



Molecular phylogeny of penaeid shrimps inferred from two mitochondrial markers

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ABSTRACT. Penaeid shrimps are an important resource in crustacean fisheries, representing more than the half of the gross production of shrimp worldwide. In the present study, we used a sample of wide-ranging diversity (41 shrimp species) and two mitochondrial markers (758 bp) to clarify the evolutionary relationships among Penaeidae genera. Three different methodologies of tree reconstruction were employed in the study: maximum likelihood, neighbor joining and Bayesian analysis. Our results suggest that the old *Penaeus* genus is monophyletic and that the inclusion of the *Solenocera* genus within the Penaeidae family remains uncertain. With respect to *Metapenaeopsis* monophyly, species of this genus appeared clustered, but with a nonsignificant bootstrap value. These results elucidate some features of the unclear evolution of Penaeidae and may contribute to the taxonomic characterization of this family.

Key words: Molecular evolution, Monophyly, Maximum likelihood, Crustacea, Old genus *Penaeus*

INTRODUCTION

Penaeid shrimps are the most important economic resource in the world's crustacean fishery industry (Holthuis, 1980; Dall et al., 1990; Pérez-Farfante and Kensley, 1997), being responsible for more than half of the gross production of shrimp (FAO, 2000). Recently, several molecular phylogenetic studies have contributed to the clarification of the evolutionary history of the group (Baldwin et al., 1998; Gusmão et al., 2000; Tong et al., 2000; Maggioni et al., 2001; Lavery et al., 2004; Vázquez-Bader et al., 2004).

Most of the studies, however, have focused on the phylogenetic issues of individual penaeid genera. Only recently, Quan et al. (2004) and Vázquez-Bader et al. (2004) examined penaeid phylogenetics, but they were severely restricted by taxon sampling (12 species; Quan et al., 2004) or sequence length (around 300 bp; Vázquez-Bader et al., 2004). Thus, it remains to be seen whether their results would change if more data were made available.

In the present study, we used a sample of wide-ranging diversity (41 shrimp species), two mitochondrial markers (758 bp) and robust phylogenetic methodologies to clarify the evolutionary relationships among Penaeidae genera.

MATERIAL AND METHODS

The taxonomic classification proposed by Pérez-Farfante and Kensley (1997) was adopted in this study. Sequences for two mitochondrial genes, namely 16S rRNA gene (16S) and cytochrome oxidase subunit 1 gene (CO1), were downloaded from GenBank for thirty-nine Penaeidae species. Additionally, two species of the closely related Solenoceridae family and the Brachyura species *Portunus trituberculatus* (as the outgroup, see Quan et al., 2004) were included (accession numbers are presented in Table 1).

The sequences were aligned using the ClustalW algorithm available at <http://www.ebi.ac.uk/clustalw> (Thompson et al., 1994) and subsequently checked by visual inspection. All gaps were excluded from the alignment, resulting in a concatenated sequence 758 bp long.

The phylogenetic tree and branch support values were estimated using three different methodologies of phylogenetic reconstruction: 1) maximum likelihood (ML), 2) distance and 3) Bayesian inference (BI). In the ML approach, a heuristic algorithm was necessary due to computer time limitations. Two initial trees, obtained by stepwise addition or by neighbor-joining (NJ), were swapped using the tree bisection and reconnection method. Because both stepwise addition and NJ approaches yielded exactly the same final topology, the NJ + tree bisection and reconnection method was used for the computation of the branch supporting values, since it is a much faster algorithm. In this case, a nonparametric bootstrap was performed with 100 pseudo-replicates. The best-fit model (GTR + Γ + I; $\alpha = 0.5995$ and I = 0.529) was selected through a hierarchical likelihood ratio test on the Modeltest 3.06 software (Posada and Crandall, 1998). All ML analyses were performed with the PAUP* 4.0 software (Swofford, 2002).

For distance analysis, a nonparametric bootstrap was performed on 1000 pseudo-replicates, using the NJ algorithm and Jukes-Cantor as the model of substitution. The choice of this model was based on Nei and Kumar (2000) (overall media: p distance = 0.141 transitions/transversions = 1.444). Distance analysis was carried out with MEGA 3 (Kumar et al., 2004).

Finally, Bayesian posterior probabilities were calculated from 9,000 sample trees, using the model selected through Modeltest 3.06. The first 100,000 generations of the Markov chain

Table 1. GenBank accession numbers for 16s rRNA and CO1 sequences for each species analyzed.

Species	GenBank accession numbers	
	16s rRNA	CO1
<i>Farfantepenaeus aztecus</i>	AF279811	AF279834
<i>Farfantepenaeus brasiliensis</i>	AF192054	AY135196
<i>Farfantepenaeus duorarum</i>	AF279812	AF279835
<i>Farfantepenaeus paulensis</i>	AF192060	AY135194
<i>Farfantepenaeus subtilis</i>	AF192068	AY135193
<i>Farfantepenaeus notialis</i>	X84350	X84350
<i>Farfantepenaeus californiensis</i>	AY046912	AY135197
<i>Litopenaeus schmitti</i>	AF192080	AY135189
<i>Litopenaeus setiferus</i>	AF279819	AF279841
<i>Litopenaeus stylirostris</i>	AF255057	AY135191
<i>Litopenaeus vannamei</i>	AF279818	AF279842
<i>Xiphopenaeus kroyeri</i>	AF192092	AY135200
<i>Fenneropenaeus chinensis</i>	AF279813	AF279836
<i>Fenneropenaeus indicus</i>	AF279815	AF279837
<i>Fenneropenaeus merguensis</i>	AF279814	AF279838
<i>Fenneropenaeus penicillatus</i>	AF279816	AF279839
<i>Fenneropenaeus silasi</i>	AF279817	AF279840
<i>Marsupenaeus japonicus</i>	AF279820	AF279832
<i>Melicertus canaliculatus</i>	AF279825	AF279843
<i>Melicertus plebejus</i>	AF279822	AF279848
<i>Melicertus merginatus</i>	AF279824	AF279847
<i>Melicertus longistylus</i>	AF279823	AF279846
<i>Melicertus latisulcatus</i>	AF279821	AF279845
<i>Melicertus kerathurus</i>	AF279826	AF279844
<i>Metapenaeopsis acclivis</i>	AF105040	AF105051
<i>Metapenaeopsis barbata</i>	AF105041	AF105052
<i>Metapenaeopsis commensalis</i>	AF105042	AF105054
<i>Metapenaeopsis lamellata</i>	AF105043	AF105055
<i>Metapenaeopsis liui</i>	AF105044	AF105057
<i>Metapenaeopsis palmesis</i>	AF105045	AF105058
<i>Metapenaeopsis provocatoria</i>	AF105047	AF105059
<i>Metapenaeus affinis</i>	AY264904	AY264886
<i>Metapenaeus ensis</i>	AF279810	AF279830
<i>Parapenaeopsis hardwickii</i>	AY264910	AY264895
<i>Parapenaeus fissuroides</i>	AY264909	AY264894
<i>Penaeus monodon</i>	AF279829	AF279833
<i>Penaeus esculentus</i>	AF279828	AF279849
<i>Penaeus semisulcatus</i>	AF279827	AF279831
<i>Trachysalambria curvirostris</i> *	AY264916	AY264903
<i>Solenocera crassicornis</i>	AY264915	AY264902
<i>Solenocera koelbeli</i>	AF105038	AF105049
<i>Portunus trituberculatus</i>	AY264913	AY264900

*This species appears as *Trachypenaeus curvirostris* in GenBank files.

Monte Carlo were not used to ensure that the chain would be sampled on a stationary portion. The other parameters were set according to the MrBayes 3.0 program default (Ronquist and Huelsenbeck, 2003).

RESULTS AND DISCUSSION

The ML analysis of the concatenated sequence of the 42 species studied resulted in the phylogenetic tree shown in Figure 1. Controversial issues of Penaeidae phylogenetics and aspects that were never properly tested are discussed below.

The first important result of our tree is that the genus *Solenocera* clustered with *Parapenaeus fissuroides* (hereafter, support values for ML: 41; BI: 63; NJ: 44), and this clade appeared to be associated with *Metapenaeopsis* spp (ML: 61; BI: 94; NJ: 39). This is an important result because it argues against the monophyletic status of the Penaeidae family, since *Solenocera* belongs to a different family.

Nonetheless, support values of this association are too low to firmly contest the monophyletic condition of Penaeidae. In fact, even in a more restricted analysis including 12 Penaeoidea species (Quan et al., 2004), the bootstrap value for the group formed by the *Solenocera* and *P. fissuroides* + *M. barbata* clade was still not significant (75%). Another study (Vázquez-Bader et al., 2004) showed that the *Solenocera* genus is included within Penaeidae, but again a non-significant bootstrap value was assigned to the branch (less than 74%). Naturally, if this outcome proves consistent, the *Solenocera* genus will not be deemed suitable for an outgroup in penaeid phylogenetics, as has been the case in some studies (Tong et al., 2000; Lavery et al., 2004). This clearly indicates an unstable condition of the group, and thus, a more detailed study is apparently needed to put these matters to rest.

The second major result considers the monophyletic condition of the *Metapenaeopsis* genus. Tong et al. (2000) have already analyzed the phylogenetic relations within species of this genus. Unfortunately, however, they used an improper outgroup (*S. koelbeli*), and in addition, a single species outside the *Metapenaeopsis* genus was included in the analysis (*P. monodon*). More recently, Quan et al. (2004) and Vázquez-Bader et al. (2004) studied the phylogenetic position of the genus within Penaeoidea, but again, a single species of *Metapenaeopsis* was included in the analyses. Consequently, the monophyletic status of the genus was never properly tested previously with molecular data. In our tree, all seven *Metapenaeopsis* species were grouped with relatively high support values (ML: 78; BI: 100; NJ: 73). With regard to morphology, the diagnostic characteristics for the genus are the asymmetrical petasma and the unpaired and uninvginated seminal receptacles (Vázquez-Bader et al., 2004).

The group formed by *Farfantepenaeus*, *Fenneropenaeus*, *Marsupenaeus*, *Melicertus*, *Litopenaeus*, and *Penaeus*, also known as the “old *Penaeus* genus,” clustered with high support values (ML: 91; BI: 98; NJ: 89). This result is very interesting and may eventually be used to justify a separate intermediate taxonomic level for the classification of this group. Our results also suggest the monophyletic condition of *Fenneropenaeus* with a high ML bootstrap value and significant supports in BI and NJ (ML: 90; BI: 100; NJ: 95). As expected, the monophyly of *Farfantepenaeus* (ML: 86; BI: 100; NJ: 99) and *Litopenaeus* (ML: 98; BI: 100; NJ: 97) was confirmed (Maggioni et al., 2001; Lavery et al., 2004) with high support values, which corroborates the classification based on thelycum morphology (Pérez-Farfante and Kensley, 1997). The group formed by *Melicertus* + *Marsupenaeus* genera (ML: 81; BI: 98; NJ: 82) is also

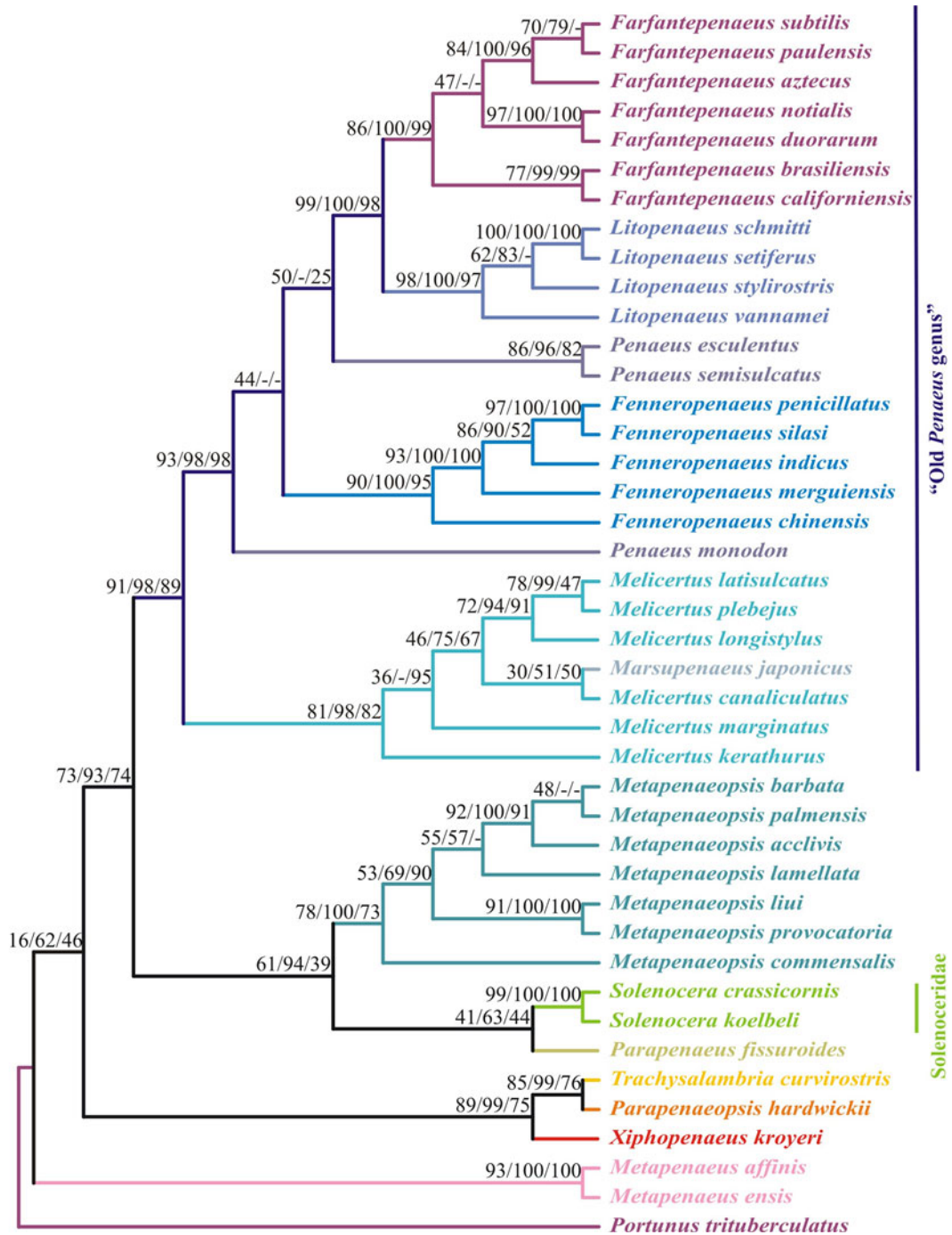


Figure 1. Maximum likelihood tree topology with the branch support values estimated by bootstrap pseudo-replicates in maximum likelihood, neighbor-joining and Bayesian inference, respectively shown above each branch. (-) indicates that the cluster was not formed in the analysis. Colors indicate species in different genera.

supported by morphological data (Pérez-Farfante and Kensley, 1997), since both subgenera usually have telson with tree pairs of movable lateral spines. In our tree, *Marsupenaeus* is not clearly included in the *Melicertus* group, but the BI support value strongly suggests that they are closely related genera.

The genus *Penaeus* appears to be paraphyletic with *Fenneropenaeus* spp, clustering between the two *Penaeus* lineages: *P. monodon* and *P. esculentus* + *P. semisulcatus*. Nonetheless, this result needs verification because the support values are particularly low (ML: 44; BI: NA; NJ: NA).

The most basal lineages that appear in the tree are the *Metapenaeus* genus, followed by a clade formed by *T. curvirostris* + *P. hardwickii* + *X. kroyeri*. The support values for the deeper branches, however, are too low to firmly establish the relation among these penaeid lineages (ML: 16; BI: 62; NJ: 46). Still, the monophyly of *Metapenaeus* is attested by significant supporting values (100%) in the Bayesian and NJ analyses (ML: 93).

This inclusive analysis of Penaeidae phylogenetics helped to examine some aspects of the unclear evolutionary relation among Penaeidae genera, which were never properly addressed until now. In the future, the phylogeny obtained here can be used for taxonomic purposes and possibly in a divergence time analysis which may also clarify the origin and diversification of the family. It is important to note that even when using the combined sequences of two markers (16S and COI) and a comprehensive sample (including 42 Penaeid species), the phylogenetic relation within the family remains unstable. Obviously, more genes must be sequenced to clarify the taxonomic issues of the family.

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