CSIRO PUBLISHING
www.publish.csiro.au/journals/is *Invertebrate Systematics*, 2007, **21**, 613–622

Systematic status of the caridean families Gnathophyllidae Dana and Hymenoceridae Ortmann (Crustacea : Decapoda): a preliminary examination based on nuclear rDNA sequences

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Abstract. The systematic positions of the caridean families Gnathophyllidae and Hymenoceridae are inferred based on analyses of nuclear 18S rRNA and 28S rRNA genes. The phylogenetic trees based on 18S rRNA and 28S rRNA from selected species of one genus of the family Gnathophyllidae, two genera of the family Hymenoceridae, one genus of the family Anchistioididae, eight genera of the subfamily Pontoniinae and five genera of the subfamily Palaemoninae show a close relationship between Hymenoceridae, Gnathophyllidae and Pontoniinae, with the last group constituting a paraphyletic assemblage. This result concurs with the morphology of maxilla in the first zoea, but not the shape of the third maxilliped in adults, based on which Gnathophyllidae and Hymenoceridae are treated as families. Molecular analysis supports the similarities in larval morphology between Hymenoceridae, Gnathophyllidae and Pontoniinae and therefore draws into question the familial status of the former two groups.

Introduction

The infraorder Caridea is a diverse group of shrimps with ~2800 species (Bauer 2004). The number of families and their relationships are controversial among taxonomists. According to Martin and Davis (2001), there are 36 families of Caridea grouped into 16 superfamilies, following largely the schemes of Chace (1992) and Holthuis (1993), which are based mainly on the similarities in the shapes of the mouth parts and pereiopods. Yet Martin and Davis (2001) stated that the scheme does not necessarily represent the natural relationships of the shrimps.

Members of the families Gnathophyllidae Dana and Hymenoceridae Ortmann are coral reef shrimps with striking coloration. They are widely distributed in tropical seas worldwide and are highly valued in the aquarium trade (Chan 1998; Calado *et al*. 2003), with photographs often appearing in underwater guidebooks (e.g. Debelius 1984, 1999; Minemizu 2000; Kato and Okuno 2001; Kawamoto and Okuno 2003). Gnathophyllidae and Hymenoceridae are generally included with six other families, namely Palaemonidae Rafinesque, Typhlocarididae Annandale & Kemp, Desmocarididae Borradaile, Anchistioididae Borradaile, Euryrhynchidae Holthuis and Kakaducarididae Bruce, in the superfamily Palaemonoidea Rafinesque (e.g. Chace 1992; Chace and Bruce 1993; Holthuis 1993; Martin and Davis 2001).

The two species-poor families Gnathophyllidae and Hymenoceridae are separated from the other families and all other caridean shrimps mainly by having a broadened third maxilliped that is sometimes leaf-like (e.g. Bruce 1986; Chace and Bruce 1993; Holthuis 1993). Nevertheless, the family Hymenoceridae had been placed in the family Gnathophyllidae for a long time until Chace (1992) separated the members into two different families based on the presence or absence of armature on the ischium and merus of the third maxilliped.

Bruce (1986), however, argued that the larval morphology of Gnathophyllidae is very similar to that of Pontoniinae Kingsley, a subfamily of Palaemonidae, and therefore proposed to synonymise these two groups. Later Bruce (1988) also reported that the larvae of Hymenoceridae show close similarity with the other members of the family Palaemonidae, and suggested that Hymenoceridae should be included in Palaemonidae. In a recent larval study, Yang and Ko (2004) also indicated that the first zoea of a pontoniine species, *Conchodytes nipponensis* (de Haan), is very similar to that of *Gnathophyllum americanum* Guérin-Méneville. The present study attempts to elucidate the phylogenetic relationships among Gnathophyllidae, Hymenoceridae and Pontoniinae based on DNA sequence analyses of nuclear 18S and 28S rRNA. In this study, we address whether the molecular analysis supports a close relationship between the three taxa and the monophyly of the subfamily Pontoniinae exclusive of the families Gnathophyllidae and Hymenoceridae.

Materials and methods

Materials

One genus in Gnathophyllidae, two genera in Hymenoceridae and eight genera in Pontoniinae were used in the present study. Two 'classical' genera, namely *Palaemon* Weber and *Macrobrachium* Bate, together with three other species with sequences from GenBank in the other subfamily, Palaemoninae Rafinesque, of Palaemonidae and another family, Anchistioididae, in Palaemonoidea were used to resolve the

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status of Gnathophyllidae and Hymenoceridae in the superfamily Palaemonoidea. Two genera, *Alpheus* Fabricius and *Eugonatonotus* Schmitt, each from a different caridean superfamily, Alpheioidea Rafinesque and Nematocarcinoidea Smith respectively, were included as outgroup taxa. One species was used to represent each of the above genera and the details on the specimens examined are listed in Table 1. Whole bodies of shrimps were fixed in 75–99% ethyl alcohol.

DNA extraction

Pleopod muscle or eggs (10–15 mg) of most of the samples were used for total DNA extraction with QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany). The DNA was eluted in 200 μL of double distilled H_2O (ddH_2O). For *Hymenocera picta* Dana, *Coralliocaris superba* (Dana) and *Periclimenes brevicarpalis* (Schenkel), DNA was extracted by mincing 1 mg of eggs or muscle and was digested using proteinase K–sodium dodecyl sulfate (SDS) solution and purified by standard phenol/chloroform extraction. DNA was dissolved in 20–100 μL of TRIS-ethylenediaminetetraacetic acid (EDTA) buffer (pH 7.6). To check the DNA quality, 5 μL of the DNA extract was subjected to 1% agarose gel electrophoresis and ethidium bromide staining.

PCR amplification and nucleotide sequencing

PCR was performed to amplify partial segments of the nuclear gene coding for 18S rRNA (~1.8 kb) and 28S rRNA (~1 kb). The 18S rRNA gene was amplified with primers 18S A and 18S B (Medlin *et al*. 1988). For 28S rRNA amplification, the primer pair 28S-RD3.3f and 28S-rD5b (Whiting 2002) was used.

The PCR amplifications were performed in 50 μL containing 5 μL of DNA extract, 0.4 μM of each primer, 0.2 μM of deoxyribonucleotide triphosphate (dNTP), 2.5 units of *Taq* polymerase (Promega, Madison, WI, USA), 1.5 mM of magnesium chloride and 1× Mg^{2+} free buffer. Thermal cycling for 18S rRNA gene amplification was performed as follows: initial denaturation for 3 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 53°C and 2 min at 72°C, with a final extension at 72°C for 3 min. For amplification of 28S rRNA genes, the cycling profiles involved 3 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 56°C and 1 min 30 s at 72°C and then 3 min of final extension. PCR products were purified by QIAquick PCR purification kit or QIAquick gel purification kit (QIAGEN) before sequencing.

The 28S rRNA gene segments were sequenced using the same forward and reverse primers for PCR amplification. For sequencing the 18S rRNA gene segment, three sets of internal primers (Apakupakul *et al*. 1999: 18S A and 18S L, 18S C and 18S Y, 18S O and 18S B) were used to amplify three ~600-bp overlapping fragments. The 20-μL cycle sequencing mix contained 8 μL of ABI Prism dRodamine terminator (Applied Biosystems, Foster City, CA, USA), 3–6 μL of purified PCR products, 0.16 μM of primer and ddH_2O to make up to 20 μL. The cycling profile involved 1 min at 96°C, followed by 25 cycles of 30 s at 96°C, 15 s at 50°C and 4 min at 60°C. The products were purified with an ethyl alcohol–sodium acetate precipitation protocol (Applied Biosystems). The purified products were loaded onto ABI 3100 Genetic Analyzer (Applied Biosystems) for analysis. The sequences were confirmed by checking the data from both strands using Sequencher Version 3.1 (Gene Codes Corporation, Ann Arbor, MI, USA).

Phylogenetic analyses

Alignments of the datasets were conducted using ClustalX-1.83.1 (Thompson *et al*. 1997). The 'slow–accurate' algorithm was used for pairwise alignment with costs of 10.0 for gap opening and 0.10 for gap extension. For multiple alignments,

Table 1. Shrimp taxa and GenBank accession numbers for 18S and 28S rRNA gene sequences in this study
Sequences with an asterisk are those not obtained by the authors.

Family/subfamily	Species	Sampling locality	GenBank Accession number	
			18S sequences	28S sequences
Gnathophyllidae	*Gnathophyllum americanum*	Panglao Island, the Philippines	DQ642848	EF540838
Hymenoceridae	*Hymenocera picta*	Aquarium (the Philippines)	DQ642855	EF540839
	Phyllognathia ceratophthalma	Panglao Island, the Philippines	DQ642847	EF540840
Palaemonidae				
Pontoniinae	*Dactylonia* sp.	Panglao Island, the Philippines	DQ642850	EF540841
	Conchodytes meleagrinae	Nakamoto, Kuroshima, Okinawa Is., Japan	EF540837	EF540842
	Izucaris masudai	Kuryo, Izu, Japan	EF540835	EF540843
	Tuleariocaris zanzibarica	Kuryo, Izu, Japan	EF540836	EF540844
	Periclimenes brevicarpalis	Iriomote Island, Japan	DQ642853	EF540845
	Anchistus miersi	Panglao Island, the Philippines	DQ642851	EF540846
	Thaumastocaris streptopus	Panglao Island, the Philippines	DQ642852	–
	Coralliocaris superba	Iriomote Island, Japan	DQ642854	–
Palaemoninae	*Palaemon macrodactylus*	Tokyo Bay, Japan	DQ642849	EF540847
	Macrobrachium rosenbergii	Hong Kong	DQ642856	EF540848
	Palaemonetes paludosus		DQ079755*	DQ079796*
	Cryphiops caementarius		DQ079747*	DQ079785*
	Creaseria morleyi		DQ079746*	DQ079784*
Anchistioidae	*Anchistioides* sp.	Iriomote Island, Japan	DQ642857	EF540849
Alpheidae	*Alpheus gracilipes*	Kenting, Taiwan	DQ642859	EF540850
Eugonatonotidae	*Eugonatonotus chacei*	Dashi, Taiwan	DQ642858	EF540851

the cost for gap opening was 10.0 and gap extension was 0.20. The alignments were adjusted by eye. Uncertain alignments were omitted from the analysis. Four methods were used to infer the phylogenetic relationships: distance (BIO neighbour-joining, BIONJ), maximum parsimony (MP) and maximum likelihood (ML) performed in PAUP 4.0b10 (Swofford 2002), and Bayesian inference (BI) using MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). They were conducted for each dataset separately (18S rRNA, 19 taxa; 28S rRNA, 17 taxa) and for the combined dataset (17 taxa).

Before analysing the combined dataset, incongruence length difference (ILD) test (Farris *et al*. 1994), referred to as a 'partition homogeneity test' in PAUP 4.0b10 (Swofford 2002) was used to examine possible incongruence between genes. Although there are still controversies on combining distinct datasets into a single analysis (Huelsenbeck *et al*. 1996), conditional combination supporters argued that testing for topological incongruence between data partitions is an important step in data exploration because it can tell whether some of the data partitions support wrong phylogeny (Huelsenbeck *et al*. 1996). The ILD test was found to be the most useful statistical test of incongruence (Cunningham 1997). Yet the use of ILD test for measurement of incongruence to act as indicator of dataset combinability has been questioned (Yoder *et al*. 2001). With such reservation in mind, we performed ILD tests as an exploratory step. One thousand replicates of the ILD test were implemented. No evidence was presented for phylogenetic conflict between 18S rRNA and 28S rRNA gene partitions ($P = 1.0$), therefore justifying a combined data approach. The best-fit model of nucleotide substitution used for BIONJ, ML and BI analyses was determined in Modeltest version 3.5 (Posada and Crandall 1998) using the hierarchical likelihood ratio test (hLRT; Huelsenbeck and Crandall 1997).

Heuristic MP and ML searches were executed using tree-bisection-reconnection (TBR) branch swapping with 10 random addition sequence replicates. Starting tree for branch-swapping was obtained by stepwise addition. Gaps were treated as a fifth character. Bootstrap analysis, based on full heuristic search of 1000 and 500 pseudoreplicates using TBR branch-swapping and as-is stepwise addition, was carried out to determine the MP and ML branch support respectively. One thousand bootstrap replicates were performed in BIONJ analysis to assess the confidence level at each branch. Bayesian inference analysis was performed with specific models and parameters assigned separately to the individual and combined dataset based on MrModeltest 2.2 (Nylander 2004). A single model was used for the combined dataset. A Markov chain Monte Carlo search was run for 2 000 000 generations with a sampling frequency of 100 generations. Trees before log-likelihood stabilisation (burn-in = 25% trees) and convergence were discarded before producing a consensus tree.

Alternative phylogenetic hypotheses were statistically tested in PAUP* using the Kishino–Hasegawa (KH) test (Kishino and Hasegawa 1989) and Shimodaira–Hasegawa (SH) test (Shimodaira and Hasegawa 1999) respectively. The null hypothesis for all topology testing is that there is no difference between topologies.

Results

Both nuclear 18S rRNA and 28S rRNA gene segments were successfully amplified from 14 of the 16 species studied (Table 1). 18S rRNA, but not 28S rRNA, sequences were obtained from *Coralliocaris superba* (Dana) and *Thaumastocaris streptopus* Kemp. All sequences determined were deposited in GenBank database (see Table 1 for accession numbers). The average length of the 18S rRNA and 28S rRNA PCR products are 1830 bp (1813–1839 bp) and 991 bp (962–1072 bp) respectively. With an aligned length of 1851 bp in the 18S rRNA sequences, there are 259 variable sites, of which 129 are parsimony informative. The nucleotide composition is 24.8% A, 23.8% T, 23.3% C and 28.2% G (A+T% = 48.6%). With an aligned length of 966 bp in the 28S rRNA sequences (257 bp of uncertain alignments were omitted from the analysis), there are 376 variable sites, of which 270 are parsimony informative. The nucleotide composition is 20.8% A, 20.4% T, 25.1% C and 33.6% G (A+T% = 41.2%).

The pairwise Kimura's 2-parameter distances between each species for the two gene segments are listed in Table 2. For each species pair, the divergence is always higher in 28S rRNA than in 18S rRNA. For instance, the sequence divergences between *Phyllognathia ceratophthalma* (Balss) and *Hymenocera picta* are 2.2% and 7.8% for 18S rRNA and 28S rRNA respectively. The mean sequence divergences among species are 2.7% and 15.0% (excluding outgroups) for 18S rRNA and 28S rRNA respectively.

Results of homogeneity test supported combined analysis of 18S rRNA and 28S rRNA genes ($P = 1.0$). Modeltest and MrModeltest both suggested the same evolutionary models for the combined or individual datasets. In the combined dataset analysis, the best-fit DNA substitution model is general time reversible incorporating invariable sites and rate variation among sites (GTR+I+G with base frequencies A = 0.237, C = 0.243, G = 0.298, T = 0.223; R(A–G) = 1.344, R(C–T) = 3.215, R(A–C) = 0.413, R(A–T) = 1.087, R(C–G) = 0.918, R(G–T) = 1; I = 0.589; G = 0.549) (Rodriguez *et al*. 1990). In the 18S dataset analysis, the best-fit model is the symmetrical model incorporating invariable sites and rate variation among sites (SYM+I+G with base frequencies A = 0.250, C = 0.250, G = 0.250, T = 0.250; R(A–G) = 1.646, R(C–T) = 2.960, R(A–C) = 1.0, R(A–T) = 1.0, R(C–G) = 1.0, R(G–T) = 1.0; I = 0.74; G = 0.682). In the 28S dataset analysis, the best-fit model is general time reversible incorporating invariable sites and rate variation among sites (GTR+I+G with base frequencies A = 0.2, C = 0.240, G = 0.338, T = 0.222; R(A–G) = 1.791, R(C–T) = 4.340, R(A–C) = 1.0, R(A–T) = 1.0, R(C–G) = 1.0, R(G–T) = 1.0; I = 0.396; G = 0.803). MP analysis of the combined dataset gives one most-parsimonious tree, with 1504 steps, a consistency index (CI) of 0.632 and a retention index (RI) of 0.526. MP analysis of the 18S and 28S datasets gives one and three most-parsimonious trees, with 502 and 1268 steps, CI of 0.691 and 0.625 and RI of 0.545 and 0.549 respectively.

In the combined gene tree (Fig. 1*a*), the two species, *Phyllognathia ceratophthalma* and *Hymenocera picta*, of the family Hymenoceridae group together with strong support (100% bootstrap (BP) support in BIONJ, MP and ML analyses; Bayesian posterior probabilities (BPP) = 1.00). *Gnathophyllum*

Table 2. Kimura's 2-parameter distances of 28S rRNA (above diagonal) and 18S rRNA (below diagonal) gene sequences among 19 species studied

	Phyllognathia ceratophthalma	*Hymenocera picta*	*Gnathophyllum americanum*	*Dactylonia* sp.	*Conchodytes meleagrinae*	*Izucaris masudai*	*Tuleariocaris zanzibarica*	*Periclimenes brevicarpalis*	*Anchistus miersi*	*Anchistioides* sp.	*Macrobrachium rosenbergii*	*Cryphiops caementarius*	*Creaseria morleyi*	*Palaemon macrodactylus*	*Palaemonetes paludosus*	*Eugonatonotus chacei*	*Alpheus gracilipes*	*Thaumastocaris streptopus*
Phyllognathia ceratophthalma		0.078	0.094	0.158	0.149	0.171	0.165	0.173	0.160	0.169	0.193	0.184	0.191	0.209	0.211	0.242	0.231	–
Hymenocera picta	0.022		0.095	0.152	0.142	0.181	0.174	0.171	0.160	0.165	0.197	0.190	0.194	0.193	0.209	0.231	0.227	–
Gnathophyllum americanum	0.033	0.031		0.142	0.133	0.160	0.167	0.134	0.138	0.138	0.174	0.170	0.188	0.182	0.190	0.233	0.220	–
Dactylonia sp.	0.034	0.031	0.015		0.069	0.153	0.153	0.117	0.100	0.120	0.188	0.148	0.165	0.171	0.168	0.213	0.196	–
Conchodytes meleagrinae	0.034	0.032	0.015	0.007		0.154	0.147	0.119	0.110	0.131	0.185	0.168	0.167	0.181	0.185	0.217	0.205	–
Izucaris masudai	0.040	0.040	0.024	0.021	0.022		0.101	0.135	0.121	0.142	0.188	0.172	0.167	0.181	0.190	0.230	0.210	–
Tuleariocaris zanzibarica	0.055	0.052	0.040	0.031	0.032	0.036		0.141	0.133	0.134	0.168	0.168	0.179	0.175	0.192	0.214	0.205	–
Periclimenes brevicarpalis	0.041	0.040	0.026	0.017	0.019	0.023	0.035		0.063	0.094	0.146	0.133	0.136	0.135	0.152	0.172	0.165	–
Anchistus miersi	0.040	0.038	0.020	0.013	0.013	0.019	0.028	0.012		0.081	0.132	0.112	0.126	0.129	0.130	0.180	0.171	–
Anchistioides sp.	0.038	0.036	0.023	0.018	0.018	0.023	0.036	0.020	0.012		0.137	0.135	0.121	0.135	0.135	0.178	0.163	–
Macrobrachium rosenbergii	0.050	0.048	0.038	0.032	0.032	0.036	0.043	0.031	0.027	0.030		0.083	0.140	0.137	0.143	0.206	0.202	–
Cryphiops caementarius	0.046	0.043	0.031	0.024	0.025	0.029	0.039	0.023	0.019	0.022	0.014		0.131	0.130	0.144	0.199	0.182	–
Creaseria morleyi	0.050	0.047	0.036	0.028	0.029	0.034	0.040	0.029	0.025	0.029	0.026	0.022		0.151	0.158	0.192	0.189	–
Palaemon macrodactylus	0.041	0.039	0.025	0.018	0.017	0.022	0.032	0.019	0.012	0.016	0.022	0.018	0.024		0.063	0.192	0.171	–
Palaemonetes paludosus	0.045	0.043	0.029	0.023	0.023	0.025	0.035	0.021	0.018	0.022	0.022	0.016	0.026	0.008		0.199	0.177	–
Eugonatonotus chacei	0.062	0.062	0.056	0.049	0.050	0.054	0.062	0.047	0.042	0.043	0.057	0.051	0.056	0.044	0.053		0.108	–
Alpheus gracilipes	0.086	0.083	0.076	0.072	0.072	0.078	0.080	0.068	0.069	0.071	0.072	0.073	0.077	0.071	0.074	0.067		–
Thaumastocaris streptopus	0.042	0.039	0.022	0.015	0.015	0.019	0.032	0.014	0.005	0.012	0.027	0.019	0.027	0.012	0.017	0.043	0.071	
Coralliocaris superba	0.038	0.037	0.021	0.014	0.014	0.018	0.030	0.011	0.004	0.012	0.025	0.018	0.025	0.010	0.016	0.043	0.068	0.004

Fig. 1. Phylogenetic trees resolved by maximum likelihood analysis of DNA sequences of (*a*) combined 18S rRNA and 28S rRNA, (*b*) 28S rRNA and (*c*) 18S rRNA. Percentage levels of support based on bootstrap replicates (1000 bootstrap for BIO neighbour-joining and maximum parsimony analyses, and 500 bootstrap for maximum likelihood analysis) are indicated on each branch for BIO neighbour-joining (in normal), maximum parsimony (in bold), maximum likelihood (in italics) and Bayesian posterior probabilities (underlined).

americanum of the family Gnathophyllidae forms a well supported clade with the family Hymenoceridae (BP values 100% in BIONJ, MP and ML analyses; BPP = 1.00). The grouping of the families Hymenoceridae and Gnathophyllidae with *Dactylonia* Fransen and *Conchodytes meleagrinae* Peters of the subfamily Pontoniinae is supported (BP values ≥ 92% in BIONJ, MP and ML analyses; BPP = 1.00). Furthermore, the six Pontoniinae species group with the families Hymenoceridae and Gnathophyllidae to form a well supported clade (BP values ≥ 82% in BIONJ, MP and ML analyses; BPP = 1.00). *Palaemon macrodactylus* Rathbun, *Macrobrachium rosenbergii* (De Man) and three species from the GenBank entries for the subfamily Palaemoninae form a distinct group with BPP = 0.79; and *Anchistioides* Paulson from the family Anchistiodidae is more closely related to the Hymenoceridae–Gnathophyllidae–Pontoniinae clade than to the Palaemoninae taxa.

Independent analysis of the 28S rRNA dataset yields a similar topology of the species studied (Fig. 1*b*) to the combined analysis with the exception that members of Palaemoninae do not form a monophyletic group. Yet the bootstrap supports for the relationships in this group are weak. In contrast, the monophyletic status of Palaemoninae is supported by the 18S rRNA gene tree (Fig. 1*c*). The close relationship of *Dactylonia* Fransen and *Conchodytes meleagrinae* to Hymenoceridae and Gnatho-

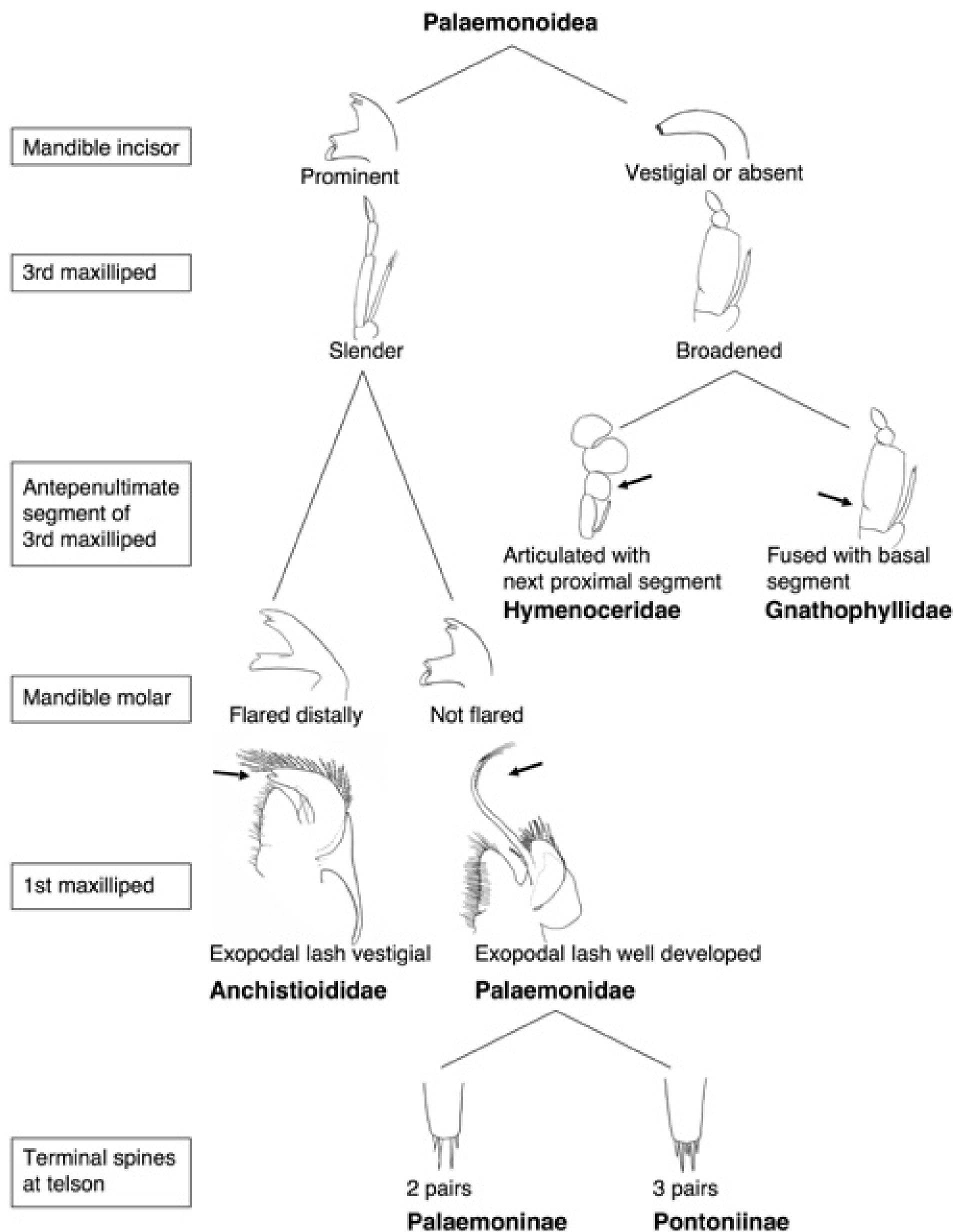

Fig. 2. Pictorial key to the families and subfamilies used in the present study from generally accepted classification scheme based on adult morphology, modified from Chace and Bruce (1993) and Holthuis (1955). Line drawings provided are schematic.

phyllidae is supported by all analyses. The two species of Pontoniinae, *Coralliocaris superba* and *Thaumastocaris streptopus*, with only the 18S rRNA sequences, cluster with the Pontoniinae–Hymenoceridae–Gnathophyllidae clade, but the BP values of the 18S rRNA gene tree supporting this clade or any of the relationships within the clade are generally much lower than those of the 28S rRNA or combined gene trees. A major discrepancy between the 18S rRNA gene tree and the 28S rRNA or combined gene tree is that in the former, all members of the families Palaemonidae, Hymenoceridae and Gnathophyllidae are grouped together (BP values ≥ 57% in BIONJ, MP and ML analyses; BPP = 0.93) so that *Anchistioides* Paulson of the family Anchistiodidae is the most distantly related taxon among the ingroup taxa studied (Fig. 1*c*). This is different from a close relationship between *Anchistioides* and the Hymenoceridae–Gnathophyllidae–Pontoniinae clade as revealed in the 28S and combined analyses.

For each of the combined or individual gene trees, the four phylogenetic analyses yielded similar topologies; the differences lie in the relationships among the pontoniine taxa, whereas the Hymenoceridae–Gnathophyllidae clade and its grouping with all the pontoniine taxa are always supported. The groupings of all the pontoniine taxa (without Hymenoceridae and Gnathophyllidae) or all the members of Palaemonidae (i.e. both Pontoniinae and Palaemoninae) are not supported by monophyly tests ($P < 0.001$). On the other hand, there are statistically significant levels of support ($P < 0.001$) based on both KH and SH tests for the grouping of Hymenoceridae and Gnathophyllidae and their clustering with Pontoniinae. Moreover, the tree that groups all ingroup taxa studied without *Anchistioides* is not significantly different in topology from the combined gene tree shown in Fig. 1*a* ($P > 0.1$ in both tests).

Discussion

The phylogenetic trees derived from the 18S rRNA and 28S rRNA sequences show that the two genera in Hymenoceridae are closely related, and they are most closely related to the family Gnathophyllidae (Fig. 1). The divergences of the 28S rRNA gene among the three species are the lowest among all the species studied (Table 2). The individual and combined gene trees show that the Hymenoceridae–Gnathophyllidae clade is grouped with all the pontoniine genera included in the present analysis to form a larger clade. Thus, species of the subfamily Pontoniinae constitute a paraphyletic group because the Hymenoceridae and Gnathophyllidae are nested within these species.

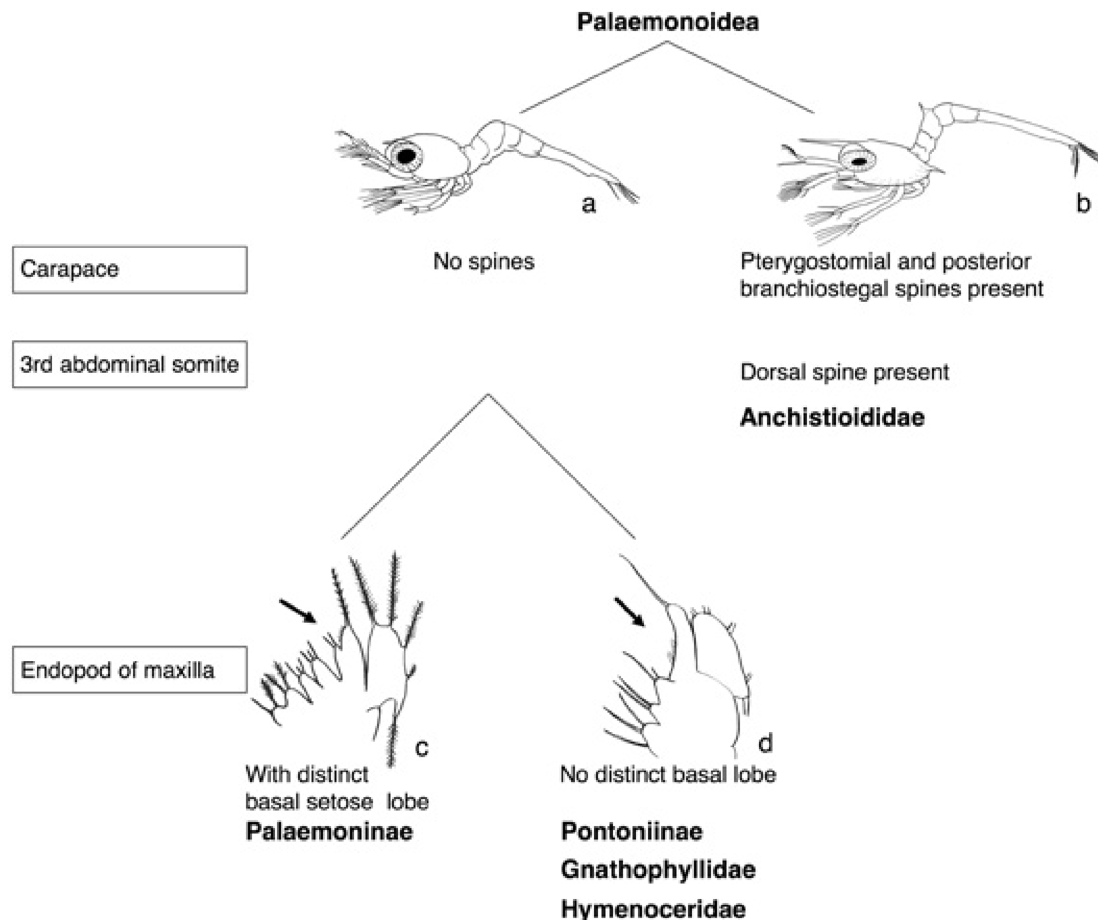

Fig. 3. Diagrammatic illustrations showing the separation of the families and subfamilies based on first zoeal morphology, modified from Bruce (1986, 1988) and Yang and Ko (2002). Line drawings (*a*) and (*d*) modified from Gurney (1938), (*b*) modified from Gurney (1936) and (*c*) modified from Shokita (1985).

Figure 2 summarises the major characters used for separating the families and subfamilies in Palaemonoidea studied in the present analysis. Figure 3 illustrates the similarities and differences reported for the larvae of these families and subfamilies as discussed by Bruce (1986, 1988) and Yang and Ko (2002). The relationships revealed in the molecular analysis are in accordance with the relationships among Hymenoceridae, Gnathophyllidae and Pontoniinae as suggested by the larval morphology (Fig. 3). Bruce (1986) suggested that the larval morphology of Gnathophyllidae is closest to that of Pontoniinae, but did not state clearly how their larvae differ from the other carideans. Yang and Ko (2002) summarised the morphology of the first zoea of Palaemoninae and Pontoniinae and concluded that the larvae of the two subfamilies may be distinguished by the number of setae on the endopod of the maxilla. Yang and Ko (2004) argued that the first zoea of the pontoniine species *Conchodytes nipponensis* is very similar to that of *Gnathophyllum americanum.* The present molecular study includes both of these two genera (i.e. *C. meleagrinae* and *G. americanum*) and they do show a very close relationship in the combined gene tree. A review of the literature (Lebour 1925; Caroli 1926; Gurney 1936, 1938; Gurney and Lebour 1941; Nayar 1947; Shokita 1977, 1985; Fincham and Williamson 1978; Gore *et al*. 1981; Calafiore *et al*. 1991; Costanzo *et al*. 1996; Gamba 1998; Yang and Ko 2002, 2004; Nagai and Shokita 2003, on 16 genera) on the first zoea with enough details reported (excluding those with abbreviated development) shows that most species of Palaemoninae and Pontoniinae can be separated as suggested by Yang and Ko (2002), except for five genera, namely *Leander* E. Desmarest and *Leandrites* Holthuis of the Palaemoninae and *Harpilius* Dana, *Cuapetes* Clark (= *Kemponia* Bruce) and *Philarius* Holthuis of the Pontoniinae. In contrast, the first zoea of both *Gnathophyllum americanum* and *Hymenocera picta* described by Bruce (1986, 1988) lacks basal setae and bears a terminal seta on the endopod of the maxilla, thus exhibiting the 'typical' features of pontoniine larvae.

Gordon (1935) regarded the family Anchistioididae (containing only a single genus *Anchistioides*) as invalid and placed this group of shrimps under Pontoniinae. However, the peculiar larval morphology of *Anchistioides* discovered later (Gurney 1936, 1938) suggests its separation from the other palaemonoid families (also see Bruce 1986, 1988; Chace 1992; Chace and Bruce 1993; Holthuis 1993). Such a separation is also supported by our 18S rRNA gene tree. Yet conflicting results on the phylogenetic position of the Anchistioididae are obtained from the 28S rRNA data and the combined analysis. As the combined gene tree could not conclusively define the phylogenetic position of Anchistioididae (as indicated by monophyly tests), the application of more genes is needed to elucidate the relationship of Anchistioididae with Palaemonidae.

The five palaemonine genera used in the present analysis are morphologically rather similar. However, some other palaemonine genera show close resemblance to certain pontoniines instead. For example, whether the above-mentioned *Leander* and *Leandrites*, as well as *Brachycarpus* Bate and *Urocaridella* Borradaile, really belong to Palaemoninae, and whether *Periclimenella* Bruce, *Manipontonia* Bruce, Okuno & Li and *Exoclimenella* Bruce really are pontoniines, are still unclear (Ďuriš and Bruce 1995; Bruce *et al*. 2005). The currently defined Palaemoninae and Pontoniinae contain a very large number of genera and species (~862 species in 115 genera, see Chace and Bruce 1993; Li 2000; Fransen 2002; Bruce *et al*. 2005), and it is often suspected that neither subfamily is monophyletic. More information on the larval morphology of the various genera (particularly for *Brachycarpus, Urocaridella*, *Periclimenella, Manipontonia* and *Exoclimenella* as mentioned above) in the family Palaemonidae is needed to determine if the difference observed at the endopod of the maxilla truly represents a natural division in these shrimps. Nevertheless, the present molecular data reveal that both Hymenoceridae and Gnathophyllidae are not distinct from the pontoniines, and the two groups should be merged with the latter. Yet given the limited number of Pontoniinae species and gene sequences examined in the present analysis, the hypotheses on the paraphyly of Pontoniinae and inclusion of Hymenoceridae and Gnathophyllidae in this group should be regarded as preliminary. Further studies on these groups as well as the inclusion of the other four families of Palaemonoidea (Desmocarididae, Euryhynchidae, Kakaducarididae and Typhlocarididae) in the analyses are necessary to ascertain their phylogenetic relationships and taxonomic ranks in the Caridea.

Acknowledgements

The first author would like to thank M. Takeda and I. Nishiumi of the National Science Museum, Tokyo for their suggestions and encouragements and Y. Ise of the University of Tokyo for collecting *Anchistioides* sp. Some of the specimens used in this study were collected by the Philippine 'Panglao 2004' expedition. The 'Panglao 2004' Marine Biodiversity Project was a collaboration between Muséum national d'Histoire naturelle, Paris (Principal Investigator, Philippe Bouchet) and University of San Carlos, Cebu City (Principal Investigator, Danilo Largo). The 'Panglao 2004' was supported by the Total Foundation for Biodiversity and the Sea, the French Ministry of Foreign Affairs, and the ASEAN Regional Center for Biodiversity Conservation (ARCBC). The Philippines Bureau of Fisheries and Aquatic Resources (BFAR) is acknowledged for issuing a research permit on the materials collected through 'Panglao 2004'. This work was supported by grants from the Research Grants Council of the Hong Kong Special Administrative Region, China (Project No. CUHK4419/04M) to KHC, the National Science Council, R.O.C. and the Center for Marine Bioscience and Biotechnology of the National Taiwan Ocean University to TYC.

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Manuscript received 1 June 2006, accepted 30 October 2007