded as burn-in, keeping 4500 trees. The maximum credibility tree was calculated and parameter values annotated with TreeAnnotator (part of the BEAST package). The GTR+Γ substitution model was applied for all partitions as suggested by PartitionFinder v1.1.1 (Lanfear *et al.,* 2012) using the Bayesian Information Criterion and considering GTR, TrN, HKY and JC models with and without gamma distributed substitution frequencies. A Yule tree prior was used and the nucleotide exchange rates for the 16S rRNA mitochondrial gene partition were adjusted after initial test runs. Unfortunately, there are no fossil gall crabs or trace fossils of their dwellings in corals available for calibration of a molecular clock, hence we estimated divergence times using external substitution rates using an uncorrelated lognormal relaxed molecular clock approach. In detail, these are:

(1) A mean rate of 1.09% per Ma (normal distribution) for the 16S mitochondrial rRNAs (SD = 0.239% per Ma; 5-95% interquantile range = 0.63-1.4% per Ma) was applied, that resulted from a phylogeny of the Old World freshwater crab family Gecarcinucidae that was dated with three fossil calibration points (for the calibration scheme, see Klaus *et al.,* 2010; for chronostratigraphy of the fossils, see Klaus and Gross, 2010). These are the MRCA of the genus *Potamon* (divergence *P. fluviatile* and *P. persicum*) calibrated with fossil *P. quenstedti*; the MRCA of the gecarcinucid genus *Sartoriana* based on fossil claws from the South Asian Siwalik formation; and the MRCA of *Potamonautes niloticus* and *Platythelphusa armata* based on Late Miocene *P.* aff. *niloticus*. The taxonomy and chronostratigraphy of the potamid and gecarcinucid fossils was recently assessed (Klaus and Gross 2010), and associated uncertainty was modelled conservatively in the study of Klaus *et al.* (2010).

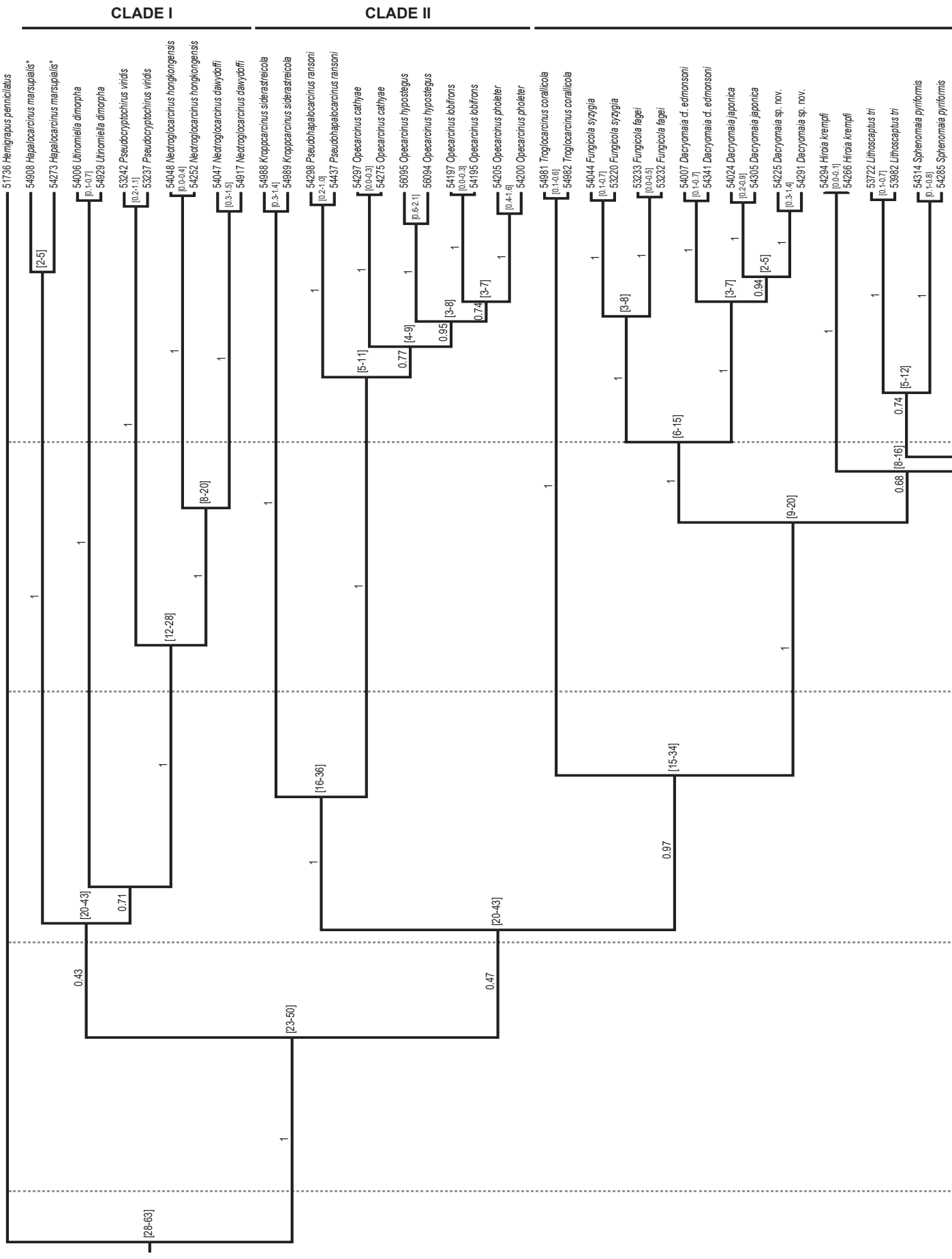
(2) 0.19% per Ma for the H3 gene (SD = 0.04% per Ma; 5-95% interquantile range = 0.12-0.26% per Ma). This rate is also derived from the study on gecarcinucid freshwater crabs of Klaus *et al.* (2010; see above).

(3) For the COI locus a substitution rate of 1.165% per Ma (SD = 0.90% per Ma with 0.00% and 2% as hard lower and upper bounds; 5-95% interquantile range = 0.20-2.69% per Ma) was used as inferred for Jamaican sesarmid freshwater crabs based on the Pliocene closure of the Isthmus of Panama (Schubart *et al.,* 1998). Similar values for the COI substitution rate have been obtained for other arthropod taxa using biogeographical calibration (Papadopoulou *et al.,* 2010; and references therein).

Results

The gall crabs are shown to be monophyletic with total support. The age of the MRCA was estimated at 50-23 Mya (Early Eocene – Early Miocene; credibility interval). Short branches at the base of the clades suggest an accelerated radiation in the last 10-2 Mya (Fig. 1).

Three distinct clades could be observed, albeit some with low support: 1) clade I (tMRCA 43-20 Mya) is comprised of the Pocilloporidae-inhabiting genera *Hapalocarcinus* and *Utinomiella*, together with the Dendrophylliidae-inhabiting genera *Neotroglocarcinus* and *Pseudocryptochirus*, however, support for this clade is low. No recent radiation was observed in this clade; 2) the well-supported clade II (tMRCA 36-16 Mya) consists of the Atlantic species *Kroppcarcinus siderastreicola*, which is the sister genus of a clade (tMRCA 11-5 Mya) containing the


CLADE I
CLADE II
51736 Hemigrapus penicillatus
54908 Hapalocarcinus marsupialis*
54273 Hapalocarcinus marsupialis*
54006 Utinomiella dimorpha [0.1-0.7]
54929 Utinomiella dimorpha
53242 Pseudocryptochirus viridis [0.2-1.1]
53237 Pseudocryptochirus viridis
54048 Neotroglocarcinus hongkongensis [0.0-0.4]
54252 Neotroglocarcinus hongkongensis
54047 Neotroglocarcinus dawydoffi [0.3-1.5]
54917 Neotroglocarcinus dawydoffi
54988 Kroppcarcinus siderastreicola [0.3-1.4]
54989 Kroppcarcinus siderastreicola
54298 Pseudohapalocarcinus ransoni [0.2-1.0]
54437 Pseudohapalocarcinus ransoni
54297 Opecarcinus cathyae [0.0-0.3]
54275 Opecarcinus cathyae
56095 Opecarcinus hypostegus [0.6-2.1]
56094 Opecarcinus hypostegus
54197 Opecarcinus lobifrons [0.0-0.3]
54195 Opecarcinus lobifrons
54205 Opecarcinus pholeter [0.4-1.6]
54200 Opecarcinus pholeter
54981 Troglocarcinus corallicola [0.1-0.6]
54982 Troglocarcinus corallicola
54044 Fungicola syzygia [0.1-0.7]
53220 Fungicola syzygia
53233 Fungicola fagei [0.0-0.5]
53232 Fungicola fagei
54007 Dacryomaia cf. edmonsoni [0.1-0.7]
54341 Dacryomaia cf. edmonsoni
54024 Dacryomaia japonica [0.2-0.9]
54305 Dacryomaia japonica
54225 Dacryomaia sp. nov. [0.3-1.4]
54291 Dacryomaia sp. nov.
54294 Hiroia krempfi [0.0-0.1]
54266 Hiroia krempfi
53722 Lithoscaptus tri [0.1-0.7]
53982 Lithoscaptus tri
54314 Sphenomaia pyriformis [0.1-0.8]
54285 Sphenomaia pyriformis
[2-5]
[8-20]
[12-28]
[5-11]
[4-9]
[3-8]
[3-7]
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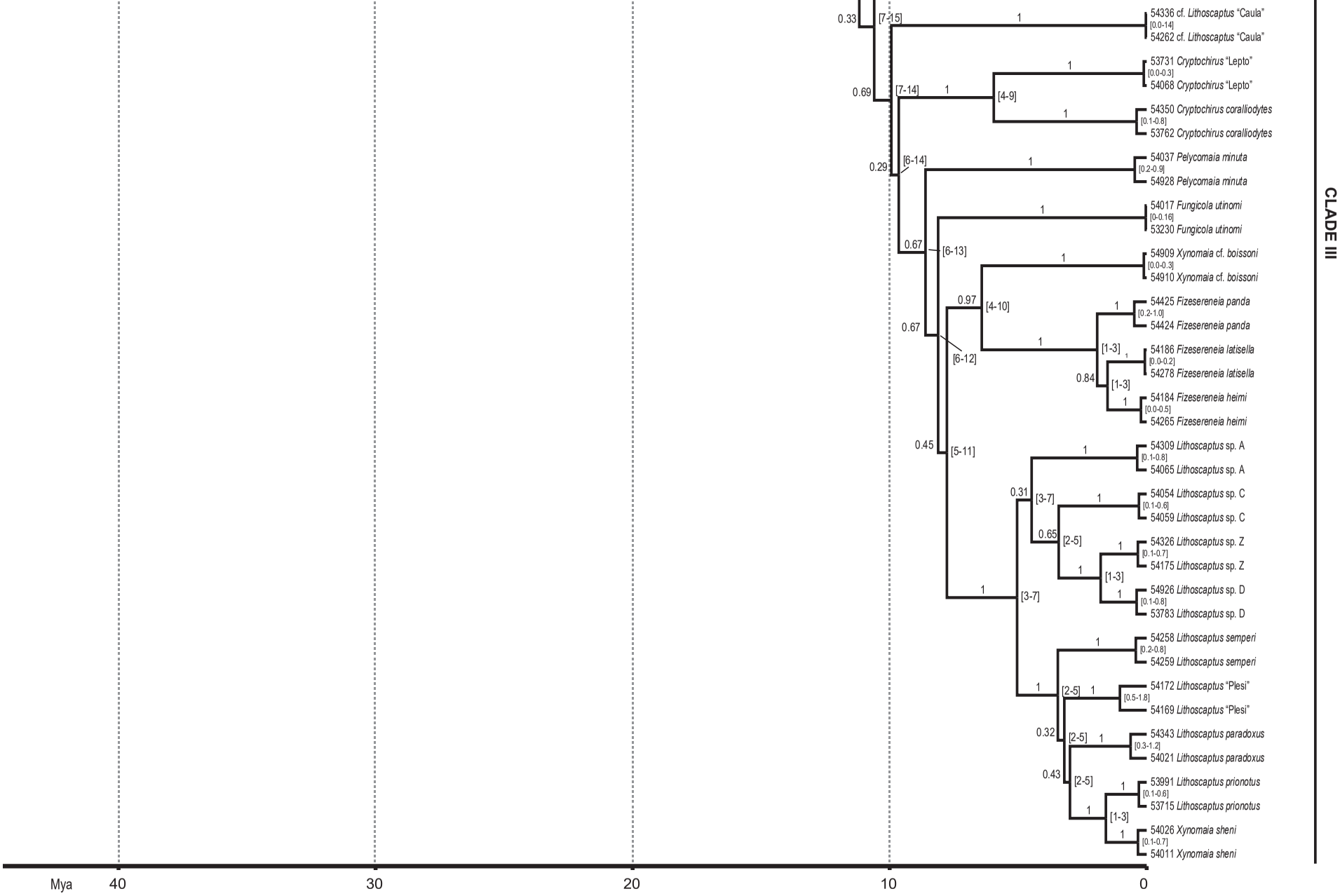

Fig. 1. Chronogram (maximum credibility tree of the Bayesian divergence time analysis in BEAST 1.7.5) with 95% highest posterior density intervals (HPD) of divergence events in million years ago (Mya). Values on branches represent their posterior probabilities, branches without values have full support. ATL = Atlantic, RS = Red Sea (Arabia, endemic), * species complex.

Agariciidae-inhabiting genera *Opecarcinus* and *Pseudohapalocarcinus.* The genus *Opecarcinus* is the only monophyletic cryptochirid genus consisting of Atlantic and Indo-Pacific species; 3) the well-supported clade III (tMRCA 34-15 Mya) comprised all remaining genera. Within clade III the Atlantic species *Troglocarcinus corallicola* diverged early from its relatives, the latter being divided in two subclades; one containing the crab species inhabiting Fungiidae, Siderastreidae, Psammocoridae and *Leptastrea*. The clades at generic level are generally well-supported (Fig. 1).

All three known West Atlantic species are included in the present analysis. They could be retrieved in two different clades. As stated above, *Kroppcarcinus siderastreicola* and *T. corallicola* diverged early within their clades. *Opecarcinus hypostegus* was retrieved as part of the (monophyletic) genus *Opecarcinus*. The only Red Sea – Arabia endemic clustered within the large clade III, together with its Indo-Malayan congeners (Fig. 1).

Discussion

Origin of the Cryptochiridae

The most recent common ancestor of the Cryptochiridae appears to have originated between 50-23 Mya, whereas Tsang *et al. (*2014) traced the divergence of Cryptochiridae from its sister group (albeit without support) into the Cretaceous. The origin of the Thoracotremata was well-supported and is estimated to have originated around 108 [± 11] Mya, which makes it the most recently originated subsection within the Brachyura (Tsang *et al*., 2014). Paulay and Starmer (2011) postulated that Thoracotremata evolved in 'safe places', such as intertidal, non-marine, deep water and endo-symbiotic habitats. Survival and diversification of thoracotreme crabs might therefore be related to their adaptability to new environments. Several other thoracotreme families – all with different lifestyles – appear to have originated around the same time as the Cryptochiridae (e.g. Sesarmidae and Glyptograpsidae) whereas other families originated earlier (Dotillidae) or later (Percnidae) (Tsang *et al.,* 2014).

Comparison with the evolution of Scleractinia

Scleractinia are much older than Cryptochiridae. The most recent common ancestor of the Scleractinia is estimated to have originated in the Triassic (ca. 250 to 200 Mya; Park *et al*., 2012). There are two main clades in the Scleractinia: the "complex" clade and the "robust" clade (Fukami *et al*., 2008; Kitahara *et al*., 2010). These clades diverged in the Triassic and the most recent common ancestor for each clade originated in the middle of the Cretaceous (ca. 145 ± 4 to 66 Mya) (Park *et al*., 2012). The phylogenetic topology of the Cryptochiridae (but not the divergence times) follows this pattern; the host corals of the gall crabs in clades I and II belong to the complex clade, whereas the host corals of the gall crabs in clade III belong to the robust clade (Fig. 1).

Clade I consists of Pocilloporidae- and Dendrophylliidae-inhabiting crabs, all of which are restricted to the Indo-Pacific (IP). West Atlantic (ATL) gall crab species are retrieved in two out of the three main clades (Fig. 1). This suggests that there have been multiple exchanges of gall crab species between what is currently recognised as the Atlantic and the Indo-Pacific. The strictly Atlantic genus *Kroppcarcinus* clusters as a sister genus to *Opecarcinus* (IP + ATL) and *Pseudohapalocarcinus* (IP) (clade II). The recovery of the Agariciidae-inhabiting genus *Opecarcinus* (with one Atlantic and several Indo-Pacific species) as monophyletic and recently divergent surprising, yet corresponds with the monophyletic family Agariciidae occurring in both basins. The origin of the West Atlantic crab species *O. hypostegus,* estimated at 8-3 Mya (Fig.1),

fits the closure of the Panamanian Isthmus. The timing of vicariance of transisthmian sister species varies among taxa, with many falling around 3.1 Mya (Malay and Paulay, 2010; and references therein). The Merulinidae is the only other coral family to host gall crabs in both oceanic basins, yet there is no evidence for a close relationship between Atlantic and Indo-Pacific Merulinidae-inhabiting gall crabs (clade III in Fig. 1). *Troglocarcinus corallicola*, like *Kroppcarcinus*, is strictly Atlantic and clusters as a sister genus to the remaining genera and species in clade III. The position of *Detocarcinus balssi,* an East Atlantic species recorded from between ca. 3 and 98 meters depth, has not yet been assessed using molecular methods, but this species appears to be closest to the Indo-Pacific species *Utinomiella dimorpha* (clade I) (Kropp and Manning, 1987; Kropp, 1988; van der Meij and Nieman, unpubl.). Analyses of this species and deep-water gall crab species could shed more light on these results, especially given the results by Kitahara *et al*. (2010) who showed that shallow-water corals originated from deep-water species.

The Red Sea – Arabia endemic *Fizesereneia panda* van der Meij, 2015 was retrieved within the large overall clade, otherwise containing Indo-Malayan species. It appears that gall crabs diversified in the Indo-Pacific Ocean and radiated from there to secondary biodiversity areas such as the Red Sea, however, the position of a single species is not enough to reach a conclusion. Such radiation is shown in a study on hermit crabs, which indicated that allopatrically distributed sister species pairs were significantly younger than sympatric sister species (Malay and Paulay, 2010). These results are also in agreement with a study on coral-dwelling gobies which diversified mostly in the last 5 Mya, supporting a hypothesis in which they diversified in the Indo-Pacific Ocean and then radiated recently, with multiple new variants found in the Red Sea (Duchene *et al*., 2013).

Van der Meij (chapter 10) suggested that the evolutionary development of the association between corals and gall crabs should be seen as sequential evolution. Sequential evolution is defined as a particular case of coevolution where the changes and the phylogeny of the symbionts are influenced by the host evolution without reciprocity. The discrepancy between the origin of the Scleractinia and Cryptochiridae supports this hypothesis. Fossil-calibrated phylogenies of reef-building corals are, however, only sparsely becoming available (Santodomingo *et al*., 2014). The first results show that within "robust" clade of Scleractinia a high diversification is observed between ca. 20 to 2 Mya among species of the families Merulinidae, Diploastreidae, Montastreidae and Lobophyllidae. Many of the gall crab species in clade III (Fig. 1), inhabit corals belonging to the Merulinidae and diversification in the Cryptochiridae is highest between approx. 10 to 2 Mya. Cenozoic climate change and tectonic events likely shaped the strong diversification in reef-associated taxa (e.g. Budd, 2000; Williams *et al*., 2013). Further analyses are needed to study the temporal pattern of diversification of both coral and gall crab species in these recently diverging clades. If the origins of taxa within these clades turn out to be synchronous, the strict coevolution vs sequential evolution paradigm needs to be revisited.

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Host specificity and coevolution

Chapter 7

Host species, range extensions, and an observation of the mating system of Atlantic shallow-water gall crabs (Decapoda: Cryptochiridae)

Sancia E.T. van der Meij

Abstract

Coral-associated invertebrates dominate the biodiversity of coral reefs. Some of the associations involving symbiotic invertebrates remain unknown or little studied. This holds true even for relatively wellstudied coral reefs, like those in the Caribbean Sea. Coral gall crabs (Cryptochiridae), obligate symbionts of stony corals, form a much-overlooked component of coral reef communities. Most recent studies on the Atlantic members of Cryptochiridae have been conducted off Brazil and little recent data have become available from the Caribbean region. During fieldwork off Curaçao (southern Caribbean Sea), eight new host coral species, belonging to four coral families, were recorded for three cryptochirid species. *Kroppcarcinus siderastreicola* Badaro, Neves, Castro and Johnsson, 2012, previously only known from Brazil, and *Opecarcinus hypostegus* (Shaw and Hopkins, 1977) are new additions to the fauna of Curaçao. Besides the new hosts and geographic range extensions, a free-living male *Troglocarcinus corallicola* Verrill, 1908 was observed visiting a female of the same species lodged in her gall in an *Orbicella annularis* (Ellis and Solander, 1786) colony. This is the first photodocumented record of the 'visiting' mating system in Cryptochiridae.

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Introduction

The biodiversity of coral reefs is predominantly composed of invertebrates, many of which live in close association with sponges, molluscs, echinoderms, ascidians, and coelenterates like sea anemones, and soft and stony corals. About 870 invertebrate species are known to be associated with stony corals (Scleractinia) alone, but the extent of these associations is only partially known (Stella *et al*., 2011; Hoeksema *et al*., 2012). Species that live in obligate symbioses with a host depend on it for their survival and, hence, are more vulnerable to extinction (McKinney, 1997). This is a concern in the light of the ongoing degradation of coral reefs, especially given that the coral-associated fauna is relatively unknown. Such associated fauna has not been subject of many surveys, even in relatively well-studied regions like the Caribbean Sea. With the exception of the overview provided by Zlatarski and Martínez-Estalella (1982), most published studies have focused on a particular geographical area, host, or symbiont (Reed *et al*., 1982; Scott 1985, 1987, 1988).

Gall crabs (Cryptochiridae; also known as pit crabs) are obligate symbionts of stony corals (see Castro, 1988) worldwide, but many regions still need to be monitored for their occurrence. Research on Atlantic gall crabs has also been sparse. Kropp and Manning (1987) studied both deep and shallow-water Atlantic cryptochirids and included many new host corals based on museum collections. All published research on Cryptochiridae conducted after 1987 has been carried out in Brazil (Noguiera, 2003; Johnsson *et al*., 2006; Oigman-Pszczol and Creed, 2006; Badaro *et al*., 2012; Noguiera *et al*., 2014), except for one publication from Mexico (Carricart-Ganivet *et al*., 2004). For the three Atlantic species of shallow-water gall crabs recognized to date, a total of 23 host species have been recorded (Kropp and Manning, 1987; Badaro *et al*., 2012). One gall crab species, *Kroppcarcinus siderastreicola* Badaro, Neves, Castro and Johnsson, 2012, is so far only known from Brazil, whereas *Troglocarcinus corallicola* Verrill, 1908 and *Opecarcinus hypostegus* (Shaw and Hopkins, 1977) have amphi-Atlantic distributions (Kropp and Manning, 1987).

The present study focuses on the gall crab fauna off Curaçao, for which previously only one gall crab had been recorded (Kropp and Manning, 1987). The present study uses the 'reversed' approach, which is to investigate the associated fauna from the perspective of the host by collecting specimens from as many coral species as possible.

Material and methods

Between 16 October and 9 November, 2013, fieldwork was conducted around Curaçao (Dutch Caribbean, Leeward Islands) in the southern Caribbean Sea. A total of 23 localities were visited, 22 on the leeward side and one on the windward side of the island. Cryptochirids were sampled from a wide range of corals to a maximum depth of 40 m. After in situ photography, crabs were collected from their coral hosts and taken to the CARMABI research station for further processing. All cryptochirids were photographed in vivo with a digital SLR camera with 50/60 mm macro lens, and subsequently fixed in 80% ethanol. The crab specimens were stored in the scientific collections of Naturalis Biodiversity Center in Leiden, the Netherlands. Identifications of cryptochirids were based on Kropp and Manning (1987) and Badaro *et al*. (2012), whereas coral identifications were based on Wells (1973), Zlatarski and Martínez-Estalella (1982), Humann and DeLoach (2002), Coralpedia (http://coralpedia.bio.warwick.ac.uk), and the reference collections of Naturalis Biodiversity Center. Coral nomenclature was updated following Budd *et al*. (2012).

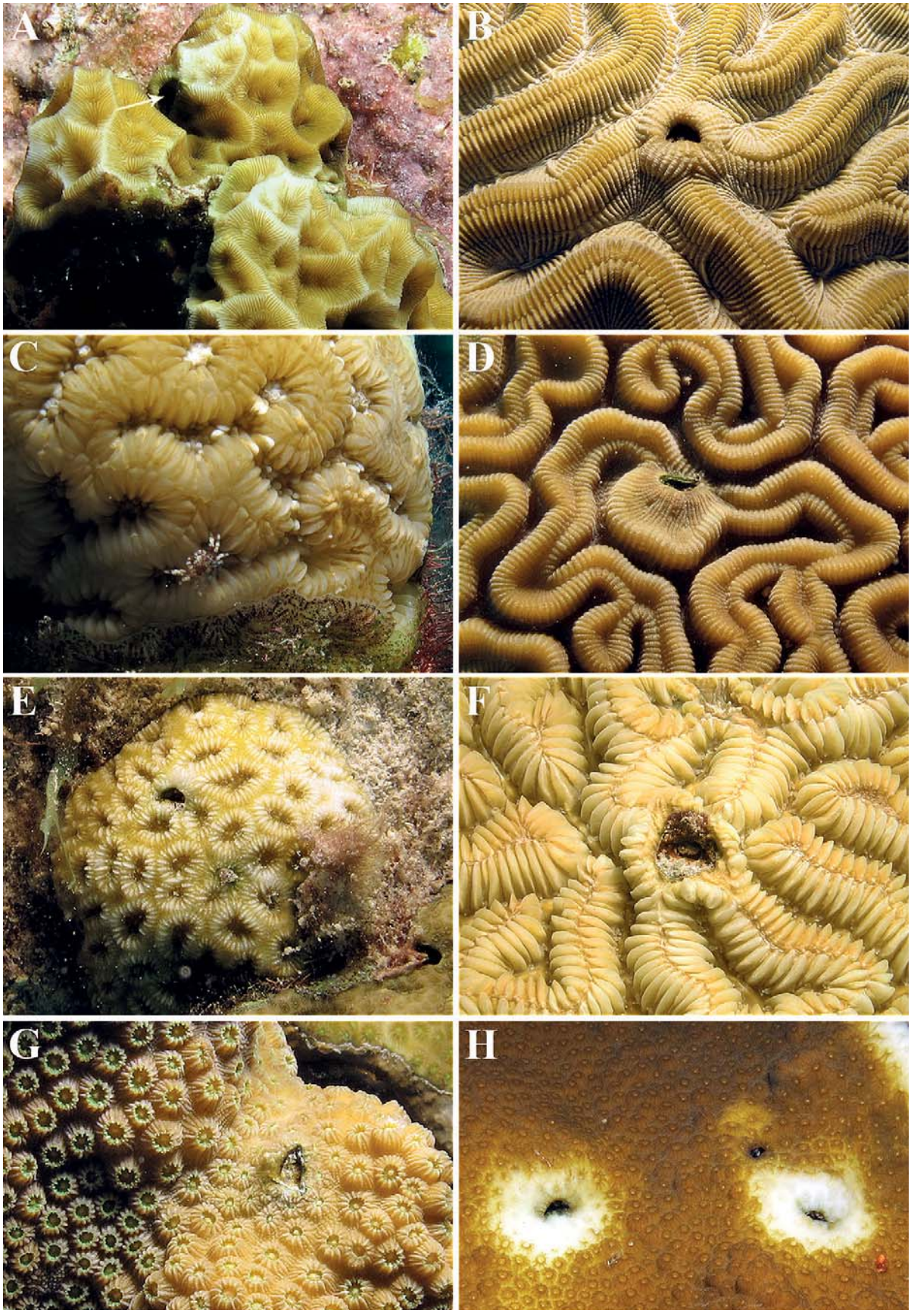

Fig. 1. Gall crab dwellings in the newly reported coral hosts. **A**, *Agaricia humilis*; **B**, *Colpophyllia natans*; **C**, *Dendrogyra cylindrus* (free-living male); **D**, *Diploria labyrinthiformis*; **E**, *Favia fragum*; **F**, *Meandrina meandrites*; **G**, *Orbicella faveolata*; **H**, *Orbicella franksi*. For the associated gall crab species, see Table 1.

Table 1. Overview of the reef coral species hosting shallow-water Atlantic cryptochirids. Names of coral species indicated **in bold** represent new host records. Tcor = *Troglocarcinus corallicola* Verrill, 1908, Ohyp = *Opecarcinus hypostegus* (Shaw and Hopkins, 1977), Ksid = *Kroppcarcinus siderastreicola* Badaro *et al*., 2012.

Coral family/species	Crab	Present study	References earlier records	Remarks
Agariciidae				
Agaricia agaricites (Linnaeus, 1758)	Ohyp	*n* = 5	Kropp and Manning, 1987, Scott, 1987	
Agaricia fragilis Dana, 1846	Ohyp	*n* = 1	Shaw and Hopkins 1977, Kropp and Manning, 1987	
Agaricia grahamae Wells, 1973	Ohyp	*n* = 1	Kropp and Manning, 1987, Scott, 1987	
Agaricia humilis Verrill, 1901	Ohyp	*n* = 1		New host for *O. hypostegus*.
Agaricia lamarcki Milne-Edwards and Haime, 1851	Ohyp	*n* = 11	Kropp and Manning, 1987, Scott, 1987	
Astrocoeniidae				
Stephanocoenia intersepta (Lamarck, 1816)	Tcor	—	Scott, 1985	As *S. michellini* by Scott (1985), considered a j.s. of *S. intersepta* (see Zlatarski and Martínez-Estalella, 1982). There is no material available to check if this record should possibly be attributed to *K. siderastreicola*.
S. intersepta	Ksid	*n* = 4		First record outside of Brazil, new host for *K. siderastreicola*.
Caryophylliidae				
Polycyathus sp.	Tcor	—	Kropp and Manning, 1987	
Meandrinidae				
? *Dendrogyra cylindrus* Ehrenberg, 1834	Tcor	*n* = 1		This is a tentative new host record. One male was collected from a *D. cylindrus* colony, but no dwelling was found (see Fig. 1).
Dichocoenia stokesii Milne-Edwards and Haime, 1848	Tcor	*n* = 2	Verrill, 1908, Shaw and Hopkins, 1977	As *Dichocoenia* sp. by Verrill (1908) and Shaw and Hopkins (1977).
Meandrina meandrites (Linnaeus, 1758)	Tcor	*n* = 7		New host for *T. corallicola*.
Merulinidae				
Orbicella annularis (Ellis and Solander, 1786)	Tcor	*n* = 2	Scott, 1985, 1987, Kropp and Manning, 1987	
Orbicella faveolata (Ellis and Solander, 1786)	Tcor	*n* = 3		New host for *T. corallicola*.
Orbicella franksi (Gregory, 1895)	Tcor	*n* = 4		New host for *T. corallicola*.
Montastraeidae				
Montastraea cavernosa (Linnaeus, 1766)	Tcor	*n* = 4	Scott, 1985, Kropp and Manning, 1987	
Mussidae				
Colpophyllia natans (Houttuyn, 1772)	Tcor	*n* = 6		New host for *T. corallicola*.
Diploria labyrinthiformis (Linnaeus, 1758)	Tcor	*n* = 5		New host for *T. corallicola*.
Favia fragum (Esper, 1795)	Tcor	*n* = 2		New host for *T. corallicola*.
Favia gravida Verrill, 1868	Tcor	—	Kropp and Manning, 1987	*Favia gravida*'s distribution range includes Brazil and the eastern Atlantic (Laborel, 1969, 1974).

Isophyllia sinuosa (Ellis and Solander, 1786)	Tcor	—	Scott, 1985, Kropp and Manning, 1987	
Manicina areolata (Linnaeus, 1758)	Tcor	$n = 2$	Rathbun, 1937, Utinomi, 1944, Shaw and Hopkins, 1977, Scott, 1985, Kropp and Manning, 1987, Carricart-Ganivet, 2004	As *Meandra areolata* by Rathbun (1937), as *Meandra areolata* and *Meandrea areolata* var. *hispida* by Utinomi (1944).
Mussa angulosa (Pallas, 1766)	Tcor	$n = 2$	Shaw and Hopkins, 1977	
Mussismilia hispida (Verrill, 1901)	Tcor	—	Utinomi, 1944, Coelho, 1966 in Kropp and Manning, 1987	*As Mussa (Isophyllia) dipsacea, Mussa (Symphyllia) hispida*, and *Mussa Harrttii* var. *conferta* by Utinomi (1944), as *M. hispida tenuisepta* by Coelho (1966). Genus endemic for Brazil.
M. hispida	? Ohyp	—	Noguiera, 2003	*Opecarcinus hypostegus* has only been recorded from *M. hispida* by Noguiera (2003). Because no other records exist, I tentatively include it here.
Mycetophyllia sp.	Tcor	—	Kropp and Manning, 1987	
Pseudodiploria clivosa (Ellis and Solander, 1786)	Tcor	$n = 3$	Verrill, 1908, Scott, 1985	As *Meandra clivosa* by Verrill (1908), as *Diploria clivosa* by Scott (1985).
Pseudodiploria strigosa (Dana, 1846)	Tcor	$n = 4$	Scott, 1985, Kropp and Manning, 1987	As *Diploria strigosa* by Scott (1985) and Kropp and Manning (1987).
Scolymia lacera (Pallas, 1766)	Tcor	—	Shaw and Hopkins, 1977, Martínez-Estalella, 1982	
Oculinidae				
Oculina sp.	Tcor	—	Kropp and Manning, 1987	
Oculina varicosa Lesueur, 1821	Tcor	—	Scotto and Gore, 1981	
Sclerhelia hirtella (Pallas, 1766)	Tcor	—	Kropp and Manning, 1987, den Hartog, 1989	
Siderastreidae				
Siderastrea siderea (Ellis and Solander, 1786)	Tcor	—	Kropp and Manning, 1987	
S. siderea	Ohyp	—	Scott, 1985, 1987	
S. siderea	Ksid	$n = 8$		First record outside of Brazil, new host for *K. siderastreicola*.
Siderastrea stellata (Verrill, 1868)	Tcor / Ohyp	—	Johnsson *et al.*, 2006	
S. stellata	Ksid	—	Noguiera *et al.*, 2014	Records from Bahia State: Tinharé-Boipeba Archipel, Todosos-Santos Bay, and the North Shore.
Siderastrea sp.	Ksid	—	Badaro *et al.*, 2012	Described from Guarajuba (type locality) and Praia do Forte (Brazil) in northern Bahia State.

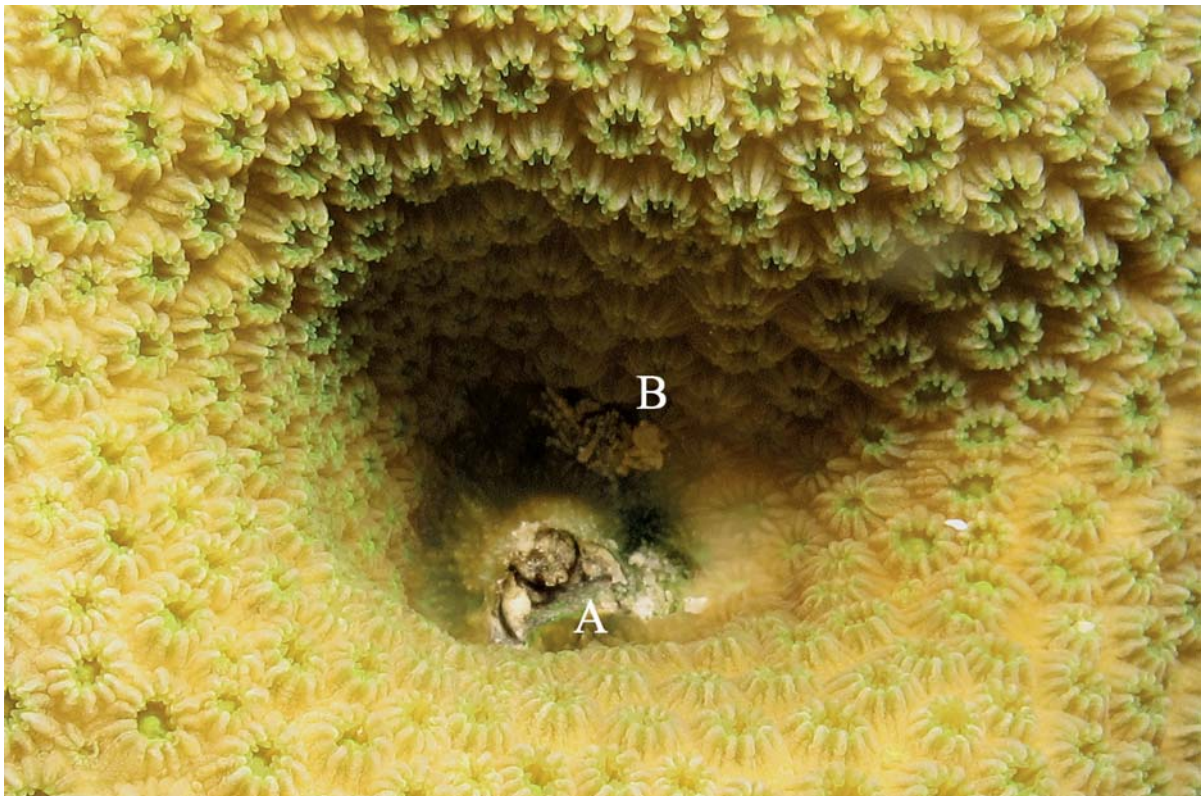

Fig. 2. A female *Troglocarcinus corallicola*. **A**, in her lodge inside a colony of the coral *Orbicella annularis*, with a free-living male; **B**, residing closely.

Results

In total, 21 coral species were recorded hosting three cryptochirid species off Curaçao. Eight of these 21 coral species represent new records as cryptochirid hosts (Fig. 1A-H). With an additional 10 host records based on literature, the number of Atlantic host coral species for gall crabs is now 31 (Table 1). The majority of the coral species housing gall crabs belong to the coral families Agariciidae and Mussidae, the latter being the Atlantic coral family with most species. *Favia fragum* (see Table 1 for species authorities), *Manicina areolata*, and *Mussa angulosa* were only recorded in low densities, yet they were found inhabited by cryptochirids on two different occasions. Some common coral species (e.g. *Colpophyllia natans* and *Meandrina meandrites*) were frequently found inhabited by gall crabs. *Mycetophyllia* sp. was previously recorded as a host in Kropp and Manning (1987), but despite extensive searches, no cryptochirid was found associated with *Mycetophyllia* off Curaçao.

Kroppcarcinus siderastreicola is recorded here outside of Brazil for the first time, with *Siderastrea siderea* and *Stephanocoenia intersepta* as new hosts. *Opecarcinus hypostegus*, representing a new record for Curaçao, was found in association with five *Agaricia* species, of which *Agaricia humilis* is a new record. The agariciid *Helioseris cucullata* was encountered on a few reefs, but was not found inhabited by cryptochirids. *Troglocarcinus corallicola* was associated with a wide range of hosts, but did not occur in association with Agariciidae (Table 1).

Male 'visiting' female gall crab

During a dive in Slangenbaai (Snake Bay) a male *T. corallicola* was observed residing close to the dwelling of a female (Fig. 2A-B). The female was partially extended from her lodge, an

uncommon sight for cryptochirids. The male was observed for approximately 5 min during which he did not move. This immobility could have been caused by the presence of the diver and/ or the flashes of the camera strobe. In the present study, cryptochirid males were collected mainly from their own dwelling on a host coral, with the exception of this record of *T. corallicola* from *Orbicella annularis*, a free-living male *T. corallicola* from *Dendrogyra cylindrus* and a free-living male *T. corallicola* from *Pseudodiploria clivosa* (Table 1).

Discussion

Previously only one published record was available for the gall crab fauna of Curaçao; LB Holthuis collected *Troglocarcinus corallicola* in 1957 from unknown coral hosts in Piscadera Baai (Piscadera Bay, record in Kropp and Manning, 1987). This record was also the only available record from the southern Caribbean Sea. The results of the present study increase the gall crab fauna of Curaçao from one to three species, and it now has the highest number of recorded cryptochirid-coral associations. *Opecarcinus hypostegus* and *T. corallicola* were already known from various localities in the Caribbean region, but the recently described *K. siderastreicola* was so far only known from off Bahia State, Brazil (Badaro *et al*., 2012; Noguiera *et al*., 2014). *Kroppcarcinus siderastreicola* is now also documented from the Caribbean Sea. It is possible that *K. siderastreicola* also occurs in the central Atlantic Ocean, like *T. corallicola* and *O. hypostegus*, because its host coral genus *Siderastrea* has a distribution range that includes western off Africa (Laborel, 1974; Neves *et al*., 2010; Nunes *et al*., 2011). *Siderastrea siderea* is now recorded to host *K. siderastreicola*, a new host for the species. This coral species was previously considered restricted to the Caribbean Sea, but was recently recorded off Brazil (Neves *et al*., 2010).

Eight new coral hosts were recorded for gall crabs, which increases the number of Atlantic host coral species from 23 to 31 (Table 1). The new host records include common coral species like *Colpohyllia natans*, *Diploria labyrinthiformis*, and *Meandrina meandrites*, all of which are inhabited by *T. corallicola*, a generalist that occurs in association with a wide variety of Atlantic coral species (Verrill, 1908; Kropp and Manning, 1987). *Opecarcinus hypostegus* is associated with Atlantic species of the coral families Agariciidae (Kropp and Manning, 1987, present study) and Siderastreidae (Scott, 1985, 1987; Johnsson *et al*., 2006), whereas *K. siderastreicola* is now known from Siderastreidae and the astrocoeniid *S. intersepta*. Consistent with previous collections, no gall crabs were encountered in corals belonging to the families Acroporidae and Poritidae (Kropp and Manning, 1987; Kropp, 1990a).

One of the newly recorded hosts, *Dendrogyra cylindrus*, is possibly not a true host of cryptochirids. A male *T. corallicola* was found on the surface of a colony, among the coral tentacles, but no dwelling was found. No other gall crabs were found on *D. cylindrus* colonies despite further searching. This single observation, also based on the fact that there are no other records of gall crabs associated with long-tentacled coral species, may reflect the wanderlust of a free-living male.

The observation of a free-living male *T. corallicola* close to the lodged female in an *Orbicella annularis* colony is consistent with Asakura (2009), who, based on anecdotal evidence and observations (see McCain and Coles, 1979; and references in Asakura, 2009), used the term 'visiting' for the mating system in which cryptochirid males 'visit' females inhabiting separate galls or pits. Baeza and Thiel (2007) used the term 'visiting' or 'pure-search polygynandry of sedentary females,' and Guinot *et al*. (2013) used 'visiting' for the mating system in which males of symbiotic species of crabs move from host to host in search of potential female mates. Baeza and Thiel (2007) presume that a 'pure-search polygynandry of sedentary females' evolves when

hosts are extremely small, which is (mostly) not the case in cryptochirids. Asakura (2009) specifically mentioned *T. corallicola*: '… the male crab normally resides outside the gall, which was constructed by the female, and is thought to visit the gall of the female for mating.' The fact that almost all other males were collected from their own dwelling, as well as the close proximity to the female, suggests that this male was indeed 'visiting.' This is the first photodocumented observation of this mating system in cryptochirids.

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Chapter 8

Host preferences, colour patterns and distribution records of *Pseudocryptochirus viridis* Hiro, 1938 (Decapoda: Cryptochiridae)

Sancia E.T. van der Meij

Abstract

The coral gall crab *Pseudocryptochirus viridis* is an obligate symbiont of some species of the Indo-West Pacific coral genus *Turbinaria*. The colour pattern variation within the species is illustrated for the first time. Overviews of the coral host species and distribution records are provided, including new records from Indonesia, Malaysia and Australia.

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Introduction

Coral gall crabs (Cryptochiridae) are obligate symbionts of stony corals (Scleractinia), residing in galls or pits in its host. Cryptochirids settle as megalopae on scleractinian corals, and somehow induce the host to grow over and around them (Utinomi, 1944; Castro, 1976). Despite their peculiar mode of life, little is known about their biology and ecology. The taxonomy of the Cryptochiridae was revised by Kropp (1990a), including a summary of all known coral host genera. Host specificity information at species level, however, remains incomplete.

This paper discusses the coral gall crab *Pseudocryptochirus viridis* Hiro, 1938, associated with stony corals of the genus *Turbinaria* (Dendrophylliidae). The colour patterns of juveniles and adults are described for the first time. An overview of the coral host species and distribution records is provided, including new records for Indonesia, Malaysia and Australia.

Material and methods

Coral gall crabs were collected in Bunaken National Marine Park (N. Sulawesi, Indonesia, Dec. 2008), around Ternate (Halmahera, Indonesia, Oct.-Nov. 2009), in Semporna (E. Sabah, Malaysia, Nov.-Dec. 2010), and around Lembeh Island (N. Sulawesi, Indonesia, Jan.-Feb. 2012). Corals of the genus *Turbinaria* were searched for specimens of *Pseudocryptochirus viridis*. Encountered gall crabs were collected and taken to the field station. After being photographed with a digital SLR camera with a 50 mm macro-lens, the crabs were preserved in 80% ethanol. All material is deposited in the Crustacea collection of Naturalis Biodiversity Center in Leiden (formerly Rijksmuseum van Natuurlijke Historie) (coded RMNH.Crus.D).

Results

Order Decapoda
Family Cryptochiridae Paul'son, 1875
Genus *Pseudocryptochirus* Hiro, 1938
Pseudocryptochirus viridis Hiro, 1938

Material examined. Indonesia: RMNH.Crus.D.53235, N Sulawesi, Bunaken, Timur II, 1°36'30.66"N 124°46'58.2"E, in *T. mesenterina*, 20 Dec. 2008, collected by S.E.T. van der Meij; RMNH.Crus.D.54109, N Sulawesi, Lembeh, Tanjung Nanas I, 1°27'40.428"N 125°13'36.408"E, 15 m depth, in *T. mesenterina*, 30 Jan. 2012, collected by S.E.T. van der Meij; RMNH.CRUS. D.54110, N Sulawesi, Lembeh, SE Sarena Kecil, 1°27'15.804"N 125°13'29.5314"E, 8 m depth, in *T. mesenterina*, 30 Jan. 2012, collected by S.E.T. van der Meij; RMNH.Crus.D.54111, N Sulawesi, Lembeh, Baturiri, 1°27'34.704"N 125°14'23.1"E, 11 m depth, in *Turbinaria* sp., 6 Feb. 2012, collected by S.E.T. Van der Meij; RMNH.Crus.D.54112-54113, N Sulawesi, Lembeh, Teluk Makawide, 1°29'5.0634"N 125°14'26.1234"E, 6 m depth, in *T.* cf. *mesenterina*, 9 Feb. 2012, collected by S.E.T. van der Meij; RMNH.Crus.D.54114, N Sulawesi, Lembeh, S Pulau Dua, 1°23'17.016"N 125°12'43.1274"E, 8 m depth, in *T.* cf. *mesenterina* (together with *Neotroglocarcinus* sp.), 13 Feb. 2012, collected by S.E.T. van der Meij; RMNH.Crus.D.53242, Tidore, Pilongga S, 0°42'44.1"N 127°28'47.3"E, 8 m depth, in *Turbinaria* cf. *reniformis*, 12 Nov. 2009, collected by S.E.T. van der Meij; RMNH.Crus.D.53236-53238, Ternate, Batu Angus, 0°50'48.5"N 127°21'58.98"E, <5 m depth, in *T. mesenterina*, 30 Oct. 2009, collected by B.W. Hoeksema; RMNH.Crus.D.53239, Ternate, Sulamadaha II, 0°52'2"N 127°19'45.8"E, 8 m depth, in *T. mesenterina*, 6 Nov. 2009, collected by

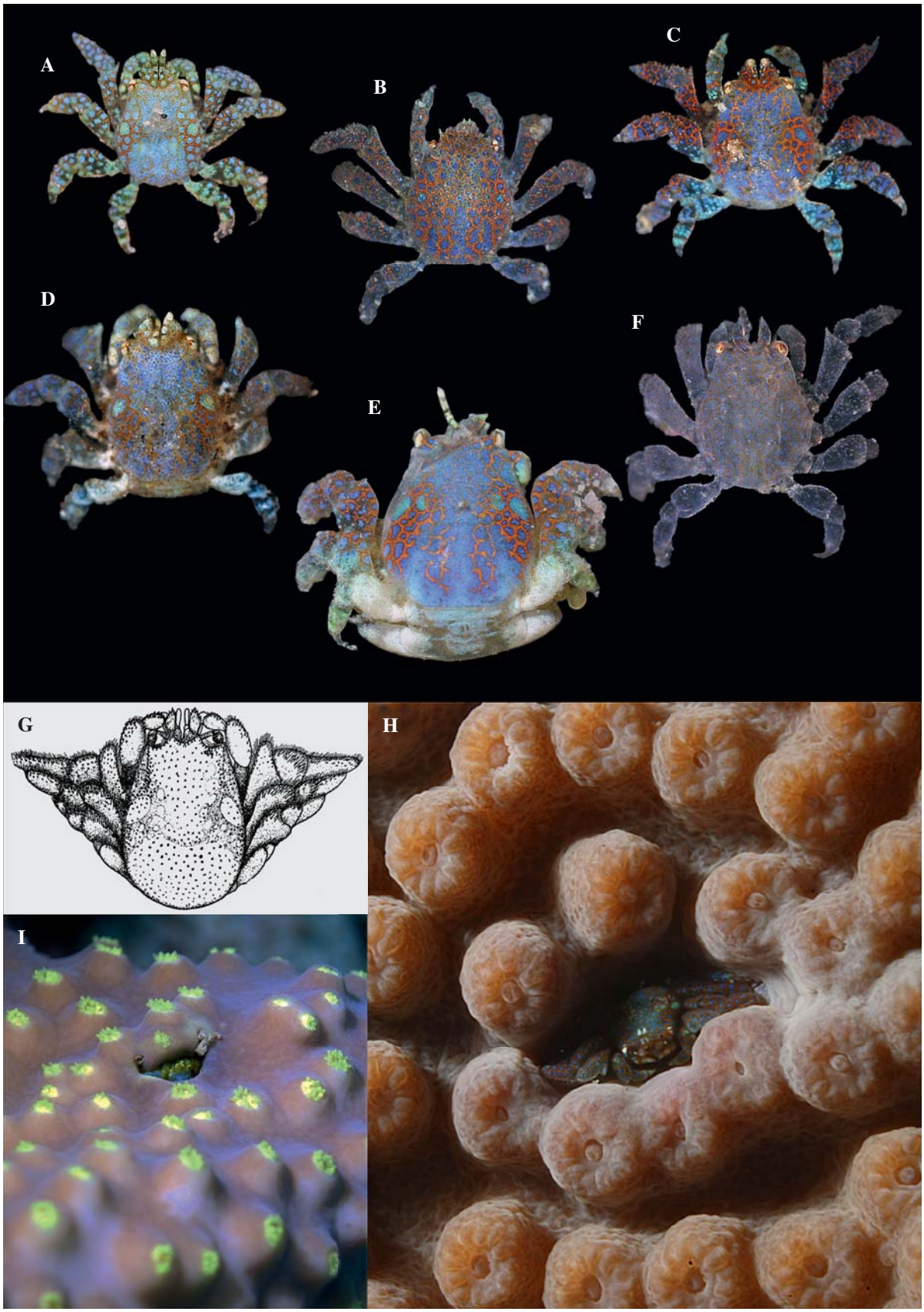

Fig. 1. A-F, colour patterns in *Pseudocryptochirus viridis* Hiro, 1938; **F**, recent moult; **G**, posture of *P. viridis* in gall, after Utinomi (1944, Fig. 3); **H-I**, in situ photographs of *P. viridis*. Photos by S.E.T. van der Meij (A-F), B.T. Reijnen (H) and B.W. Hoeksema (I).

S.E.T. van der Meij; RMNH.Crus.D.53240, Halmahera, Pasir Lamo W, 0°53'20.5"N 127°27'34.2"E, 14 m depth, in *T. mesenterina*, 8 Nov. 2009, collected by S.E.T. van der Meij; RMNH.Crus.D.53243, Halmahera, Teluk Dodinga E-N of Jere, 0°50'47.8"N 127°37'48.7"E, 3 m depth, in *T.* cf. *frondens* (Dana, 1846), 13 Nov. 2009, collected by S.E.T. van der Meij; RMNH.Crus.D.53244, Halmahera, Teluk Dodinga - Karang Galiasa Besar E, 0°50'45.6"N 127°35'7.4"E, 10 m depth, in *T. mesenterina*, 14 Nov. 2009, collected by S.E.T. van der Meij; Malaysia: RMNH.Crus.D. 53983, Semporna, SE of Tawau, Darby Rock, 04°06'42.8"N 118°13'39.7"E, 15 m depth, in *Turbinaria* sp., 30 Nov. 2010, collected by S.E.T. van der Meij; RMNH.Crus.D.53984, Semporna, SE of Tawau, Darby Rock, 04°06'42.8"N 118°13'39.7"E, 15-18 m depth, in *Turbinaria* sp., 30 Nov. 2010, collected by B.W. Hoeksema; RMNH.Crus.D.53985, Semporna, SE of Tawau, Hand Rock, 04°08'24.5"N 118°10'44.3"E, 20 m depth, in *Turbinaria* sp., 30 Nov. 2010, collected by S.E.T. van der Meij; RMNH.Crus.D.53986, Semporna, Ligitan Isl., Ligitan 3, 04°12'43.0"N 118°54'36.6"E, 15 m depth, in *T. mesenterina*, 03 Dec. 2010, collected by S.E.T. van der Meij; RMNH.Crus.D.53987, Semporna, Ligitan Isl., Ligitan 3, 04°12'43.0"N 118°54'36.6"E, 10-20 m depth, in *T. reniformis*, 03 Dec. 2010, collected by S.E.T. van der Meij; RMNH.Crus.D. 54049, Semporna, Tg. Pantau Pantau, Bumbun Isl., 04°26'54.1"N 118°46'31.0"E, 10 m depth, in *T. mesenterina* (together with *Neotroglocarcinus* sp.), 07 Dec. 2010, collected by S.E.T. van der Meij; RMNH.Crus.D. 53988, Semporna, NW Gaya Island, 04°38'32.5"N 118°44'6.0"E, shallow, in *T.* cf. *reniformis*, 10 Dec. 2010, collected by B.W. Hoeksema; RMNH.Crus.D. 53710, Semporna, S Boheydulang Isl., outer reef, 04°35'00.3"N 118°46'39.1"E, in *T. mesenterina*, 11 Dec. 2010, collected by S.E.T. van der Meij; RMNH.Crus.D.53709, Semporna, S Boheydulang Isl., outer reef, 04°35'00.3"N 118°46'39.1"E, in *T. mesenterina*, 11 Dec. 2010, collected by B.W. Hoeksema; RMNH.Crus.D.54050, Semporna, Church Reef 1, 04°40'54.9"N 118°39'28.4"E, 3 m depth, in *T. mesenterina*, 13 Dec. 2010, collected by S.E.T. van der Meij; RMNH.Crus.D. 53713, Semporna, Bakungan Isl., 04°45'11.1"N 118°29'16.0"E, in *Turbinaria* sp., 16 Dec. 2010, collected by S.E.T. van der Meij.

Coral host
Order Scleractinia
Family Dendrophylliidae Gray, 1847
Genus *Turbinaria* Oken, 1815

The genus *Turbinaria* is in serious need of a revision. Bernard's (1896) monograph of *Turbinaria* was a turning point in the study of this genus, and was highly criticized by later coral taxonomists for recognizing too many species that actually represent various morphotypes resulting from ecophenotypical variation. Current authors (Veron and Pichon, 1980; Cairns *et al*., 1999; Cairns, 2001) recognize 13 to 15 valid species of *Turbinaria* compared to the 58 listed by Bernard (1896), many of which are now considered to be either junior synonyms or species of uncertain status. The identifications of the *Turbinaria* in this paper should therefore be treated with some caution, although the majority of *Turbinaria* corals from which *P. viridis* was collected seem to belong to two species currently regarded as *T. reniformis* Bernard, 1896 and *T. mesenterina* (de Lamarck, 1816).

Fize and Serène (1957) list many *Turbinaria* species as hosts for *P. viridis*. Some of these have been synonymized and the identity of other *Turbinaria* species remains unresolved (table I). Fize and Serène (1957) did remark that all hosts of *P. viridis* consisted of *Turbinaria* corals with small polyps (up to approximately 3 mm), which excludes *T. peltata* (Esper, 1794) and most likely *T. patula* (Dana, 1846). Besides *P. viridis*, *Turbinaria* corals also host the gall crabs *Neotroglocarcinus dawydoffi* (Fize and Serène, 1956) and *N. hongkongensis* (Shen, 1936).

Table 1. Overview of the coral hosts of *Pseudocryptochirus viridis* Hiro, 1938.

Coral host	Reference
Turbinaria frondens (Dana, 1846) (as *T. contorta* Bernard, 1896; *T. danae* Bernard, 1896; *T. Edwarsii* [*edwardsi*] Bernard, 1896; *T. pustulosa* Bernard, 1896)	Hiro, 1938; Utinomi, 1944; Fize and Serène, 1957; Garth, 1964; this study
T. mesenterina (de Lamarck, 1816) (as *Turbinaria tubifera* Bernard, 1896)	Utinomi, 1944; Wei *et al*., 2006; this study
T. cf. *patula* (Dana, 1846)	Kropp, 1988
T. reniformis Bernard, 1896 (as *T. veluta* Bernard, 1896)	Fize and Serène, 1957; this study
T. stellulata (de Lamarck, 1816)	Kropp, 1988
T. agaricia Bernard, 1896 (identity unclear)	Fize and Serène, 1957
T. mollis Bernard, 1896 (identity unclear)	Fize and Serène, 1957
T. crater (Pallas, 1766) (identity unclear)	Fize and Serène, 1957

Turbinaria often occurs in protected environments with turbid water. Because of these conditions the colour of *Turbinaria* corals may appear greyish-brown, but in fact the colour ranges from orange-grey (Maerz and Paul, 1950, pl. 11, B7) to more purple-grey (Maerz and Paul, 1950, pl. 4, D2). Often a yellowish growth line along the coral edge is visible. In some species polyp tentacles are yellow.

Colour pattern

Hiro (1938) named the species *Pseudocryptochirus viridis* for its bluish-green colour. The colour pattern of *P. viridis* (Fig. 1A-F) is rather uniform, with juvenile crabs appearing more cyan-green, especially on the legs. The eyestalks are bluish with four brown longitudinal stripes, whereas the eyes themselves are off-white with a horizontal red band. Antennules have white bands on an overall transparent background. The eye region, including the antennules, sometimes appears yellowish (Fig. 1I). The light blue background of the carapace seems to deepen to azure when the individual matures. The mesobranchial region of the carapace is marked on both sides with an emerald green spot, or sometimes two or three smaller spots clustered together. In some specimens the reddish-brown marbled pattern is more pronounced than in others (Fig. 1B). The colour of the dorsal surface of the walking legs is like the carapace. One specimen (Fig. 1F) appears to have recently moulted or is in an intermoult stage. Closer examination shows the reddish-brown dotted pattern, including azure blue spots, on the generally transparent carapace. No sexual dimorphism has been observed in carapace colouration.

The maximum carapace length of *P. viridis* according to Fize and Serène (1957) is 4.5 mm for females and 2.0 mm for males. Utinomi (1944) mentions maximum carapace length/breadth dimensions of 5.8/5.2 for females and 2.5/2.1 mm for males.

Distribution

Gall crabs were collected from *Turbinaria* corals at depths between 3 and 20 m. An infested coral usually hosts many crabs, mostly (ovigerous) females. An overview of published distribution records is given in table II. Bunaken, Lembeh and Ternate are new Indonesian records for *P. viridis*, and Semporna is the first record for Malaysia. An additional specimen was observed in *T. reniformis* on Hastings Reef off Cairns (Great Barrier Reef, Australia), which is a new record for Australia. The holotype of *T. reniformis* Bernard, 1896 (NHM 1892.12.1.374), from the Great Barrier Reef, has an empty gall (Bernard, 1896). Based on the shape of the pit, the coral was most

Table 2. Distribution records of *Pseudocryptochirus viridis* Hiro, 1938.

Country	Location	Reference
Australia	Hastings Reef, off Cairns	This study
China	Hong Kong (gall only)	Scott, 1984
Indonesia	Banda Neira, Banda Island	Kropp, 1994
	Moluccas	Serène *et al*., 1974
	Bunaken, Lembeh (N Sulawesi), Ternate (Halmahera)	This study
Japan	Tanabe Bay	Hiro, 1938
	Yaeyama Islands; Ryukyu Islands	Utinomi, 1944
Malaysia	Semporna (E Sabah)	This study
Marshall Islands	Eniwetok Atoll	Garth, 1964
Micronesia	Palau (Palao)	Utinomi, 1944
	Guam, Palau, Pohnpei	Kropp, 1990a
New Caledonia	Loyalty Islands	Juncker and Poupin, 2009
Taiwan	Hung-Chung Peninsula; Orchid Island	Wei *et al*., 2006
	Penghu Island (the Pescadores); ?Pratas Island (Dongsha Island)	Utinomi, 1944
Vietnam	Nha Trang	Fize and Serène, 1957

likely inhabited by *P. viridis*. This distribution record corresponds with the herein reported observation of *P. viridis* on Hastings Reef.

Discussion

Females of many species of gall crabs are permanently confined by their host, e.g., *Hapalocarcinus marsupialis* Stimpson, 1859 and *Fungicola* spp. associated with Pocilloporidae and Fungiidae, respectively. Unlike their congeners, the females of *Pseudocryptochirus viridis* can leave their pit, which is merely a shallow, crescent-shaped depression within the coral. Specimens of *P. viridis* show a characteristic position when lodged in their gall, with most of the carapace and the anterior three pereiopods exposed (Fig. 1G). They are positioned on roughly the same level as the surface of the host coral.

Their bright colours could make the gall crabs more detectable for predators. Figure 1I shows how the eye region of *P. viridis* appears to be yellow, just like the polyp tentacles of its *Turbinaria* host, making the gall crab visually blend in the coral. There is only one published record of a gall crab in a fish stomach (Kropp and Manning, 1987), which the authors consider a doubtful record in terms of actual predation.

The currently known distribution of *P. viridis* ranges from Vietnam in the west to the Marshall Islands and New Caledonia in the east (table II). The distribution ranges of some of its host species (e.g., *T. mesenterina* and *T. reniformis*) also include the east coast of Africa and the southern Red Sea (Pichon *et al*., 2010), but so far no records of *Turbinaria*-associated cryptochirid fauna are available from those regions.

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I am grateful to Prof. Farnis Boneka and Prof. Markus Lasut (Universitas Sam Ratulangi) for their help during fieldwork in Bunaken. Dr Bert W. Hoeksema (NCB Naturalis) is gratefully acknowledged for his efforts in organizing the 2009 expedition to Ternate, under the umbrella of E-win (EkspedisiWidya Nusantara) of the Indonesian Institute of Sciences (LIPI), as well as the 2012 Marine Biodiversity Workshop in Lembeh Strait. Dr James D. Thomas (NOVA University) allowed me to join his Australia course in May 2010. The 2010 Semporna Marine Ecological Expedition (SMEE2010) was jointly organized by WWF-Malaysia, Universiti Malaysia Sabah's Borneo Marine Research Institute, Universiti Malaya's Institute of Biological Sciences and the Netherlands Centre for Biodiversity Naturalis. The research permits for Indonesia were granted by RISTEK, and in Malaysia by the Prime Minister's Department, Economic Planning Unit Sabah, Sabah Parks and Department of Fisheries Sabah. The director of the Seto Marine Biological Laboratory (Kyoto University), Dr Akira Asakura, granted permission for reproducing Fig. 3 of Utinomi (1944). Bastian T. Reijnen (NCB Naturalis) assisted with photography in the field. Nadia Santodomingo (Natural History Museum) helped with the Spanish abstract. Funding was provided by the Schure- Beijerinck-Poppingfonds (KNAW), A. M. Buitendijkfonds, L. B. Holthuisfonds, J. J. ter Pelkwijkfonds (all Naturalis) and the LUF International Study Fund (Leiden University). Dr Charles H. J. M. Fransen and Dr Bert W. Hoeksema (both Naturalis) are acknowledged for useful comments on an early draft of the manuscript. Comments by four anonymous reviewers greatly improved the manuscript.

Chapter 9

Phylogenetic ecology of gall crabs (Brachyura: Cryptochiridae) and their mushroom host corals (Scleractinia: Fungiidae)

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Abstract

Coral-associated fauna is a relatively understudied topic, hence the nature of the relationship between an associated organism and its host is frequently unknown. In the present study the obligate associations between gall crabs (Cryptochiridae) and mushroom corals (Fungiidae) are reviewed from a phylogenetic perspective. Based on field surveys, examination of museum material and a literature review, a total of 35 fungiid species have been found that act as hosts for four gall crab species. Fungiid-associated gall crabs appear to be more geographically widespread than previously known, with new records showing their occurrences from the Red Sea and western Indian Ocean all the way to the central Pacific Ocean. The obligate nature of the association between cryptochirids and their hosts makes them an ideal model taxon to test for possible cospeciation events. The congruence between their phylogenies was tested by using the programme Jane 4.0, resulting in cospeciation and duplication events between the crabs and their host corals. The sharing of several closely related coral host species by a gall crab species or genus may provide support to models indicating phylogenetic relationships within the Scleractinia.

Manuscript under review

Introduction

The integration of molecular analyses with skeleton microstructure data in recent phylogeny reconstructions of stony corals (Scleractinia), has initiated large changes in scleractinian systematics (i.e. Benzoni *et al.,* 2007; Budd *et al.,* 2012; Huang *et al.,* 2014). For the mushroom coral family Fungiidae this approach has resulted in various changes at genus level and the inclusion of two additional species (Gittenberger *et al.,* 2011; Benzoni *et al.,* 2012a). Fungiidae occur in the Indo-Pacific with a distribution ranging from the Red Sea and eastern Africa to the west coast of central America (Hoeksema, 1989). Several species have been recorded to live in association with fungiids. Most of the associated fauna consists of crustaceans and molluscs, but also includes acoel flatworms and fishes (e.g., Hoeksema *et al.,* 2012; van der Meij, 2015a; Bos and Hoeksema, in press).

Gall crabs (Brachyura: Cryptochiridae) are obligate associates of stony corals, living in dwellings inside their coral hosts. They are common inhabitants of coral reefs, but are easily overlooked because of their small size and hidden life inside their coral hosts (Hoeksema and van der Meij, 2013). Gall crab genera used to be defined by host specificity (Fize and Serène, 1957), a scheme that worked for some crab genera but proved to be unreliable for other genera (Kropp and Manning, 1987).

According to the last taxonomic revision of Indo-Pacific gall crabs (Kropp, 1990a), two species are known to live in association with mushroom corals: *Fungicola fagei* (Fize and Serène, 1956) and *F. utinomi* (Fize and Serène, 1956). Hoeksema *et al*. (2012) reported on a *Dacryomaia* species as a third cryptochirid species associated with Fungiidae, whereas Van der Meij and Hoeksema (2013) reported on the fourth. The latter concerned a cryptic species closely related to *F. fagei*, described as *Fungicola syzygia* van der Meij, 2015.

The obligate nature of the association between cryptochirids and their hosts raises questions about possible cospeciation between the two. Studies on the associated fauna of stony corals, however, have so far largely been focused on the symbiont. In this study the following questions are addressed. Is there an overlap between the geographical distribution of the corals and their associated gall crabs? Are common coral species more likely to be inhabited by gall crabs than less commonly occurring corals? Are the phylogenetic relationships of the host corals reflected in the phylogenetic relationships of the crabs, hence is there some kind of cospeciation between the two?

To answer these questions fungiid-associated gall crabs were studied from the perspective of the host by collecting crabs from as many coral species as possible. Fieldwork in various parts of the Indo-Pacific, examination of museum collections, and a review of available literature were carried out in order to obtain host, distribution and occurrence records. The gall crab-coral associations and occurrence rates were projected on a cladogram of the Fungiidae in order to reconstruct the evolutionary history of the associations of the crabs and their host species. The congruence between the fungiid and gall crab phylogenies was tested for cospeciation events with the help of the programme Jane 4.0.

Material and methods

Historical records

In order to examine the distribution of fungiid associated gall crabs the coral collections of Naturalis Biodiversity Center (RMNH) in Leiden, the Netherlands, and the Royal Belgian Institute of Natural Sciences (IRSNB) in Brussels, Belgium, were searched for the presence of gall crabs or their vacated pits. Additional records were obtained from the coral collections of the

Table 1. Distribution of gall crab species based on museum records of Fungiidae containing coral gall crabs (indicated by species name) or their pits (+), literature, and incidental observations (photo vouchers). Coral names updated according to Gittenberger *et al*. (2011). Localities of the listed host species: A = Israel (Eilat, Red Sea); B = Kenya (western Indian Ocean); C = Gulf of Aden, Yemen; D = Seychelles (western Indian Ocean); E = Maldives (central Indian Ocean); F = Thailand (Phuket); G = Indonesia; H = Vietnam (Nha Trang); I = Malaysia (Tioman Isl.).; J = Malaysia (Sabah); K = Taiwan; L = Palau; M = Papua New Guinea (Bismarck Sea); N = Japan (Yaeyama Isl.,); O = Australia (GBR, off Cairns); P = Samoa Isl. (western Pacific Ocean); Q = Tahiti (central Pacific Ocean); R = Hawaii; S = Vanuatu. Museum records: [1] = RMNH, [2] = IRSNB, [3] = UNIMIB, [4] = AMNH. In bold, localities based on literature references and/or incidental observations. **Fungicola utinomi* without host record was reported from Indonesia (Moluccas – Kropp, 1994) and Micronesia (Mariana Isl. – Paulay *et al.,* 2003).

Coral host	Museum records	Localities	Reference for locality data
Cycloseris costulata (Ortmann, 1889)	*Fungicola syzygia*[1]	B, G, J, S	
C. curvata (Hoeksema, 1989)	+[3]	C	
C. cyclolites (Lamarck, 1815)	+[1]	F, G	
C. fragilis (Alcock, 1893)	+[1]	G	
C. mokai (Hoeksema, 1989)	+[1]	G	
C. sinensis (M. Edwards & Haime, 1851)	+[1]	G	
C. tenuis (Dana, 1846)	+[1]	F, K	
Danafungia horrida (Dana, 1846)	-	**H**	Fize and Serène, 1957 (*F. utinomi*)
Fungia fungites (Linnaeus, 1758)	+[1], *F. utinomi*[2]	G, **H**, M	Fize and Serène, 1957 (*F. utinomi*)
Herpolitha limax (Esper, 1797)	-	**O**	
Lithophyllon concinna (Verrill, 1864)	+[1]	G, K	
L. ranjithi Ditlev, 2003	+[1]	J	
L. repanda (Dana, 1846)	+[1], *F. utinomi*[2]	**H**, K, M, **N**, **O**	Takeda and Tamura, 1979 (*F. utinomi*); Fize and Serène, 1957 (*F. utinomi*)
L. scabra (Döderlein, 1901)	*Dacryomaia* sp., *Fungicola* sp.	G	
L. undulatum Rehberg, 1892	*Dacryomaia* sp.	G, **I**	
Lobactis scutaria (Lamarck, 1801)	*Fungicola* sp.[4]	R	
Pleuractis granulosa (Klunzinger, 1879)	*Fungicola syzygia* [1, 2]	A, E, G, L, P, S	
P. gravis (Nemenzo, 1956)	*Fungicola syzygia* [1]	G	
P. moluccensis (Van der Horst, 1919)	+[1]	G, K	
P. paumotensis (Stutchbury, 1833)	*Fungicola syzygia* [1, 2]	E, G, **H**, **N**, **O**, Q, S	Fize and Serène, 1957 (? *F. syzygia*); Takeda and Tamura, 1979 (? *F. syzygia*)
P. seychellensis (Hoeksema, 1993)	*Fungicola syzygia* [1]	D	
P. taiwanensis (Hoeksema & Dai, 1991)	+[1]	G	
Podabacia crustacea (Pallas, 1766)	*F. fagei*[1]	G	
P. motuporensis Veron, 1990	+[1]	L	
P. sinai Veron, 2000	*F. fagei*[1]	L	
Sandalolitha dentata Quelch, 1884	+[1]	G, **H**	Fize and Serène, 1957 (*F. fagei*)
S. robusta (Quelch, 1886)	*F. fagei*[1, 2]	M, S	

University of Milano-Bicocca (UNIMIB) in Milan, Italy, and the American Museum of Natural History (AMNH) in New York, USA. Some pits contained (dried) gall crab carapaces which were examined for identification (Table 1). Gall crab identifications were based on Fize and Serène (1957), Kropp (1990a) and van der Meij (2015a), whereas coral identifications were based on Hoeksema (1989), Gittenberger *et al*. (2011) and Benzoni *et al*. (2012a). Literature was studied to obtain further distribution records. Host species data provided by Fize and Serène (1957) were taken from the main text (p. 122, 130, 134, 156, 171) because these were assumed to be more correct than those listed on p. 13. In addition, a few field observations are included (photo vouchered).

Fieldwork

A large part of the fieldwork was carried out in Spermonde Archipelago – SW Sulawesi, in the southern part of the Makassar Strait (1994), where belt quadrats of 50 × 2m^2 were used to study gall crab – fungiid occurrences. Per quadrat the density of mushroom coral species and the percentage of inhabited corals was recorded. Transect work was mostly carried out on the western reef slopes as mushroom coral species are most abundant at these sides of the reefs, which are the most exposed to wind and wave action. Additionally, inhabited mushroom corals were collected to obtain the gall crab specimens. The corals were split by use of a hammer and chisel and coral fragments containing the gall crabs were immersed in 80% ethanol for at least one hour to immobilize the crabs, which were subsequently transferred to labelled vials. All specimens are deposited in the collections of Naturalis in Leiden, The Netherlands (collection coded as RMNH.Crus.D).

Further data on fungiid-gall crab associations were collected during fieldwork (2007-2012) in Indonesia (Raja Ampat – W Papua, Bunaken – N Sulawesi, Ternate – N Moluccas, Lembeh Strait – N Sulawesi) and Malaysia (Semporna – N Borneo, Kudat – N Borneo). Mushroom corals from various reef sites were sampled for gall crabs, attempting to sample as many host species as possible from deep to shallow reef zones. Mushroom corals containing gall crabs were collected until a representative collection of the Fungiidae species was reached. The corals were sampled in the same way as described above after being photographed with a Canon 400D camera equipped with a 50 mm Sigma macro-lens.

Additional records were obtained from Vietnam (Nha Trang – 2006), Australia (Great Barrier Reef – off Cairns (2010), New Caledonia (2012, Loyalty Is. – 2013), Malaysia (Payar Isl, Tioman Isl – 2013), and the Maldives (2014).

Cophylogenetic analyses based on host preference data

The phylogenetic congruence of hosts and associates was tested by using the programme Jane 4.0 (Conow *et al*., 2010), based on the phylogenies in Gittenberger *et al*. (2011), Benzoni *et al*. (2012a), and Van der Meij (2015a). The programme is based on an event-based model which considers cospeciation as the most parsimonious explanation for congruence between host and associate trees. Detection of coevolutionary relationships are easily obstructed by the complex interplay of events, i.e., cospeciation, duplication (intrahost speciation), host switching, sorting (extinction) and inertia (lack of parasite speciation). For definitions we refer to Paterson and Banks (2001) and Conow *et al*. (2010). The evolutionary events are used to superimpose phylogeny reconstruction of the associated taxon on that of the host taxon. Jane 4.0 assigns a cost to each evolutionary event, after which it seeks to find mappings minimizing the total cost. The default costs settings of Jane were used, as follows: cospeciation (0), duplication (1), duplication – host switching (2), loss (1) and failure to diverge (1). Statistical analyses are performed by comparing the best (minimum) costs found for the host parasite data set against randomized data sets (Cruaud *et al*., 2012).

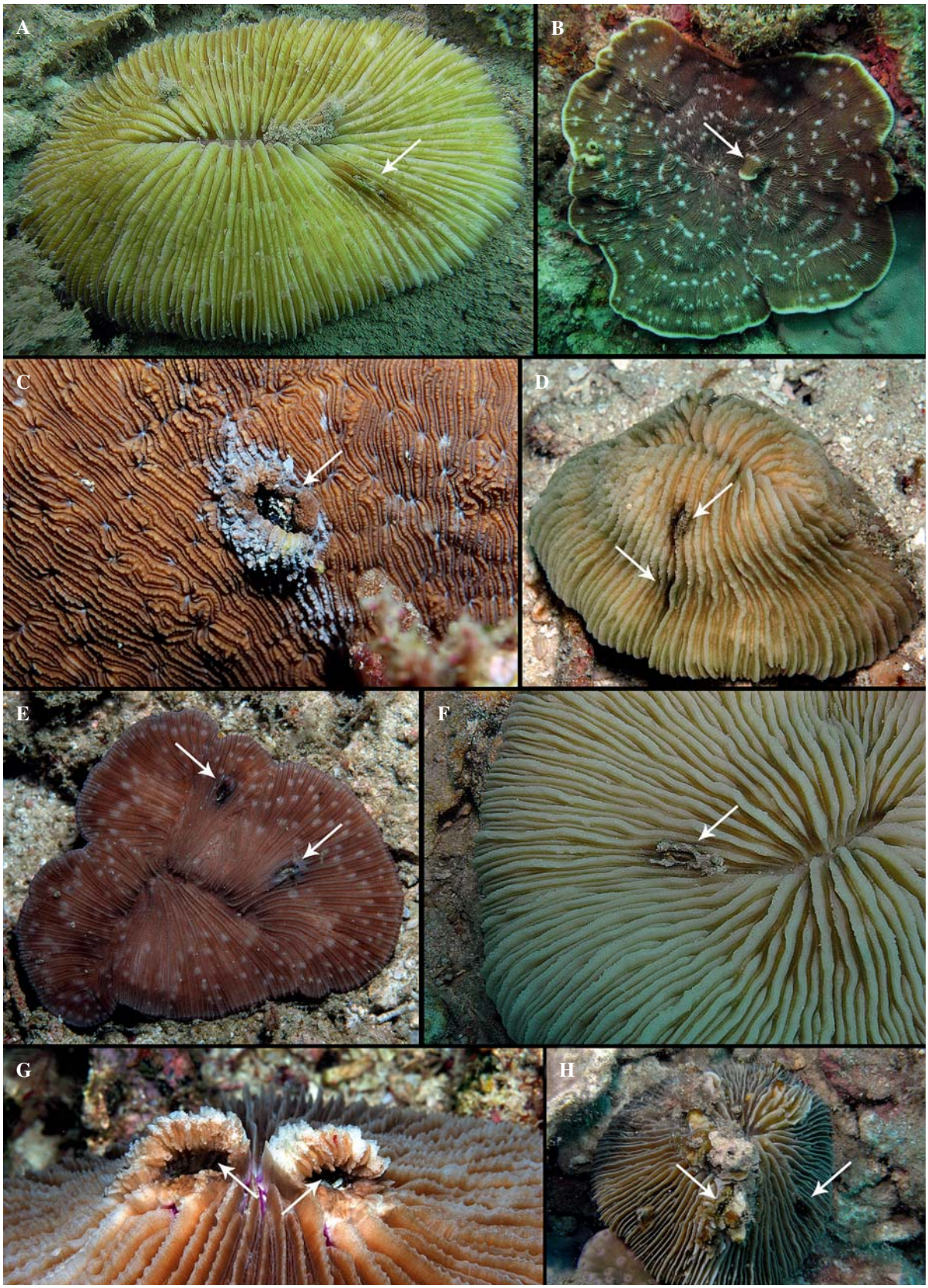

Fig. 1. Mushroom coral hosts with crab galls and their pits (arrows). **A**, *Pleuractis paumotensis* (Nha Trang, Vietnam); **B**, *Lithophyllon undulatum* (Nha Trang, Vietnam); **C**, *Podabacia crustacea* (Raja Ampat, Indonesia); **D**, *Pleuractis moluccensis* (Nha Trang, Vietnam); **E**, *Cycloseris sinensis* (Raja Ampat, Indonesia); **F**, *Pleuractis granulosa* (Ternate, Indonesia); **G**, *Lithophyllon repanda* (Raja Ampat, Indonesia); **H**, *L. scabra* (Nha Trang, Vietnam). Photographs not to scale.

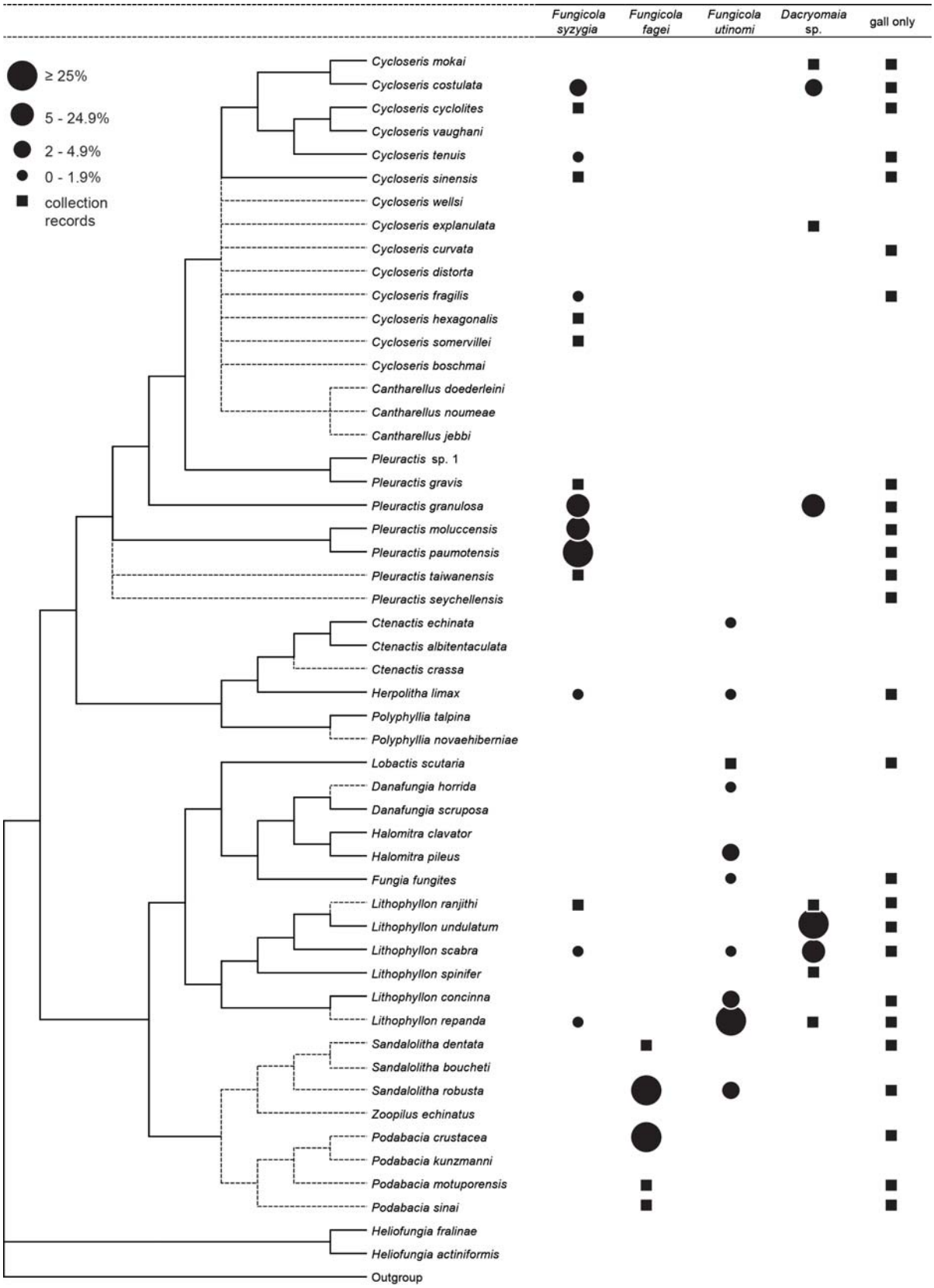

Fig. 2. Cladogram of the Fungiidae (based on Gittenberger *et al*., 2011; Benzoni *et al*., 2012), combined with gall crab associations. Percentages portray how the gall crabs are distributed over their coral hosts: *Fungicola syzygia* (n = 316), *F. fagei* (n = 4), *F. utinomi* (n = 82), and *Dacryomaia* sp. (n = 29). Other records based on collection data (Table 1) and fieldwork other than SW Sulawesi. All fungiid-gall crab associations resulting from fieldwork after 1994 are included as squares.

Table 2. Mushroom coral species (Fungiidae) acting as host for gall crab species in the Spermonde Archipelago, SW Sulawesi The number of collected coral specimens hosting specified gall crab species is given.

Coral host	*Fungicola syzygia*	*Fungicola fagei*	*Fungicola utinomi*	*Dacryomaia* sp.
Ctenactis echinata (Pallas, 1766)			1	
Cycloseris costulata (Ortmann, 1889)	8			1
C. fragilis (Alcock, 1893)	2			
C. tenuis (Dana, 1846)	1			
Danafungia horrida (Dana, 1846)			1	
Fungia fungites (Linnaeus, 1758)			1	
Halomitra pileus (Linnaeus, 1758)			3	
Herpolitha limax (Esper, 1797)	1		1	
Lithophyllon concinna (Verrill, 1864)			4	
L. repanda (Dana, 1846)	1		68	
L. scabra (Döderlein, 1901)	1		1	7
L. undulatum Rehberg, 1892				15
Pleuractis granulosa (Klunzinger, 1879)	49			6
P. moluccensis (Van der Horst, 1919)	40			
P. paumotensis (Stutchbury, 1833)	213			
Podabacia crustacea (Pallas, 1766)		1		
Sandalolitha robusta (Quelch, 1886)		3	2	

The programme can take multiple host associations into account, but occurrence levels are not supported, and therefore it was run twice: 1) on the complete dataset including all host specificity data, 2) on a dataset comprising only the common hosts (see Norton and Carpenter, 1998). In this second dataset sporadic host occurrences (singletons) were removed. In both runs the following settings were used (stats mode): 100 generations, population size 500, sample size 100. All other settings were left unchanged.

Results

Distribution based on historical records

Based on museum and literature records, distributions of Fungiidae-associated gall crabs range from Eilat in the Red Sea, and Kenya in the western Indian Ocean, towards Hawaii and Tahiti in the central Pacific Ocean (Table 1).

Occurrence records

Data on crab occurrences obtained from the belt quadrats in the Spermonde Archipelago, are projected on a cladogram of the Fungiidae (Table 2, Fig. 2). Percentages per host species are based on the number of encountered coral specimens per gall crab species. Fig. 1 shows gall crab dwellings in eight of their common gall crab hosts. *Fungicola fagei* was only found inhabiting corals belonging to the genera *Podabacia* and *Sandalolitha*, *F. syzygia* was predominantly found in corals of the genus *Pleuractis* and to a lesser extent in *Cycloseris*, whereas *Fungicola utinomi* was predominantly found in *Lithophyllon repanda*. *Dacryomaia* sp. mainly inhabits corals of the genera *Lithophyllon*, and was primarily associated with *L. undulatum*. It also occurs in the genera *Cycloseris* and *Pleuractis*. In the belt quadrats only one specimen of *Dacryomaia* sp. was recorded from the genus *Cycloseris*.

Host preferences and cophylogenetic analyses

The total number of Fungiidae associated with gall crabs is 35 (Fig. 2, Table S1). *Fungicola utinomi* is found to be associated with 10 mushroom corals species, *F. fagei* with five fungiids, and *F. syzygia* with 15 hosts. *Dacryomaia* sp., appears to be associated with nine fungiid species (Fig. 2, Table 2). *Cycloseris curvata* and *C. explanulata* are new host records. Hoeksema *et al*. (2012) recorded *Polyphyllia talpina* as a gall crab host. Further inspection of the material in the Naturalis collections revealed that this is likely not a gall crab dwelling, because the two pits in the host coral are interconnected and the surface of the dwelling is not smooth. These characteristics argue against a gall crab dwelling, and we therefore remove this coral species from the list of fungiid gall crab hosts until more evidence becomes available.

Based on the analysis in Jane 4.0 the complete dataset (Fig. S2) shows two duplication events, one cospeciation event, 34 losses and 37 failures to diverges. The smaller dataset (Fig. S4), comprised of only the common hosts, resulted in one duplication event, one duplication plus host switch event, one cospeciation event, 20 losses and 11 failures to diverge. Both results show that the costs of the random sample solutions are higher than the optimal [= cospeciation] solution (Figs S3, 5).

Discussion

Invertebrate taxa account for the greatest numerical abundance and diversity on coral reefs, yet have received rather little attention. Our awareness of coral reef ecosystem functioning is derived from what we know about a relative small proportion of coral reef species. Animals so closely associated with their habitat may be vital to the maintenance of critical ecological systems pertaining to coral health (Stella *et al.,* 2010), and as such could be potentially useful as environmental indicators (Thomas, 1993; Scaps and Denis, 2008).

In this study we used a phylogeny of the Fungiidae corals to map host preferences and occurrence rates. Using phylogenies to map ecologically meaningful traits of species is a fusion between ecology and evolution, also known as phylogenetic ecology or phylo-ecology (Westoby, 2006; Hoeksema, 2012a).

Distribution records

Until the late 1960s, the genus *Fungicola* was only known from Vietnam and since then just a few records became available from elsewhere (Takeda and Tamura, 1979; Kropp, 1990a, 1994). Van der Meij and Hoeksema (2013) and Van der Meij (2015a) added several new records of the genus in Indonesia and Malaysia. The present research on museum collections resulted in the availability of many additional records for all three *Fungicola* species (Table 1). During a short survey on the Great Barrier Reef off Cairns in May 2010 one specimen of *F. utinomi* was observed in *Lithophyllon repanda*, and individuals of *Fungicola* sp. were observed in *Pleuractis paumotensis* and *Herpolitha limax*. *Fungicola syzygia* is now reported from the Red Sea and Kenya in the west, to Japan and Vanuatu in the east, while *F. fagei* and *F. utinomi* are now recorded from Vietnam and Indonesia in the west, to Japan and possibly Australia (GBR) in the east. *Dacryomaia* sp. is recorded from the heart of the Coral Triangle: Indonesia and Malaysia (Table 1-2). The Indo-Pacific mushroom coral *Lobactis scutaria*, host to *Fungicola utinomi*, was brought to Jamaica from Eilat in 1966 and has established an apparently viable population (Bush *et al.,* 2004). So far no gall crabs have been reported for this population, which seems unlikely given current day ocean currents.

Hoeksema and Gittenberger (2008) report that coral gall crabs appear to be abundant in Nha Trang, Vietnam, especially in *Podabacia crustacea* and *Lithophyllon repanda*. Based on their results, the gall crab fauna in Vietnam likely consists of *Fungicola fagei* and *F. utinomi*, which is in agreement with the reports by Fize and Serène (1956a, b; 1957). According to Takeda and Tamura (1979), *F. utinomi* is more common in Japan than *F. fagei*, of which only two specimens are known. Based on Van der Meij (2015a) the identification of *F. fagei* by Takeda and Tamura (1979) should most likely be corrected to *F. syzygia*. The main hosts of *F. fagei* are, however, also present in Japan (Hoeksema, 1989). It is unclear whether the findings of Takeda and Tamura (1979) are caused by undersampling of particular species of mushroom coral hosts or by lower occurrence rates of *F. fagei* and *F. syzygia*. The genus *Dacryomaia* has been recorded from non-fungiid corals at the Ryukyu Islands (Japan), Caroline Isl. (Kiribati), Guam and other Mariana Isl. (Table 1), however, these records most likely concern *D. japonica*, *D. edmonsoni* and/or further undescribed species (Paulay *et al.,* 2003, van der Meij unpubl. data).

There appears to be much overlap in the geographical distribution of the mushroom corals and fungiid-associated gall crabs (Hoeksema, 1989; Table 2). The distribution ranges of the gall crab species is likely even more extensive. Presumably rare species, or species with a disjunct distribution, may be represented in scientific coral collections without being noticed. This confirms the value of historical collection material for biogeographical research, since museum specimens may show that species display a greater distribution range than previously assumed (Drew, 2011; Hoeksema *et al.,* 2011; van der Meij and Visser, 2011).

Occurrence records

The results of the belt quadrats in the Spermonde Archipelago show that the percentage of encountered gall crabs appears to be linked to the relative occurrence of their host corals. The coral species for which most gall crabs are reported are also among the most commonly occurring mushroom corals, i.e. *Lithophyllon repanda*, *Pleuractis granulosa*, *P. moluccensis* and *P. paumotensis* (see Hoeksema, 2012b). However, some common mushroom corals are not frequently inhabited by gall crabs (e.g. *Halomitra pileus*, *Lobactis scutaria*, *Sandalolitha dentata*), whereas others appear to be associated with one or more species (Table 2). Small and/or thin species (e.g. *Cycloseris boschmai*, *C. distorta*, *Halomitra clavator, Zoopilus echinatus*), those with fleshy polyps and permanently extending tentacles (e.g. *Heliofungia* spp., *Polyphyllia* spp.), or rarely observed species (e.g. *Cantharellus* spp., *Podabacia kunzmanni*, *Sandalolitha boucheti*) are not yet found to be associated with gall crabs.

Host preferences and cophylogenetic analyses

The total number of fungiid species inhabited by gall crabs is now 35 (Table S1). *Cycloseris explanulata* and *C. wellsi* were not yet included in the Fungiidae (Benzoni *et al.,* 2012) during most of the present research and were therefore also not considered as potential host for fungiid-associated gall crabs. This likely lead to under-sampling of these coral hosts. *Polyphyllia talpina* is no longer considered to be a gall crab host. This is in line with previous observations that gall crabs are mostly not observed in coral species with fleshy polyps and large tentacles (e.g. Van der Meij, 2014a).

Recently the coral family Fungiidae was revised based on a molecular analysis (Gittenberger *et al.,* 2011). The majority (95%) of *Fungicola syzygia* specimens was encountered in *Pleuractis* corals, i.e. *P. paumotensis*, *P. granulosa*, and *P. moluccensis* (Fig. 2). Apart from the genus *Pleuractis*, this gall crab species also occurs in the closely related genus *Cycloseris*. *Fungicola utinomi* is in almost all cases associated with *Lithophyllon repanda*, but occurs to a lesser extent in corals belonging to other genera. None of the inhabited fungiids were simultaneously occupied

by more than one gall crab species, but one host species, *Lithophyllon scabra*, was found inhabited by either one of the three gall crab species. The sporadic selection of certain corals as a host might be related to a low availability of the common or 'preferred' host species at a certain locality. It might also be the result of a collecting artefact, as it remains possible that host occurrence has geographic variability.

Dacryomaia sp. mostly targets *Lithophyllon undulatum*, and to a lesser extent *L. scabra*, and *Pleuractis granulosa*. Other species in the genus *Dacryomaia* are associated with the genera *Coscinaraea* (Coscinaraeidae), *Leptastrea* (Scleractinia *incertae sedis*), and *Psammocora* (Psammocoridae) (Kropp, 1990a, van der Meij, unpubl. data). This is likely not a coincidence, since these genera are closely related to the Fungiidae (Fukami *et al.,* 2008; Kitahara *et al*., 2010; Huang, 2012). The genus *Dacryomaia*, which contains undescribed species, is in need of a taxonomic revision (Paulay *et al.,* 2003, van der Meij, unpubl. data). Further research on the gall crabs of this genus and their host preferences may be used to verify congruencies of the phylogenetic relationships of the associated fauna and their hosts as support for reconstructed phylogenetic relationships within the Scleractinia.

The analyses in Jane 4.0 show that there have been cospeciation and duplication events between fungiids and their gall crab inhabitants, as well as several losses and failures to diverge. Differences between the outcomes of the analysis on the complete dataset vs the common host dataset can be explained by the settings of the programme Jane. Associations between host and symbiont are not weighed, hence single recorded associations are given the same value in the analysis, obscuring the overall patterns between host and symbiont. Both analyses show that even within a moderately small coral family like the Fungiidae with just over 50 species (Gittenberger *et al.,* 2011; Benzoni *et al.,* 2012a), four gall crab associates occupy their own niche and are host-specific to a certain degree. *Fungicola fagei* appears to be more strict in its host preference than the other three species. The large-scale phylogeny reconstruction of all gall crabs and their coral hosts provides more insight in the cospeciation between these associates and their hosts. Gall crabs are mostly host specific on coral genus level, which explains the high number of losses and failures to divergence in the Jane analysis. The relationship between Scleractinia and Cryptochiridae appears to be so tight that gall crabs can be used as phylogenetic indicators of scleractinian evolution (van der Meij, 2015a), which contradicts the hypothesis of Kropp and Manning (1987) that the generic identity of coral hosts is an unreliable character for defining gall crab genera.

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Appendices S1-5 are available upon request.

Chapter 10

Adaptive divergence in coral-dwelling gall crabs: signature of host driven evolution

Sancia E.T. van der Meij

Abstract

Intimate interactions between host organisms and their symbionts can, on a long time scale, lead to impact on the evolution of the partner. Within the theoretical context of host-parasite evolution, coevolution is only considered appropriate for a given host-symbiont assemblage if the hosts and their symbionts show similar patterns of phylogenetic differentiation. Many studies on coevolutionary relationships focus on terrestrial organisms and involve vertebrates as hosts. The present research on the association between stony corals (Scleractinia) and gall crabs (Cryptochiridae) concerns an invertebrate-invertebrate association in the marine realm. For the Cryptochiridae the phylogenetic relationships within the family were reconstructed based on 16S, COI and H3 markers, whereas information on the phylogenetic relationships within the Scleractinia was already largely available in the literature. The congruence between both phylogeny reconstructions was tested using the programme Jane 4.0, which tests for the occurrence of coevolutionary events. The phylogram of the Cryptochiridae shows three large clades and multiple paraphyletic genera. Further taxonomic work is needed to find out whether some genera are monophyletic. The test for congruency resulted in 20 cospeciation events, three duplication events, 14 duplication - host switching events, eight losses and 10 failures between the gall crab phylogeny and coral phylogeny. The statistics show that coevolution is the most likely scenario for the observed congruence, as the outcome is significantly higher than it would have been as expected by chance alone. The observed events should most probably be ascribed to sequential evolution, which indicates that the phylogeny of the Cryptochiridae has been directed by the evolution of the Scleractinia.

Manuscript in preparation

Introduction

Evolutionary diversification among close associations between heterospecific species (symbiosis), as an alternative to direct competition between associated species for the same host, is an important strategy for survival in biotic communities. Symbioses include a broad category of heterospecific associations embracing various degrees of adaptive interactions that involve intimate physiological and ecological interactions (Castro, 1988). If interactions between species are close enough, the organisms involved may have speciated synchronously, so a reconstruction of their evolutionary histories would show congruent events of speciation (Paterson and Banks, 2001). Nonetheless, the impact of these interactions on the evolution of each partner depends on the time-scale considered. Only macroevolutionary patterns will be considered here, i.e., the long-term evolutionary dynamics of speciation following host shifts. These are differentiated from studies at a shorter time scale (e.g. changes in allele frequencies over successive generations, Red Queen driven processes) (Desdevises, 2007; de Vienne *et al.*, 2013).

Many studies on coevolutionary relationships focus on mammal, bird and (to a lesser extent) fish hosts and their parasites but cophylogenetic analyses have also been carried out in a diverse range of other systems, including non-symbiotic ones such as plants – pollinator and vertebrate – virus systems (for overviews see Lanterbecq *et al.*, 2010; Duchene *et al.*, 2013). A well-known symbiotic coevolution example is that of gophers and lice (Hafner and Nadler, 1988; Hafner *et al.*, 1994), but studies of intimate evolutionary associations between hosts and parasites started with avian hosts and their parasites (Hoberg *et al.*, 1997).

Parasite speciation and specificity is based on their host group, hence the phylogenies of parasites are considered to have great predictive value in elucidating the associated host phylogeny (Eichler, 1942). A series of parasitological rules were developed of which Fahernholz's rule – *parasite phylogeny mirrors host phylogeny* – is the most well-known. Indeed, phylogenetic studies of interacting organisms often reveal congruence between the phylogenies of the interacting taxa. Congruence between host and parasite phylogenies is seen as evidence for coevolution (e.g. Hafner and Nadler, 1988; Hafner *et al.*, 1994; Patterson and Banks, 2001). Within a theoretical context of host-parasite evolution, coevolution is only considered appropriate for a given host-parasite assemblage if the hosts and their parasites show identical patterns of phylogenetic differentiation. In contrast, identical patterns in host organisms and their parasites are only rarely observed and certain levels of discordance between host and parasite phylogenies are considered the norm (Hafner and Nadler, 1990). Moreover, parasites can vary in their host specificity. Groups of parasites occupy a spectrum from highly host-specific to host generalist. There is a general tendency among parasites that infect more than one host species to infect hosts that are phylogenetically closely related - that is, usually species within the same genus or family – which appears to be an important factor in speciation (Norton and Carpenter, 1998).

Coevolution is the universally accepted term for the process involving two or more lineages that reciprocally influence each other's evolution. This is, however, a general term that encompasses strict coevolution and sequential coevolution. Strict coevolution implies that two separate taxa mutually influence the evolution of the other, the two taxa tending to i) change together (coadaptation), or ii) speciate together (cospeciation) (Ridley, 1996). It has been assumed that coadaptation favours cospeciation, but it appears that the critical factor may be the rate at which the symbiont or parasite encounters potential new host species (Ronquist, 1997). Sequential evolution is a particular case of coevolution where the changes (morphological, physiological or behavioural) and the phylogeny of the symbionts are influenced by the host evolution, but it is not reciprocal (Ridley, 1996).

Documentation of widespread coevolution in a host-parasite assemblages requires statistical evidence that the congruence observed between the host and parasite phylogenies exceeds that expected by chance (Huelsenbeck *et al.*, 1997; Hafner and Nadler, 1990). Two kinds of evidence are necessary to document coevolution in a host-parasite assemblage: evidence that the host and parasite phylogenies are derived independently and statistical evidence that the topological similarity of the host and parasite trees exceeds chance expectations (Hafner and Nadler, 1990). By comparing the phylogenies of host species and their associates, it is possible to detect if a statistically significant cophylogenetic signal is present and estimate the role played by the different historical events (Paterson and Gray, 1997). Analyses of coevolutionary relationships, however, are obstructed by the complex interplay of coevolutionary events. Four types of basic coevolutionary events were defined, here applied to parasitic relationships (Page, 1994; Page and Charleston, 1998): cospeciation (concomitant host and parasite speciation), host switching (colonization of a new host by a parasite), duplication (parasite speciation on a single host lineage), and sorting event (disappearance of a parasite lineage from a host). Some authors define more types of events (e.g. Paterson and Banks, 2001; Johnson *et al.*, 2003), but they broadly fall into the four basic categories described above (Desdevises, 2007). These coevolutionary events may all produce incongruence between host and parasite phylogenies (Patterson and Banks, 2001). Speciation of the symbiont can occur independently of host speciation, often through host shifts as the symbiont comes to occupy a new host environment in isolation from the ancestral lineage (de Vienne *et al.*, 2013).

Only few taxa received much of the attention in studies on cophylogenies. Marine models have not been extensively studied, especially not models in which marine invertebrates are involved, yet their difference compared to more known terrestrial systems may shed light on processes concerning the generation of cophylogenetic patterns (Desdevises, 2007; Duchene *et al.*, 2013). This chapter studies the relationship between gall crabs (Cryptochiridae) and their stony coral hosts (Scleractinia). Cryptochiridae is a family of coral-inhabiting crabs occurring on reefs worldwide. These crabs depend on their hosts for food and shelter (Kropp, 1986, 1990a). The observed host-specificity patterns of gall crabs (e.g. Fize and Serène, 1957; van der Meij, 2015a) triggers questions about the nature of the association. The relatively small size and worldwide occurrence of the Cryptochiridae (approx. 50 described species – Davie, 2014) allows to study coevolutionary patterns between a monophyletic family (van der Meij and Schubart, 2014) and their scleractinian hosts across the whole family, as well as between oceanic basins. Cophylogenetic approaches in coevolution and biogeography studies ask for a whole new set of analytical methods (Ronquist, 1997). The combination of a high species diversity in certain crab genera, biogeographic patterns, host specificity, and (presumably) millions of years of association, prompts many questions about the underlying mechanisms causing diversification. In order to study these mechanisms the following questions need to be answered first. 1. Does the phylogeny of the Cryptochiridae mirror the phylogeny of the corals (Fahernholz's rule) or are there incongruences between the two? 2. Is there coevolution (in the broad sense) between the crabs and their hosts, and if so, i) which type of coevolution can be distinguished, and ii) which coevolutionary events are expected to have occurred? To study these questions the phylogenetic relationships within the Cryptochiridae are reconstructed and compared with a phylogeny reconstruction of the Scleractinia.

Material and methods

The material used in this study has been collected from 2007 to 2013 in Indonesia, Malaysia and the Saudi Arabian part of the Red Sea in the Indo-Pacific and in Curaçao, Dutch Caribbean, in

the Atlantic. Corals from many different families were searched for galls and pits, and subsequently split with hammer and chisel. The gall crabs were preserved in 80% ethanol, after being photographed with a digital SLR camera equipped with a 50 mm macro lens. The crab specimens are deposited in the collections of Naturalis Biodiversity Center in Leiden, The Netherlands (formerly Rijksmuseum van Natuurlijke Historie), collection-coded as RMNH.Crus.D).

Molecular analyses

For the reconstruction of relationships within the Cryptochiridae, 38 shallow-water species belonging to 17 genera were selected. The type species of each genus was included. Material from the Atlantic (ATL) and Indo-Pacific (IP) was used. Unfortunately, deep sea gall crab species were not available for molecular study. The *Hemigrapsus pennicilatus* (Varunidae) was selected as an outgroup (van der Meij and Schubart, 2014).

Gall crabs were sequenced for three markers (16S, COI mtDNA, H3 nDNA). DNA extraction was performed following the protocols specified in Van der Meij (2015a). For each marker, sequences were trimmed to be of equal length and aligned in Guidance using the Prank algorithm (Penn *et al.*, 2010a, b), resulting in scores of 0.98 for 16S (minimally adjusted by eye in BioEdit (Hall, 1999)), 0.99 for COI, and 1.0 for H3. The 16S dataset contained 383 constant, 169 parsimony-informative and 33 uninformative characters. The COI dataset contained 396 constant, 238 parsimony informative and nine uninformative variable characters. The H3 dataset contained 203 constant, 75 parsimony-informative and eight uninformative characters.

The appropriate model of evolution was determined using jModeltest 2.1.3 (Darriba *et al.*, 2012) using the Akaike Information Criterion (AIC). For COI this resulted in TrN+I+G (Tamura and Nei, 1993), for 16S in TIM2+I+G (Posada, 2008), and for H3 in GTR+I+G (Tavaré, 1986). Sequences were concatenated in Sequence Matrix (Vaidya *et al.*, 2011), converted to nexus and partitioned as follows: 16S bp 1-585, COI bp 586-1228, H3 bp 1229-1514.

Phylogeny reconstructions

Bayesian inferences were estimated in MrBayes (Ronquist and Huelsenbeck, 2003). The programme was run for 5,000,000 generations using the most complex GTR+I+G model. The analysis stabilized at 0.004865, burnin was set to 25%. Maximum Likelihood (ML) analyses were carried out in Garli 2.0 (Zwickl, 2006) on the partitioned dataset, with the evolutionary models as specified earlier. Two search replicates were carried out with 250 bootstrap replicates. The bootstrap consensus tree was visualised with the SumTrees 3.3.1 package of the DendroPy 3.12.0 package in the Phyton library (Sukumaran and Holder 2010). Scleractinian phylogeny, for the coevolutionary analyses, was reconstructed based on literature. The main groupings were based on Fukami *et al.* (2008), supplemented by data from Budd *et al.* (2012) and Huang *et al.* (2014).

Coevolutionary analyses

The congruence between coral and gall crab phylogenies was tested by using the programme Jane 4.0 (Conow *et al.*, 2010). The programme is based on an event-based model which considers cospeciation as the most parsimonious explanation for congruence between host and parasite trees. Coevolutionary relationships are obstructed by the complex interplay of cospeciation, duplication (intrahost speciation), host switching, sorting (extinction) and inertia (lack of parasite speciation). For definitions see Paterson and Banks (2001) and Conow *et al.* (2010). The evolutionary events are used to superimpose phylogeny reconstruction of the associated taxon on that of the host taxon. Jane 4.0 assigns a cost to each evolutionary event, after which it seeks to find mappings minimizing the total cost. The default costs settings of Jane were used, as follows:

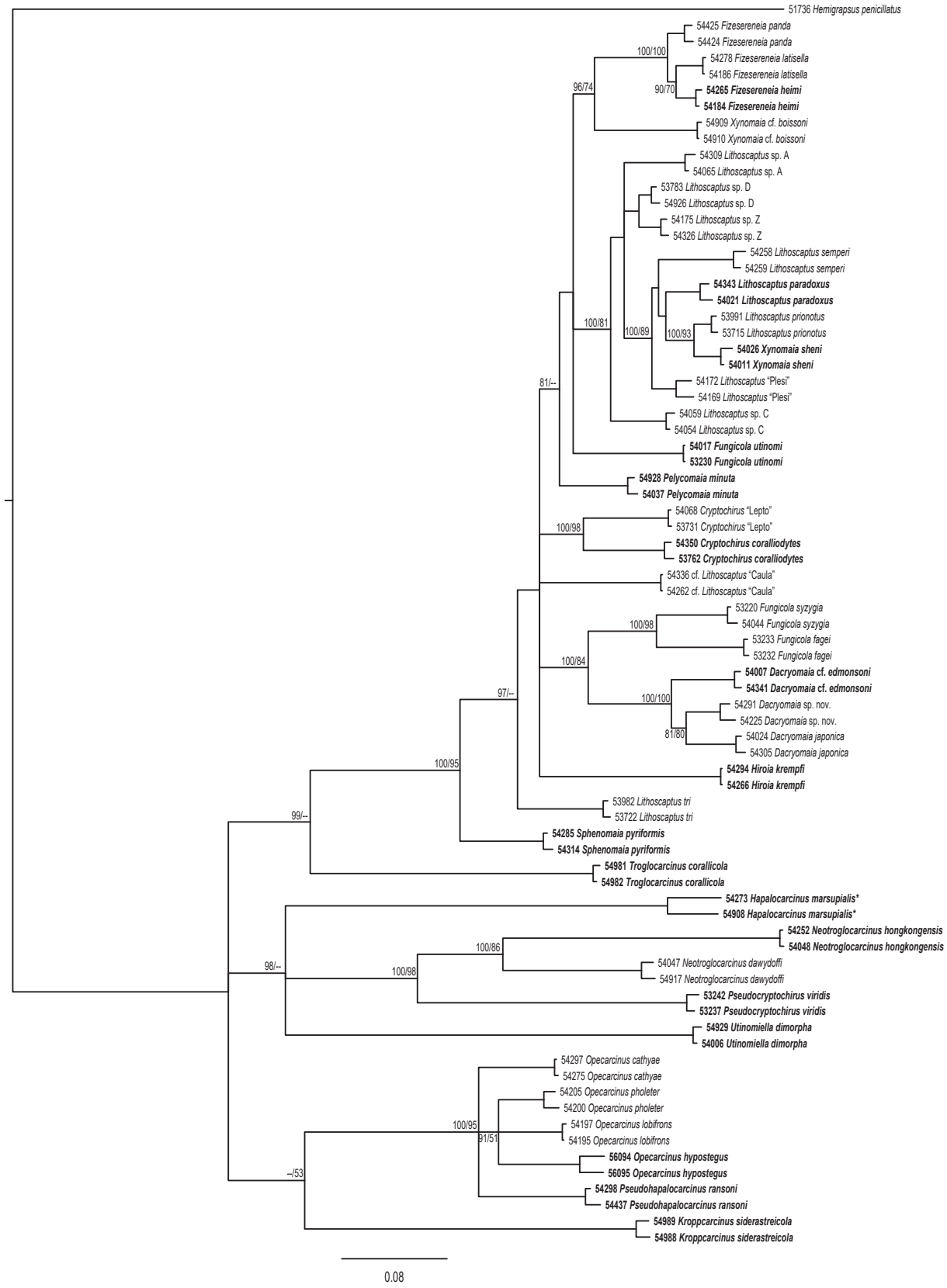


Fig. 1. Bayesian inference (BI) tree based on the concatenated dataset of 16S, COI and H3, with the varunid *Hemigrapsus penicillatus* as outgroup. Maximum likelihood (ML) values resulting from the Garli run are plotted on the BI tree. BI values <80 and ML values < 50 are not provided. Type species are printed in bold, * represents a species complex.

cospeciation (0), duplication (1), duplication – host switching (2), loss (1) and failure to diverge (1). Statistical analyses are performed by comparing the best (minimum) costs found for the host parasite data set against randomized data sets (Cruaud *et al.*, 2012). The following settings were used in stats mode: generations 500, population size 2500, sample size 100. All other settings were left unchanged.

Results

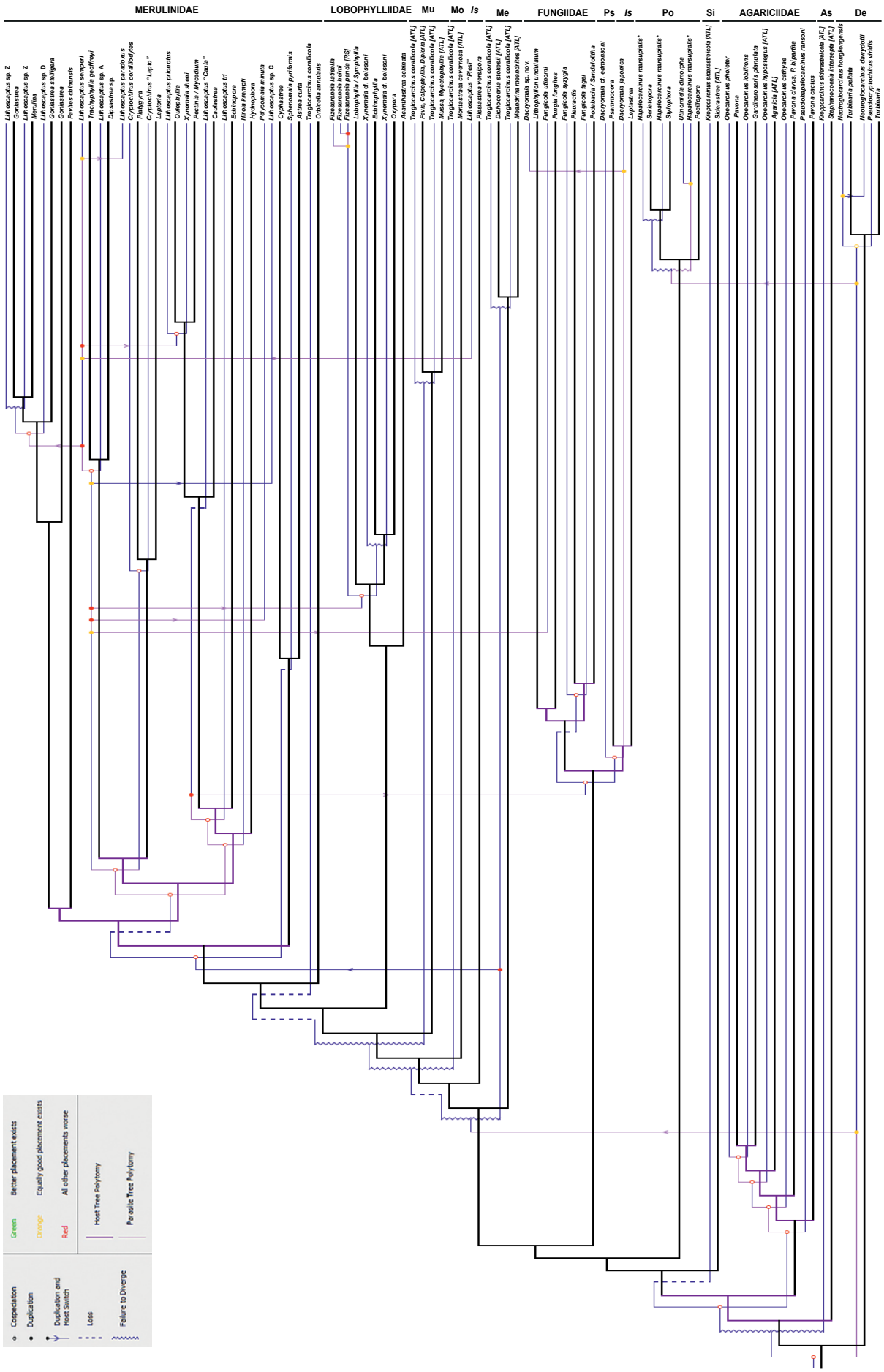
Phylogenetic tree

The topology of the phylogeny reconstruction (Fig. 1) is derived from the Bayesian inference 50% majority rule consensus of the trees remaining after the burnin, with high support values in the basal part as well as in the distal phylogenetic branches. The outgroup is separated by a long branch. Within the Cryptochiridae, three major clades can be distinguished, but the relationships between these clades are unclear. The first large clade has *Troglocarcinus corallicola* (ATL) as the most basal clade (not supported by the ML analysis), followed by *Sphenomaia pyriformis* (IP) and *Lithoscaptus tri* (IP). Several subclades can be discerned within this clade; 1) *Fungicola fagei* and *F. syzygia* are closely related to the genus *Dacryomaia*. The type species of the genus *Fungicola* does not cluster in the same subclade. *Cryptochirus coralliodytes* is closely related to a presumably undescribed species associated with the coral genus *Leptoria*. A larger clade is formed by several species (including undescribed species) of *Lithoscaptus*, including the type species *L. paradoxus*. This clade also contains the type species of *Xynomaia*. Another clade is formed by *Fizesereneia*, with another *Xynomaia* species clustering basally. A second clade is formed by the Indo-Pacific genera *Hapalocarcinus*, *Utinomiella*, *Neotroglocarcinus* and *Pseudocryptochirus*, however, this clade is not supported by the ML analysis. The latter two genera form a well-supported subclade within this clade. The third clade is formed by the genera *Opecarcinus* (IP+ATL) and *Pseudohapalocarcinus* (IP), with *Kroppcarcinus* (ATL) in a basal position (albeit with low support and long branch length).

Coevolution analyses

Based on the analysis in Jane 4.0, the following events can be discerned: 20 cospeciation events, three duplication events, 14 duplication – host switching events, eight losses, and 10 failures to diverge between Cryptochiridae and Scleractinia (Fig. 2). The majority of the cospeciation events were recorded in associations of gall crabs and hosts species belonging to the Agariciidae, Dendrophyllidae, Fungiidae and Merulinidae. The results of the stats run show that the costs of the random sample solutions are higher than the optimal [= coevolution] solution, for which the costs are 49 (Fig. 3). For all the isomorphic optimal solutions provided by Jane 4.0 the costs and number of estimated coevolutionary events were the same.

Fig. 2. Tree resulting from analysis in Jane 4.0 showing the different coevolutionary events between Scleractinia (black lines) and Cryptochiridae (blue lines). ATL = Atlantic, RS = Red Sea, all other species are from the Indo-Malay region. * indicates species complex. Letters in bold refer to the host coral family of the gall crabs specimens: As = Astrocoeniidae, De = Dendrophylliidae, *Is* = Insertae sedis, Me = Meandrinidae, Mo = Montastreidae, Mu = Mussidae, Po = Pocilloporidae, Ps = Psammocoridae, Si = Siderastreidae (classification after Budd *et al.*, 2012; Huang *et al.*, 2014). ▶


MERULINIDAE
LOBOPHYLLIIDAE
Mu
Mo
Is
Me
FUNGIIDAE
Ps
Is
Po
Si
AGARICIIDAE
As
De
Lithoseptus sp. Z
Goniastrea
Lithoseptus sp. Z
Merulina
Lithoseptus sp. D
Goniastrea stelligera
Goniastrea
Favites chinensis
Lithoseptus semperi
Tracyphyllia geoffroyi
Lithoseptus sp. A
Dipsastrea sp.
Lithoseptus paradoxus
Cyphochirus coralliodytes
Hetopsyra
Cryptochirus "Lepto"
Leptoria
Lithoseptus prionotus
Oulophyllia
Xynomaia sheni
Pectinia / Mycedium
Lithoseptus "Caula"
Caulastrea
Lithoseptus tri
Echinopora
Hiroia khempfi
Hydnophora
Pectinia paeonia minuta
Lithoseptus sp. C
Cyphastrea
Sphenomaia pyriformis
Astrea curta
Troglocarcinus corallicola
Orbicella annularis
Fizesereneia latsella
Fizesereneia helmi
Fizesereneia panda [RS]
Lobophyllia / Symphyllia
Xynomaia cf. boasoni
Echinophyllia
Xynomaia cf. boasoni
Oxypora
Acanthastrea echinata
Troglocarcinus corallicola [ATL]
Favia, Colpophyllia, Diploria [ATL]
Troglocarcinus corallicola [ATL]
Mussa, Mycetophyllia [ATL]
Troglocarcinus corallicola [ATL]
Montastraea cavernosa [ATL]
Lithoseptus "Pisa"
Pleastrea versipora
Troglocarcinus corallicola [ATL]
Dichocoenia stokesii [ATL]
Troglocarcinus corallicola [ATL]
Meandrina meandrites [ATL]
Dacryomaia sp. nov.
Lithophyllon undulatum
Fungicola utinomi
Fungia fungites
Fungicola syzygia
Pleuractis
Fungicola fagei
Podabacia / Sandalolitha
Dacryomaia cf. edmonsoni
Psammocora
Dacryomaia japonica
Lobactis
Hapalocarcinus marsupialis*
Scuriatopora
Hapalocarcinus marsupialis*
Stylophora
Utinomiella dimorpha
Hapalocarcinus marsupialis*
Pocillopora
Kroppcarcinus sideraetricola [ATL]
Siderastrea [ATL]
Opecarcinus pholeter
Pavona
Opecarcinus lobifrons
Gardineroseris planulata
Opecarcinus hypostegus [ATL]
Agaricia [ATL]
Opecarcinus cardiyae
Pavona clavus, P. bipartita
Pseudohaplocarcinus ransoni
Pavona cactus
Kroppcarcinus sideraetricola [ATL]
Stephanocoenia intersepta [ATL]
Neotroglocarcinus hongkongensis
Turbinaria peltata
Neotroglocarcinus dawydoffi
Pseudocryptochirus viridis
Turbinaria
Green
Better placement exists
Orange
Equally good placement exists
Red
All other placements worse
Host Tree Polytomy
Parasite Tree Polytomy
Cospeciation
Duplication
Duplication and Host Switch
Loss
Failure to Diverge

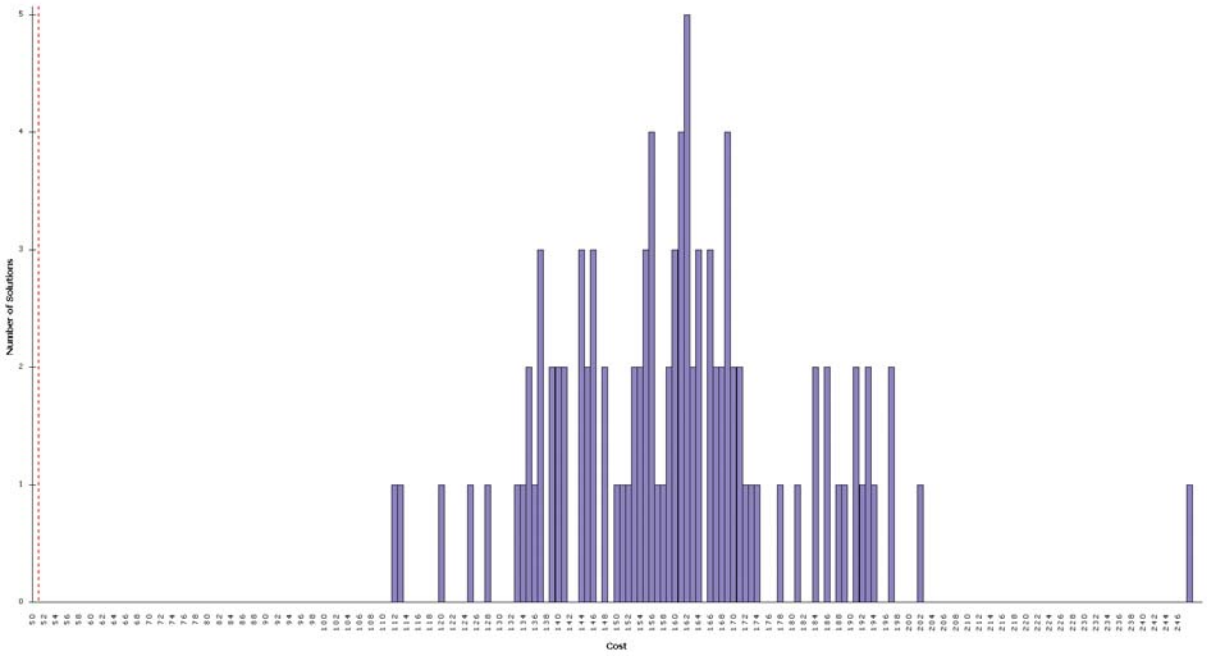


Fig. 3. Histogram resulting from a stats run in Jane 4.0, showing the distributions of costs of the random sample solutions. The costs of the optimal [= coevolution] solution is indicated by the red dotted line.

Discussion

Relationships within the Cryptochiridae

There are three major clades within the Cryptochiridae, similar to the results of van der Meij and Reijnen (2014), which was based on 16S and COI mtDNA, and the results of Wei *et al.* (2013) that were based on the morphological data of Kropp (1988). The first large clade shows the Atlantic genus *Troglocarcinus* in a basal position, which is not supported by the ML analysis. The remainder of the clade consists of Indo-Pacific species, of which one species (*Fizesereneia panda*) is endemic to the Red Sea and to other waters around the Arabian peninsula (van der Meij *et al.* in press). The genera *Fungicola*, *Lithoscaptus* and *Xynomaia* appear to be paraphyletic. Based on their host specificity (Fungiidae) and overall morphology this result is especially surprising for the genus *Fungicola*. The type species, *F. utinomi* clusters in a subclade with four other genera, whereas *F. fagei* and *F. syzygia* cluster with the genus *Dacryomaia*. The second clade, which is formed by Dendrophylliidae-associated genera *Neotroglocarcinus* and *Pseudocryptochirus,* is very well supported, whereas the clustering of *Hapalocarcinus* and *Utinomiella* with this clade is only supported by Bayesian inference. The clade containing *Opecarcinus* and *Pseudohapalocarcinus*, two genera associated with Agariciidae, is very well supported. *Kroppcarcinus* clusters weakly with this clade. This genus is strictly Atlantic, whereas *Opecarcinus* occurs in the Atlantic and the Indo-Pacific (Kropp, 1989) and *Pseudohapalocarcinus* only in the Indo-Pacific (Kropp, 1990a). The position of *Hapalocarcinus* and *Utinomiella* is so far not consistent, and with low support (see Van der Meij and Reijnen, 2014). Again their position (Fig. 1) is only supported by the Bayesian analysis, in the ML analysis the resulting tree ended in a polytomy. Interestingly, these genera are both associated with Pocilloporidae corals.

More species need to be added for certain genera, especially for *Lithoscaptus*, to understand the relationships within the paraphyletic genera. It is however clear that taxonomic revisions of certain genera are needed in order to become monophyletic genera.

Coevolution

Two kinds of evidence are necessary (and sufficient) to document widespread cospeciation in a host-parasite assemblage: evidence that the host and parasite phylogenies are derived independently and statistical evidence that the topological similarity of the host and parasite trees exceeds chance expectations (Hafner and Nadler, 1990). They furthermore warn that the taxonomy of either host or parasite may have been influenced, explicitly or implicitly, by knowledge of relationships within the other. They further their statement by mentioning that systematic investigations of parasites generally postdate systematic studies of their hosts. The latter is not true for gall crabs. The recent overhaul in scleractinian systematics (e.g. Gittenberger *et al.*, 2011; Arrigoni *et al.,* 2014a; Huang *et al.,* 2014) will have undone any implicit influence of scleractinian systematics on gall crab systematics, in addition to a molecular approach to reconstruct the Cryptochiridae relationships. The present analysis supports the hypothesis that the topological congruence between the gall crab and coral trees is not due to chance alone, hence speciation of stony corals may have induced speciation in gall crabs. The Cryptochiridae and corals, however, do not have strict parallel phylogenies and evolutionary events other than cospeciation are needed to explain the topological incongruence found in the gall crab-coral tree pairs. Sorting events, host-switches, losses and, to a lower degree, duplications, were present all along the twin history of these organisms.

An important aspect in determining whether there are mutual events between the crabs and hosts is the origin of the Cryptochiridae compared to the origin of the Scleractinia. The most recent common ancestor of the gall crabs appeared between 48-23 Ma, with a strong diversification roughly around 10 Ma (van der Meij and Klaus, chapter 6). This preliminary data shows that gall crabs likely diversified in a later stage than their host corals (Budd, 2000; Duchene *et al.*, 2013; Santodomingo *et al.*, 2014). Also, the common ancestor of the gall crabs does not necessarily have the same symbiotic lifestyle of the extant Cryptochiridae (i.e. this ancestor may not have constructed clear pits and may not have shown a strict host specificity). It appears that the observed coevolutionary event should be ascribed to sequential evolution – the phylogeny of the symbionts are influenced by the host evolution, but it is not reciprocal.

Based on the present results, it appears that the coral-cryptochirid system is a good model of marine cophylogeny involving symbionts. It is difficult to compare the present results with those presented in literature, which exclusively involve either parasites or mutualists, because (i) the number of hosts and symbionts used in the various existing studies is extremely variable, and (ii) the taxonomical range of symbionts and hosts is also extremely different from one study to another. Only one study is known that deals with such coevolutionary relationships in the marine environment, i.e., by looking at the relationship between crinoids and their myzostomid commensals (Lanterbecq *et al.*, 2010). This study showed a minimum of eight cospeciation events between 16 Myzostomida worms and their Crinoidea hosts. This is comparable with the gall crabs, which showed 20 events between 38 Cryptochiridae and their coral hosts. However, the study of Lanterbecq *et al.* (2010) only comprised a small subset of the known associations between myzostomids and crinoids, whereas the present study includes about half the number of known associations between gall crabs and corals (van der Meij *et al.*, chapter 12; van der Meij, unpublished data). The importance of one evolutionary event on another within a host-symbiont system can vary from case to case, based on the type of association (parasitism, commensalism, mutualism) (Lanterbecq *et al.*, 2010). The association between Cryptochiridae and Scleractinia is mostly considered to be a symbiotic relationship (Kropp, 1986; Castro, 1988).

Limitations of this study

Since information on the host specificity of certain gall crabs is now limited to genus level, the resolution of the test would be improved by adding more specific data on their hosts. Also the addition of more species, especially for species rich genera such as *Lithoscaptus*, and the inclusion of known cryptic species would shed more light on coevolutionary events in these associations. The coevolutionary analysis used in this paper is an event-based method, which would ideally be supplemented by a topology- and distance-based methods (de Vienne *et al.* 2013). For the majority of the programmes that can perform such analyses the Scleractinia phylogeny has to be reconstructed based on molecular data, an exercise that is now hampered by large datasets, a lack of suitable markers and missing species. Preferably additional testing would also include a test of biogeography.

Gall crabs as phylogenetic indicators of scleractinian evolution

The relationship between corals and gall crabs is a tight one, with at least 20 cospeciation events according to Jane 4.0. Also when comparing the phylogenies by eye, several similarities between the large overall clades become apparent. Within the Scleractinia two main clades are recognized: a 'complex' clade and a 'robust' clade (Fukami *et al.* 2008). A third basal clade (containing the Gardineriidae and Micrabaciidae) can be recognized, representatives of the most basal lineage of modern scleractinians (Kitahara *et al.* 2010). No gall crabs have so far been recorded from this basal clade. Within the 'complex' and 'robust' clades several main clades can be distinguished. In the complex clade we find the gall crab hosting families Dendrophylliidae, Agariciidae, and Pocilloporidae, whereas the robust clade is comprised of a subclade containing the Fungiidae, Psammocoridae and *Leptastrea*, and a large subclade (again with several subclades) consisting of Merulinidae, Lobophylliidae and several smaller families. Several Atlantic species cluster basal to this large subclade.

The Cryptochiridae show a similar pattern with the Dendrophylliidae and Agariciidae associated gall crabs in separate clades. Two gall crab genera inhabit corals of the Pocilloporidae. The position of these genera within the Cryptochiridae is somewhat equivocal. Support for the position of these genera is low and so far they have 'jumped' through the different trees resulting from phylogeny reconstructions. Two *Fungicola* species and *Dacryomaia* inhabit corals from the Fungiidae, Psammocoridae and *Leptastrea* which perfectly matches the coral phylogeny. The types species of *Fungicola*, however, clusters in a different clade. Like with the corals, the remaining gall crabs, associated mostly with Merulinidae and Lobophylliidae, form a large clade, and, like the corals, the Atlantic species *Troglocarcinus corallicola* clusters basally to this clade. In a more narrow framework of one family, gall crabs have shown to be good indicators of their host relationships, especially at generic level (van der Meij, 2015). Recent results from recent molecular studies on Lobophyllidae and Merulinidae, such as the close relationship between the coral genera *Lobophyllia* and *Symphyllia*, and *Oxypora* or between *Oulophyllia* and *Mycedium* are mirrored in the gall crab phylogeny (Arrigoni *et al.*, 2014b; Huang *et al.*, 2014). The presence of deep-water species in the Cryptochiridae allows for future studies on the relationship between deep-water corals and shallow-water reef corals (Kitahara *et al.*, 2010).

There are other groups of symbionts 'predicting' systematic relationships, in the case of cryptic sympatric sponges the food preferences of predatory starfish proved to be a good indicator of the different species (Wulff, 2006). Similarly, based on the results of this study, gall crabs could serve as phylogenetic indicators of scleractinian relationships. Especially for scleractinian species and genera that are currently classified as *insertae cedis*, for example *Leptastrea* spp. or *Plesiastrea versipora*, gall crabs could provide an indication of their closest coral relatives. This could be somewhat weakened by apparent host shifts.

Concluding remarks

The two kinds of evidence as required according to Hafner and Nadler (1990) are met. The host and parasite phylogeny reconstructions were derived independently and the cospeciation analysis in Jane 4.0 showed that the topological similarity of the trees exceeds chance expectations, and thus the observed coevolutionary events should be ascribed to sequential evolution. The relationship between Scleractinia and Cryptochiridae appears to be so tight that gall crabs can be used as phylogenetic indicators of scleractinian evolution.

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Biogeography

Chapter 11

The curious case of *Neotroglocarcinus dawydoffi* (Decapoda: Cryptochiridae): unforeseen biogeographic patterns resulting from isolation

Sancia E.T. van der Meij & Bastian T. Reijnen

Abstract

Coral gall crabs form a commonly overlooked component of the associated fauna of shallow-water reef corals and therefore little is known about their ecology and biogeography. This study investigated the biogeography and phylogenetic position of the informal 'Detocarcini' species group within the Cryptochiridae. We used molecular data for two mitochondrial markers (COI and 16S) obtained from gall crabs covering (part of) a wide geographic range: the Red Sea, Malaysia, Indonesia and New Caledonia. Our phylogeny reconstructions portrayed the 'Detocarcini' as paraphyletic within the monophyletic Cryptochiridae. A phylogeographic clustering was noticed in *Neotroglocarcinus dawydoffi* that was absent in its sister species, *N. hongkongensis*, and the closely related species *Pseudocryptochirus viridis*. A Neighbour Network was estimated for the *N. dawydoffi* dataset to visualize the similarity between sequences from different biogeographic areas, resulting in three groupings: (1) New Caledonia with Lembeh/Ternate (eastern Indonesia), (2) Semporna/Kudat (eastern Malaysia), and (3) Red Sea (Saudi Arabia). Cryptic speciation rather than isolation is discussed and rejected as an alternative explanation for the observed biogeographic pattern.

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Introduction

Cryptochirids (Cryptochiridae), commonly known as coral gall crabs, may occur in high densities in coral reefs, but form a commonly overlooked component of the coral associated reef fauna (Hoeksema and van der Meij, 2013). Many aspects of their evolution, distribution and ecology have remained largely unknown, but the taxonomy of this monophyletic family (Guinot *et al*., 2013; van der Meij and Schubart, 2014) has been studied in several series of papers by Fize and Serène (1956a, b, 1957), Takeda and Tamura (e.g. 1980, 1981, 1985), and Kropp (e.g. 1989, 1990a, 1994). Within the Cryptochiridae, Kropp (1988a) recognized four informal species groups based on morphology: (1) *Hapalocarcinus*, (2) *Pseudohapalocarcinus*, (3) 'Detocarcini' and (4) 'Cryptochirini'. The 'Detocarcini' group consists of the Atlantic deep-water genus *Cecidocarcinus*, and four shallow- water genera, one of which is restricted to East Africa (*Detocarcinus*) and three to the Indo-West Pacific (*Neotroglocarcinus*, *Pseudocryptochirus*, *Utinomiella*). The 'Cryptochirini' species group includes all the remaining gall crab genera, including the Atlantic *Troglocarcinus*.

The informal 'Detocarcini' species group is noteworthy because all members of this clade (except *Utinomiella*) live in association with dendrophylliid corals (family Dendrophylliidae). Dendrophylliids are most common at depths between 50-300 metres, but also known from the intertidal and depths of up to 2165 metres (Cairns, 2001). The close relatives of this coral family remain unclear to some extent, but include the hermatypic Poritidae (Cairns, 2001; Fukami *et al*., 2008; Kitahara *et al*., 2010; Huang, 2012), which is not known to host gall crabs (Kropp, 1990a). *Utinomiella* is associated with the coral genera *Pocillopora* and *Stylophora*, which belong to the Pocilloporidae (Kropp, 1990a). Studies on higher-level relationships among the Scleractinia show that the Pocilloporidae is part of the 'robust clade' that is highly divergent from the 'complex' clade containing the Dendrophylliidae and Poritidae (Fukami *et al*., 2008; Huang, 2012). Based on the close association between gall crabs and their coral hosts, paraphyly of the 'Detocarcini' within the Cryptochiridae is expected.

We analysed the relationships between the species included in 'Detocarcini' (we had no fresh material from *Cecidocarcinus* and *Detocarcinus*): *Utinomiella dimorpha* (Henderson, 1906) associated with pocilloporids, and *Neotroglocarcinus* and *Pseudocryptochirus* associated with the dendrophylliid *Turbinaria* (Fize and Serène, 1957; van der Meij, 2012). The latter two cryptochirid genera comprise three species: *Pseudocryptochirus viridis* Hiro, 1938; *Neotroglocarcinus hongkongensis* (Shen, 1936) and *N. dawydoffi* (Fize and Serène, 1956). A third *Neotroglocarcinus* species, *N. monodi* (Fize and Serène, 1956), was synonymised with *N. hongkongensis* by Kropp (1988b). To study the position of the 'Detocarcini' species group within the Cryptochiridae we analysed a dataset based on two mitochondrial markers (16S, COI) that included 12 out of the 15 known Indo-West Pacific gall crab genera. Specimens from a wide biogeographic range (Red Sea to New Caledonia) were used for the molecular phylogeny reconstructions.

Material and methods

Collecting

Gall crabs were collected (2007-2012) from various locations in Indonesia (Manado [Menado], Sulawesi; Lembeh Strait, Sulawesi; Ternate, Halmahera; Raja Ampat, Papua) and Malaysian Borneo (Kudat, Sabah; Semporna, Sabah). Additional specimens were collected in 2012 in New Caledonia and in 2013 in the Red Sea (Saudi Arabia). In the figures and table, localities are coded as the first three letters of the full locality names, except that specimens from Kudat (Tun Mustapha

Park), New Caledonia and Saudi Arabia are coded as TMP, NC and SA, respectively. Corals were searched for gall crabs and subsequently split with hammer and chisel to isolate the crabs. After being photographed with an SLR camera equipped with macro lens to register colour patterns the gall crabs were preserved in 80% ethanol. All specimens are deposited in the collections of Naturalis Biodiversity Center in Leiden, the Netherlands (formerly Rijksmuseum van Natuurlijke Historie, collection coded as RMNH.Crus.D).

DNA analyses

For all specimens used in this study DNA was isolated from muscle tissue of the fifth pereiopod, using the DNeasy 96 Blood and Tissue Kit (Qiagen) and the Nucleo-Mag 96 Kit (Machery-Nagel) according to the manufacturer's protocol for animal tissue. Incubation took place overnight for approximately 18 h. The final elution step was performed with subsequently 100 mL or 150 mL elution buffer. Polymerase chain reaction was carried out with standard PCR conditions: PCR CoralLoad Buffer (containing 15 mM MgCl2), 0.5 mL dNTPs (2.5 mM), 1.0 mL of each primer (LCO-1490 and HCO-2198, see Folmer *et al*. (1994), 16L2 and 16H10, see Schubart (2009)), 0.3 mL Taq polymerase (15 units per mL), 18.7 mL of extra pure PCR water and 1.0 mL DNA template. Thermal cycling was performed for COI and 16S by: initial denaturation at 95°C for 5 min, followed by 39 cycles of 95°C for 5 s, 47°C for 1 min and 72°C for 1 min. The initial elongation steps were followed by an additional elongation step of 10 min at 72°C. Sequences were assembled and edited in Sequencher 4.10.1. Voucher data and GenBank accession numbers (KJ923643-KJ923766) are provided in Appendix 1.

Phylogenetic analyses

Phylogenetic analyses were carried out on three datasets: (1) a dataset containing 12 cryptochirid genera, with the heterotreme domeciid *Cherusius triunguiculatus* (Borradaile, 1902) and the thoracotreme varunid *Hemigrapsus penicillatus* (de Haan, 1835) as outgroup species (see van der Meij and Schubart, 2014); (2) a dataset containing members of the 'Detocarcini' group (*P. viridis*, *N. hongkongensis* and *N. dawydoffi*) from various localities. Based on the phylogeny reconstruction of the first dataset, *Utinomiella dimorpha* (Henderson, 1906) was selected as the outgroup; (3) a dataset solely containing *N. dawydoffi* specimens from different locations to study the biogeographic patterns resulting from the second dataset. *Neotroglocarcinus hongkongensis* was used as the outgroup.

Sequences were aligned using Guidance (ClustalW, removal of unreliable columns below 0.93) (Penn *et al*., 2010a). The alignment score for dataset 1 was 0.992 and for datasets 2 and 3 0.999. A model selection analysis was carried out in jModelTest (Posada, 2008) to select the best-fit models based on the corrected Akaike Information Criterion (AICc), resulting in TVM+I+G as the best-fit model and GTR+I+G as second best-fit model for all datasets.

Maximum likelihood analyses (1000 bootstraps) were carried out in MEGA 5.20 (Tamura *et al*., 2011), and Bayesian inferences were estimated in MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003) based on the GTR+I+G model. Six Markov Monte Carlo chains were run for 3 000 000 generations, with a sample tree saved every 1000 generations. Likelihood scores stabilized at a value of 0.006 for dataset 1, at 0.010 for dataset 2 and at 0.002 for dataset 3. Consensus trees were constructed in MrBayes with a burnin of 25%, and visualized in FigTree 1.3.1 (http://tree.bio.ed.ac.uk/software/;figtree/). Moreover, tests for maximum parsimony were conducted in MEGA 5.20 (1000 bootstraps, Close-Neighbour-Interchange (CNI), 10 initial trees with random addition).

For the species belonging to *Neotroglocarcinus* and *Pseudocryptochirus*, the evolutionary divergence over sequence pairs, between a priori determined groups based on their respective localities, was estimated in MEGA 5.20 (p-distance; Table 1).

A Neighbour Network was estimated for the *N. dawydoffi* dataset to visualize the similarity between sequences from different biogeographic areas, using the Neighbor-Net algorithm in SplitsTree4 (Huson and Bryant, 2006). This algorithm is based on a distance method estimating the splits between the nodes. Inconsistencies in the splits are shown by using multiple lines connecting the nodes, when the algorithm does not fully resolve the network. All possible splits between the nodes are shown. The analysis produced a consensus network based on 10 000 bootstraps and 95% confidence.

Species delimitation

To check for potential cryptic speciation within *Neotroglocarcinus* and *Pseudocryptochirus*, we used the web version of ABGD (Automatic Barcode Gap Discovery; Puillandre *et al*., 2012) on a concatenated dataset (16S and COI) and on the single marker sequence datasets of *N. dawydoffi*, *N. hongkongensis* and *P. viridis* using the standard settings (Pmin: 0.001; Pmax: 0.1; steps: 10; X: 1.5; Nb bins: 20) and the Kimura (K80) Ts/Tv model (mode: 2,0). ABGD identifies the barcoding gap within a given dataset based on pairwise distances between the sequences without a priori species hypotheses and sorts sequences into putative species. The advantage of this approach is that no such hypotheses are needed, nor any of the proposed presets that were established as a standard limit between intra- and interspecific species divergence, e.g. 3% of divergence (Smith *et al*., 2005) or the '10 times' rule (Hebert *et al*., 2004).

Results

New geographic records of gall crabs belonging to the 'Detocarcini'

Neotroglocarcinus dawydoffi: This species has so far been recorded from Micronesia (Enewetak Atoll, Marshall Islands - Garth *et al*., 1987; Palau, Guam, Pohnpei - Kropp, 1990a), and Vietnam (Nha Trang - Fize and Serène, 1957). New records reported herein include: Red Sea (Saudi Arabian coast), Malaysia (Kudat, Semporna), Indonesia (Lembeh Strait, Ternate), and New Caledonia. One specimen was observed in the Maldives (Lankanfinolhu, N Male Atoll - photo voucher SETvdM). Appendix 2 shows the intraspecific variation in colour pattern in specimens from various localities. Specimens of *N. dawydoffi* collected during this study were mostly associated with the corals *Turbinaria mesenterina* (de Lamarck, 1816) or *Turbinaria* sp. (see van der Meij (2012) for a discussion of coral host identifications). The specimens of *N. dawydoffi* from New Caledonia were collected from *T. heronensis* Wells, 1958. *Neotroglocarcinus dawydoffi* co-occurred with *P. viridis* in one of the colonies. Fize and Serène (1957) described *N. dawydoffi* from *Turbinaria elegans* Bernard, 1896, a possible junior synonym of *Turbinaria stellulata* (de Lamarck, 1816). The latter species appears similar to *T. mesenterina* and *T. reniformis*.

Neotroglocarcinus hongkongensis: This species has so far been recorded from Oman (Sadh - Hogarth, 1989), Vietnam (Nhatrang - Fize and Serène, 1957; Rocher Noir, Bai Miew - Kropp, 1988b), Singapore (Serène, 1966), Indonesia (Banda, Moluccas - Kropp, 1994), Hong Kong (Shen, 1936), Taiwan (Penghu Islands [Pescadores] - Utinomi, 1944; Orchid Isl. - Wei *et al*., 2006), Japan (Tanabe Bay, Central Japan - Hiro, 1938; Yaeyama Group, Ryukyu Isls - Utinomi, 1944) and Micronesia (Palau, Guam, Pohnpei - Kropp, 1990a). New records: Malaysia (Kudat, Semporna) and Indonesia (Lembeh Strait). All *N. hongkongensis* specimens in this study were associated with *Turbinaria peltata* (Esper, 1794); except for one specimen from *T.* cf. *patula* (Dana, 1846).

The host of *N. hongkongensis* was not recorded by Shen (1936), but the host of *N. monodi* [= *N. hongkongensis*], was recorded as *T. peltata* by Fize and Serène (1957).

Pseudocryptochirus viridis: Records for *P. viridis* range from Vietnam to New Caledonia (see van der Meij, 2012). All specimens of *P. viridis* were collected from *T.* (cf.) *reniformis*, *T.* (cf.) *mesenterina* or *Turbinaria* sp. One specimen from New Caledonia was collected from *T. heronensis*, where it co-occurred with *N. dawydoffi*. Despite search efforts by the first author, *N. hongkongensis* and *P. viridis* were not encountered along the Saudi Arabian coast of the Red Sea.

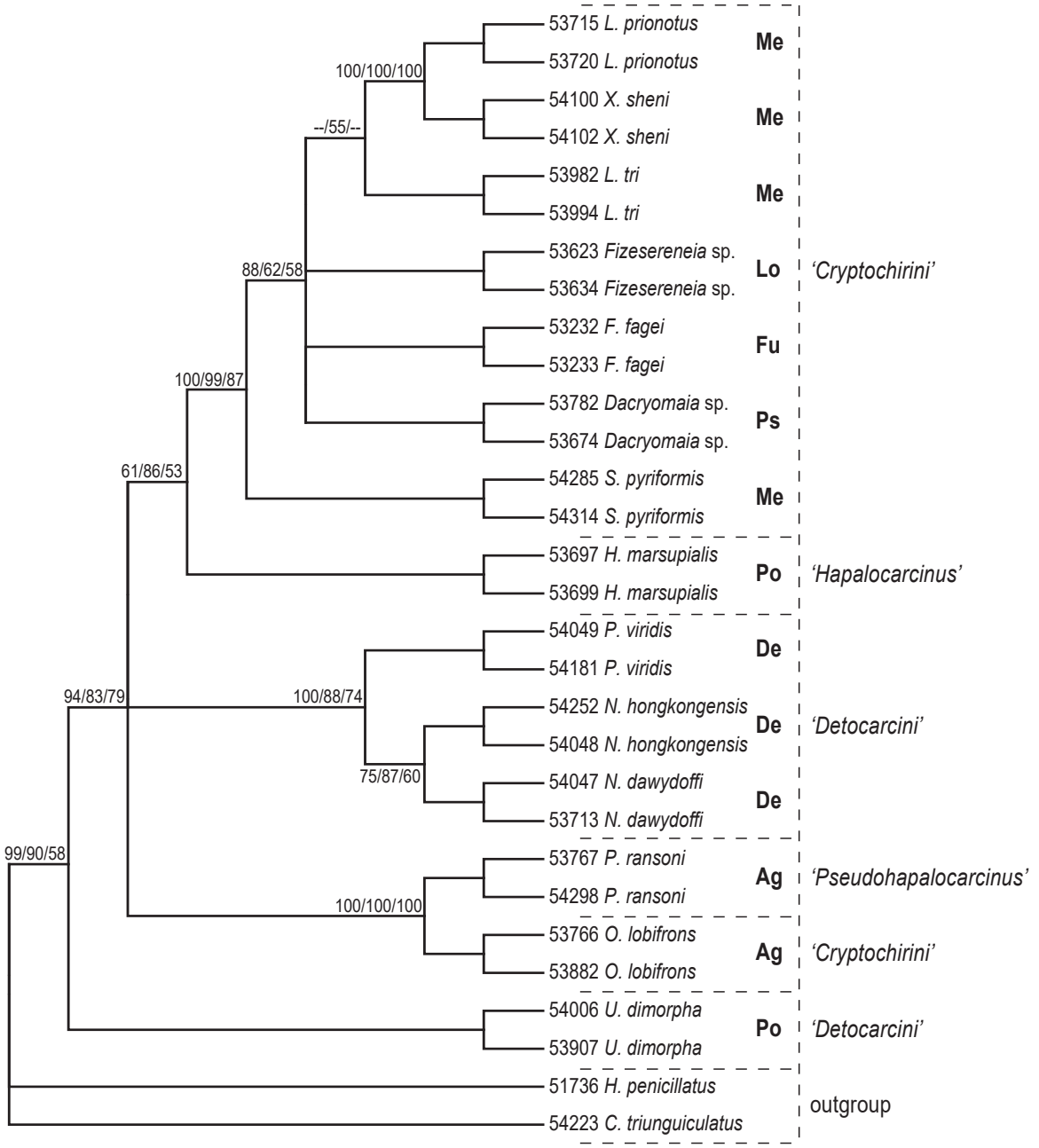

Fig. 1. Cladogram (ML analysis 1000 bootstrap iterations; 16S and COI) of the Cryptochiridae. Support values represent from left to right: Bayesian posterior probabilities/ML/MP. Numbers refer to collection codes (RMNH. CRUS.D). Letters in bold refer to the host coral family of the gall crabs specimens: Me = Merulinidae, Lo = Lobophylliidae, Fu = Fungiidae, Ps = Psammocoridae, Ag = Agariciidae, Po = Pocilloporidae, De = Dendrophylliidae (classification after Budd *et al.*, 2012; Huang *et al.*, 2014). The groupings of the gall crabs are based on Kropp (1988a).

Fig. 2. Cladogram of the genera *Pseudocryptochirus* and *Neotroglocarcinus*, based on 16S and COI, topology derived from Bayesian analysis. Support values from left to right: Bayesian posterior probabilities/ML/MP. NC = New Caledonia, TER = Ternate (Indonesia), LEM = Lembeh Strait (Indonesia), MEN = Manado (Indonesia), SEM = Semporna (Malaysia), TMP = Kudat (Malaysia), SA = Red Sea (Saudi Arabia). Stars represent nodes with Bayesian probabilities of >90 and high ML/MP values.

Relationships within the Cryptochiridae

The first molecular phylogeny reconstruction of the Cryptochiridae resulted in identical topologies for the ML (MEGA) and Bayesian analyses (Fig. 1). Five large clades can be distinguished. The first large clade contains most genera considered by Kropp (1988a) to form the 'Cryptochirini'. Most notably, *Sphenomaia pyriformis* (Edmonson, 1933) clusters as a sister species to the other genera, and *Lithoscaptus prionotus* Kropp, 1994 and *Xynomaia sheni* (Fize and Serène, 1956) appear to be closely related. *Hapalocarcinus marsupialis* Stimpson, 1859 clusters as a sister clade (Fig. 1) to the 'Cryptochirini', but support values are low. The third clade consists of *Pseudocryptochirus* and *Neotroglocarcinus* as sister genera. The fourth clade is formed by *Pseudohapalocarcinus ransoni* Fize and Serène, 1956 and *Opecarcinus lobifrons* Kropp, 1989. *Utinomiella dimorpha* clusters basally to all other genera.

Patterns within the 'Detocarcini', with a focus on N. dawydoffi

The topologies of the ML, MP and Bayesian consensus trees were congruent and the three clades are highly supported (Fig. 2). Within the *P. viridis* clade and the *N. hongkongensis* clade, little to no sequence variation was observed between specimens, resulting in short branch lengths. In contrast, subclades can be discerned within the *N. dawydoffi* clade. Specimens from New Caledonia cluster together, as well as specimens from eastern Indonesia (Lembeh/Ternate). Little variation can be observed among specimens from eastern Malaysia and Saudi Arabia, and support for separate grouping is absent (Fig. 2). Yet, in a separate analysis containing only data of *N. dawydoffi* five clades can be discerned (Fig. 3). The split between clade I and II has low support values.

Based on the geographic clustering in Fig. 3, a Neighbour Network analysis was conducted on the *N. dawydoffi* specimens to visualise sequence similarities (Fig. 4). The box-like shapes in the network indicate data incompatibilities, shown as parallel lines, which cannot be explained by tree-like evolution scenarios. The distinction between these two groups is unclear and therefore their affinity is considered high when the edges (= branches) between groups are short and connected by several parallel lines. There are more conflicts (parallel lines) within the observed clusters than in the main network. The network shows three clusters: (1) New Caledonia with Lembeh/Ternate (eastern Indonesia), (2) Semporna and Kudat (eastern Malaysia) and (3) Red Sea (Saudi Arabia). The edges between Saudi Arabia and Malaysia are shorter than those between Malaysia and Indonesia, despite the geographic distance. Groupings in the network (Fig. 4) are similar to those in the cladogram (Fig. 3) and therefore considered to be robust.

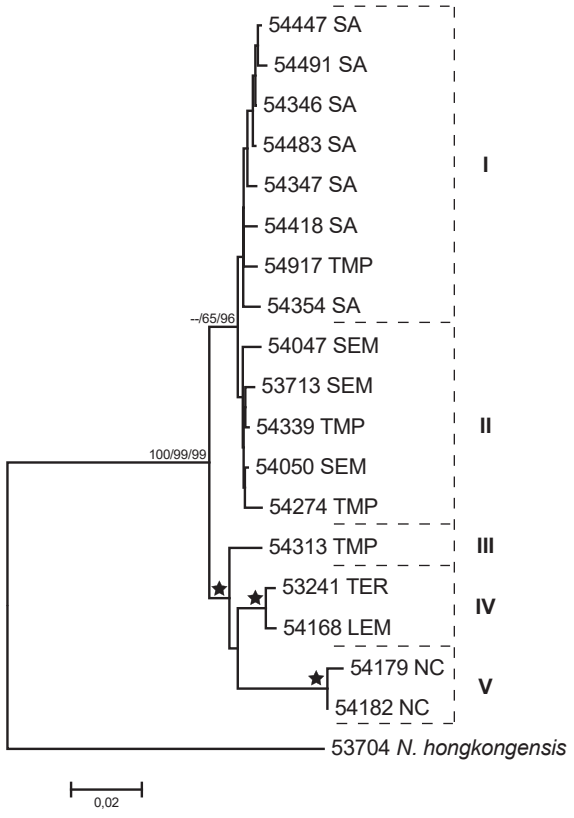

Fig. 3. Cladogram showing variation within *N. dawydoffi*, with *N. hongkongensis* as outgroup. Groupings I-V are also reflected in Fig. 4. Support values from left to right: Bayesian posterior probabilities/ML/MP. Stars represent nodes with Bayesian probabilities of >90 and high ML/MP values.

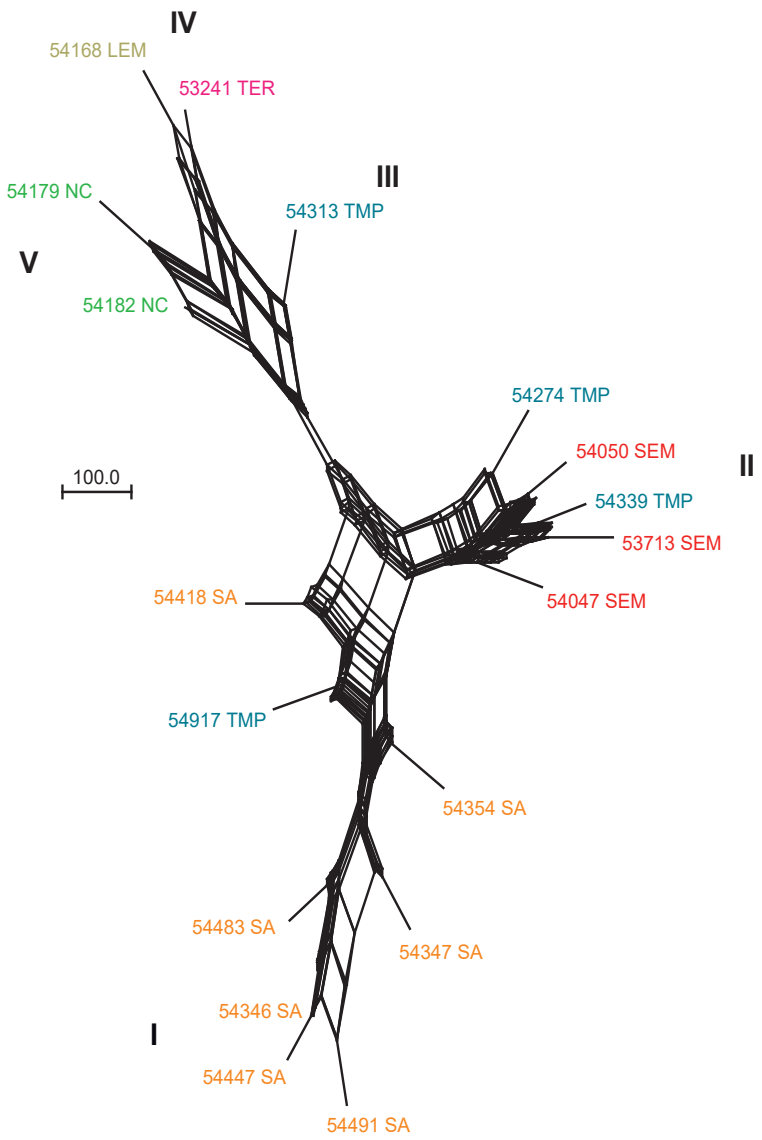

Fig. 4. Neighbour Network analysis by SplitsTree4 to visualize the geographic clustering in *N. dawydoffi*, based on 16S and COI. NC = New Caledonia, TER = Ternate (Indonesia), LEM = Lembeh Strait (Indonesia), SEM = Semporna (Malaysia), TMP = Kudat (Malaysia), SA = Red Sea (Saudi Arabia). Groupings I-V are based on Fig. 3.

High sequence heterogeneity is observed in *N. dawydoffi* from Kudat (TMP; Appendix 3). These specimens do not show a clear biogeographic pattern (Fig. 3) and 'wander' through the Neighbour Network (Fig. 4). They are most similar to specimens from nearby Semporna (Table 1).

Based on the estimates of evolutionary divergence (Table 1), the similarity between *N. dawydoffi* specimens from Malaysia and Saudi Arabia was investigated further. There is a 3.7-3.8% sequence difference between specimens of *N. dawydoffi* from New Caledonia and Lembeh/Ternate and almost 5% sequence difference between the New Caledonian and Malaysian/Saudi Arabian specimens (Table 1; see also Appendix 3). The same test for evolutionary divergence was conducted for *N. hongkongensis* and *P. viridis*. These tests showed almost no difference between *N. hongkongensis* from three different localities (LEM/SEM/TMP), contrary to *N. dawydoffi* for the same three localities. For *P. viridis* the largest sequence difference (1.6%) was observed between New Caledonia and Ternate.

Species delimitation and speciation

The concatenated sequence dataset (16S and COI) and single marker datasets subjected to ABGD resulted in prior maximal intraspecific divergences of 0.077 for the concatenated and 16S dataset.

N. dawydoffi		NC	TER	LEM	SEM	TMP	SA
	NC		0.006	0.006	0.006	0.006	0.006
	TER	0.038		0.002	0.005	0.004	0.004
	LEM	0.037	0.005		0.005	0.004	0.004
	SEM	0.045	0.032	0.032		0.002	0.002
	TMP	0.044	0.028	0.028	0.012		0.002
	SA	0.049	0.032	0.033	0.010	0.014	

N. hongkongensis		LEM	SEM	TMP
	LEM		0.001	0.000
	SEM	0.002		0.000
	TMP	0.000	0.001	

P. viridis		NC	TER	LEM	MEN	SEM
	NC		0.003	0.002	0.003	0.003
	TER	0.016		0.002	0.002	0.002
	LEM	0.010	0.013		0.002	0.002
	MEN	0.015	0.006	0.012		0.002
	SEM	0.014	0.010	0.013	0.010	

Table 1. Estimates of evolutionary divergence over sequence pairs between a priori determined groups based on 16S and COI (10 000 bootstraps). The number of base differences per site from averaging over all sequence pairs between groups are shown below the diagonal, SE estimates are shown above the diagonal. NC = New Caledonia, TER = Ternate (Indonesia), LEM = Lembeh Strait (Indonesia), MEN = Manado (Indonesia), SEM = Semporna (Malaysia), TMP = Kudat (Malaysia), SA = Red Sea (Saudi Arabia).

For COI, the prior maximal intraspecific divergence was 0.129. Values higher than the maximal intraspecific divergence resulted in three Molecular Operational Taxonomic Units (MOTUs) in both the recursive and initial partition. Each of these MOTUs corresponded to one of the three nominal species (*Neotroglocarcinus dawydoffi*, *N. hongkongensis*, *Pseudocryptochirus viridis*). Changing the Kimura model to the Jukes-Cantor model or changing the relative gap width did not have an effect on the outcome of the analysis.

Discussion

Relationships within the Cryptochiridae, with a focus on the 'Detocarcini'

The phylogeny reconstruction of the Cryptochiridae reflects the informal groupings based on morphology used by Kropp (1988a) to a degree, but some substantial differences are observed (Fig. 1). The 'Detocarcini' was found to be paraphyletic, with representatives retrieved in two clades. *Pseudocryptochirus* is here positioned basally to *Neotroglocarcinus* and should be considered a sister genus of *Neotroglocarcinus*, which is in agreement with Kropp (1988a). The presumed close affinity of *Pseudocryptochirus* and *Neotroglocarcinus* with *Cecidocarcinus* and *Detocarcinus* based on morphology could not be tested, owing to a lack of DNA material for the latter two genera. *Utinomiella dimorpha*, the other species belonging to the 'Detocarcini' group according to Kropp (1988a), was retrieved basally to all other cryptochirids and does not seem to be affiliated with its supposed relatives *Pseudocryptochirus* and *Neotroglocarcinus* (see Kropp, 1988a: 340). Like *Neotroglocarcinus* and *Pseudocryptochirus*, *Cecidocarcinus* is only known to be associated with the Dendrophylliidae, whereas *Detocarcinus* is associated with corals belonging to the Rhizangiidae, Oculinidae and Caryophyllidae, and possibly Dendrophylliidae (Kropp and Manning, 1987). In comparison, *U. dimorpha* is found inhabiting the Pocilloporidae.

Almost all other gall crab genera are represented in the 'Cryptochirini' group recognised by Kropp (1988a). Kropp considered *Pseudohapalocarcinus* a separate group, yet the DNA results show a very close affinity with *Opecarcinus lobifrons*, which was placed in the 'Cryptochirini' group. *Pseudohapalocarcinus* and *Opecarcinus* exclusively inhabit corals of the Agariciidae, but there are obvious morphological differences between the two, of which carapace shape is the most notable. The position of *Hapalocarcinus marsupialis* remains to some extent unclear.

Neotroglocarcinus dawydoffi: *biogeographic isolation or cryptic speciation?*

Numerous molecular phylogenetic and population genetic studies on different marine organisms have revealed a genetic discontinuity between the Indian and Pacific Ocean, which is explained by low sea-level stands during Pliocene and Pleistocene glaciations (Hoeksema, 2007; Timm *et al*., 2008; Kochzius *et al*., 2009). Studies on stony and soft corals showed strong clustering in biogeographic regions (Keshavmurthy *et al*., 2013; Reijnen *et al*., 2014). These studies found a high genetic divergence between Indian Ocean and Indo-West Pacific clades, and often lacked morphological characters to explain this divergence. Such biogeographic clustering further complicates already present uncertainties in the taxonomy and systematics of marine invertebrates.

Within the informal 'Detocarcini' species group, *Neotroglocarcinus dawydoffi* shows a biogeographic pattern. Surprisingly, and contrary to the studies mentioned above, *N. dawydoffi* shows a closer relationship between Malaysian Borneo and the Red Sea than between Malaysian Borneo and Lembeh/Ternate in eastern Indonesia (Table 1, Figs 3-4), despite the shorter distance between the latter. Isolation by distance can be a reason for high genetic differentiation (Timm and Kochzius, 2008), but this does not appear to be the case in *N. dawydoffi*. The observed biogeographic pattern does not correspond with current patterns of ocean circulation. Palumbi (1996) showed similar differences in average sequence heterogeneity between sea urchin populations from the central Indo-West Pacific (Bali, Papua New Guinea) compared with the area north and east of the central Indo-West Pacific. The lack of congruence between our results and the results of Palumbi (1996) and present-day surface circulation does not imply that genetic structure always has to result from past events (e.g. dispersal from the Indian Ocean towards the Indo-West Pacific). It may suggest that present-day mechanisms of dispersal are different from those assumed to have been the case so far (Benzie, 1999), or that in *N. dawydoffi* specimens from Borneo and the Red Sea went through a similar bottleneck event resulting in sequence homogeneity.

A correct assessment of species boundaries is fundamental to biogeographic hypothesis testing (e.g. Palumbi, 1996, 1997). No cryptic species are expected within *N. dawydoffi* based on the results from our ABGD analysis. When comparing the values of Table 1 (largest genetic difference 4.9%) with those obtained from the ABGD analysis, in which values exceeding 7.7% were considered to identify interspecific differences between specimens, it can be concluded that these values fit within the limits of intraspecific variation. Therefore we adopt the hypothesis that the observed difference is related to biogeographic variation within a single species and not a result of cryptic speciation. Moreover, in the genus *Fungicola* cryptic speciation is observed to be strongly linked to host specificity and branch lengths between the known species and newly discovered cryptic species were much longer, and no geographic clustering was observed (van der Meij and Hoeksema, 2013; van der Meij, unpubl). Compared with *Fungicola* the branch lengths are much shorter in *Neotroglocarcinus*. Possible host specificity is obscured by taxonomic difficulties concerning the host genus *Turbinaria* (Cairns, 2001; van der Meij, 2012).

It remains possible that the selected markers are too conservative for the analyses, although (parts of the) Cytochrome Oxidase I gene of the mtDNA are frequently used for studies on

biogeographic patterns and population genetics (e.g. Palumbi, 1996; Benzie, 1999; Kochzius *et al*., 2009; Ahrens *et al*., 2013). Moreover, the present study was limited by the number of specimens available per locality. Nonetheless, it is noteworthy that (i) closely related sister species show such large differences in sequence variation, and (ii) that the observed variation in *N. dawydoffi* appears to be linked to biogeographic patterns, which has so far not been observed in other gall crab species.

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Chapter 12

The Red Sea and Arabia are a diversity and endemism hotspot for coral-dwelling gall crabs (Cryptochiridae)

Sancia E.T. van der Meij, Michael L. Berumen & Gustav Paulay

Abstract

The Red Sea and Arabia are renowned for marine endemism and diversity, mostly based on studies of the coral and fish fauna. To place the Red Sea in a broader biogeographic context we aim to study: 1) the proportional diversity of coral-dwelling gall crabs by comparing their composition and diversity in the Indo-Malayan area and the Red Sea; and 2) to determine if endemic species are present in the Red Sea (or just outside the basin) and what their approximate age is. Gall crabs were collected from a wide range of scleractinian corals in the Red Sea and Arabian peninsula and compared with collections from the Indo-Malayan area. Arabian occurrence records from the literature were also assembled. Sequence data (COI), morphology, and distribution were used to delineate Evolutionary Significant Units (ESUs). Pairwise genetic divergence between Red Sea and Indo-Malayan populations of wide ranging species, and between endemic species and their sister species was calculated as patristic distance. We recorded 36 species of Cryptochiridae from the Red Sea and Arabia, of which 11 appear to be endemic. Most of the wide-ranging species occupied the same host(s) in the Red Sea and Indo-Malaya, although with some exceptions. Genetic divergence between endemics and their closest relatives ranged between 2.3-11.4% p-distance. Wide-ranging species tend to show little differentiation: nine share haplotypes, and 17 have <1% sequence divergence between Red Sea and Indo-Malaya. The Red Sea has the second highest diversity of gall crabs after Indo-Malaya. Deep divergence of endemics pre-date the Last Glacial, when Red Sea habitats were impacted by highly saline waters in the restricted basin. The wider range of several endemics in Arabia suggests that the Gulf of Aden was a key area in the origin and survival of endemics.

Manuscript under review

Introduction

The Indo-West Pacific (IWP), the largest and most diverse marine biogeographic region, extends across well over half the tropics from the Red Sea and South Africa to Hawaii and Easter Island. Despite this vast geographic extent, it has a relatively homogeneous fauna, with many species ranging from Africa to Polynesia, not infrequently with considerable genetic homogeneity (e.g. Lessios *et al.,* 1999; 2003). Nevertheless most species have narrower ranges, limited by environmental conditions or dispersal barriers, giving rise to prominent patterns in diversity and endemism that derive from dynamics of origination, extinction, and distributional change. The most striking patterns relate to the distribution of diversity and endemism.

The ranges of many tropical marine species overlap in a centre of maximum marine biodiversity in the Indo-Malayan area (IM) – also referred to as the Coral Triangle – which supports the greatest diversity in the marine biosphere (Ekman, 1935, 1953; Hoeksema, 2007; Briggs and Bowen, 2013). Diversity falls eastward across the Pacific and more unevenly westward, with secondary peaks in the SW Indian Ocean and Red Sea. Endemism within the IWP is high in peripheral areas, such as the Red Sea, Hawaii, and other remote islands in Oceania. Endemics in these areas are typically sister species of more wide-ranging species, and are thought to have a role in diversification (Ladd, 1960; Kay, 1980; Malay and Paulay, 2010).

The Red Sea and neighbouring waters around the Arabian Peninsula are a diversity and endemism hotspot (DiBattista *et al*., review 1, 2). The area harbours diverse coral reefs across a broad range of environmental conditions, ranging from oligotrophic, coral-dominated systems in the northern Red Sea to mixed kelp-coral communities in southern Oman (Sheppard *et al*., 1992; Ngugi *et al*., 2012; Raitsos *et al*., 2013). It is separated from other Indian Ocean reefs to the south by coasts under the influence of intense monsoonal upwelling that largely lack reefs and reef corals from Somalia to India. This barrier, together with unusual reef systems in many areas of Arabia, provides isolation and selection that facilitate the evolution of endemics.

The Red Sea is an especially well-known area of endemism (e.g. Guinot, 1966; Briggs and Bowen, 2012), yet paradoxically most its biota is expected to be very young, of Holocene age. During glacial periods the basin may have become so hypersaline that many or most species could not survive (Braithwaite, 1987; Siddall *et al*., 2003). Three hypotheses may resolve this paradox: endemics are young, not strictly restricted to the basin, or survived within the basin in refugia.

Research into the processes responsible for the pattern of diversity in the IWP have taken two approaches – top down, where predictions from general drivers are tested against patterns of distribution (e.g. Rosen, 1978; Bellwood and Hughes, 2001; Bellwood *et al*., 2005) or bottom up, where phylogenetic reconstruction dissects how species arose and distributions developed in a particular clade (e.g. Westneat and Alfaro, 2005; Hodge *et al*., 2014). Both the power and limitation of the first approach comes from its breadth - the pattern is most evident and robust at the biotic level, but it is challenging to dissect multiple drivers from a single data set (but see Bellwood and Hughes, 2001; Bellwood *et al*., 2005) and the evolutionary origin of diversity is not directly investigated. Indeed, reviews of alternative hypotheses about IWP diversity and diversification have found that multiple processes are responsible, rather than single drivers as proposed by several hypotheses (Paulay, 1997; DiBattista *et al*., 2013). The second approach allows a more direct investigation of diversification, but its narrower scope limits generalization. The accumulation of bottom up studies across taxa is bridging the gap between these approaches, allowing an evaluation of the generality vs. variability of patterns and processes. Comparisons of taxa with different traits allow exploration of how these impact the dynamics of diversification.

Fig. 1. Gall crab dwellings in **A**, *Stylophora* **B**, *Pavona explanulata* **C**, *Oulophyllia crispa* **D**, *Dipsastraea* **E**, *Astrea curta* **F**, *Platygyra*; dwellings indicated by arrows. All pictures are from the Red Sea.

Thorough sampling and phylogenetic analysis of diverse clades is an effective method for investigating diversification. We focus on the gall crab family Cryptochiridae. They are an especially interesting group because they 1) live obligately in corals, providing a comparative evolutionary context against their hosts, one of the most investigated taxa in IWP biogeography, 2) are symbiotic, thus allowing discrimination of the role played by geographical setting (dispersal) and ecology (host) in diversification, and 3) can be efficiently sampled because they are visible on the reef surface (Fig. 1), like corals and fish, two taxa that have been the mainstay of reef ecological and biogeographical analyses partly for this reason.

The Cryptochiridae is a monophyletic family of obligate symbionts, residing in galls, tunnels, or pits in the skeleton of scleractinian corals (van der Meij and Schubart, 2014). The family consists of 21 genera, 49 described species (Ng *et al*., 2008; Davie, 2014), and numerous undescribed taxa under study (van der Meij, unpubl.). They are recorded from shallow to deeper waters (to 512 m), but the majority of known species live in the photic zone (Kropp and Manning, 1987; Kropp, 1990a). Although gall crabs occur in almost all of the world's tropical oceans, they are most diverse in the IWP, like their coral hosts (Fize and Serène, 1957; Kropp, 1990a; Hoeksema, 2007). Much of their ecology, life history, and biogeography are virtually unstudied (Kropp, 1990a; van der Meij and Schubart, 2014).

Focused collecting, taxonomic, and phylogenetic study by SETvdM in Indo-Malaya has created a new basis for analysing cryptochirid diversity and evolution. With the fauna of the IWP diversity centre now well documented, we collected gall crabs in Arabia for comparison. Cryptochirids, like most non-coral invertebrates, are very much understudied in the Red Sea (Berumen *et al*., 2013). The first gall crab species was described from Hawaii by Stimpson (1859), but the second, *Cryptochirus coralliodytes* Heller, 1861, was described from the Red Sea. Since then only one gall crab has been described from the Red Sea and three additional species recorded (see van der Meij and Reijnen, 2014; van der Meij *et al*., 2015)). This is surprising considering the proximity to Europe and substantial attention the Red Sea fauna has received from early

taxonomists like Klunzinger and Ehrenberg. Five species have been recorded from Oman by Hogarth (1989): *Cryptochirus coralliodytes*, *Lithoscaptus paradoxus*, *Hapalocarcinus marsupialis* [species complex], *Hiroia sheni* [= *Xynomaia sheni*], and *Neotroglocarcinus monodi* [= *Neotroglocarcinus hongkongensis*], identified by Roy Kropp, without host records and no mention of coral vouchers. Gall crabs have not been previously recorded from the Gulf of Aden.

The obligate nature of the symbioses between gall crabs and corals suggests that differences in diversity and endemism may in part be related to changes in the host coral fauna. Red Sea corals have been reviewed by Scheer and Pillai (1983), Sheppard and Sheppard (1991), and Veron (2000), with 307 species recorded, including 20 endemics (DiBattista *et al*., review 1). The range of some 'endemics' extends to the Gulf of Aden or further around the Arabian peninsula. About 600 reef coral species are known from Indo-Malaya (Huang *et al*., 2014a), with up to 581, including 31 Indo-Malayan endemics, recorded from a single locality (Veron, 2000). The Arabian Gulf hosts 66 corals, while 126 species are recorded in the Gulf of Oman (Sheppard and Sheppard, 1991; DiBattista *et al*., review 1).

We identify and genetically compare gall crab from the Red Sea and neighbouring Arabian seas to address the following questions. How diverse are cryptochirids in the region? Does the region appear to be a diversity hotspot? Does the proportional diversity of different gall crab genera differ between Indo-Malaya and the Red Sea? How many species are endemic, and how are these distributed among regional basins and seas? Are there likely Red Sea endemics and what is their age? Is there a relationship between coral and crab endemism – do endemic corals tend to have endemic cryptochirids? Answers to these questions will help place the Red Sea in a broader biogeographic context (Bowen *et al*., 2013).

Material and Methods

Gall crabs were collected in the southern Red Sea from Al-Lith to Jizan during a biodiversity cruise in March 2013, and from reefs offshore from Thuwal, in the central Red Sea, in March 2013 and November 2014. Gall crabs were collected in Oman on a series of expeditions between 1999-2008, in the Gulf of Oman and Masirah Island. Collections were made in Djibouti during a biodiversity cruise in Feb.-March 2012. Indo-Malayan collections for comparison came from extensive studies by SETvdM in Indonesia (Raja Ampat, Bunaken, Ternate, Lembeh) and Malaysia (Semporna, Kudat) between 2007 and 2012.

Corals were searched for galls and pits, photographed, and split with hammer and chisel. Crabs were photographed and then preserved in 80% ethanol. The material is deposited in the Naturalis Biodiversity Center (RMNH), Leiden, the Netherlands, and Florida Museum of Natural History, University of Florida (UF), Gainesville, USA. Gall crabs were identified based on Fize and Serène (1957) and Kropp (1990a). Recognized species from ongoing taxonomic revisions (e.g. van der Meij, 2015a), are referred to as sp. A, B, C, etc. Host corals in the Red Sea were identified following Scheer and Pillai (1983) and Sheppard and Sheppard (1991), and with the help of Francesca Benzoni who was present on some expeditions; in the IM following Veron and Pichon (1976), Veron *et al*., (1977), Veron and Pichon (1980), Hoeksema (1989), and Veron (2000). Nomenclature for crabs and corals follows Cairns (2014) and Davie (2014).

We follow DiBattista *et al*. (review 1)'s terminology for endemism, with slight modifications: 1) Red Sea endemic: species found only in the Red Sea (including Gulf of Aqaba), 2) Red Sea to Gulf of Aden endemic: species found only in the Red Sea and Gulf of Aden (including Djibouti), 3) Arabian endemic: species found in the Red Sea and beyond the Gulf of Aden in the waters surrounding the Arabian peninsula. Literature records from the Arabian area were evaluated

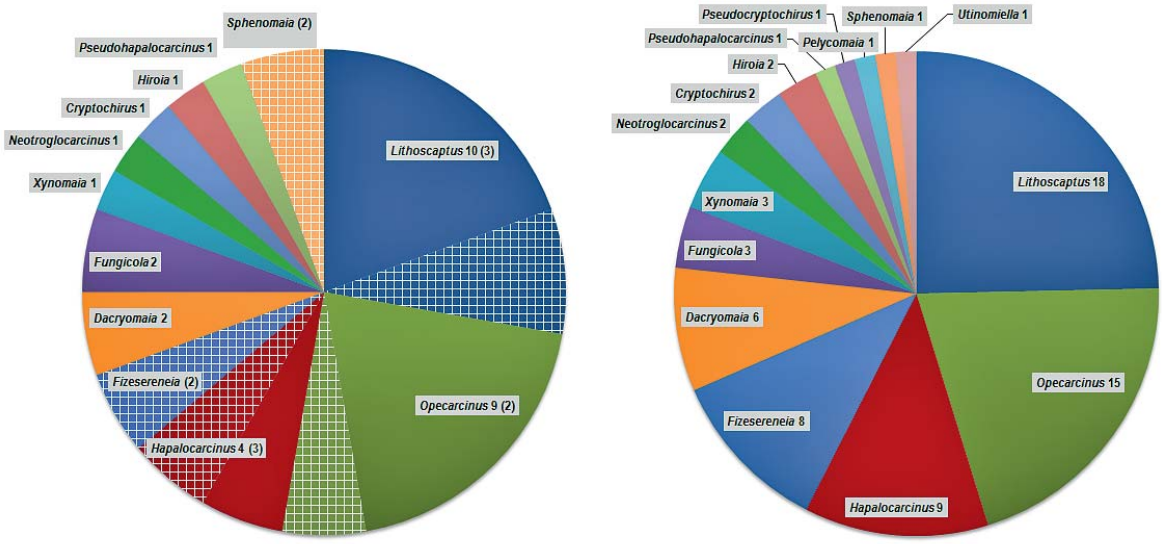

Fig. 2. Generic composition of Cryptochiridae in Red Sea (left) and Indo-Malaya (right). Numbers of species (endemics) provided per genus; endemic species also indicated by the chequered pattern.

based on current species concepts, synonymies (Davie, 2014), and consideration of nomenclatural concepts at the time of their publications.

Sequence data, morphology, and distribution were used to delineate Evolutionary Significant Units (ESUs). We defined ESUs as populations that are reciprocally monophyletic in two or more independent characters, thus demonstrating lack of gene flow. All Red Sea, Oman, and Djibouti material, as well as representative specimens of the same putative species (based on morphology and coral hosts) from the IM, were sequenced for the Folmer region of cytochrome c oxidase subunit I (HC02198 and LCO1490 primers (RMNH) and dgHCO2198 and dgLCO1490 (UF) (Folmer *et al*., 1994; Meyer, 2003); see van der Meij, 2015a). Pairwise genetic divergence between Red Sea and IM populations of wide ranging species, and between endemic species and their sister species, was calculated as patristic distance in MEGA 6.06 (Tamura *et al*., 2013). Gall crabs were considered to be endemic when specimens were morphologically distinct and/or were divergent and reciprocally monophyletic in COI sequences from other populations sampled.

Results

Diversity and composition of fauna

We recorded 36 species from the Red Sea, an 86% increase over what was previously known. IM harbours at least 73 species, thus about twice as many as the Red Sea (Fize and Serene, 1957; Kropp 1990; van der Meij, unpubl. data; Fig. 2). Ten and six species are now recorded from Oman and Djibouti, respectively, but these faunas are too incompletely known to allow meaningful comparisons (Table 1).

The Red Sea fauna is dominated by *Lithoscaptus* (10 species) and *Opecarcinus* (9 species), respectively associated with Merulinidae (former Faviidae) and Agariciidae. These genera comprise more than half the Red Sea diversity. Similarly, 33 of 73 species in the IM belong to these genera. Three quarters of the gall crabs in both areas are species of *Lithoscaptus*, *Opecarcinus*, *Hapalocarcinus*, *Fizesereneia* and *Dacryomaia* (Fig. 2).

Eleven species, or almost 1/3 of the fauna, appear to be endemic to the basin or Arabia. Two of these 11 were recorded from Djibouti and five from Oman, leaving six species known only

Table 1. Pairwise genetic divergence (as patristic distance) between Red Sea and Indo-Malayan populations of wide ranging species, and between endemic species (bold) and their sister species.

Genus	Species	Host Red Sea / Arabia	Red Sea/Arabia (endemic)	Red Sea	Oman	Djibouti	Indo-Malaya	COI divergence	
								RS - IM	Sister species
								p-distance	p-distance
Cryptochirus	*coralliodytes*	*Platygyra*	0	1a	1d	1	1	0.0-1.7%	
Dacryomaia	sp. A	*Leptastrea*	0	1			1	1.8-2.9%	
Dacryomaia	sp. B	*Leptastrea*	0	1			1	0.0-1.8%	
Fizesereneia	***panda***	***Lobophyllia ehrenbergi,*** *L. hemprichii*	1	1	1				2.3%
Fizesereneia	**aff. *panda***	*Acanthastrea* sp.	1	1	1				unknown
Fungicola	*syzygia*	*Pleuractis, Cycloseris*	0	1b			1	0.0-0.8%	
Fungicola	*utinomi*	*Fungia, Lithophyllon*	0	1			1	0.0-1.1%	
Hapalocarcinus	**aff. *marsupialis 1****	*Pocillopora damicornis*	1	1c	d	1			9.0%
Hapalocarcinus	**aff. *marsupialis 2****	*Pocillopora*	1	1					3.3%
Hapalocarcinus	aff. *marsupialis 3**	*Pocillopora,* ***Stylophora wellsi,*** *Stylophora* sp.	0	1			1	0.3-1.7%	
Hapalocarcinus	aff. *marsupialis 4**	*Pocillopora* cf. *verrucosa*	0	1			1	0.6-1.3%	
Hiroia	cf. *krempfi**	*Hydnophora exesa*	0	1			1	1.2-2.0%	
Lithoscaptus	cf. *helleri**	*Favites* cf. *flexuosa*	0	1			1	2.5-2.7%	
Lithoscaptus	*nami [R]*	*Hydnophora* cf. *exesa*	0	1			1	0.5-2.3%	
Lithoscaptus	*paradoxus*	*Platygyra*	0	1	1d	1	1	1.1-2.0%	
Lithoscaptus	aff. *paradoxus*	*Leptoria phrygia*	0	1			1	1.4-2.3%	
Lithoscaptus	cf. *prionotus**	*Oulophyllia crispa*	0	1		1	1	0.8%	
Lithoscaptus	**sp. S**	***Echinopora fructiculosa, E. forskaliana***	1	1	1	1			6.8%
Lithoscaptus	sp. A	*Dipsastraea* spp.	0	1		1	1	0.0-1.2%	
Lithoscaptus	sp. B	*Plesiastrea versipora*	0	1			1	1.5-2.1%	
Lithoscaptus	**sp. K**	***Merulina scheeri*** *(and Goniastrea pectinata?)*	1	1	1				2.8%, 2.3%
Lithoscaptus	**sp. L**	*Dipsastraea*	1	1					3.5%
Neotroglocarcinus	*hongkongensis*	*Turbinaria*	0		d		1		
Neotroglocarcinus	*dawydoffi*	*Turbinaria*	0	1			1	1.0-4.8%	
Opecarcinus	*cathyae*	*Pavona clavus*	0	1			1	0.0-1.1%	
Opecarcinus	*lobifrons*	*Gardineroseris planulata*	0	1			1	0.0-0.6%	
Opecarcinus	sp. A*	*Leptoseris yabei*	0	1			1	1.7-2.4%	
Opecarcinus	sp. B	*Pavona* cf. *explanulata*	0	1			1	0.2-1.4%	

Opecarcinus	sp. C	*Leptoseris* cf. *incrustans*	0	1		1	0.0-1.5%	
Opecarcinus	sp. F	*Leptoseris incrustans*	0	1		1	0.6-2.9%	
Opecarcinus	**sp. R**	*Pavona* cf. *varians / venosa*	1	1				11,40%
Opecarcinus	**sp. S**	*Pavona* cf. *explanulata*	1	1				4.5%, 5.4%, 4.2%
Opecarcinus	sp. V	*Pavona*	0	1		1	0.5-1.5%	
Pseudo-hapalocarcinus	*ransoni*	*Pavona cactus, P.* cf. *decussata* (sensu Scheer and Pilai, 1983)	0	1		1	0.8-1.7%	
Sphenomaia	**aff. *pyriformis* 1***	*Dipsastraea* cf. *laxa*	1	1				3.1%
Sphenomaia	**aff. *pyriformis* 2***	*Astrea curta, Paramontastraea peresi*	1	1				3.2%
Xynomaia	cf. *boissoni***	*Mycedium elephantotus, Echinophyllia*	0	1	1	1	0.0-0.1%	
Xynomaia	*sheni***	unknown	0		d	1		
			11	36	10	6	27	

In bold endemic for the Red Sea (and Arabia); n/a = not applicable; a = Heller, 1961; b = Kramarsky-Winter *et al*., 1995; c = Abelson *et al*., 1991; d = Hogarth, 1989; * cryptic species present; ** Xynomaia spp. can be difficult to identify, hence an identification error cannot be ruled out. For the literature record, however, there is no material deposit in a natural history museum.

from the Red Sea basin (Table 1). Two gall crab genera (*Fizesereneia*, *Sphenomaia*) do not share species between the Red Sea and IM, despite occupying the same hosts.

Host relationships

Most cryptochirid species were found only a in single species or genus of host. Four were collected in two confamilial genera each (Table 1). Five endemic corals had gall crabs: *Lobophyllia ehrenbergi*, *Echinopora forskaliana*, *E. fruticulosa*, *Merulina scheeri*, and *Stylophora wellsi* (Table 1). Four of these five hosted endemic gall crabs. The endemic crabs, however, sometimes utilized a mixture of endemic and wide-ranging hosts, e.g. *Fizesereneia panda* inhabited the endemic *Lobophyllia ehrenbergi* as well as the wide-ranging *L. hemprichii*. The wide-ranging crab *Hapalocarcinus* aff. *marsupialis 3* inhabited the endemic coral *S. wellsi*, as well as wide-ranging *Pocillopora* species.

Most crabs that occur both in the RS and IM occupy the same coral host(s) in both. Consistent with previous observations, no gall crabs were encountered in corals of the Acroporidae, Poritidae (Kropp, 1990a), or Euphylliidae (van der Meij, pers. obs). *Dacryomaia* sp. 1, occupied different hosts *Leptastrea* in the RS, but the endemic *Lithophyllon undulatum* in the IM (Hoeksema *et al*., 2012; van der Meij and Hoeksema, 2013). Some coral genera (e.g. *Cyphastrea*, *Podabacia*) are inhabited by gall crabs in the IM but no gall crabs were found in them in the Red Sea despite search efforts. Some species, e.g. *Astrea curta*, *Dipsastraea laxa* and *Lobophyllia hemprichii*, are inhabited by different gall crabs in the RS than in IM. Some genera (*Pocillopora*, *Turbinaria*) are inhabited by multiple gall crab genera in IM, but only one species in the RS. The genera *Pelycomaia*, *Pseudocryptochirus*, and *Utinomiella* appear to be absent in the Red Sea, despite the presence of their host. The diversity in host/symbiont specificity and distributional patterns in the gall crab-coral system provides an ideal system for future work to investigate questions of co-evolution and specialist symbioses.

Genetic distance
Genetic divergence between the 11 endemics and their closest relatives ranged between 2.3-11.4%. Genetic distance between RS and IM specimens of wide-ranging species ranged from 0-2.9%. Nine species shared haplotypes across this ~10,000 km span.

Discussion

Diversity and endemism
The 36 species recorded from the Red Sea represent the second largest diversity for gall crabs after Indo-Malaya. Although this is in part a consequence of limited sampling in other areas, the Red Sea nevertheless stands out as a centre of diversity. In comparison, Guam, extensively studied by Kropp, has 28 species (Paulay *et al*., 2003), while 23 are recorded from Japan, 18 from Vietnam (Fize and Serène, 1957), 17 from Moorea in Polynesia, (GP, unpubl.), and 5 from Hawaii (Castro, 2011).

Over 30% of the Red Sea species are known at present only from the basin or Arabia. This is substantially higher than the typical 10-15% endemism recorded for the marine fauna of the Red Sea (DiBattista *et al*., in review 1). However studies considering the evolutionary history of several fishes using genetic data suggest that endemism in the Red Sea may be higher than currently thought (DiBattista *et al*., 2013). Similarly, an integrative study of Red Sea sea cucumbers found endemism to be much higher than suggested by morphological taxonomy alone (39% vs 21%, Paulay *et al*., in review). These results suggest that integrative taxonomic study combining morphological and molecular approaches for detecting cryptic species will substantially augment estimates of endemism.

Five of 10 species of gall crabs we collected (and thus could sequence) from Djibouti (2 of 6) and Oman (4 of 6) are 'endemic' to Arabia. All five were also encountered in the Red Sea. While the Gulf of Aden and Arabian Sea remain quite undersampled, the high endemism and sharing of endemics across the region suggests that most, if not all endemics, documented from the Red Sea will be found outside the basin, if their hosts occur there. The non-Arabian western Indian Ocean is yet (mostly) unstudied for gall crabs, thus it is possible that some of these putative Red Sea – Arabia endemics will be found to range into these neighbouring areas. The Red Sea – Arabia endemics appear to be absent from the IM.

The sharing of endemics between the Red Sea and Oman, suggests that gall crab endemics are broadly distributed across Arabia. This contrasts with holothuroids, where endemics in the Red Sea tend to range to the Gulf of Aden, but tend to drop out from the productive, upwelled coasts of southeastern Arabia. Corals also show a major faunal break between the Red Sea and Oman (Claereboudt, 2006; DiBattista *et al*., review 1). This potentially broader range of gall crab endemics may be related to their tolerance of high planktonic productivity.

Gall crabs were much more abundant in the southern than northern Red Sea, and were also abundant in Djibouti and Oman. A similar general pattern is apparent in coral barnacles (Pyrgomatidae) (GP, pers. obs.; Malay, unpubl.). The latter areas are characterized by high algal biomass and productivity, murkier waters, an abundance of suspension feeders, including many that live in corals (Sheppard and Salm, 1987). Consequently rates of bioerosion are high, especially in comparison with the oligotrophic northern Red Sea (Glynn, 1993; Paulay *et al*., in review). High productivity appears to favour symbiotic gall crabs and barnacles, as observed by Highsmith (1980) broadly for coral bioeroders. The southern Red Sea and Gulf of Aden may be the most diverse area in Arabia for cryptochirids, because both coral diversity and planktonic productivity are high. Low productivity in the northern Red Sea, while associated with a diverse coral fauna,

may limit abundance and possibly diversity of gall crabs, while high productivity in Oman limits coral diversity, although with the addition of several endemic scleractinians (Claereboudt, 2006).

Red Sea-Arabian endemics are separated from their sister species by 2.3-11.4% p-distance in COI, indicating that they substantially predate the Holocene. Six of the endemics cluster at 2.3-3.5% p-distance separation, suggesting Pleistocene origin, and suggestive of an important vicariant event.

Genetic divergence

The lack of coral habitat along the 2200-km coastline from Djibouti to southern Somalia may inhibit gene flow between the Red Sea and western Indian Ocean by limiting opportunities for steppingstone dispersal (Kemp, 1998). Similarly, lack of reef development and paucity of corals along the shores of Pakistan and eastern India (except for the Gulf of Kuch), create a dispersal barrier toward the central Indian Ocean (UNEP/IUCN, 1988).

Genetic divergence between Red Sea and IM populations of broadly distributed gall crabs is low. Nine of 25 species sampled share haplotypes, and 17 have p-distances <1%. IWP-wide connections at the haplotype-level are not uncommon among strong dispersers (e.g. Lessios *et al*., 1998; Fratini and Vannini, 2002; Holland *et al*., 2004).

Benthic marine organisms move little as juveniles and adults, so connectivity is largely through pelagic (oceanic) stages such as larvae, and in some cases larval dispersal can be extensive (Scheltema, 1986). Larval development for Cryptochiridae is practically unknown, described only for the Atlantic *Troglocarcinus corallicola*, which has typical brachyuran development with at least 5 zoeal stages and a megalopa (Scotto and Gore, 1981). Larvae of four cryptochirid species were also identified in plankton samples from Moorea, Polynesia, using DNA-sequence data (Moorea Biocode project, unpublished data). Xanthid crabs, typically with 4 zoeal stages, show substantial homogeneity across the IWP in contrast with majoid crabs, typically with 2 zoeal stages, which tend to have restricted distributions (Moore and Paulay, in press). The extended larval development and limited genetic differentiation suggests that cryptochirids are capable of substantial dispersal, concordant with the hypothesis that species that require specialised or scarce habitats (such as specific hosts) should have competent larval stages of long duration (Miller and Hadfield, 1990). Gall crabs also appear to be able to store sperm for later use (Vehof *et al*., in press).

Number of corals predicts number of cryptochirids

Gall crabs are host specific to species, genera, or families of corals. The change in diversity between the Red Sea and IM of crabs and hosts is comparable; the Red Sea has ~49% and ~51% as many gall crabs and reef corals as the IM. Whether the proportional diversity of gall crabs and corals are relatively stable across the IWP is an interesting question for further research.

Conclusion - Origin of Red Sea fauna

data from numerous bottom-up studies in IWP biogeography allow comparisons of patterns and tests generalities of patterns and processes. The wealth of specific studies focused on the Red Sea-Arabian region in this volume provide interesting comparisons. Gall crabs are particularly interesting, as the first obligately-symbiotic group studied in detail.

The Red Sea and Arabia are renowned for marine endemism and diversity, and gall crabs exemplify both. Regional endemism in reef-associated organisms is likely caused by isolation of Arabia from the rest of the Indian Ocean by unsuitable habitats; notably soft bottoms and productive shores, with upwelling limiting reef development. Within Arabia the diverse physiographic

and oceanographic setting further structures marine habitats, from the oligotrophic northern Red Sea to the macroalgal-dominated communities of central southern Arabia. The location of the major distributional and diversity breaks in these complex environments vary among taxa. Corals are most diverse in the north and central Red Sea, and also have high endemism there (DiBattista *et al*., review 1). Sea cucumbers are also most diverse in the Red Sea, but most endemics range across to the Gulf of Aden, with a major break in diversity and distribution lying between the Gulf of Aden and the Omani Arabian Sea coast. Data for gall crabs is more limited, but evidence suggests that their endemics are broad ranging from the Red Sea to Oman at least. These contrasts highlight the interplay between environment and phylogeny – oligotrophic areas favour photosymbiotic diversity and endemism, while productive waters that still support corals and some reef development favour coral inquilines. The contrast between coral and cryptochirid patterns is striking, surprising, and warrants further investigation.

The Red Sea has undergone drastic environmental changes during glacial times when low sea levels led to hypersaline conditions that may have killed much of the marine biota (Klausewitz, 1989; but see DiBattista *et al*., 2013). Cryptochirids and holothuroids (Paulay *et al*., in review), two groups investigated genetically in their entirety, both suggest that strict Red Sea endemics are rare. In both groups, all regional endemics pre-date the last glacial (and several previous glacial) periods, and most species extend to at least the Gulf of Aden.

There is also no evidence, for old, relictual endemism dating from the Tethys in gall crabs. The age of cryptochirids is unclear; current estimates put the origin of the clade (but not necessarily the symbiotic life style) between 83 [+/-11] Ma (Tsang *et al*., 2014), and 36 [+/-13] Ma (van der Meij and Klaus, chapter 6). Diversification of the living gall crabs may have started as recently as < 13 [+/-6] Ma (van der Meij and Klaus, chapter 6) substantially lagging behind the Cenozoic diversification of the reef fauna. A similar late origin and diversification is evident in the coral-symbiotic fish genus *Gobiodon* (Duchene *et al*., 2013). Arabia holds no endemic cryptochirid genera, and most species are relatively young, at 2-11% COI p-distance.

Taken together, these findings suggest that the Gulf of Aden was a key area in the evolution of the Arabian marine biota, and may have served as a refuge during late Cenozoic glacial periods. The occurrence of numerous regional endemics outside the Red Sea basin in both gall crabs and sea cucumbers suggest that survival inside the Red Sea during low sea level periods is not necessary to explain their distribution. As individual case studies emerge, it is apparent that the evolutionary history of Arabian and Red Sea fauna is complex, with no 'one size fits all' explanation.

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Distributions over reefs and shelves

Chapter 13

Cross-shelf distribution patterns of gall crabs in the Makassar Strait (SW Sulawesi, Indonesia)

Sancia E.T. van der Meij, Leon R. Pasman & Bert W. Hoeksema

Abstract

Coral reef cryptofauna forms an important component of tropical marine biodiversity, consisting primarily of invertebrates dwelling in and on corals and other sessile organisms. Distribution patterns of associated organisms are, however, poorly understood. During fieldwork on reefs in the Spermonde archipelago, the cross-shelf distribution patterns of gall crabs (Cryptochiridae) associated with mushroom corals (Fungiidae) were studied from near-shore to offshore over the 40 km wide Spermonde Shelf. Occurrence rates of crabs was measured in four parallel shelf zones along the shore with the use of belt quadrats at 5 m depth intervals over the reef bottom down to a maximum of 40 m. Four gall crab species were encountered, of which *Fungicola syzygia* was the most abundant and inhabited the widest range of mushroom coral hosts. The primary factor determining gall crab distributions was host coral availability. Host shifts were observed when the preferred host was absent in certain shelf zones or at certain depths. The mid- and outer shelf reefs had the highest occurrence rates of gall crabs, while those near-shore had lower occurrence rates. Highest occurrence rates of gall crabs were observed from 5 to 15 m depth, and mostly at 10 m depth.

Manuscript under review

Introduction

Coral-associated organisms contribute highly to the species richness of coral reefs, especially in the Coral Triangle, where the highest concentrations of coral host species can be found (Hoeksema, 2007). Nonetheless, such associated faunas are relatively understudied, possibly because many symbionts that seek shelter in their host are 'cryptic' owing to their small size, camouflage, or endosymbiotic lifestyle (e.g. Scott, 1987; Bickford *et al*., 2007). The size of the coral host may be important for the composition of the associated fauna (Schiemer *et al*., 2009; Carvalho *et al*., 2014). The nature of such associations is often uncertain, implying that they can be either commensals or parasites (Castro, 1988; Buhl-Mortensen and Mortensen, 2004).

Reef habitats support abundant and diverse assemblages of small crustaceans; a large portion of the more than 500 (out of nearly 2,000) brachyuran crab species dwelling on Indo-Pacific coral reefs live in close association with scleractinian corals (Serène, 1972). This includes both motile species such as copepods and amphipods, as well as (mostly) sessile species such as *Paguritta* hermit crabs (Paguridae) and gall crabs (Cryptochiridae). The associations between corals and crustaceans range from facultative arrangements to obligate dependencies (Stella *et al*., 2011; Hoeksema *et al*., 2012).

Gall crabs are obligate associates of stony corals, living in enclosed galls or pits in their coral hosts. Although cryptochirids have been known to science for over 150 years, little is known about their ecology and biology. They are common inhabitants of coral reefs, but easily overlooked because they are small and reside inside holes (Hoeksema and van der Meij, 2013). According to the last taxonomic revision of Indo-Pacific gall crabs (Kropp, 1990a), two species are known to live in association with Fungiidae corals: *Fungicola fagei* (Fize and Serène, 1956) and *F. utinomi* (Fize and Serène, 1956). Hoeksema *et al*. (2012) reported on a *Dacryomaia* species as a third cryptochirid species associated with Fungiidae, and van der Meij and Hoeksema (2013) reported on an undescribed species, closely related to *F. fagei*, which is now described as *F. syzygia* van der Meij, 2015.

Literature on distribution patterns of coral-associated organisms is scarce (Preston and Doherty, 1994; Oigman-Pszczol and Creed, 2006; Gittenberger and Hoeksema, 2013; van der Meij and Hoeksema, 2013). The presence of coral-associated organisms evidently depends on host availability, which may be related to various environmental factors, such as distance offshore, exposure to winds, and depth (Cleary *et al*., 2005; Hoeksema, 2012a, b). It is not entirely understood how these environmental factors interact with occurrence rates (Gittenberger and Hoeksema, 2013; van der Meij and Hoeksema, 2013), with the possible exception of sedimentation. Sediment is expected to hinder gall crabs and other endosymbiotic invertebrates because it may clog their burrows (Kramarsky-Winter *et al*., 1995), whereas the host itself may be well equipped to shed sediments (Bongaerts *et al*., 2012; Erftemeijer *et al*., 2012).

To examine which factors may control gall crab occurrences, a good knowledge of the host species and their distributions is conditional. Ideally, the research should be undertaken in an area where clear environmental gradients can be discerned that affect both the host species and the associated organisms. This area should also be species-rich regarding host assemblages and associated fauna in order to distinguish the effects of host preference and inter-specific competition among the crabs.

In this paper the focus is on the cross-shelf distribution patterns of gall crab species associated with mushroom corals (Fungiidae) in the Spermonde archipelago in SW Sulawesi (Indonesia), which is situated in the Coral Triangle. A total of 37 fungiid species has been observed in this archipelago, some of which show wide cross-shelf distribution ranges (Hoeksema, 2012a, b).

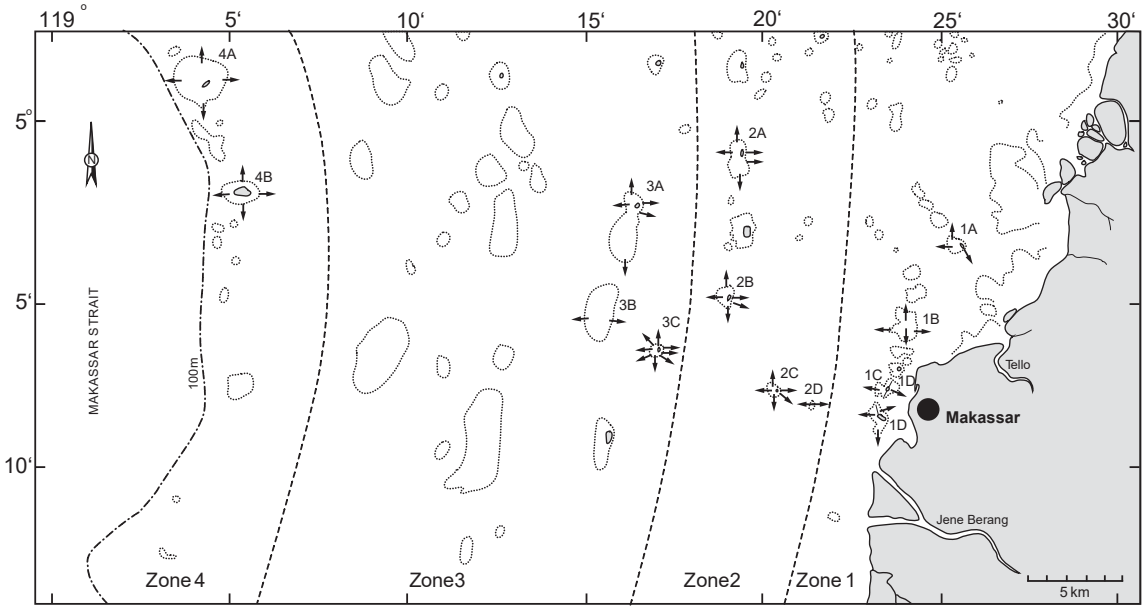

Fig. 1. Map of the Spermonde archipelago, showing zone I-IV.

Most fungiid species are free-living (Hoeksema, 1989; Gittenberger *et al*., 2011) and may co-exist in dense multi-species aggregations within their depth range overlaps (Hoeksema, 2012a, b; Hoeksema and Benzoni, 2013). Because of these ecological traits, mushroom corals and their associates can easily be counted and used in quantitative comparative studies using quadrats over reef transects dealing with co-occurrence in both host species assemblages and their associated fauna.

Material and methods

The fieldwork was carried out in the Spermonde archipelago, off SW Sulawesi in 1994 (Fig. 1). The Spermonde Archipelago is situated on a well-documented carbonate coastal shelf, approximately 40 km across, with several environmental influences that vary along on-to-offshore gradients (Cornils *et al*., 2010; Hoeksema, 2012a; Sawall *et al*., 2013; references therein). These influences are related to sewage seepage and pollution from the city of Makassar and to fluvial discharge with land-eroded sediments and sewage from the mouths of the nearby Jene Berang river to the south and smaller rivers to the north. Makassar city, the capital of South Sulawesi province, is a major port with a population of over one million inhabitants (Hoeksema, 2012a, b; references therein).

The reefs are arranged in rows parallel to the coast line, which is reflected in their distribution in four shelf zones (Fig. 2). The reefs are rich in coral species, which is related to the various reef environments (Umbgrove, 1930; Moll, 1983; Best *et al*., 1989). The distribution of mushroom corals off SW Sulawesi varies with: 1) the arrangement of reefs along cross-shelf gradients, from onshore to offshore, 2) the circum-reef variation in wind exposure and subsequent wave action, and 3) the depth range, from the shallow reef flat down to the reef slope and the sandy bottom of the reef base below (Hoeksema, 2012a, b).

A total of 11 reefs divided over four zones were surveyed (Figs 1-2). Data collection consisted of two parts. Firstly, mushroom corals were collected at various depths (down to 40 m) in four shelf zones parallel to the shoreline as a preliminary inventory of crab-infested mushroom coral species. Secondly, belt quadrats of 50 x 2m^2 at isobaths across depth gradients in the transects sites (at 1, 5,

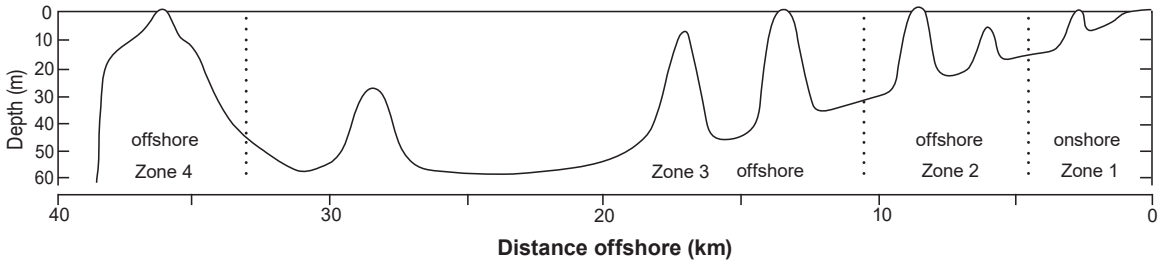

Fig. 2. Schematic cross-section of the central Spermonde Shelf from the Makassar Strait to the mainland (after Hoeksema, 2012a).

10, 15, 20 and 25 m) were monitored for mushroom corals containing gall crabs at 27 sites. In each quadrat an area of 100 m^2 was searched for gall crab species, except in zone 4 at a depth of 5 meters where this was 50 m^2. Transect work was predominantly carried out on the wave-exposed west sides of the reefs, as mushroom coral species are most abundant here (Fig. 1, Table 1).

For the identification of the host corals, a taxonomic revision of the Fungiidae (Hoeksema, 1989) was used, combined with a classification based on a molecular phylogeny reconstruction (Gittenberger *et al*., 2011). The corals were split with a hammer and chisel and the gall crab was extracted for identification. All gall crab samples were eventually stored in 70% ethanol and deposited in the collections of Naturalis in Leiden (collection coded as RMNH.Crus.D). Gall crab identifications and associations are based on literature (Fize and Serène, 1957; Takeda and Tamura, 1979; Kropp, 1990a; Hoeksema *et al*., 2012; van der Meij and Hoeksema, 2013; van der Meij, 2015a). The gall crab-host associations reported in Hoeksema *et al*. (2012) were largely derived from this survey. *Dacryomaia* sp. is possibly new to science, which is currently being studied by the first author (see also Paulay *et al*., 2003).

Results

The percentage of corals inhabited by gall crabs was highest on Samalona reef in zone II (Fig. 1, Table 2). Barang Caddi (zone II), Bone Tambung and Kudingareng Keke (both zone III) also had

Table 1. List of 11 reefs in four reef zones on the Spermonde Shelf with the position of 27 transects (Fig 1), maximum depth (m) are provided.

Shelf zone	Reef	Transect direction with maximum depth (m)						
		N	NW	W	SW	S	SE	E
Zone I	Lae-Lae	-	-	10	-	-	-	10
	Lae-Lae Keke	-	-	10	-	-	-	10
Zone II	Barang Caddi	-	-	25	-	25	20	-
	Barang Lompo	-	25	25	-	-	-	-
	Bone Baku	-	-	20	-	-	-	-
	Samalona	20	-	25	-	25	20	25
Zone III	Badi	-	-	40	-	-	-	-
	Bone Tambung	35	35	35	-	-	-	-
	Kudingareng Keke	-	30	30	-	35	25	25
	Lumu Lumu	-	-	40	-	-	-	-
Zone IV	Langkai	5	-	15	-	-	-	-

Table 2. Cross-shelf distribution in the Spermonde archipelago. Coral presence/absence data and zonations I-IV after Hoeksema (2012a). All Fungiidae identifications updated after Gittenberger et al. (2011). Abbreviations of localities: B = Badi; BB = Bone Baku; BC = Barang Caddi; BL = Barang Lompo; BT = Bone Tambung; KK = Kudingareng Keke; LA = Langkai: LL = Lae Lae; LLK = Lae Lae Keke; LU = Lumu Lumu; S = Samalona. Symbols: ♦ = species inhabited by *Dacryomaia* sp.; ▲ = species inhabited by *Fungicola fagei*; ● = species inhabited by *F. syzygia* ■ = species inhabited by *F. utinomi*; ○ = species present, not inhabited by gall crab; - = coral species absent; ? = no species presence/absence data available.

	I		II				III				IV	
Coral host	LLK	LL	BB	S	BL	BC	KK	B	BT	LU	LA	%
Ctenactis albitentaculata Hoeksema, 1989	-	-	-	○	?	○	○	?	○	?	○	0
C. crassa (Dana, 1846)	-	-	○	○	?	○	○	?	○	?	○	0
C. echinata (Pallas, 1766)	○	○	○	■	?	○	○	?	○	?	○	13
Cycloseris costulata (Ortmann, 1889)	○	○	○	○	●	●	●♦	?	○	●	○	40
C. cyclolites (de Lamarck, 1816)	-	-	○	○	?	-	○	?	○	?	-	0
C. distorta (Michelin, 1842)	-	-	-	-	?	-	○	?	-	?	-	0
C. fragilis (Alcock, 1893)	-	-	●	○	?	○	○	?	●	?	-	40
C. mokai (Hoeksema, 1989)	-	-	○	○	?	○	○	?	○	?	○	0
C. sinensis (Milne Edwards and Haime, 1851)	-	-	-	○	?	○	○	?	○	?	○	0
C. somervillei (Gardiner, 1909)	-	-	-	○	?	○	○	?	○	?	-	0
C. tenuis (Dana, 1846)	-	-	○	●	?	○	○	?	○	?	○	17
C. vaughani (Boschma, 1923)	-	-	-	○	?	-	○	?	○	?	-	0
Danafungia horrida (Dana, 1846)	○	○	○	■	?	○	○	?	○	?	○	13
D. scruposa (Klunzinger, 1879)	○	○	○	○	?	○	○	○	○	?	○	0
Fungia fungites (Linnaeus, 1758)	○	○	○	○	?	○	○	?	■	?	○	13
Halomitra pileus (Linnaeus, 1758)	-	-	○	■	?	○	○	?	■	?	○	33
Heliofungia actiniformis (Quoy and Gaimard, 1833)	○	○	○	○	?	○	○	?	○	?	○	0
H. fralinae (Nemenzo, 1955)	-	-	-	○	?	○	○	?	○	?	○	0
Herpolitha limax (Esper, 1797)	○	○	○	○	?	■	○	?	○	?	●	13
Lithophyllon concinna (Verrill, 1864)	○	○	○	○	■	■	■	?	○	■	○	40
L. repanda (Dana, 1846)	○	○	■	■	■	■	●■	■	■	■	■	82
L. scabra (Döderlein, 1901)	○	○	○	●■♦	♦	○	♦	?	♦	?	○	44
L. spinifer (Claereboudt and Hoeksema, 1987)	-	-	-	-	?	-	-	?	-	?	-	0
L. undulatum Rehberg, 1892	-	-	-	○	♦	♦	♦	♦	♦	?	-	83
Lobactis scutaria (de Lamarck, 1801)	-	-	○	○	?	○	○	?	○	?	○	0
Pleuractis granulosa (Klunzinger, 1879)	-	-	○	●♦	●	●♦	●♦	●	●♦	●♦	●	89
P. gravis (Nemenzo, 1955)	○	-	○	○	?	○	○	?	○	?	○	0
P. moluccensis (van der Horst, 1919)	○	○	○	●	●	●	●	●	●	●	○	64
P. paumotensis (Stutchbury, 1833)	●	○	●	●	●	●	●	●	●	●	●	91
Podabacia crustacea (Pallas, 1766)	○	○	-	▲	?	○	○	?	○	?	-	17
Polyphyllia talpina (Lamarck, 1801)	○	○	○	○	?	○	○	?	○	?	○	0
Sandolitha dentata Quelch, 1884	-	-	-	○	?	○	○	?	○	?	-	0
S. robusta (Quelch, 1886)	○	○	○	○	?	○	■	?	○	■	▲	33
Zoopilus echinatus Dana, 1846	-	-	-	-	?	-	○	?	○	?	-	0
% inhabited	6	0	13	32	-	28	27	-	28	-	21	

Table 3. Number of commonly inhabited fungiid coral individuals present per zone and transect depth (number of inhabited corals between brackets in **bold**). % indicates the occurrence rate. In zone I no reef was present below 10 m depth, in zone IV no reefs were present below 15 m (indicated by -).

crab species coral species	zone	1 m n	%	5 m n	%	10 m n	%	15 m n	%	20 m n	%	25 m n	%
		Dacryomaia sp.											
Pleuractis granulosa	I	0	0	0	0	0	0	-	-	-	-	-	-
	II	0	0	1(0)	0	28(0)	0	16(0)	0	1(0)	0	0	0
	III	0	0	5(0)	0	87(**1**)	0	80(0)	0	3(0)	0	5(0)	0
	IV	0	0	0	0	3(0)	0	1(0)	0	-	-	-	-
Lithophyllon scabra	I	2(0)	0	3(0)	0	0	0	-	-	-	-	-	-
	II	0	0	5(0)	0	10(0)	0	3(0)	0	0	0	0	0
	III	0	0	4(0)	0	20(0)	0	11(0)	0	0	0	0	0
	IV	1(0)	0	0	0	0	0	0	0	-	-	-	-
L. undulatum	I	0	0	0	0	0	0	-	-	-	-	-	-
	II	0	0	1(0)	0	1(0)	0	2(0)	0	0	0	0	0
	III	0	0	4(0)	0	7(0)	0	5(0)	0	1(0)	0	0	0
	IV	0	0	0	0	1(0)	0	0	0	-	-	-	-
		Fungicola fagei											
Sandalolitha robusta	I	2(0)	0	4(0)	0	0	0	-	-	-	-	-	-
	II	0	0	15(0)	0	16(0)	0	11(0)	0	0	0	0	0
	III	0	0	15(0)	0	26(0)	0	3(0)	0	0	0	0	0
	IV	0	0	5(**1**)	20	1(0)	0	0	0	-	-	-	-
		F. syzygia											
Cycloseris costulata	I	0	0	3(0)	0	2(0)	0	-	-	-	-	-	-
	II	0	0	4(0)	0	56(**1**)	1.8	47(**1**)	2.1	1(0)	0	0	0
	III	0	0	6(0)	0	99(0)	0	129(0)	0	16(0)	0	18(0)	0
	IV	0	0	0	0	0	0	0	0	-	-	-	-
Pleuractis granulosa	I	0	0	0	0	0	0	-	-	-	-	-	-
	II	0	0	1(0)	0	28(**2**)	7.1	16(**1**)	6.3	1(0)	0	0	0
	III	0	0	5(0)	0	87(**6**)	6.9	80(0)	0	3(0)	0	5(0)	0
	IV	0	0	0	0	3(0)	0	1(0)	0	-	-	-	-
P. moluccensis	I	0	0	30	0	8(0)	0	-	-	-	-	-	-
	II	0	0	0	0	50(**2**)	4.0	56(**1**)	1.8	8(0)	0	1(0)	0
	III	0	0	0	0	0	0	14(0)	0	1(**1**)	100	14(**1**)	7.1
	IV	0	0	0	0	0	0	0	0	-	-	-	-
P. paumotensis	I	27(0)	0	20(0)	0	0	0	-	-	-	-	-	-
	II	1(0)	0	43(**2**)	4.7	95(**9**)	9.5	46(**4**)	8.7	2(0)	0	0	0
	III	0	0	67(**3**)	4.8	109(**9**)	8.3	27(**4**)	14.8	1(0)	0	0	0
	IV	0	0	26(0)	0	4(0)	0	0	0	-	-	-	-
		F. utinomi											
Halomitra pileus	I	0	0	0	0	0	0	-	-	-	-	-	-
	II	0	0	1(0)	0	2(0)	0	4(0)	0	0	0	0	0
	III	0	0	5(0)	0	12(0)	0	4(0)	0	0	0	0	0
	IV	0	0	5(0)	0	1(0)	0	0	0	-	-	-	-

cont. Table 3

crab species coral species	zone	1 m n	%	5 m n	%	10 m n	%	15 m n	%	20 m n	%	25 m n	%
Lithophyllon concinna	I	0	0	0	0	0	0	-	-	-	-	-	-
	II	0	0	26(0)	0	56(0)	0	45(0)	0	2(0)	0	0	0
	III	1(0)	0	51(0)	0	234(0)	0	62(0)	0	1(0)	0	2(0)	0
	IV	0	0	78(0)	0	4(0)	0	0	0	-	-	-	-
L. repanda	I	1(0)	0	0	0	0	0	-	-	-	-	-	-
	II	2(0)	0	98(**1**)	1.0	126(**2**)	1.6	64(0)	0	2(0)	0	0	0
	III	11(0)	0	428(**6**)	1.4	628(**4**)	0.6	180(0)	0	2(0)	0	3(0)	0
	IV	0	0	474(**1**)	0.2	52(0)	0	2(0)	0	-	-	-	-
Sandalolitha robusta	I	2(0)	0	4(0)	0	0	0	-	-	-	-	-	-
	II	0	0	15(0)	0	16(0)	0	11(0)	0	0	0	0	0
	III	0	0	15(**1**)	6.7	26(0)	0	3(0)	0	0	0	0	0
	IV	0	0	5(0)	0	1(0)	0	0	0	-	-	-	-
Infested corals per depth		50(0)	-	1462 (**15**)	-	1865 (**36**)	-	923 (**11**)	-	45 (**1**)	-	30 (**1**)	-

high rates of corals inhabited by gall crabs, followed closely by Langkai (zone IV). Despite the abundant presence of mushroom corals, gall crabs were absent in the onshore zone I, as well as at 1 m depth in the other three zones.

Fungicola syzygia was the most abundant gall crab species inhabiting Fungiidae over the whole shelf area, despite its near-absence close to the shore line. This species was only encountered once on an on-shore reef (outside transects), but was found abundantly on the mid-shelf reefs in zones II and III (Tables 2-3). The single specimen in zone I was found in a coral of *Pleuractis paumotensis*. This mushroom coral species hosted *F. syzygia* across the whole shelf, including the most offshore reefs. It also showed its highest abundance near-shore, and was common elsewhere on the shelf (Hoeksema, 2012a). *Pleuractis granulosa, P. moluccensis* and *P. paumotensis* were regularly found inhabited on reefs in zone II-IV, just like *Cycloseris costulata*. This latter species was inhabited by both *F. syzygia* and *Dacryomaia* sp. *Fungicola utinomi* was observed inhabiting *Lithophyllon repanda* on all reefs in zones II-IV, and also frequently observed in *L. concinna* on the same reefs. *Dacryomaia* sp. inhabited *Lithophyllon scabra*, *L. undulatum* and *P. granulosa* in zones II-III. *Fungicola fagei* was only observed on two reefs, inhabiting the phylogenetically closely related species *Podabacia crustacea* and *Sandalolitha robusta* (Table 2).

The most frequently inhabited coral species were *Lithophyllon repanda*, *L. undulatum*, *Pleuractis granulosa* and *P. paumotensis*, which housed three out of the four known gall crabs inhabiting fungiids. *Fungicola fagei*, encountered on only two Spermonde reefs, is associated with fungiids belonging to the genera *Podabacia* and *Sandalolitha*, which were observed in all zones.

Occurrence rates

In most fungiid host corals, gall crabs reside in pits between the septae with a narrow opening for water circulation. However, crab species associated with free-living corals of *Lithophyllon repanda* reside in gall-like structures with overhangs near the coral mouth. However, such overhangs can also be observed in pits of *Dacryomaia* sp. in corals of the attached *L. undulatum*.

Occurrence rates can be obtained based on transect data (Table 3). For example, in zone II at 5 m depth, *F. syzygia* inhabited two out of 43 available *Pleuractis paumotensis* corals, resulting in an occurrence rate of 4.7%. At 10 and 15 m depth, the respective occurrence rates for the same coral host were 9.5% and 8.7%, respectively.

If outliers are ignored (*F. fagei* infesting one out of five *S. robusta* corals and *F. syzygia* inhabiting a single available *P. moluccensis* coral), the occurrence rates range between 0.2 and 14.8 %. Data for *Dacryomaia* sp. and *F. fagei* is scarce, relating to a lower abundance in comparison to *F. syzygia* and *F. utinomi*. Of the latter two, *F. syzygia* has a higher overall abundance in its respective hosts than *F. utinomi* (Table 3).

Depth distributions

Data on the depth distribution of gall crabs were obtained from belt quadrats of 50 × 2m^2 along depth gradients (1, 5, 10, 15, 20 and 25 m; Table 3). Only results concerning the preferred coral host species of the gall crabs during the research efforts are mentioned here (Table 2; Hoeksema *et al*., 2012; van der Meij and Hoeksema, 2013).

No inhabited fungiid species were observed in the belt transects of zone I, as well as in all the 1-m depth belt quadrats. Most gall crabs were found at 10 m depth, where also the highest density of host corals was found. The depth with the highest concentrations of fungiids increased with distance from the coast (except for Langkai in zone IV).

Fungicola syzygia was present at depths with high densities of available host coral species. The highest occurrence rates were found in zones II and III at 10 and 15 m depth in its preferred host *Pleuractis paumotensis,* which was also present in zone IV, but to a lesser extent (Table 3). Its sister species *P. moluccensis* (see Gittenberger *et al*., 2011) prefers greater depths (> 15 m), where it hosts *F. syzygia*. *Fungicola fagei* was only observed in zone IV, where it inhabited one out of the five observed *Sandalolitha robusta* individuals. Zones II and III had many available host corals belonging to the genera *Podabacia* and *Sandalolitha*, but these were not inhabited by gall crabs. *Fungicola utinomi* was only found at 5 and 10 m depth in zones II and III, where its preferred host species *Lithophyllon repanda* also showed its highest abundance. The occurrence rates are much lower than for *F. syzygia*. Only one specimen of *Dacryomaia* sp. was found at 10 m depth in a colony of *Pleuractis granulosa* (zone III).

Discussion

The distribution of mushroom corals on the Spermonde shelf varies with: 1) the distance of reefs offshore, 2) the circum-reef variation in exposure to wave action, and 3) the depth range (Hoeksema, 2012a, b). Mushroom corals of the mid-shelf reefs Barang Caddi, Samalona, Bone Tambung, and Kudingareng Keke (zones II and III) show the highest occurrence rates (> 30%). These four mid-shelf reefs are more remote from terrigenous impact than reefs in the near-shore zone I, and also less affected by *Halimeda* dust, upwelling and wave impact as on the offshore reefs of zone IV (Hoeksema, 2012a).

The near-shore reefs in zone I contain fewer fungiid species than those in zones II – IV because they are the most influenced by sediments, river discharge, sewage and harbour activities, and also because the surrounding sea floor is shallow, which implies that the depth ranges of onshore reefs offer less available space for some mushroom coral habitats than those on the deeper offshore reefs (Hoeksema, 2012a, b). Evidently, low host coral availability offers less potential habitat for gall crabs. Nevertheless, the percentage of crab-inhabited corals is also lower on near-shore reefs than in the other zones. Van der Meij and Hoeksema (2013) showed that reefs in the

Semporna area that were under influence of natural or anthropogenic disturbances had lower occurrence rates of gall crabs. Stress has a negative effects on coral assemblages and hence on their associated cryptofauna (Risk *et al*., 2001; van der Meij *et al*., 2010). Similarly, Preston and Doherty (1990, 1994) showed that coral-dwelling crustacea on the Great Barrier Reef had a maximum abundance on the mid-shelf reefs, and that their total abundance was significantly lower on the inner shelf reefs.

Occurrence rates

Van der Meij and Hoeksema (2013) discussed various studies on occurrence rates in gall crabs, and show that low occurrence rates are possibly linked to natural and anthropogenic stress. Apart from this study, only one study (in Brazil) used belt quadrats to determine occurrence rates, with occurrence rates ranging between 17 and 21% (Oigman-Pszczol and Creed, 2006). However, the quadrats were haphazardly placed in areas where at least one of the studied coral species occurred, whereas the in the present study they were placed over the reef at depth intervals regardless of the presence of fungiid corals. This might explain the higher observed occurrence rates in the Brazilian study, in addition to differences caused by the discrepancy in coral fauna composition.

The present study shows much variation in occurrence rates among crab species and within species among preferred host corals. The most abundant corals are not necessarily the most commonly inhabited (Scott, 1987; Norton and Carpenter, 1998), which is related to the host preference of the gall crabs (van der Meij and Hoeksema, 2013: Table 1). So far, it is unclear why the crabs show specific host preferences. For mushroom corals such preferences are also known from several wentletrap snails (Epitoniidae) and parasitic *Leptoconchus* snails (Gittenberger and Gittenberger, 2011; Gittenberger and Hoeksema, 2013) and some commensal shrimp species (Hoeksema *et al*., 2012). In comparison, some species of boring mussels (Mytilidae) living inside fungiid corals may have a much broader host spectrum (Owada and Hoeksema, 2011), while information on host-specific composition of crypobenthic fish fauna is hardly available for fungiids (Bos, 2012; Hoeksema *et al*., 2012) and other corals (Schiemer *et al*., 2009; Reijnen *et al*., 2011; Duchene *et al*., 2013; Tornabene *et al*., 2013). Preference for a particular host may be advantageous when many potential hosts are abundantly available. Moreover, host corals may produce bioactive compounds influencing settlement of gall crab larvae in some species. No direct cause may be present when host preferences have been derived from ancestral associated species in which the association was more advantageous than in descendant species.

Depth distributions

Depth, so far, does not seem to be a limiting factor for gall crabs, which inhabit their fungiid hosts in wide depth ranges. The maximum depth record for gall crabs in this study was 32 m (in *Pleuractis granulosa* during a reconnaissance survey on the sandy reef base of Pulau Badi), while there are also shallow records of 1 m depth (host *P. granulosa*, Papua New Guinea, Institut Royal des Sciences Naturelles de Belgique (IRNSB) coll. nr. 26862/84-46). Mushroom corals at greater depths are usually dwelling on sand (Hoeksema, 2012a), but this does not appear to affect the presence of crabs as long as their hosts are also able to survive in sandy habitats.

Several fungiid species show a downward shift in depth range with increasing distance offshore (Table 3; Hoeksema, 2012a). At depths outside the preferred depth ranges of the preferred host coral, gall crabs appear to shift to the second-preferred host coral. *Fungicola syzygia* shifts from *Pleuractis paumotensis* to *P. moluccensis* at depths > 15 m. On the other hand, *Fungicola utinomi* in *L. repanda* was predominantly observed at 5 and 10 m depth, despite the host's occurrence at 15 m depth. This indicates that the depth ranges of gall crabs are not necessarily strictly

related to those of their hosts and that some gall crab species might show more restricted depth ranges than others regardless of their host coral.

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Chapter 14

Distribution of gall crabs inhabiting mushroom corals on Semporna reefs, Malaysia

Sancia E.T. van der Meij & Bert W. Hoeksema

Abstract

Coral reef cryptofauna forms an important component of tropical marine biodiversity, consisting primarily of invertebrates dwelling in and on corals. During a survey carried out around the Semporna peninsula (Sabah, NE Borneo), the occurrence of gall crabs inhabiting mushroom corals was examined on reefs ranging from sheltered to exposed conditions. Out of 44 fungiid species, 19 were found to be associated with gall crabs. The gall crabs were observed at 85% of the 62 studied sites, and their occurrence rates per site ranged between 0 and 25%. High occupancy rates were almost equally distributed over the northern (sheltered) and southern (exposed) sites. Sites without gall crabs were all wave-exposed and predominantly under the influence of disturbances, such as blast fishing or relatively high nutrient loads.

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Introduction

Coral reefs are well known for their high biodiversity. An important component of reef biota is formed by cryptofauna, predominantly consisting of endozoic and epizoic invertebrates associated with corals (Stella *et al.*, 2011). Although reef corals are known to act as hosts to a wide range of invertebrates (e.g. Oigman-Pszczol and Creed, 2006; Stella *et al.*, 2010; Hoeksema *et al.*, 2012), little information is available on environmental factors that affect the species composition of the associated fauna.

Mushroom corals (Scleractinia: Fungiidae) are common Indo-Pacific reef corals. They have been used as a model taxon for a variety of studies, including research on regional and local biodiversity patterns (Hoeksema and Moka, 1989; Hoeksema, 1991a, 2007, 2012b; Hoeksema and Koh, 2009) as well as research on their associated fauna (Bos, 2012; Hoeksema *et al.*, 2012). Coral gall crabs (Brachyura: Cryptochiridae) are obligate associates of living stony corals, residing in galls or pits in their hosts. Their taxonomy has been revised (Kropp and Manning, 1987; Kropp, 1990a), but many aspects of the distribution and ecology of the species have so far remained unknown (e.g. van der Meij, 2012).

This study provides information on the occurrence of gall crab fauna in association with mushroom corals on reef assemblages in eastern Sabah, ranging from inshore sheltered conditions to exposed oceanic environments.

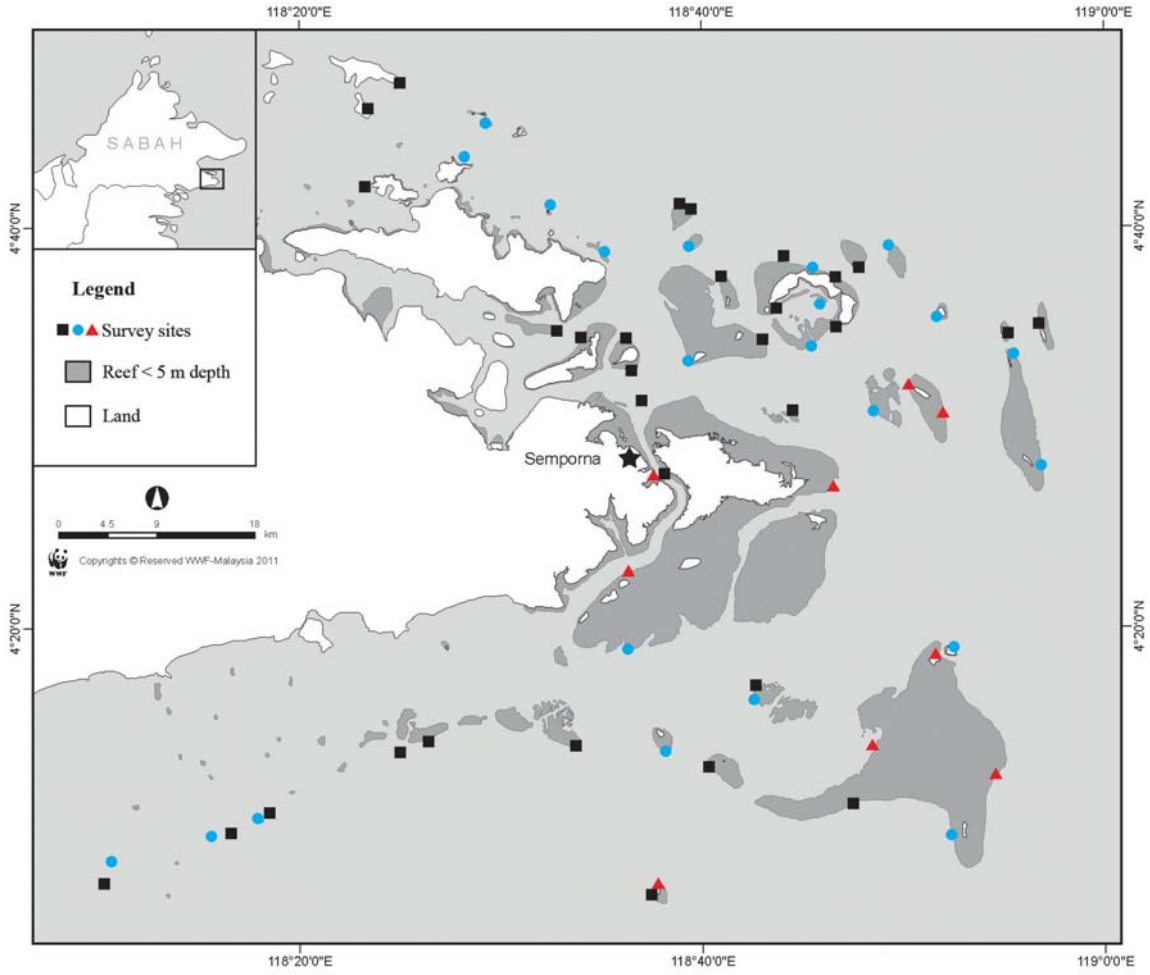

Fig. 1. Semporna, showing the various degrees of gall crab occurrence per site: red triangles n=0%; black squares 0<n≤15%; blue circles n >15%. Percentages are based on the occurrence rates listed in Table 2.

Material and methods

Mushroom coral and gall crab data were collected during fieldwork on coral reefs in eastern Sabah (Malaysia) as part of the Semporna Marine Ecological Expedition in December 2010 (SMEE2010), using the roving diver technique (Hoeksema and Koh, 2009). Data collection started around the deepest part of the reef (max. 30-40 m), and continued to the shallow part. For the identification of the host corals, a taxonomic revision of the Fungiidae (Hoeksema, 1989) was used, combined with a classification based on a molecular phylogeny reconstruction (Gittenberger *et al.*, 2011).

Fungiids were searched for gall crabs at 62 sites (Fig. 1), and the presence (or absence) of gall crab species was noted per mushroom coral host. The gall crabs were not identified to species level, as this would have required collecting all the gall crabs and their coral hosts, which was impossible in the given time frame. The gall crab-mushroom coral associations were taken from Hoeksema *et al.* (2012), and unpublished data (van der Meij, unpubl.).

The gall crab occurrence rate per site was plotted on a map of the research area (Fig. 1; see also Waheed and Hoeksema: fig. 1). Multivariate analyses of the coral species composition were conducted for the survey sites using PRIMER 6 (Clarke and Gorley, 2006). A resemblance matrix based on the Bray-Curtis similarity measure was used to determine the similarity between sites. Subsequently, a group-averaged cluster dendrogram was generated (Fig. 2), with the significant clusters derived by similarity profiles (SIMPROF). In addition, an MDS ordination of only the inhabited fungiid corals was performed. Lastly, a Pearson's chi-squared test was carried out to test for differences in the distribution patterns of the crab-inhabited fungiids between the more sheltered northern reefs and more exposed southern reefs.

Table 1. Overview of mushroom corals inhabited by gall crabs in the Semporna area. Hosts: no. of sites where fungiid is present, gall crabs: no. of sites where fungiid is inhabited by gall crabs, % percentage of sites where fungiid is gall crab inhabited.

Mushroom coral species acting as host	Hosts	Gall crabs	%
Pleuractis granulosa (Klunzinger, 1879)	62	30	48
Lithophyllon repanda (Dana, 1846)	62	26	42
Podabacia crustacea (Pallas, 1766)	62	13	21
Herpolitha limax (Esper, 1797)	62	1	2
Pleuractis paumotensis (Stutchbury, 1833)	61	41	67
Cycloseris costulata (Ortmann, 1889)	58	6	10
Sandalolitha robusta (Quelch, 1886)	58	5	9
Pleuractis gravis (Nemenzo, 1955)	53	1	2
Pleuractis moluccensis (van der Horst, 1919)	51	13	25
Lithophyllon scabra (Döderlein, 1901)	37	4	11
Cycloseris mokai (Hoeksema, 1989)	37	1	3
Cycloseris tenuis (Dana, 1846)	36	1	3
Lithophyllon undulatum Rehberg, 1892	15	4	27
Sandalolitha dentata Quelch, 1884	15	1	7
Lithophyllon ranjithi Ditlev, 2003	12	3	25
Lithophyllon spinifer (Claereboudt and Hoeksema, 1987)	7	2	29
Cycloseris somervillei (Gardiner, 1909)	4	1	25
Cycloseris hexagonalis (Milne Edwards and Haime, 1848)	3	1	33
Cycloseris vaughani (Boschma, 1923)	2	1	50

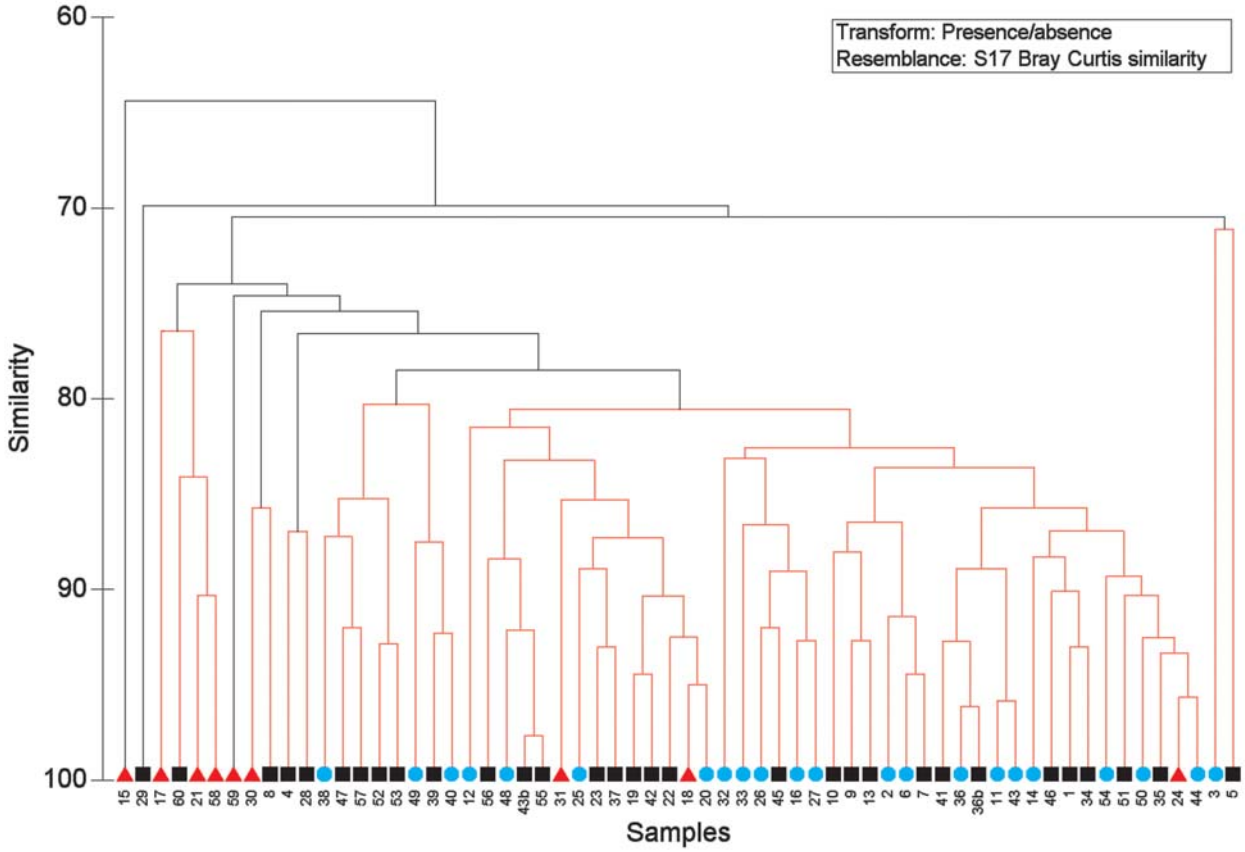

Fig. 2. The group-averaged hierarchical clustering of 62 sites, based on the species composition (presence/absence) of mushroom corals in Semporna. Significant clusters derived by SIMPROF are indicated by the bold solid line. The gall crab occurrence rates are indicated as follows: red triangles n=0%; black squares 0<n≤15%; blue circles n>15%, see also Fig. 1.

Table 2. Locality data of the sites visited during SMEE2010. Hosts: no. of mushroom coral species, gall crabs: no. of gall crab-inhabited mushroom corals, % occurrence rate.

Date	Site	Locality name	Latitude (N)	Longitude (E)	Hosts	Gall crabs	%
29 Nov	SEM.01	Roach reef, Mid rock/Tyre reef	04°10’39.0”	118°18’12.1”	22	2	9
	SEM.02	NW Roach reef, Second reef	04°10’31.5”	118°17’53.5”	17	3	18
30 Nov	SEM.03	SE of Tawau, Hand rock	04°08’24.5”	118°10’44.3”	28	6	21
	SEM.04	SE of Tawau, Darby rock	04°06’42.8”	118°13’39.7”	23	2	9
	SEM.05	SE of Tawau, Alert patches 2	04°09’38.5”	118°15’36.3”	17	1	6
	SEM.06	SE of Tawau, Alert patches 3	04°09’46.7”	118°16’35.8”	18	3	17
1 Dec	SEM.07	Erzherzog reef	04°14’26.5”	118°23’35.2”	18	2	11
	SEM.08	Horn reef	04°14’31.9”	118°26’25.0”	19	2	11
	SEM.09	Ligitan reef 1 S / Yoshi point	04°14’05.8”	118°33’26.7”	22	2	9
2 Dec	SEM.10	SE of Mabul Isl., Kapalai	04°13’05.4”	118°40’20.0”	17	1	6
	SEM.11	NE of Mabul Isl, W Cust reef	04°16’27.5”	118°42’32.9”	23	5	22
	SEM.12	Mabul Isl., Eel garden	04°13’49.8”	118°38’12.3”	22	4	18
3 Dec	SEM.13	Ligitan Isl., Ligitan 1	04°11’13.8”	118°47’27.9”	19	1	5

cont. Table 2

Date	Site	Locality name	Latitude (N)	Longitude (E)	Hosts	Gall crabs	%
	SEM.14	Ligitan Isl., Ligitan 2	04°09’35.8”	118°52’22.2”	23	4	17
	SEM.15	Ligitan Isl., Ligitan 3	04°12’43.0”	118°54’36.6”	14	0	0
4 Dec	SEM.16	Si Amil Isl., Second beach	04°18’56.9”	118°52’33.8”	20	4	20
	SEM.17	Wof Si Amil Isl., Denawan Isl.	04°18’55.9”	118°51’03.6”	15	0	0
	SEM.18	Ligitan Isl., Ligitan 4	04°14’06.5”	118°48’26.5”	20	0	0
5 Dec	SEM.19	Cust reef 2	04°17’08,3”	118°42’40.7”	17	2	12
	SEM.20	Creach reef	04°18’58.8”	118°36’17.3”	20	5	25
	SEM.21	Sipanggau Isl.	04°22’51.4”	118°36’20.3”	15	0	0
	SEM.22	Bumbun Isl. W (channel)	04°27’40.7”	118°38’09.1”	20	1	5
7 Dec	SEM.23	Pasalat reef	04°30’47.8”	118°44’07.8”	22	2	9
	SEM.24	Tg. Pantau Pantau, Bumbun Isl.	04°26’54.1”	118°46’31.0”	23	0	0
	SEM.25	Batura reef	04°30’48.6”	118°48’31.2”	19	4	21
8 Dec	SEM.26	Bohayen Isl.	04°28’00.9”	118°56’51.6”	24	4	17
	SEM.27	Timba Timba Isl.	04°33’37.7”	118°55’30.4”	21	4	19
	SEM.28	Pandanan Isl.	04°34’36.0”	118°55’14.1”	23	2	9
	SEM.29	Mataking Isl.	04°34’57.6”	118°56’46.5”	15	1	7
9 Dec	SEM.30	S Kulapuan Isl.	04°30’41.3”	118°51’58.4”	16	0	0
	SEM.31	N Kulapuan Isl.	04°32’09.6”	118°50’18.6”	21	0	0
	SEM.32	Pom Pom Isl.	04°35’29.8”	118°51’43.1”	17	4	24
	SEM.33	Kapikan reef	04°38’56.5”	118°49’15.0”	24	4	17
10 Dec	SEM.34	Mantabuan Isl.	04°37’56.0”	118°47’48.6”	22	1	5
	SEM.35	Gaya Isl.	04°37’29.0”	118°46’38.9”	22	2	9
	SEM.36	N Gaya Isl. (back)	04°37’57.6”	118°45’32.3”	27	5	19
	SEM.36B	NW Gaya Isl.	04°38’32.5”	118°44’6.0”	25	3	12
11 Dec	SEM.37	S Boheydulang Isl., outer reef	04°35’00.3”	118°46’39.1”	19	2	11
	SEM.38	Boheydulang Isl., outer reef lagoon	04°34’01.8”	118°45’27.5”	22	5	23
	SEM.39	Tetagan Isl., inner lagoon	04°35’55.4”	118°43’43.2”	21	3	14
	SEM.40	Ribbon reef	04°36’10.0”	118°45’53.6”	18	3	17
12 Dec	SEM.41	Maiga Isl.	04°37’32.2”	118°40’58.0”	29	2	7
	SEM.42	Selakan Isl.	04°34’22.1”	118°43’04.3”	19	2	11
	SEM.43	Sebangkat Isl.	04°33’19.9”	118°39’17.3”	25	5	20
	SEM.43B	Singamata Pancang	04°31’21.0”	118°37’00.7”	21	1	5
13 Dec	SEM.44	Sibuan Isl.	04°39’01.9”	118°39’22.6”	23	4	17
	SEM.45	Church reef 1	04°40’54.9”	118°39’28.4”	26	3	12
	SEM.46	Church reef 2	04°41’10.5”	118°38’56.5”	24	3	13
	SEM.47	Larapan Isl.	04°34’27.5”	118°36’15.0”	25	3	12
15 Dec	SEM.48	Timbun Mata Isl.	04°37’59.6”	118°35’21.6”	23	4	17
	SEM.49	Balusuan Isl.	04°41’07.9”	118°32’29.6”	25	4	16
	SEM.50	Batik Isl.	04°43’09.2”	118°28’22.0”	22	4	18
16 Dec	SEM.51	Tabawan Isl.	04°47’15.6”	118°25’00.8”	24	3	13
	SEM.52	Silumpat Isl.	04°45’58.7”	118°23’25.6”	27	3	11
	SEM.53	Batik Kulambu Isl.	04°42’02.1”	118°23’18.4”	29	1	3
	SEM.54	Bakungan Isl.	04°45’11.1”	118°29’16.0”	22	4	18
17 Dec	SEM.55	Silawa Isl.	04°34’29.8”	118°33’59.6”	21	1	5
	SEM.56	Mata Pahi Isl.	04°34’50.9”	118°32’49.4”	21	2	10
	SEM.57	S Larapan Isl. 2	04°32’51.1”	118°36’31.3”	25	3	12
	SEM.58	Semporna town, mangrove	04°27’35.6”	118°37’33.6”	16	0	0
18 Dec	SEM.59	Sipadan Isl., Baracuda point	04°07’12.0”	118°37’44.9”	17	0	0
	SEM.60	Sipadan Isl., Hanging gardens	04°06’45.3”	118°37’29.3”	13	1	8

Results

Occurrence rates

Out of 44 fungiid species recorded from the area (Waheed and Hoeksema, 2013), 19 were found to be inhabited by at least a single gall crab (Table 1). The most frequently recorded fungiid hosts were *Lithophyllon repanda, Pleuractis granulosa,* and *P. paumotensis. Cycloseris vaughani* also showed a high occupancy rate, but this was based on low absolute numbers; only two specimens of this host species were found, one of which was inhabited. For five host species that were encountered on nearly all localities (61-62 out of 62), the number of sites with gall crabs differed considerably. Crabs inhabiting *P. paumotensis* were observed in 41 out of 62 localities, whereas gall crab in *Herpolitha limax* was observed only once (Table 1).

The occupancy rate ranged from 0 to 25% inhabited host species per site. In general, sites with rates >15 % (Fig. 2) have relatively high numbers of recorded mushroom coral species. Seventeen of the 22 high gall crab occurrence sites had ≥20 recorded fungiid species (Table 2).

Distribution patterns

Gall crabs were observed at 85% of the 62 studied reef sites, but the occurrence rates differed between the sheltered northern sites and the exposed southern ones. High host occupancy by gall crabs was almost equally distributed over the northern and southern sites, but sites without gall crabs were all located in the exposed southern area (Fig. 1). The reef sites richest in fungiid species were found in the northernmost part of the research area (Waheed and Hoeksema, 2013: fig. 5), whilst the southern, more wind exposed sites on average displayed lower numbers.

In a dendrogram showing clusters of localities based on the mushroom coral species composition (Q-mode clustering), nine significant clusters can be distinguished with three outliers. The majority of the sites grouped in one large cluster. After plotting the occurrence data on the dendrogram it becomes apparent that the outliers and the small clusters had the lowest gall crab occupancy (Fig. 2).

The MDS ordination of the inhabited fungiids (thus excluding the presence/absence data on non-inhabited fungiids) show no grouping based on locality.

Discussion

Occurrence rates

The results of the present study are difficult to compare with previous studies on gall crab occupancy rates, owing to different methods of data collection. In this study, occurrence rates per locality are compared, whereas in previous studies they were recorded per species. Three earlier studies used transects to determine the occurrence in various species of host corals. In the Red Sea, the gall crab *Cryptochirus coralliodytes* Heller, 1861 inhabited four faviid genera along transect lines (between 2 and 7 m) with 25% infected hosts (Simon-Blecher and Achituv, 1997), whereas at 10-20 m depth, 20% of the individuals of the mushroom coral *Pleuractis granulosa* appeared to act as host for *Fungicola fagei* (Fize and Serène, 1956) (Kramarsky-Winter *et al.*, 1995). A study in Brazil using belt transects showed that gall crabs infested 17-21% of the host species *Mussismilia hispida* (Verril, 1902) and *Siderastrea stellata* Verril, 1868 (Oigman-Pszczol and Creed, 2006). Another Brazilian study by Johnsson *et al.* (2006) recorded occurrence percentages for *S. stellata*, which ranged between 10 and 37% depending on the survey site. In the Mexican Caribbean, Carricart-Ganivet *et al.* (2004) found that 21%

of *Manicina areolata* (Linnaeus, 1758) corals (n=160) were inhabited by *Troglocarcinus corallicola* Verrill, 1908. Lastly, in Vietnam, Fize and Serène (1957) encountered *F. fagei* in three out of 30 fungiids identified as *Parahalomitra* [= *Sandalolitha*] *robusta*, which most likely consisted of both *Sandalolitha dentata* (see Fize and Serène, 1957: pl. XIII, DEF) and *S. robusta*.

Of the 31 recorded fungiid species that can act as gall crab hosts (Hoeksema *et al.*, 2012), 30 were found in Semporna. Nineteen of these 31 were observed to be associated with gall crabs. *Cycloseris vaughani* is now recorded as a new host. Fungiids with the highest numbers of gall crabs are *Lithophyllon repanda, Pleuractis granulosa*, and *P. paumotensis*. *Cycloseris* species are not frequently observed inhabiting gall crabs, which may be restricted by the relatively small sizes of the hosts (see Hoeksema, 1991b; Gittenberger *et al.*, 2011). *Pleuractis granulosa* and *P. paumotensis* are hosts to the gall crab *Fungicola fagei*. In addition, *P. granulosa* is also known to be inhabited by *Dacryomaia* sp. *Lithophyllon repand*a is also associated with *F. fagei*, but is most frequently inhabited by *Fungicola utinomi* (Fize and Serène, 1956) (Hoeksema *et al.*, 2012; van der Meij *et al.*, in preparation). Molecular studies on gall crabs inhabiting Fungiidae indicate the presence of a cryptic species closely related to *F. fagei*, which is currently studied in more detail (van der Meij, in preparation).

Herpolitha limax, host to both *F. fagei* and *F. utinomi*, occurred at all Semporna localities, but it was found only once to be inhabited by a gall crab. This mushroom coral can nevertheless be considered a hospitable species as it is host to a wide range of other associated organisms (Hoeksema *et al.*, 2012). *Cycloseris* spp. that are hosts to *F. fagei* and *Dacryomaia* sp., were inhabited at only a few sites. These differences in occupancy rate indicate a host preference in certain gall crab species.

Distribution patterns

So far little information is available on the distribution patterns of coral-associated organisms (see e.g. Gittenberger and Hoeksema, 2013), in particular gall crabs. The present results show that localities with relatively high occupancy rates are distributed quite evenly over the research area, but that low occurrence rates are only found in the southern part of the research area. Apart from having few or no gall crab-inhabited mushroom coral species, those sites also differed in fungiid species composition. Some sites with low occurrence numbers can be related to oceanic conditions (SEM.59-60). Other sites were heavily damaged by blast fishing (SEM.15) or affected by high nutrient impact (SEM.58) (Waheed and Hoeksema, 2013). Natural and anthropogenic stress have negative effects on coral assemblages and hence on their associated cryptofauna (Sebens, 1994; van der Meij *et al.*, 2010).

Near-shore sites show lower numbers of inhabited corals compared to offshore sites (Fig. 1). Cross-shelf distribution data of gall crab-inhabited mushroom corals in the Spermonde Archipelago (SW Sulawesi) show a similar presence/absence pattern, with the near-shore reefs having the lowest occurrence rates (0-6% of the available host species) (van der Meij *et al.*, in preparation). In the Spermonde, where mushroom coral distributions have been studied extensively (Hoeksema, 2012b), no clear differences in occurrence rates between the mid-shelf and outer-shelf reefs can be discerned (van der Meij *et al.*, in preparation). Near-shore reefs can have a higher sediment load, especially close to river outlets (van der Meij *et al.*, 2010; Erftemeijer *et al.*, 2012). Kramarsky-Winter *et al.* (1995) mention that no crab-inhabited fungiids were found in sandy areas, and relate this to the fact that sediments may fill the crab's burrow, hence smothering the crab.

Concluding remarks

Previous research involving gall crab occurrence data was conducted in the western Atlantic (Mexican Caribbean and Brazil) and the Red Sea, making the present study the first in the central Indo-Pacific. The number of inhabited fungiid species recorded per locality showed occurrence rates from 0 to 25%. In general, sites with higher infestation rates had higher numbers of host species. The distribution patterns of inhabited host species (at the different sites) show that there is little difference (chi-squared test, df01, $p>0.05$) between the more sheltered reefs in the northern section of the study area and the more exposed reefs in the south, where all the low crab occurrences were recorded. The majority of these sites have low fungiid species richness and a different fungiid species composition (Waheed and Hoeksema, 2013).

Cryptochirids are diminutive crabs that may occur in high densities but are usually overlooked on coral reefs (Hoeksema and van der Meij, 2013). A little over 85% (53 out of 62) of the reef sites around the Semporna peninsula harbour mushroom corals inhabited by gall crabs. Non-inhabited sites can be related to disturbances. Future studies will tell more about their diversity and the evolution of their host specificity (van der Meij, in preparation).

Acknowledgements

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Reproductive morphology

Chapter 15

Female reproductive morphology of coral-inhabiting gall crabs (Crustacea: Decapoda: Brachyura: Cryptochiridae)

Juliane Vehof, Sancia E.T. van der Meij, Michael Türkay & Carola Becker

Abstract

Gall crabs are obligate associates of stony corals in which they induce skeletal modifications. In some cryptochirid species, females live in open depressions accessible to males; while in others, females are rather isolated in semi-closed galls, which necessitates elaborate sperm storage capabilities by the female. In this study we investigate the female gross morphology and reproductive systems of *Fungicola syzygia* lodged in semiclosed flattened pits, *Opecarcinus cathyae* with semiopen pits and *Pseudocryptochirus viridis* from shallow open depressions using line drawings and histological methods. The general morphology of the cryptochirids' reproductive systems is uniform and conforms to other thoracotreme brachyurans: paired muscular vaginae of the concave pattern lead from the sternal gonopores into paired seminal receptacles where sperm is stored. The seminal receptacle is internally lined by distinct types of epithelia: a cuticle underlined by a columnar epithelium ventrally, a monolayered secretory epithelium dorsally and a multilayered transfer tissue where the oviducts enter the seminal receptacle. In all studied specimens, the seminal receptacle contained free spermatozoa; however, in specimens of *Pseudocryptochirus viridis* it also contained spermatophores, indicating a recent insemination. In contrast to most other brachyurans ovaries of the investigated cryptochirids extend into the pleon. The specific degree of ovary extension differs between the studied species and is closely related to female body shape.

Introduction

Gall crabs of the family Cryptochiridae are minute-sized obligate associates of stony corals, in which they induce skeletal modifications. These modifications are used as dwellings (see Fig. 1). Cryptochirids show a certain level of host specificity, insofar that each gall crab species is restricted to a specific group of corals. Distinct types of dwellings are recognized such as shallow depressions (Fize and Serène, 1957; van der Meij, 2012; Wei *et al.*, 2013; Fig. 1I, J), cylindrical pits (Fize and Serène, 1957; Simon-Blecher and Achituv, 1997; Wei *et al.*, 2013) and more or less closed galls (Potts, 1915; Fize and Serène, 1957; Wei *et al.*, 2013). Due to their small size and their hidden lifestyle within the coral skeleton, gall crabs are one of the least studied eubrachyuran groups (Potts, 1915; Kotb and Hartnoll, 2002). The actual impact on the coral host by the gall crab is not determined, and the characterization of cryptochirids as either commensals or parasites is debated. Furthermore, their mode of feeding is still under discussion (see Potts, 1915; Kropp, 1986; Castro, 1988; Abelson *et al.*, 1991; Simon-Blecher and Archituv, 1997); however, the obligate character of the association is undisputed.

Morphologically, Cryptochiridae differ from other brachyurans in several aspects. The carapace, that is the cephalothorax, is elongated in most species and longer than wide in both sexes. Apart from that, a strong sexual dimorphism is recognized, with the sedentary females being considerably larger than the mobile males. Adult females have developed a large brood pouch where embryos mature until hatching (Fig. 1D, G). This so-called marsupium, formed by the abdomen [= pleon] (Potts, 1915), is a synapomorphy of the Cryptochiridae. The breeding female is sedentary and in some species completely isolated by the coral skeleton except for fine pores for water circulation (Potts, 1915). In other species, the female inhabits a pit that remains open so that free-living males can enter, and occasionally, males and females were found in adjoining pits on the same coral (McCain and Coles, 1979; Carricart-Ganivet *et al.*, 2004).

In several aspects, Cryptochiridae resemble Pinnotheridae, another symbiotic brachyuran group. Both families have a similar lifestyle: adult pinnotherid females live sedentary in their hosts (e.g. bivalves, tunicates) and have a strongly widened pleon to carry their large broods (Becker, 2010). Their reproductive investment (Hartnoll, 2006) and output (Hines, 1992) are very high compared to other brachyurans. Analogous to pinnotherids, the reproductive investment of cryptochirids is also considered high (Kotb and Hartnoll, 2002). No data, however, are so far available on their reproductive morphology. Herein, we investigate the female reproductive systems of *Fungicola syzygia* van der Meij, 2015, *Opecarcinus cathyae* van der Meij, 2014 and *Pseudocryptochirus viridis* Hiro, 1938, by histological methods, and present descriptions on their gross morphology. We incorporate these results with data obtained from live observations during fieldwork on species' ecology and cavity types to reveal possible reproductive strategies among Cryptochiridae.

Material and methods

Collection of specimens and fixation

Gall crabs were hand-collected by scuba diving on coral reefs in the Semporna district (eastern Sabah, Malaysia) in December 2010. The research area is described in detail in Waheed and Hoeksema (2013) and Van der Meij and Hoeksema (2013).

Corals were searched for galls and pits, photographed and subsequently split with hammer and chisel. The following gall crab species were included in this study: one male and four females (three ovigerous) of *Fungicola syzygia* collected from Pom Pom Isl. (04°35”29.8’N 118°51”43.1’E)

in *Pleuractis paumotensis* (Stutchbury, 1833) from 0 to 10 m depth; five ovigerous females of *Opecarcinus cathyae* collected at Creach Reef (04°18”58.8’N 118°36”17.3’E) in *Pavona clavus* (Dana, 1846) from 10 to 14 m depth; one male and four females (two ovigerous) of *Pseudocryptochirus viridis* collected from Bakungan Isl. (04°45”11.1’N 118°29”16.0’E) in *Turbinaria* sp. from ca. 15 m depth. Whole specimens were fixed in a mixture of formalin, acetic acid, mercuric chloride and trichloroacetic acid (‘Susa Heidenhain’ after Romeis, 1989), washed with 100%, 90% and 80% ethanol for two hours each and subsequently stored in 70% ethanol. Samples and slides are stored at Senckenberg Research Institute in Frankfurt. Further gall crab specimens belonging to the same series were fixed in 80% ethanol and are stored in the collections of the Naturalis Biodiversity Center in Leiden, the Netherlands (collection coded as RMNH.Crus.D).

Gross morphology and histology

Line drawings were prepared with the help of a stereo microscope Leica MZ8 equipped with a camera lucida and subsequently digitized using Adobe Illustrator and Photoshop (after Coleman, 2003).

For decalcification, samples were incubated in 20% EDTA (ethylenediaminetetraacetic acid) for 72 h. In a graded ascending series of ethanol with steps of 80%, 90%, 96% and 100% (p. A.) ethanol (each step for 2 h), samples were dehydrated and then infiltrated with paraffin overnight (Leica TP1020 Histokinette). Subsequently, samples were embedded in paraffin blocks, and histological sections were prepared with a Leica RM2165 microtome at 6-8 μm. A trichromatic Masson–Goldner staining ‘light green’ (after Romeis, 1989), performed with a Leica Autostainer XL, was used for general tissue differentiation. Covered slides were studied and photographed with a Zeiss microscope equipped with a digital camera (CamScan Prog Res).

Results

Species and gall types

Field pictures of gall crabs inside their hosts and line drawings of each species with its specific gall type are presented in Fig. 1A-L.

Fungicola syzygia is associated with fungiid corals (Fungiidae) and lives in flattened pits similar to those of *F. fagei* (Fize and Serène, 1956). These pits have a very small ‘slit-like’ opening and are lodged between the septae of the corals (Fig. 1A, B). Mature females cannot leave the galls, while males are sometimes found in separate pits on the same coral or holding onto the septae, without a gall.

The studied specimens of *Opecarcinus cathyae* were collected from *P. clavus* (Agariciidae). The pit has a crescent shaped opening, which allows some movement by the crabs (Fig. 1E, F). Crabs were not observed to freely move around on the coral. *Pavona clavus* can occur in large monospecific stands housing many individuals, both males and females, of *O. cathyae* (Hoeksema and van der Meij, 2013).

Pseudocryptochirus viridis is associated with *Turbinaria* sp. (Dendrophylliidae), and often a large coral houses many males and females, each in their own pit. The pits shown in Fig. 1I and J are shallow crescent-shaped depressions, which allow full movement for both male and female, nevertheless, moving crabs were only observed when disturbed.

Gross morphology

The studied species have a body form typical for cryptochirids with a cephalothorax that is longer than wide, short walking legs and chelae equal in size (Fig. 1). The cephalothorax is extremely

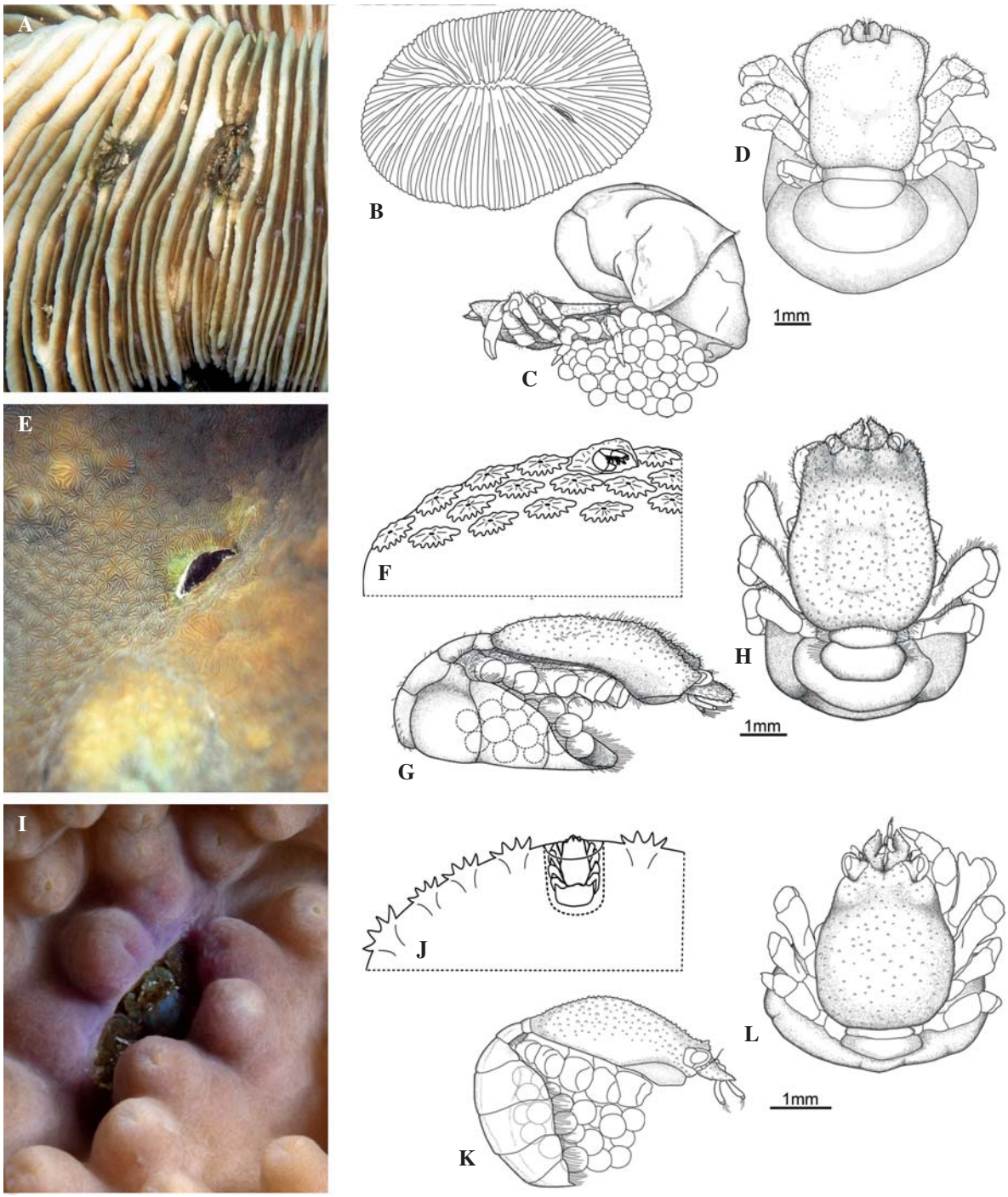

Fig. 1. Morphology of the investigated gall crab females and their different gall types **A-D**, *Fungicola syzygia*; **E-H**, *Opecarcinus cathyae*; **I-L**, *Pseudocryptochirus viridis*. **A**, opening of flattened pit including a specimen of *F. syzygia* in *Pleuractis paumotensis*, with only the middle part of the carapace front visible. Photographed by BW Hoeksema; **B**, Schematic drawing of a mushroom coral with a pit lodged between the septae; **C**, Lateral view of *F. syzygia*, the rectangular cephalothorax of this species is extremely flattened, the marsupium of this ovigerous female is turned to the outside by the rough fixation process; **D**, Dorsal view of *F. syzygia* with a well- developed and regular-formed marsupium; **E**, Gall with an overhang and a crescent-shaped opening, induced by *O. cathyae*, on a large colony of *Pavona clavus*. Photographed by SET van der Meij; **F**, Schematic drawing of a gall with overhang; **G**, Lateral view of an ovigerous female of *O. cathyae;* **H**, dorsal view of *O. cathyae;* **I**, Opening of a shallow crescent-shaped depression including a specimen of *P. viridis* in *Turbinaria* sp., the anterior part of carapace and front parts of the chelae are visible. Photographed by BT Reijnen/SET van der Meij; **J**, Schematic longitudinal section of a typical pit; **K**, Lateral view of an ovigerous female of *P. viridis;* **L**, Dorsal view of a female of *P. viridis*.

flattened in *F. syzygia* (Fig. 1C, D), in contrast to the more bulbous form with a anteriorly deflected part in *O. cathyae* (Fig. 1G, H)and *P. viridis* (Fig. 1K, L).The marsupium is built by the strongly widened pleon, which is developed in all studied specimens irrespective of whether females were ovigerous or not. In *F. syzygia* (Fig. 1C, D), the marsupium is more voluminous than in *O. cathyae* (Fig. 1G, H) and *P. viridis* (Fig. 1K, L) so that the walking legs of the female can barely reach ground.

Morphology of the female reproductive system

Overview

The general morphology of the female reproductive system in cryptochirids conforms to other eubrachyuran crabs. Paired sternal gonopores (vulvae) lead via cuticular vaginae into the likewise paired sac-shaped, oval to round storage organs: the seminal receptacles (Figs 2, 3A). Both seminal receptacles are connected to the ovaries of each body side – left and right through an oviduct. The receptacle is coated on the outside by connective tissue (Fig. 3A, B); internally, it is lined by three different types of epithelia: a monolayered columnar epithelium with an overlying cuticle ventrally that is continuous with the also cuticular vagina, a monolayered glandular epithelium dorsally (Fig. 3B-D) and a multilayered tissue where the oviduct runs into the seminal receptacle (Fig. 3E, F). Species differed, however, from each other in histological details of the secretory epithelia, in the seminal receptacle content (secretion and male sperm) and in the location and extension of the ovaries.

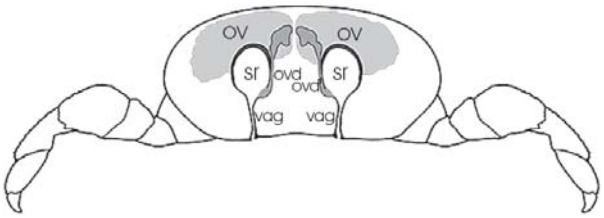

Fig. 2. Schematic transverse section through the cephalothorax of a female gall crab. Oocytes are produced by the ovary consisting of several lobes whose extension could differ around the light grey area. During spawning, the oviduct leads mature oocytes from the ovary lobes into the seminal receptacles, and subsequently, they leave the body through the vaginae. ov, ovary; ovd, oviduct; sr, seminal receptacle; vag, vagina.

Vaginae

The paired vaginae are cuticle-lined narrowed ducts running perpendicularly from the sternal gonopores into the also paired seminal receptacles (Fig. 2, 3A). The vagina lumen is crescent-shaped in transverse sections ('concave pattern' sensu Hartnoll, 1968) because the lateral half of its wall ('inner vagina wall') is longitudinally folded into the mesial side ('outer vagina wall') (Fig. 3G). The inner vagina wall (Fig. 3H) exhibits strong musculature running to the sternum.

Seminal receptacle and secretion

Monolayered glandular epithelium

The epithelium lining the seminal receptacle dorsally is monolayered and produces secretions into the lumen of the seminal receptacle. Slight differences are present in these dorsal epithelia among the investigated species. In *O. cathyae* the glandular epithelium is composed of slim elongated cells that are irregularly shaped and apically lined by microvilli (Fig. 3B). Rounded nuclei are located in the cells basally; some cells have several nuclei (Fig. 3B). Many secretions are present nearby the epithelium (Fig. 3B, E). In *F. syzygia* (Fig. 3C), the cells of the epithelium are comparatively smaller than those in *O. cathyae* so that the nuclei occupy almost the entire cells, with plenty secretions nearby as in *O. cathyae*. The surface of the epithelium of *P. viridis* is regular and

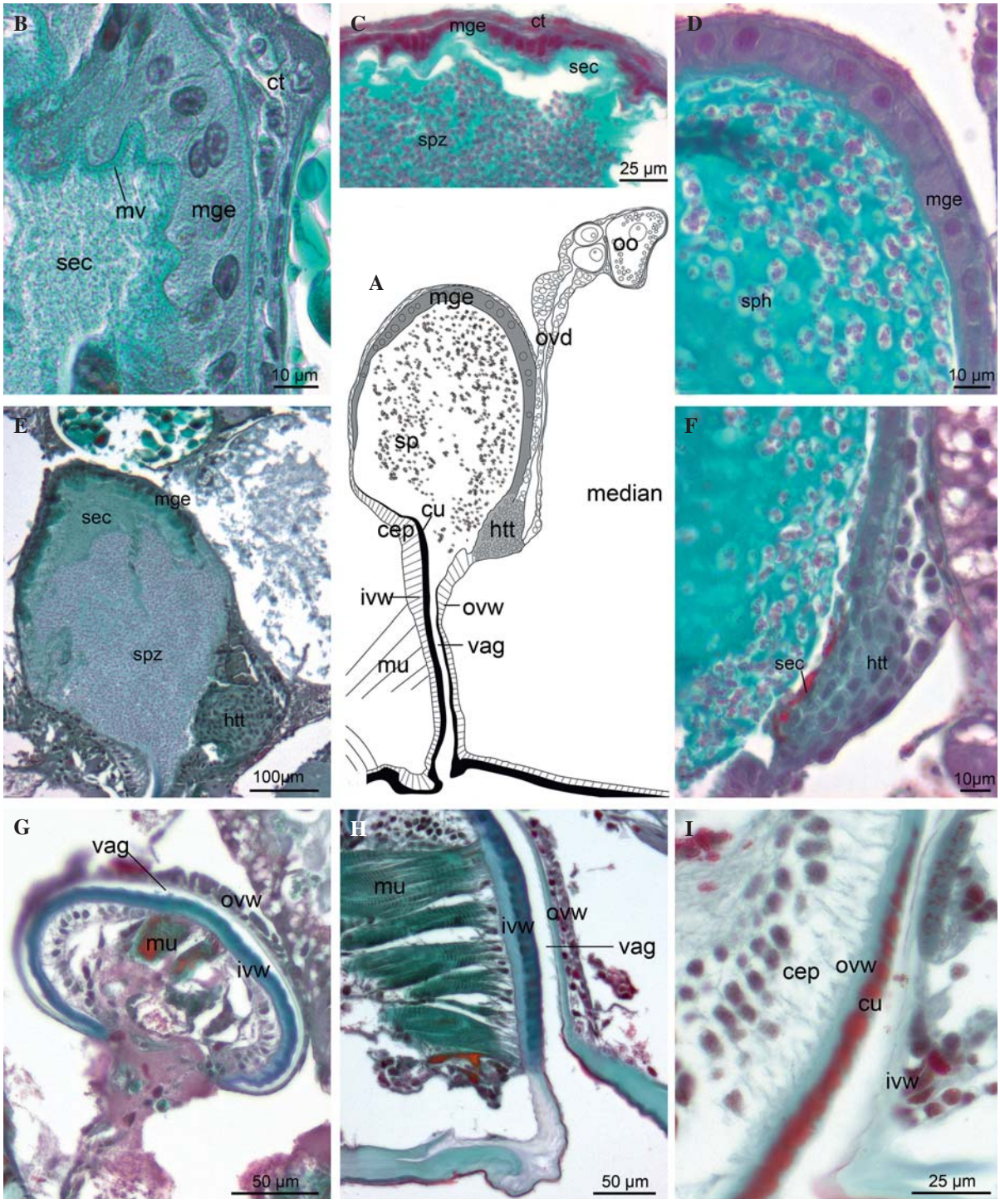

Fig. 3. Schematic view of seminal receptacle and vagina of Cryptochiridae with histological details; **A**, schematic drawing of seminal receptacle and vagina, main parts of the seminal receptacle are lined by glandular epithelium; **B**, Glandular epithelium of *Opecarcinus cathyae*, apical parts of the cells with microvilli seem; **C**, Glandular epithelium of *Fungicola syzygia;* **D***,* Glandular epithelium of *Pseudocryptochirus viridis*; **E**, Ventral localisation of the orifice of oviduct in *O. cathyae;* **F**, Details of the transfer tissue at the orifice of oviduct with orange staining secretion in *P. viridis;* **G**, Vagina of *P. viridis*, crescent-shaped in transverse sections; **H**, Longitudinal section through the vagina of *O. cathyae*, musculature inserts at the inner vagina wall; **I**, The cuticle of the flexible muscular vagina wall differs from the general cuticle and stains orange to red in trichromatic Masson-Goldner staining, well visible here in *F. syzygia* cep, cuticular epithelium; cu, cuticle; ct, connective tissue; htt, holocrine transfer tissue; ivw, inner vagina wall; mge, monolayered glandular epithelium; mu, musculature; mv, microvilli; ovw, outer vagina wall; sec, secretion; sph, spermatophore; spz, spermatozoa; vag, vagina.

rather even, consisting of square-shaped cells, while fluids are homogenously distributed in the seminal receptacle (Fig. 3D).

Secretory transfer tissue
At the junction with the seminal receptacles, the oviduct runs into a multilayered tissue that can protrude into the lumen of the receptacle (Fig. 3E, F). Because of its position at the location where oocytes are transferred from the ovary through the oviduct into the seminal receptacle, this tissue is referred to as 'transfer tissue'. Its cells are irregularly shaped and oriented. Towards the lumen of the seminal receptacle, cells flatten and their plasma becomes increasingly dense; in the periphery, cells are shed as secretion. In the applied Masson-Goldner staining, these secretions stain different from the ones of the dorsal epithelium (Fig. 3F). The transfer tissue is histologically uniform among the investigated species, but differs slightly in the extension towards the lumen of the seminal receptacle.

Seminal receptacle content
All the studied females (ovigerous and non-ovigerous) had their seminal receptacles filled with spermatozoa, either free or enclosed in spermatophores (Fig. 4A-D). The amount of sperm present within the seminal receptacle varied slightly and, therefore, the size of the seminal receptacle – sometimes even among the right and left seminal receptacle within one specimen. In *F. syzygia* and *O. cathyae* spermatozoa were free (Fig. 4A, B). In *O. cathyae* spermatozoa and fluids were evenly distributed throughout the seminal receptacle and also present close to the vagina. In contrast to this, the sperm mass was concentrated in the dorsal part of the seminal receptacle in *F. syzygia*, while the ventral part around the vagina was free of spermatozoa (Fig. 4B). Only in *P. viridis* were the spermatozoa still enclosed within spermatophores in all studied specimens (Fig. 4C, D).

Ovary
The ovaries of the investigated cryptochirids species, consisted of several lobes, are not restricted to the cephalothorax but extend into the pleon in various degrees. In the ovary, mature oocytes were present next to previtellogenic stages in all three species. In *O. cathyae* and *P. viridis*, ovaries contained mature oocytes centrally in the cephalothorax (Fig. 5A) and in the pleon; while in *F. syzygia*, the main part of the ovary was located in the pleon (Fig. 5B) and reached beyond the sixth pleon segment In histological sections of the cephalothorax, we identified only a few immature oocytes. The different developmental stages of oocytes were best detectable in the ovary of *F. syzygia* (Fig. 5C). In germinative zones, shown in Fig. 5C, germ cells proliferate by mitosis and develop into oogonia (Fig. 5D). The germinative zones are surrounded by maturation zones in which oogonia undergo the following stages of vitellogenesis. Most of the ovary is filled with mature oocytes in their final stage of vitellogenesis (Fig. 5C).

Discussion

Overview and systematic position
The main characteristics of the female reproductive systems (a concave vagina, the general construction of the seminal receptacle, its ventral connection with the oviduct, and the types and localisation of epithelia) are uniform among the investigated gall crab species and conform to the reproductive systems of so far investigated representatives of thoracotreme brachyurans from the families Ocypodidae (Sant'Anna *et al.*, 2007; López-Greco *et al.*, 2009; Lautenschlager *et al.*,

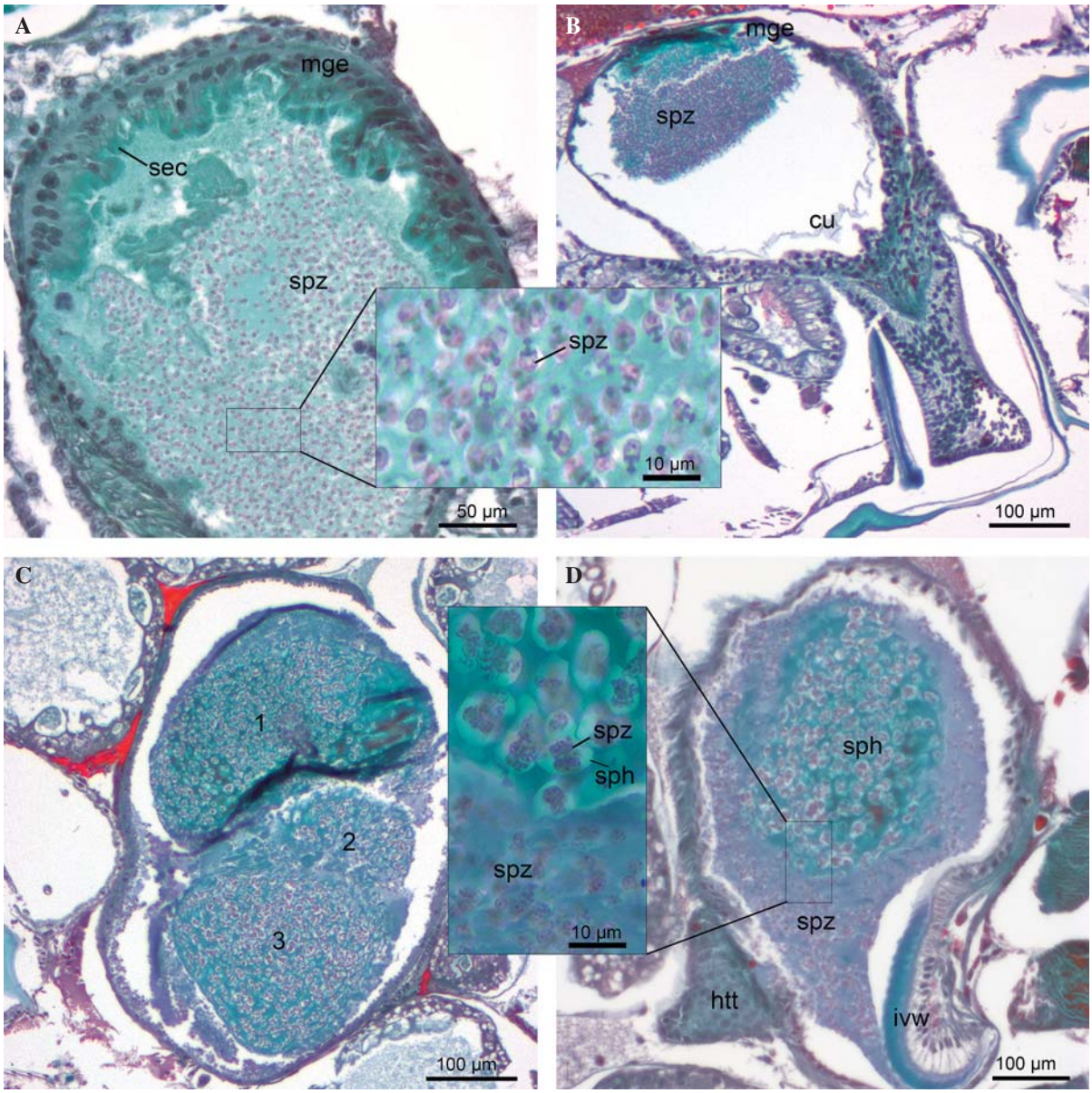

Fig. 4. Contents of seminal receptacles; **A**, Longitudinal section through seminal receptacle of *Opecarcinus cathyae* filled with free spermatozoa, male seminal plasma and secretions produced by the dorsal glandular epithelium; **B**, Longitudinal section through seminal receptacle of *Fungicola syzygia* with free spermatozoa in its dorsal region; **C**, Transversal section through seminal receptacle of *Pseudocryptochirus viridis* with sperm mass forming distinct portions; **D**, Transversal section through seminal receptacle of *P. viridis* with spermatophores centrally and free spermatozoa close to the periphery. cu, cuticle; htt, holocrine transfer tissue; ivw, inner vagina wall; mge, monolayered glandular epithelium; sph, spermatophore; spz, spermatozoa; vag, vagina.

2010), Varunidae (López-Greco *et al.*, 1999); Pinnotheridae (Becker *et al.*, 2011) and Gecarcinidae (de Souza *et al.*, 2013). Hence, a systematic position of the Cryptochiridae within the Thoracotremata as proposed by Ng *et al.* (2008), Wetzer *et al.* (2009) and Van der Meij and Schubart (2014) is supported by the female reproductive morphology.

Vagina and seminal receptacle

The cryptochirids' concave vagina which conforms to the 'concave pattern' sensu Hartnoll (1968) is characteristic for thoracotreme crabs and has been found in a number of thoracotreme crab

taxa, for example Varunidae (Lee and Yamazaki, 1990), Ocypodidae (Lautenschlager *et al.*, 2010), Pinnotheridae (Becker *et al.*, 2011) and Gecarcinidae (de Souza *et al.*, 2013). However, several 'advanced' heterotreme groups also have a vagina of the 'concave pattern', while heterotremes with many basal characters often have vaginae of the 'simple pattern' sensu Hartnoll (1968). These are simple, round ducts closed by a cuticle, wherein mating is often linked to moult cycles (Hartnoll, 1968). The vagina is closed to the outside by a convex bulge of the inner vagina wall, similar to the operculum described by Hartnoll (1968), and the width of the vagina lumen is actively controlled by the female's musculature. The histology of the cryptochirid concave vagina corresponds to those of *Uca* (Lautenschlager *et al.*, 2010) and Pinnotheridae (Becker *et al.*, 2011). The width of the vaginal lumen could be actively controlled by the female's musculature, and no structures were found that necessitate moulting before copulation. This musculature control might play an important role during copulation and oviposition.

Inside the seminal receptacle, the two types of epithelia, the monolayered glandular epithelium and the secretory transfer tissue, produce secretions, which are supposed to be involved in reproductive processes in a way that is not entirely understood (see below).

A specialized transfer tissue at the connection of the seminal receptacle with the oviduct was first described in *Eriocheir sinensis* H. Milne-Edwards, 1853 termed as 'valve-like tissue' (Lee and Yamazaki, 1990). The authors assumed that it has a barrier function inhibiting the intrusion of spermatozoa into oviducts and ovaries. Only at the time of ovulation, they observed an open passage in this tissue within the oviduct and seminal receptacle. Beyond a possible barrier function, the transfer tissue produces secretions. Based on its histological characteristics and structural similarities with the tissue in pinnotherids (Becker *et al.*, 2011), we assume a holocrine secretory mechanism in Cryptochiridae, which is characterized by the dissolving of whole cells into secretion (Plattner and Hentschel, 2006). Structurally similar glandular epithelia of the holocrine type are common in eubrachyuran seminal receptacles, for example in portunids (Johnson, 1980), cancrids (Jensen *et al.*, 1996) and majoids (Diesel, 1989; Benninger *et al.*, 1993). In these groups, multilayered holocrine glandular epithelia line the dorsal part of the seminal receptacles; while in cryptochirids and other thoracotremes, for example ocypodids (Lautenschlager *et al.*, 2010), pinnotherids (Becker *et al.*, 2011) and gecarcinids (de Souza *et al.*, 2013), the holocrine secretion is restricted to the transfer tissue at the connection to the oviduct. Whether the holocrine transfer tissue in the Thoracotremata might be homologous to the multilayered holocrine epithelia of the Heterotremata is currently uncertain.

The highly secretory non-holocrine glandular epithelium of the dorsal seminal receptacle is also present in other thoracotreme taxa, for example Ocypodidae (Sant'Anna *et al.*, 2007; López-Greco *et al.*, 2009; Lautenschlager *et al.*, 2010), Pinnotheridae (Becker *et al.*, 2011) and Gecarcinidae (de Souza *et al.*, 2013). Several possible functions, mostly based on studies of heterotreme crabs, have been proposed for these secretions in general: antibacterial activity (Benninger *et al.*, 1993), providing a milieu that allows the growth of useful bacteria (Jensen *et al.*, 1996), dehiscence of spermatophores (Adiyodi and Anilkumar, 1988; Diesel, 1989) or nourishment of spermatozoa inside the seminal receptacle (Anilkumar *et al.*, 1996). To what extent the secretions might contribute to the maintenance of sperm could not be investigated in this study. Involvement in the formation of a sperm plug that closes the female genital ducts after copulation (Bawab and El-Sherief, 1989) or in the dissolution of the sperm plug (Spalding, 1942) is not likely in cryptochirids, as sperm plugs were not present inside the vaginae and/ or seminal receptacles.

The presence of intact spermatophores inside the seminal receptacle of the *Pseudocryptochirus viridis* specimens suggests a recent insemination of the female as the permanence of transmitted spermatophores is usually short (Anilkumar *et al.*, 1999; Jennings *et al.*, 2000). Moreover,

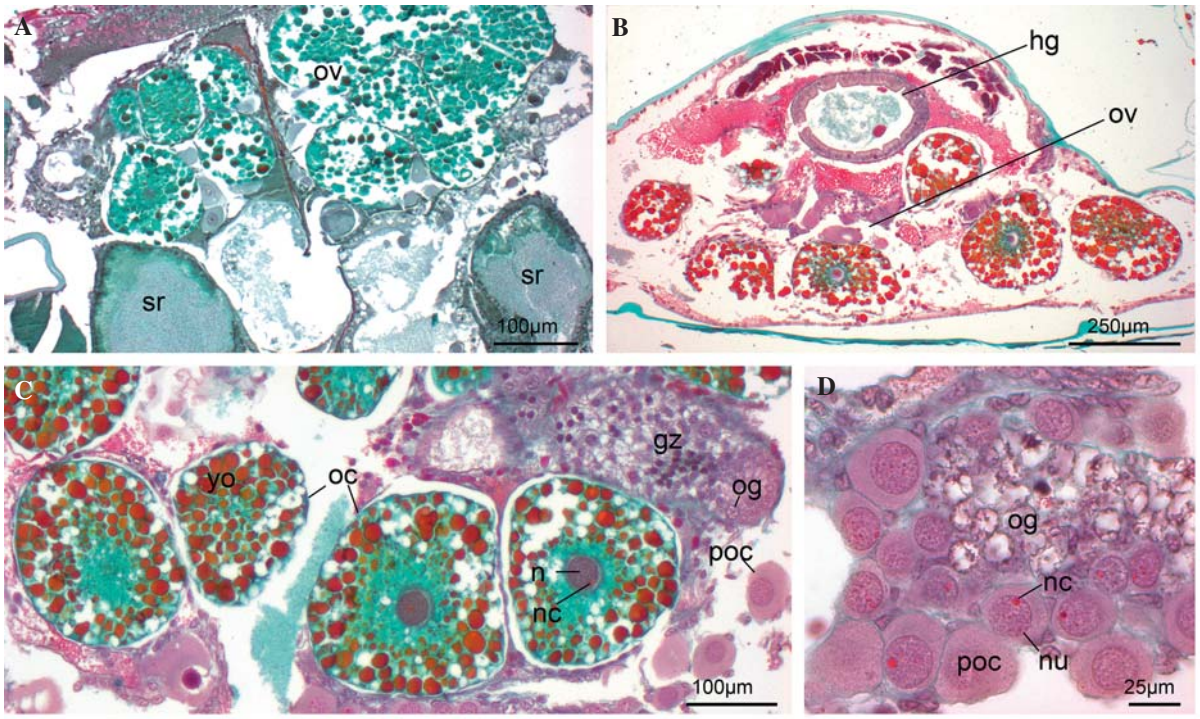

Fig. 5. Histology of the ovary; **A**, Transverse section through mature ovary of *Opecarcinus cathyae* within cephalothorax; **B**, Transverse section of ovary inside the pleon of *Fungicola syzygia* with mature oocytes ventrally to the hindgut; **C**, Section through ovary of *F. syzygia* showing germinative zone and mature oocytes (up to 200 µm in diameter) filled with yolk vesicles staining orange to red in Masson-Goldner staining; within mature oocytes the size of the yolk droplets increases from the centre of the cell to its periphery; **D**, Details of oogonia (about 15 µm in diameter) and previtellogenic oocytes (25-30 µm) which are present at the periphery of germinative zones. gz, germinative zone; hg, hindgut; nc, nucleolus; nu, nucleus; oc, oocyte; og, oogonia; ov, ovary; poc, previtellogenic oocyte; yo, yolk.

the sperm mass agglomeration (containing spermatophores) in several distinct clusters suggests recent multiple mating of the female. In contrast to that, in *Fungicola syzygia* and *Opecarcinus cathyae*, the seminal receptacle content was a homogenous mass of free spermatozoa with no indication of a recent insemination.

Ovaries and reproductive investment

A postoviposition regeneration of the ovary in ovigerous females or clearly detectable ovary developmental stages (see de Souza and Silva, 2009; Castilho-Westphal *et al.*, 2013) were not observed in the ovaries of cryptochirids. This supports the observation of Kotb and Hartnoll (2002) that brood production lacks seasonality in gall crabs, which is expected considering their tropical distribution.

The extension of the ovaries into the pleon is unusual among brachyuran crabs, in which ovaries are normally restricted to the cephalothorax (Adiyodi and Subramoniam, 1983; Krol *et al.*, 1992). Only few exceptions are known; for example, mature ovaries of *Cardisoma guanhumi* Latreille, 1828 and *Goniopsis cruentata* (Latreille, 1803) reach from the cephalothorax into the second or third pleomer, respectively (de Souza and Silva, 2009; de Souza *et al.*, 2013). Only in pinnotherids were ovaries found to extend into the last segments of the strongly broadened pleon (Becker *et al.*, 2011), as it is the case in the cryptochirid species investigated here. The extreme expansion of ovaries in pinnotherids in cephalothorax and pleon enables brood weights from 66% up to 97% of the female body weight (Hines, 1992). No data on brood weights are available for

the species investigated in the present study, but in the gall crab *Hapalocarcinus marsupialis* Stimpson, 1859 a similarly high reproductive investment has been shown: the brood weight is 59% of the female body weight (Kotb and Hartnoll, 2002). In comparison with free-living crabs with mean brood weights of 11-20%of the female body weight (Hines, 1992; Hartnoll, 2006), the reproductive investment of gall crabs is much higher than average. Just like pinnotherids, gall crabs benefit from the host-related lifestyle, for example in being protected against predators. However, this delimits body size and hence influences the potential space for yolk accumulation in the cephalothorax, which in turn influences the production of embryonic mass (Hines, 1992). At the same time, associated living animals (especially small ones) need a larger number of offspring, because the free-living larval life phase, including the search for a suitable host, is such a critical phase in their life cycle (Bush *et al.*, 2001). In cryptochirids, reproduction can be maximized at cost of mobility (marsupium), as females do not have to leave their dwelling.

Gall type and possible mating strategies

From the observations of the gall type in the field and the histological results, we conclude that the entrance of the crescent- shaped gall in *P. viridis* is wide enough to allow males to enter for repetitive mating, even with already ovigerous females. In this species, several females and males live together on a coral host, and therefore, repetitive mating and promiscuity are likely to occur (see 'visiting type' in Asakura, 2009). On the contrary, in *F. syzygia*, the entrance to the gall wherein the female lives is a narrow slit that is unlikely to allow the female to leave or a male to enter while an ovigerous female is inside. In this case, we suggest that copulation only takes place in a specific (early) life stage of the female when the pit is still accessible. Therefore, sperm storage over a longer period of time is crucial, and probably, several broods are fertilised by spermatozoa received in an earlier life stage. The gall crab *H. marsupialis* lives in semiclosed galls in corals of the family Pocilloporidae and may have a similar mating strategy: females of this species presumably copulate when the gall opening is still wide enough for males to enter. After the gall closes the female produces eight or more egg batches within the following 10 months (Kotb and Hartnoll, 2002).

Conclusions

The investigated species have a generally uniform reproductive system of the thoracotreme type and a high investment in reproduction apparent from the enlargement of ovaries extending into the pleon. In *Fungicola syzygia*, inhabiting narrow slit-like pits, the marsupium and the displacement of the ovaries was most developed with the broadened pleon seriously hampering locomotion of ovigerous females. According to our study, this is the species most adjusted to the symbiotic lifestyle and possibly isolated from mating as an adult, similar to *Hapalocarcinus marsupialis* (Kotb and Hartnoll, 2002). *Pseudocryptochirus viridis* and *Opecarcinus cathyae* living in open to (semi)open dwellings are seemingly less hampered in locomotion by the broadened pleon, and the ovaries in the pleon are less developed.

Only in *P. viridis*, with dwellings most accessible to males was a recent copulation confirmed. According to our results, cryptochirids might have different mating strategies from each other, and knowledge of the specific gall/pit type and the coral host is crucial for understanding reproduction in gall crabs.

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Summary

Gall crabs (Brachyura: Thoracotremata: Cryptochiridae) are small, coral-dwelling crabs that live in obligate association with their host corals, on which they rely for food and shelter. They commonly occur on coral reefs, but are often overlooked because of their diminutive size and hidden lifestyle. To date most research on gall crabs has focused on their systematics and taxonomy. This PhD thesis deals with various aspects of Cryptochiridae evolution and diversification. It is divided into five sections, each containing one or more chapters.

In the section **Phylogeny and taxonomy** the classification of the Cryptochiridae within the Thoracotremata is discussed first, based on 16S mtDNA sequences of 68 thoracotreme crab species, and sequence data obtained from 10 gall crab species belonging to nine genera. The monophyly of the Cryptochiridae is confirmed, which was debated by Wetzer *et al*. (2009). Data from additional molecular markers are needed to define the position of the Cryptochiridae within the Thoracotremata more precisely (**chapter 1**). In the following chapters several gall crab species are described as new to science. *Opecarcinus cathyae* is described from Indonesia and Malaysia, where it inhabits the coral species *Pavona bipartita* and *P. clavus,* both belonging to the Agariciidae (**chapter 2**). An endemic new species from the Red Sea and Oman is described as *Fizesereneia panda*; it inhabits the corals *Lobophyllia corymbosa*, *L. hemprichii* and *Symphyllia recta*, all belonging to the Lobophylliidae (**chapter 3**). *Lithoscaptus semperi* is described from Indonesia and Malaysia, where it lives in association with the free-living coral species *Trachyphyllia geoffroyi*, belonging to the Merulinidae (**chapter 4**). *Fungicola syzygia* is a cryptic new species closely related to *F. fagei*. This species was discovered by its host specificity in combination with molecular and morphological data. Both *Fungicola* species live in association with mushroom corals (Fungiidae). Based on morphology alone they are difficult to separate (**chapter 5**). In the last chapter of this section, a molecular clock approach is used to estimate the origin of the Cryptochiridae based on nucleotide substitution rates. The age of the most recent common ancestor is estimated at 50-23 Mya (early Eocene – early Miocene). Accelerated diversification is observed during the last 10-2 Mya (**chapter 6**).

The section **Host specificity and coevolution** starts with a study on the host associations of Atlantic gall crabs, resulting in eight newly recorded host corals. These new records include common Atlantic coral species like *Colpohyllia natans*, *Diploria labyrinthiformis*, and *Meandrina meandrites*. In addition, the gall crab *Kroppcarcinus siderastreicola* is newly recorded from the Caribbean Sea. An observation of the 'visiting' mating system is documented for the gall crab *Troglocarcinus corallicola*. In this mating system males of symbiotic crab species move from host to host in search of potential female mates (**chapter 7**). In **chapter 8** the host preferences and colour pattern of the Indo-Pacific species *Pseudocryptochirus viridis* are described, and new distribution records for Australia, Indonesia and Malaysia are presented. The evolution of the association between mushroom corals and their associated gall crabs is discussed in **chapter 9**. Based on museum collections, many new Indo-Pacific distribution records are documented. A phylogeny reconstruction of the Fungiidae is used to infer the evolution of crab-coral associations. These associations were tested for coevolutionary events in the programme Jane 4.0. This analysis reveals that cospeciation (in wide sense) is a likely scenario for the observed host specificity between mushroom corals and gall crabs. In **chapter 10** the coevolution scenario is tested on extended coral and gall crab phylogenies. Phylogeny reconstructions of the corals are based on studies already published in the literature, whereas the gall crab phylogeny is reconstructed by

using 16S, COI and histone H3 molecular markers. Coevolutionary analyses on the observed associations shows a wide range of cospeciation, host switching and duplication events between gall crabs and corals.

In the section **Biogeography**, the species *Neotroglocarcinus dawydoffi* is discussed, which shows (based on molecular data) an unforeseen biogeographic clustering between specimens from the Red Sea, eastern Indonesia, eastern Malaysia and New Caledonia. These groupings could not be explained by host specificity or cryptic speciation, and are not observed in closely related species. The observed patterns are therefore attributed to geographic isolation (**chapter 11**). The next chapter deals with the gall crab diversity in the Red Sea – a well-recognized biogeographic region of endemism – and uses gall crabs and their distribution in the Indo-Malayan region as a model. Marine biodiversity is highest in the latter area, however. The Red Sea is a secondary diversity centre with around 30% endemic species (**chapter 12**).

The section **Distributions over reefs and continental shelves** discusses the cross-shelf distribution of mushroom coral-associated gall crabs across the Spermonde shelf in Indonesia. Their occurrence rate is highest on mid- and offshore shelf zones. In addition, their host preference switches with varying depths, mostly when their common host is not present (**chapter 13**). The next chapter discusses the distribution of gall crabs in mushroom corals on reefs in Semporna, Malaysia. The results show that gall crabs commonly occur on non-disturbed reefs, without a clear preference for sheltered or exposed sites. Near-shore reefs that are impacted by natural or anthropogenic stress were not, or only sparsely, inhabited by gall crabs (**chapter 14**).

The last section of this PhD thesis is on **Reproductive morphology**. The female reproductive morphology is described for three gall crab species, using histological methods. The species have different dwelling morphologies, that range from shallow depressions to semi-closed pits. The results suggest that different gall crab species may have different reproductive strategies, which is possibly linked to the type of dwelling they inhabit. The results also confirm that, based on the female reproductive system, gall crabs should be classified within the Thoracotremata (**chapter 15**).

Nederlandse samenvatting

Galkrabben (Brachyura: Thoracotremata: Cryptochiridae) zijn kleine, koraalbewonende krabben die in een vaste associatie met koralen leven, waarvan ze afhankelijk zijn voor hun voedsel en onderkomen. Ze komen algemeen voor op riffen, maar worden vaak over het hoofd gezien vanwege hun geringe afmeting en verborgen leefwijze. Het meeste onderzoek aan galkrabben heeft zich gericht op hun systematiek en taxonomie. Dit proefschrift behandelt verschillende aspecten van de evolutie en diversificatie van Cryptochiridae. Het is verdeeld in vijf delen, die elk een of meer hoofdstukken bevatten.

In het deel **Fylogenie en taxonomie** wordt eerst de classificatie van de Cryptochiridae binnen de Thoracotremata besproken, op basis van 16S mtDNA sequenties van 68 thoracotreme krabbensoorten en sequenties van 10 galkrabbensoorten behorende tot negen genera. De monofylie van de Cryptochiridae wordt daarbij bevestigd, wat betwist werd door Wetzer *et al*. (2009). Gegevens van additionele moleculaire markers zijn nodig om de positie van de Cryptochiridae binnen de Thoracotremata nauwkeuriger te bepalen (**hoofdstuk 1**). In de volgende hoofdstukken worden diverse galkrabbensoorten als nieuw voor de wetenschap beschreven. *Opecarcinus cathyae* wordt beschreven van Indonesië en Maleisië, waar de koraalsoorten *Pavona bipartita* en *P. clavus* bewoond worden, die beide tot de Agariciidae behoren (**hoofdstuk 2**). Een endemische nieuwe soort voor de Rode Zee en Oman wordt beschreven als *Fizesereneia panda*; deze soort leeft in de koralen *Lobophyllia corymbosa*, *L. hemprichii* en *Symphyllia recta*, die tot de Lobophylliidae behoren (**hoofdstuk 3**). *Lithoscaptus semperi* wordt beschreven van Indonesië en Maleisië, waar de soort in associatie leeft met de vrijlevende koraalsoort *Trachyphyllia geoffroyi*, behorende tot de Merulinidae (**hoofdstuk 4**). *Fungicola syzygia* is een cryptische nieuwe soort, nauw verwant aan *F. fagei*. Deze soort werd ontdekt door haar gastheerspecificiteit in combinatie met moleculaire en morfologische gegevens. Beide *Fungicola* soorten leven in associatie met paddenstoelkoralen (Fungiidae). Enkel gebaseerd op morfologie zijn ze moeilijk van elkaar te onderscheiden (**hoofdstuk 5**). In het laatste hoofdstuk van dit deel wordt een moleculaire klok analyse gebruikt om de oorsprong van de Cryptochiridae te bepalen gebaseerd op *substitution rates* van nucleotiden. De leeftijd van de meest recente gemeenschappelijke voorouder wordt geschat op 50-23 Mya (vroeg Eoceen – vroeg Mioceen). In de laatste 10-2 Mya heeft zich een versnelde diversificatie voorgedaan (**hoofdstuk 6**).

Het deel **Gastheerspecificiteit en co-evolutie** begint met een onderzoek naar de gastheerassociaties van Atlantische galkrabben, wat resulteert in acht nieuwe gastheerkoralen. Deze nieuwe meldingen betreffen algemeen voorkomende Atlantische koraalsoorten, zoals *Colpophyllia natans, Diploria labyrinthiformis* en *Meandrina meandrites*. Daarnaast wordt de galkrab *Kroppcarcinus siderastreicola* voor het eerst gemeld uit de Caraïbische Zee. Een observatie van het '*visiting*' voortplantingssysteem is gedocumenteerd voor de galkrab *Troglocarcinus corallicola*. Hierbij verplaatsen mannetjes van symbiotische krabbensoorten zich tussen diverse gastheren op zoek naar een vrouwelijke partner (**hoofdstuk 7**). In **hoofdstuk 8** worden de gastheervoorkeuren en kleurpatronen van de Indo-Pacifische soort *Pseudocryptochirus viridis* beschreven, met nieuwe verspreidingsgegevens voor Australië, Indonesië en Maleisië. De evolutie van de associatie tussen paddenstoelkoralen en hun geassocieerde galkrabben wordt besproken in **hoofdstuk 9**. Gebaseerd op museumcollecties zijn vele nieuwe Indo-Pacifische verspreidingsgegevens voor de galkrabben verkregen. Een fylogeniereconstructie van de Fungiidae is gebruikt om de evolutie van de krab-koraalassociaties af te leiden. De geobserveerde associaties zijn getest voor co-evo-

lutionaire patronen in het programma Jane 4.0. Deze analyse laat zien dat cospeciatie (in de brede zin van het woord) een aannemelijk scenario is voor de waargenomen gastheerspecificiteit tussen paddenstoelkoralen en galkrabben. In **hoofdstuk 10** wordt het co-evolutiescenario getest op uitgebreide koraal- en galkrabfylogeniereconstructies. De reconstructie van de koraalfylogenie is gebaseerd op onderzoeken al gepubliceerd in de literatuur, terwijl de galkrabfylogenie gereconstrueerd is met behulp van 16S, COI en histone H3 merkers. Co-evolutionaire analyses van de uiteenlopende associaties brengen diverse vormen van 'co-evolutie' tussen galkrabben en koralen aan het licht, zoals cospeciatie, gastheerwisseling en duplicatie.

In het deel **Biogeografie** wordt de soort *Neotroglocarcinus dawydoffi* besproken, die een onvoorziene groepering laat zien (gebaseerd op moleculaire gegevens) tussen exemplaren uit de Rode Zee, oostelijk Indonesië, oostelijk Maleisië en Nieuw-Caledonië. Deze groepering kan niet worden uitgelegd door gastheerspecificiteit of cryptische soortvorming en wordt niet geobserveerd in nauwverwante soorten. De geobserveerde patronen worden daarom toegeschreven aan geografische isolatie (**hoofdstuk 11**). Het volgende hoofdstuk bespreekt de galkrabbendiversiteit in de Rode Zee – een welbekende biogeografische regio met een hoge mate van endemisme – en gebruikt galkrabben en hun verspreiding in de Indo-Maleisische regio als vergelijking voor de diversiteit. De mariene biodiversiteit is het hoogst in de Indo-Maleisische regio, terwijl de Rode Zee met ongeveer 30% endemische soorten als een secundair centrum van diversiteit wordt gezien (**hoofdstuk 12**).

Het deel **Verspreiding over riffen en continentale plateaus** behandelt de *cross-shelf* verspreiding van paddenstoelkoraalgeassocieerde galkrabben over het Spermonde plateau in Indonesië. De krabben zijn het talrijkst in de midden- en *offshore*-zones. Daarnaast blijkt de gastheervoorkeur van de krabben te wisselen afhankelijk van de diepte, vooral wanneer hun algemene gastheer niet aanwezig is (**hoofdstuk 13**). Het volgende hoofdstuk bespreekt de verspreiding van galkrabben in paddenstoelkoralen op riffen in Semporna, Maleisië. De resultaten laten zien dat galkrabben voornamelijk voorkomen op niet verstoorde riffen, maar geen duidelijke voorkeur hebben voor beschutte of onbeschutte locaties. Riffen vlakbij de kust, die onder invloed staan van natuurlijke of antropogene stressfactoren, waren niet of in lage aantallen bewoond door galkrabben (**hoofdstuk 14**).

Het laatste deel van dit proefschrift gaat over **Reproductieve morfologie**. De vrouwelijk reproductieve morfologie is beschreven voor drie galkrabbensoorten door middel van histologische technieken. De bestudeerde soorten hebben verschillende galvormen, variërend van ondiepe (open) tot halfgesloten structuren. De resultaten suggereren dat verschillende galkrabbensoorten mogelijk verschillende reproductieve strategieën hebben, wellicht gerelateerd aan het type gal dat ze bewonen. De resultaten bevestigen daarnaast dat, gebaseerd op het vrouwelijk reproductieve systeem, galkrabben tot de Thoracotremata gerekend moeten worden (**hoofdstuk 15**)

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My parents never questioned my decision to aspire a career in science, for which I am grateful. My friends were kind enough to always listen to my stories and support me.

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Curriculum vitae

Born in Groningen (The Netherlands), Sancia Esmeralda Theonilla van der Meij grew up in the land-locked province of Drenthe. She attended high school [Atheneum] in Groningen before moving to Costa Rica in 1999 to study Spanish, where she worked in a wide array of jobs and travelled all over Central America. After a travel companion suggested that she would probably enjoy diving, she obtained her first dive certificate at Utila (Islas de la Bahía, Honduras). A few years later, after obtaining her BSc in Leeuwarden at the Van Hall Institute, she moved to Leiden in 2004 to pursue her MSc degree in Biology at Leiden University with a focus on everything ocean-related.

Her first MSc internship was carried out at the NIOZ (Royal Netherlands Institute for Sea Research - Texel) on the diversity and distribution of cetaceans in the southern North Sea. She continued with a second MSc internship at Naturalis in Leiden on historical changes in the species composition of stony corals and molluscs in Jakarta Bay (Indonesia). Ever since, marine invertebrates of coral reefs have been a topic of her interest. Sancia graduated *cum laude* from Leiden University in 2007.

During her studies she worked as a freelance translator of movie subtitles for a variety of different genres, but mostly science fiction and (romantic) comedies. In addition, she worked as a museum host at Naturalis between 2006 and 2009. All of 2008 she worked at the secretariat of the Pelagic Regional Advisory Council, to prepare and provide advice on the management of pelagic fish stocks in European waters for the European Commission. Since 2008 she also held several (part-time) research, project management, and collections manager positions at Naturalis. She is the managing editor of the scientific journal *Contributions to Zoology* from 2009 onwards. In her spare time she worked on various scientific projects, mostly on gall crabs and/or corals, which eventually lead to the formal start of her PhD trajectory in 2012.

Over the years fieldwork was carried out at beautiful locations all over the world, including Australia, Curaçao, Indonesia, Malaysia, and the Red Sea, the results of which are included in the various chapters of this thesis.

Publications

Publications resulting from this thesis

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