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

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Abstract

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

The other studied genera remained in the original ancestral Indo-West Pacific range. We hypothesize that similar internal environments of shrimp hosts from different phyla will function as hot spots for interphylum host switching in various lineages of symbionts.

Keywords

ancestral biogeography – ancestral character state analysis – morphological phylogeny – symbiosis – total evidence

Introduction

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

Interphylum host switches are common within marine palaemonid shrimps (Kou

et al., 2015; Horká et al., 2016; Chow et al., 2021). To obtain insight into host-switching events during the evolution of the marine Palaemonidae, Horká et al. (2016) performed a molecular phylogenetic analysis based on mitochondrial and nuclear genes. They recognized two distinct clades in which marine palaemonid shrimps radiated over bivalve mollusc and solitary ascidian hosts. From their analyses, they concluded that there were at least two lineages associated with solitary ascidians and two with bivalve molluscs, which were consistently related to each other (Horká et al., 2016). Additionally, two species of *Periclimenaeus* Borradaile, 1915 in the analysis were associated with compound ascidians, but clustered with sponge-endosymbionts (Horká et al., 2016). Similar results were found by a more recent study focussing on host spectrum and morphological adaptations (Chow et al., 2021). The number of host switches found in these two studies was higher than previous estimates, which were based on smaller datasets comprising less genera and species (Kou et al., 2015).

The ancestral host association of the clade containing *Conchodytes* Peters, 1852 and related species (fig. 1) is unresolved at present (Horká et al., 2016: fig. 4, clade 6; Chow et al., 2021: fig. 3, upper branch of clade IIIC). Previous phylogenetic analyses by Fransen & Reijnen (2012), Kou et al. (2015), Horká et al. (2016), and Chow et al. (2021) did not comprise

the entire generic and species diversity of these two clades known at that time and new species have been discovered since (e.g., De Gier & Fransen, 2018; Anker & De Grave, 2021; Fransen et al., 2021). It is hypothesized that the number of host switches is underestimated due to the limited number of taxa included in previous analyses. Consequently, there is a need for a more comprehensive and detailed phylogenetic analysis combined with the reconstruction of ancestral host-associations.

In this study, we extended the molecular alignment and included a morphological dataset, similar to those of Fransen (2002) and De Gier & Fransen (2018), to include all species that are hypothesized to be part of the aforementioned clade 6 from Horká et al. (2016) (table 1). Our assemblage of genera thus consisted of *Anchiopontonia* Bruce, 1992, *Bruceonia* Fransen, 2002, *Cainonia* Bruce, 2005, *Conchodytes* Peters, 1852, *Pinnotherotonia* Marin & Paulay, 2010, *Platypontonia* Bruce, 1968, and *Pseudopontonia* Bruce, 1992, which are associated with bivalve molluscs, and the genera *Ascidonia* Fransen, 2002, *Colemonia* Bruce, 2005, *Dactylonia* Fransen, 2002, *Notopontonia* Bruce, 1991, and *Rostronia* Fransen, 2002, of which all species are associated with solitary ascidians.

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

the exception of *P. chimaera* Holthuis 1951, which lives inside the gastropod *Strombus galeatus* Swainson, 1823. The recently designated monotypic genus *Opaepupu* Anker & De Grave, 2021 is also included in the analysis. This genus also associates with bivalves, with the only currently recognised species living in symbiosis with *Trapezium oblongum* (Linnaeus, 1758). Although the systematic position of this genus may not be immediately clear due to its distinct unique morphology and lack of obvious similarity with other species, Anker & De Grave (2021) argued it is morphologically most similar to several IWP genera that also associate with bivalves, namely *Anchiopontonia*, *Bruceonia*, *Cainonia*, *Pinnotherotonia*, *Platypontonia* and *Pontonia*. *Opaepupu huna* Anker & De Grave, 2021 may thus also fall into the aforementioned clade and is therefore included in the present study.

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

Material and methods

Taxon sampling

A total of 55 ingroup and five outgroup species were included in this study (supplementary

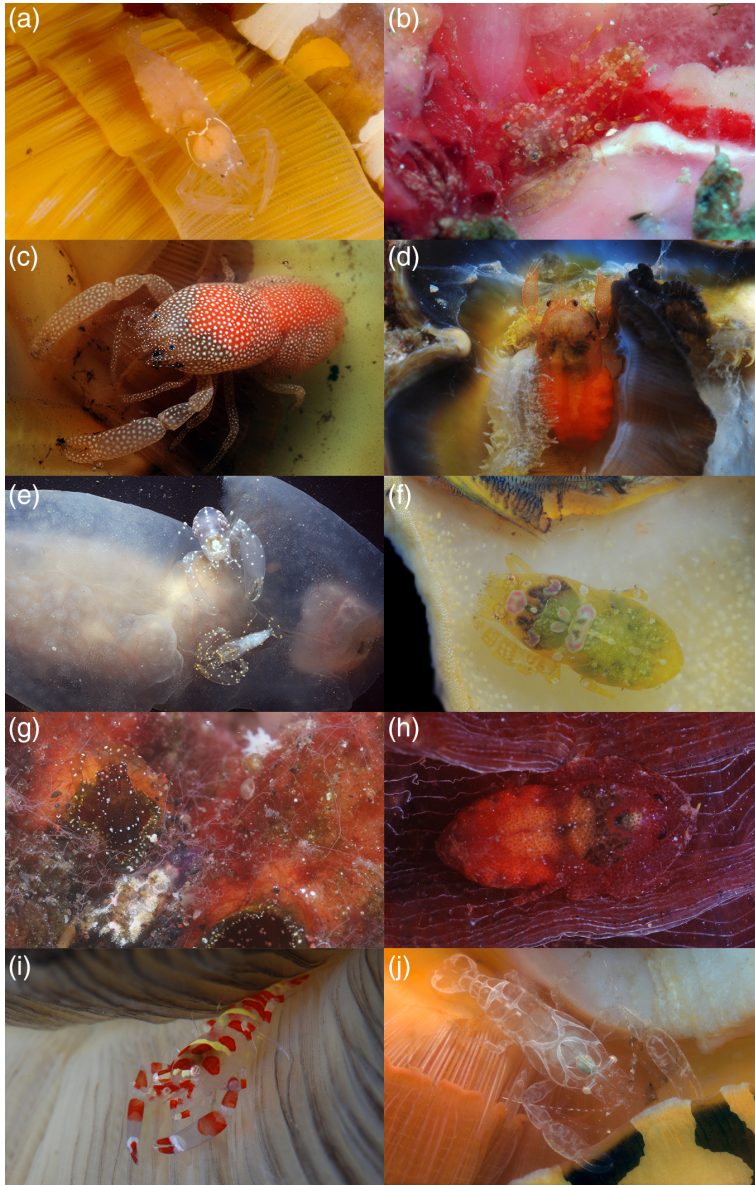


FIGURE 1 An overview of the diversity in morphology, colouration, and host-associations of the studied clade. A, *Anchiopontonia hurii* (Holthuis, 1981) in the spiny oyster *Spondylus* sp.; B, *Ascidonia quasipusilla* (Chace, 1972) in a solitary ascidian; C, *Conchodytes meleagrinae* Peters, 1852 in the spiny oyster *Spondylus* sp.; D, *Conchodytes pteriae* Fransen, 1994 in the pearl oyster *Pteria loventi* (Dunker, 1879); E, male-female pair of *Dactyлонia ascidicola* (Borradaile, 1898) from the solitary ascidian *Ascidia* sp.; F, *Odontonia katoi* (Kubo, 1940) in the solitary ascidian *Polycarpa aurata* (Quoy & Gaimard, 1834); G, *Odontonia plurellicola* De Gier & Fransen, 2018 in the colonial ascidian *Plurella* sp.; H, *Odontonia sibogae* (Bruce, 1973) in the solitary ascidian *Polycarpa* sp.; I, *Platypontonia hyotis* Hipeau-Jacquotte, 1971 in the giant honeycomb oyster *Hyotissa hyotis* (Linnaeus, 1758); J, *Pontonia manningi* Fransen, 2000 in the spiny oyster *Spondylus americanus* Hermann, 1781.

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appendix S1; table 1, supplementary table S1). Of those included in the ingroup, 39 species have their distribution in the Indo-West Pacific region (IWP), eight in the East Pacific region (EP), and another eight in either the West and/or East Atlantic Ocean (W/E Atl) (table 1). Five outgroup taxa were selected to span across the spectrum of hosts within the symbiotic Palaemonidae, representing echinoderm, sponge and cnidarian associates and one mostly free-living species (supplementary table S1). These are *Actinimenes inornatus* (Kemp, 1922), *Actinimenes ornatus* (Bruce, 1969), *Cuapetes tenuipes* (Borradaile, 1898), *Periclimenes colemani* Bruce, 1975 and *Typton wasini* Bruce, 1977. With the exception of *C. tenuipes*, all these outgroup species fall within Clade 5 of Horká et al. (2016), which is the sister clade of the present clade of focus. All outgroup species have an IWP distribution.

Fresh specimens were collected by SCUBA diving or other standard collection methods (supplementary table S1). Recently collected specimens were directly stored in 96% ethanol for DNA barcoding and identification. Subsampling was done by removing the left second and third pleopod or left third and fourth pereopod of each specimen. After subsampling, specimens were moved to 70% ethanol for morphological analysis and long-term storage in the Naturalis Biodiversity Center decapod collection (RMNH.CRUS.D.; Leiden, The Netherlands) or the Museum Zoologicum Bogoriense (MZB.; Bogor, Indonesia). The newly sequenced material consists of 24 species, spread over seven genera. In addition to the newly generated sequences, sequence data from GenBank (Sayers et al., 2020) was used (supplementary table S1).

DNA extraction, amplification and sequencing

Total genomic DNA was extracted from the subsampled tissue using the DNeasy Blood

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

TABLE 1 Overview of all species within the studied clade, their general distribution range and preferred host group. Abbreviations: IWP = Indo-West Pacific, EP = East Pacific region, E Atl = East Atlantic Ocean, W Atl = West Atlantic Ocean. Detailed information and literature sources are provided in the appendices (supplementary appendix S1)

| Ingroup species | Region | Host group(s) |
|--|-----------|---|
| <i>Anchiopontonia</i> Bruce, 1992 | | |
| <i>A. hurii</i> (Holthuis, 1981) | IWP | Mollusca: Bivalvia: Pectinida: Spondylidae |
| <i>Ascidonia</i> Fransen, 2002 | | |
| <i>A. californiensis</i> (Rathbun, 1902) | EP | Chordata: Ascidiacea: Phlebobranchia: Ascidiidae |
| <i>A. flavomaculata</i> (Heller, 1864) | E Atl | Chordata: Ascidiacea: Phlebobranchia: Ascidiidae |
| <i>A. miserabilis</i> (Holthuis, 1951) | W Atl | Chordata: Ascidiacea: Phlebobranchia: Ascidiidae |
| <i>A. pusilla</i> (Holthuis, 1951) | EP | Chordata: Ascidiacea: Stolidobranchia: Pyuridae – Aplousobranchia: Diazonidae |
| <i>A. quasipusilla</i> (Chace, 1972) | E + W Atl | Chordata: Ascidiacea: Solidobranchia: Pyuridae |
| <i>Bruceonia</i> Fransen, 2002 | | |
| <i>B. ardeae</i> (Bruce, 1981) | IWP | Mollusca: Bivalvia: Venerida: Chamidae |
| <i>Cainonia</i> Bruce, 2005 | | |
| <i>C. medipacifica</i> (Edmondson, 1935) | IWP | Mollusca: Bivalvia: Pectinida: Spondylidae – Venerida: Chamidae |
| <i>Colemonia</i> Bruce, 2005 | | |
| <i>C. litodactylus</i> Bruce, 2005 | IWP | Chordata: Ascidiacea indet. |
| <i>Conchodytes</i> Peters, 1852 | | |
| <i>C. biunguiculatus</i> (Paulson, 1875) | IWP | Mollusca: Bivalvia: Ostreida: Pinnidae |
| <i>C. chadi</i> (Marin, 2011) | IWP | Mollusca: Bivalvia: Ostreida: Ostreidae |
| <i>C. kempoides</i> Bruce, 2013 | IWP | Mollusca: Bivalvia: Ostreida: Isognomonidae |
| <i>C. maculatus</i> Bruce, 1989 | IWP | Mollusca: Bivalvia: Ostreida: Margaritidae |
| <i>C. meleagrinae</i> Peters, 1852 | IWP | Mollusca: Bivalvia: Ostreida: Margaritidae |
| <i>C. monodactylus</i> Holthuis, 1952 | IWP | Mollusca: Bivalvia: Ostreida: Pteriidae, Pinnidae |
| <i>C. nipponensis</i> (De Haan, 1844) | IWP | Mollusca: Bivalvia: Ostreida: Pinnidae |
| <i>C. philippinensis</i> Bruce, 1996 | IWP | Unknown, probably Mollusca: Bivalvia |
| <i>C. placunae</i> (D.S. Johnson, 1967) | IWP | Mollusca: Bivalvia: Pectinida: Placunidae |
| <i>C. pteriae</i> Fransen, 1994 | IWP | Mollusca: Bivalvia: Ostreida: Pteriidae |
| <i>C. tridacnae</i> Peters, 1852 | IWP | Mollusca: Bivalvia: Cardiida: Cardiidae |
| <i>Dactylonia</i> Fransen, 2002 | | |
| <i>D. anachoreta</i> (Kemp, 1922) | IWP | Chordata: Ascidiacea: Stolidobranchia: Styelidae |
| <i>D. ascidicola</i> (Borradaile, 1898) | IWP | Chordata: Ascidiacea: Phlebobranchia: Ascidiidae |

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| Ingroup species | Region | Host group(s) |
|---|--------|--|
| <i>D. borradalei</i> Bruce, 2005 | IWP | Chordata: Ascidiacea: Phlebobranchia: Ascidiidae |
| <i>D. carinacula</i> Bruce, 2006 | IWP | Unknown, probably from encrusting Chordata: Ascidiacea |
| <i>D. franseni</i> Bruce, 2003 | IWP | Chordata: Ascidiacea: Phlebobranchia: Ascidiidae |
| <i>D. holthuisi</i> Fransen, 2002 | IWP | Chordata: Ascidiacea: Phlebobranchia: Plurellidae |
| <i>D. monnioti</i> (Bruce, 1990) | IWP | Chordata: Ascidiacea: Phlebobranchia: Ascidiidae |
| <i>D. okai</i> (Kemp, 1922) | IWP | Chordata: Ascidiacea: Phlebobranchia: Ascidiidae |
| <i>Notopontonia</i> Bruce, 1991 | | |
| <i>N. platycheles</i> Bruce, 1991 | IWP | Chordata: Ascidiacea: Stolidobranchia: Pyuridae |
| <i>Odontonia</i> Fransen, 2002 | | |
| <i>O. bagginsi</i> De Gier & Fransen, 2018 | IWP | Chordata: Ascidiacea indet. |
| <i>O. compacta</i> (Bruce, 1996) | IWP | Chordata: Ascidiacea: Stolidobranchia: Pyuridae |
| <i>O. katoi</i> (Kubo, 1940) | IWP | Chordata: Ascidiacea: Stolidobranchia: Styelidae, Pyuridae |
| <i>O. kerangcaris</i> Fransen, Groenhof & De Gier, 2021 | IWP | Mollusca: Bivalvia: Venerida: Chamidae |
| <i>O. plurellicola</i> De Gier & Fransen, 2018 | IWP | Chordata: Ascidiacea: Phlebobranchia: Plurellidae |
| <i>O. rufopunctata</i> Fransen, 2002 | IWP | Chordata: Ascidiacea: Stolidobranchia: Styelidae |
| <i>O. seychellensis</i> Fransen, 2002 | IWP | Unidentified Chordata: Ascidiacea |
| <i>O. sibogae</i> (Bruce, 1973) | IWP | Chordata: Ascidiacea: Stolidobranchia: Styelidae, Pyuridae |
| <i>O. simplicipes</i> (Bruce, 1996) | IWP | Chordata: Ascidiacea: Stolidobranchia: Styelidae |
| <i>Opaepupu</i> Anker & De Grave, 2021 | | |
| <i>O. huna</i> Anker & De Grave, 2021 | IWP | Mollusca: Bivalvia: Venerida: Trapezidae |
| <i>Pinnotherotonia</i> Marin & Paulay, 2010 | | |
| <i>P. rumphiusi</i> Marin & Paulay, 2010 | IWP | Mollusca: Bivalvia: Venerida: Veneridae |

TABLE 1 Overview of all species within the studied clade, their general distribution range and preferred host group. Abbreviations: IWP = Indo-West Pacific, EP = East Pacific region, E Atl = East Atlantic Ocean, W Atl = West Atlantic Ocean. Detailed information and literature sources are provided in the appendices (supplementary appendix S1) (*cont.*)

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

lengths were checked using Thermofisher SYBR Safe DNA stained 2% agarose gels. Sanger sequencing reactions were performed by BaseClear B.V., Leiden, The Netherlands.

Morphological character state analysis

All ingroup and outgroup species were scored morphologically, based on descriptions and illustrations in previous literature, as well as specimens present in the Naturalis collections

(supplementary table S1). Specimens were studied with a dissecting stereomicroscope (Zeiss Discovery.V8) and a compound microscope (Olympus BX53). The morphological data matrix contains 85 characters (supplementary appendix S2, supplementary table S2), and was formatted based on previous analyses by Fransen (2002) and De Gier & Fransen (2018) on a subset of the species in the current analysis. Multistate characters 3, 4,

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

Phylogeny reconstructions

Multiple sequence alignments were obtained using the ClustalW (Thompson et al., 1994) algorithm (default settings), and their ends were trimmed manually in MEGA v10.2.5 (Kumar et al., 2018). Alignments of protein-coding genes COI and H3 were subjected

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

A concatenated dataset containing all molecular markers was constructed in MEGA v10.2.5 (Kumar et al., 2018). This alignment consisted of 105 sequences and 1791 positions (table 2). Phylogenetic analyses were performed under the maximum likelihood (ML) and Bayesian Inference (BI) criteria using RAxML via the on-line CIPRES (Miller et al., 2010) with the RAxML-NG BlackBox v1.0.0 tool (Kozlov et al., 2019) and MrBayes v3.2.7 (Ronquist & Huelsenbeck, 2003), respectively. For the single-marker ML analyses

TABLE 2 Analytical methods and the used models for sequence evolution.

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

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

default settings were used and automatic bootstrapping with a cut-off of 0.03, under an unpartitioned model. The concatenated four-marker dataset used a partitioned model with scaled branch length linkage and automatic bootstrapping with a cut-off of 0.03. Rest of the settings were default. The BI analyses were conducted using a Markov Chain Monte Carlo (MCMC) method with two independent runs and four chains. Analyses were run with a minimum of 5,000,000 generations to ensure that the average standard deviation of split frequencies (ASDSF) reached a value < 0.01 , which would indicate the two runs have converged to a stationary distribution. The BI analysis on the concatenated molecular dataset did not reach this threshold after an additional increase of 15,000,000 generations (making a total of 20,000,000 generations) and was terminated with a ASDSF value of 0.010234. Trees were sampled each 500 generations. The initial 25% of trees was discarded as burn-in and the remaining trees were used to generate consensus trees and to estimate Bayesian posterior probabilities (PP). The BI analysis was consequently examined using MCMC Convergence Diagnostics in Tracer v1.7.1 (Rambaut et al., 2018), where the 0.25 burn-in fraction was taken into account. Closer inspection of the trace plots of the log-likelihood indicated that convergence was reached, with an effective sample size (ESS) of 3477.3.

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

A total evidence (TE) analysis was done, similar to the one used in Van Der Wal et al. (2019). For this approach, concatenation of the molecular and morphological data was

performed manually using a standard text editor. This alignment consisted of 66 sequences and 1876 positions (see table 2). All species were represented by morphological data, and where possible, with a set of sequences of all four available markers. If a species was found to be represented in multiple lineages in the previous single-markers analyses, all lineages were represented with their own sequences, in addition to a copy of the morphological data for this species. The selected sequences used in the TE analysis can be found in the appendices (supplementary table S1). Models for sequence evolution were as defined previously (table 2). Ordered and irreversible states were treated accordingly in MrBayes (Ronquist & Huelsenbeck, 2003), using the command ctype (table 2).

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

Biogeographical analysis

In order to reconstruct the ancestral biogeographic ranges of the studied ingroup, broad current geographical distributions were obtained from taxonomic and ecological literature (supplementary appendix S1). Distributions were classified as mentioned above (EP, IWP, E Atl, W Atl). The resulting ML tree from the TE analysis was used as a backbone for figures, but an ultrametric version of the tree was used during the analyses. The ultrametric version of the phylogenetic tree was made using the force.ultrametric() function of the R-package phytools (Revell, 2012).

Three different models of biogeographic evolution were assessed using the R-package BioGeoBEARS (Matzke, 2013), similar to other studies focussing on other marine organisms (e.g., Baraf et al., 2019). The following models were used for comparison: Dispersal-Extinction-Cladogenesis (DEC) (Ree & Smith, 2008); Dispersal-Vicariance Analysis (DIVA) (Ronquist, 1997); and Bayesian inference of historical biogeography for discrete areas (BAYAREA) (Landis et al., 2013). AIC scores were used to choose the best-fitting model, and can be found in the appendices (supplementary table S3). Given the recent concerns regarding the addition of the founder speciation event parameter “J” (Ree & Sanmartín, 2018), the current analyses resort to the most commonly used models for comparison without considering “jump” (e.g., Baraf et al., 2019). For all analyses, a restricted model was used to limit the movement of ranges to only adjacent areas. This was done using a matrix file, as described in the BioGeoBEARS manual (Matzke, 2013). The resulting pie-charts displaying the ML values were edited and added to the phylogeny in Adobe Illustrator CC (Adobe Systems, USA). ML-values for all probabilities can be found in the appendices (supplementary table S4).

Ancestral state reconstruction of host associations

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

Results

Molecular phylogeny reconstructions

Three major clades can be distinguished in the ingroup, with most deeper nodes bearing support values lower than 50 (fig. 2). Various intrageneric branches within *Conchodytes*, *Ascidonia* and *Pontonia* were recovered with both high bootstrap and Bayesian PP support values. All outgroups were separated from the ingroup, except for one *Periclimenes colemani* specimen of which only a 18S sequence was available, which nested within a clade of *Dactyлонia* (see discussion). One of two branches of *Platypontonia hyotis* Hipeau-Jacquotte, 1971 was the most basal ingroup lineage, while the other branch seems to be a basal branch within the first clade (see below). The placement of *P. hyotis* is debated in the discussion.

The first clade contains the Atl/EP genera *Ascidonia* and *Pontonia*, the IWP genus *Anchiopontonia*, and one branch of *Platypontonia hyotis*. Although some of the intrageneric species relations of this clade are resolved with high bootstrap or Bayesian PP values, the topology of the deeper nodes is resolved with very low bootstrap values and different topologies in the Bayesian analysis. Basally, the second branch of *Platypontonia hyotis* and a branch with five sequences of *Anchiopontonia hurii* (Holthuis, 1981) are split off from the first clade respectively, after which the monophyletic genus *Ascidonia* (consisting of *Ascidonia miserabilis* (Holthuis, 1951), and four sequences of *Ascidonia quasipusilla* (Chace, 1972)) appears to be sister of the monophyletic genus *Pontonia*. Within *Pontonia*, various species seem to be phylogenetically paraphyletic: *Pontonia manningi* Fransen, 2000 can be found in a basal branch also containing one sequence of *Pontonia margarita* Smith, 1869, and in more derived branches, of which one is also containing an

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

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

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

Morphological phylogeny

The morphological analysis recovered all the genera as monophyletic (fig. 3). *Cuapetes tenuipes*, *Actinimenes inornatus*, *A. ornatus* and *Periclimenes colemani* were recovered as outgroup species, while *Typton wasini* clustered with the ingroup species *Colemonia*

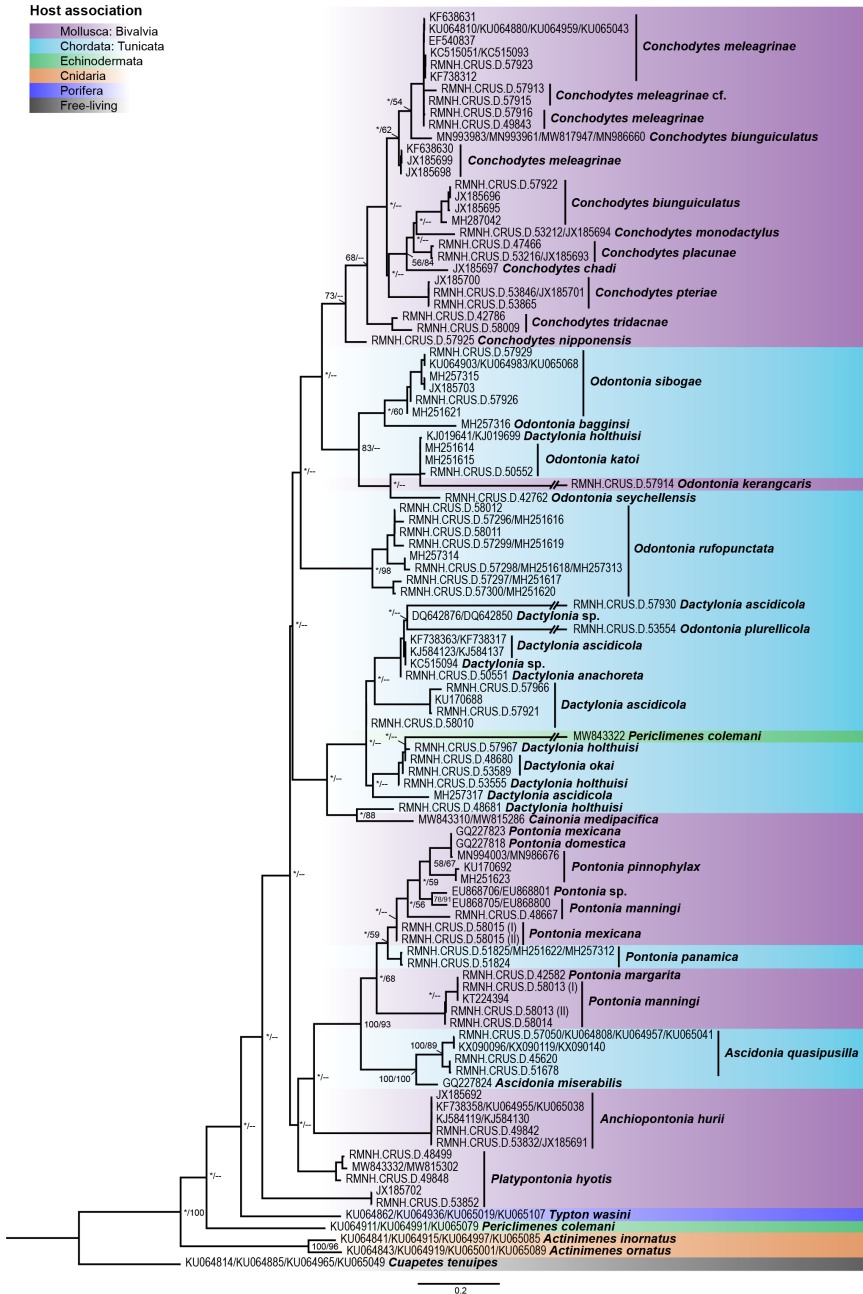


FIGURE 2 Phylogeny based on the RAxML tree topology of the concatenated molecular dataset (CO1, H3, 16S, 18S). RAxML bootstrap support and Bayesian posterior probabilities expressed as percentages are indicated respectively. Dashes (–) indicate values <50; asterisk (*) indicates different topology of RAxML or MrBayes tree. Four branches are shortened for convenience. Three major clades can be recognized. Newly acquired barcodes are indicated with a collection accession number (RMNH.CRUS.D., MZB.), otherwise GenBank accession numbers are given. The selection of species can be found in the appendices (supplementary table S1). Colours indicate various host associations.

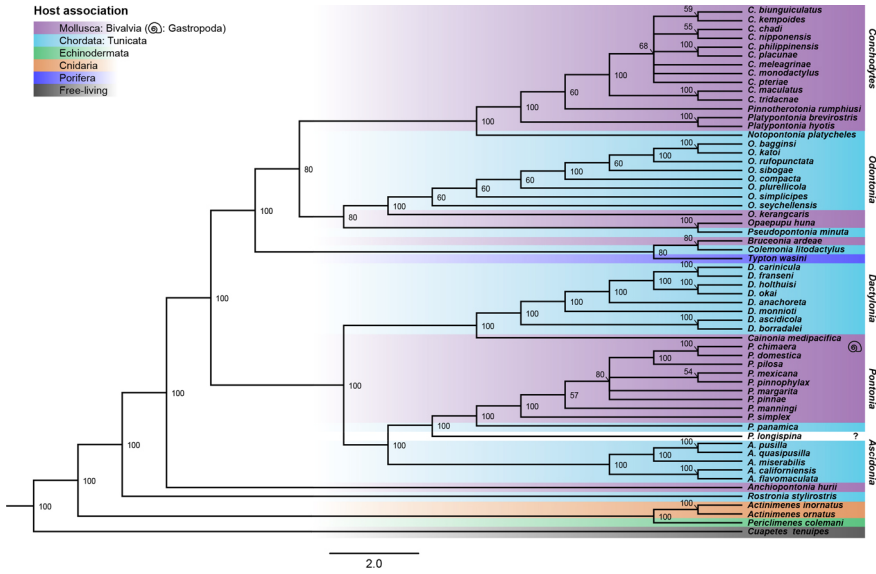


FIGURE 3 The 50% majority rule consensus tree of the morphological analysis in PAUP. Five distinct clades can be recognized. Support values are given at every dichotomous or polytomous branching. Colours indicate various host associations. The host association of *Pontonia longispina* Holthuis, 1951 is unknown, indicated with a question mark (?), and the known host association of *Pontonia chimaera* Holthuis, 1951 is with gastropod molluscs, which is indicated with an outline of a shell.

litodactylus Bruce, 2005 and *Bruceonia ardeae* (Bruce, 1981) in the 50% majority consensus tree (fig. 3). Five distinct, well-supported clades could be recognized, with the monotypic IWP genera *Rostronia* and *Anchiopontonia* branching off basally, accompanied by high (100) support values. The first clade contains the Atl/EP genera *Pontonia* and *Ascidonia* that together form a well-supported lineage (100). Branching off this first clade is the second well-supported (100) clade, containing the IWP genera *Cainonia* and *Dactylonia*. Sister of the first two clades are the following three clades, in succession: the third well supported (100) clade containing the outgroup species *Typton wasini* basally, and the two monotypic IWP genera *Bruceonia* and *Colemonia*; the fourth well supported (100) clade contains the two IWP genera *Pseudopontonia* and *Opaepupu* basally, and all species of *Odontonia*; the fifth and last clade contains *Notopontonia* most basally, after which *Platypontonia* and later

Pinnotherotonia branched off, the latter forming a direct sister to *Conchodytes*.

Two somewhat supported polytomies can be found in the morphological phylogeny: one in the genus *Conchodytes*, branching off into six lineages (of which three contain two species); and one in the genus *Pontonia*, branching off to four lineages (of which one contains two species, and one three species).

Total Evidence analysis

Four clades can be distinguished based on the TE approach (fig. 4). Unfortunately, the deeper nodes in the phylogeny had support values < 50 and inferences from these nodes should thus be made with caution. The in- and outgroup were confidently separated, illustrated by both high bootstrap and Bayesian PP support values. *Colemonia litodactylus* was the most basal ingroup lineage.

The first clade contains the Atl/EP genera *Ascidonia* and *Pontonia*. Within *Pontonia*, *Pontonia panamica* was basal to the rest of

the genus. While the multiple sequences of *Ascidonia quasipusilla* clustered together nicely, the two sequences of *P. pinnophyllax* were divided by a branch containing *P. mexicana*.

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

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

basal species in *Conchodytes*, with somewhat high support values.

Historical biogeography analysis

An ancestral state reconstruction analysis was performed to identify ancestral biogeographic ranges of the studied ingroup taxa. Of the studied models, the DEC model was retrieved with the lowest AIC score (see supplementary table S3). The clade with the genera *Pontonia* and *Ascidonia* has a distribution outside Indo-West Pacific area (see table 1). The common ancestor of this clade has an EP/W Atl distribution (fig. 5: node A, $P = 0.77$). *Pontonia* has maintained an EP distribution until the common ancestor of *P. manningi* evolved with an ancestral range in the EP/W Atl (fig. 5: node B, $P = 0.98$).

The common ancestor of the genus *Ascidonia* was recovered to have an EP/W Atl distribution (fig. 5: node C, $P = 0.91$). This remains the ancestral range for the common ancestor of the clade containing *Ascidonia pusilla* (Holthuis, 1951) and *A. quasipusilla*. The ancestor of the other clade with sister species *Ascidonia californiensis* (Rathbun, 1902) and *Ascidonia flavomaculata* (Heller, 1864) was recovered as mainly W Atl (fig. 5: node D, $P = 0.81$).

Ancestral state reconstruction of host associations

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

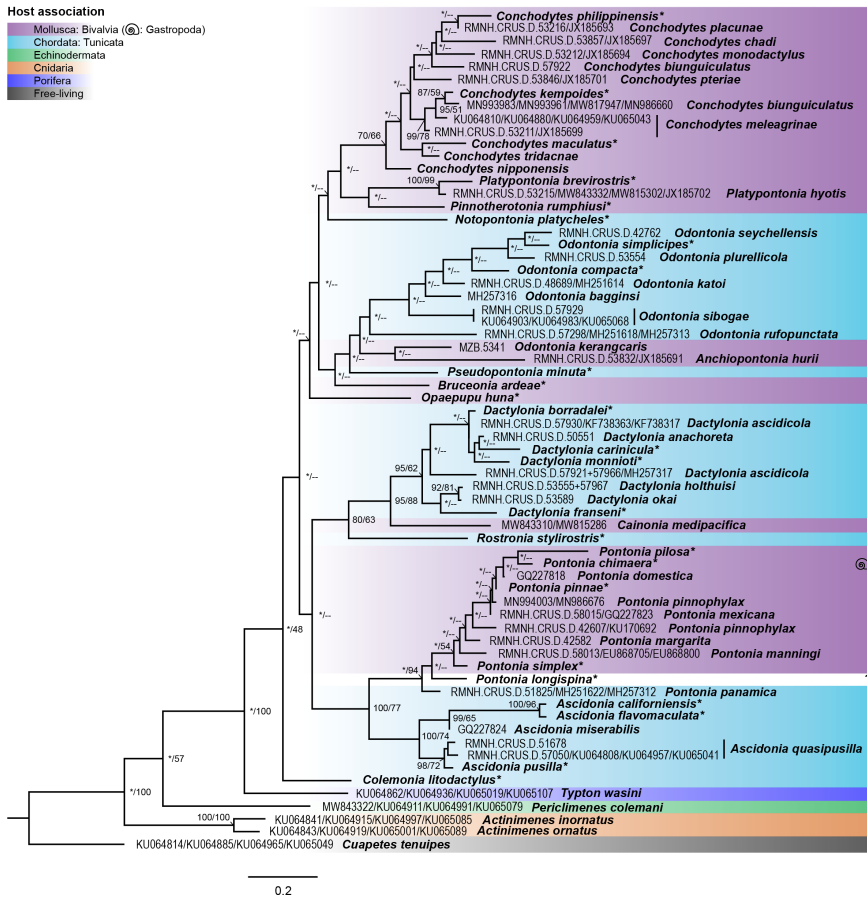


FIGURE 4 Phylogeny based on the RAxML tree topology of the TE approach. RAxML bootstrap support and Bayesian posterior probabilities expressed as percentages are indicated respectively. Dashes (--) indicate values <50; asterisk (*) indicates different topology of RAxML or MrBayes tree. Four major clades can be recognized. Newly acquired barcodes are indicated with a collection accession number (RMNH.CRUS.D., MZB.), otherwise GenBank accession numbers are given. The selection of species can be found in the appendices (supplementary table S1), and species of which only morphological data was analysed are indicated with an asterisk (*) and no accession number. Colours indicate various host associations. The host association of *Pontonia longispina* Holthuis, 1951 is unknown, indicated with a question mark (?), and the known host association of *Pontonia chimaera* Holthuis, 1951 is with gastropod molluscs, which is indicated with an outline of a shell.

containing *Ascidonia*, *Dactyлонia* and most of *Odontonia* are fully resolved to be associated with ascidians.

Most interesting are the ancestral character states of the entire ingroup, and nodes where interphylum host-switches seem to occur. The character state of the ancestral node of the entire ingroup is recovered to be an ascidian-associated species, both with or

without the inclusion of the basally branching *Colemonia* (fig. 6: node I and II, $P = 0.99$ and $P = 1.00$). While the ancestral host association of the first major clade, containing *Conchodytes*, *Odontonia* and related genera, is recovered as a bivalve associate (fig. 6: node V, $P = 0.62$), the character state of the ancestor when including *Opeapupu* is still recovered as an ascidian-associate (fig. 6, node III,

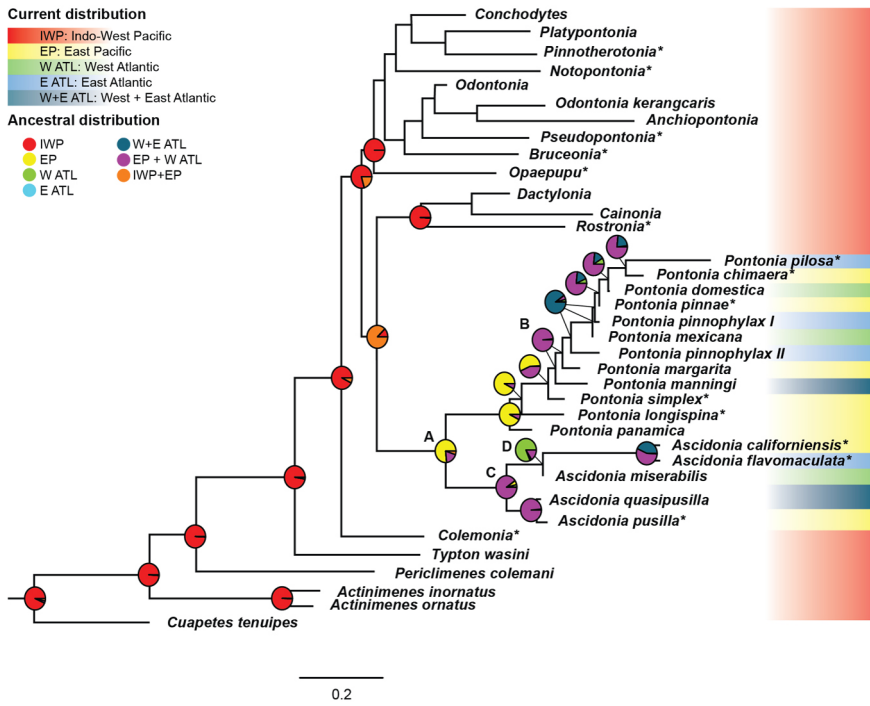


FIGURE 5 Phylogeny based on the RAxML tree topology of the TE approach (fig. 4), with ancestral biogeographic range reconstructions on the internal nodes (probabilities are shown as pie charts). Indo-West Pacific genera simplified as genus names, except for *Odontonia kerangcaris* Fransen, Groenhof & De Gier, 2021 due to its position outside of the genus. Colours indicate distribution ranges, both for the species as well as the ancestral distribution ranges. Species/genera of which only morphological data was analysed are indicated with an asterisk (*).

$P = 0.95$). A host switch has occurred from an ascidian to a bivalve mollusc host at the base of this clade, but also separately in the branch containing *Opeapupu*. In addition, the ancestral character state of the clade including *Conchodytes*, *Platypontonia*, *Pinnotherontonia*, and *Notopontonia*, was recovered to be a bivalve-associate, even when taking the ascidian-associated *Notopontonia* into account (fig. 6: node VI, $P = 0.64$). This means that there has occurred a similar switch, albeit now from a bivalve mollusc to an ascidian host, within the branch of *Notopontonia*. Within the other part of the clade, comprised of *Odontonia*, *Anchiopontonia*, *Pseudopontonia*, and *Bruceonia*, two to three host switches, again from a bivalve mollusc to an ascidian host, can be observed. First one in the single branch of

Pseudopontonia, and later in the branch containing *O. rufopunctata*, and in the other lineage of *Odontonia* (excluding *O. kerangcaris*).

The ancestral state of the clade containing *Pontonia*, *Ascidonia*, *Dactylonia* and related species is recovered as an ascidian-associated lineage (fig. 6: node IV, $P = 1.00$). Within this clade, two host switches from an ascidian host to a bivalve mollusc, and one within-phyllum (from a bivalve mollusc to a gastropod mollusc) host switch can be found. The subclade containing *Dactylonia*, *Cainonia* and *Rostronia* can be observed to have an inter-phyllum host switch at a basal branch of the clade (after *Rostronia* splits off, at the branching with *Cainonia*): the ancestral character state of this node is recovered as an ascidian associated species (fig. 6: node IX, $P = 0.99$). In

contrast to the monotypic *Cainonia*, the various bivalve-associated *Pontonia* species form a separate clade after splitting off from their ascidian associated congener, *P. panamica*. The unknown host association of *P. longispina* causes the ancestral state reconstruction of their joint node to have a lower probability in favour of an ascidian-associated ancestor (fig. 5: node X, $P = 0.67$). The intergeneric host switch of *P. chimaera* from a bivalve to a gastropod mollusc, results in a fully resolved ancestral character reconstruction in favour of a bivalve mollusc host (fig. 5: node XI, $P = 1.00$).

Discussion & Conclusions

Uncertain placement of ingroup species among different tree topologies

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

Anchiopontonia hurii can be observed to be most closely related to the recently described species *Odontonia kerangcaris* in the TE analysis (fig. 4), while it was placed basally to

Ascidonia and *Pontonia* in the concatenated and morphological alignment (figs. 2, 3). Bruce (1992) argued that *A. hurii* was closely related to some other species that have long dorsal telson spines, but this character can be found in various lineages of the studied ingroup. Previous phylogenetic studies have at least confirmed the basal placement of the species, but if the species is basal to some IWP genera (e.g., Fransen & Reijnen, 2013, Chow et al., 2021) or to the entire, or a part of the ingroup (e.g., Gan et al., 2015, Horká et al., 2016), remains elusive.

While the placement of *Conchodytes* as a genus is fairly well resolved in all phylogeny reconstructions, the placement of its species is far from similar in all analyses. In addition, previous literature based on morphological data does not seem to fully comply with the current results based on molecular data (e.g., Holthuis, 1952; Bruce, 1989; Fransen, 1994b; Bruce, 2013). In the current analyses, there seems to be a consensus about the basal placement of *C. nipponensis* and *C. tridacnae* (and the closely related *Conchodytes maculatus* Bruce, 1989, which is only represented by morphological data) to the rest of the genus, but the support values resulting from the TE and concatenated alignment are rather low (figs. 2 and 4). The deviation in placements might be related to the morphological similarity of *C. nipponensis* to the other members of the genus (fig. 3) (e.g., Fransen, 1994b). Bruce (1989) mentioned that *C. maculatus* is morphologically quite distinct, suggesting a basal placement too in the genus. The remaining species of *Conchodytes* seem to cluster in two lineages in the TE and concatenated approach (figs. 2 and 4), similar to the previous phylogenetic studies on the genus (Fransen & Reijnen, 2012, 2013): *C. chadi*, *C. placunae*, *C. monodactylus* and (one lineage of) *C. biunguiculatus* seem to cluster together in all analyses, complemented by one species

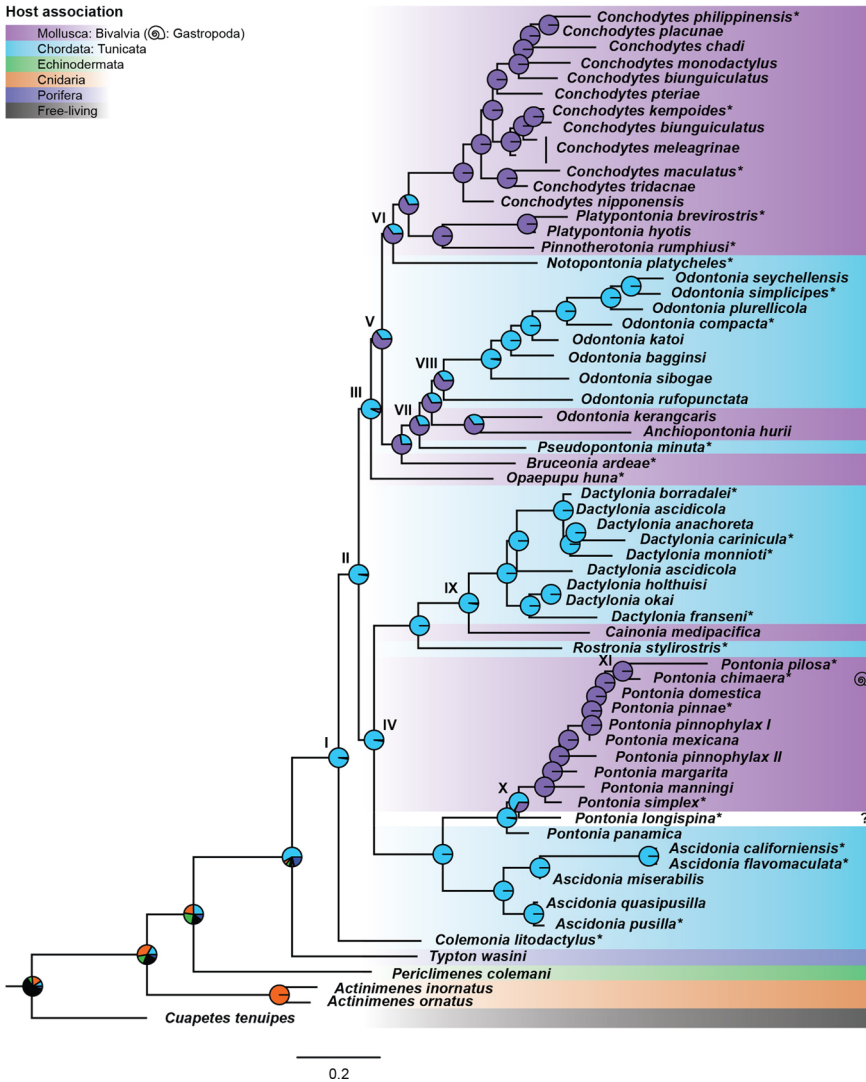


FIGURE 6 Phylogeny based on the RAxML tree topology of the TE approach (fig. 4), with ancestral character state reconstructions on the internal nodes (probabilities are shown as pie charts). Colours indicate various host associations, both for the species as well as the ancestral character states. The host association of *Pontonia longispina* Holthuis, 1951 is unknown, indicated with a question mark (?), and the known host association of *Pontonia chimaera* Holthuis, 1951 is with gastropod molluscs, which is indicated with an outline of a shell. Species of which only morphological data was analysed are indicated with an asterisk (*).

which is only represented by morphological data (*C. philippinensis* Bruce, 1996). It is worth noting that the previous phylogenetic studies of this genus used only one molecular marker (COI). Interestingly, Bruce (1996) mentions *C. philippinensis* to be most related to *C. nipponensis* and *C. maculatus*, which are

here recovered as one of the basal species within *Conchodytes* (fig. 4). While in both analyses *C. pteriae* is represented as a basal species related to this cluster, previous literature states *C. pteriae* to be a sister to *C. meleagrinae*, a species from the other group in the present analyses.

The second group in the present analyses is a cluster of two lineages of *C. meleagrinae*, and one lineage of *C. biunguiculatus*. In addition, this cluster also includes *C. kempoides*, which is only represented by morphological data from the limited published illustrations and description (Bruce, 1989, 2013). As mentioned above, *C. meleagrinae* and *C. biunguiculatus* are represented by two lineages each, the latter lineages even found in both *Conchodytes* groups. The possible explanations for these may be deduced from the localities and host of the species (supplementary table S1): the *C. biunguiculatus* specimen from Chow et al. (2020, 2021) which does not comply with the phylogenetic position found in previous studies (Fransen & Reijnen, 2012, 2013) comes from the Red Sea (Eilat, Israel), from a *Pinctada margaritifera* (Linnaeus, 1758). The other specimens are from more Eastern waters (Indian Ocean, IWP; supplementary table S1), presumably from different species of bivalves (presumably all *Pinna* spp.). The genetic distance between the two lineages of *C. biunguiculatus* can partially be explained by the geographical distance between both populations, although a placement as sister species would be expected (as can be seen in the genus *Alpheus* Fabricius, 1798 in Williams et al., 2001). Similarly, a difference in host species would possibly not affect the genetic distance between populations, as was proven in *Z. soror* by Antokhina & Sorokin (2010). This was however just proven in one species, and is worth examining in other palaemonid shrimp species with a smaller host-range. Physical examination of this specimen is needed to find out if this was correctly identified or whether it belonged to an undescribed species of *Conchodytes*. Holthuis (1952) already noted a resemblance of *C. monodactylus* to *C. biunguiculatus*, which is herein also confirmed based on molecular data of the “true” *C. biunguiculatus* (fig. 4). Similarly, there are

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

The two lineages recovered in the TE analysis (fig. 4) within *Dactylonia* are hard to identify in the other molecular analysis (fig. 2). The first lineage seems to partially correspond to the previous study by Fransen (2002) and includes *D. okai*, *D. holthuisi*, and one species that is only represented by morphological data (*Dactylonia franseni* Bruce, 2003). The second lineage seems to be represented by *D. anochoreta*, *Dactylonia borradalei* Bruce,

2005, *Dactylonia carinicula* Bruce, 2006, *Dactylonia monnioti* (Bruce, 1990) (the latter three only represented by morphological data), and two lineages of *D. ascidicola*. Interestingly, Bruce (2003) already thought *D. franseni* to be related to *D. okai*, but also to *D. ascidicola*, which is in part congruent with the current analyses. In a similar situation, Bruce (2006) thought *D. carinicula* to be a close relative to *D. ascidicola*, *D. anachoreta*, or *D. monnioti*, which seems to correspond as well. At least two species with uncertain placements cause the phylogeny reconstruction to be far from resolved within the genus. As mentioned above, *D. ascidicola* is represented by two specimens in two lineages in the TE analysis, and by eight specimens (as five separate lineages) in the concatenated alignment. Assuming the specimen identified as *D. anachoreta* is correctly sequenced, an indication of the true relations between the specimens identified as *D. ascidicola* can be seen. Due to both lineages being examined for their morphology, and the high genetic similarity, and therefore low intrageneric support values, the “true” lineage of *D. ascidicola* cannot be identified. However, there seems to be a consensus that *D. ascidicola* is closely related to *D. anachoreta* (and possibly to *D. borradalei*, as this species was described by Bruce (2005) on the basis of specimens previously attributed to *D. ascidicola*). The only known COI sequence of *D. ascidicola* is located in the other *Dactylonia* lineage (fig. 2: MH257317) and was later used in the TE alignment (fig. 4), where it clustered with the above-mentioned clade. The other problematic species, *D. holthuisi*, can be observed to be represented in four lineages in the concatenated alignment: the presumably correct placement as two clustering with *D. okai*, one basally to *Dactylonia* clustering with *Cainonia*, and one within *Odontonia*. The first three lineages were correctly identified by physical examination, while the Taiwanese

material from Gan et al. (2015) from the fourth lineage could not be examined. This specimen might have been mistaken for *Odontonia katoi* due to the shrimp’s small size and ascidian-inhabiting behaviour (the host of Gan et al.’s (2015) specimen is however unknown). The specimen related to *Cainonia* is represented by a single 16S sequence, potentially causing this placement (supplementary fig. S1C). Undersampling of genetic markers in the available specimens, as well as marker choice, can influence the resulting placement in a phylogenetic tree (e.g., da Silva et al., 2011). Similar to *Conchodytes*, more molecular data is needed in order to correctly place the species of *Dactylonia* in a phylogenetic framework.

Although the species within *Odontonia* are placed in a different order in all three tree topologies, there seems to be some kind of consensus on which species are more basal, and which species are more related to one another. The recovered topologies are largely similar to those of Fransen (2002). *Odontonia* seems to be split up into two main lineages in the analysis using only molecular markers, with *O. rufopunctata* being placed basal to the *Odontonia* and *Conchodytes* groups (fig. 2). *Odontonia rufopunctata* is placed in a basal position to the other ascidian-associated *Odontonia* species in the TE analysis as well (fig. 4). Similarly, the bivalve-associated *O. kerangcaris* is placed basally to the other *Odontonia* species with *Anchiopontonia huri* in the TE analysis (fig. 4), while it is placed in *Odontonia* next to *O. katoi* in the tree resulting from the concatenated alignment (fig. 2). *Odontonia kerangcaris* is also placed basally in the morphological analysis, however, *O. rufopunctata* is placed in a derived branch of *Odontonia* in this tree topology. The basal placement of *O. kerangcaris* is as expected, since the species’ host and morphological features were found by Fransen et al. (2021)

to resemble that of related, bivalve-associated genera such as *Conchodytes*. The placement of *O. rufopunctata* is less expected, but the placement away from the other species within the genus was also recovered in a previous study based on DNA and morphological features (De Gier & Franssen, 2018). As mentioned above, the other species seem to be grouping in two separate lineages in the concatenated analysis: one lineage including *O. katoi*, *O. seychellensis* and *O. kerangcaris* (and one specimen of *Dactylonia holthuisi*); and one lineage including *O. bagginsi* and *O. sibogae*. The placement of the relatively basal *O. katoi* is interesting, since it here clusters with *O. seychellensis*, which was recovered to be a very derived species in the TE analysis. The potential explanation for this can be found in the only successfully sequenced marker of *O. katoi*, 16S. *Odontonia katoi* can therefore only be compared to other 16S sequences, in this case being limited to only *O. sibogae* and *O. rufopunctata* (see De Gier & Franssen, 2018). Due to *O. simplicipes* and *O. compacta* being only represented by morphological data, and *O. katoi*, *O. kerangcaris*, *O. plurellicola*, *O. bagginsi*, and *O. seychellensis* only being represented by one molecular marker, the relation between these species and the other species in *Odontonia* is far from resolved.

The inclusion of the recently described genus *Opaepupu* is only based on similarities in morphological characters, and a similar host association (with a bivalve mollusc). Anker & De Grave (2021) even remarked that this monotypic genus might not necessarily be closely related to the other bivalve endosymbionts. Nonetheless, *O. huna* seems to bear various morphological characters that resulted in the current analyses in a basal placement to *Conchodytes* (fig. 3) or to a clade including *Conchodytes*, *Odontonia*, and other related genera (fig. 4). These morphological characters include: slender, seemingly

unadapted dactyli on the ambulatory legs; a well-developed, acute antennal spine and dorsal and rostral carina; and a slender third maxilliped (Anker & De Grave, 2021; supplementary appendix S2, supplementary table S2). In a similar situation the sole member of *Notopontonia*, *N. platycheles* Bruce, 1991, was also included based on the morphological similarities of the described specimens in Bruce (1991) and Berggren (1999). Based on external characters, Bruce (1991) argued *Notopontonia* to be closely related to *Pontonia*. However, Bruce (1991) did not discover a host for the species, and suggested that the species was a bivalve associate due to its depressed body shape and the horizontal disposition of the second pereopods (Berggren, 1999). Berggren (1999) found the species in the ascidian *Herdmania momus* (Savigny, 1816), but did not mention anything about its systematic placement. The ascidian-associated habit of *N. platycheles*, as well as its previously thought relatedness with *Pontonia*, lead us to believe the species is a member of the currently studied clade.

Platyponotonia hyotis is represented by five individuals in the concatenated alignment, clustered in two groups of two and three barcodes respectively. The basal placement of the first group (fig. 2) raises questions about the similarities of these specimens, compared to the ones being basally placed next to *Anchiopontonia*, *Ascidonia* and *Pontonia*. The possible explanation for this aberrant placement can be found in the selection of the barcodes (supplementary table S1): the two basally placed barcodes are both COI-barcodes, while the other three clustered barcodes are a combination of 16S and H3 barcodes. Physical examinations were done on four specimens, eliminating the chance of misidentifications. If the barcodes are combined and supplemented with morphological data (fig. 4), *P. hyotis* clusters together

with *Platypontonia brevirostris* (Miers, 1884) (represented only with morphological data), as a sister to *Pinnotherotonia* (and together with *Pinnotherotonia* with *Conchodytes*). In contrast, previous analyses by Fransen & Reijnen (2012) and Chow et al. (2021) placed *Platypontonia* respectively basal to *Conchodytes*, and next to *Anchiopontonia*. Bruce (1968) even suggested a relatedness to *Pontonia*. Based on previous analyses and our data, it is likely that the genus is at least part of a clade containing *Conchodytes*, *Odontonia* and also *Dactylonia*.

Although the recovered topologies are largely similar to those of Fransen (2002), various species within *Pontonia* cause problems when studying the phylogeny reconstructions. Most problematic placements can be traced back to potential misidentifications and limited genetic information of some species, causing low intrageneric support values. Interestingly, the genetic distances between various species of *Pontonia* is very small in the concatenated alignment. These species groups are: *P. domestica*, one lineage of *P. mexicana*, and one lineage of *P. pinnophylax*; and *P. margarita* and one lineage of *P. manningi* (fig. 2). The placement of these specimens and more obvious problems are discussed below. First up, the placement of the *Pontonia* species with an aberrant host association (or potentially aberrant host association) (resp. *P. panamica* and *P. longispina*) are generally placed more basally to the bivalve associated species. This is the case in the morphological, as well as the TE analysis. This is in congruence with previous remarks by Marin & Anker (2008). In the tree resulting from the concatenated alignment, a branch containing *P. margarita* and two lineages of *P. manningi* is placed basally to all other species of *Pontonia*. The possible explanation to this different topology can be found in the list of successfully sequenced molecular

markers (supplementary table S1): only COI and H3 have successfully been amplified for these specimens. Therefore, these specimens could only be compared to the other species of *Pontonia* using these markers, which do not give well-resolved topologies on their own (supplementary fig. S1A, B). In addition, *P. manningi* can be represented by two other, more derived, lineages: one related to an unidentified species of *Pontonia*, and one basal to this cluster and *P. pinnophylax*, *P. domestica*, and one lineage of *P. mexicana*. As mentioned above, the reason for this aberrant placement can be found in the successfully sequenced markers (supplementary table S1), as these two lineages of *P. manningi* are represented by the two ribosomal markers (16S and 18S). Both resulting lineages of *P. manningi* were physically examined, so misidentifications are ruled out. Similarly, *P. mexicana* is also represented by two lineages, one of which was checked for its morphology. The other specimen included in the concatenated alignment (fig. 2: GQ227823; Baeza, 2010) could not be checked, and is represented by only one 16S sequence.

Inclusion of unidentified species

As can be observed in the phylogenetic tree resulting from the concatenated alignment, various unidentified species were included in the study (fig. 2). The inclusion of the species resulted in tentative identifications to the two unidentified species of *Dactylonia* and one of *Pontonia*. Both unidentified specimens of *Dactylonia* (from Kou et al., 2013 and Mitsuhashi et al., 2007) seem to group together with a large clade of *D. ascidicola*, which seems to be the best guess to the correct identification of the species. Both specimens have a distribution indicated as “Philippines, Panglao Island” (supplementary table S1), which corresponds with the known distribution of *D. ascidicola* (see Fransen,

2002). The host of these specimens are however unknown.

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

Outgroup species placement

Cuapetes tenuipes was assigned as outgroup, followed by the recovery of *Actinimenes* as being most related to this outgroup. The other two outgroup species were correctly recovered in the TE analysis, but their placement varied in the molecular and morphological analyses. The four molecular sequences of *Periclimenes colemani* were split in two in the phylogeny reconstruction based on the concatenated alignment, due to the 16S sequence of this species being from a different sample (see supplementary table S1). The 16S sequence clusters together with members of *Dactyлонia* (fig. 2). The 16S marker, compared to 18S, resolves the intrageneric relatedness quite well for the studied clade (supplementary fig. S1C), so the placement of this sequence of *P. colemani* is not expected. Since physical examination of the misplaced specimen used in Chow et al. (2021) was not performed, it is not possible to find an explanation for its placement in *Dactyлонia*.

In both the analyses using the TE and concatenated alignment, *Typton wasini* is recovered as the outgroup species most related to the ingroup. However, in the morphological dataset, the phylogenetic analysis recovered the species as a close relative to *Colemonia* and

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

Historical biogeography

Combining the published and previously unpublished distribution data (supplementary appendix S1; table 1, supplementary table S1) with the presented phylogeny reconstruction from the TE approach (fig. 4), gives an indication of potential evolutionary distribution patterns of the studied ingroup (fig. 6). Almost all studied genera can be found in tropical coral reefs in the IWP, ranging from the Red Sea to Vanuatu and Hawaii (supplementary appendix S1). The EP/Atl lineage, containing all members of *Ascidonia* and *Pontonia*, forms a monophyletic clade (fig. 4). This is in congruence with a previous study using morphological characters (Fransen, 2002). It reflects the combination of the Terminal Tethyan Event (TTE, 12–18 Myr ago), forming a 'hard' barrier, and the East Pacific Barrier (EPB, 5000 km open ocean, in

effect throughout the past 65 Myr), forming a 'soft' barrier, separating IWP and EP populations. The internal architecture of the clade is mirrored in the subsequent closure of the Isthmus of Panama (IOP, 3.1 Myr ago) separating the EP and Atl regions, as well as the 'soft' barrier caused by the opening of the Atlantic Ocean (OAO, in effect throughout the past ca. 60 Myr) which can be observed in several marine groups (e.g., Fransen, 2002; Anker & Baeza, 2012; Cowman & Bellwood, 2013; Anker et al. 2017; Baraf et al., 2019). Examples of closely related paraphyletic or sister species pairs that possibly evolved as a consequence of the IOP are: *P. manningi*/*P. margarita* and *A. pusilla*/*A. quasipusilla*. This also holds for the sister species *A. flavomaculata* and *A. californiensis*, although no representative of this clade has been recorded from the W Atl. This could indicate subsequent extinction in that region or it remains to be discovered there. The OAO is best reflected in the species *P. pinnophylax* and *P. mexicana*. As indicated by Fransen (2002), morphological differences between these species are minute and not reliable. The genetic distance between the W Atl populations of *P. mexicana* and those of the E Atl *P. pinnophylax* could be seen as an indication of speciation in progress.

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

The host switch from an ascidian host in *P. panamica* to a mollusc host in the remaining *Pontonia* species parallels the IOP in the biogeographical analysis (fig. 5). The interactions between host switching and biogeographical

events have been studied in detail before (e.g., Hoberg & Brooks, 2008). While this is the only apparent matching between the two analyses, it might be worth studying this in more detail.

Host switching and notes on ecomorphological characters

The ancestral character state with regard to the host choice of the entire monophyletic ingroup was recovered to be an ascidian-associated shrimp species (fig. 6). Interestingly, previous studies featuring ancestral state reconstruction methods resulted in an unresolved host association (Horká et al., 2016) or a bivalve-associated shrimp species (Chow et al., 2021) for the common ancestor. The explanation for this mismatch in ancestral character state recovery should be sought in the limited number of included species in the previous studies (Horká et al., 2016: $n = 4$; Chow et al., 2021: $n = 10$), causing ambiguous results for the presently studied clade.

As mentioned before, there seem to be at least six to seven different instances of host switches between phyla (from an ascidian to a bivalve host, or from a bivalve to an ascidian host), and one instance of a host switch within a phylum (from a bivalve to a gastropod mollusc). It is worth noting that the number of host switches presented in this study is based on the tree topology resulting from the TE-approach, which unfortunately is accompanied with low support values on various branches (figs. 4 and 6). Once more (complete) molecular data becomes available, the tree topologies and support values might change, revealing a different number of host switches. Thus, the number of host switches is tentatively identified as six to seven. Most of the host switches in the studied clades are characterized as a single occurrence: the host switch from an ascidian to a bivalve host in the genus *Pontonia* happened once after the branching of the ascidian-associated *P.*

panamica, or after the branching of *P. longispina*, of which a host association remains uncertain (fig. 6). Similarly, within the clade containing *Rostronia*, *Cainonia* and *Dactylonia*, one host switch from an ascidian to a bivalve host occurred in the branch represented by the only species of *Cainonia* (fig. 6). In previous morphological analyses by Fransen (2002), *Cainonia* was considered to be part of *Dactylonia*, although the genus was later founded partly based on the aberrant host-association of the species (Bruce, 2005). Within the clade containing *Conchodytes*, *Odontonia* and all other smaller related genera, a switch from an ascidian-associated ancestor to a bivalve associated ancestor can be observed basally in the phylogeny reconstruction (fig. 6), after *Opeapupu* splits off. The ancestral character state of the clade including *Opeapupu* is still an ascidian association, meaning that in the branch of *Opeapupu* a host-switch has occurred from an ascidian to a bivalve host. After the initial switch, several unique host switching events occur in the clade, first in the branch containing *Notopontonia*, which seems to have switched back from a bivalve- to an ascidian-associated lifestyle. Similarly, in the clade containing *Pseudopontonia*, *Anchiopontonia*, and *Odontonia*, the bivalve-associated ancestors do not switch once, but twice back to an ascidian-associated species in *Pseudopontonia*, and later in *Odontonia* (fig. 6). Within *Odontonia*, the ancestor of the genus (excluding *O. kerangcaris*) is recovered as a bivalve-associate, suggesting that the ascidian-associated *O. rufopunctata* has switched to an ascidian-associated lifestyle independently from the rest of the genus.

It has been hypothesized that the switches from an ectosymbiotic to an endosymbiotic host association resulted in various species-specific associations with bivalve, ascidian and sponge hosts in multiple clades

within the Palaemonidae (Horká et al., 2016). This can be observed in the high level of host-specificity in the studied endosymbiotic clade, exploiting 19 confirmed families (table 1; Chow et al., 2021). Chow et al. (2021), however, hypothesized that these clades of endosymbiotic shrimp do make smaller taxonomic jumps than ectosymbiotic shrimp, when colonizing new hosts. This is congruent with the host choices of the species in most of the studied genera (supplementary appendix S1), but this does not explain the seemingly difficult switch from one phylum to another. One explanation for these seemingly unexpected host switching events can be found in the internal structures of the host phyla: the studied shrimp species living inside bivalve molluscs are reported as inhabitants of the mantle cavity (e.g., Fransen, 1994), feeding from the mucus and pseudofeces which are built up near the gills (e.g., Kennedy et al., 2001; Ashelby et al., 2015; De Grave et al., 2021). This soft, mucus-producing internal cavity might be morphologically similar to the pharyngeal basket of phlebobranch and stolidobranch solitary ascidians (Horká et al., 2016, for comparison, see Monniot, 1991). A seemingly similar host switch between phyla has occurred in a lineage of the mainly sponge-inhabiting genus *Periclimenaeus*, where various species can be found in the sponge-like tunnelling systems of colonial (or compound) ascidian species (e.g., Fransen, 2006; Horká et al., 2016).

The ancestor of the gastropod-associated *Pontonia chimaera* and the closely related bivalve-associated congener was recovered to be a bivalve-associate. Although this intrageneric host switch can easily be distinguished in the currently presented phylogeny reconstruction, there are two other minor host switches present in the current study. De Gier and Fransen (2018) reported that *Odontonia plurellicola* was found in a species of *Plurella* Kott, 1973, an aggregating phlebobranch

species. Similarly, *Dactyлонia holthuisi* can also be found in the same and other *Plurella* species (Fransen, 2006; C. Fransen, pers. obs.). Members of *Plurella*, like all other members of the Plurellidae, are thought to have evolved from a solitary ancestor, resulting in an aggregating species characterized by a non-shared branchial sac. Contrary to the above-mentioned ecology of *Periclimenaeus*, this means that if individuals of *O. plurellicola* and *D. holthuisi* want to move to another ascidian in the aggregate, they have to enter it from the outside (De Gier & Fransen, 2018).

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

As we detected multiple host switching events from one phylum to the other and vice versa in this lineage of symbiotic shrimp, we conclude that bivalves and solitary ascidians with their similar internal microenvironments function as hot spots for interphylum host switching. It is expected that interphylum host switches will be observed more often between phyla with matching internal

microenvironments than between phyla without these matching internal microenvironments in various lineages of crustaceans and other symbionts.

Future perspectives

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

To test the hypothesis that interphylum host switches will be observed more often between phyla with matching internal microenvironments than between phyla without these matching internal microenvironments, similar analyses could be performed with for instance pinnotherine crabs and amphipods that inhabit the same host phyla. It is expected

that for instance the matching internal microhabitats of compound ascidians and sponges will be another hot spot for inter phylum host switching. A comparative study of inter phylum host switching throughout nature could reveal more of these hot spots.

Supplementary material

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

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

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References

- Anker, A. & Baeza, J.A. (2012) Molecular and morphological phylogeny of hooded shrimps, genera *Betaeus* and *Betaeopsis* (Decapoda, Alpheidae): Testing the center of origin biogeographic model and evolution of life history traits. *Mol. Phylogenet. Evol.*, 64, 401–415.
- Anker, A. & De Grave, S. (2021) *Opaepupu*, a new genus and species of bivalve-associated shrimp (Decapoda: Caridea: Palaemonidae) from Hawai'i. *Zootaxa*, 4903, 55–70.
- Anker, A., Hultgren, K.M. & De Grave, S. (2017) *Synalpheus pinkfloydi* sp. nov., a new pistol shrimp from the tropical eastern Pacific (Decapoda: Alpheidae). *Zootaxa*, 4254, 111–119.
- Antokhina, T.I. & Sorokin, P.A. (2010) Molecular Genetic Analysis of the Two Morphs of Sea Star Shrimp *Periclimenes soror* Nobili, 1904, the Symbionts of Tropic Sea Stars. *Russ. J. Genet.*, 46, 852–857.
- Ashelby, C.W., De Grave, S. & Johnson, M.L. (2015) Preliminary observations on the mandibles of palaemonoid shrimp (Crustacea: Decapoda: Caridea: Palaemonoidea). *PeerJ*, 3: e846; doi: 10.7717/peerj.846.
- Baeza, J.A. (2010) Molecular systematics of peppermint and cleaner shrimps: phylogeny and taxonomy of the genera *Lysmata* and *Exhippolysmata* (Crustacea: Caridea: Hippolytidae). *Zool. J. Linn. Soc.*, 160, 254–265.
- Bagge, L.E., Kinsey, S.T., Gladman & J., Johnsen, S. (2017) Transparent anemone shrimp (*Ancylomenes pedersoni*) become opaque after exercise and physiological stress in correlation with increased hemolymph perfusion. *J. Exp. Biol.*, 220, 4225–4233. doi: 10.1242/jeb.162362.

- Baraf, L.M., Pratchett, M.S. & Cowman, P.F. (2019) Ancestral biogeography and ecology of marine angelfishes (F: Pomacanthidae). *Mol. Phylogenet. Evol.*, 140, 106596. doi: 10.1016/j.ympev.2019.106596.
- Berggren, M. (1999) *Notopontonia platycheles*, a little-known shallow-water shrimp from Western Australia (Decapoda: Pontoniinae). *J. Crust. Biol.*, 19, 180–187.
- Bininda-Emonds, O.R.P., Cardillo, M., Jones, K.E., MacPhee, R.D.E., Beck, R.M.D., Grenyer, R., Price, S.A., Vos, R.A., Gittleman, J.L. & Purvis, A. (2007) The delayed rise of present-day mammals. *Nature*, 446, 507–512. doi: 10.1038/nature05634.
- Bracken, H.D., De Grave, S. & Felder, D.L. (2009) Phylogeny of the Infraorder Caridea Based on Mitochondrial and Nuclear Genes (Crustacea: Decapoda). In: Martin, J.W., Crandall, K.A., Felder, D.L. (Eds.) *Decapod Crustacean Phylogenetics*. Taylor and Francis/CRC Press, Boca Raton.
- Brinkmann, B.W. & Fransen, C.H.J.M. (2016) Identification of a new stony coral host for the anemone shrimp *Periclimenes rathbunae* Schmitt, 1924 with notes on the host-use pattern. *Contrib. Zool.*, 85, 437–456.
- Bruce, A.J. (1968) Notes on some Indo-Pacific Pontoniinae. XII. The re-examination of the types of *Pontonia? brevirostris* Miers, 1884, with the designation of a new genus, *Platypontonia* (Decapoda, Natantia). *Crustaceana*, 15, 289–297.
- Bruce, A.J. (1976) Coral Reef Caridea and 'Commensalism.' *Micronesica*, 12, 83–98.
- Bruce, A.J. (1977) Pontoniine shrimps in the collections of the Australian Museum. *Rec. Aust. Mus.*, 31, 39–81. doi:10.3853/j.0067-1975.31.1977.233.
- Bruce, A.J. (1989) Notes on some Indo-Pacific Pontoniinae, XLV. *Conchodytes maculatus* sp. nov., a new bivalve associate from the Australian northwest shelf. *Crustaceana*, 56, 182–192.
- Bruce, A.J. (1991) *Notopontonia platycheles*, new genus, new species (Decapoda: Pontoniinae) from South Australia, with remarks on *Pontonia pinnophylax* (Otto), the type species of *Pontonia* Latreille. *J. Crust. Biol.*, 11 (4), 607–628.
- Bruce, A.J. (1992) Designation of two new pontoniine shrimp genera (Decapoda: Palaemonidae). *J. Nat. Hist.*, 26, 1273–1282.
- Bruce, A.J. (1994) A synopsis of the Indo-West Pacific genera of the Pontoniinae (Crustacea, Decapoda, Palaemonidae). Koeltz Scientific Books, Königstein.
- Bruce, A.J. (1996) Crustacea Decapoda: Palaemonoid shrimps from the Indo-West Pacific region mainly from New Caledonia. In: Crosnier, A. (Ed.) *Résultats des Campagnes MUSORSTOM*, 15. *Mém. Mus. Natl. Hist. Nat.*, 168, 197–267.
- Bruce, A.J. (2003) A new species of *Dactyлонia* Fransen (Crustacea: Decapoda: Pontoniinae) from East Africa. *Cah. Biol. Mar.*, 44, 299–306.
- Bruce, A.J. (2005) Pontoniinae shrimps from Papua New Guinea, with designation of two new genera, *Cainonia* and *Colemonia* (Crustacea: Decapoda: Palaemonidae). *Mem. Queensl. Mus.*, 51, 333–383.
- Bruce, A.J. (2006) Pontoniine shrimps (Decapoda: Palaemonidae) from the island of Socotra, with descriptions of new species of *Dactyлонia* Fransen, 2002 and *Periclimenoides* Bruce, 1990. *Zootaxa*, 1137, 1–36.
- Bruce, A.J. (2013) A note on *Conchodytes kempii* Bruce 1989 (Crustacea, Palaemonidae, Pontoniinae), with the designation of *C. kempoides* sp. nov. *Zootaxa*, 3664, 92–94.
- Caldas, I.V. & Schrago, C.G. (2018) Data partitioning and correction for ascertainment bias reduce the uncertainty of placental mammal divergence times inferred from the morphological clock. *Ecol. Evol.*, 9, 2255–2262.
- Camin, J.H. & Sokal, R.R. (1965) A method for deducing branching sequences in phylogeny. *Evolution*, 19, 311–326.
- Chow, L.H., De Grave, S. & Tsang, L.M. (2020) The family Anchistioididae Borradaile, 1915 (Decapoda: Caridea) is a synonym of

- Palaemonidae Rafinesque, 1815 based on molecular and morphological evidence. *J. Crust. Biol.*, 40, 277–287. doi: 10.1093/jcabi/ruaa012.
- Chow, L.H., De Grave, S. & Tsang, L.M. (2021) Evolution of protective symbiosis in palaemonid shrimps (Decapoda: Caridea) with emphases on host spectrum and morphological adaptations. *Mol. Phylogenet. Evol.*, 162, 107201.
- Colgan, D.J., Hutchings, P.A. & Beacham, E. (2008) Multi-gene analyses of the phylogenetic relationships among the Mollusca, Annelida, and Arthropoda. *Zool. Stud.*, 47, 338–351.
- Cowman, P.F. & Bellwood, D.R. (2013) Vicariance across major marine biogeographic barriers: temporal concordance and the relative intensity of hard versus soft barriers. *Proc. R. Soc. B*, 280, 20131541. doi: 10.1098/rspb.2013.1541.
- da Silva, J.M., dos Santos, A., Cunha, M.R., Costa, F.O., Creer, S. & Carvalho, G.R. (2011) Multigene Molecular Systematics Confirm Species Status of Morphologically Convergent *Pagurus* Hermit Crabs. *PLoS ONE*, 6, e28233. doi: 10.1371/journal.pone.0028233.
- de Gier, W. & Franssen, C.H.J.M. (2018) *Odontonia phurellicola* sp. n. and *Odontonia bagginsi* sp. n., two new ascidian-associated shrimp from Ternate and Tidore, Indonesia, with a phylogenetic reconstruction of the genus (Crustacea, Decapoda, Palaemonidae). *ZooKeys*, 765, 123–160.
- de Gier, W. & Becker, C. (2020) A review of the ecomorphology of pinnotherine pea crabs (Brachyura: Pinnotheridae), with an updated list of symbiont-host associations. *Diversity*, 12, 1–42.
- De Grave, S. (1999) Pontoniinae (Crustacea: Decapoda: Palaemonidae) associated with bivalve molluscs from Hansa Bay, Papua New Guinea. *Bull. Inst. Royal Sci. Nat. Belg. Biol.*, 69, 125–141.
- De Grave, S., Struck, U. & Johnson, M.L. (2021) Preliminary study into the trophic position of symbiotic palaemonid shrimps (Decapoda, Palaemonidae) using stable isotopes. *Crustaceana*, 94, 1145–1153.
- Dobson, N.C., De Grave, S. & Johnson, M.L. (2014) Linking eye design with host symbiont relationships in pontonine shrimps (Crustacea, Decapoda, Palaemonidae). *PLoS ONE*, 9, e99505.
- Dobson, N.C., Johnson, M.L. & De Grave, S. (2016) Insights into the morphology of symbiotic shrimp eyes (Crustacea, Decapoda, Palaemonidae); the effects of habitat demands. *PeerJ*, 4, e1926.
- Đuriš, Z., Horká, I., Juračka, P.J., Petrušek, A. & Sandford, F. (2011) These squatters are not innocent: the evidence of parasitism in sponge-inhabiting shrimps. *PLoS ONE*, 6, e21987.
- Farris, J.S. (1970) Methods for computing Wagner Trees. *Syst. Zool.*, 19, 83–92.
- Fitch, W.M. (1971) Toward defining the course of evolution: Minimal change for a specific tree topology. *Syst. Zool.*, 20, 375–379.
- Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol.*, 3, 294–299.
- Franssen, C.H.J.M. (1994a) Shrimps and Molluscs. *Vita Marina*, 42, 105–113.
- Franssen, C.H.J.M. (1994b) Marine palaemonid shrimps of the Netherlands Seychelles Expedition 1992–1993. *Zool. Verh. Leiden*, 297, 85–152.
- Franssen, C.H.J.M. (2002) Taxonomy, phylogeny, historical biogeography, and historical ecology of the genus *Pontonia* (Crustacea: Decapoda: Caridea: Palaemonidae). *Zool. Verh. Leiden*, 336, 1–433.
- Franssen, C.H.J.M. (2006) On Pontoniinae (Crustacea, Decapoda, Palaemonidae) collected from ascidians. *Zoosystema*, 28, 713–746.
- Franssen, C.H.J.M. & Reijnen, B.T. (2012) A second discovery of *Lacertopontonia chadi* Marin, 2011 (Crustacea: Decapoda: Palaemonidae), with

- remarks on its systematic position. *Zootaxa*, 3437, 43–50.
- Fransen, C.H.J.M. & Reijnen, B.T. (2013) Caught in speciation? A new host for *Conchodytes meleagrinae* Peters, 1852 (Decapoda, Caridea, Palaemonidae). *Zootaxa*, 3721, 265–280.
- Fransen, C.H.J.M., Groenhof, M. & de Gier, W. (2021) *Odontonia kerangcaris* sp. nov., a new bivalve-associated shrimp (Crustacea, Decapoda, Palaemonidae) from East Kalimantan, revealing intrageneric host switching. *Zootaxa*, 5801, 275–280.
- Galil, B.S., Marchini, A. & Occhipinti-Ambrogi, A. (2018) East is east and West is west? Management of marine bioinvasions in the Mediterranean Sea. *Estuar. Coast.*, 201, 7–16. doi: 10.1016/j.ecss.2015.12.021.
- Gan, Z.B., Li, X.Z., Chan, T.-Y., Chu, K.H. & Kou, Q. (2015) Phylogeny of Indo-West Pacific pontoniine shrimps (Crustacea: Decapoda: Caridea) based on multilocus analysis. *J. Zool. Syst. Evol. Res.*, 53, 282–290.
- Hoberg, E.P. & Brooks, D.R. (2008) A macroevolutionary mosaic: episodic host-switching, geographical colonization and diversification in complex host-parasite systems. *J. Biogeogr.*, 35, 1533–1550.
- Holthuis, L.B. (1952) The Decapoda of the Siboga Expedition. Part XI. The Palaemonidae collected by the Siboga and Snellius Expeditions with remarks on other species II. Subfamily Pontoniinae. *Siboga Exped.* 39a10, 1–253.
- Horká, I., De Grave, S., Fransen, C.H.J.M., Petrusek, A. & Ďuriš, Z. (2016) Multiple host switching events shape the evolution of symbiotic palaemonid shrimps (Crustacea: Decapoda). *Sci. Rep.*, 6, 26486.
- Kennedy, H., Richardson, C.A., Duarte, C.M. & Kennedy, D.P. (2001) Diet and association of *Pontonia pinnophylax* occurring in *Pinna nobilis*: insights from stable isotope analysis. *J. Mar. Biol. Ass. U.K.*, 81, 177–178.
- Kou, Q., Li, X.Z., Chan, T.Y., Chu, K.H. & Gan, Z. (2013) Molecular phylogeny of the superfamily Palamonoidea (Crustacea: Decapoda: Caridea) based on mitochondrial and nuclear DNA reveals discrepancies with the current classification. *Invertebr. Syst.*, 27, 502–514.
- Kou, Q., Li, X.Z., Chan, T.Y. & Chu, K.H. (2015) Divergent evolutionary pathways and host shifts among the commensal pontoniine shrimps: a preliminary analysis based on selected Indo-Pacific species. *Org. Divers. Evol.*, 15, 369–377.
- Kozlov, A.M., Darriba, D., Flouri, T., Morel, B. & Stamatakis, A. (2019) RAXML-NG: a fast, scalable and user-friendly tool for maximum likelihood phylogenetic inference. *Bioinformatics*, 35, 4453–4455.
- Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. (2018) MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Mol. Biol. Evol.*, 35, 1547–1549.
- Landis, M.J., Matzke, N.J., Moore, B.R. & Huelsenbeck, J.P. (2013) Bayesian analysis of biogeography when the numbers of areas is large. *Syst. Biol.*, 62, 789–804.
- Lee, M.S.Y. & Palci, A. (2015) Morphological phylogenetics in the genomic age. *Curr. Biol.*, 25, R922–R929.
- Levitt-Barmats, Y. & Shenkar, N. (2018) Observations on the symbiotic relationship between the caridean shrimp *Odontonia sibogae* (Bruce, 1972) and its ascidian host *Herdmania momus* (Savigny, 1816). *PLoS ONE*, 13, e0192045.
- Marin, I. & Anker, A. (2008) A new species of *Pontonia* Latreille, 1829 (Crustacea, Decapoda, Palaemonidae) associated with sea squirts (Tunicata, Ascidiacea) from the Pacific coast of Panama. *Zoosystema*, 30, 501–515.
- Matzke, N.J. (2013) Probabilistic historical biogeography: new models for founder-event speciation, imperfect detection, and fossils allow improved accuracy and model-testing. *Front. Biogeogr.*, 5, 242–248.
- Miller, M.A., Pfeiffer, W. & Schwartz, T. (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: *Proceedings of the Gateway Computing*

- Environments Workshop (GCE), 14 Nov. 2010, New Orleans, LA, 1–8.
- Mitsuhashi, M., Sin, Y.W., Lei, H.C., Chan, T.-Y. & Chu, K.H. (2007) Systematic status of the caridean families Gnathophyllidae Dana and Hymenoceridae Ortmann (Crustacea: Decapoda): a preliminary examination based on nuclear rDNA sequences. *Invertebr. Syst.*, 21, 613–622.
- Monniot, C., Monniot, F. & Laboute, P. (1991) Coral reef ascidians of New Caledonia. ORSTOM, Paris.
- Palumbi, S., Martin, A., Romano, S., McMillan, W.O., Stice, L. & Grabowski, G. (1991) The Simple simple Fool's fool's Guide guide to PCR version 2.0. University of Hawaii, Honolulu.
- Paradis, E. & Schliep, K. (2019) ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R (v. 5.5). *Bioinformatics*, 35, 526–528.
- Penell, M., Eastman, J., Slater, G., Brown, J., Uyeda, J., Fitzjohn, R., Alfaro, M. & Harmon, L. (2014) geiger v2.0: an expanded suite of methods for fitting macroevolutionary models to phylogenetic trees (v. 2.0.7). *Bioinformatics*, 30, 2216–2218.
- Rambaut, A. (2009) FigTree version 1.4.2. Available at: <http://tree.bio.ed.ac.uk> (Accessed: 14th November 2020).
- Rambaut, A., Drummond, A.J., Xie, D., Baele, G. & Suchard, M.A. (2018) Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Syst. Biol.*, 67, 901–904.
- Raupach, M.J. & Radulovici, A. (2015) Looking back on a decade of barcoding crustaceans. *Zookeys*, 539, 53–81. doi: 10.3897/zookeys.539.6530.
- Ree, R.H. & Smith, S.A. (2008) Maximum likelihood interference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Syst. Biol.*, 57, 4–14. doi: 10.1080/10635150701883881.
- Ree, R.H. & Sanmartín, I. (2018) Conceptual and statistical problems with the DEC+J model of founder-event speciation and its comparison with DEC via model selection. *J. Biogeogr.*, 45, 741–749. doi: 10.1111/jbl.13173.
- Revell, L.J. (2012) phytools: an R package for phylogenetic comparative biology (and other things). *Methods Ecol. Evol.*, 3, 217–223. doi: 10.1111/j.2041-210X.2011.00169.x.
- Ronquist, F. (1997) Dispersal-vicariance analysis: a new approach to the quantification of historical biogeography. *Syst. Biol.*, 46, 195–203.
- Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19, 1572–1574.
- Salis, P., Roux, N., Soulat, O., Lecchini, D., Laudet, V. & Frédérick, B. (2018) Ontogenetic and phylogenetic simplification during white stripe evolution in clownfishes. *BMC Biol.*, 16, 1–14. doi: 10.1186/s12915-018-0559-7.
- Sayers, E.W., Cavanaugh, M., Clark, K., Ostell, J., Pruitt, K.D. & Karsch-Mizrachi, I. (2020) GenBank. *Nucleic Acids Res.*, 48, D84–D86.
- Scotland, R.W., Olmstead, R.G. & Bennett, J.R. (2003) Phylogeny reconstruction: the role of morphology. *Syst. Biol.*, 52, 539–548.
- Swofford, D.L. (2003) Paup*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland, MA.
- Talavera, G. & Castresana, J. (2007) Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Syst. Biol.*, 56, 564–577.
- Thompson, J.D., Higgins, D.G. & Gibson, T.J. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.*, 22, 4673–4680.
- Van Der Wal, C., Ayhong, S.T., Ho, S.Y.W., Lins, L.S.F. & Lo, N. (2019) Combining morphological and molecular data resolves the phylogeny of Squilloidea (Crustacea: Malacostraca). *Invertebr. Syst.*, 33, 89–100. doi: 10.1071/is18035.
- Whiting M.F. (2002) Mecoptera is paraphyletic: multiple genes and phylogeny of Mecoptera and Siphonaptera. *Zool. Scr.*, 31, 93–104.

- Williams, S.T., Knowlton, N., Weigt, L.A. & Jara, J.A. (2001) Evidence for three major clades within the snapping shrimp genus *Alpheus* inferred from nuclear and mitochondrial gene sequence data. *Mol. Phylogenet. Evol.*, 20, 375–389. doi: 10.1006/mpev.2001.0976.
- Wood, L.E., De Grave, S. & Daniels, S.R. (2017) Phylogeographic patterning among two codistributed shrimp species (Crustacea: Decapoda: Palaemonidae) reveals high levels of connectivity across biogeographic regions along the South African coast. *PLoS ONE*, 12, e0173356. doi:10.1371/journal.pone.0173356.
- Xia, X., Xie, Z., Salemi, M., Chen, L. & Wang, Y. (2003) An index of substitution saturation and its application. *Mol. Phyl. Evol.*, 26, 1–7.
- Xia, X. (2009) Assessing substitution saturation with DAMBE. In: P. Lemey, M. Salemi & A.-M. Vandamme (Eds) *The Phylogenetic Handbook: A Practical Approach to Phylogenetic Analysis and Hypothesis Testing*, pp. 615–630. Cambridge University Press, Cambridge, UK.