



Phylogeny of penaeoid shrimps (Decapoda: Penaeoidea) inferred from nuclear protein-coding genes

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ABSTRACT

Penaeoidea is a diverse group of economically important marine shrimps. Attention to the evolutionary history of the penaeoids has been raised since studies using mitochondrial DNA markers and sperm ultrastructure contradict classification of the penaeoid families based on morphology and hence challenge the long standing taxonomy of this superfamily. In this study, DNA sequences of two nuclear protein-coding genes, phosphoenolpyruvate carboxykinase and sodium–potassium ATPase α -subunit, were determined from 37 penaeoid genera to reconstruct the evolutionary relationships and to estimate divergence ages of the penaeoid shrimps. Phylogenetic analyses using maximum likelihood and Bayesian approaches strongly support the monophyly of Solenoceridae, Aristeidae and Benthescymidae, but find Sicyoniidae nested within Penaeidae, making this family paraphyletic. Penaeoidea comprises two lineages: the former three families in one while the latter two in another. The diversification of these lineages may be related to bathymetry. The penaeid-like lineage diversified in the Triassic, earlier than the aristeid-like lineage with an origin in the Jurassic. Taxonomic revisions within Penaeoidea are also proposed for further investigation. Due to the paraphyly of Penaeidae and the high genetic divergence among the three penaeid tribes of Burkenroad [Burkenroad, M.D., 1983. Natural classification of Dendrobranchiata, with a key to recent genera. In: Schram, F.R. (Ed.), *Crustacean Issues I. Crustacean Phylogeny*. A.A. Balkema, Rotterdam, pp. 279–290], these tribes should be treated as having the same taxonomic rank as Sicyoniidae, while the family ranking of Benthescymidae has to be re-considered owing to the low genetic divergence between the benthescymids and the aristeids.

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1. Introduction

The penaeoid shrimps (superfamily Penaeoidea) constitute a diverse group of marine decapods with over 400 species. Globally distributed, and inhabiting both shallow waters and abyssal zones below 5000 m, they occupy different trophic levels of the food chain at various water depths in the ocean (Pérez Farfante and Kensley, 1997). This group includes most of the shrimps of high economic value that account for over one-third of the annual wild crustacean catch (FAO, 2008). Given the commercial importance of its members, it is surprising that no consensus on the phylogeny of Penaeoidea has been reached to put an end to uncertainties surrounding its higher taxonomy.

Penaeoidea is considered by most authors to have four families, namely, Aristeidae, Solenoceridae, Penaeidae and Sicyoniidae (e.g., Holthuis, 1980; Liu and Zhong, 1986; Yu and Chan, 1986; Dall et al., 1990; Hayashi, 1992; Chan, 1998). However, the most recent classification scheme gives Penaeoidea five families, by adding the

family Benthescymidae (Pérez Farfante and Kensley, 1997; Martin and Davis, 2001). Benthescymidae was traditionally considered as a subgroup (i.e., series, tribe or subfamily) of Aristeidae, and the suggestion that it should be ranked as a family, first made by Crosnier in 1985 went unheeded until recently. As for the other four families, Sicyoniidae is commonly believed to be close to Penaeidae while Solenoceridae is allied with Aristeidae. Such a subdivision of the superfamily coincides with the distinct adult habitat choices of the families: the penaeids and sicyoniids usually inhabit littoral waters while the aristeids and solenocerids are mostly deep-sea species (Burkenroad, 1934, 1936; Pérez Farfante, 1977; Dall et al., 1990). However, detailed discussions of the overall phylogenetic relationships amongst the penaeoid families and genera have been limited, and only two comprehensive schemes have been proposed by Kubo (1949) and Burkenroad (1983). Kubo's (1949, Fig. 1a) scheme, although deduced from a very complicated set of characters (19 body parts including even appendix masculina, ptero-cardiac, zygocardiac and prepyloric ossicles), was based on rather limited genera. He proposed that Sicyoniidae (as Eusicyoninae) was the most primitive while Penaeidae (as Penaeinae) was the most advanced group, with Solenoceridae (as Solenocerinae) being

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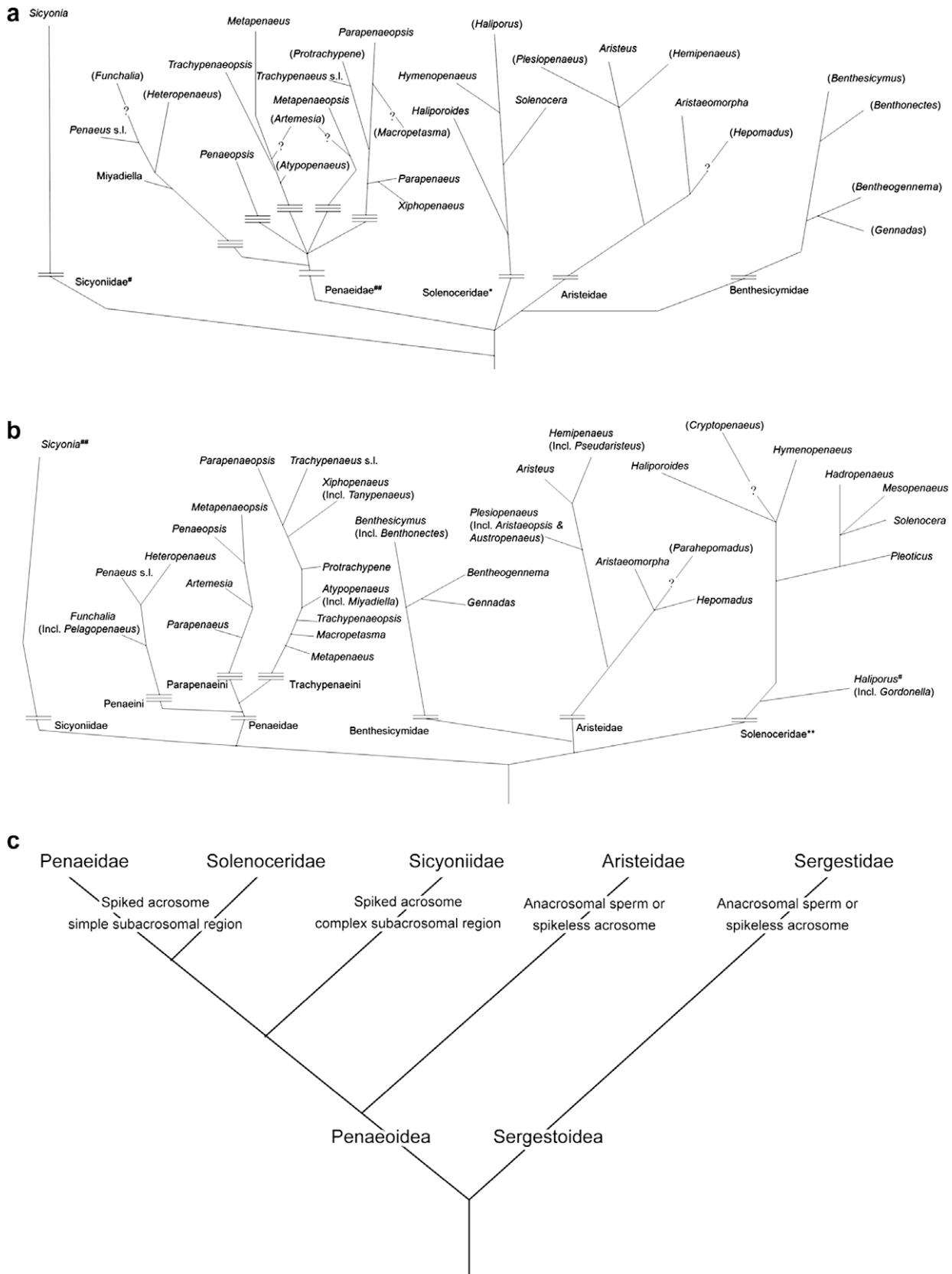


Fig. 1. Morphological phylogeny of the penaeoid genera proposed by (a) Kubo (1949), reconstructed from text, genera in brackets were not fully analyzed, and (b) Burkenroad (1983), reconstructed from key (mentioned by the author as "...a natural key down to the level of genus", genera in brackets were recently discovered or split from existing genera. (c) Phylogeny of the dendrobranchiate families based on sperm ultrastructure proposed by Scelzo and Medina (2004) and Medina et al. (2006a, b). * Considered as intermediate between Penaeidae and Aristeidae. ** Relationships of solenoceric genera after Pérez Farfante (1977) who mostly based on the grouping of Burkenroad (1936). # Considered as the most primitive group. ## Considered as the most advanced group. "?" refers to uncertain relationship. Noted that all names used here follow Pérez Farfante and Kensley (1997), with many of them different from those used by Kubo (1949) and Burkenroad (1983), and they both did not recognize the five-family scheme in Penaeoidea.

intermediate between Aristeidae (as Aristaeinae) and Penaeidae. Burkenroad (1983, Fig. 1b) constructed a scheme of all the genera known at that time and considered that Solenoceridae was the most primitive group based on fossil findings (even perhaps the ancestor of Penaeoidea and likely also Sergestoidea, see also Burkenroad, 1963), and Sicyoniidae (as Sicyoninae) was the most advanced. The relationships amongst the penaeoid genera are also very different between the schemes of Kubo and Burkenroad (Fig. 1, see also Chan et al., 2008). Although, Burkenroad (1983) only considered three genera in Solenoceridae (as Solenocerinae), Pérez Farfante (1977) elaborated the phylogenetic relationships of the then established seven solenocerid genera based mostly on Burkenroad's (1936) earlier groupings. Several other genera later discovered or split from the existing genera can also be readily incorporated into Burkenroad's (1983) scheme (i.e., those genera in parentheses in Fig. 1b). It should also be noted that the rare genus *Gordonella* was originally established by Tirmizi (1960) under Benthescymidae but was later re-assigned to Solenoceridae by Crosnier (1988).

The above morphologically-inferred phylogenies were challenged by recent phylogenetic studies with noticeably contrasting conclusions. Analyses of spermatozoa ultrastructure suggest a close relationship between Penaeidae and Solenoceridae, both with spiked acrosome and simple subacrosomal region (Scelzo and Medina, 2004; Medina et al., 2006a, b; see Fig. 1c). In common with these two families, Sicyoniidae also has spiked spermatozoa but differs by having an elaborated subacrosomal region. Aristeidae is placed in a basal position because the spikeless spermatozoa is regarded by Scelzo and Medina (2004) as an ancestral character shared by the sergestoids shrimps (the sister superfamily of Penaeoidea). However, owing to the limited data available on spermatozoa ultrastructure and the relatively few characters assessed, these results should be viewed as preliminary in terms of phylogenetic reconstruction. On the other hand, molecular phylogenetic studies have produced controversial results that partly refute the monophyly and long-established classification of the penaeoid families. A study based on mitochondrial 16S rRNA gene sequences found Penaeidae paraphyletic, with Solenoceridae nested within it, whereas the other three families are closely related and not reciprocally monophyletic (Vázquez-Bader et al., 2004). Subsequent phylogenetic analysis using both 16S and another mitochondrial gene COI consistently showed the insertion of Solenoceridae into Penaeidae (Quan et al., 2004; Voloch et al., 2005). Nonetheless, bootstrap supports for the inferred topologies are weak and the taxon sampling is limited in these studies. It therefore remains unclear whether the contrasting results represent discrepancies between character evolution and speciation or artifacts of gene tree reconstruction. A more comprehensive study using markers with better resolution is needed to decide between these alternative hypotheses on the evolution of Penaeoidea.

A thorough understanding of evolutionary history requires knowledge not only of phylogenetic relationships but also of the origin and diversification time of the taxa, which is essential for determining whether and how major geological or ecological events impacted on the evolution of organisms. However, owing to their rare and incomplete fossil records, little is known about when penaeoid shrimps diversified. The first trace of penaeoids appeared in the Permo-Triassic period (Burkenroad, 1963; Glaessner, 1969), while the Triassic (206–248 MYA) and Jurassic era (144–206 MYA) were dominated by the family Penaeidae, which began to diversify in the Cretaceous (Glaessner, 1969; Garassino, 1994; Garassino and Teruzzi, 1994). Fossils of Sicyoniidae and Benthescymidae have been discovered, although rarely, in Cretaceous (65–144 MYA) deposits, but no relics of Aristeidae and Solenoceridae are recorded from the Mesozoic (Glaessner, 1969). Based on the observation that some recent solenocerids (e.g., *Haliporus*) possess several

characters similar to the Jurassic fossil *Aeger* (Burkenroad, 1936, 1945, 1963), Burkenroad (1983) hypothesized that the Solenoceridae, and its alliance Aristeidae, have a longer evolutionary history than the other families. Unfortunately, the absence of fossil aristeids and solenocerids prohibits a direct delineation of when these families diversified. On the other hand, the discovery of the Triassic fossil *Antrimpos*, which closely resembles the extant *Penaeus* (Burkenroad, 1963; Glaessner, 1969), may suggest that the family Penaeidae was established at a somewhat earlier period. Rapid diversification and radiation have also commonly been observed in crustaceans based on their fossil records (Schram et al., 1978) but the scarcity of Penaeoidea fossils makes it difficult to determine whether this also applies to them. Application of the relaxed molecular clock method, which permits variation of evolutionary rates across the tree and incorporation of fossil constraints in divergence time estimations, may shed more light on the problem of diversification (Drummond et al., 2006; Rutschmann, 2006). Nonetheless, a reliable phylogenetic tree is a prerequisite for accurate estimations.

The utility of two nuclear protein-coding genes, phosphoenolpyruvate carboxykinase (PEPCK) and sodium–potassium ATPase α -subunit (NaK), as molecular markers for decapod infra-ordinal phylogenetics has been demonstrated (Tsang et al., 2008). These two genes participate in fundamental cellular functions in the animal kingdom and are well-conserved throughout evolution. Presumably, these genes only exist as single-copy in decapods. Previously, they have been applied successfully to resolve the deep-level phylogeny of insects (e.g., Friedlander et al., 1996; Leys et al., 2002) and bilateral metazoans (Anderson et al., 2004). Given that these genes are informative across a wide range of taxonomic levels, we attempted to utilize PEPCK and NaK gene sequences to investigate phylogenetic relationships within Penaeoidea. We aimed to test the alternative hypotheses on the familial relationships of the penaeoids which should, we anticipate, provide new insights to the evolution and classification of the group. We also estimated the divergence ages of the major taxa of Penaeoidea.

2. Materials and methods

2.1. Taxon sampling

We collected the Penaeoid shrimps for this study either by trawling directly from the sea or by purchasing from local fish markets. We followed the most recent classification scheme proposed by Pérez Farfante and Kensley (1997) throughout the study. Representatives from 37 of the 49 genera of the five families in Pérez Farfante and Kensley (1997) were analyzed, including 7 of 9 genera in Aristeidae, 2 of 4 genera in Benthescymidae, 19 of 26 genera in Penaeidae, the single genus of Sicyoniidae, and 8 of 9 genera in Solenoceridae, in total to 46 species (Table 1). The specimen of *Heteropenaeus longimanus*, which was used in our previous phylogenetic study using mitochondrial genes (Chan et al., 2008), was also available for analyses, but PCR amplifications of the target genes in this sample were not successful. Three members of Sergestidae (*Acetes* sp., *Sergestes* sp. and *Sergia maxima*) which is the sister superfamily of Penaeoidea in the sub-order Dendrobranchiata, together with a caridean *Rhynchocinetes durbanensis*, and an euphausiidean *Euphausia superba*, were used as outgroup taxa. Species identification followed the keys of Crosnier (1978, 1988, 2003); Crosnier et al. (2007); Yu and Chan (1986); Liu and Zhong (1986); Pérez Farfante (1988); Dall et al. (1990); Pérez Farfante and Kensley (1997) and Chan (1998). Identification of some aristeids, solenocerids and *Sicyonia* was verified by Crosnier. Samples were either frozen at -70°C or preserved in 95% ethanol prior to DNA extraction.

Table 1

Classification, sampling locations and voucher ID of the species and GenBank accession number of the gene sequences of this study.

Superfamily	Family	Species	Sampling location	Voucher ID	NaK	PEPCK	
<i>Dendrobranchiata</i>							
<i>Penaeoidea</i>							
	Aristeidae	<i>Aristaeomorpha foliacea</i>	Taiwan	NTOU M00747	FJ441125	FJ441170	
		<i>Aristaeopsis edwardsiana</i>	Taiwan	NTOU M00749	FJ441126	FJ441171	
		<i>Aristeus mabahissae</i>	Taiwan	NTOU M00750	FJ441127	FJ441172	
		<i>Aristeus pallidicauda</i>	Taiwan	NTOU M00751	FJ441128	FJ441173	
		<i>Aristeus virilis</i>	Taiwan	NTOU M00703	EU427143	EU427212	
		<i>Hemipenaeus carpenteri</i>	Taiwan	NTOU M00753	FJ441140	FJ441185	
		<i>Hepomadus glacialis</i>	Taiwan	NTOU M00754	FJ441141	FJ441186	
		<i>Parahepomadus vaubani</i>	Taiwan	NTOU M00755	FJ441152	FJ441197	
		<i>Plesiopenaeus armatus</i>	Taiwan	NTOU M00756	FJ441160	FJ441205	
		Benthescymidae	<i>Benthescymus investigatoris</i>	Taiwan	NTOU M00757	FJ441131	FJ441176
			<i>Benthonectes filipes</i>	Is. Wallis	MNHN 1	FJ441130	FJ441175
		Penaeidae	<i>Atypopenaeus dearmatus</i>	Philippines	NTOU M00699	FJ441129	FJ441174
			<i>Farfantepenaeus aztecus</i>	Gulf of Mexico	NTOU M00573	FJ441156	FJ441201
			<i>Fenneropenaeus chinensis</i>	Zhujiang estuary, China	MSLKHC-Fechi	FJ441134	FJ441179
			<i>Fenneropenaeus merguensis</i>	Fish market, Hong Kong	MSLKHC-Femer	FJ441159	FJ441204
			<i>Funchalia</i> sp.	Philippines	NTOU M00758	FJ441135	FJ441180
			<i>Litopenaeus setiferus</i>	Gulf of Mexico	NTOU M00759	FJ441143	FJ441188
			<i>Litopenaeus vannamei</i>	Fish market, Hong Kong	MSLKHC-Livan	FJ441144	FJ441189
			<i>Marsupenaeus japonicus</i>	Singapore	ZRC 2002.0583	FJ441145	FJ441190
			<i>Megokris pescadorensis</i>	Taiwan	NTOU M00760	FJ441146	FJ441191
	<i>Melicertus latisulcatus</i>		Taiwan	NTOU M00513	FJ441147	FJ441192	
	<i>Metapenaeopsis palmensis</i>		Fish market, Hong Kong	MSLKHC-Mepal	FJ441153	FJ441198	
	<i>Metapenaeopsis provocatoria longirostris</i>		Taiwan	NTOU M00761	FJ441149	FJ441194	
	<i>Metapenaeus affinis</i>		Fish market, Hong Kong	MSLKHC-Meaff	FJ441150	FJ441195	
	<i>Metapenaeus ensis</i>		Fish market, Hong Kong	MSLKHC-Meens	FJ441151	FJ441196	
	<i>Parapenaeopsis cornuta</i>		Taiwan	NTOU M00762	FJ441154	FJ441199	
	<i>Parapenaeus sextuberculatus</i>		Taiwan	NTOU M00763	FJ441155	FJ441200	
	<i>Pelagopenaeus balboae</i>		Indian Ocean	NTOU M00764	FJ441157	FJ441202	
	<i>Penaeopsis eduardoi</i>		Taiwan	NTOU M00765	FJ441158	FJ441203	
	<i>Penaeus monodon</i>		Fish market, Hong Kong	MSLKHC-Pemon	EU427144	EU427213	
	<i>Rimapenaeus pacificus</i>		Panama	MNHN 2	FJ441161	FJ441206	
	<i>Trachypenaeopsis mobilispinis</i>	Taiwan	NTOU M00787	FJ905029	FJ905030		
	<i>Trachysalambria starobogatovi</i>	Natal, S. Africa	MNHN Na16746	FJ441167	FJ441212		
	Sicyoniidae	<i>Xiphopenaeus kroyeri</i>	French Guiana	MNHN Na12785	FJ441168	FJ441213	
		<i>Sicyonia lancifer</i>	Taiwan	NTOU M00704	FJ441163	FJ441208	
		<i>Sicyonia curvirostris</i>	Taiwan	NTOU M00766	FJ441164	FJ441209	
	Solenoceridae	<i>Sicyonia fallax</i>	Taiwan	NTOU M00767	FJ441165	FJ441210	
		<i>Cryptopenaeus clevai</i>	Taiwan	NTOU M00768	FJ441132	FJ441177	
		<i>Gordonella paravillosa</i>	Taiwan	NTOU M00769	FJ441136	FJ441181	
		<i>Hadropenaeus lucasii</i>	Taiwan	NTOU M00770	FJ441137	FJ441182	
		<i>Haliporoides sibogae</i>	Taiwan	NTOU M00771	FJ441138	FJ441183	
		<i>Haliporus taprobanensis</i>	Taiwan	NTOU M00772	FJ441139	FJ441184	
		<i>Hymenopenaeus equalis</i>	Taiwan	NTOU M00773	FJ441142	FJ441187	
		<i>Mesopenaeus brucei</i>	Taiwan	NTOU M00774	FJ441148	FJ441193	
		<i>Solenocera melantho</i>	Taiwan	NTOU M00705	EU427147	EU427216	
		<i>Solenocera crassicornis</i>	Fish market, Hong Kong	NTOU M00781	FJ441166	FJ441211	
<i>Sergestoidea</i>	Sergestidae	<i>Acetes</i> sp.	Fish market, Hong Kong	MSLKHC-Acsp	FJ441124	FJ441169	
		<i>Sergestes</i> sp.	Philippines	NTOU M00775	FJ441162	FJ441207	
		<i>Sergia maxima</i>	Taiwan	NTOU M00702	EU427145	EU427214	
<i>Caridea</i>							
<i>Nematocaricinoidea</i>	Rhynchocinetidae	<i>Rhynchocinetes durbanensis</i>	Aquarium shop, Hong Kong	MSLKHC-Rhdur	EU427177	EU427246	
<i>Euphausiacea</i>							
<i>Euphausiidea</i>	Euphausiidae	<i>Euphausia superba</i>	Fish market, Hong Kong	MSLKHC-Eusup	FJ441133	FJ441178	

2.2. DNA extraction, PCR and sequencing

Total genomic DNA was extracted from pleopod muscle using the commercial QIAamp Tissue Kit (QIAGEN). Primers for amplification of PEPCK and NaK were based on Tsang et al. (2008). Amplifications were carried out in a reaction mix containing 1–5 µl of template DNA, 1X PCR reaction buffer, 3 mM MgCl₂, 200 µM dNTPs, 200 nM of each primer, 1.5 units of *Taq* polymerase (Amersham) and ddH₂O to a total volume of 50 µl. The PCR profile for both genes was as follows: 3 min at 94 °C for initial denaturation, followed by 35 cycles of 30 s at 94 °C, 30 s at 55–60 °C (depending on individual samples), 1 min 30 s at 72 °C with a final extension for 10 min at 72 °C. The PCR products were purified using the QIAquick gel purification kit (QIAGEN), in accordance with the manufacturer's instructions. The same sets of primers were used in

sequencing reactions conducted by an Applied Biosystems (ABI) 3100 automated sequencer using the ABI Big-dye Ready-Reaction mix kit, following standard cycle sequencing protocol.

2.3. Phylogenetic analyses

Nucleotide sequences were aligned using CLUSTAL W (Thompson et al., 1994) using default parameters, manually adjusted and confirmed by translating into amino acid sequences using MEGA 4.0 (Tamura et al., 2007). The best-fit model of nucleotide substitution for each gene was determined by Modeltest 3.7 (Posada and Crandall, 1998). Maximum likelihood (ML) analysis was performed with RAxML 7.0.3 (Stamatakis, 2006). The model GTRGAMMAI was used for the two partitions (genes), with individual α -shape parameters, GTR-rates and base frequencies estimated and opti-

mized for each partition. We conducted 1000 bootstrap runs and searched for the best-scoring ML tree. The data was partitioned by gene and separate models were assigned to each partition in the Bayesian inference (BI) analysis implemented in MrBayes v.3.12 (Ronquist and Huelsenbeck, 2003). Three independent runs were carried out with four differentially heated Metropolis coupled Monte Carlo Markov Chains for 5000,000 generations started from a random tree. Model parameters were estimated during analyses. Chains were sampled every 500 generations and the trees before convergence were discarded as burn-in to ensure that analysis had stabilized (determined using Tracer v1.4, Rambaut and Drummond, 2004). Convergence was confirmed by monitoring likelihood values graphically. A 50% majority-rule consensus tree was constructed from the remaining trees to estimate posterior probabilities (PP).

Alternative phylogenetic hypotheses from previous morphological and molecular studies were tested using the Kishino–Hasegawa (KH) test (Kishino and Hasegawa, 1989) and Shimodaira–Hasegawa (SH) test (Shimodaira and Hasegawa, 1999) implemented in PAUP*4.0b10 (Swofford, 2002). Alternative tree topologies were constructed using MacClade 3.0 (Maddison and Maddison 1992) by rearranging the branches showing conflicting relationships between the ML tree and the a priori hypotheses. The tests were carried out with REL optimization and 1000 bootstrap pseudoreplicates.

2.4. Divergence time estimation

BEAST v1.4.7 (Drummond and Rambaut, 2007) was used to estimate the divergence times of all nodes. This Bayesian method employs a relaxed molecular clock model, allowing evolutionary rates to vary in different lineages, and permits multiple constraints deduced from fossil records. The standard deviation of the uncorrelated log-normal relaxed clock parameter (ucl.d.stdev) was estimated to be ~0.45 in preliminary analyses, justifying the use of the relaxed clock model.

Fossil records of the penaeoids were limited. Only three fossil constraints could be placed on the analysis of divergence times. (1) The earliest dendrobranchiate fossils were found in the Triassic deposits in Europe and Madagascar and these included two families of Penaeoidea (Glaessner, 1969; Burkenroad, 1963, 1981). The *Antrimpos* fossils are “quite indistinguishable from the living *Penaeus*” (Burkenroad, 1981) but many fossil species not showing diagnostic characters of recent Penaeidae have tended to be assigned to this genus (Balss, 1922). For a cautious estimation, we regard it as an ancestral stock of Penaeidae. The *Aeger* fossils constitute an extinct family, Aegeridae (Burkenroad, 1963), that once existed from the Triassic to the late Cretaceous era (Glaessner, 1969; Feldmann et al., 2007). The existence of two distinct families of Penaeoidea in the Triassic implies that the superfamily had diverged prior to that period. Therefore, a log normal (zero offset = 248 MYA, SD = 1) prior distribution was implemented to place the most recent common ancestor (MRCA) of Penaeoidea at the end of the Permian era (2 and 3). The oldest fossil of *Sicyonia* and Benthescymidae were discovered in the Cretaceous shales (Glaessner, 1969). Constraints of 65 MYA with SD = 1 were set on the divergence of both *Sicyonia* and Benthescymidae. The log normal prior distribution was chosen for all fossil constraints because it assumes that the divergence time should predate the fossil occurrence, and that the probability of divergence should be highest on the fossil age and decrease towards earlier period (Leaché and Mulcahy, 2007).

The model for the gene-partitioned dataset was chosen by Modeltest 3.7. The uncorrelated log-normal relaxed molecular clock model with a Yule prior distribution for branching rates was employed as suggested by the BEAST user manual. All of the Markov

chain Monte Carlo analyses were run for 10 million generations with a burn-in of one million generations and sampled every 1000 generations. The analyses were repeated to refine the tuning operators to improve efficiency using the auto-optimize function in BEAST. Two separate runs were then combined using LogCombiner v1.4.5 (Drummond et al., 2006) and Tracer v1.4 was used to determine the effective sample size of each parameter (Rambaut and Drummond, 2004).

3. Results

3.1. Phylogenetic analyses

The aligned partial sequences of PEPCK gene included 570 nucleotide positions with 202 parsimony informative sites. The NaK gene included 582 positions in which 211 were parsimony informative (Table 2). No introns or indels were observed. Ambiguous sites (double peaks in chromatograms), probably due to heterozygosity of individuals, were coded as ambiguous using the IUB symbols. Sequences of PEPCK were slightly GC rich (56.5%) while those of NaK showed small AT bias (51.4%). However, there was no significant base heterogeneity across all codon positions of the two genes (Chi-square $p = 0.51$) (Table 2). The Kimura 2-parameter distance matrices of PEPCK and NaK sequence data are shown in Tables 3 and 4. Average interfamily distances of PEPCK and NaK ranged from 0.034 to 0.161 and 0.089 to 0.168, respectively. The pairwise distances within and among Aristeidae, Benthescymidae and Solenoceridae appeared higher in NaK than in PEPCK while the opposite occurred in Sicyoniidae and Penaeidae. It was interesting to note that the interfamilial genetic distance was lowest between Aristeidae and Benthescymidae (only 0.034 in PEPK and 0.089 in NaK), while distances between tribes of Penaeidae (0.111–0.133 in PEPCK and 0.106–0.118 in NaK) were comparable or even higher than those among Aristeidae, Benthescymidae and Solenoceridae (0.034–0.061 in PEPCK and 0.089–0.127 in NaK).

The Akaike Information Criterion implemented in ModelTest selected HKY + I + G for the PEPCK dataset while a SYM + I + G model was selected for the NaK dataset.

The tree topologies resulting from ML and BI approaches were largely congruent and received high supports in most nodes. Only the relationships of several closely related genera were poorly resolved and received low supports for their grouping. Here, only the BI tree was presented (Fig. 2) with support values for both BI and ML analyses. The most significant difference between the two tree topologies was that in the ML tree *Funchalia* sp. and *Pelagopenaeus balboae* were distantly related to *Penaeus* s.l. (not shown), while in the BI tree these two species nested with

Table 2
Summary of parsimony results.

Gene	No. of sites	No. of variable sites	No. of parsimony informative sites	% A/T	Chi-square (p)
<i>PEPCK</i>					
nt1	190	56	31	46.7	1
nt2	190	34	17	51.5	1
nt3	190	175	154	32.1	<0.001
All sites	570	265	202	43.5	0.703
<i>NaK</i>					
nt1	194	56	37	44.4	1
nt2	194	33	12	61.6	1
nt3	194	176	162	48.0	<0.001
All sites	582	265	211	51.4	0.9998
<i>Overall</i>					
Nucleotide	1152	530	413	47.5	0.51

Table 3
Ranges of K2P distances of PEPCK gene within (bold, on diagonal) and between (below diagonal) families (and tribes of Penaeidae) of Penaeoidea with the average values in parentheses.

Family/tribe	Aristeidae	Benthescymidae	Solenoceridae	Sicyoniidae	Penaeidae	Trachypenaeini (Penaeidae)	Parapenaeini (Penaeidae)	Penaeini (Penaeidae)
Aristeidae	0.002–0.036 (0.021)							
Benthescymidae	0.031–0.038 (0.034)	0.025–0.025 (0.025)						
Solenoceridae	0.038–0.094 (0.061)	0.046–0.082 (0.061)	0.017–0.074 (0.044)					
Sicyoniidae	0.127–0.186 (0.161)	0.144–0.169 (0.156)	0.137–0.178 (0.155)	0.007–0.152 (0.099)				
Penaeidae	0.086–0.189 (0.113)	0.09–0.18 (0.114)	0.076–0.194 (0.108)	0.108–0.175 (0.148)	0.015–0.199 (0.105)			
Trachypenaeini (Penaeidae)	0.096–0.189 (0.124)	0.096–0.18 (0.12)	0.079–0.194 (0.113)	0.108–0.173 (0.144)		0.034–0.198 (0.101)		
Parapenaeini (Penaeidae)	0.087–0.116 (0.102)	0.09–0.11 (0.102)	0.076–0.104 (0.09)	0.133–0.175 (0.149)		0.08–0.199 (0.111)	0.025–0.078 (0.059)	
Penaeini (Penaeidae)	0.086–0.126 (0.107)	0.097–0.126 (0.114)	0.086–0.13 (0.11)	0.137–0.164 (0.151)		0.103–0.198 (0.133)	0.082–0.129 (0.11)	0.015–0.084 (0.05)

Table 4
Ranges of K2P distances of NaK gene within (bold, on diagonal) and between (below diagonal) families (and tribes of Penaeidae) of Penaeoidea with the average values in parentheses.

Family/tribe	Aristeidae	Benthescymidae	Solenoceridae	Sicyoniidae	Penaeidae	Trachypenaeini (Penaeidae)	Parapenaeini (Penaeidae)	Penaeini (Penaeidae)
Aristeidae	0.01–0.099 (0.053)							
Benthescymidae	0.069–0.112 (0.089)	0.061–0.061 (0.061)						
Solenoceridae	0.098–0.138 (0.119)	0.106–0.141 (0.127)	0.023–0.102 (0.076)					
Sicyoniidae	0.153–0.186 (0.166)	0.16–0.18 (0.168)	0.132–0.196 (0.158)	0–0.047 (0.031)				
Penaeidae	0.123–0.179 (0.144)	0.117–0.165 (0.141)	0.1–0.198 (0.135)	0.084–0.158 (0.123)	0.008–0.153 (0.096)			
Trachypenaeini (Penaeidae)	0.132–0.179 (0.149)	0.139–0.165 (0.152)	0.123–0.198 (0.149)	0.084–0.129 (0.111)		0.026–0.116 (0.086)		
Parapenaeini (Penaeidae)	0.126–0.165 (0.144)	0.13–0.154 (0.142)	0.114–0.172 (0.134)	0.105–0.131 (0.116)		0.086–0.135 (0.109)	0.046–0.083 (0.069)	
Penaeini (Penaeidae)	0.123–0.157 (0.14)	0.117–0.142 (0.131)	0.1–0.166 (0.123)	0.122–0.158 (0.137)		0.096–0.153 (0.118)	0.088–0.124 (0.106)	0.008–0.076 (0.045)

a clade composed of *Marsupenaeus japonicus* and *Melicertus latissulcatus*, making *Penaeus s.l.* paraphyletic (Fig. 2). However, the supports at this position were low (54 in ML and 0.76 in BI) and further study incorporating more markers and taxa is necessary to resolve this issue. Monophyly was evident with strong nodal support for the superfamily Penaeoidea (91 in ML and 1.0 in BI) and four of its families (Fig. 2), Aristeidae (74 in ML and 0.98 in BI), Benthescymidae (98 in ML and 1.0 in BI) and Sicyoniidae (97 in ML and 1.0 in BI). The support for Solenoceridae monophyly was high only in BI analyses (1.0). However, Penaeidae was paraphyletic with Sicyoniidae nested within it and the a priori hypothesis of Penaeidae monophyly was rejected by SH test ($P < 0.05$). Our results did not support the close relationship among Aristeidae, Benthescymidae and Sicyoniidae (KH and SH $P < 0.001$) that was suggested by mitochondrial markers, nor did they agree with the affinity of Solenoceridae to Penaeidae (without the insertion of Sicyoniidae, as proposed according to mt DNA and sperm morphology; see Quan et al., 2004; Scelzo and Medina, 2004; Vázquez-Bader et al., 2004; Voloch et al., 2005; Medina et al., 2006a, b) (KH and SH $P < 0.001$). The five families were grouped into two clades, with clade A consisting of Solenoceridae, Aristeidae and Benthescymidi-

dae (the latter two being sister taxa), and clade B including Penaeidae and Sicyoniidae (Fig. 2).

3.1.1. Solenoceridae, Aristeidae and Benthescymidae

It was strongly supported that Solenoceridae was distantly related to the other two families in clade A, but the relationships among the solenocerid genera were not well resolved (Fig. 2). The family was divided into two lineages, in which *Haliporoides*, *Haliporus* and *Cryptopenaeus* appeared to be closely related. In the other lineage, *Gordonella* may be most distantly related while *Hymenopenaeus* and *Hadropenaeus* were sister genera although the ML support was only moderate (72 in ML but 0.99 in BI). Nonetheless, the position of *Mesopenaeus* was unclear, and required further investigation. Benthescymidae and Aristeidae were closely related as indicated by their low interfamily divergence (lowest among all the major clades), but they are reciprocally monophyletic (Fig. 2). Within Aristeidae, *Aristaeomorpha* and *Aristaeopsis* were closely related. The phylogeny of the remaining genera was obscure. *Parahepomadus* appeared to be sister to *Aristeus* while *Hemipenaeus* seemed to be sister to *Plesiopenaeus*. Nonetheless, ML bootstrap supports on these relationships were low (<62) despite the high PP from BI (>0.89).

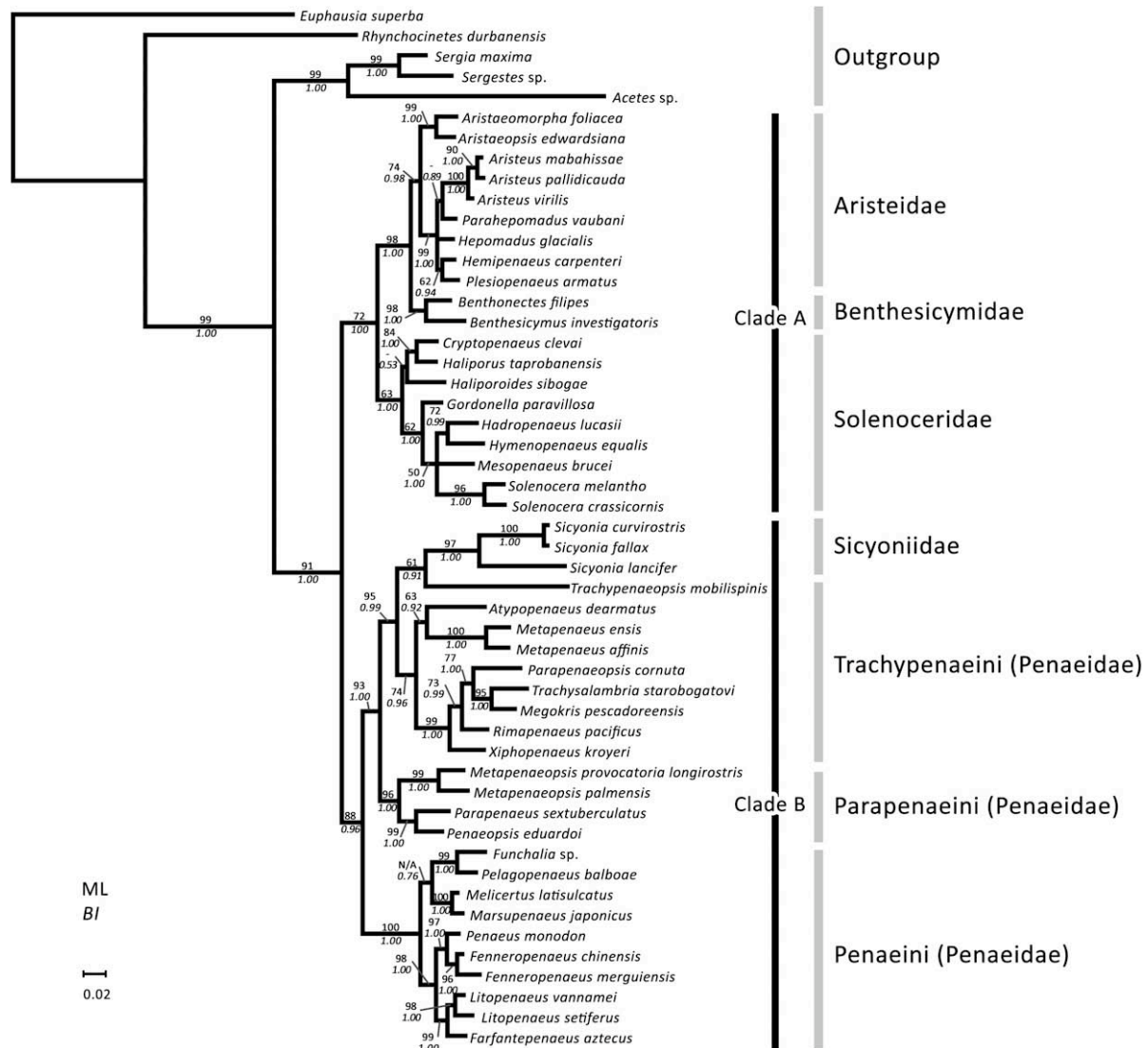


Fig. 2. Bayesian inference tree from combined PEPC and NaK analysis. Numbers above branches indicate bootstrap values from maximum likelihood while posterior probabilities from BI are indicated below branches. Values below 50 are not shown.

3.1.2. Penaeidae and Sicyoniidae

Clade B consisted of four lineages that received moderate (61 in ML and 0.91 in BI) to strong (100 and 1.00) nodal supports (Fig. 2). The most distantly related lineage corresponded to the tribe Penaeini (as Penaeini) established by Burkenroad (1983). Our results also found tribe Parapenaeini (as Parapeneini in Burkenroad, 1983), which contains only four genera, to be monophyletic. However, tribe Trachypenaeini (as Trachypeneini in Burkenroad, 1983) was paraphyletic as *Trachypenaeopsis mobilispinis* clustered with Sicyoniidae but with only moderate support (61 in ML and 0.91 in BI). Yet, monophyly of Trachypenaeini was not rejected by SH ($P = 0.197$) and KH tests ($P = 0.373$). The close association between Sicyoniidae and Trachypenaeini was rigorously supported (95 and 0.99 in ML and BI, respectively). These four lineages in clade B were genetically highly differentiated (Table 3), with divergence levels comparable to those among the families in clade A.

3.2. Divergence time estimation

In each MCMC run of 10 million generations in BEAST v1.4.7, the effective sample sizes of all parameters were well higher than optimal (data not shown). Molecular divergence dates estimated are shown in Fig. 3 with 95% credibility intervals and the posterior

mean ages. The results showed that clade B diversified earlier at about 224 MYA than clade A at about 174 MYA. The three tribes of Penaeidae were old, all with histories of over 95 MY. Their ages were comparable to the families Aristeidae (~73 MY) and Solenoceridae (~112 MY). Our data showed that diversification events in Solenoceridae and Aristeidae occurred at high frequency during the late Cretaceous and Paleogene period, respectively (Fig. 3). On the contrary, lineages in clade B radiated slowly over a period of more than 100 million years since the Jurassic. Although, beyond the scope of this study, it may worth mentioning that Dendrobranchiata was estimated to have diverged from the rest of Decapoda at about 458 MYA.

4. Discussion

4.1. Evolutionary relationships of the penaeoid shrimps

Our study presents the most comprehensive and robust molecular phylogenetic study of Penaeoidea to date. It is also the first molecular phylogenetic study to incorporate an extensive number of genera from Aristeidae and Solenoceridae, and thus can provide new fundamental information on the evolution of these families. The resulting phylogenetic tree is very different from those ob-

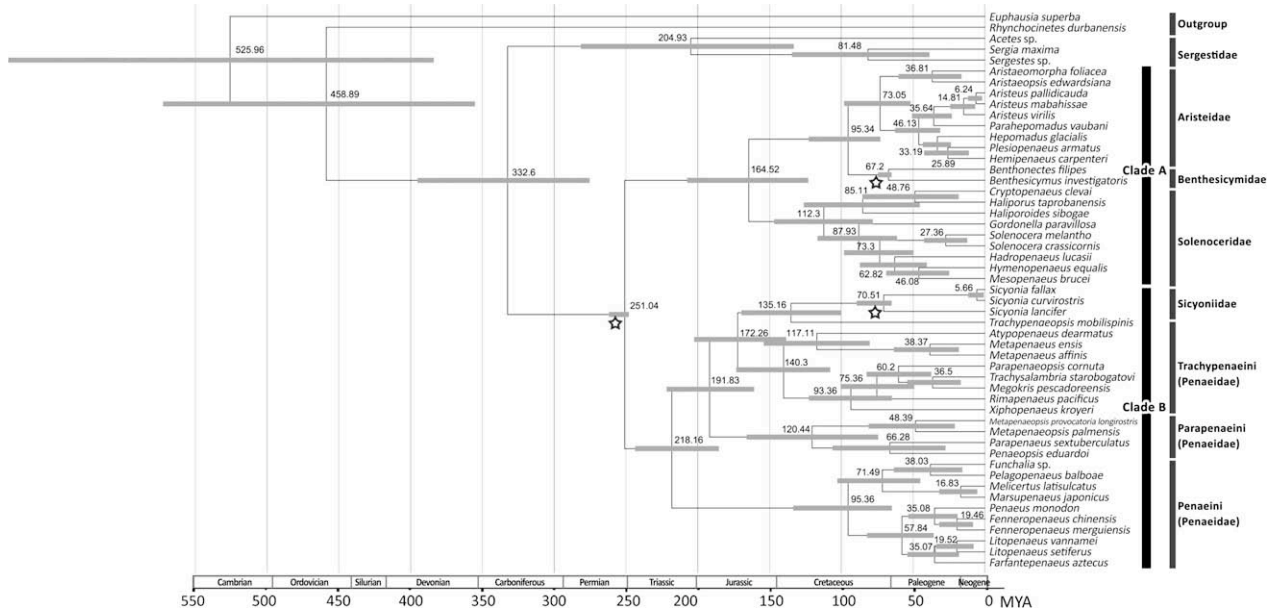


Fig. 3. Phylogenetic tree showing molecular divergence estimates in million years based on a relaxed phylogenetic analysis of combined PEPCK and NaK sequence data with gray bars showing 95% credibility intervals and posterior mean age adjacent to each node. Stars near nodes indicate fossil calibration points.

tained from mitochondrial markers, which suggest a close relationship between Aristeidae, Benthescymidae and Sicyoniidae (Vázquez-Bader et al., 2004), in addition to the insertion of Solenoceridae within Penaeidae (Vázquez-Bader et al., 2004; Quan et al., 2004; Voloch et al., 2005). However, the data supplied by mitochondrial genes, although suitable for inferring phylogenetics of closely related taxa, must be used with caution in resolving deep nodes because mitochondrial genes are subject to a high level of homoplasy resulting from extreme compositional biases, asymmetry of transformation-rate matrices and rapid substitution saturation (Springer et al., 2001; Lin and Danforth, 2004). By contrast, nuclear protein-coding genes, such as PEPCK and NaK used in this study, were demonstrated to be informative across taxonomic levels (Rokas et al., 2002) and can provide good resolution to Mesozoic to Paleozoic-age systematics (Friedlander et al., 1996). Moreover, a more extensive taxon sampling in this study (when compared to only two genera from each family other than Penaeidae in previous molecular studies) gives better phylogenetic accuracy (Pollock et al., 2002; Zwickl and Hillis, 2002) and hence tree stability and statistical support, than previous studies using mitochondrial markers, particularly at deeper branches. Our results, though fairly similar to Burkenroad's (1983) morphology-inferred phylogeny of the penaeoids, propose yet another classification scheme for these shrimps.

The close associations of Penaeidae with Sicyoniidae (clade B), and Aristeidae with Benthescymidae and Solenoceridae (clade A), have long been recognized in traditional taxonomy (e.g., Burkenroad, 1934, 1936, 1983; Crosnier, 1978). Crosnier (1978) and Burkenroad (1983) even thought that Penaeoidea had only two families, namely Aristeidae and Penaeidae. The two groups differ in the shape of the arthrobranch on the fourth pereopod and male second pleopod, as well as the development of the proximal endite of the maxilla and thoracic exopods (see Burkenroad, 1983). Although the association of Solenoceridae with Aristeidae (including Benthescymidae) has been commonly accepted, phylogenetic studies based on sperm morphology and mitochondrial markers have found that Solenoceridae is closely related to Penaeidae. However, the limited taxon sampling of solenocerids might have caused erroneous results in these studies. Moreover, the phyloge-

netic inference based on mitochondrial DNA (Quan et al., 2004; Vázquez-Bader et al., 2004; Voloch et al., 2005) might also be flawed due to mutational saturation as a result of the high mutation rates of these genes. On the other hand, the gain of spiked acrosome in sperms might have occurred several times independently throughout the evolution of dendrobranchiates, and hence might not necessarily be a synapomorphic character uniting Solenoceridae and Penaeidae.

The relationships among the penaeid and sicyoniid genera are intriguing. The penaeid genera appear to cluster according to the traditional three-tribe scheme of Burkenroad (1983): Penaeini, Trachypenaeni and Parapenaeni, with Penaeini diverged the earliest. A previous study using mitochondrial 16S rRNA gene sequences (Chan et al., 2008) also provides some supports for this scheme. Yet the 16S gene tree places two Trachypenaeni genera, *Atypopenaeus* and *Trachypenaopsis* into tribes Parapenaeni and Penaeini, respectively with weak support (<56 in ML). This study clearly shows that *Atypopenaeus* belongs to the Trachypenaeni, but *Trachypenaopsis* groups with Sicyoniidae with moderate support (61 in ML and 0.91 in BI), making the tribe, and also Penaeidae, paraphyletic. Sicyoniidae is unique in Penaeoidea in that the three posterior pleopods are uniramous (vs. normal biramous pleopods in other penaeoids, as well as in carideans and lobsters), in addition to other distinctive characters (see Burkenroad, 1983; Pérez Farfante and Kensley, 1997; Crosnier, 2003). However, the shape of the genitalia of Sicyoniidae, particularly the very rigid and strongly ridged petasma of males, is quite similar to many genera of Trachypenaeni. Burkenroad (1983) argued that Sicyoniidae have genitalia resembling those of Penaeini, but the petasma of the latter is lamella-like and rather thin. Another character often used to separate Sicyoniidae from the other penaeoids is their rigid and stony integument (see Pérez Farfante and Kensley, 1997), but actually quite a few *Sicyonia* species have their integument not very rigid (see Yu and Chan, 1986; Crosnier, 2003). The genus *Trachypenaopsis* is rather rare and contains only two species (Crosnier et al., 2007), and its relationships with the other penaeid genera is generally obscure (see Chan et al., 2008). This result shows that *Trachypenaopsis* appears to be more related to Sicyoniidae than to other genera of Trachypenaeni. As the support for grouping *Trac-*

hypenaeopsis with Sicyoniidae is only moderate and there are more than 50 species with high morphological diversity in the single genus of Sicyoniidae (Pérez Farfante and Kensley, 1997; Crosnier et al., 2007), further study is necessary to determine whether the aberrant genus *Trachypenaeopsis* belongs to Trachypenaeini or allies with *Sicyonia*.

4.2. Divergence dating and evolution of Penaeoidea

This study presents the first molecular dating of divergence events within Penaeoidea, and thus suggests new hypotheses on how paleo-geography, climate and ecology might have shaped the evolution of the superfamily. Fossil record suggests that the two lineages of Penaeoidea (clades A and B) might have diverged in the late Permian (before 250 MYA). These lineages have different preferences in adult habitats: the aristeid-like lineage (clade A) mostly inhabits deep ocean floor whereas the penaeid-like lineage (clade B) prefers shallow continental shelves (Burkenroad, 1934, 1936; Pérez Farfante, 1977; Dall et al., 1990). As its sister superfamily Sergestoidea also includes both deep-water and epipelagic shrimps, it is difficult to determine conclusively whether the ancestors of penaeoids lived in littoral or bathyal zone. However, since fossil records indicate that Paleozoic crustaceans predominantly inhabited shallow marine environment in the tropical Laurentia region (Schram, 1977), it is likely that penaeoids also have a shallow-water origin in Laurentia, from which the aristeid-like lineage evolved to offshore environment. A similar “onshore-innovation, offshore-archaic” evolutionary shift has been postulated for various marine organisms such as the Cambrian-Ordovician marine benthic communities, late Cretaceous shelf fauna and the echinoderms (Jablonski et al., 1983; Jablonski and Bottjer, 1990). Populations inhabiting different depths might have experienced local selection pressures that isolate gene pools, such as differential effect of hydrostatic pressure on enzyme structure and function (Hochachka and Somero, 1984; Somero, 1990) and protein conformation, especially for those present on sperm and egg surfaces that influence reproductive compatibility (Chase et al., 1998). These local selection pressures might have led to ecological speciation in the ancestral stock of Penaeoidea in the Permian, resulting in lineages with dissimilar bathymetric affinity. Another plausible scenario is that the ancestral stock was sundered geographically, possibly due to the suturing of Pangea in the mid Permian and low sea level during most of the Permian and Triassic periods (Schram, 1977; Miller et al., 2005), resulting in allopatric speciation. In this case, the development of bathymetric adaptation might have occurred during population isolation or after re-mixing of the populations as the sea level rose in the Jurassic period. In either case, the acquisition of new adaptation should have played an important role in the evolution of the penaeoid lineages.

The two existing phylogenetic schemes for penaeoids disagree on the origin of the group (Fig. 1). Kubo (1949) regarded Sicyoniidae as the most primitive and Penaeidae as the most advanced, with Solenoceridae being intermediate between Penaeidae and Aristeidae. Burkenroad (1983), on the other hand, suggested that Solenoceridae (more precisely *Haliporus*) was the most primitive and *Sicyonia* (and hence Sicyoniidae) the most derived in Penaeoidea. Besides refuting the family grouping proposed by Kubo (1949), our results indicate that sicyoniids represent one of the most recent clade. Although, these results support Burkenroad's view (1983) that Sicyoniidae were the most advanced, they refute his hypothesis that solenocerids and aristeids diverged earlier than penaeids and sicyoniids. Our study established that the penaeid-like lineage (clade B) started to radiate in the middle Triassic (218 MYA), preceding the aristeid-like lineage (clade A) which began diversification in the middle Jurassic (164 MYA). The radiations of the five penaeoid families and the three tribes of

Penaeidae do not seem to be rapid. The time of diversifications in the penaeid-like lineage corresponds to the recovery period after the Permo-Triassic mass extinction, during which almost the entire Paleozoic fossil malacostracan fauna disappeared and might hence have created empty habitats for the radiation of the more advanced marine decapods that have dominated the oceans in the present day (Schram, 1977; López-Gómez and Taylor, 2005). It has been proposed that unfavorable climatic and oceanographic conditions such as widespread anoxia and accumulation of greenhouse gases sustained for a long period after mass extinction, resulting in a lengthy recovery period when compared to other extinction events in the earth history (Hallam, 1991; Kidder and Worsley, 2004). This may explain why the major groups in clade B slowly radiated. On the other hand, the divergence of Solenoceridae from the Aristeidae-Benthescymidae lineage in the middle Jurassic coincides with the splitting of Pangea.

The estimated divergence times of Penaeoidea and its families are comparable to those of other decapod taxa computed using similar methods. Superfamilies of other decapod infraorders are estimated to have radiated in the Permian (Porter et al., 2005), as Penaeoidea has been so estimated in this study. Porter et al. (2005) also noticed that the diversification of the astacid families occurred in the Cretaceous, and therefore shared the same time frame as the radiation of the penaeoid families Aristeidae and Solenoceridae based on BEAST estimation. In addition, there is no significant difference between estimations of the age of divergence of Dendrobranchiata from Pleocyemata obtained by Porter et al. (2005) and the present study. We date the divergence back to the Ordovician period (459 MYA) which is slightly earlier than the Silurian radiation (437 MYA) estimated by Porter et al. (2005). The slight disparity may be due to the differences in fossil calibrations used or because only one dendrobranchiate species was analyzed in Porter's study so that the divergence between Dendrobranchiata and Pleocyemata might have been underestimated.

Although, our results deduced from divergence age estimations are in agreement with the other molecular studies of decapods, they must be treated with a degree of caution due to several limitations. For instance, some inherent inaccuracies associated with fossil ages have not been taken into account, such as misidentifications of the taxonomy of the fossils and inaccuracies in assigning the fossils to geological strata (Graur and Martin, 2004). Errors might have crept into our calculations because we incorporated only a relatively small number of calibration points and used a single estimation method. Nevertheless, we do not believe that limitations of this kind would significantly affect our argument, and are confident that our main findings, viz. that the penaeid-like lineage was established earlier than the aristeid-like lineage and that Penaeoidea did not undergo rapid radiation, are unlikely to be challenged.

4.3. Taxonomic revision

Given the paraphyly of Penaeidae demonstrated in this study, its conventional classification as a family can scarcely be sustained. Penaeidae can be maintained either by synonymizing it with Sicyoniidae, or raising the three penaeid tribes to the familial rank. The two major clades in our results correspond to the two-family scheme of Burkenroad (1983) with only Aristeidae and Penaeidae. However, the reciprocal monophyly of the three tribes demonstrated in this study merits their recognition as distinct taxa. The levels of genetic divergence among the tribes and Sicyoniidae are comparable to those among Aristeidae, Benthescymidae and Solenoceridae. Therefore, the tribes in Penaeidae warrant at least the same taxonomic rank as the latter. To maintain Sicyoniidae, and even Solenoceridae, the three tribes of Penaeidae should also be

recognized as separate families, namely, Penaeidae Rafinesque-Schmaltz, 1815, Parapenaeidae Ortmann, 1898 and Trachypenaeidae Burkenroad, 1983 (yet with the exclusion of *Trachypenaeopsis*). Even if the two-family scheme of Burkenroad (1983) is followed, these three tribes should be regarded as subfamilies equivalent to Solenocerinae, Aristeinae and Sicyoniinae, though synapomorphies of these three tribes have not yet been fully comprehended (see Burkenroad, 1983; Chan et al., 2008).

It is less clear whether Benthescymidae warrants a family status. Crosnier (1985) treated it as a separate family from Aristeidae but several later studies did not follow his lead (e.g., Liu and Zhong, 1986; Dall et al., 1990; Hayashi, 1992; Chan, 1998). Pérez Farfante and Kensley (1997), however, revived the notion of Benthescymidae as a separate family, and have been followed by Martin and Davis (2001). Unfortunately, neither Crosnier (1985) nor his supporters have provided any detailed rationale for elevating benthescymids into the family rank. This results suggest that benthescymids constitute a monophyletic group sister to aristeids. However, the sequence divergences between benthescymids and aristeids (0.034 in NaK and 0.089 in PEPCK) are the lowest among all the major clades even including Burkenroad's (1983) penaeid tribes (Tables 3 and 4). The level of divergence in NaK is lower than the values among family members except for Aristeidae and Benthescymidae, while the divergence in PEPCK is lower than those among penaeid genera. Nevertheless, given the limited sampling of benthescymids in this work, and as the two genera used have generally been considered to be very close, it would be more prudent to carry out a more comprehensive molecular study of these two families to determine if the family or even subfamily rank of benthescymids can be justified.

Although the present molecular analyses have effectively resolved the familial and tribal relationships in Penaeoidea, the relationships amongst the genera within each family and tribe remain mostly unresolved. Nevertheless, the results of this work provide strong genetic evidence to clarify the taxonomic status of several genera. The aristeid genus *Aristaeopsis*, containing only the monotypic species *A. edwardsiana*, has generally been regarded as a synonym of *Plesiopenaeus*, and it is only recently that a separate status has been proposed (Pérez Farfante and Kensley, 1997). Our gene tree shows that *Aristaeopsis* is distinct from *Plesiopenaeus* but close to *Aristaeomorpha* instead. Therefore, the generic status of *Aristaeopsis* is supported. On the other hand, *Hepomadus* and *Parahepomadus*, usually considered allies to *Aristaeomorpha* (Fig. 1), are genetically distinct from the latter. The molecular data confirm that the rare genus *Gordonella* is not a benthescymid but belongs to Solenoceridae, and it is not close to *Haliporus* as suggested by Crosnier (1988). Moreover, our gene tree does not support all the phylogenetic groupings of the solenocerid genera as proposed by Pérez Farfante (1977) and Kubo (1949). For Penaeidae, the splitting (i.e., polyphyly) of *Trachypenaeus s.l.* by Pérez Farfante and Kensley (1997) is strongly supported by our nuclear gene analysis, which in turn is consistent with our results based on mitochondrial DNA (Chan et al., 2008).

5. Conclusion

The phylogenies of Penaeoidea inferred from morphology and molecular markers have been controversial. The present phylogenetic analysis using sequences of two nuclear protein-coding genes have yielded results, with high statistical support, which are largely consistent with the groupings of the morphology-inferred phylogeny above the genus level proposed by Burkenroad (1983). These have provided new insights into the mode of diversification of the superfamily, age of divergence events, and arguments for taxonomic revision in Penaeoidea. The paraphyly of Penaeidae

and the large genetic divergence amongst the three penaeid tribes of Burkenroad (1983) and the other penaeid families justify assigning the same taxonomic rank as Aristeidae, Solenoceridae and Sicyoniidae to the three tribes. The low genetic divergence between Aristeidae and Benthescymidae suggests a re-evaluation of the family status of the latter. Our results also show that the penaeid-like lineage diverged earlier than the aristeid-like lineage. The use of nuclear protein-coding genes and more comprehensive taxon sampling of Sicyoniidae, Aristeidae and Solenoceridae than in the previous molecular studies have generated novel hypotheses for the evolution of genera or species in these families.

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