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Barcoding reveals a remarkable genetic diversity of pontarachnid mites (Pontarachnidae, Hydrachnidia) in the Adriatic Sea, including the description of a new species from Slovenia

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ABSTRACT

The family Pontarachnidae is found worldwide, mainly in coastal marine habitats; however, the diversity (and especially the ecology) of this poorly studied and mostly neglected group of marine meiofauna still awaits discovery. In this study, we present data on Mediterranean pontarachnids based on extensive material from the Adriatic and Tyrrhenian seas. Up to now, only one sequence of a pontarachnid mite from the Mediterranean was present in GenBank; we are adding 81 new pontarachnid mite barcodes from these Mediterranean areas to the DNA barcode library. Our study, using the DNA barcode fragment of the mitochondrial *cytochrome c oxidase subunit I* (*COI*) gene, shows that the coast of Slovenia harbours at least six genetic lineages representing distinct Molecular Operational Taxonomic Units (MOTUs), corresponding with six species of pontarachnid mites (three *Litarachna* Walter, 1925 and three *Pontarachna*; Philippi, 1840). The material from the Adriatic Sea (Piran, Slovenia) also revealed a new species, *Litarachna cursusmaritima* sp. nov., clearly separated from the hitherto known species by *COI* and by a very conspicuous dorsal colour pattern. Finally, the finding of two species, *L. communis* and *P. punctulum,* each represented by two Barcode Index Numbers (BINs) with relatively high genetic distance between morphologically similar populations from the Adriatic and the Tyrrhenian Sea, clearly shows the necessity of finding and using new morphological characters, such as colour pattern and applying new markers such as nuclear genes, to reveal possible cryptic or pseudocryptic diversity in pontarachnid mites.

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Introduction

The family Pontarachnidae Koenike, 1910 comprises two genera, *Pontarachna* Philippi 1840 and *Litarachna* Walter 1925. The latter genus was split into two subgenera by Cook (1958), but the division was later withdrawn (Cook 1986).

Most of the known pontarachnid species inhabit the marine littoral zone (Smit 2003; Pešić et al. 2012; Chatterjee et al. 2019), although some have been recorded at depths of nearly 70 m (Pešić et al. 2012, 2014). In addition, there are also (very few) records from freshwater habitats in proximity to the sea (Cook 1986, 1996; Smit 2007; Pešić 2013).

The research on pontarachnid mites in the Mediterranean Sea has a long tradition, dating back to Philippi (1840), who described the first species, *Pontarachna punctulum* Philippi 1840, from the Gulf of Naples, Tyrrhenian Sea, Italy. To date, 10 species of pontarachnid mites have been reported from the Mediterranean Sea; nine were found in the eastern part, while four were reported from the western sector of the Mediterranean basin – some species occur in both sectors (Pešić et al. 2019). Furthermore, Pešić et al.

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(2019) provide a comprehensive overview of the diversity of Pontarachnidae in the Mediterranean Sea, including a morphological key to species identification.

A worldwide checklist of pontarachnid mites provided by Chatterjee et al. (2019) listed 53 species. Meanwhile, three more species have been described (Pešić et al. 2019; Montes-Ortiz et al. 2021). Hence, 31 species of the genus *Pontarachna* are currently known, while 25 belong to the genus *Litarachna*. Nearly 19% of these are found in the Mediterranean Sea, making the pontarachnid fauna of this region the second most well-known, after the tropical western Pacific (which houses 30% of all described species) (Chatterjee et al. 2019). However, most species are still known from only a few specimens from the type locality. Even though our knowledge of this family has improved in the last several years, we are still far from a clear picture of the distribution patterns (not to mention the ecology) of the individual species, as pontarachnids are still widely neglected in surveys of marine meiofauna (Pešić et al. 2019).

In recent years, the use of the DNA barcode fragment of the mitochondrial *cytochrome c oxidase subunit I* (*COI*) gene has been shown to have great potential in the identification of water mites, which led to the formation of public reference libraries of DNA barcodes of water mites (e.g. Pešić et al. 2017, 2022, 2023, 2024, 2025, 2025; Blattner et al. 2019; Carew et al. 2022). Pontarachnid mites are sparsely represented in the two major public databases, GenBank and BOLD; at the time of this study, GenBank contained only a single *COI* sequence for this group.

This study provides a first DNA barcode reference library for the pontarachnid fauna of the Mediterranean, laying the foundation for future taxonomic, ecological and biogeographical research on this neglected group of marine meiofauna.

Material and methods

The first and second authors collected 81 pontarachnid individuals in 2023 and 2024. Coastal samples were obtained by snorkelling or wading, using hand nets for sea grass and 500 mL Kautex bottles for algae.

Afterwards, the material was washed through a coarse (~2 mm mesh size) sieve into a fine net (250 µm mesh size) and sorted alive in the laboratory or on the spot. Specimens were picked up individually with fine tweezers, immediately preserved in 96% EtOH, and stored in a fridge for molecular analyses.

DNA barcode amplification and sequencing

DNA extraction was carried out at the Institute of Biology, University of Szczecin (IoB-UoS) using the GeneMATRIX Tissue DNA Purification Kit (EurX, Gdańsk, Poland) according to the manufacturer's instructions. The procedure was non-destructive (DNA was extracted from the whole specimens; exoskeletons remained intact), and vouchers were retained in 96% EtOH for later morphological study. We removed the exoskeletons from the DNA extract, and moved each to a new vial with 96% EtOH for further morphological examination. The *COI* region (Hebert et al. 2003) was amplified using the primer pair LCO1490-JJ and HCO2198-JJ (Astrin & Stüben 2008), each attributed with 9 bp tags (Srivathsan et al. 2024). Primer tags were generated with Barcode Generator (Comai & Howell 2012), following the guidelines in Srivathsan et al. (2019, 2021). Polymerase Chain Reaction (PCR) amplification was performed using the following thermal cycling profile: an initial denaturation at 95°C for 5 min; 5 cycles of 95°C for 50 s, 45°C for 50 s, and 72°C for 60 s; followed by 35 cycles of 95°C for 50 s, 51°C for 50 s, and 72°C for 60 s; and a final extension at 72°C for 2 min. A subset of PCR products was visualized on a 1% agarose gel to verify successful amplification. From each PCR product, 5 µL were pooled into a single 1.5 mL Eppendorf tube. The pool was purified using Sera-Mag™ Select (Cytiva Life Sciences) and checked on a 1% agarose gel to confirm effective cleanup. The final DNA concentration of the pool was measured using a Qubit 4 Fluorometer with the Qubit dsDNA HS Assay Kit (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA). The sequencing library was prepared with the Oxford Nanopore Ligation Sequencing Kit v. 14 (SQK-LSK114) according to the manufacturer's instructions and sequenced on a Flongle flow cell (R10.4.1; FLOFLG114). Demultiplexing and DNA barcode generation were conducted using ONT Barcoder 2.0 (Srivathsan et al. 2024) at the University of Lodz (UniLodz), Łódź, Poland.

DNA data assembly and barcode analyses

The newly obtained sequences were edited, aligned with MUSCLE (Edgar 2004), and made available in the Barcode of Life Data Systems (BOLD, Ratnasingham & Hebert 2007). Relevant voucher information, and recently generated DNA barcodes are publicly accessible through the Dataset Contribution to the knowledge on DNA barcodes of pontarachnid mites of the Mediterranean (DS-MEDPO; dx.doi.org/10.5883/DS-MEDPO) in BOLD. Four sequences from France, produced by Harry Smit (so far unpublished), and one obtained from GenBank were added to the dataset (details in Table 1).

The haplotype relationship was visualized through median-joining networks (MJN) using PopART (Leigh & Bryant 2015). Networks were prepared separately for *Pontarachna punctulum* and *Litarachna communis*.

Intra- and interspecific genetic distances were calculated using the Kimura 2-parameter (K2P) model (Kimura 1980) in MEGA X (Kumar et al. 2018). We assigned sequences to groups based on species delimitation results. The latter software was used to produce a maximum likelihood (ML) tree (model selected using the Bayesian information criterion (BIC) implemented in MEGA X: T92 + G for *Pontarachna*, HKY+I for *Litarachna*, TN93 + G + I for supplementary data tree) with an initial neighbour-joining (NJ) tree and using the SubtreePruning-Regrafting – Extensive heuristic search (SPR level 5). Support for tree branches was calculated using the nonparametric bootstrap method (Felsenstein 1985) with 1000 replicates and is shown next to the branches. Codon positions included were 1st + 2nd + 3rd + Noncoding. To root the supplementary tree we used three *Unionicola* spp. COI sequences available from GenBank and BOLD (NC_011036, FJ218017, MARBN1314-23).

Species delimitation

We applied three species delimitation methods representing two methodological approaches. First, we used two distance-based approaches. The Barcode Index Number (BIN) system (Ratnasingham & Hebert 2013), implemented in BOLD clusters, assigns both newly submitted and existing *COI* sequences to operational units based on genetic distance. We then applied Assemble Species by Automatic Partitioning (ASAP; Puillandre et al. 2021), a hierarchical clustering algorithm specifically developed for species partitioning based on pairwise distance distributions. We used the online ASAP version (https://bioinfo.mnhn.fr/abi/public/asap/asapweb.html) with default settings and the K2P distance model.

For the phylogeny-based approach, we used the Bayesian implementation of the multi-rate Poisson Tree Processes (PTP) model (mPTP; Kapli et al. 2017) available at https://mcmc-mptp.h-its.org/mcmc/. This method uses Markov Chain Monte Carlo (MCMC) sampling to provide a fast, robust assessment of species boundaries. ML trees generated in MEGA X (Kumar et al. 2018) were used as input for the mPTP analyses.

Delimitation methods were applied independently for *Pontarachna* and *Litarachna* alignments.

Morphology

After DNA extraction, the analysed specimens were stored in 96% EtOH and morphologically examined (81 vouchers of pontarachnid mites from Slovenia ($n = 24$), Italy ($n = 53$) and France ($n = 4$)). Morphological nomenclature follows Wiles et al. (2002) and Pešić et al. (2019). Some specimens were dissected and slide-mounted in Faure's medium, while the rest were transferred to Koenike's fluid.

The following abbreviations are used: Cx-I to -IV – first to fourth coxae; Cxgl-4 – coxoglandulare 4 (E4 according to Wiles et al. 2002); dL – dorsal length; H – height; I-leg-1 to –6 – first to sixth segments of first leg; L – length; *p*-1 to -5 – palp segments 1 to 5; W – width. All measurements are given in µm.

Microphotographs (Figure 12A–M) were taken with a Nikon V1 camera connected to a Leica Z16 APO and a 2.0× objective. Images were stacked using Helicon Focus 5.3. Further photos (Figure 12N–R) were taken with an Olympus TG4 camera.

Results

In this study, DNA barcodes of 81 specimens of pontarachnid mites from Slovenia ($n = 24$), Italy ($n = 53$) and France ($n = 4$), were generated (Table 1). Together with one sequence from GenBank (KM101025, France), they are morphologically assigned to six species. The genus *Pontarachna* (22 sequences) is represented by

Table 1. Details on the newly DNA barcoded specimens, including localities and coordinates of sampling sites, sample codes and the barcode index number codes. BOLD data presented here was last accessed on 20 June 2025. The sequence downloaded from GenBank is indicated by an asterisk.

Locality	Coordinates	Specimen ID	BOLD/GenBank IDs
Litarachna communis **BOLD: ADD6045**			
Italy, Sardinia	39°17′52.7″N, 9°37′09.8″E	K80_2	HYDPT002-24/PX634135
Italy, Sardinia	39°17′52.7″N, 9°37′09.8″E	K80_3	HYDPT003-24/PX634110
Italy, Sardinia	39°17′52.7″N, 9°37′09.8″E	K80_4	HYDPT004-24/PX634085
Italy, Sardinia	39°17′52.7″N, 9°37′09.8″E	K80_5	HYDPT005-24/PX634068
Italy, Sardinia	39°17′52.7″N, 9°37′09.8″E	K80_6	HYDPT006-24/PX634101
Italy, Sardinia	39°17′54.3″N, 9°37′18.5″E	K80_7	HYDPT007-24/PX634109
Italy, Sardinia	39°17′54.3″N, 9°37′18.5″E	K80_8	HYDPT008-24/PX634098
Italy, Sardinia	39°18′41.0″N, 9°36′49.1″E	K80_10	HYDPT010-24/PX634102
Italy, Sardinia	39°18′41.0″N, 9°36′49.1″E	K80_11	HYDPT011-24/PX634117
Italy, Sardinia	39°18′41.0″N, 9°36′49.1″E	K80_12	HYDPT012-24/PX634104
Italy, Sardinia	39°18′55.4″N, 9°36′29.6″E	K80_13	HYDPT013-24/PX634072
Italy, Sardinia	39°18′55.4″N, 9°36′29.6″E	K80_14	HYDPT014-24/PX634132
Italy, Sardinia	39°18′55.4″N, 9°36′29.6″E	K80_15	HYDPT015-24/PX634144
Italy, Sardinia	39°18′55.4″N, 9°36′29.6″E	K80_16	HYDPT016-24/PX634124
Italy, Sardinia	39°18′55.4″N, 9°36′29.6″E	K80_17	HYDPT017-24/PX634086
Italy, Sardinia	39°18′55.4″N, 9°36′29.6″E	K80_18	HYDPT018-24/PX634075
Italy, Sardinia	39°18′55.4″N, 9°36′29.6″E	K80_19	HYDPT019-24/PX634148
Italy, Sardinia	39°17′49.1″N, 9°37′00.7″E	K80_20	HYDPT020-24/PX634128
Italy, Sardinia	39°17′49.1″N, 9°37′00.7″E	K80_22	HYDPT022-24/PX634141
Italy, Sardinia	39°17′49.1″N, 9°37′00.7″E	K80_24	HYDPT024-24/PX634103
Italy, Sardinia	39°17′49.1″N, 9°37′00.7″E	K80_25	HYDPT025-24/PX634107
Italy, Sardinia	39°17′49.1″N, 9°37′00.7″E	K80_26	HYDPT026-24/PX634081
Italy, Sardinia	39°21′50.7″N, 9°36′05.4″E	K80_27	HYDPT027-24/PX634070
Italy, Sardinia	39°21′50.7″N, 9°36′05.4″E	K80_28	HYDPT028-24/PX634119
Italy, Sardinia	39°21′50.7″N, 9°36′05.4″E	K80_29	HYDPT029-24/PX634111
Italy, Sardinia	39°21′50.7″N, 9°36′05.4″E	K80_31	HYDPT031-24/PX634146
Italy, Sicily	37°29′25.5″N, 13°10′18.8″E	K82_38	HYDPT134-24/PX634113
Italy, Sicily	37°29′25.5″N, 13°10′18.8″E	K82_39	HYDPT135-24/PX634130
Italy, Sicily	37°29′26.1″N, 13°10′19.3″E	K82_40	HYDPT136-24/PX634091
Italy, Sicily	37°29′26.1″N, 13°10′19.3″E	K82_42	HYDPT138-24/PX634116
Italy, Sicily	37°29′26.1″N, 13°10′19.3″E	K82_43	HYDPT139-24/PX634105
Italy, Sicily	37°29′26.1″N, 13°10′19.3″E	K82_46	HYDPT142-24/PX634083
Italy, Sicily	37°29′26.1″N, 13°10′19.3″E	K82_47	HYDPT143-24/PX634087
Italy, Sicily	37°29′26.1″N, 13°10′19.3″E	K83_2	HYDPT146-24/PX634096
Italy, Sicily	37°17′23.7″N, 13°28′21.7″E	K83_19	HYDPT163-24/PX634069
Italy, Sicily	37°17′23.7″N, 13°28′21.7″E	K83_20	HYDPT164-24/PX634120
Italy, Sicily	37°17′23.7″N, 13°28′21.7″E	K83_21	HYDPT165-24/PX634077
Italy, Sicily	37°17′23.7″N, 13°28′21.7″E	K83_22	HYDPT166-24/PX634134
France	43°11′32.09″N, 6°39′10.37″E	OSAL 0014405	KM101025*
France, Corsica	42°35′16.5″N, 8°48′23.2″E	RMNH.5070758	NLACA1000-17/PX634129
France, Corsica	42°35′16.5″N, 8°48′23.2″E	RMNH.5070756	NLACA998-17/PX634099
Litarachna communis **AGH8517 (Slovenia)**			
Slovenia	45°31′12.0″N, 13°34′01.0″E	K80_34	HYDPT034-24/PX634123
Slovenia	45°31′12.0″N, 13°34′01.0″E	K80_35	HYDPT035-24/PX634126
Slovenia	45°31′12.0″N, 13°34′01.0″E	K80_36	HYDPT036-24/PX634089
Slovenia	45°31′12.0″N, 13°34′01.0″E	K80_37	HYDPT037-24/PX634122
Slovenia	45°31′12.0″N, 13°34′01.0″E	K80_43	HYDPT043-24/PX634084
Slovenia	45°31′12.0″N, 13°34′01.0″E	K80_44	HYDPT044-24/PX634140
Slovenia	45°31′12.0″N, 13°34′01.0″E	K80_46	HYDPT046-24/PX634121
Slovenia	45°31′38.5″N, 13°34′00.9″E	K81_29	HYDPT077-24/PX634097
Slovenia	45°31′38.5″N, 13°34′00.9″E	K81_33	HYDPT081-24/PX634092
Slovenia	45°31′38.5″N, 13°34′00.9″E	K81_35	HYDPT083-24/PX634071
Slovenia	45°31′38.5″N, 13°34′00.9″E	K81_37	HYDPT085-24/PX634147
Slovenia	45°31′38.5″N, 13°34′00.9″E	K82_3	HYDPT099-24/PX634112
Slovenia	45°31′34.0″N, 13°34′43.7″E	K82_14	HYDPT110-24/PX634143

(*Continued*)

Table 1. (Continued).

Locality	Coordinates	Specimen ID	BOLD/GenBank IDs
Slovenia	45°31′59.8″N, 13°36′03.2″E	K82_33	HYDPT129-24/PX634115
***Litarachna cursusmaritima* sp. nov. BOLD: AGH8823**			
Slovenia	45°31′59.8″N, 13°36′03.2″E	K82_31	HYDPT127-24/PX634078
Slovenia	45°31′59.8″N, 13°36′03.2″E	K82_32	HYDPT128-24/PX634100
***Litarachna duboscqi* BOLD: AGH9552**			
Slovenia	45°31′12.0″N, 13°34′01.0″E	K80_38	HYDPT038-24/PX634082
Slovenia	45°31′38.5″N, 13°34′00.9″E	K81_21	HYDPT069-24/PX634139
Slovenia	45°31′34.0″N, 13°34′43.7″E	K82_13	HYDPT109-24/PX634133
***Pontarchna aenariensis* BOLD: ADF7263**			
Slovenia	45°30′46.0″N, 13°35′11.4″E	K82_18	HYDPT114-24/PX634131
Slovenia	45°31′59.8″N, 13°36′03.2″E	K82_30	HYDPT126-24/PX634093
France, Corsica	42°35′16.5″N, 8°48′23.2″E	RMNH.5070759	NLACA1001-17/PX634118
***Pontarachna* cf. *adriatica* BOLD: AGH8822**			
Slovenia	45°30′46.0″N, 13°35′11.4″E	K82_19	HYDPT115-24/PX634125
***Pontarchna punctulum* BOLD: ADF7262**			
Italy, Sicily	37°29′26.1″N, 13°10′19.3″E	K82_44	HYDPT140-24/PX634106
Italy, Sicily	37°29′26.1″N, 13°10′19.3″E	K82_45	HYDPT141-24/PX634127
Italy, Sicily	37°29′26.1″N, 13°10′19.3″E	K83_3	HYDPT147-24/PX634145
Italy, Sicily	37°29′26.1″N, 13°10′19.3″E	K83_4	HYDPT148-24/PX634074
Italy, Sicily	37°29′26.1″N, 13°10′19.3″E	K83_5	HYDPT149-24/PX634073
Italy, Sicily	37°29′26.1″N, 13°10′19.3″E	K83_6	HYDPT150-24/PX634079
Italy, Sicily	37°29′26.1″N, 13°10′19.3″E	K83_7	HYDPT151-24/PX634138
Italy, Sicily	37°29′26.1″N, 13°10′19.3″E	K83_8	HYDPT152-24/PX634142
Italy, Sicily	37°17′23.7″N, 13°28′21.7″E	K83_9	HYDPT153-24/PX634088
Italy, Sicily	37°17′23.7″N, 13°28′21.7″E	K83_10	HYDPT154-24/PX634136
Italy, Sicily	37°17′23.7″N, 13°28′21.7″E	K83_11	HYDPT155-24/PX634093
Italy, Sicily	37°17′23.7″N, 13°28′21.7″E	K83_12	HYDPT156-24/PX634090
Italy, Sicily	37°17′23.7″N, 13°28′21.7″E	K83_13	HYDPT157-24/PX634137
Italy, Sicily	37°17′23.7″N, 13°28′21.7″E	K83_14	HYDPT158-24/PX634076
Italy, Sicily	37°17′23.7″N, 13°28′21.7″E	K83_18	HYDPT162-24/PX634114
France, Corsica	42°35′16.5″N, 8°48′23.2″E	RMNH.5070760	NLACA1002-17/PX634080
***Pontarchna punctulum* BOLD: AGH9553**			
Slovenia	45°31′12.0″N, 13°34′01.0″E	K81_9	HYDPT057-24/PX634093
Slovenia	45°31′12.0″N, 13°34′01.0″E	K81_12	HYDPT060-24/PX634108

three species: *P. punctulum*, *P. aenariensis* and *P. adriatica*. The genus *Litarachna* (60 sequences) is also represented by three species: *L. communis*, *L. duboscqi*, and the newly described *L. cursusmaritima* sp. nov. Except for the sequence from GenBank – KM101025, which is 570 bp, and NLACA998-17, which is 611 bp, the whole dataset was 658 bp. Except for BIN: BOLD: ADD6045, seven BINs assigned to the studied sequences were unique and are provided for the first time in the BOLD database.

Molecular analysis of the genus Pontarachna

The *COI* phylogenetic analyses revealed four clades in the examined *Pontarachna* dataset. The specimens from Italy (Sardinia and Sicily) and France (Corsica), morphologically identified as *P. punctulum*, form a well-supported clade which is placed as the sister clade to *P. punctulum* specimens from Slovenia (Figure 1). The third lineage, morphologically identified as *P.* cf. *aenariensis*, groups two specimens from Slovenia and one

specimen from France (Tyrrhenian Sea), which, in the tree, is placed as the sister group to the two aforementioned *P. punctulum* clades. The fourth lineage, which includes a single female from Slovenia, provisionally identified as *P.* cf. *adriatica*, forms a separate clade (Figure 1).

The ASAP, mPTP, and BIN delimitation methods were congruent and assigned sequences to the four clades (see Figure 1). For readability, we will use BINs as equivalents of clades, as each has a unique identifier, which will ease the presentation of results and discussion for the reader. Within *Pontarachna punctulum* we have two BINs: one, BOLD: ADF7262, from Italy and France; and the other, BOLD: AGH9553, from Slovenia (see Figure 2).

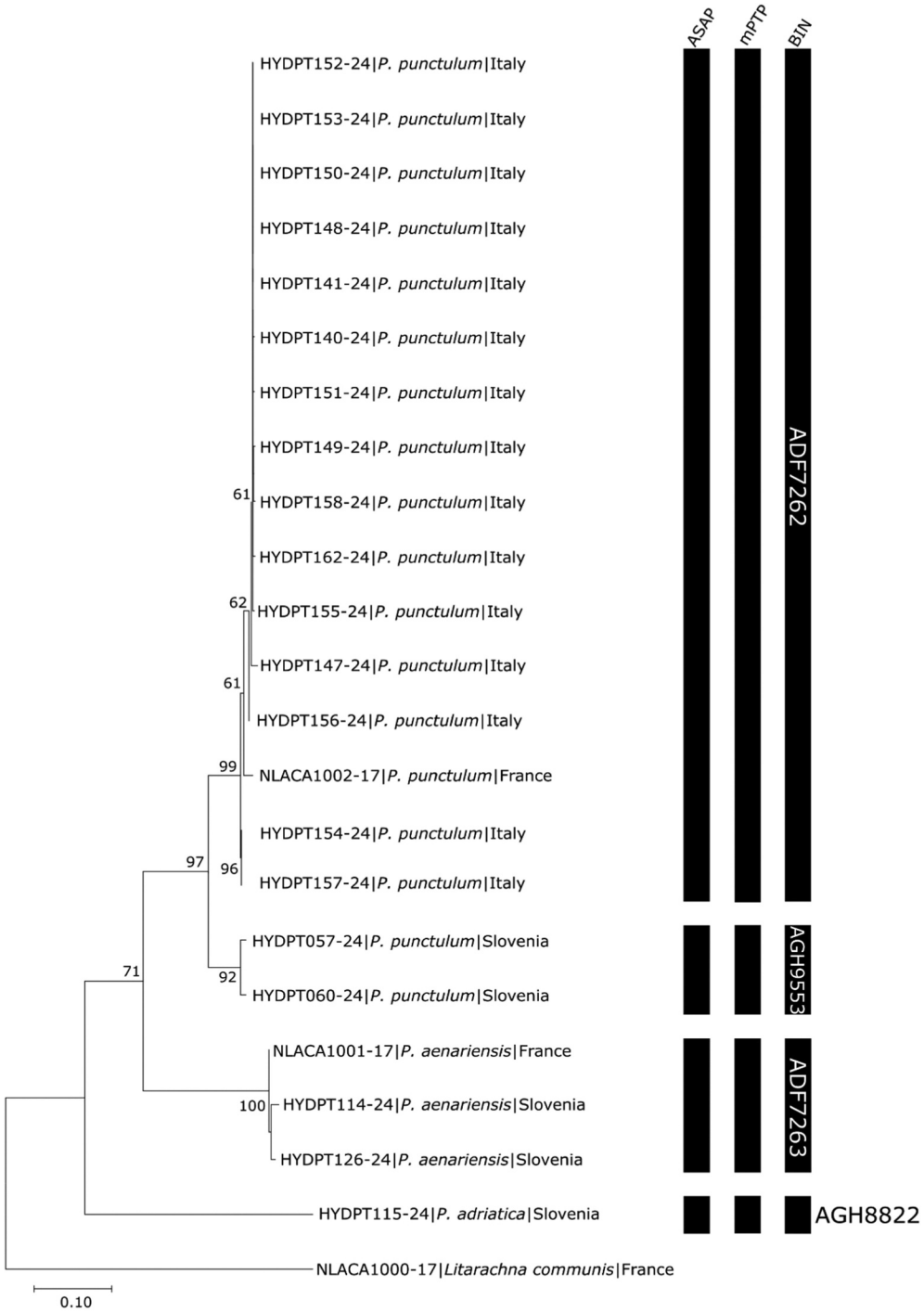

Figure 1. Maximum likelihood tree (T92 + G model) of the 22 sequences of *Pontarachna* spp. complex used in our study. The outgroup is *Litarachna communis*. The results of the assemble species by automatic partitioning (ASAP), the multi-rate Poisson tree processes model (mPTP) and barcode index number (BIN) species delimitation are indicated by vertical bars, and BIN number. Only bootstrap values with support greater than 50% are reported. BINs are based on the DNA barcode analysis from 20 June 2025.

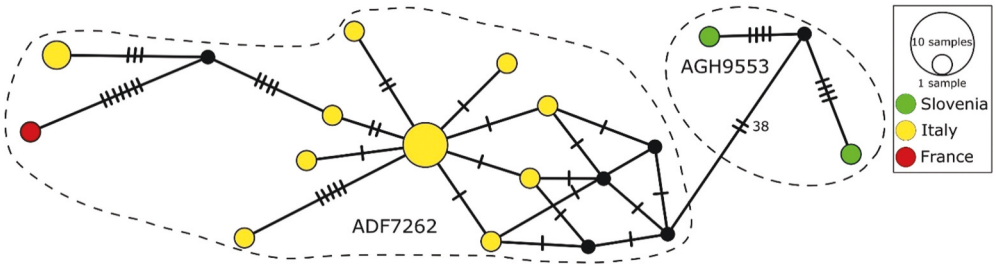

Figure 2. Median-joining haplotype networks showing relationships between haplotypes for *Pontarachna punctulum* based on the *COI* dataset. Each bar represents a single mutational step, with small black dots indicating undetected intermediate haplotype states. Circle sizes are proportionate to haplotype frequencies, as illustrated by the open circles with accompanying numbers. In all panels, colours represent individuals from different countries. Dashed lines encircle distinct barcode index numbers (BINs), each indicated by its respective code.

Table 2. Estimates of average genetic distance Kimura 2-parameter (K2P) within (intragroup) and between clades (intergroup) of examined species of *Pontarachna* sequence pairs. Abbreviation. n/c – not calculated.

		Intergroup	
MOTU	Intragroup	(1)	(2)
(1) *Pontarachna punctulum* – (BOLD: ADF7262)	0.0076		
(2) *Pontarachna punctulum* – (BOLD: AGH9553)	0.0123	0.0729	
(3) *Pontarachna aenariensis* (BOLD: ADF7263)	0.0112	0.1459	0.1499
(4) *Pontarachna* cf. *adriatica* (BOLD: AGH8822)	n/c	0.1924	0.1951

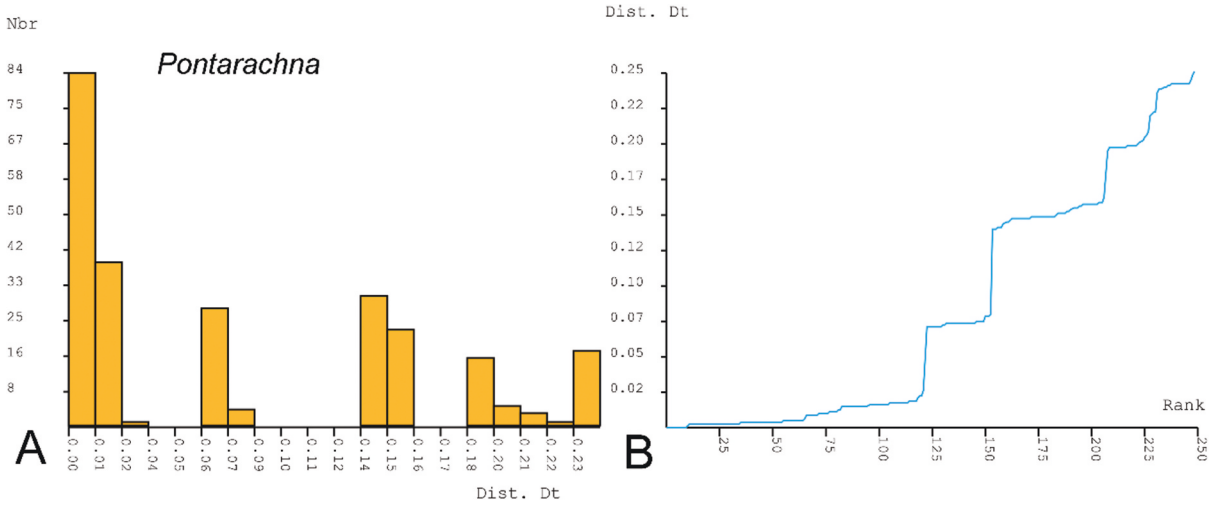

Figure 3. Results of ASAP analysis for *COI* sequences of the studied species of the genus *Pontarachna*. A – distribution of pairwise differences; B – ranked pairwise differences.

The mean genetic K2P distance between and among clades ranged from 7.29% between *Pontarachna punctulum* – BOLD: ADF7262 (Tyrrhenian Sea) and *P. punctulum* – BOLD: AGH9553 (Adriatic Sea), to 19.89% between *P. aenariensis* and *P.* cf. *adriatica* (see Table 2, Figure 3). In contrast, the mean intraspecific distance within clades was low, ranging from 0.76% in *P. punctulum* – BOLD: ADF7262 to 1.23% in *P. punctulum* – BOLD: AGH9553.

Molecular analysis of the genus Litarachna

The *COI* phylogenetic analyses revealed four species-level clades in *Litarachna*. Three of these clades morphologically belong to the *L. communis* group, while one lineage corresponds morphologically to

L. duboscqi Walter, 1925. The three lineages within the *L. communis* group show support values above 50% (see Figure 4). All applied species delimitation methods were congruent and consistently identified four clades within the available dataset of *Litarachna* sequences (Figure 4).

The *Litarachna communis* lineage from France (Corsica) and Italy (Sardinia and Sicily), which shares the same BIN (BOLD: ADD6045), was recovered as a sister clade to the *L. communis* lineage from Slovenia, which forms a separate BIN (BOLD: AGH8517) (see Figure 5). A third lineage, comprising two specimens from Slovenia and forming another distinct BIN (BOLD: AGH8823), is described here as *L. cursusmaritima* sp. nov., and is also represented in the tree (Figure 4).

The mean genetic K2P distance between and among clades ranged from 16% between *Litarachna communis* – BIN BOLD: ADD6045 and *L. communis* – BIN BOLD: AGH8517, to 25.7% between *L. duboscqi* and *L. communis* – BOLD: ADD6045 (see Table 3). These genetic distances exceed the barcoding gap determined by the ASAP method (3% to 14%; see Figure 6). In contrast, the mean intraspecific distance within clades was very low, ranging from 0.06% in *L. communis* – BOLD: AGH8517 to 1.13% in *L. communis* – BOLD: ADD6045.

The ML analysis does not support the monophyly of the genus *Litarachna*. In particular, *Litarachna duboscqi* does not cluster with the remaining representatives of *Litarachna* but instead forms a well-supported clade separate from the core *Litarachna* lineage. This placement renders *Litarachna* paraphyletic in the combined dataset (Figure 4). In contrast, the genus *Pontarachna* is recovered as monophyletic with strong bootstrap support.

Morphological analysis of the genus *Pontarachna*

Pontarachna punctulum Philippi, 1840

The examined specimens from the Tyrrhenian Sea (Corsica, Sardinia and Sicily) which share the same BIN (BOLD) and the specimens from the Adriatic (Slovenia) which belong to a separate BIN (BOLD), were keyed to *Pontarachna punctulum* following Pešić et al. (2019). The latter species was originally described by Philippi (1840), who collected it from the Gulf of Naples, Tyrrhenian Sea. *Pontarachna punctulum* is characterized by the combination of the following features: A pair of glandularium-like structures without setae located anterior to a pair of platelets bearing two "wheels" and a pore and postero-lateral to Lgl-3, and a sclerotized ring around the male gonopore with 40–50 pairs of setae (Pešić et al. 2019).

The examined specimens from our study generally match the description of *Pontarachna punctulum*. We did not find clear morphological differences (see Figure 7) between the specimens from Slovenia, which form a distinct genetic lineage (Figure 1), and specimens from France and Italy, which we assume represent the true *P. punctulum* lineage. Further studies, including of nuclear genes, are necessary to clarify the taxonomic position of the Slovenian lineage and the possibility that it represents a cryptic species within the *P. punctulum* complex.

Pontarachna aenariensis Mari and Morselli, 1983

Two examined females from Slovenia were keyed to *Pontarachna aenariensis* following Pešić et al. (2019). These specimens form a separate BIN (BOLD: ADF7263), including one specimen from France (Figure 1).

Pontarachna aenariensis was originally described by Mari and Morselli (1983) from the Gulf of Naples, Tyrrhenian Sea. Following the original description and Pešić et al. (2019), *P. aenariensis* is diagnosed by the combination of the following features: A pair of glandularium-like structures without setae located anterior to a pair of platelets with two "wheels" and a pore and between E4 and L3; a sclerotized ring around the male gonopore with 18–30 pairs of setae.

Additional material, including males, would be necessary to confirm the taxonomic assignment of the barcoded material from Slovenia.

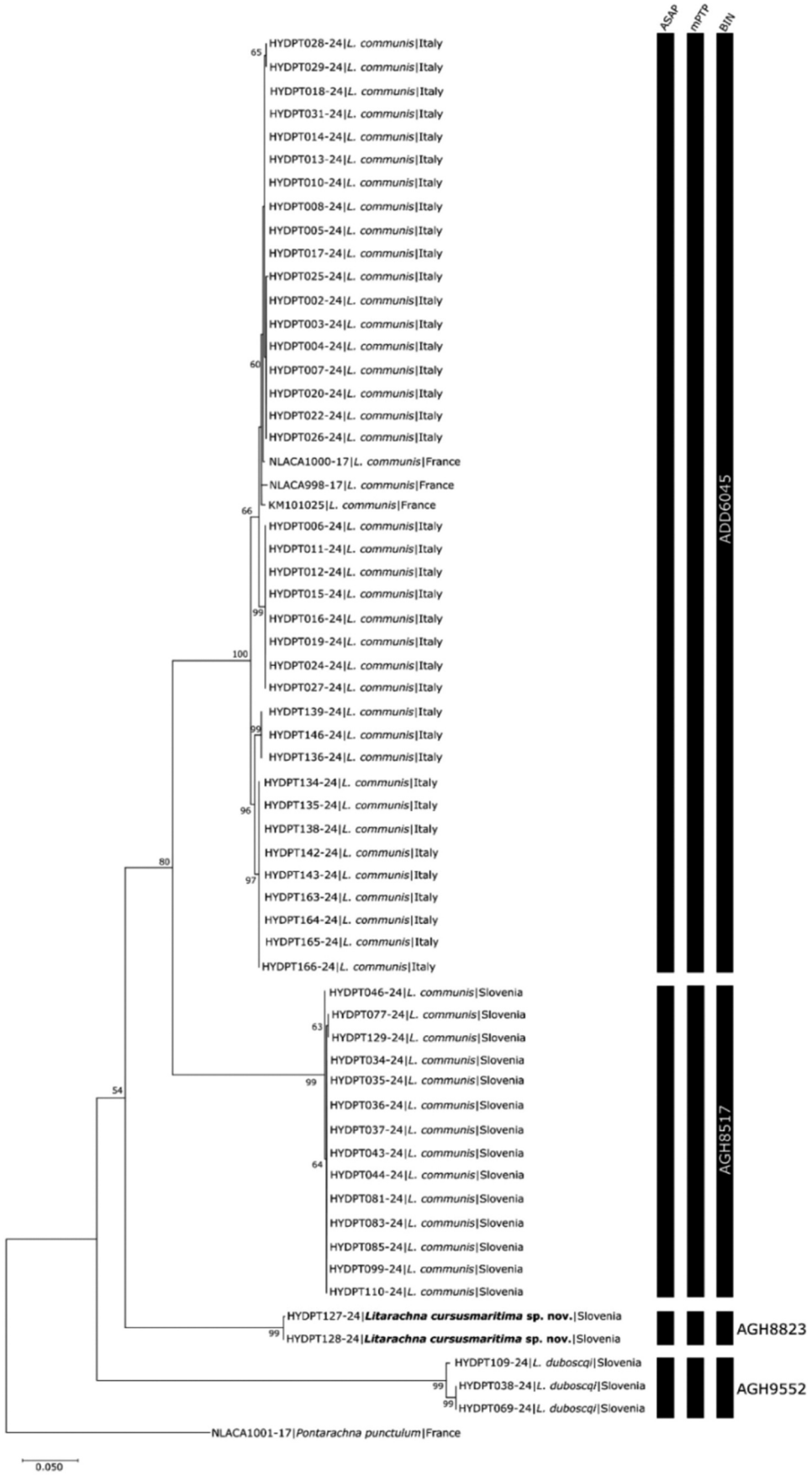

Figure 4. Maximum likelihood tree (HKY+I model) of the 60 sequences of *Litarachna* spp. complex used in our study. The outgroup is *Pontarachna punctulum*. The results of the assemble species by automatic partitioning (ASAP), the multi-rate Poisson tree processes model (mPTP) and barcode index number (BIN) species delimitation are indicated by vertical bars, and BIN numbers. Only bootstrap values with support greater than 50% are reported. BINs are based on the DNA barcode analysis from 20 June 2025.

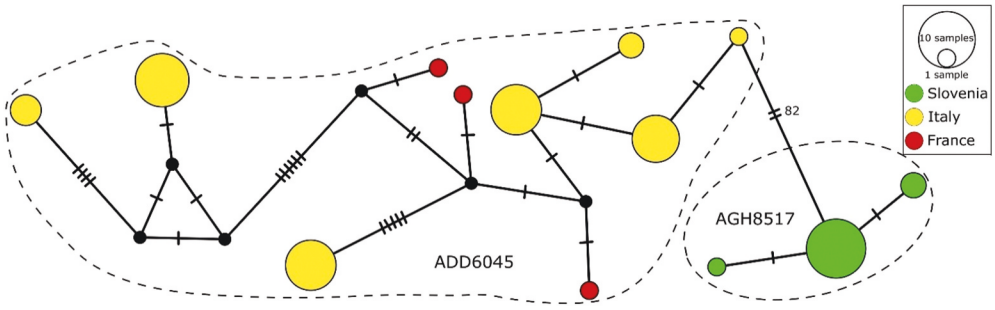

Figure 5. Median-joining haplotype networks showing relationships between haplotypes for *Litarachna communis* based on the *COI* dataset. Each bar represents a single mutational step, with small black dots indicating undetected intermediate haplotype states. Circle sizes are proportionate to haplotype frequencies, as illustrated by the open circles with accompanying numbers. In all panels, colours represent individuals from different countries. Dashed lines encircle distinct barcode index numbers (BINs), each indicated by its respective code.

Table 3. Estimates of average genetic distance (K2P) within (intragroup) and between clades (intergroup) of examined species of *Litarachna* sequence pairs.

		Intergroup	
MOTU	Intragroup	(1)	(2)
(1) *Litarachna communis* – (BOLD: ADD6045)	0.0113		
(2) *Litarachna communis* – (BOLD: AGH8517)	0.0006	0.160	
(3) *Litarachna. cursusmaritima* sp. nov. (BOLD: AGH8823)	0.0015	0.166	0.194
(4) *Litarachna duboscqi* (BOLD: AGH9552)	0.0082	0.239	0.257

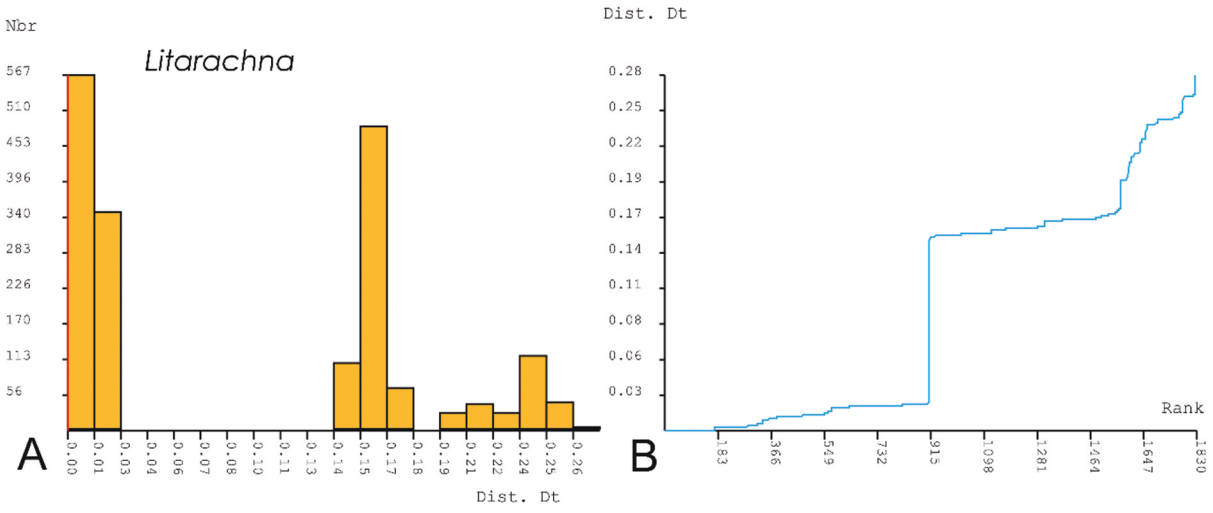

Figure 6. Results of the ASAP analysis for *COI* sequences of the studied species of the genus *Litarachna*. A – distribution of pairwise differences; B – ranked pairwise differences.

Pontarachna cf. *adriatica* Morselli, 1980

The examined single female from Slovenia was keyed to *Pontarachna adriatica* following Pešić et al. (2019). This specimen forms a separate BIN (BOLD: AGH8822).

Pontarachna adriatica was originally described by Morselli (1980) from lagoons in the Northern Adriatic Sea. Following the original description and Pešić et al. (2019), *P. adriatica* is diagnosed by the combination of the following features: a pair of glandularium-like structures without setae (= V3 gland sensu Wiles et al. 2002) is located posterior to a pair of platelets with two "wheels" and a pore; in the male there is a sclerotized ring around the gonopore with 28–37 pairs of setae.

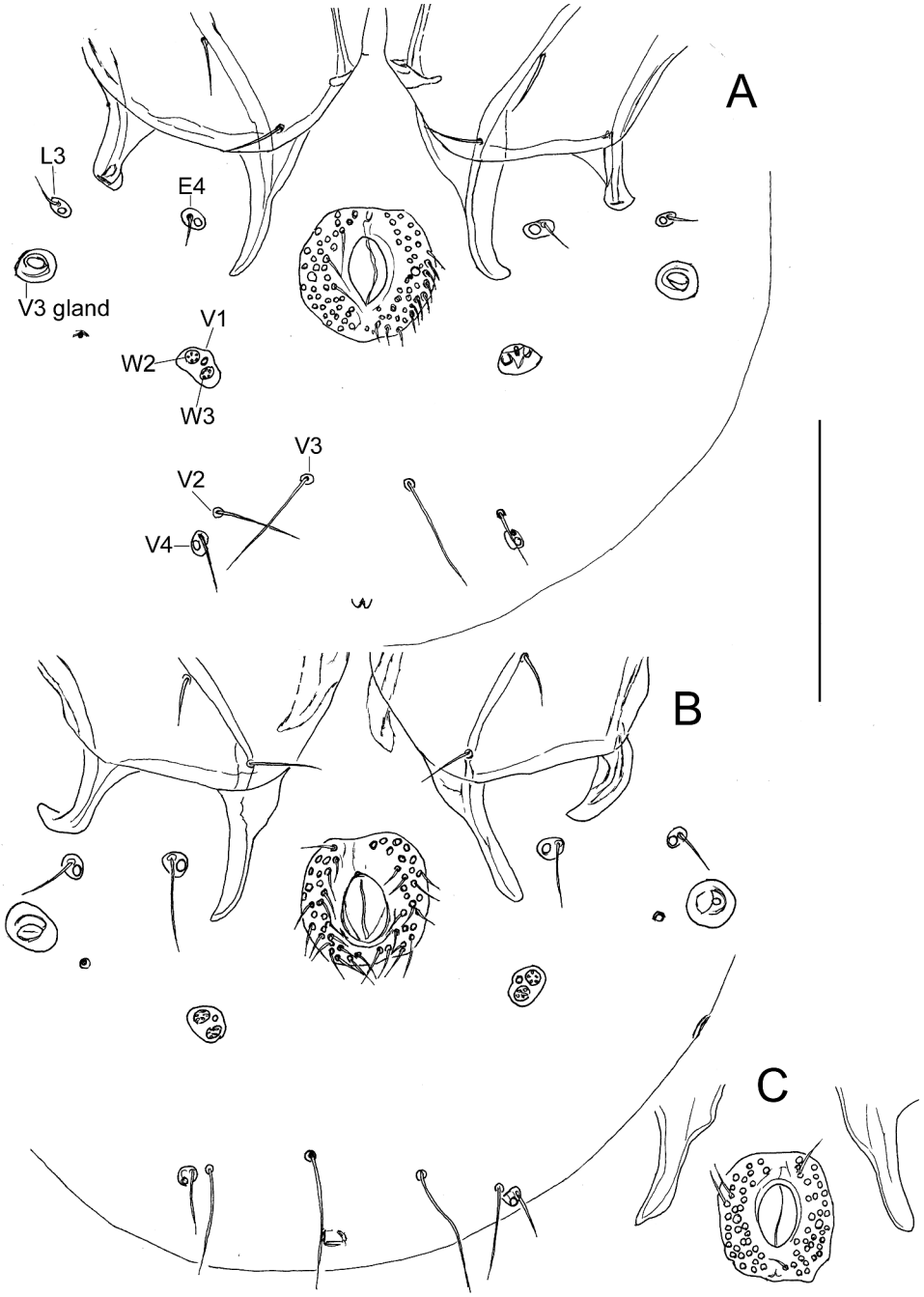

Figure 7. *Pontarachna punctulum*, ♂ (A – clade 1, K83_11, Italy, Sicily; B – clade 2, K81_9 Slovenia; C – clade 2, K81_12, Slovenia): A–B – posterior ventral surface of idiosoma (mounted); C – genital field (unmounted). Scale bar = 100 µm. W2 and W3 are "wheel-like acetabula". Terminology after Wiles et al. (2002).

The single female from Slovenia matches the description of *Pontarachna adriatica*, except the platelet with two "wheels" and a pore, on each side, is split into two adpressed platelets each with a "wheel", one also bearing a glandularium (see Figure 8). Additional material including males would be necessary to confirm the taxonomic assignment of the specimen from Slovenia.

Analysis of the colour patterns in *Pontarachna*

Unfortunately, we have information only on the dorsal colour patterns for two of the three *Pontarachna* species found in the present study. Both *P. punctulum* and *P. aenariensis* bear a slightly irregular dorsal white

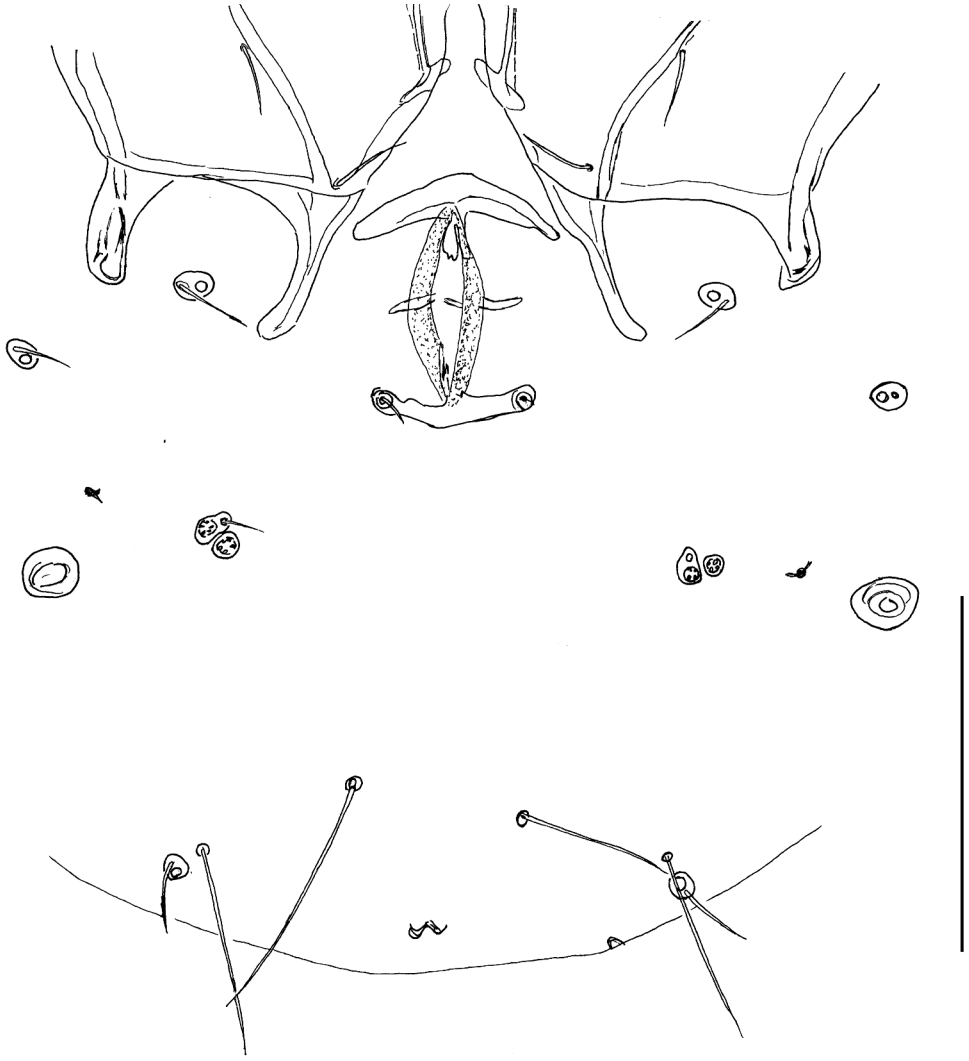

Figure 8. *Pontarachna* cf. a*driatica*, ♀, K82_19, Slovenia: posterior ventral surface of idiosoma. Scale bar = 100 µm.

cross with the anterior arm clearly thinner than the others; in both species the colour between the arms of the cross is reddish-orange (Figure 12J–M, R).

Morphological analysis of the genus *Litarachna*

Litarachna communis Walter, 1925

The examined specimens from France (Corsica, three specimens) and Italy (Sardinia, 26 specimens and Sicily, 12 specimens) which belong to BIN BOLD: ADD6045, and 14 specimens from Slovenia which belong to BOLD: AGH8517 were determined to be *Litarachna communis*, following Viets (1957), Moto and Abé (2014) and Pešić et al. (2019).

Litarachna communis was originally described by Walter (1925) based on specimens collected from algae on the coast of Banyuls-sur-Mer in France. In the original description, Walter (1925) did not specify the type designation and type depository of *L. communis*. Recently, Moto and Abé (2014) redescribed the latter species based on newly designated types from material stored in the Natural History Museum of Basel and provided additional information on the morphological characteristics of *L. communis*.

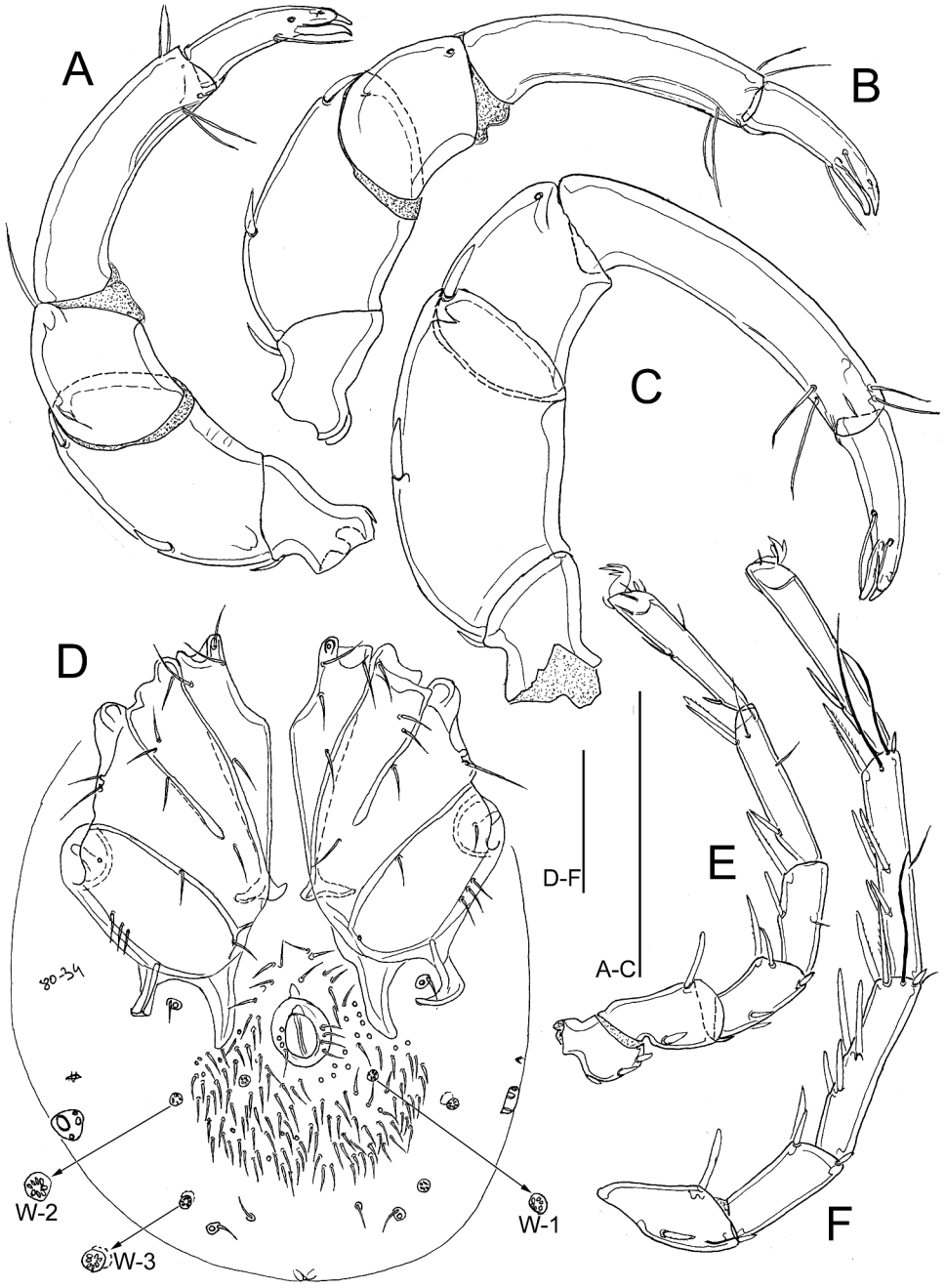

Figure 9. A – *Litarachna communis* clade 1, ♂, K80_32, Italy, Sardinia. **B** – *L. communis* clade 2, ♂, K80_34, Slovenia. **C–F.** *L. cursusmaritima* sp. nov., ♂ holotype, Slovenia: A–C – palp; D – idiosoma, ventral view; E – I-L; F – IV-L-2 to -6. Scale bars = 100 µm. W1, W2 and W3 are "wheel-like acetabula".

Here we provide the diagnosis of *Litarachna communis* following Moto and Abé (2014): ventral tubercle on *p*-2 absent; ventral tubercle and peg-like seta on *p*-4 absent; *p*-2 as long as *p*-4 in dorsal length; *p*-5 longer than half of *p*-4 in dorsal length (Figures 9A–B); genital field in female located between left and right apodemes of Cx-I.

The examined specimens from our study generally match the description of *Litarachna communis*, with *p*-5 distinctly longer than half of *p*-4 (see Figures 9A–B). We did not find clear morphological differences between specimens from Slovenia, which form a distinct genetic lineage, and specimens from France and Italy, which we assume represent the true *L. communis* lineage. Including additional nuclear genes is

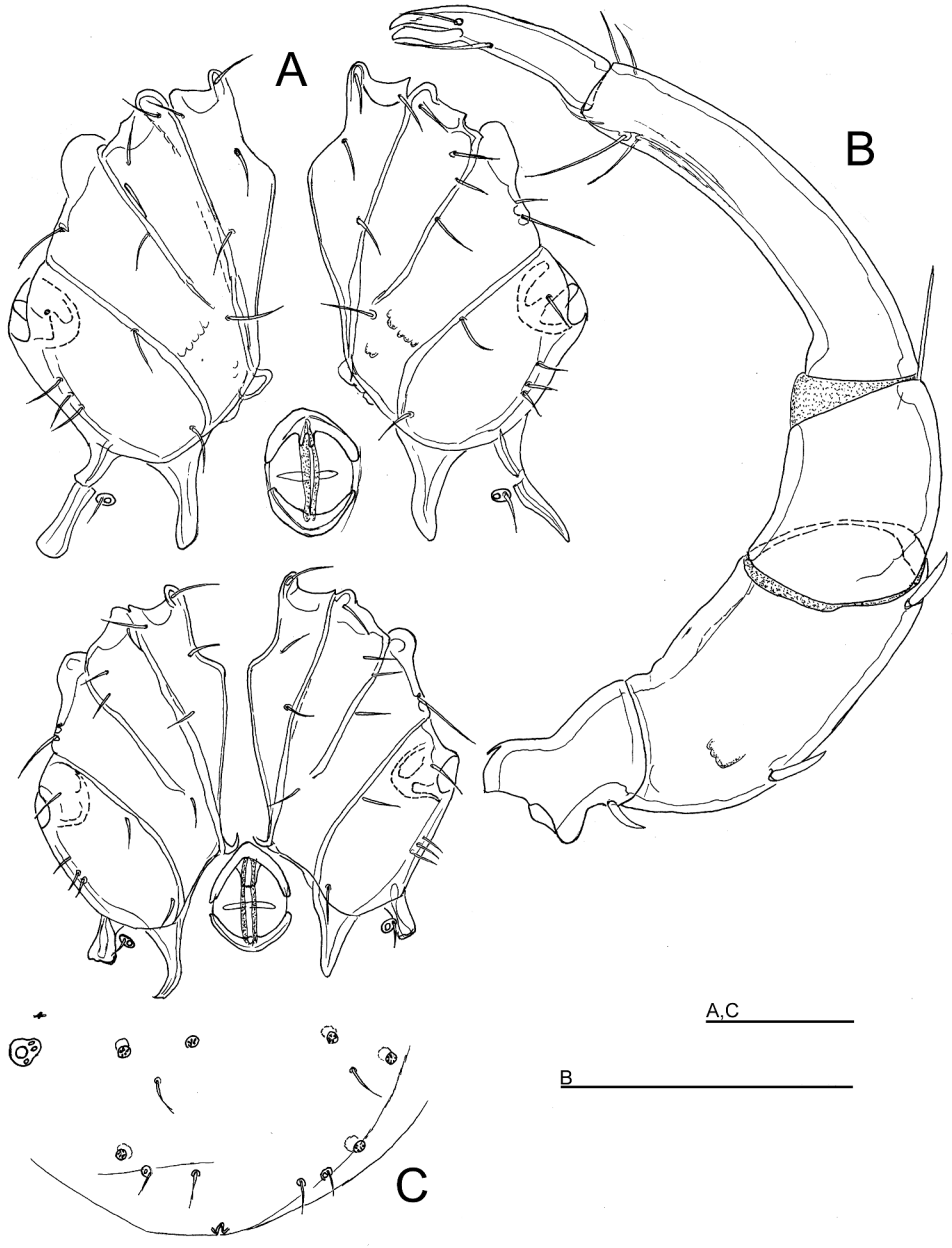

Figure 10. **A–B** – *Litarachna cursusmaritima* sp. nov., ♀ paratype, K82_31, Slovenia. **C** – *L. communis* clade 1, ♀, RMNH.5070756, France, Corse: A – coxal and genital field, B – palp; C – idiosoma, ventral view. Scale bars = 100 µm.

necessary to clarify the taxonomic position of the Slovenian lineage and to assess the possibility that it is a cryptic species within the *L. communis* complex.

Litarachna cursusmaritima Goldschmidt and Pešić sp. nov.

Figures 9C–F, 10A–B, 12E–F

Material examined. Holotype ♂ (RMNH), dissected and slide mounted, sequenced (voucher ID: K82_32, BOLD ID: HYDPT128-24), Slovenia, Adriatic Sea, Piran, Strunjan, seagrass, ~6 m depth, leg. Goldschmidt. Paratype: 1 ♀, same data as holotype, dissected and slide mounted (RMNH), sequenced (voucher ID: K82_31, BOLD ID: HYDPT127-24).

Diagnosis. Colour pattern: Dorsum brownish-salmon with six to eight irregularly serrated white crossbars. *p*-5 nearly as long as half of *p*-4, *p*-4 ventral setae not lying at the same level, female genital field shifted posteriorly, reaching the level of posterior margin of the medial apodemes of Cx-IV.

Description. Male: Medial apodemes of Cx-IV extending beyond the anterior margin of genital field, genital field consisting of a sclerotized ring with four pairs of setae; many perigenital setae (around 50 pairs) free in the integument around the genital field (Figure 9D). Female: Medial apodemes of Cx-IV extending to the posterior margin of the genital field, pre- and post-genital sclerites bowed (Figure 10A).

Measurements – Male (holotype; K82_32): Idiosoma L 453, W 363; coxal field L 234, W 311; Cx-III W 298; genital field L/W 55/43. Ejaculatory complex L 88. Palp: dL/H: *p*-1, 25/28; *p*-2, 123/60; *p*-3, 61/50; *p*-4, 138/36; *p*-5, 67/19; dL *p*-4/P-5 ratio 2.06.
dL of I-L-1 to –6: 58, 55, 73, 81, 118, 134; dL of II-L-1 to –6: 62, 55, 77, 89, 134, 139. dL of III-L-1 to –6: 73, 56, 78, 95, 142, 150; dL of IV-L-2 to –6: 74, 96, 148, 164, 163.

Female (paratype; K82_31): Idiosoma L 647; coxal field L 273, W 398; Cx-III W 341; genital field L 94, pre-genital sclerite W 67, post-genital sclerite W 61. Palp: dL/H: *p*-1, 25/28; *p*-2, 138/63; *p*-3, 72/55; *p*-4, 150/39; *p*-5, 77/20; dL *p*-4/P-5 ratio 1.95.
dL of I-L-1 to –6: 63, 61, 86, 98, 135, 142; dL of II-L-1 to –6: 63, 55, 86, 99, 145, 156; dL of III-L-1 to –6: 73, 58, 85, 106, 164, 164; dL of IV-L-1 to –6: 132, 80, 106, 167, 186, 177.

Etymology. The species name is dedicated to the students of the University of Munich's Mediterranean marine biology course held in Piran in 2023, and to all courses in marine biology.

Type depository. The types of the new species will be deposited in the mite collection of the Bavarian State Collection of Zoology, Section Arthropoda varia, Munich, Germany.

Discussion. The specimens from Slovenia described here as the new species *Litarachna cursusmaritima* sp. nov. differ from the specimens of both *L. communis* clades in the following characters: In both sexes *p*-4 is comparatively longer and *p*-5 nearly half as long as *p*-4 (in *L. communis p*-4 comparatively less elongated and *p*-5 distinctly longer than half of *p*-4 – see Figures 9C and 10B); the female genital field is shifted more posteriorly from the coxal field, reaching the level of the posterior margin of the medial apodemes of Cx-IV (in *L. communis* the genital field is shifted close to the apodeme of the Cx-I/II suture line, and the end of the medial apodemes of Cx-IV strongly exceeds the posterior margin of the genital field – compare Figure 10A with 10C).

Remarks on *Litarachna divergens* Walter, 1925

Litarachna divergens was originally described by Walter (1925) based on Von Schaub's (1889) figures of specimens collected from Trieste Bay in the northern Adriatic Sea. In the original description Walter (1925) stated that *L. divergens* is morphologically close to *L. communis* and may even present the same form. In the same paper, Walter (1925) assigned a specimen from the Black Sea recorded by Sernow (1913) to *L. divergens*. Further records of this species were reported from the Suez Canal by Soar (1927), followed by Uchida (1935), who provided a detailed description of both sexes of *L. divergens* based on material from the Sea of Japan.

Karl Viets (1956) was the first to propose *Litarachna divergens* as a species incerta, but later on, he included it in his key to *Litarachna* species (see Viets 1957). It is worth noting that Viets's (1957) attempt to retain *L. divergens* was based on Uchida's (1935) specimens from Japan.

As the main diagnostic characters for separating *Litarachna divergens* from *L. communis*, Viets (1957) listed differences in the palps, i.e. *p*-2 ventral margin with a kink, *p*-5 shorter than half of *p*-4 (*p*-2 ventral margin slightly concave, *p*-5 longer than half of *p*-4 in *L. communis*), ventral setae on *p*-4 slightly shifted to the distal edge and distanced from each other (ventral setae lying on the same level in *L. communis*). Moreover, the genital field of the female of *L. communis* is located between the posterior apodemes of the first coxae, while in *L. divergens* it is located clearly posterior to these apodemes.

Recently, the specimens from the Sea of Japan previously reported under the name *Litarachna divergens* have been described by Pešić and Smit (2016) as a new species, *L. thetis* Pešić and Smit 2016. The same authors (Pešić & Smit 2016) considered *L. divergens* a species incerta.

The specimens from Slovenia described in this study as a new species *Litarachna cursusmaritima* sp. nov., differ from *L. divergens* in the colour pattern (brownish-salmon with irregular white crossbars in the new species vs. a large white cross with light red-brownish in between in *L. divergens* (according to Von Schaub 1889) and the shape of *p*-2 (ventrally nearly straight in the new species vs. strongly concave in *L. divergens* (see Von Schaub 1889, fig. 1).

Litarachna duboscqi Walter, 1925

Two examined specimens from Slovenia were keyed to *Litarachna duboscqi*, following Viets (1957) and Pešić et al. (2019). These specimens form a separate BIN (BOLD: AGH9552).

Litarachna duboscqi is a species widely distributed in the Mediterranean Sea (see Pešić et al. 2019 for an overview). This species can easily be distinguished from all other Mediterranean species of the genus by the combination of the following features: Suture line Cx-III/IV incomplete, medially obliterated, *p*-4 with a pointed projection near seta insertions (Figure 11) and a pair of large glandularium-like structures without a seta (V3 glands sensu Wiles et al. 2002) located between posterior apodemes of Cx-IV fused with the platelets bearing Cxgl-4.

Analysis of the colour patterns in *Litarachna*

In addition to the morphological characteristics discussed and illustrated above, the three species found in the present study also show clear differences in their colour pattern.

Both clades of *Litarachna communis* are characterized by a clear white cross on the dorsal side, with bright red patches in between the branches of the latter (Figure 12A–D, N, O). In *Litarachna duboscqi*, the white dorsal pattern rather forms a T- or Y-shaped cross with four antero-lateral lobes and a posterior, terminally

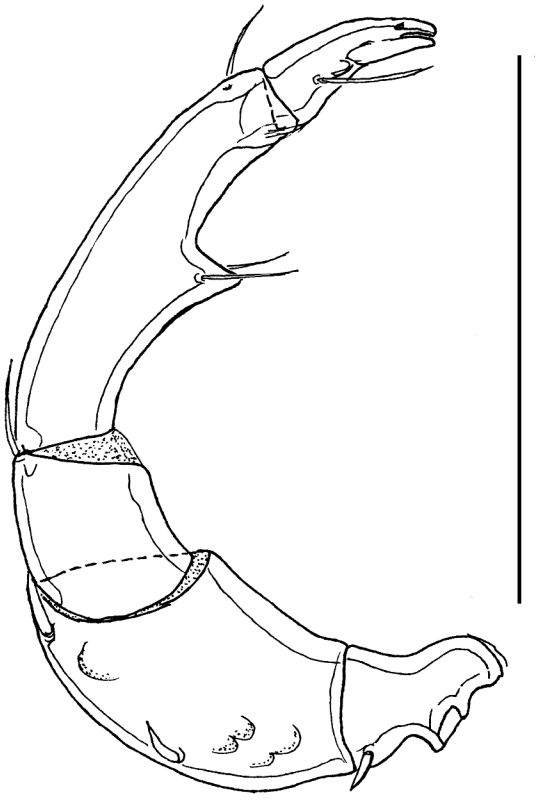

Figure 11. *Litarachna duboscqi*, ♀, K82_13, Slovenia: palp. Scale bar = 100 µm.

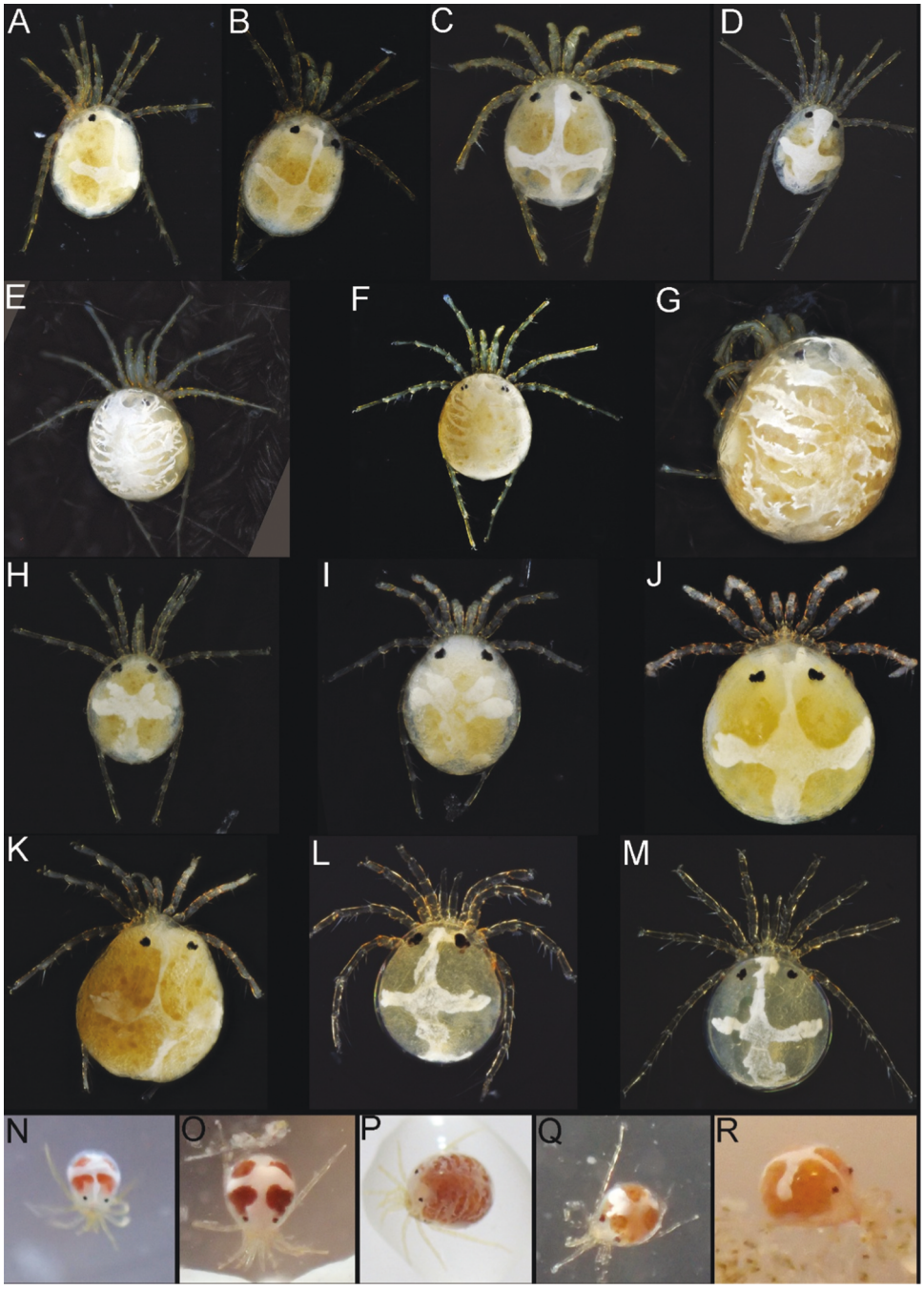

Figure 12. A–M. Photographs of dorsal views of selected Pontarachnidae species (specimens conserved in 96% EtOH): A – *Litarachna communis* clade 1, Sardinia, ♂, K80_25; B – *Litarachna communis* clade 1, Sardinia, ♀, K80_26; C – *Litarachna communis* clade 2, Slovenia, ♂, K81_33; D – *Litarachna communis* clade 2, Slovenia, ♀, K81_35; E – *Litarachna cursusmaritima* sp. nov., Slovenia, ♂, K82_31; F – *Litarachna cursusmaritima* sp. nov., Slovenia, ♂, K82_31; G – *Litarachna cursusmaritima* sp. nov., Slovenia, ♀, K82_32; H – *Litarachna duboscqi*, Slovenia, ♂, K81_21; I – *Litarachna duboscqi*, Slovenia, ♀, not barcoded; J – *Pontarachna aenariensis*, Slovenia, ♂, K82_18; K – *Pontarachna aenariensis*, Slovenia, ♀, not barcoded; L – *Pontarachna punctulum*, Slovenia, ♂, K81_9; *M* – *Pontarachna punctulum*, Slovenia, ♀, K81_12. **N–R**. photographs of live specimens of selected Pontarachnidae species: N – *Litarachna communis* clade 1, Sardinia; O – *Litarachna communis* clade 2, Slovenia; P – *Litarachna cursusmaritima* sp. nov., Slovenia; Q – *Litarachna duboscqi*, Slovenia; R – *Pontarachna aenariensis*, Slovenia. Photos by T. Goldschmidt.

widened "stem". The patches in between are more orange-reddish (Figure 12H–I, Q). Finally, the new species described above, *L. cursusmaritima* sp. nov., provides a very strikingly different colour pattern (compared to rather small differences to *L. communis* in the morphology): Instead of a white cross, the dorsum is covered

by six to eight very irregularly serrate white horizontal beams, with the interspace brownish-salmon (Figure 12E–G, P).

As well, the two clades of *Litarachna communis* found in the present study show slight differences in their colour pattern – in the illustrated specimens from Sardinia (*L. communis* clade 1), the anterior arm of the dorsal white cross is thinner than in the specimens from Slovenia (*L. communis* clade 2) (see Figure 12N vs. O and 12A, B vs. C, D).

Discussion

In the latest overview on Mediterranean pontarachnids (Pešić et al. 2019), the authors already reported a clearly higher species diversity in the eastern compared to the western part of the Mediterranean Sea – documented by four vs. eight recorded species. Out of the eight species in the eastern Mediterranean, four were listed for the Adriatic Sea. The authors hypothesized that this might reflect differences in sampling effort rather than a real biogeographic trend (Pešić et al. 2019).

In the present study, comparable sampling efforts across the Tyrrhenian (Sicily, Sardinia, Corsica) and the Adriatic Sea (Slovenia), combined with DNA barcoding of the mitochondrial *COI* gene, revealed consistently higher molecular diversity in the eastern basin. In the Tyrrhenian, we detected *Litarachna communis* (BOLD: ADD6045), *Pontarachna punctulum* (BOLD: ADF7262) and *P. aenariensis*, whereas the Adriatic Sea harboured *Litarachna communis* (BOLD: AGH8517), *L. duboscqi*, *L. cursusmaritima* sp. nov., as well as *Pontarachna punctulum* (BOLD: AGH9553), *P. aenariensis* and *P.* cf. *adriatica*. Both *Litarachna communis* and *Pontarachna punctulum* were represented by two BINs each, with relatively high genetic divergence between Adriatic and Tyrrhenian populations indicating strong genetic isolation.

The ML phylogenetic reconstruction based on the combined dataset fails to recover *Litarachna* as a monophyletic group. While most *Litarachna* species (e.g. *L. communis* and *L. cursusmaritima* sp. nov.) form a distinct and well-supported clade, *L. duboscqi* is recovered as a separate lineage. The relationship between these two lineages is poorly supported (bootstrap value: 43%), indicating that *Litarachna*, as currently defined, is likely paraphyletic. These results suggest that the taxonomic placement of *L. duboscqi* may require formal re-evaluation (Figure 4).

The species delimitation methods applied (ASAP, mPTP, and BIN) consistently supported the distinctiveness of the recovered clades in both genera, with mean interclade genetic distances comparable to those reported for cryptic species in other water mite lineages (Martin et al. 2010; Stålstedt et al. 2013; Pešić et al. 2017). These lineages may represent either cryptic species or deeply divergent conspecific populations. Resolving their taxonomic status will require additional sampling and detailed morphological examination. Should the hypothesis of cryptic species be confirmed, the formal description of new species using DNA-based diagnostic characters is recommended (Grabowski et al. 2017).

In the study mentioned above (Pešić et al. 2019), the authors assumed that a relatively high diversity of pontarachnids could be expected if meiofauna from different habitats is sampled with a particular focus on mites. Our results precisely confirm this assumption – at 10 sites (one to five subsamples each) near the city of Piran (Slovenia), different algae and seaweeds were sampled and systematically examined for water mites; in total, 194 Pontarachnidae were found. So far, just 24 specimens from 11 subsamples have been barcoded – and these already represent six different species (three *Pontarachna*, three *Litarachna*).

So far, the only record of Pontarachnidae from Slovenia had been the chance find of a single female of *Pontarachna adriatica* in the gut of a fish caught in the Piran Bay (Siokou et al. 2013). Now, as just a few specimens from some sample sites near Piran have already revealed six different species of Pontarachnidae, we can assume this is just the tip of the iceberg in terms of the diversity of this group of marine mites on the Slovenian coast.

Up to now, no attention has been paid to the colour patterns of different species (Pešić et al. 2019). Even though many Hydrachnidia exhibit very striking colours, they are widely ignored in species identification (Fisher et al. 2017). These colour patterns are primarily produced by inner organs (mainly the excretory organ and the midgut lobes (Bartsch et al. 2006). Details of their shape vary among individuals and with the age of the specimens. Nevertheless, the example of the three different *Litarachna* species treated in the present study shows that, even though the variability of colours and colour patterns remains largely unknown, focusing on structures that remain stable beyond individual differences can provide valuable additional information that should not be ignored. As colour patterns may vary with age and between specimens, more

data are needed to provide reliable information on individual variability and usefulness for species determination. Therefore, in future studies on pontarachnids, much more attention should be paid to this character as well. Unfortunately, the actual colour is largely lost during ethanol fixation of the mites; however, as Figure 12 shows, the white pattern is still clearly visible in most cases, even though the bright reddish colours turn brownish-yellow (see Figures 12N, 12O vs. 12A–D). However, as these patterns are also lost after DNA extraction, it is necessary to study and document these characters before molecular analysis.

Our findings highlight the underestimated species diversity of Pontarachnidae in the Mediterranean, particularly along the Slovenian coast, where even limited sampling revealed a surprisingly high number of distinct lineages. The observed genetic divergence between Adriatic and Tyrrhenian populations supports the existence of cryptic (or pseudocryptic) species, underlining the need for integrative taxonomic approaches combining molecular data with detailed morphological and ecological analyses. Future studies focusing on overlooked characters, such as colour patterns, may further enhance species delimitation in this understudied group.

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Disclosure statement

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Data availability statement

DNA sequences prepared during this study are deposited in the Barcode of Life Data Systems (doi number dx.doi.org/10.5883/DS-MEDPO) and GenBank.

References

Astrin JJ, Stüben PE. 2008. Phylogeny in cryptic weevils: Molecules, morphology and new genera of western Palaearctic Cryptorhynchinae (Coleoptera: Curculionidae). Invertebrate Systematics 22(5):503–522. DOI: 10.1071/IS07057

Bartsch I, Davids C, Deichsel R, Di Sabatino A, Gabrys G, Gerecke R, Gledhill T, Jäger P, Makol J, Smit H, van der Hammen H, Weigmann G, Wohltmann A, Wurst E. 2006. Chelicerata: Acari I. In: Gerecke R, editor. Süßwasserfauna von Mitteleuropa 7/2-1. Heidelberg (Spektrum Akademischer Verlag) pp. 1–388.

Blattner L, Gerecke R, von Fumetti S. 2019. Hidden biodiversity revealed by integrated morphology and genetic species delimitation of spring dwelling water mite species (Acari, Parasitengona: Hydrachnidia). Parasites and Vectors 12 (1):492. DOI: 10.1186/s13071-019-3750-y

Carew ME, Yow WK, Robinson KL, Coleman RA, Hoffmann AA. 2022. DNA barcoding and metabarcoding of highly diverse aquatic mites (Acarina) can improve their use in routine biological monitoring. Marine & Freshwater Research 73 (7):900–914. DOI: 10.1071/MF21291

Chatterjee T, Schizas NV, Pešić V. 2019. A checklist of Pontarachnidae (Acari: Hydrachni-dia) and notes on distributional patterns of the species. Zootaxa 4619(3):527–544. DOI: 10.11646/zootaxa.4619.3.6

Comai L, Howell T 2012. Barcode generator v. 2.8.http://comailab.genomecenter.ucdavis.edu/index.php/Barcode_generator (accessed 15 June 2024).

Cook DR 1958. A new species of Litarachna from the British West Indies (Acarina: Pontarachnidae). Proceedings of the Entomological Society of Washington 60:19–22.

Cook DR. 1986. Water mites from Australia. Memoirs of the American Entomological Institute 40:1–568.

Cook DR. 1996. A freshwater species of *Pontarachna* (Acari, Pontarachnidae) from South Africa, with a discussion of genital acetabula in the family. Anales Instituto de Biología Universidad Nacional Autónoma de México, Seria Zoología 67:259–264.

Edgar RC. 2004. MUSCLE: Multiple sequence alignment with high accuracy and high 679 throughput. Nucleic Acids Research 32(5):1792–1797. DOI: 10.1093/nar/gkh340

Felsenstein J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39(4):783–791. DOI: 10.1111/j.1558-5646.1985.tb00420.x

Fisher JR, Fisher DM, Skvarla MJ, Nelson WA, Dowling APG. 2017. Revision of torrent mites (Parasitengona, Torrenticolidae, *Torrenticola*) of the United States and Canada: 90 descriptions, molecular phylogenetics, and a key to species. ZooKeys 701:1–496. DOI: 10.3897/zookeys.701.13261

Grabowski M, Wysocka A, Mamos T. 2017. Molecular species delimitation methods provide new insight into taxonomy of the endemic gammarid species flock from the ancient Lake Ohrid. Zoological Journal of the Linnean Society 181 (2):272–285. DOI: 10.1093/zoolinnean/zlw025

Hebert PD, Cywinska A, Ball SL, DeWaard JR. 2003. Biological identifications through DNA barcodes. Proceedings of the Royal Society B: Biological Sciences 270(1512):313–321. DOI: 10.1098/rspb.2002.2218

Kapli P, Lutteropp S, Zhang J, Kobert K, Pavlidis P, Stamatakis A, Flouri T. 2017. Multi-rate poisson tree processes for single-locus species delimitation under maximum likelihood and Markov chain Monte Carlo. Bioinformatics 33 (11):1630–1638. DOI: 10.1093/bioinformatics/btx025

Kimura M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16(2):111–120. DOI: 10.1007/BF01731581

Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution 35(6):1547–1549. DOI: 10.1093/molbev/msy096

Leigh JW, Bryant D. 2015. POPART: Full-feature software for haplotype network construction. Methods in Ecology and Evolution 6(9):1110–1116. DOI: 10.1111/2041-210X.12410

Mari M, Morselli I. 1983. Osservazione su due specie di Hydrachnellae (Acari) marine: *Pontarachna aenariensis* n.sp.e Litarachna communis Walter. Atti della Società Toscana di Scienze Naturali: Memorie, Serie B 90:91–98.

Martin P, Dabert M, Dabert J. 2010. Molecular evidence for species separation in the water mite *Hygrobates nigromaculatus* Lebert, 1879 (Acari, Hydrachnidia): Evolutionary consequences of the loss of larval parasitism. Aquatic Sciences 72 (3):347–360. DOI: 10.1007/s00027-010-0135-x

Montes-Ortiz L, Goldschmidt T, Vásquez-Yeomans L, Elías-Gutiérrez M. 2021. A new species of *Litarachna* Walter, 1925 (Acari: Hydrachnidia: Pontarachnidae) from Corozal Bay (Belize), described based upon morphology and DNA barcodes. Acarologia 61(3):1–12. DOI: 10.24349/r7no-Ludg

Morselli I. 1980. Su tre Acari Prostigmati di acque salmastre dell'alto Adriatico. Atti della Società Toscana di Scienze Naturali: Memorie Serie B 87:181–195.

Moto A, Abé H. 2014. *Litarachna communis* Walter, 1925 (Acari, Hydrachnidae: Pontarachnidae): Taxonomic status, lectotype and paralectotype designation and redescription. Acarologia 54(2):201–220. DOI: 10.1051/acarologia/20142128

Pešić V. 2013. A new marine water mite species (Acari, Hydrachnidia, Pontarachnidae) from a coastal lake in Southeast Madagascar. Marine Biology Research 9(3):312–315. DOI: 10.1080/17451000.2012.739697

Pešić V, Asadi M, Cimpean M, Dabert M, Esen Y, Gerecke R, Martin P, Savić A, Smit H, Stur E. 2017. Six species in one: Evidence of cryptic speciation in the *Hygrobates fluviatilis* complex (Acariformes, Hydrachnidia, Hygrobatidae). Systematic & Applied Acarology 22(9):1327–1377. DOI: 10.11158/saa.22.9.4

Pešić V, Bankowska A, Goldschmidt T, Hårsaker K, Jovanović M, Kaitetzidou E, Krakowiak M, Kozłowska A, Michaloudi E, Michoński G, Miliša M, Pozojević I, Rewicz T, Rusiniak O, Sobolak K, Szućko I, Stryjecki R, Stur E, Szlauer-Łukaszewska A, Szenejko M, Zawal A. 2025. Checklist of water mites from the Balkan Peninsula: Second supplement, new DNA barcodes and description of a new species. Zootaxa 5676(1):1–74. DOI: 10.11646/zootaxa.5676.1.1

Pešić V, Chatterjee T, Alfaro M, Schizas N. 2014. A new species of *Litarachna* (Acari, Hydrachnidia, Pontarachnidae) from a Caribbean mesophotic coral ecosystem. ZooKeys 425:89–97. DOI: 10.3897/zookeys.425.8110

Pešić V, Chatterjee T, Ingole B, Velip D, Pavićević A. 2012. A new species of *Litarachna* Walter, 1925 (Acari: Hydrachnidia) from the West Indian coast, with a discussion on the diversity of the family Pontarachnidae Koenike, 1910. Cahiers de Biologie Marine 53:547–553.

Pešić V, Chatterjee T, Schizas N. 2012. A new species of *Pontarachna* (Acari, Hydrachnidia, Pontarachnidae) from a mesophotic coral ecosystem off Vieques Island, Puerto rico, Caribbean Sea. Zootaxa 3440(1):63–67. DOI: 10.11646/zootaxa.3440.1.3

Pešić V, Durucan F, Zawal A. 2019. Marine mites (Acari: Hydrachnidia) of the Mediterranean Sea: Descriptions of two new species, key for identification and future prospects. Zootaxa 4585(3):501–516. DOI: 10.11646/zootaxa.4585.3.6

Pešić V, Esen Y, Gerecke R, Goldschmidt T, Mumladze L, Smit H, Zawal A. 2022. Evidence of cryptic speciation in the *Hygrobates calliger* complex (Acariformes, Hydrachnidia, Hygrobatidae) with the description of two new species. Ecologica Montenegrina 59:101–122. DOI: 10.37828/em.2022.59.10

Pešić V, Smit H. 2016. On the identity of *Litarachna divergens* Walter, 1925 (Acari, Hydrachnidia: Pontarachnidae), with description of one new species. Marine Biodiversity 46(1):51–57. DOI: 10.1007/s12526-015-0316-x

Pešić V, Smit H, Konopleva ES. 2023. Molecular phylogeny and description of *Hygrobates cyrnusensis* sp. nov. reveals multiple colonization of Corsica by water mites of the *H. fluviatilis*-complex (Acariformes, Hydrachnidia, Hygrobatidae). Acarologia 63(1):262–274. DOI: 10.24349/h12f-rs8x

Pešić V, Zawal A, Bankowska A, Araújo R, Sługocki Ł, Rewicz T, Krakowiak M, Michoński G, Girão D, Silva LPD, Órfão I, Raposeiro PM, Ballini L, Stryjecki R, Ekrem T, Ferreira S. 2025. Exploring the water mite fauna (Acari, Hydrachnidia) of the Madeira archipelago: DNA barcoding reveals a remarkable species endemicity. Zootaxa 5621(5):501–513. DOI: 10.11646/zootaxa.5621.5.1

Pešić V, Zawal A, Ferreira S, Benitez-Bosco L, Cruz-Oliveira A, Girão D, Padilha A, Turaccio P, Rossini S, Ballini L, Staffoni G, Fratini S, Ciof C, Iannucci A, Ekrem T, Stur E. 2024. DNA barcode library of Portuguese water mites, with the descriptions of two new species (Acari, Hydrachnidia). ZooKeys 1217:119–171. DOI: 10.3897/zookeys.1217.131730

Philippi RA. 1840. Zoologische Bemerkungen. VI. *Pontarachna punctulum* Ph. eine Hydrachnide des Meeres. Archiv für Naturgeschichte 6:181–195.

Puillandre N, Brouillet S, Achaz G. 2021. ASAP: Assemble species by automatic partitioning. Molecular Ecology Resources 21(2):609–620. DOI: 10.1111/1755-0998.13281

Ratnasingham S, Hebert PD. 2013. A DNA-based registry for all animal species: The barcode index number (BIN) system. PLOS ONE 8(7):e66213. DOI: 10.1371/journal.pone.0066213

Ratnasingham S, Hebert PDN. 2007. BOLD: The barcode of Life data System. Molecular Ecology Notes 7(3):355–364. DOI: https://doi.org/10.1111/j.1471-8286.2007.01678.x

Sernow SA. 1913. Zur Frage über die Erforschung des Lebens im Schwarzen Meere. Mémoires de l'Academie impériale des sciences de St.-Pétersbourg 32(1):1–299.

Siokou AS, Ateş D, Ayas J, Ben Souissi T, Chatterjee T, Dimiza M, Durgham H, Dogrammatzi K, Erguden D, Gerakaris V, Grego M, Issaris Y, Kadis K, Katağan T, Kapiris K, Katsanevakis S, Kerkhof F, Papastergiadou E, Pešić V, Polychronidis L, Rifi M, Salomidi M, Sezgin M, Triantaphyllou M, Tsiamis K, Turan C, Tziortzis I, D'udekem D'Acoz C, Yaglioglu D, Zaouali J, Zenetos A. 2013. New Mediterranean Marine biodiversity records (June 2013). Mediterranean Marine Science 14(1):238–249.

Smit H. 2003. Five new species of the water mite family Pontarachnidae from western Australia (Acari: Hydrachnidia). In: Wells FE, Walker DI, Jones DS, editors The Marine Flora and fauna of Dampier, Western Australia 2. Perth: Western Australian Museum. pp. 547–562.

Smit H. 2007. *Litarachna brasiliensis* n. sp. the first member of the water mite family Pontarachnidae (Acari: Hydrachnidia) from South America. Systematic & Applied Acarology 12:141–146. DOI: 10.11158/saa.12.2.8

Soar CD. 1927. Report on a Hydrarachnid. In: Zoological results of the Cambridge expedition to the Suez Canal 1924. Transactions of the Zoological Society of London 22(3):287. DOI: 10.1111/j.1096-3642.1927.tb00377.x

Srivathsan A, Feng V, Suárez D, Emerson B, Meier R. 2024. Ontbarcoder 2.0: Rapid species discovery and identification with real-time barcoding facilitated by Oxford Nanopore R10.4. Cladistics 40(2):192–203. DOI: 10.1111/cla.12566

Srivathsan A, Hartop E, Puniamoorthy J, Lee WT, Kutty SN, Kurina O, Meier R. 2019. Rapid, large-scale species discovery in hyperdiverse taxa using 1D MinION sequencing. BMC Biology 17(1):96. DOI: 10.1186/s12915-019-0706-9

Srivathsan A, Lee L, Katoh K, Hartop E, Kutty SN, Wong J, Yeo D, Meier R. 2021. Ontbarcoder and MinION barcodes aid biodiversity discovery and identification by everyone, for everyone. BMC Biology 19(1):217. DOI: 10.1186/s12915-021-01141-x

Stålstedt J, Bergsten J, Ronquist F. 2013. Forms" of water mites (Acari: Hydrachnidia): Intraspecific variation or valid species? Ecology and Evolution 3(10):3415–3435. DOI: 10.1002/ece3.704

Uchida T. 1935. Marine water mites from Japan. Journal of the Faculty of Science, Hokkaido University. Series 6, Zoology 4 (4):183–195.

Viets K. 1956. Die Milben des Süßwassers und des Meeres. Hydrachnellae et Halacaridae (Acari) II. und III. Teil: Katalog und Nomenklator. Jena: Gustav Fischer. pp. 1–870.

Viets K. 1957. Die Wassermilben-Gattungen *Pontarachna* und *Litarachna* (Hydrachnellae, Acari). Journal of the Faculty of Science, Hokkaido University. Series 6, Zoology 13:147–155.

Von Schaub R. 1889. Über marine Hydrachniden nebst einigen Bemerkungen über *Midea* (BRUZ.). Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften. Mathematisch-Naturwissenschaftliche Classe 98:163–179.

Walter C. 1925. Marine Hygrobatidae. Revision der Wassermilben-Genera *Pontarachna* Philippi und *Nautarachna* Moniez. Internationale Revue der gesamten Hydrobiologie und Hydrographie 14(1–2):1–54. DOI: 10.1002/iroh.19250140102

Wiles PR, Chatterjee T, De Troch M. 2002. Two new and one known marine water mite (Acari: Hydrachnidia: Pontarachnidae) from South-East Africa. Journal of Natural History 36(16):1987–1994. DOI: 10.1080/00222930110068583