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Molecular phylogeny of Arcoidea with emphasis on Arcidae species (Bivalvia: Pteriomorphia) along the coast of China: Challenges to current classification of arcoids

Yanwei Feng, Qi Li *, Lingfeng Kong

Key Laboratory of Mariculture, Ministry of Education, Ocean University of China, Qingdao 266003, China

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ABSTRACT

The current classifications of arcoids are based on phenetic similarity, which display considerable convergence in several shell and anatomical characters, challenging phylogenetic analysis. Independent molecular analysis of DNA sequences is often necessary for accurate taxonomic assignments of arcoids, especially when morphological characters are equivocal. Here we present molecular evidence of the phylogenetic relationships among arcoid species based on Bayesian inference and Maximum Likelihood analyses of three nuclear genes (18S rRNA, 28S rRNA, and histone H3) and two mitochondrial genes (COI and 12S). Tree topologies are discussed by considering traditional arrangements of taxonomic units and previous molecular studies. The results confirm the monophyly of the order Arcoida, the family Noetiidae, and the subfamilies Anadarinae and Striarcinae, with support for the inclusion of the Glycymerididae in the Arcoidea. The subfamily Arcinae and the genera *Arca*, *Barbatia*, *Scapharca*, *Anadara*, and *Glycymeris* are non-monophyletic, suggesting that taxonomic issues still remain. The families Noetiidae, Cucullaeidae, and Glycymerididae appear as subgroups within, rather than sister groups to, the Arcidae. This study strongly suggests the need to carry out a taxonomic revision of the Arcoidea, especially the Arcidae, through combined analysis of morphological, paleontological, and molecular data.

1. Introduction

Arcoid bivalves (Bivalvia: Pteriomorphia: Arcoida) are abundant and diverse in modern seawater across all latitudes and depths, ranging from the low water mark up to 5000 m offshore. Living and extinct arcoids are (or were) epibyssate endobyssate, or shallow burrowers with a wide range of shell forms, which reflect adaptations to their life habits (Thomas, 1978).

Primitive arcoid bivalves have a long geological history stemming from the early Paleozoic era (Waller, 1978). Phenetic characters form a basis for the present classification of arcoids and it remains so for the analysis of extensive fossil records (Oliver and Holmes, 2006). Extant arcoid bivalves comprise two superfamilies, Arcoidea and Limopsoidea. The former superfamily contains five families: Arcidae, Noetiidae, Parallelodontidae, Cucullaeidae, and Glycymerididae; the latter superfamily embraces two families, Limopsidae and Philobryidae. There are a number of conflicting classifications at the superfamily level, especially with respect to the position of the family Glycymerididae (Oliver and Holmes, 2006).

Different classification systems of the most diverse family being Arcidae are proposed on the basis of shell characters. Because most taxonomic studies are limited to a certain region, one is forced to select those aspects of each system to best fit local fauna until a consensus is reached (Kilburn, 1983). Arcidae species are divided into two subfamilies, Arcinae and Anadarinae, based on the strength of the byssus in the attached or free-living forms (Newell, 1969). This split corresponds to separate adaptive radiations, one epibyssate and one endobyssate (Oliver and Holmes, 2006). The generic and subgeneric divisions of the subfamily are inconsistent between authors because of different interpretations of such important morphological features as shell sculpture and inaequivalve or equivalve state.

The taxonomic status of noetiid species is varying. Stewart (1930) first defined the noetiids to the subfamily Noetiinae in the family Glycymeridae, whereas Reinhart (1935), Bouchet et al. (2010) and Carter et al. (2011) later placed the Noetiinae into the Arcidae. Additionally, Frizzell (1946) and Newell (1969) gave the noetiids family rank and retained it in the Arcoidea, which is the

* Corresponding author. Fax: +86 532 82032773.
E-mail address: qili66@ouc.edu.cn (Q. Li).

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Table 1
Molecular phylogeny of Arcoidea with emphasis on Arcidae species (Bivalvia: Pteriomorphia) along the coast of China: challenges to current classification of arcoids Yanwei Feng, Qi Li, Lingfeng Kong.

List of taxa with the classification, source/locality data and GenBank accession numbers. Accession numbers in bold were previously published (Marko, 2002; Matsumoto, 2003)

Classification	Species	Authority	Museum voucher no.	Source/locality	GenBank accession no.				
					COI	12S	H3	28S	18S
Arcidae									
Anadarinae									
	*Scapharca broughtonii*1	(Schrenck, 1867)	LSGB4060201	Lianyungang, Jiangsu	HQ258854	JN974652	JN974600	JN974550	JN974499
	*Scapharca broughtonii*2	(Schrenck, 1867)	LSGB4060202	Panjin, Liaoning	HQ258855	JN974653	JN974601	JN974551	JN974500
	*Scapharca subcrenata*1	(Lischke, 1869)	LSGB4060101	Beihai, Guangxi	HQ258852	JN974654	JN974602	JN974552	JN974501
	*Scapharca subcrenata*2	(Lischke, 1869)	LSGB4060102	Ganyu, Jiangsu	HQ258851	JN974655	JN974603	JN974553	JN974502
	*Scapharca inaequivalvis*1	(Bruguiere, 1789)	LSGB4060301	Sanya, Hainan	HQ258858	JN974650	JN974598	JN974548	JN974497
	*Scapharca inaequivalvis*2	(Bruguiere, 1789)	LSGB4060302	Beihai, Guangxi	HQ258858	JN974651	JN974599	JN974549	JN974498
	*Scapharca cornea*1	(Reeve, 1844)	LSGB4060401	Lingao, Hainan	HQ258859	JN974648	JN974596	JN974546	JN974495
	*Scapharca cornea*2	(Reeve, 1844)	LSGB4060402	Lingao, Hainan	HQ258859	JN974649	JN974597	JN974547	JN974496
	*Scapharca gubernaculum*1	(Reeve, 1844)	LSGB4060501	Lingao, Hainan	HQ258857	JN974646	JN974594	JN974544	JN974493
	*Scapharca gubernaculum*2	(Reeve, 1844)	LSGB4060502	Lingao, Hainan	HQ258857	JN974647	JN974595	JN974545	JN974494
	*Anadara crebricostata*1	(Reeve, 1844)	LSGB4060801	Beihai, Guangxi	HQ258847	JN974642	JN974590	JN974540	JN974489
	*Anadara crebricostata*2	(Reeve, 1844)	LSGB4060802	Beihai, Guangxi	HQ258847	JN974643	JN974591	JN974541	JN974490
	*Anadara vellicata*1	(Reeve, 1844)	LSGB4060901	Beihai, Guangxi	HQ258848	JN974640	JN974588	JN974538	JN974487
	*Anadara vellicata*2	(Reeve, 1844)	LSGB4060902	Beihai, Guangxi	HQ258848	JN974641	JN974589	JN974539	JN974488
	*Anadara antiquata*1	(Linnaeus, 1758)	LSGB4061001	Lingao, Hainan	HQ258849	JN974644	JN974592	JN974542	JN974491
	*Anadara antiquata*2	(Linnaeus, 1758)	LSGB4061002	Sanya, Hainan	HQ258849	JN974645	JN974593	JN974543	JN974492
	Anadara grandis	(Broderip and Sowerby, 1829)	–	–	–	–	AF416841	–	–
	Anadara tuberculosa	(Sowerby 1833)	–	–	–	–	AF416842	–	–
	Anadara similis	(Adams, 1852)	–	–	–	–	AF416843	–	–
	Anadara ovalis	(Bruguiere, 1789)	–	–	–	–	AF416844	–	–
	Anadara transversa	(Say, 1822)	–	–	–	–	AF416845	–	–
	Anadara nux	(Sowerby, 1833)	–	–	–	–	AF416846	–	–
	Anadara chemnitzii	(Philippi, 1851)	–	–	–	–	AF416847	–	–
	*Scapharca globosa*1	(Reeve, 1844)	LSGB4060601	Sanya, Hainan	HQ258861	JN974636	JN974584	JN974534	JN974484
	*Scapharca globosa*2	(Reeve, 1844)	LSGB4060602	Sanya, Hainan	HQ258861	JN974637	JN974585	JN974535	–
	Scapharca sp.1	–	LSGB4060701	Sanya, Hainan	HQ258863	JN974638	JN974586	JN974536	JN974485
	Scapharca sp.2	–	LSGB4060702	Beihai, Guangxi	HQ258863	JN974639	JN974587	JN974537	JN974486
	Scapharca satowi	(Dunker, 1882)	–	–	AB050898	–	–	–	–
	*Tegillarca granosa*1	(Linnaeus, 1758)	LSGB4061101	Wenchang, Hainan	HQ258866	JN974658	JN974606	JN974556	JN974505
	*Tegillarca granosa*2	(Linnaeus, 1758)	LSGB4061102	Yueqing, Wenzhou	HQ258867	JN974659	JN974607	JN974557	JN974506
	*Tegillarca nodifera*1	(v. Martens, 1860)	LSGB4061201	Ganyu, Jiangsu	HQ258869	JN974656	JN974604	JN974554	JN974503
	*Tegillarca nodifera*2	(v. Martens, 1860)	LSGB4061202	Ganyu, Jiangsu	HQ258869	JN974657	JN974605	JN974555	JN974504
	Diluvarca ferruginea	(Reeve, 1844)	–	–	AB050896	–	–	–	–
	Potiarca pilula	(Reeve, 1844)	LSGB4061301	Sanya, Hainan	HQ258862	JN974660	JN974608	JN974558	JN974507
Arcinae									
	*Barbatia decussata*1	(Sowerby, 1833)	LSGB4062001	Weizhou, Guangxi	HQ258830	JN974662	JN974610	JN974560	JN974509
	*Barbatia decussata*2	(Sowerby, 1833)	LSGB4062002	Weizhou, Guangxi	HQ258827	JN974663	JN974611	JN974561	JN974510
	*Barbatia decussata*3	(Sowerby, 1833)	LSGB4062003	Sanya, Hainan	HQ258839	JN974661	JN974609	JN974559	JN974508
	*Barbatia trapezina*1	(Lamarck, 1819)	LSGB4062101	Fangchenggang, Guangxi	HQ258837	JN974664	–	JN974562	JN974511
	*Barbatia trapezina*2	(Lamarck, 1819)	LSGB4062102	Pingtan, Fujian	HQ258837	JN974665	JN974613	JN974563	JN974512
	Barbatia candida	(Helbling, 1779)	–	–	–	–	AF416849	–	–
	Barbatia reeveana	(d'Orbigny, 1846)	–	–	–	–	AF416850	–	–
	Barbatia	(Lamarck, 1819)	–	–	–	–	AF416855	–	–

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Table 1 (*continued*)

List of taxa with the classification, source/locality data and GenBank accession numbers. Accession numbers in bold were previously published (Marko, 2002; Matsumoto, 2003)

Classification	Species	Authority	Museum voucher no.	Source/locality	GenBank accession no.				
					COI	12S	H3	28S	18S
	domingensis								
	Barbatia plicata	(Dillwyn, 1817)	–	–	–	–	AF416856	–	–
	Barbatia gradata	(Broderip and Sowerby, 1829)	–	–	–	–	AF416857	–	–
	*Arca navicularis*1	(Bruguière, 1792)	LSGB4061401	Weizhou, Guangxi	HQ258822	JN974669	–	–	JN974517
	*Arca navicularis*2	(Bruguière, 1792)	LSGB4061402	Beihai, Guangxi	HQ258824	JN974670	JN974618	JN974567	JN974518
	*Barbatia virescens*1	(Reeve, 1844)	LSGB4061801	Shengsi, Zhejiang	HQ258840	JN974676	JN974624	JN974573	JN974524
	*Barbatia virescens*2	(Reeve, 1844)	LSGB4061802	Xiapu, Fujian	HQ258840	JN974677	JN974625	JN974574	JN974525
	*Trisidos kiyonoi*1	(Kuroda, 1930)	LSGB4062201	Wenchang, Hainan	HQ258842	JN974674	JN974622	JN974571	JN974522
	*Trisidos kiyonoi*2	(Kuroda, 1930)	LSGB4062202	Beihai, Guangxi	HQ258843	JN974675	JN974623	JN974572	JN974523
	*Arca avellana*1	(Lamarck, 1819)	LSGB4061501	Fangchenggang, Guangxi	–	JN974680	JN974627	JN974576	JN974527
	*Arca avellana*2	(Lamarck, 1819)	LSGB4061502	Fangchenggang, Guangxi	–	JN974681	JN974628	–	JN974528
	*Arca boucardi*1	(Jousseaume, 1894)	LSGB4061701	Rizhao, Shandong	–	JN974682	JN974629	JN974577	JN974529
	*Arca boucardi*2	(Jousseaume, 1894)	LSGB4061702	Nanji, Zhejiang	–	–	JN974630	–	–
	Arca ventricosa	(Lamarck, 1819)	–	–	AB076935	–	–	–	–
	Arca sp.2	–	LSGB4061601	Nanji, Zhejiang	–	–	JN974631	–	–
	Arca imbricata	(Bruguiere, 1789)	–	–	–	–	AF416851	–	–
	Arca mutabilis	(Sowerby, 1833)	–	–	–	–	AF416852	–	–
	Arca pacifica	(Sowerby, 1833)	–	–	–	–	AF416853	–	–
	Arca zebra	(Swainson, 1833)	–	–	–	–	AF416864	–	–
	*Barbatia fusca*1	(Bruguière, 1789)	LSGB4061901	Lingao, Hainan	–	JN974678	JN974626	JN974575	JN974526
	*Barbatia fusca*2	(Bruguière, 1789)	LSGB4061902	Weizhou, Guangxi	–	JN974679	–	–	–
	Nipponarca bistrigata	(Dunker, 1866)	–	–	AB076936	–	–	–	–
	Bentharca sp.	–	–	–	AB076938	–	–	–	–
Noetiidae									
Striarcinae									
	*Arcopsis interplicata*1	(Grabau and King, 1928)	LSGB4090201	Rizhao, Shandong	HQ258875	JN974672	JN974620	JN974569	JN974520
	*Arcopsis interplicata*2	(Grabau and King, 1928)	LSGB4090202	Rizhao, Shandong	HQ258876	JN974673	JN974621	JN974570	JN974521
	Arcopsis sp.	–	LSGB4090301	Fangchenggang, Guangxi	HQ258872	JN974671	JN974619	JN974568	JN974519
	Arcopsis adamsi	(Dall, 1886)	–	–	–	–	AF416861	–	–
	Arcopsis solida	(Sowerby, 1833)	–	–	–	–	AF416862	–	–
	*Didimacar tenebrica*1	(Reeve, 1844)	LSGB4090101	Fangchenggang, Guangxi	HQ258870	–	JN974616	–	JN974515
	*Didimacar tenebrica*2	(Reeve, 1844)	LSGB4090102	Nanji, Zhejiang	HQ258871	JN974668	JN974617	JN974566	JN974516
Noetiinae									
	Noetia olssoni	(Sheldon and Maury, 1922)	–	–	–	–	AF416859	–	–
	Noetia ponderosa	(Say, 1822)	–	–	–	–	AF416860	–	–
Cucullaeidae									
	Cucullaea labiata	(Lightfoot, 1786)	–	–	AB050892	–	–	–	–
	*Cucullaea labiata*1	(Lightfoot, 1786)	LSGB4080101	Beihai, Guangxi	HQ258880	JN974666	JN974614	JN974564	JN974513
	*Cucullaea labiata*2	(Lightfoot, 1786)	LSGB4080102	Lingshui, Hainan	HQ258880	JN974667	JN974615	JN974565	JN974514
Glycymerididae									
Glycymeridinae									
	Glycymeris reevei	(Mayer, 1868)	–	–	AB076933	–	–	–	–
	Glycymeris rotunda	(Dunker, 1882)	–	–	AB076934	–	–	–	–
	Glycymeris sp.1	–	LSGB4110101	Beihai, Guangxi	HQ258873	–	JN974632	JN974578	JN974530
	Glycymeris sp.2	–	LSGB4110102	Beihai, Guangxi	HQ258874	–	–	JN974579	JN974531
	Glycymeris sp.	–	–	–	–	–	AF416863	–	–
Limopsidae									
	Empleconia cumingii	(Adams, 1863)	–	–	AB076930	–	–	–	–
Philobryidae									
	Cosa waikikia	(Dall, Bartsch, and Rehder, 1939)		–	AB084107	–	–	–	–
Outgroup									
	Mimachlamys nobilis	(Reeve, 1852)	LSGB4180201	Sanya, Hainan	JN974583	JN974684	JN974635	JN974581	JN974533
	Pinctada martensii	(Dunker, 1873)	LSGB4140101	Beihai, Guangxi	JN974582	JN974683	JN974634	JN974580	JN974532

most widely accepted classification. The family Noetiidae embraces two living subfamilies, Noetiinae and Striarcinae.

At present, the family Cucullaeidae, which flourished in the late Mesozoic, is represented by a single, widely distributed, Indo-Pacific species, *Cucullaea labiata* (Boss, 1982). A number of taxonomic studies have been undertaken on the family Glycymerididae, but its classification is still complicated (Matsukuma, 1986). Nicol (1956) divided living members of the Glycymerididae into the *Glycymeris* (*s.s.*) and *Tucetona* groups based on smooth-shelled or strongly ribbed character. Habe (1977) erected the subfamily Melaxinaeinae based on split-ribbed species whose characters also occur in the *Tucetona* group. Matsukuma (1986) recognized Melaxinaeinae in preference to the *Tucetona* group, but transferred *Tucetilla* to the subfamily Glycymeridinae.

Several arcoid characters are known to have convergently arisen and display pervasive homoplasy (Oliver and Holmes, 2006). The dividing line between the subfamilies Anadarinae and Arcinae is blurred in the presence of species with intermediate features, such as *Anadara mosambicana*, *A. erythraeonensis* [Lutaenko, 1994; the two species possess a narrow byssal gape and a rather flattened shell similar to the shells of some *Barbatia* (Arcinae)], and *Trisidos* species (Oliver and Holmes, 2006; the form is epibyssate, but the habit is endobyssate owing to the twist of the shell). A similar case is present in the subfamilies Noetiinae and Striarcinae. In the genus *Arca*, *A. tetragona* has a myophoric flange, which is a defining character of the family Noetiidae given by Newell (1969). The previous findings indicate that the phenetic characters of arcoids are associated with their mode of life and habitat, which are possibly unable to resolve subfamilial and generic relationships.

Nucleotide sequences form datasets independent from the morphology. The former are especially useful in phylogenetic reconstruction when morphological characters and their underlying homology decisions are equivocal (Steiner and Hammer, 2000). To date, there have been few studies to reconstruct an arcoid phylogeny using molecular data. Available data indicate that the phylogenetic relationships within the Arcoidea are far from explicit (e.g., Marko, 2002; Matsumoto, 2003).

In the present study, we aimed to elucidate the evolutionary relationships among arcoid species based on molecular evidence. Three nuclear genes and two mitochondrial genes were used to analyze the phylogeny of the Arcoidea, with emphasis on Arcidae species sampled along the coast of China. The results will provide a large phylogenetic framework for arcoids and thus contribute to better understanding of their evolution.

2. Materials and methods

2.1. Taxon sampling

Fifty-nine taxa were analyzed for the phylogenetic relationships of arcoids in this paper. Species name and sampling locality are given in Table 1. Samples were collected from two individuals of each species, where possible at geographically distant sites. *Mimachlamys nobilis* (Reeve, 1852) and *Pinctada martensii* (Dunker, 1873) were used as outgroups. Specimens were identified based on a review of the literature (e.g., Newell, 1969; Evseev and Lutaenko, 1998) and then ethanol-preserved until extraction.

2.2. DNA extraction, amplification, and sequencing

Total genomic DNA was extracted from adductor muscle following a modification of the standard phenol–chloroform procedure described by Li et al. (2002). Partial or complete fragments of the mitochondrial cytochrome oxidase I (COI) and 12S rRNA genes, as well as the nuclear 28S rRNA, 18S rRNA, and histone H3 genes were amplified via polymerase chain reactions (PCR). Amplification primers are given in Supplementary Fig. 1.

PCR amplifications were carried out in a 50-μL reaction volume containing 2 U *Taq* DNA polymerase (TaKaRa, Dalian, China), approx. 100 ng template DNA, 1 μM forward and reverse primers, 200 μM each dNTP, 1 × PCR buffer, and 2 mM $MgCl_2$. The amplification was performed with an initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing for 1 min, and extension at 72 °C for 1 min.

The PCR results were visualized on 1.5% agarose gels stained with ethidium bromide and then purified with an EZ-10 Spin Column DNA Gel Extraction Kit (Sangon BioTechnologies, Shanghai, China). All purified products were sequenced using an ABI PRISM 3730 automatic sequencer (Applied Biosystems, Foster City, CA, USA).

2.3. Data analyses

The forward and reverse sequences of each individual were assembled and reciprocally edited using SeqMan (DNAstar Lasergene, Madison, WI, USA). GenBank accession numbers are listed in Table 1. In order to increase taxon sampling, we downloaded H3 sequences from Marko (2002) and COI sequences from Matsumoto (2003). The H3 and COI sequences were aligned using Clustal W module (Thompson et al., 1994) in BioEdit 7.0.9 (Hall, 1999) and other sequences were aligned using MUSCLE (Edgar, 2004). All alignments were checked manually in BioEdit. Ribosomal 12S, 28S, and 18S sequences were characterized by highly variable stretches, resulting in gap-rich regions with ambiguous alignments. These variable regions were removed using Gblocks 0.91b (Castresana, 2000). Genetic distances were calculated using Kimura 2-parameter in MEGA 4 (Tamura et al., 2007).

Datasets were partitioned by gene. For the H3 and COI genes, further partition by codon position was performed to account for heterogeneous evolution at each codon position. A total of nine partitions were obtained. To test sequence saturation, the transition/transversion ratio against sequence divergence was plotted separately for each partition in DAMBE (Xia and Xie, 2001). The third codon positions of COI showed substitutional saturation, while the second codon positions of H3 were highly conserved with few variable sites. Thus, only the remaining seven partitions were included in further analysis. Because single H3 gene was found unable to resolve the relationships effectively, additional trees were constructed from the simultaneous dataset by excluding the sequences having only the H3 gene.

2.4. Phylogenetic reconstructions

Phylogenetic reconstructions were performed using Bayesian inference (BI) and Maximum Likelihood (ML) methods. Best-fit models of nucleotide substitution were selected for each partitioned and combined dataset using jModelTest 0.1.1 (Posada, 2008). The results of model analyses are detailed in Supplementary Fig. 2.

The BI and ML analyses were conducted on seven single-gene partitions and three combined datasets (mtDNA, nuDNA, and simultaneous). Sequence data were concatenated using Seaview 4.2 (Galtier et al., 1996). To test whether the combined datasets deviate significantly from the assumption of homogeneity, the incongruence length differences (ILD) was implemented in PAUP* 4.0b10 (Swofford, 2003).

The BI analyses were performed using MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003). Each analysis included four Markov Chain Monte Carlo chains and was run twice in parallel for 10^7 generations with trees sampled every 100 generations. Stationarity

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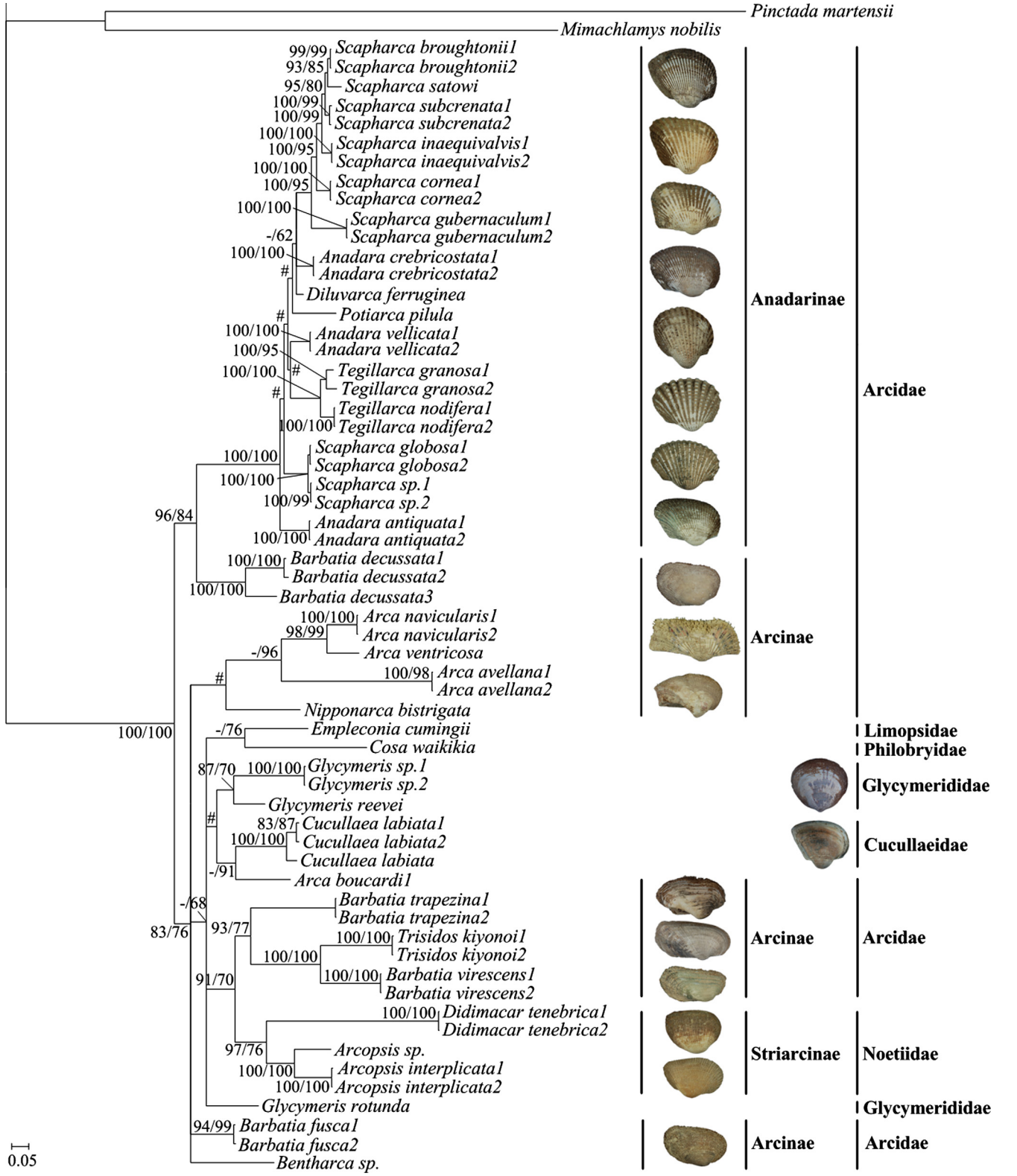

Fig. 1. Bayesian inference tree obtained from simultaneous analysis of COI, 12S, H3, 28S, and 18S genes (excluding sequences only having the H3 gene). Bayesian posterior probability values (⩾80%)/Maximum Likelihood bootstraps (⩾60%) are shown at nodes. Several nodes not occurring in the Maximum Likelihood tree are indicated by pound symbols (#).

was defined as mean standard deviation of split frequency less than 0.01. The first 25% sampled trees determined by Tracer were discarded as burn-in. A majority-rule consensus tree was constructed using the remaining trees with support calculated by Bayesian posterior probabilities (BPP).

The ML analyses were performed using PhyML 3.0 (online server: http://www.atgc-montpellier.fr/phyml/). For starting trees of ML analyses, one BioNJ tree and four additional random trees improved with both subtree pruning & regrafting and Nearest Neighbor Interchange approaches were calculated. The ML trees

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were estimated using the heuristic search algorithm of the best-fit models with four substitution rate categories. Support for nodes was assessed by analyses of 1000 bootstrap replicates.

3. Results

3.1. DNA sequence variation

Sequences were not obtained from all the five genes for ingroup specimens. The COI and H3 alignments involved 55 and 69 specimens, 874 and 319 bp long, respectively. These alignments were compared with translated open reading frames and no insertions or deletions were found. The COI gene had 485 variable sites and 451 phylogenetically informative sites, while the H3 gene had 64 variable sites and 55 phylogenetically informative sites. Poor resolution of the H3 gene could be attributed to high sequence conservation of histone H3 in arcoids.

Sequences of ribosomal gene fragments were of variable lengths. After removal of ambiguously-aligned sites, 375 bp remained in the 12S alignment, of which 191 sites were phylogenetically informative; 679 bp remained in the 28S alignment, of which 129 sites were phylogenetically informative; and 1757 bp remained in the 18S alignment, of which 103 sites were phylogenetically informative.

Examination of genetic divergences among ingroup individuals showed that the mean and range of divergence increased with relative taxonomic rank. When compared across loci, both parameters were smallest for the 18S gene and largest for the 12S gene.

3.2. Partitioned molecular analyses

3.2.1. Single-gene datasets

The BI and ML trees obtained from each gene are shown in Supplementary Fig. 3. All the trees showed resolution to a certain degree and provided support at different taxonomic levels.

At the superfamily level, the Arcoidea was monophyletic with high support in all the trees. An exception was COI trees in which two species (*Empleconia cumingii* and *Cosa waikikia*) of the Limopsoidea appeared within the Arcoidea at low support (Supplementary Fig. 3A and F). The monophyly of the Arcidae was not supported in all the trees. The Noetiidae was monophyletic in H3 ML tree (giving low support, Supplementary Fig. 3H) as well as 28S BI and ML trees (Supplementary Fig. 3D and I). The Glycymerididae was monophyletic in the H3 trees (Supplementary Fig. 3C and H), while it was polyphyletic in COI trees with respect to the position of *Glycymeris rotunda* (Supplementary Fig. 3A and F).

At the subfamily level, the Anadarinae was monophyletic, receiving strong support in the BI and ML trees of COI, 28S and 12S, as well as the BI tree of 18S (Supplementary Fig. 3A–G and I). The Arcinae was paraphyletic or polyphyletic in all the analyses. The monophyly of the Striarcinae was only observed in 28S trees (Supplementary Fig. 3D and I), while the included Noetiinae species formed a monophyletic cluster in H3 trees (Supplementary Fig. 3C and H). The genera *Scapharca*, *Anadara*, *Tegillarca*, and *Potiarca* formed the group Anadarinae in all the single-gene trees except for H3. Among all the genera that contained more than one species, only *Tegillarca* and *Noetia* were monophyletic.

3.2.2. Combined datasets

The ILD test data showed that the combined datasets were homogeneous ($P > 0.05$). This result suggests that combining molecular partitions in a phylogenetic analysis is unlikely to reduce phylogenetic accuracy. In the present study, the combined datasets did not produce more robust phylogenetic resolution than partitioned single-gene datasets (trees not shown). The BI tree constructed from the simultaneous dataset by excluding sequences having only the H3 gene is shown in Fig. 1.

The order Arcoida was well supported, in which two major clades were recovered. The first Arcoida clade (96% BPP, 84% bootstrap) was composed of anadarines and one *Barbatia* taxa, while the second (83% BPP, 76% bootstrap) contained all the remaining lineages. Within the first Arcoida clade, Anadarinae were grouped together with high support (100%). The *Barbatia decussata* clade of Arcinae represented the Arcinae as a sister group to anadarines with high support values. *Scapharca* and *Anadara* were recovered as polyphyletic groups.

Within the second Arcoida clade, *Arca* was not monophyletic and its major clade was well supported (96%) in ML analysis. *A. boucardi* formed a single clade and appeared as a sister group to the family Cucullaeidae. The *Barbatia trapezina*/*Trisidos kiyonoi*/*Barbatia virescens* clade of Arcinae formed a sister group to the family Noetiidae and received moderate support (91% BPP, 70% bootstrap). The Noetiidae formed an expected single cluster given the distinctive growth pattern of ligaments. The Glycymerididae was represented by the genus *Glycymeris* which did not form a monophyletic group. Although the monophyly of the Limopsoidea received a moderate support in ML analysis, it appeared within the Arcoidea and disrupted the arcoidean monophyly.

4. Discussion

4.1. Classification of the superfamily Arcoidea

The monophyly of the Arcoida is well supported here (100% BPP, 100% bootstrap), suggesting that this order is a valid taxonomic group. The contentious problem at the superfamily level is mainly related to the position of the Glycymerididae. Vokes (1967) and Newell (1969) placed the Glycymerididae in the Limopsoidea and it is this classification that is most widely accepted. However, Amler (1999) suggested the Glycymerididae be included in the Arcoidea. Oliver and Holmes (2006) supported the view of Amler, basing their decision on shell and anatomical features, the fossil record, and published molecular studies. In the present study, two Limopsoidea taxa appeared as a subgroup within, rather than a sister group to, the Arcoidea (Fig. 1), leading to the paraphyly of the Arcoidea. Nonetheless, these two taxa formed a single clade with moderate support (ML analysis), suggesting that the Limopsoidea is a monophyletic group. If the Glycymerididae is placed in the Limopsoidea, the monophyly of the Limopsoidea would be lost. Therefore, the Arcoidea should preferably include the family Glycymerididae. It is not surprising to observe the Limopsoidea taxa within the Arcoidea, because ligament structure suggested that the former were derived from the latter (Waller, 1978).

4.2. Classification of the subfamily Arcinae

In the present study, the Arcinae was not recovered as a monophyletic group, consistent with previous finding by Marko (2002). The genus *Arca* formed three small clades, which corresponded to three morphotypes (*A. noae*, *A. avellana*, and *A. tetragona*) as identified by Oliver and Holmes (2006), and three groups (*A. zebra*, *A. imbricata*, and *A. boucardi*) as suggested by Vermeij (2013). Among those, groups *A. noae*/*A. zebra* (*A. navicularis* and *A. ventricosa*) and *A. avellana*/*A. imbricata* clustered together with high support values, whereas *A. tetragona*/*A. boucardi* formed a sister group to the family Cucullaeidae (Fig. 1). This result raises doubts about the position of the *A. tetragona*/*A. boucardi* group in *Arca*, and it supported the morphological work of Vermeij (2013). Furthermore, the *A. tetragona*/*A. boucardi* group is confined to colder temperate

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waters (Oliver and Holmes, 2006), inconsistent with the diagnostic characters of *Arca*. Although the combination of the *A. tetragona*/*A. boucardi* group and the Cucullaeidae is surprising, these two share a common morphological character with respect to the location of adductor muscle on the flange structure (Oliver and Holmes, 2006).

Nipponarca and *Trisidos* have intermediate features between the Arcinae and Anadarinae. The present study provided evidence that both the genera probably should not be assigned to the Anadarinae. Newell (1969) previously included *Bentharca* in the Anadarinae, although it was earlier considered as a subgenus of the Arcinae (Reinhart, 1935). Results from the present study support the viewpoint of Reinhart (1935) that *Bentharca* is a member of the Arcinae, but as an independent genus. Owing to the limitations of sampling, the conclusions regarding the position of these taxa need further examination by increasing taxon sample size. The genus *Barbatia* was polyphyletic and its taxonomic status needs revision.

4.3. Classification of the subfamily Anadarinae

According to data from the present study and Matsumoto and Hayami (2001), the Anadarinae should be recognized as a valid subfamily. However, the traditional subfamily is split into the Arcinae and Anadarinae based on the strength of byssus (Newell, 1969), which needs revision owing to the existence of a group with an intermediate set of features between the Arcinae and Anadarinae. In order to preserve the validity of the Anadarinae, we may consider to accept the new genus *Mosambicarca* as proposed by Lutaenko (1994), or the new subfamily Hawaiarcinae as established by Noda (1986), for species with intermediate features (e.g., *Trisidos kiyonoi*, Fig. 1). The monophyly of *Scapharca* and *Anadara* was not supported in the present study. This result was contradictory to the conclusions of Matsumoto and Hayami (2001) and Marko (2002), likely because of different sample sizes (number of taxa). *Tegillarca* was recovered as a valid group.

4.4. Taxonomic status of the families Noetiidae, Cucullaeidae, and Glycymerididae

The noetiids have been raised to the rank of family and are widely accepted as members of the Arcoidea according to distinctive growth pattern of ligaments (Frizzell, 1946; Newell, 1969). However, Thomas et al. (2000) have shown that the derived characters on which the family Noetiidae is based may not be uniquely shared. Thus, it is thought that the Noetiidae can well be polyphyletic. In the present study, the Noetiidae formed its own clade and received good support (Fig. 1). This result suggests that the Noetiidae is a monophyletic group, although only three taxa of the subfamily Striarcinae were included. The nesting of the Noetiidae within the Arcidae indicates that the former is a younger group derived from the latter. This finding is supported by the fossil record that the Arcidae has arisen by the Jurassic, while the Noetiidae extends back only to the Cretaceous.

The Cucullaeidae is thought to be contemporary with the Arcidae, both of which have their origins in the Jurassic (Oliver and Holmes, 2006). However, the Cucullaeidae formed a clade with *Arca boucardi*1 and appeared within the Arcidae (Fig. 1), indicating that it may be younger than the Arcidae. Although there are numerous fossils available for the Arcoids, it is difficult to date our phylogenetic tree. Our results showed that the Arcidae, the Arcinae, and the *Arca*, *Barbatia*, *Scapharca*, and *Anadara* are not a monophyletic group. This finding indicates that a number of problems exist in the current classifications of arcoids. Consequently, choosing appropriate fossil calibration points is more difficult when dating the phylogenetic tree. The origin of the Cucullaeidae and its taxonomic status as a family merit further studies.

It is not surprising to find that the Glycymerididae nested within the Arcidae. The former, which originated from the Cretaceous, is younger than the latter. The viewpoint of Nicol (1950) regarding the evolution of Glycymeridae from a cucullaeid stock was not supported in the present undated tree. The validity of the genus *Glycymeris* was not tested, but taxonomic revision should be considered by combining morphological and molecular analyses with increased taxon sample size.

5. Conclusions

This study provided the first large molecular phylogenetic framework for arcoids sampled from the vast coast of China. Evidence showed that the family Arcidae is not a monophyletic group. Within the Arcidae, Anadarinae species cluster together with high support. The non-monophyletic nature of the Arcinae questions its validity as an individual subfamily. The monophyly of the genera *Arca*, *Barbatia*, *Scapharca*, and *Anadara* are not supported. The families Noetiidae, Cucullaeidae, and Glycymerididae appear as subgroups within, rather than sister groups to, the Arcidae. The phylogenetic relationships within the Arcoidea inferred from mitochondrial and nuclear gene sequences show strong conflicts with the current morphological hypothesis. In order to effectively reflect the evolutionary relationships of arcoids, a reliable classification system should be established based on a combination of morphological, paleontological, and molecular data.

6. Uncited references

Barucca et al. (2004), Colgan et al. (2000), Giribet et al. (1996), Hassouna et al. (1984), and Okusu et al. (2003).

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2015.02.006.

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