

Phylogenetic Analyses of Economically Important Shrimp Species in Taiwan Based on DNA Sequences of Mitochondrial 16S rRNA

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ABSTRACT

The molecular phylogeny of economically important shrimp species of Taiwan was revealed by partial mitochondrial 16S rRNA sequences amplified from 20 species (belonging to five families) in this study. Alignment of the sequences showed considerable variation in 209 nucleotide sites and 154 parsimoniously informative sites. Phylogenetic trees were created by Neighbor-joining and maximum parsimony methods. We determined that (1) the family Solenoceridae is included within the Penaeoidea, and it seems to be more suitable to be classified in the tribe Peneini than the tribe Parapeneini of the family Penaeidae; (2) the family Penaeidae appeared to have a paraphyletic status that formed at least three clades which support the opinion of Burkenroad from morphological studies and previous molecular studies; and (3) the species *Metapenaeus ensis* was shown to form a clade by itself, which clearly indicates that *M. ensis* is outside the tribe Trachypeneini.

Key words: molecular phylogeny, 16S rRNA, shrimp, Penaeoidea.

INTRODUCTION

The Penaeoidea is one of the most economically important marine families used for seafood production worldwide (Holthuis, 1980; Chan, 1998; Rosenberry, 2001). Most of the commercial shrimp utilized in Taiwan belong to the superfamily Penaeoidea. Because their commercial, evolutionary, and taxonomical statuses are significant, there have been a number of studies on the molecular phylogeny and population genetics of the Penaeoidea (Palumbi and Benzie 1991; Bouchon *et al.*, 1994; Garcia-Machado *et al.*, 1996; Baldwin *et al.*, 1998; Maggioni *et al.*, 2001; Lavery *et al.*, 2004; Quan *et al.*, 2004; Vázquez-Bader *et al.*, 2004; Voloch *et al.*, 2005).

Mitochondrial 16S ribosomal RNA has been shown to be a useful genetic marker for phylogenetic

and population studies of crustaceans (Schubart *et al.*, 2000). In this study, we compared the DNA sequences of the mitochondrial 16S rRNA gene among some economically valuable shrimp species in Taiwan. The aim was to provide a preliminary molecular phylogeny for valuable shrimp in Taiwan and elucidate the taxonomy of the superfamily Penaeoidea.

MATERIALS AND METHODS

1. Collection of Specimens

The specimens used in this study were caught along the coast of Taiwan. The 20 species collected, which belong to five families of shrimp, are listed in Table 1. All samples were either frozen at -70 °C or preserved in 95% ethanol upon arrival in the lab. DNA sequences of partial mitochondrial 16S rRNA were amplified from the collected shrimp samples.

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Table 1 List of the economically valuable shrimp used in this study

Family	Species	Chinese name	Sampling locality
Penaeoidea			
Aristeidae			
	<i>Aristeus virilis</i> (AV)*	雄壯鬚蝦	North East
	<i>Aristaeomorpha foliacea</i> (AF)*	葉狀擬鬚蝦	North East
Solenoceridae			
	<i>Solenocera melantho</i> (SM)*	大管鞭蝦	North East
	<i>Haliporoides sibogae</i> (HI)*	東方擬海蝦	North East
	<i>Hymenopenaeus aequalis</i> (HA)*	等似膜對蝦	North East
Penaeidae			
	<i>Marsupenaeus japonicus</i> (MJ)	日本囊對蝦	North East
	<i>Melicertus marginatus</i> (MM)	緣溝對蝦	North East
	<i>Metapenaeopsis longirostris</i> (ML)*	長額赤對蝦	North East
	<i>Metapenaeopsis barbata</i> (MB)	鬚赤蝦	North East
	<i>Metapenaeus affinis</i> (MA)	近緣新對蝦	West
	<i>Metapenaeus ensis</i> (ME)	劍額新對蝦	North East
	<i>Parapenaeopsis hardwickii</i> (PH)	長角仿對蝦 (哈氏仿對蝦)	West
	<i>Parapenaeus fissuroides</i> (PF)	長縫側對蝦	North East
	<i>Parapenaeus longipes</i> (PL)*	長足側對蝦	North East
	<i>Penaeus monodon</i> (PM)	草對蝦	West
	<i>Penaeus semisulcatus</i> (PS)	熊對蝦	South West
	<i>Trachypenaeus curvirostris</i> (TC)	彎角鷹爪對蝦	North East
	<i>Fenneropenaeus penicillatus</i> (FP)	多毛明對蝦	West
Sergestoidea			
Sergestidae			
	<i>Sergia talismani</i> (ST)*	日本櫻蝦	North East
Caridea			
Pandalidae			
	<i>Heterocarpus sibogae</i> (HS)*	東方異腕蝦	North East

Abbreviation for the species names are shown in brackets.

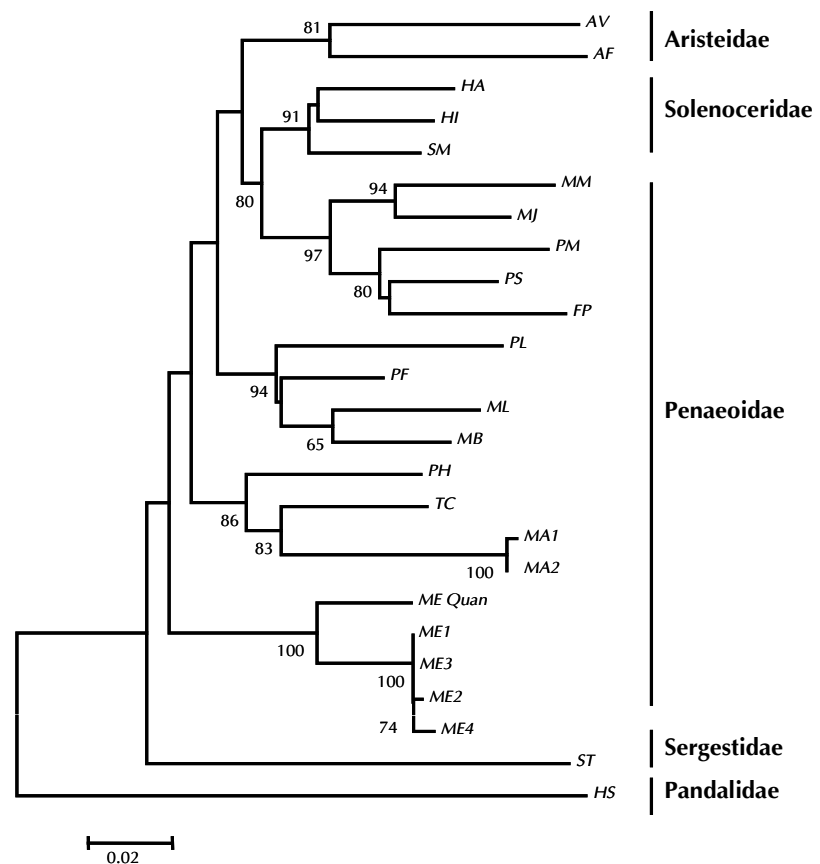
*: indicates that the species was the first time to be used for phylogenetic analyses.

2. PCR Amplification and Sequencing

Abdominal muscle tissues (20 mg) of each specimen were excised and preserved in 95% alcohol. Total genomic DNA was extracted from these tissues using a commercial DNA-binding kit (Gentra, Minneapolis, MN). A fragment of the large subunit ribosomal RNA (16S rRNA) gene was amplified from total DNA by a polymerase chain reaction (PCR) using the universal oligonucleotide primers, 16Sar-L (5'-CGCCTGTTTATCAAAAACAT-3') and 16Sbr-H (5'-CCGGTCTGAACTCAGATCACGT-3') (Palumbi,

1996). Approximately 100 ng of genomic DNA provided a template for double-stranded reactions via the PCR in 50 µl of a reaction solution which contained 35.5 µL of sterile, distilled water, 5 µL of 10x PCR buffer (Perkin-Elmer, Wellesley, MA), 4 µL of dNTP (2.5 mM each), 2 µL of each primer (10 µM), 0.5 µL of 1.25 unit Taq polymerase (Takara, Japan), and 1.0 µL of template. DNA was amplified for 30 cycles, each involving denaturation at 92 °C for 30 s, annealing at 50 °C for 45 s, and extension at 72 °C for 60 s. The PCR products were electrophoresed on a 1.0% agarose gel and later stained with ethidium

Fig. 1 Neighbor-joining (NJ) tree from mitochondrial 16S rRNA data of the shrimp. Bootstrap values are shown as percentages and are based on 1000 replicates. Only bootstrap values > 50% are shown on the branches.



bromide for band characterization via ultraviolet transillumination. PCR products were purified with a QIAquick gel extraction kit (Hilden, Germany) and sequenced with the ABI BigDye Terminator Mix (PE Biosystems, Wellesley, MA) in an ABI Prism 3730 Genetic Analyzer (Applied Biosystems, Foster City, CA). The sequence data were manually edited and automatically assembled using the DNASTAR software package (Madison, WI). The nucleotide sequences were aligned using the ClustalX program. *Heterocarpus sibogae* was used as the outgroup. Evolutionary distances were computed by Kimura's two-parameter method (Kimura, 1980), and the phylogenetic analysis used the Neighbor-joining (NJ) method (Saitou and Nei, 1987) and version 3.1 of the MEGA (Molecular Evolutionary Genetic Analysis) software (Kumar *et al.*, 2004). As a second phylogenetic method, a maximum parsimony (MP) analysis was carried out with a heuristic search with 1000 random sequence additions using the program PAUP (Swofford, 2002).

RESULTS AND DISCUSSION

The 482-bp fragment of the 16S rRNA mtDNA gene was amplified and aligned for all 20 species. The fragment of 16S rRNA sequences was AT rich (64.2%) (T, 32.0%; A, 34.2%; C, 21.3%; and G, 12.5%), which was found to be compatible with descriptions of other arthropod mtDNA genomes (Shih *et al.*, 2004; Spicer, 1995) as well as other *Penaeus* mtDNA sequences (Garcia-Machado *et al.*, 1996; Baldwin *et al.*, 1998). Alignment of the sequences showed a considerable variation in 209 nucleotide sites and 154 parsimoniously informative sites.

A comparison of the distance matrix obtained from the analysis of the alignment of all sequences of species is shown in Table 2. Values of the pairwise Kimura two-parameter distance among these shrimp ranged from 0.011 to 1.166. The Neighbor-joining trees constructed from the Kimura two-parameter distances are shown in Fig. 1. The single most-parsimonious tree resulting from heuristic

Table 2 Pairwise two-parameter distance matrix from the 482-bp portion of the mitochondrial 16S rRNA gene among the 20 species of economically important shrimp

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1 TC	–																			
2 AF	0.170	–																		
3 AV	0.780	0.275	–																	
4 FP	0.245	0.252	0.739	–																
5 HI	0.018	0.234	0.807	0.318	–															
6 HS	0.334	0.107	0.266	0.675	0.370	–														
7 HA	0.025	0.095	0.634	0.134	0.070	0.307	–													
8 MJ	0.266	0.027	0.152	0.266	0.311	0.143	0.168	–												
9 MM	0.407	0.116	0.150	0.230	0.452	0.320	0.266	0.039	–											
10 ML	0.234	0.111	0.307	0.602	0.234	0.027	0.239	0.139	0.307	–										
11 MB	0.061	0.102	0.473	0.348	0.052	0.157	0.080	0.143	0.277	0.070	–									
12 PH	0.132	0.157	0.607	0.595	0.150	0.098	0.175	0.261	0.489	0.061	0.070	–								
13 PF	0.118	0.125	0.493	0.527	0.118	0.084	0.152	0.193	0.384	0.030	0.030	0.014	–							
14 PL	0.289	0.198	0.452	0.793	0.289	0.041	0.325	0.261	0.489	0.023	0.125	0.043	0.039	–						
15 PM	0.132	0.143	0.602	0.023	0.186	0.470	0.052	0.166	0.175	0.398	0.193	0.391	0.332	0.552	–					
16 PS	0.152	0.055	0.511	0.125	0.252	0.289	0.059	0.118	0.189	0.293	0.193	0.266	0.252	0.398	0.070	–				
17 ST	0.184	0.041	0.330	0.102	0.248	0.270	0.082	0.041	0.061	0.248	0.157	0.307	0.248	0.389	0.048	0.041	–			
18 ME	0.648	0.377	0.325	1.166	0.620	0.102	0.664	0.368	0.575	0.107	0.334	0.261	0.220	0.093	0.884	0.705	0.609	–		
19 MA	0.225	0.193	0.382	0.620	0.180	0.120	0.261	0.198	0.350	0.034	0.055	0.107	0.048	0.070	0.411	0.393	0.302	0.152	–	
20 SM	0.064	0.048	0.493	0.118	0.118	0.252	0.011	0.098	0.175	0.207	0.084	0.186	0.155	0.307	0.041	0.030	0.034	0.598	0.248	–

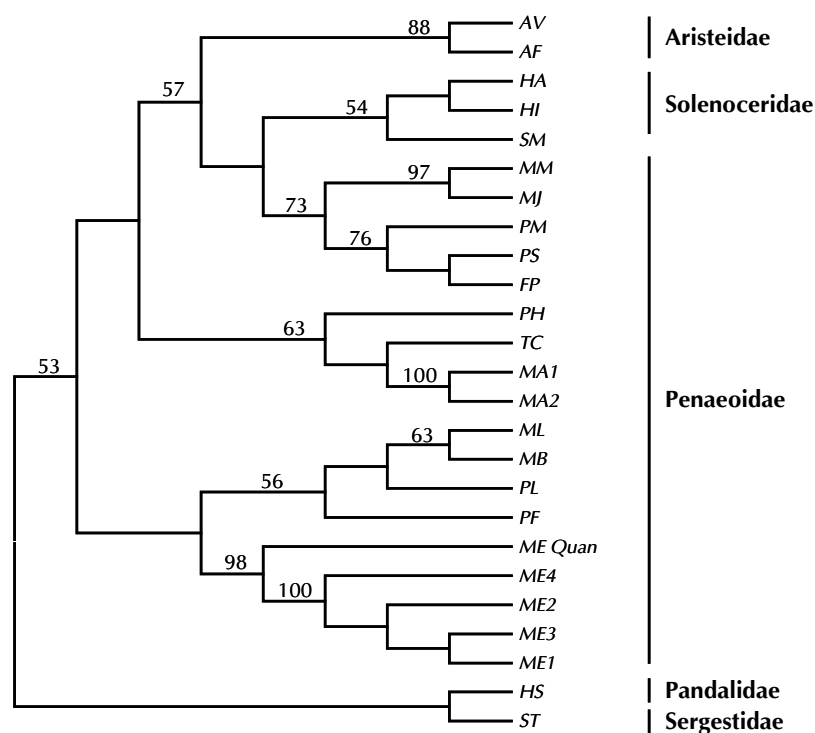
TC: *Trachypenaeus curvirostris*; AF: *Aristaeomorpha foliacea*; AV: *Aristeus virilis*; FP: *Fenneropenaeus penicillatus*; HI: *Haliporoides sibogae*; HS: *Heterocarpus sibogae*; HA: *Hymenopenaeus aequalis*; MJ: *Marsupenaeus japonicus*; MM: *Melicertus marginatus*; ML: *Metapenaeopsis longirostris*; MB: *Metapenaeopsis barbata*; PH: *Parapenaeopsis hardwickii*; PF: *Parapenaeus fissuroides*; PL: *Parapenaeus longipes*; PM: *Penaeus monodon*; PS: *Penaeus semisulcatus*; ST: *Sergia talismani*; ME: *Metapenaeus ensis*; MA: *Metapenaeus affinis*; SM: *Solenocera melanthero*.

searches is shown in Fig. 2 (with a length of 672 steps, a consistency index (CI) excluding uninformative sites of 0.49, and a retention index (RI) of 0.55).

The 16S rRNA data generated similar tree topologies for the distance and parsimony analyses. The 20 species were clustered into five obvious clades in both trees. The trees indicate that the families Aristeidae, Solenoceridae, and Penaeoidea are closely related. *Aristeus virilis* and *Aristaeomorpha foliacea* formed a single clade with the support of high bootstrap values (81 for the NJ

and 88 for the MP analysis). One of the most important results of this study is that the family Solenoceridae formed a single group and was clustered with the “old *Penaeus* genus”. The bootstrap value (of 80) of NJ for this clade in this study was higher than that reported in previous studies, which showed a clade formed by the *Solenocera*, *P. fissuroides*, and *M. barbata* (with NJ values of 44 and 74) (Vázquez-Bader *et al.*, 2004; Voloch *et al.*, 2005). Our study results show that the family Solenoceridae is included within the Penaeoidea with a significant bootstrap value;

Fig. 2 Maximum parsimony (MP) tree from mitochondrial 16S rRNA data of the shrimp. Bootstrap values are shown as percentages and are based on 1000 replicates. Only bootstrap values of > 50% are shown on the branches.



moreover, it seems more suitable to classify the family Solenoceridae into the tribe Peneini than the tribe Parapeneini of the family Penaeidae.

According to our results, the family Penaeoidea appeared to be paraphyletic and to be formed by at least three clades. *Penaeus* belongs to the tribe Peneini, *Parapenaeus* and *Metapenaeopsis* are related to the tribe Parapeneini, while *Metapenaeus*, *Parapenaeopsis*, and *Trachypenaeus* belong to the tribe Trachypeneini. This outcome is consistent with the opinion of Burkenroad (1983) based on the morphological characteristics and also previous studies using molecular data (Quan *et al.*, 2004; Vázquez-Bader *et al.*, 2004; Voloch *et al.*, 2005), which clearly indicated that the family Penaeidae is paraphyletic. Nevertheless, *Metapenaeus ensis* was shown to form a clade by itself, which was closely related to the tribe Trachypeneini. This result differs from previous phylogenetic studies (Vázquez-Bader *et al.*, 2004; Voloch *et al.*, 2005). Voloch *et al.* (2005) showed that a group was formed by *Metapenaeus affinis* and *M. ensis* with high support value (100 for the NJ analysis). We tried to align those two sequences with our data and we found that *M. affinis*

(GenBank accession no.: AY264904) was lumped together into our *M. ensis* group. In our tree, both *M. ensis* and *M. affinis* were grouped separately with relatively high support values (100 for both the NJ and MP analyses). This outcome clearly indicates that *M. ensis* appears outside the tribe Trachypeneini, although it was classified into the tribe based on morphological characters. Thus, a more-detailed study of the tribe Trachypeneini is obviously warranted.

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利用粒線體 16S rRNA DNA 序列探討台灣重要經濟蝦類之分子類緣關係

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摘要

本研究利用粒線體 16S rRNA 部份片段來探討台灣產五科二十種之類緣關係。序列經過排序後長度為 482bp，其中 209 個變異位置裡有 154 個最大儉約法資訊位置。以 Neighbor-joining 及 maximum parsimony 法來重新建構其類緣關係樹狀圖。我們的研究發現以下三項現象：(1) 管鞭蝦科 Solenoceridae 包含在對蝦科裡面，並且把他放在 Peneini 族比放在 Parapeneini 較為恰當；(2) 對蝦科 Penaeoidae 在本研究中呈現出並系群的狀態，並且至少分出三大支系；(3) 劍額新對蝦 *Metapenaeus ensis* 呈現一獨立分支並不包含在 Trachypeneini 族，這與以往形態上的結果有異。且本研究發現劍額新對蝦和近緣新對蝦 *M. affinis* 的分子親緣關係與前人所發表的結果並不相符合。因此對於 Trachypeneini 族及 *Metapenaeus* 屬有必要再做更進一步的研究。

關鍵字：分子類緣關係、對蝦科、16S rRNA

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