

Divergent evolutionary pathways and host shifts among the commensal pontoniine shrimps: a preliminary analysis based on selected Indo-Pacific species

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Abstract As the most species-rich subfamily of Caridea, Pontoniinae Kingsley, 1878 is well-known for its great diversity in morphology, lifestyle and habitat. However, the phylogeny of Pontoniinae has been poorly studied since it was erected, and there are many taxonomic and evolutionary controversies remained unresolved. Among these controversies, the relationship between the commensal pontoniine shrimps and their hosts is the issue of most concern. Herein, a total of 26 Indo-Pacific pontoniine species from 23 genera, including both free-living and commensal taxa associated with different marine organisms from five phyla, are selected to preliminarily explore the differentiation process accompanied with the reciprocal natural selections between them and their hosts. Based on the molecular phylogenetic analyses, two major clades, representing the ‘primitive groups’ and ‘derived groups’, and several subgroups related to the hosts are well recovered, which are also supported in morphology. Additionally, several possible evolutionary pathways of those

commensal pontoniine shrimps with similar hosts or niches are identified, with a host-shifting hypothesis proposed for cavity-inhabiting pontoniines based on molecular data in conjunction with morphological and ecological evidence.

Keywords Palaemonidae · Pontoniinae · Phylogeny · Evolution · Commensalism · Host shift

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Introduction

The family Palaemonidae Rafinesque, 1815, which currently comprises more than 980 species, is the most species-rich family in Caridea (De Grave and Fransen 2011). Palaemonidae consists of two subfamilies, Palaemoninae Rafinesque, 1815 and Pontoniinae Kingsley, 1878. Compared with the Palaemoninae, which is widespread in marine, brackish and freshwater in the tropical and temperate regions (Ashelby et al. 2012), the Pontoniinae is mostly restricted to tropical and subtropical marine habitats but with much more species. To date, the Pontoniinae includes over 110 genera and 600+ species, distributed from intertidal zone to deep sea, and the exact diversity is supposed to be far more than this (Li et al. 2007). Apart from a limited number of free-living species, pontoniine shrimps are generally associated with various marine organisms (Li 1993). Their hosts contain sponges, cnidarians, echinoderms, molluscs, polychaetes, ascidians and even other crustaceans and are sometimes highly specific (Bruce 1994; Hayashi and Ohtomi 2001; Bruce and Okuno 2006; Komai et al. 2010). Presumably, such a variety of commensal lifestyles and habitats in Pontoniinae contributes to the enormous morphological diversity nowadays (Li and Liu 1997; Kou et al. 2013a).

On account of the extremely high diversity, the classification of the subfamily Pontoniinae based solely on morphological characters is inevitably subjective and probably inaccurate. Consequently, additional host and habitat data are used to assist in delineating the growing pontoniine taxa. However, for a number of the commensal pontoniine shrimps sharing similar niches, their morphology appears evidently different, and they are speculated to arise from divergent evolutionary pathways (Li and Liu 1997; Marin and Chan 2006). Besides, many homoplastic and specialized morphological characters of the commensal pontoniine shrimps also perplex the taxonomists, making the traditional taxonomic studies a tough work.

As a resultful and hopeful approach to resolving the knotty problems of systematics, however, the molecular phylogenetics on the pontoniines has been limited within only a small number of taxa included. Mitsuhashi et al. (2007) examined the systematic status of the two species-poor palaemonoidean families Gnathophyllidae Dana, 1852 and Hymenoceridae Ortmann, 1890 with eight pontoniine genera, indicating an affinity between these two families and Pontoniinae. Fransen and Reijnen (2012) reevaluated the systematic position of two bivalve-associated pontoniine genera *Lacertopontonia* Marin, 2011 and *Chernocaris* Johnson, 1967 based on morphological and molecular data, suggesting that both genera should be considered as synonyms of the genera *Conchodytes* Peters, 1852. Kou et al. (2013a) investigated the phylogeny of the *Periclimenes* complex, focusing on elucidating the polyphyletic status of the genus *Periclimenes* and the phylogenetic relationships among *Periclimenes* and 15 related genera. Except for the meager studies mentioned above, the systematics of the Pontoniinae was rarely touched upon, leaving the subfamily a highly controversial taxon in phylogeny and evolution.

Kou et al. (2013a) preliminarily deduced the probable differentiation process of the pontoniine shrimps and suggested that a comprehensive molecular phylogenetic study ideally in conjunction with the morphological, lifestyle and habitat characteristics is required to infer the natural phylogeny. Thus, in this study, 26 Indo-Pacific species from 23 affiliated genera of the Pontoniinae are selected as the subject investigated, including both free-living species and commensal species associated with different marine organisms from five phyla. Firstly, we will explore their phylogeny utilizing three loci, viz. the mitochondrial 12S and 16S ribosomal RNA (rRNA) and the nuclear histone three genes, which are suggested suitable for the phylogenetic analysis at the generic level of decapod (Toon et al. 2009) and proved to be capable of solving a series of evolutionary problems in the Palaemoninae (Ashelby et al. 2012; Kou et al. 2013b). Secondly, we will attempt to answer the question, ‘Did those commensal pontoniine shrimps sharing similar hosts evolve through divergent evolutionary pathways or not?’ and find

morphological evidence to support the conclusion. Finally, we will try to preliminarily elucidate the diversification of the studied pontoniine taxa, which is often accompanied with host shifts, by integrated morphological, ecological and the current molecular data, and provide new insights to the classification of this controversial subfamily.

Materials and methods

Sample collection

A total of 26 Indo-Pacific species from 23 genera of the Pontoniinae, plus one out-group species *Macrobrachium nipponense* (De Haan, 1849 [in De Haan, 1833–1850]) from the Palaemoninae were included in this study. The ingroup taxa included both free-living and those species which are commensally associated with different marine organisms, aiming to achieve a comprehensive coverage on the different lifestyles and habitats of the pontoniine shrimps. All specimens were identified by the second or third authors, who are the experts on caridean taxonomy. Then, the specimens were preserved in absolute alcohol prior to DNA extraction. The details of all the specimens are listed in Table 1.

DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted from abdomen muscle or pleopod (5–20 mg) using a QIAamp DNA Mini Kit (Qiagen). The DNA was eluted in 100 μ l of sterile distilled H₂O (RNase free) and stored at –20 °C. The extracted DNA was checked by 1–1.5 % agarose gel electrophoresis and ethidium bromide staining.

Although effective, the nuclear protein coding genes were performed to be a bit too conservative at the generic level, and sometimes, the phylogenetic analysis could not obtain a strongly supported topology at several subbranches in our previous work (see Kou et al. 2013a). In view of this, nuclear genes were not mainly used in the present study. Instead, two mitochondrial genes (12S and 16S rRNA) and one nuclear gene (histone 3) were utilized to infer the phylogeny.

Partial segments of 12S rRNA (~350 bp), 16S rRNA (~480 bp) and histone 3 (~350 bp) genes were amplified by polymerase chain reaction (PCR). Amplifications were carried out in a reaction mix containing 1–5 μ l of template DNA, 5 μ l of 10 \times PCR buffer (Mg²⁺ plus, Takara), 4 μ l of dNTP (10 mM each), 1.5 μ l of each primer (10 mM), 1 μ l of Taq polymerase (5 U ml⁻¹, Takara) and sterile distilled H₂O to a total volume of 50 μ l. For the 12S rRNA segments, PCR amplifications were carried out using primers 12S-f/r (Mokady et al. 1994), with the following cycling profile: initial denaturation for 10 min at 94 °C, followed by 35 to 40 cycles of denaturation at 94 °C for 30 s, annealing at 48 °C for 40 s, extension at 72 °C for 30 s and a final extension at 72 °C for 10 min. For the amplification of the 16S

Table 1 Details of the specimens and GenBank accession numbers in this study

Subfamily	Genus	Species	Voucher ID	Sampling locality	GenBank accession number		
					12S	16S	Histone 3
Pontoniinae	<i>Anchiopontonia</i>	<i>Anchiopontonia hurii</i>	J2009-P	Okinawa, Japan	KJ584100	KJ584119	KJ584130
	<i>Anchistus</i>	<i>Anchistus custos</i>	A2005-D	Moreton Bay, Australia	KJ584101	KJ584120	KJ584131
	<i>Ancylomenes</i>	<i>Ancylomenes holthuisi</i>	A2005-B	Moreton Bay, Australia	KJ584102	JX025220	KJ584132
	<i>Anisomenaeus</i>	<i>Anisomenaeus spinimanus</i>	S2010-AS	Ras Afir, Somalia	KJ584103	KJ584121	N/A
	<i>Conchodytes</i>	<i>Conchodytes meleagrinae</i>	A2005-L	Moreton Bay, Australia	N/A	KC515051	KC515093
	<i>Coralliocaris</i>	<i>Coralliocaris graminea</i>	NTOUM-00935	Keelung, Taiwan	KJ584104	KJ584122	KJ584133
	<i>Cuapetes</i>	<i>Cuapetes amymone</i>	A2005-I	Moreton Bay, Australia	KJ584105	JX025216	KJ584134
		<i>Cuapetes elegans</i>	A2005-G	Moreton Bay, Australia	KJ584106	JX025213	KJ584135
		<i>Cuapetes grandis</i>	A2005-H	Moreton Bay, Australia	KJ584107	JX025211	KJ584136
	<i>Dactylonia</i>	<i>Dactylonia ascidicola</i>	T2010-D	Keelung, Taiwan	KJ584108	KJ584123	KJ584137
	<i>Harpiliopsis</i>	<i>Harpiliopsis beaupresii</i>	J2000-M33	Okinawa, Japan	KJ584109	JX025207	KJ584138
	<i>Harpilius</i>	<i>Harpilius lutescens</i>	J2000-M55	Okinawa, Japan	N/A	JX025205	KJ584139
	<i>Ischnopontonia</i>	<i>Ischnopontonia lophos</i>	J2000-M18	Okinawa, Japan	N/A	KJ584124	KJ584140
	<i>Jocaste</i>	<i>Jocaste lucina</i>	NTOUM-00958	Keelung, Taiwan	KJ584110	KJ584125	KJ584141
	<i>Laomenes</i>	<i>Laomenes nudirostris</i>	A2005-M	Moreton Bay, Australia	KJ584111	KJ584126	KJ584142
		<i>Laomenes pardus</i>	P2004-R16	Panglao, Philippines	KJ584112	JX025202	N/A
	<i>Lipkemenes</i>	<i>Lipkemenes lanipes</i>	M2010-N	Fort Dauphin, Madagascar	KJ584113	KJ584127	KJ584143
	<i>Palaemonella</i>	<i>Palaemonella rotumana</i>	MBM-108251	Hainan, China	N/A	JX025197	KJ584144
	<i>Periclimenaeus</i>	<i>Periclimenaeus bidentatus</i>	A2005-C	Moreton Bay, Australia	N/A	KJ584128	KJ584145
	<i>Periclimenella</i>	<i>Periclimenella spinifera</i>	J2009-M47	Okinawa, Japan	N/A	JX025194	KJ584146
	<i>Periclimenes</i>	<i>Periclimenes brevicarpalis</i>	A2005-A	Moreton Bay, Australia	KJ584114	JX025191	KJ584147
		<i>Periclimenes soror</i>	T2010-J	Keelung, Taiwan	KJ584115	JX025178	N/A
	<i>Phycomenes</i>	<i>Phycomenes cobourgi</i>	A2005-S	Moreton Bay, Australia	KJ584116	JX025174	N/A
	<i>Platycaris</i>	<i>Platycaris latirostris</i>	J1997-M19	Okinawa, Japan	N/A	KJ584129	KJ584148
	<i>Thaumastocaris</i>	<i>Thaumastocaris streptopus</i>	P2004-L	Panglao, Philippines	DQ642865	DQ642878	KJ584149
	<i>Unguicaris</i>	<i>Unguicaris panglaonis</i>	P2004-R38	Panglao, Philippines	KJ584117	JX025172	N/A
	Palaemoninae	<i>Macrobrachium</i>	<i>Macrobrachium nipponense</i>	C2010-O	Qingdao, China	KJ584118	JX435435

An 'N/A' indicates unobtainable sequence data

rRNA and histone 3 segments, the primers 16Sar (Simon et al. 1994)/16S-1472 (Crandall and Fitzpatrick 1996) and histone 3 AF/AR (Colgan et al. 1998) were used respectively, and the thermal cycle used was similar to that above.

The PCR products were purified using the QIAquick PCR purification kit or the QIAquick Gel extraction kit (Qiagen) before sequencing. The purified PCR products were bidirectionally sequenced using the same forward and reverse primers for PCR amplification with ABI 3730xl DNA Analyzer (Applied Biosystems). Then, the sequence chromatograms were checked using Chromas 2.23 (Technelysium Pty Ltd.) by eye.

Phylogenetic analyses

Prior to analyzing, forward and reverse sequence fragments were assembled and edited by ContigExpress (a component of Vector NTI Suite 6.0, Life Technologies). Then, the multiple sequence alignments were conducted using MUSCLE 3.8

(Edgar 2004) under the default parameters and adjusted manually. GBlocks v0.91b (Castresana 2000) was used to eliminate the highly divergent and poorly aligned segments in the 12S and 16S rRNA datasets before subsequent analyses (GBlocks parameters optimized for datasets: minimum number of sequences for a conserved position (12S/16S)=11/14; minimum number of sequences for a flanking position (12S/16S)=17/23; maximum number of contiguous non-conserved positions (12S/16S)=8/8; minimum length of a block (12S/16S)=5/5; allowed gap positions=half/half). The pruned alignments were then concatenated to a single dataset consisting of the three gene fragments end to end.

Phylogenies were inferred from the concatenated dataset using both maximum likelihood (ML) and Bayesian inference (BI) methods. ModelTest 3.7 (Posada and Crandall 1998) was used to select the best-fit DNA substitution models implemented in ML and BI analyses. The ML analysis was carried out using PhyML 3.0 (Guindon et al. 2010) performed on the ATGC

bioinformatics platform with the best-fit model for the combined dataset determined by ModelTest. The node support was evaluated by performing bootstrapping (BP, Felsenstein 1985) with 1000 replicates. The BI analysis was conducted using MrBayes 3.2 (Huelsenbeck and Ronquist 2001) while the combined dataset was partitioned and analysed according to the optimum models selected from individual dataset by ModelTest. The Markov chains were run for 10,000,000 generations, with sampling every 1000 generations. After the first 25 % trees were discarded as burn-in, the remaining trees were used to construct the 50 % majority rule consensus tree and to estimate the posterior probabilities (PP). The effective sample size (ESS) values for all sampled parameters were diagnosed by Tracer v1.5 (Rambaut and Drummond 2007) to make sure that convergence was reached.

Results

Sequences

In total, 20 12S rRNA, 27 16S rRNA and 22 histone 3 sequences were included in our analyses. The combined dataset consisted of 1163 bp (~90.6 % of the original 1284 bp alignment) after the poorly aligned and hyper-variable regions of the 12S and 16S rRNA sequences were removed using Gblocks (original alignment (12S/16S)=421/518 bp; trimmed alignment (12S/16S)=351/467 bp). The alignment gaps were represented as '-' and the missing data were designated as '?' in the analyses. The characteristics of the three individual and the combined datasets, their empirical base frequencies, rate matrix, gamma shape parameter, proportion of invariable sites and the substitution models selected by ModelTest are listed in Table 2.

Phylogenetic analyses

After 10 million iterations, the average standard deviation of split frequencies in the BI analysis reached below 0.003. Except for a few internal nodes, the phylogenies inferred from

both ML and BI analyses were highly congruent and generally well supported (Fig. 1).

All the ingroup taxa are recovered in a well-supported clade (PP=1.00, BP=100 %). Furthermore, the ingroup taxa form two major clades (I and II, shown in Fig. 1) with high support values in both analyses (PP=1.00, BP=100 %; PP=1.00, BP=68 %, respectively).

Within clade I, the basal position of the genus *Palaemonella* Dana, 1852 is strongly supported (PP=1.00, BP=100 %) as the other six species from four genera cluster together (PP=1.00, BP=88 %). *Harpilius* Dana, 1852 is the sister group to *Ischnopontonia* Bruce 1966 with high support (PP=1.00, BP=100 %). Three species of the genus *Cuapetes* Clark, 1919 group together with the incursion of *Periclimenella* Duris & Bruce, 1995 (PP=0.88, BP<50 %).

Clade II and several subordinate branches are also strongly supported by both analyses. *Anchistus* Borradaile, 1898, *Lipkemenes* Bruce & Okuno, 2010 and *Periclimenes soror* Nobili, 1904 form a branch with high support (PP=1.00, BP=100 %). *Anisomenaeus* Bruce, 2010, *Periclimenaeus* Borradaile, 1915 and *Thaumastocaris* Kemp, 1922 constitute another branch (PP=1.00, BP=77 %), which is sister to the branch consisting of *Anchiopontonia* Bruce, 1992, *Conchodytes* Peters, 1852 and *Dactylonia* Franssen 2002 (PP=1.00, BP=98 %). Four madreporine-inhabiting genera, *Harpiliopsis* Borradaile, 1917, *Platycaris* Holthuis 1952, *Coralliocaris* Stimpson, 1860 and *Jocaste* Holthuis 1952, cluster as a group, which is well recovered by BI analysis (PP=0.98) but with moderate ML support (BP=46 %). In addition, *Ancylomenes* Okuno & Bruce, 2009, *Laomenes* Clark, 1919, *Phycomenes* Bruce 2008, *Unguicaris* Marin and Chan 2006, plus *Periclimenes brevicarpalis* (Schenkel, 1902) group together with high support (PP=1.00, BP=99 %). However, the relationships among these aforementioned branches and a few of their internal relationships have not been firmly resolved in our analyses.

Discussion

In the phylogenetic tree, 23 pontonine genera are separated into two major clades. Both clades are firmly supported not

Table 2 The information of different datasets and the substitution model selected by ModelTest in this study

Dataset	Number of sites	Base frequencies nA/C/G/T)	Rmat	Gamma shape parameter	Proportion of invariable sites	Model selected by ModelTest
12S	351	0.3193, 0.0899, 0.1877, 0.4031	0.6707, 3.1825, 0.8615, 0.0505, 5.5268, 1.0000	0.7654	0.2231	GTR+I+G
16S	467	0.3251, 0.0843, 0.1824, 0.4082	1.0000, 4.8839, 1.0000, 1.0000, 9.0745, 1.0000	0.4561	0.2729	TrN+I+G
Histone 3	345	0.2574, 0.2908, 0.2487, 0.2032	1.0000, 3.7897, 1.8337, 1.8337, 8.1853, 1.0000	1.2053	0.6044	TIM+I+G
Combined	1163	0.2942, 0.1532, 0.1890, 0.3636	0.4992, 3.9678, 1.1966, 0.5467, 3.1265, 1.0000	0.8328	0.3946	GTR+I+G

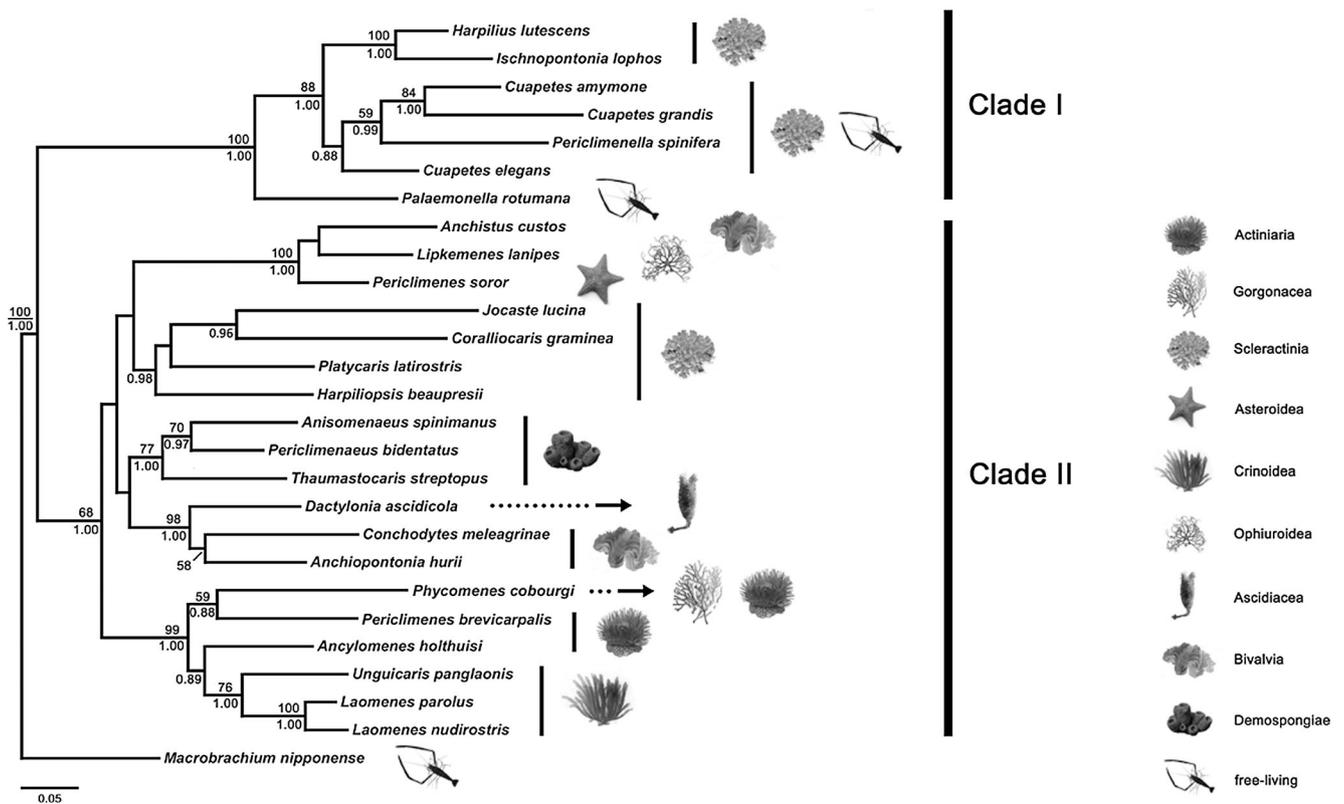


Fig. 1 Phylogenetic tree obtained by Bayesian inference analysis of DNA sequences based on the combined dataset. Bayesian posterior probabilities (*upper*) and bootstrap values (based on 1000

pseudoreplicates) from ML analysis (*lower*) are indicated on each branch. The values under 0.85 (for BI) or 50 (for ML) are not shown. The habitats of the studied species are marked behind branches

only by the current molecular data but also the morphological and ecological evidences.

Clade I consists of genera *Cuapetes*, *Harpilius*, *Palaemonella*, *Periclimenella* and *Ischnopontonia*. The first four genera, coupled with *Exoclimenella* Bruce, 1995, *Eupontonia* Bruce, 1971, *Philarius* Holthuis 1952 and *Vir* Holthuis 1952, are the putative ‘primitive groups’ of the Pontoniinae, as they share the character of the presence of the median process on the fourth thoracic sternite, which is also present in a number of palaemonine genera and could be considered as a plesiomorphy (Bruce 2008). However, judging by this presumption, two notable exceptions occur in the current phylogeny. One is the genus *Ischnopontonia*, which lacks the process structure but is nested in clade I, sister to *Harpilius*. *Ischnopontonia* was separated from *Philarius* based on its extremely compressed body, peculiar second pereiopod form, laterally situated dorsal spines of the telson and the absence of antennal spine (Bruce 1966). But in common with *Philarius* and many other taxa of the primitive groups, *Ischnopontonia* lives commensally with madreporian coral, which is considered to be the initial host of the commensal pontoniines (Li and Liu 1997; Kou et al. 2013a), and they share a suite of homoplastic characters adapting to this habitat, such as the simple strongly hooked dactyls of the last

three ambulatory pereiopods. The other exception, the genus *Phycomenes*, falls outside of clade I and is incorporated in clade II in which all the other members lack a median process on the fourth thoracic sternite. In contrast to *Ischnopontonia*, though *Phycomenes* possesses a distinct transverse median process on the fourth thoracic sternite, the median process has a different shape compared to those genera of the primitive groups, which is more triangular with a much broader base. Perhaps, it suggests that the median process structures are not homologous and *Phycomenes* might have a different origin from the primitive groups. Besides, in general morphology, *Phycomenes* appears most closely related to the species of *Periclimenes* Costa, 1844 (Bruce 2008), and it is not in association with madreporian corals but usually with sea anemones or gorgonian corals (Bruce 1983, 2008, 2010a; Bruce and Coombes 1995), and some members of *Phycomenes* are reported to be free-living from seagrass habitat (Martínez-Mayén and Román-Contreras 2006; Āuriš et al. 2008). Accordingly, *Phycomenes* has slender and biunguiculate dactyls of the last three ambulatory pereiopods rather than the typical simple hooked dactyls. Thus, according to the current phylogenetic analyses and the result from Kou et al. (2013a), the presumption about the primitive groups of the Pontoniinae is largely supported. Nevertheless, the

putative criterion whether a taxon belongs to the primitive groups or not solely rests with the median process on the fourth thoracic sternite is inaccurate, neither necessary nor sufficient.

In contrast to the vast majority of genera in clade I, the median process on the fourth thoracic sternite is absent in the members of clade II except in *Phycomenes*. More significantly, several subordinate branches which are correlative with their corresponding hosts are clearly implied from the present topology.

Four other genera in association with madreporians, *Coralliocaris*, *Harpiliopsis*, *Jocaste* and *Platycaris*, form a relatively independent group distinct from the madre-pore-associated group within clade I. Judging from the above discussion, separating these two groups merely by the presence or absence of the median process on the fourth thoracic sternite is imperfect. In our opinion, the body shape is another significant morphological difference between these two groups. The madre-pore-associated species belonging to the primitive groups have a subcylindrical to slightly laterally compressed (i.e. *Cuapetes*, *Harpilius*, *Philarius*, *Vir*) or strongly laterally compressed (*Ischnopontonia*) body. On the contrary, the four genera of the madre-pore-associated group in clade II have a dorsoventrally depressed body without exception. Therefore, this dissimilarity in body shape probably reveals the divergent evolutionary pathways between these two groups. The former represents the relatively primitive and less-specialized group, as their body shape resembles those free-living pontoniine shrimps (e.g. *Eupontonia*, *Exoclimenella*, *Palaemonella*, *Periclimenella*) and their palaemonine relatives. Other relevant characters are their well-developed rostrum and simple dactyls of the last three ambulatory pereopods. In contrast, the latter stands for the obligatorily commensal and more specialized group. Their depressed body is successfully adapted for residing in the pore spaces formed by madre-pore. Besides, the specially modified dactyls (e.g. *Coralliocaris* and *Jocaste* with a specialized hoof-shaped basal protuberance on the dactyl; *Harpiliopsis* with dactyl twisted laterally in a unique way) and the degenerate rostrum (e.g. *Coralliocaris brevirostris* and *Platycaris latirostris* have a broad, short and unarmed rostrum) could reinforce this argument, indicating that they are a more recently diverged group in evolution.

The above interpretations suggest that primitive pontoniines would have the following features: free-living or associate with madreporians, subcylindrical or laterally compressed body, fourth thoracic sternite with median process (except for *Ischnopontonia*) and simple or hooked dactyls of last three ambulatory pereopods. It is very likely that our idea of primitive pontoniines will be further enhanced after the genera *Exoclimenella*, *Eupontonia*, other genera commensally associated with madreporians (e.g. *Anapontonia* Bruce 1966, *Hamopontonia* Bruce, 1970) and more free-living

Periclimenes species are incorporated in subsequent phylogenetic studies.

In our tree, another cnidarian commensal branch is recovered within clade II, remote from the madre-pore-associated group but with two crinoid-inhabiting genera nested within. *Ancylomenes*, *Phycomenes* and *Periclimenes brevicarpalis* are included in the branch. There are considerable similarities among them in morphology: the subcylindrical body form, well-developed palaemonoid rostrum, non-ovate inferior orbital angle, feeble biunguiculate dactyls of the last three ambulatory pereopods, slightly humpbacked abdomen in profile and the generally transparent body with mottled spots. Among this group, *Phycomenes* has been reported as facultative symbiont of a gorgonian or actinarian host (see above); the *Ancylomenes* species are generally in association with actinarians (Okuno and Bruce 2010), and many Indo-West Pacific species have also often been recorded from madreporians, especially from fungid and euphyllid corals (Hoeksema et al. 2012); while *Periclimenes brevicarpalis* is a well-known shallow-water pontoniine shrimp associated with sea anemones. Thus, inferred from the present phylogeny, the species of clades I and II branched early in evolution, and the two cnidarian commensal groups in clade II appear to have a separate evolutionary pathway subsequently (Fig. 2), while the half free-living *Phycomenes*-like species with a rudimental transverse triangular median process on the fourth thoracic sternite probably occupy an intermediate position between these two clades.

Similar situation arises in the pontoniines associated with the hosts belonging to phyla other than Cnidaria. Also within clade II, the pontoniine genera associated with Echinodermata are divided into two groups. The crinoid commensal group, *Laomenes* and *Unguicaris*, is remotely separated from the ophiuroid-associated monotypic genus *Lipkemenes* and the asteroid-associated species, *Periclimenes soror*.

The shallow-water crinoid commensal pontoniine genera, *Laomenes* and *Unguicaris*, share considerable similarities in morphology: stout body and appendages, well-developed rostral midrib and conoidally produced cornea. Their common biunguiculate dactyls of ambulatory pereopods are considered helpful when they are clinging to the host's surface (Bruce 1982). Besides, their bodies usually show the same colour pattern to their host with mottled bands or stripes, making them almost undetectable when still on the pinnules of the crinoids. These modifications in structure and morphology suggest that this group is of a successful adaption to the concealed niches that are provided by the crinoid hosts after a long-term evolution.

Compared with crinoids, *Lipkemenes lanipes* and *Periclimenes soror*'s hosts are not only movable but also much more similar in size and shape. Thus, it is reasonable to deduce that the close relationship and similar habitat results in their resemblance in coloration and morphology, such as

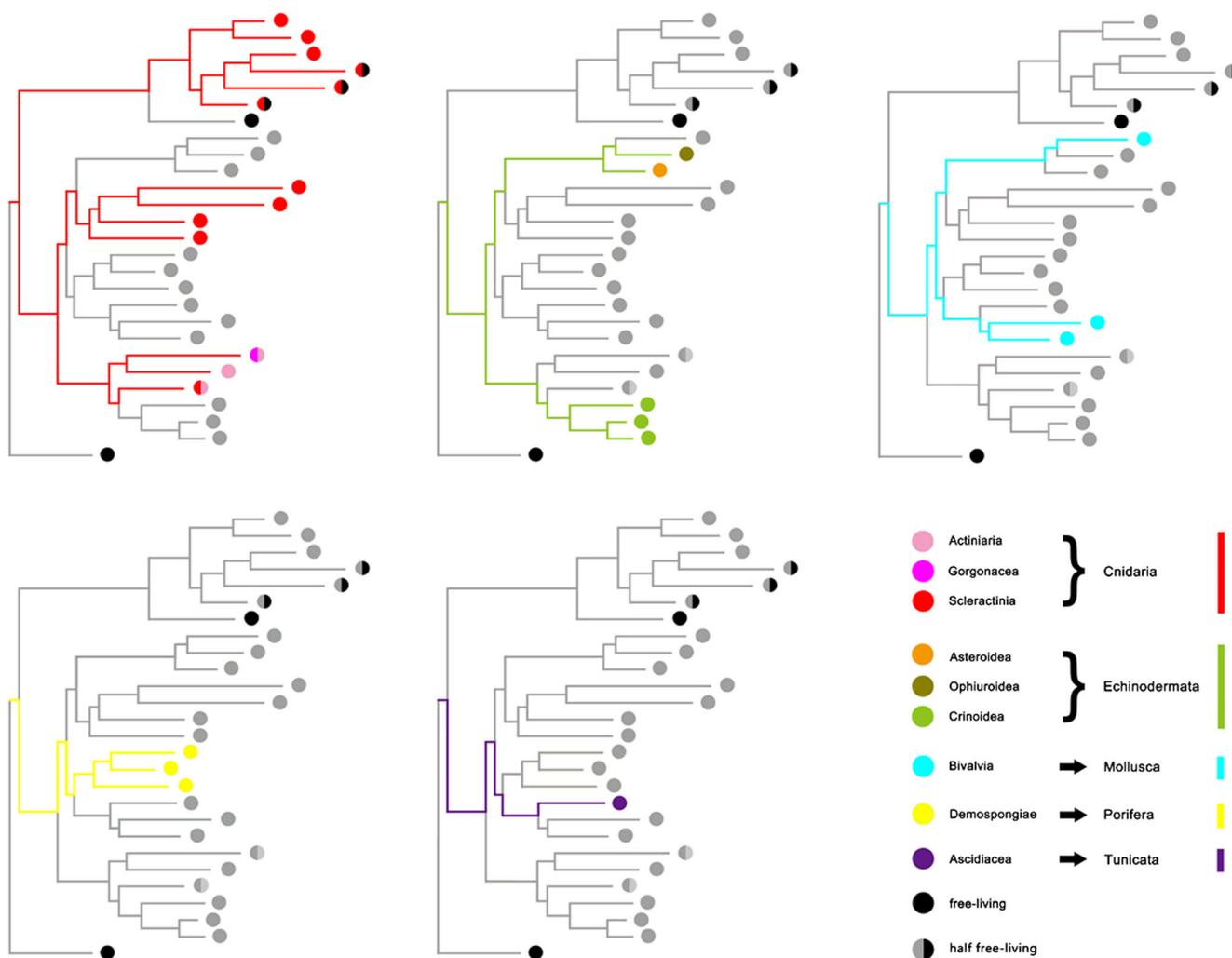


Fig. 2 The inferred evolutionary pathways of the studied pontoniine species based on the current phylogenetic analysis. The species commensally associated with different hosts are marked in colours.

References for the commensal hosts and their corresponding given colours are included in the figure

orange and white colour body; well developed, arched, directed ventrally rostrum; the first pereopod with dactyl subspatulate, cutting edges pectinate; the propodus of the ambulatory pereopods with subterminal spines on the ventral surface and feeble biunguiculate dactyls.

Apart from the cnidarian and echinoderm commensal pontoniines, the taxa associated with molluscs are also arisen from two different evolutionary pathways as suggested in the present topology. The genus *Anchistus* clusters with *L. lanipes* and *P. soror*, forming a relative independent branch. The other mollusc-associated lineage, comprising the genera *Anchiopontonia* and *Conchodytes*, is located in a branch with *Dactylonia*, separated from *Anchistus*. Although having similar hosts of bivalves, *Anchiopontonia* and *Conchodytes* are distinctly different from *Anchistus* in morphology and could be readily distinguished by the depressed body form, triangular and depressed rostrum, large telson dorsal spines and

highly specialized biunguiculate dactyls of the last three ambulatory pereopods. However, these morphological features could find parallels in *Pontonia* Latreille, 1829 and most ascidian-associated pontoniine shrimps. *Pontonia* sensu lato was considered as a polyphyletic group for its disparities in morphology and association by Bruce (1991a), and a series of revisionary work was carried out since then. Currently, the restricted *Pontonia* comprises 11 species mainly associated with molluscs (De Grave and Fransen 2011). Several ascidian-associated groups (i.e. *Pseudopontonia* Bruce, 1992, *Ascidonia* Fransen 2002, *Dactylonia* Fransen 2002, *Odontonia* Fransen 2002 and *Rostronia* Fransen 2002) and mollusc-associated groups (i.e. *Anchiopontonia* Bruce, 1992, *Bruceonia* Fransen 2002 and *Cainonia* Bruce, 2005) were given generic status recently. In addition, deduced from the result of the cladistic analysis performed by Fransen (2002), a host shift from ascidians to molluscs might occur in the early

evolutionary history of the *Pontonia* complex. This viewpoint was largely accepted by Marin and Anker (2008), and they further proposed the possibility of double host switch scenario (e.g. ascidian to mollusc to ascidian). This hypothesis is also partly supported by the present analysis, and the genus *Conchodytes* is suggested to be closely related to *Pontonia* sensu lato. However, in view of the present limited dataset, a more extensive analysis is necessary to resolve the phylogeny of the *Pontonia* complex and to verify this host-shift hypothesis.

In addition, a clade consisting of three sponge-associated pontoniine genera are well recovered in the current analysis. Apart from their hosts, these two monotypic genera *Anisomenaeus* and *Thaumastocaris* closely resemble *Periclimenaeus* in body shape and morphology. They share a small, subcylindrical body form, well-developed rostrum and are characterized by the unequal second pereopods. The most distinct morphological difference between *Thaumastocaris* and *Periclimenaeus* is the segmented carpus of the first pereopods. On the other hand, *Anisomenaeus* was originally placed in *Periclimenaeus* and merely distinguishes from *Periclimenaeus* in the absence of a sound-producing structure on the major second pereopod chela. Although our analyses demonstrate the affinities among the sponge-inhabiting pontoniine shrimps, on account of the limited taxa herein presented, it would be imprudent to further discuss the monophyly of this group. A broader analysis of this peculiar group (see Bruce 2010b) is necessary to clarify their origin and evolution in future.

In contrast, the monophyly of the genus *Periclimenaeus* is problematic suggested by its variety of hosts and morphological diversity for a long time. *Periclimenaeus* currently comprises more than 70 species and is widely distributed in all oceans and occurs from the intertidal zone to 450 m deep water (Bruce 1991b). It associates with sponges, ascidians and alcyonarians and shows remarkable variability in some diagnostic characters, particularly the shape of the dactyls of the last three pereopods (Holthuis 1952). Such a parallel exists in *Periclimenes*, the largest genus of the Pontoniinae, which is suggested to be polyphyletic by recent studies (Bruce 2007; Li 2009; Kou et al. 2013a). Perhaps just like *Periclimenes*, a comprehensive revision and subdivision of *Periclimenaeus* according to different habitats and subtle morphological distinctions are prompted as well. In addition, according to the differentiation hypothesis proposed by Kou et al. (2013a), *Periclimenes* could be considered as an intermediate group between the free-living and various commensal pontoniine shrimps. Based on the molecular and ecological evidence, we analogize and tentatively suggest that the genus *Periclimenaeus* might be an intermediate group among the cavity-inhabiting pontoniine shrimps. That is, a transition in the ‘sponge–ascidian–mollusc’ host-shift chain. Despite that the existing molecular data was still insufficient to verify this

host-shift hypothesis, some morphological characters such as the more and more specialized dactyls of the last three pereopods, the increasingly depressed body form and degenerate rostrum appear to indicate this evolutionary direction. Moreover, the robust telson dorsal spines seem to be a synapomorphy of this peculiar group.

Conclusion

Our study utilizing three gene loci infers a phylogeny of 26 Indo-Pacific pontoniine species from 23 genera with emphasis on the commensal relationships between them and their hosts as well as the differentiation process. Although the taxon coverage presented was limited, we still obtained some significant conclusions supported by the morphological and ecological evidence. Firstly, the studied pontoniine species are divided into two major clades, which are composed of the primitive groups and the ‘derived groups’, respectively. However, the median process on the fourth thoracic sternite is inadequate to be a division criterion, as exceptions exist in both clades. Secondly, the pontoniines associated with Cnidaria, Echinodermata and Mollusca are inferred to have evolved through at least two different pathways independently, even some of them share similar hosts and ecological niche. Furthermore, a host-shift process within the cavity-inhabiting pontoniine groups is revealed in this study, despite that the phylogenetic relationships among several sub-branches has not been explicitly resolved.

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