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Phylogeny and evolutionary radiation of the marine mussels (Bivalvia: Mytilidae) based on mitochondrial and nuclear genes

Jun Liu, Helu Liu, Haibin Zhang*

Institute of Deep-Sea Science and Engineering, Chinese Academy of Sciences (CAS), Sanya, China

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ABSTRACT

The marine mussels (Mytilidae) are distributed in the oceans worldwide and occupy various habitats with diverse life styles. However, their taxonomy and phylogeny remain unclear from genus to family level due to equivocal morphological and anatomical characters among some taxa. In this study, we inferred the deep phylogenetic relationships among 42 mytiloid species, 19 genera, and five subfamilies of the extant marine mussels by using two mitochondrial (COI and 16S rRNA) and three nuclear (18S and 28S rRNA, and histone H3) genes. Phylogeny was reconstructed with a combination of five genes using Bayesian inference and maximum likelihood method, and divergence time was estimated for the major nodes using a relaxed clock model with three fossil calibrations. Phylogenetic trees revealed two major clades (Clades 1 and 2). In Clade 1, the deep-sea mussels (subfamily Bathymodiolinae) were sister to subfamily Modiolinae (represented by *Modiolus*), and then was clustered with *Leiosolenus* (subfamily Lithophaginae). Clade 2 comprised *Lithophaga* (Lithophaginae) and subfamily Mytilinae. Additionally, a *Modiolus* species and *Musculus senhousia* (subfamily Crenellinae) were positioned within the subfamily Mytilinae. The phylogenetic results strongly indicated monophyly of Mytilidae and Bathymodiolinae, polyphyly of Modiolinae and Lithophaginae, and paraphyly of Mytilinae. Divergence time estimation showed an ancient and gradual divergence in most mussel groups, whereas the deep-sea mussels originated recently and diverged rapidly during the Paleogene. The present study provides new insight into the evolutionary history of the marine mussels, and supports taxonomic revision for this important bivalve group.

1. Introduction

The extant marine mussels (Mytilidae) are bivalves commonly found in the oceans worldwide. Most of the mussels inhabit coastal waters, and some are distributed in the deep sea (Wang, 1997; Distel et al., 2000). Ecologically, the mussel species play an important role in the coastal and deep-sea ecosystems as engineer species by aggregating into beds and modifying the nature and complexity of the substrate (Borthagaray and Carranza, 2007). Besides, mussel beds are one of the most productive assemblages like tropical rain forests and kelp beds (Seed et al., 2000). Economically, most marine mussels are food sources for human beings (Wang, 1997) with some species of great economic importance (e.g. *Mytilus edulis/galloprovincialis*, FAO FishStat), and mussels dominate over half of the global bivalve trade in terms of quantity (Pawiro, 2010). Additionally, some mussels are best-known fouling organisms in coastal and estuarine power plant cooling systems (Jenner et al., 1998).

The mussel species have evolved a variety of lifestyles for adapting to different habitats. They are typically attached to rocks and other hard substrates by byssus threads (referred as epifaunal), (partially) buried in soft sediments (referred as semi-infaunal), or burrow into wood, limestone or corals (referred as rock boring; Stanley, 1970). Their modes of life and habitat preferences are thought to be primarily reflected by shell shapes (Stanley, 1970). Epifaunal species are usually mytiliform, and assigned to the subfamily Mytilinae; semi-infaunal species are modioliform and mainly include subfamilies Modiolinae and Crendellinae (Stanley, 1970; Distel, 2000). A third functional shell form "lithophagiform" has been recognized for rock-boring species (Owada, 2007). These species bore into dead and live corals (e.g., Glynn and Manzello, 2015), which make them a unique group from those epibyssate mussels. In addition, a group of mussel species (subfamily Bathymodiolinae) have been found to inhabit deep waters, including hydrothermal vents, cold seeps or sunken wood and whale falls (Distel et al., 2000; Duperron et al., 2013; Smith et al., 2015). These taxa depend on chemosynthetic bacterial symbionts for nutrition in deep-sea extreme environments (deChaine and Cavanaugh, 2005; Stewart et al., 2005; Smith et al., 2015).

Among mussels, their various lifestyles and habitats probably lead

* Corresponding author at: Institute of Deep-Sea Science and Engineering, CAS, Sanya 57200, China.
E-mail address: hzhang@sidsse.ac.cn (H. Zhang).

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to variable morphology. Since the taxonomy of marine mussels is mainly based on shell morphological characters and identified diagnostic differences are few (Distel, 2000; Morton, 2015), taxonomy of Mytilidae is problematic and a number of classification systems are proposed as briefly reviewed in Wang (1997), Chichvarkhin (2002) and Morton (2015). One classification system following Soot-Ryen (1969) based on paleontological data divided the extant mussel family Mytilidae (superfamily Mytiloidea) into four subfamilies: Mytilinae Rafinesque, 1815; Crenellinae Gray, 1840; Lithophaginae H. Adams & A. Adams, 1857; and Modiolinae Keen, 1958. This system of four subfamilies was applied in Newell (1969), Boss (1982), Bernard (1983), and Wang (1997). Then, new subfamilies and genera were recognized and added to the list: subfamilies Musculinae Iredale, 1939 (Habe, 1977), and Bathymodiolinae (Kenk and Wilson, 1985). Bathymodiolinae was described based on mussels inhabiting deep-sea vents and seeps (Kenk and Wilson, 1985; Gustafson et al., 1998). Bernard et al. (1993) recognized seven subfamilies: Mytilinae, Modiolinae, Bathymodiolinae, Crenellinae, Dacrydiinae, Musculinae, and Lithophaginae. Later, Coan et al. (2000) also recognized seven subfamilies, but replaced the subfamily Musculinae as in Bernard et al. (1993) with Septiniferae. A recent classification system divided the family into eight subfamilies, by adding the subfamily Limnoperninae to Coan et al.'s (2000) list (Bieler et al., 2010). In another different classification system, the superfamily Mytiloidea was divided into four families: Mytilidae, Crenellidae, Lithophagidae, and Septiferidae (Scarlato and Starobogatov, 1979; Starobogatov, 1992). Recently, three families, Mytilidae, Crenellidae and Septiferidae, were recognized within the superfamily Mytiloidea (Carter et al., 2011). A latest taxonomic revision based on the anatomical data divided Mytiloidea into four families: Mytilidae, Crenellidae, Modiolidae, and Musculide (Morton, 2015). It is noted that recognition of subfamilies in the family or genera in the subfamily remains inconsistent in different taxonomic systems. In particular, the taxonomic status of several genera and subfamilies are the most unstable. For example, the genus *Septifer* Recluz, 1848 was placed in the subfamily Mytilinae (family Mytilidae) in some systems (Bernard, 1983; Wang, 1997), but was in the subfamily Septiniferae (family Mytilidae or Septiferidae) in other systems (Bieler et al., 2010; Carter et al., 2011; Morton, 2015). The genus *Musculus* Röding, 1798 was included in the subfamily Crenellinae (family Mytilidae) in some systems (Bernard, 1983; Wang, 1997), but was placed in the subfamily Musculinae (family Crenellidae or Musculidae) in others (Carter et al., 2011; Morton, 2015). The uncertain taxonomic systems suggest that more evidence is required to determine the relationships among the marine mussels.

In the past decades, only a few molecular studies have investigated the deep phylogenetic relationships for marine mussels, mostly based on the 18S ribosomal RNA (18S rRNA) gene (Distel, 2000; Distel et al., 2000; Owada, 2007; Samadi et al., 2007). However, many more studies have focused on the relationships among the deep-sea mussels of the subfamily Bathymodiolinae (e.g. Lorion et al., 2010, 2013; Thubaut et al., 2013). Deep phylogenetic relationships among the marine mussels have not being well resolved by the single gene analysis, and controversial findings are presented between studies with different molecular markers or taxa. For example, phylogenetic trees reconstructed by mitochondrial COI (Samadi et al., 2007) showed different topologies from 18S rRNA gene trees (Distel, 2000). Studies based on the 18S rRNA gene indicated monophyly of the subfamily Lithophaginae (Distel, 2000; Distel et al., 2000). However, a latter study using 18S rRNA gene and more Lithophaginae species implied polyphyly of Lithophaginae (Owada, 2007). To some extent, these disagreements may reflect phylogenetic inaccuracy caused by limited samples and/or sequence information.

In the present work, we aimed to revisit the deep phylogenetic relationships among 42 mytilid species (family Mytilidae) and test evolutionary hypotheses with a combination of two mitochondrial (COI and 16S rRNA) and three nuclear (18S and 28S rRNA, and histone H3) genes. By reconstructing multilocus phylogenetic trees and estimating divergence time for the main nodes, we (1) tested hypotheses referring the deep phylogenetic relationships among the marine mussels; (2) inferred the evolutionary histories of the subfamilies; and (3) discussed its implication in the taxonomy of the marine mussels. This study provides the most comprehensive molecular phylogenetic analyses of the Mytilidae mussels, and thus will support their taxonomic revision. The findings will shed light on the evolutionary history of this important bivalve group.

2. Material and methods

2.1. Taxon sampling

A total of 42 mytilid species belonging to 19 genera and five subfamilies (Mytilinae, Modiolinae, Bathymodiolinae, Lithophaginae and Crenellinae) were used in the phylogenetic analyses. Five species within Pteriomorphia including *Pinna muricata* in Pinnidae (Pinnoidea), *Crassostrea angulata* and *Saccostrea* sp. in Ostreidae (Ostreoidea) , *Isogomon* cf. *ephippium* in Isognomonidae, and *Barbatia lima* in Arcidae were used as outgroups. These outgroup taxa were selected based on previous phylogenetic studies (Distel, 2000; Steiner and Hammer, 2000). It was noted that the classification system used here followed Wang (1997), that is the extant marine mussels included only one family (Mytilidae), and four subfamilies (Mytilinae, Modiolinae, Lithophaginae and Crenellinae). The deep-sea mussel group was referred to as the subfamily Bathymodiolinae following all the previous molecular studies (e.g. Distel et al., 2000; Jones et al., 2006; Lorion et al., 2013; Thubaut et al., 2013). Morphological identification of the sampled specimens in this work followed the keys of Wang (1997). Genomic DNA was extracted from samples preserved in 95% ethanol. For all the species and sequences, see Table 1 and Table S1 (Supplementary material).

2.2. DNA extraction, PCR amplification and sequencing

The genomic DNA was extracted from adductor muscle or mantle tissue by using TIANamp Marine Animals DNA kit (TianGen, Beijing) following the manufacturer's instructions. Partial fragments of the mitochondrial cytochrome *c* oxidase subunit I (COI) and 16S rRNA, and nuclear 18S rRNA, 28S rRNA and histone H3 genes were amplified by using the Polymerase Chain Reaction (PCR) on an ABI Veriti Thermal Cycler (Applied Biosystems, USA). PCR amplification was performed in a 50-μL volume, containing 25μL Premix Taq with 1.25 U Taq, 0.4 mM of each dNTP and 4 mM Mg^{2+} (Ex Taq version, Takara, Dalian, China), 0.5 μM each of the primers and approximately 100 ng template DNA. Cycling conditions included an initial denaturation at 94 °C for 3 min, followed by 30–35 cycles of denaturation at 94 °C for 30 s, annealing temperature for 45 s, and extension at 72 °C for 45 s; and a final extension at 72 °C for 10 min. For the primers and annealing temperatures, see Table S2 (Supplementary material). PCR products were sequenced in both directions on ABI3730. The raw sequences were assembled and trimmed by the program SeqMan v7.2.1 (DNAStar Inc., Madison, WI, USA).

2.3. Phylogenetic analyses

Sequences of COI and H3 genes were aligned with the online software TranslatorX (Abascal et al., 2010; http://www.translatorx.co.uk/) based on the corresponding amino acid translations. The rRNA gene sequences were aligned using Muscle (Edgar, 2004). Ambiguous regions of the alignment of the rRNA genes were removed by using Gblocks v0.91b (Castresana, 2000), with half of the gap positions allowed. Best-fit substitution model of each gene was estimated using the Akaike information criterion (AIC), as implemented in jModeltest v0.1.1 (Guindon and Gascuel, 2003).