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# Clarifying phylogenetic relationships and the evolutionary history of the bivalve order Arcida (Mollusca: Bivalvia: Pteriomorphia) ☆

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ABSTRACT

The systematics of the bivalve order Arcida constitutes an unresolved conundrum in bivalve systematics. The current definition of Arcida encompasses two superfamilies: Limopsoidea, which includes the recent families Philobryidae and Limopsidae, and Arcoidea, which encompasses the families Arcidae, Cucullaeidae, Noetiidae, Glycymerididae and Parallelodontidae. This classification, however, is controversial particularly with respect to the position and taxonomic status of Glycymerididae. Previous molecular phylogenies were limited either by the use of only a single molecular marker or by including only a few limopsoid and glycymeridid taxa. The challenging nature of Arcida taxonomy and the controversial results of some of the previous studies, prompted us to use a broad range of taxa (55 species), three nuclear markers (18S rRNA, 28S rRNA and histone H3) and a wide range of algorithmic approaches. This broad but stringent approach led to a number of results that differ significantly from previous studies. We provide the first molecular evidence that supports the separation of Arcoidea from Limopsoidea, although the exact position of Glycymerididae remains unresolved, and the monophyly of Limopsoidea is algorithm-dependent. In addition, we present the first time-calibrated evolutionary tree of Arcida relationships, indicating a significant increase in the diversification of arcidan lineages at the beginning of the Cretaceous, around 140 Ma. The monophyly of Arcida, which has been supported previously, was confirmed in all our analyses. Although relationships among families remain somehow unresolved we found support for the monophyly of most arcidan families, at least under some analytical conditions (i.e., Glycymerididae, Noetiidae, Philobryidae, and Limopsidae). However, Arcidae, and particularly Arcinae, remain a major source of inconsistency in the current system of Arcida classification and are in dire need of taxonomic revision.



## 1. Introduction

Bivalvia is the second most speciose class of Mollusca (after Gastropoda) with approximately 10,000 described species (Huber, 2010; Bieler et al., 2013). While Bivalvia is clearly monophyletic, its internal classification has been and continues to be subject of debate and controversy. Morphological and recent molecular phylogenetic research has clarified relationships among most high-level bivalve lineages and clades (e.g., Campbell et al., 1998; Waller, 1998; Carter et al., 2000; Steiner and Hammer, 2000; Giribet, 2008; Giribet and Wheeler, 2002; Sharma et al., 2012; Bieler et al., 2014; González et al., 2015). Amongst the least controversial is the monophyly of the subclass Pteriomorphia Beurlen 1944, which comprises approximately 20% of all bivalves, including some of the most economically important marine taxa, like oysters, mussels and scallops.

A well-known, economically-important group of pteriomorphs are the ark clams or ark shells (order Arcida Gray, 1854). Ark shells are amongst the oldest bivalve lineages, reaching back to the lower Ordovician (~450 Mya; Morton et al., 1998). Today, species of Arcida are globally distributed, predominantly in shallow tropical marine and brackish waters, where they are often abundant. Several species have significant economic value. For example, *Tegillarca granosa* is cultivated on mudflats in South-East Asia (China, Taiwan, Korea, Malaysia and Thailand) and has been consumed by humans for centuries (Beesley et al., 1998); *Scapharca* species are harvested in Japan and China; *Senilia senilis* in West Africa; and *Glycymeris glycymeris* and *G. violascens* in Europe and the Mediterranean region (Oliver and Holmes, 2006). Their common name, blood cockles, refers to the presence of intracellular hemoglobin, a rare, albeit homoplastic trait among bivalves, which distinguishes members of the families Arcidae and Glycymerididae (Boyd, 1998).

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Although several ark shells display highly unusual shell morphologies, they share a straight hinge, which gives the order its common name, since the remainder of the shell now resembles the hull of a boat or an ark. Both shells and adductor muscles are usually of sub-equal size (isomyarian), equivalved and have a characteristic inner and outer crossed-lamellar microstructure (Taylor et al., 1969). At the same time, arcidans share several plesiomorphic characteristic like the taxodont dentition, or homoplastic ones, like the presence of hemoglobin, which occurs as well in the thermal vent clam *Calyptogena magnifica* (Terwilliger et al., 1983) and in several members of the families Carditidae and Crassatellidae (Slack-Smith, 1998a,b; Taylor et al., 2005). The straight hinge line can be somewhat curved among members of the superfamily Limopsidae.

Today, the order Arcida Gray, 1854 encompasses approximately 250–350 extant species of ark shells (e.g., Coan et al., 2000; Oliver and Holmes, 2006). It is monophyletic (e.g., Steiner and Hammer, 2000; Giribet and Wheeler, 2002; Matsumoto, 2003; Bieler et al., 2014) but its internal relationships remain poorly understood and represent a conundrum in bivalve systematics (Giribet and Distel, 2003; Giribet, 2008; Bieler et al., 2014).

The current definition of Arcida encompasses two superfamilies: Limopsoidea Dall, 1895, which include the recent families Philobryidae Bernard, 1897 and Limopsidae Dall, 1895, and Arcoidea Lamarck, 1809, which encompass the families Arcidae Lamarck, 1809, Cucullaeidae Stewart, 1930, Noetiidae Stewart, 1930, Glycymeridae Dall, 1908 and Parallelodontidae Dall, 1898 (e.g., WoRMS Editorial Board, 2015). This classification is controversial and particularly the position and taxonomic status of Glycymeridae is contentious. Their orbicular (ovate) shell form is characteristic of Limopsoidea (Arcoidea are usually trapezoidal, elongate or quadratic), and lead several authors to consider glycymeridids as part of the superfamily Limopsoidea (e.g., Vokes, 1968; Newell, 1969; Tevesz, 1977; Oliver, 1981; Beesley et al., 1998; WoRMS Editorial Board, 2015). On the other hand, glycymeridids have duplivincular ligaments, an important synapomorphy of Arcoidea (Limopsoidea have alivincular ligaments), which testifies to their current status as supported by most recent studies (e.g., Amler, 1989; Bieler and Mikkelsen, 2006; Oliver and Holmes, 2006; Coan and Valentich-Scott, 2012; Bieler et al., 2014; WoRMS Editorial Board, 2015). Still others have placed glycymeridids in their own superfamily, Glycymeridoidea Newton 1916, based on stomach morphology and other anatomic particularities (e.g., Coan et al., 2000). And finally, the most recent phylogenetic study of Arcoidea found no support for glycymeridid monophyly, based on four *Glycymeris* specimens representing three species (Feng et al., 2015; but see Jackson et al., 2015).

The position of glycymeridids remains controversial and has further fueled speculations about the delimitation and monophyly of the two superfamilies. For example, Coan et al. (2000) listed four superfamilies: Arcoidea, Glycymeridoidea, Limopsoidea and Philobryoidea (but see Coan and Valentich-Scott, 2012). Oliver and Holmes (2006) noted that only a few synapomorphic characters support the current classification of superfamilies. Moreover, two recent phylogenetic studies found no support for the superfamily Limopsoidea (Bieler et al., 2014; Feng et al., 2015). Although the two studies included one and two limopsoid samples only, they were all deeply nested with Arcoidea (Waller, 1998; Giribet and Wheeler, 2002). A more recent study by Jackson et al. (2015) included a dense sampling of Phylobryidae and Limopsidae and found monophyly of Limopsoidea, but nested within Arcoidea.

The situation is similarly unresolved within several Arcida families. The largest and most diverse family is Arcidae, which comprises approximately 250 extant species in 31 genera. Arcidae is subdivided in two subfamilies, Anadarinae and Arcinae, based on the strength of the byssus (Newell, 1969). Arcinae contains some of the best-known and most widely distributed genera, like *Arca* Linnaeus, 1758 and *Barbatia* Gray, 1842.

Another particularly controversial family is Noetiidae, which was originally described as a glycymeridid subfamily, Noetiinae Stewart, 1930. Soon after, the subfamily was transferred to Arcoidea (Reinhardt, 1935), a classification followed in several recent catalogues (e.g., Bouchet and Rocroi, 2010; Carter et al., 2011). Today, it is most commonly considered to be a family of Arcoidea, with the subfamilies Noetiinae and Striarcinae MacNeil, 1937 (Frizzell, 1946; Newell, 1969). The family is defined by their unique noetiid ligament, which has particular vertical strips instead of the V-shaped chevron strips of other duplivincular ligaments (MacNeil, 1937). The synapomorphic value of this prominent character, however, has recently been questioned, and due to the absence of other synapomorphies, the validity of Noetiidae has been challenged (Thomas et al., 2000).

The glycymeridids are distinguished by their strongly arched hinge line and large anterior adductor muscles (Oliver and Holmes, 2006). Their internal taxonomy is as complicated as their superfamily affiliation. No subfamilies are currently recognized and the ~100 species are organized in five genera, dominated by *Glycymeris* and *Tucetona*.

The other two arcid families, Parallelodontidae and Cucullaeidae, are represented today by a single extant species each (WoRMS Editorial Board, 2015). In fact, Parallelodontidae is considered extinct by several authors (e.g., Oliver and Holmes, 2006; Bieler et al., 2014), while others content that *Porterius dalli* from Japan is the last living parallelodontiid (Newell, 1969; WoRMS Editorial Board, 2015). Both families have a rich fossil record in the Paleozoic/Mesozoic (Amler, 1989) and in the Jurassic/Cretaceous, respectively (Nicol, 1950).

In order to evaluate the monophyly of the above-mentioned taxa and to reassess the troubled systematics of Arcida, we assembled a multi-locus dataset including a broad sample of Arcida species. Our dataset encompasses representatives of all Arcida families but the elusive Parallelodontidae. Multiple members of both limopsoidean families were analyzed and we significantly expanded the sampling, especially of the controversial families Glycymeridae and Noetiidae. In addition, we estimated the divergence times of major Arcida clades, using multiple Paleozoic and Mesozoic fossils, to analyze the speciation rates of arcid lineages over time. Our results therefore provide the basis for a refined, time-calibrated taxonomy of this ancient group of pteriomorphian bivalves.

## 2. Materials and methods

### 2.1. Species and samples

Analyzed specimens consist mostly of samples from the Museum of Comparative Zoology (MCZ), Harvard, preserved in 96% ethanol and stored at −20 °C. Table 1 gives an overview of all analyzed samples including MCZ voucher numbers, GenBank access codes, and sampling locations. Additional collection information can be obtained from the MCZbase (http://mczbase.mcz.harvard.edu). Taxonomic information follows WoRMS (2015) and differences compared to previous publications and/or GenBank are indicated.

The 93 ingroup samples cover both Arcida superfamilies, 6 out of 7 extant families, 19 genera, and 55 species. The 5 outgroup samples consist of 4 pteriomorph species from different orders and the protobranch *Nucula atacellana*.

**Table 1**
List of samples and molecular markers. Virtually all external sequences are from Feng et al. (2015), i.e. from one single specimen per sample. Additional information about MCZ specimen can be found at www.mczbase.mczharvard.edu.

| Sample | Reference | Source | 18S rRNA | 28S rRNA | Histone H3 | Sampling location |
|---|---|---|---|---|---|---|
| **Order ARCOIDA** | | | | | | |
| **Superfamily ARCOIDEA** | | | | | | |
| ***Family ARCIDAE*** | | | | | | |
| *Subfamily ARCINAE* | | | | | | |
| *Acar bailyi* | Bartsch, 1931 | MCZ 378814 | KT757765 | KT757813 | KT757860 | Baja California, Mexico |
| *Acar domingensis* | Lamarck, 1819 | MCZ 378870 | KT757766 | KT757814 | KT757861 | Bahamas |
| *Arca imbricata (1)* | Bruguière, 1789 | MCZ 378837 | KT757771 | KT757818 | KT757866 | Florida, USA |
| *Arca imbricata (2)* | Bruguière, 1789 | MCZ 378837 | KT757772 | KT757819 | KT757867 | Florida, USA |
| *Arca imbricata (3)* | Bruguière, 1789 | MCZ 378831 | KT757773 | KT757820 | KT757868 | Bahamas |
| *Arca navicularis (1)* | Bruguière, 1789 | MCZ 378833 | KT757774 | KT757821 | KT757869 | Queensland, Australia |
| *Arca navicularis (2)* | Bruguière, 1789 | GenBank/Feng et al. (2015) | JN974518 | JN974567 | JN974618 | Guangxi, China |
| *Arca noae (1)* | Linnaeus, 1758 | MCZ 378834 | KT757775 | KT757822 | KT757870 | Roses, Spain |
| *Arca noae (2)* | Linnaeus, 1758 | BivAToL-116 | KC429325 | KC429416 | KC429160 | Blanes, Spain |
| *Arca patriarchalis*[1] *(1)* | Röding, 1798 | GenBank/Feng et al. (2015) | JN974527 | JN974576 | JN974627 | Guangxi, China |
| *Arca patriarchalis*[1] *(2)* | Röding, 1798 | GenBank/Feng et al. (2015) | JN974528 | | JN974628 | Guangxi, China |
| *Arca boucardi*[2] | Jousseaume, 1894 | GenBank/Feng et al. (2015) | JN974529 | JN974577 | JN974629 | Shandong, China |
| *Arca zebra (1)* | Swainson, 1833 | MCZ 376726 | KT757776 | KT757823 | KT757871 | Bocas, Panama |
| *Arca zebra (2)* | Swainson, 1833 | MCZ 378836 | KT757777 | KT757824 | KT757872 | Florida, USA |
| *Barbatia amygdalumtostum*[3] | Röding, 1798 | GenBank/Feng et al. (2015) | JN974526 | JN974575 | JN974626 | Hainan, China |
| *Barbatia barbata (1)* | Linnaeus, 1758 | BivAToL-123 | KC429326 | KC429417 | KC429161 | Blanes, Spain |
| *Barbatia barbata (2)* | Linnaeus, 1758 | MCZ 378867 | KT757778 | KT757825 | | Roses, Spain |
| *Barbatia candida (1)* | Helbling, 1779 | MCZ 376684 | KT757782 | KT757829 | KT757873 | Bocas, Panama |
| *Barbatia candida (2)* | Helbling, 1779 | MCZ 376684 | KT757783 | KT757830 | KT757874 | Bocas, Panama |
| *Barbatia candida (3)* | Helbling, 1779 | MCZ 376686 | KT757784 | KT757831 | KT757875 | Bocas, Panama |
| *Barbatia cancellaria (1)* | Lamarck, 1819 | MCZ 378868 | KT757779 | KT757826 | | Florida, USA |
| *Barbatia cancellaria (2)* | Lamarck, 1819 | MCZ 378869 | KT757780 | KT757827 | | Florida, USA |
| *Barbatia cancellaria (3)* | Lamarck, 1819 | MCZ 378869 | KT757781 | KT757828 | | Florida, USA |
| *Barbatia foliata*[4] *(1)* | Lamarck, 1819 | GenBank/Feng et al. (2015) | JN974511 | JN974562 | JN974612 | Guangxi, China |
| *Barbatia foliata*[4] *(2)* | Lamarck, 1819 | GenBank/Feng et al. (2015) | JN974512 | JN974563 | JN974613 | Fujian, China |
| *Barbatia lacerata*[5] *(1)* | Lamarck, 1819 | GenBank/Feng et al. (2015) | JN974509 | JN974560 | JN974610 | Guangxi, China |
| *Barbatia lacerata*[5] *(2)* | Lamarck, 1819 | GenBank/Feng et al. (2015) | JN974510 | JN974561 | JN974611 | Guangxi, China |
| *Barbatia lacerata*[5] *(3)* | Lamarck, 1819 | GenBank/Feng et al. (2015) | JN974508 | JN974559 | JN974609 | Hainan, Japan |
| *Barbatia* sp. *(1)* | | MCZ 378875 | KT757785 | KT757832 | | Queensland, Australia |
| *Barbatia* sp. *(2)* | | MCZ 378875 | KT757786 | KT757833 | KT757876 | Queensland, Australia |
| *Barbatia* sp. *(3)* | | MCZ 378830 | KT757787 | KT757834 | KT757877 | Japan |
| *Barbatia virescens (1)* | Reeve, 1844 | GenBank/Feng et al. (2015) | JN974524 | JN974573 | JN974624 | Zhejiang, China |
| *Barbatia virescens (2)* | Reeve, 1844 | GenBank/Feng et al. (2015) | JN974525 | JN974574 | JN974625 | Fujian, China |
| *Barbatia virescens (3)* | Reeve, 1844 | MCZ 378874 | KT757788 | KT757835 | KT757878 | Japan |
| *Barbatia virescens (4)* | Reeve, 1844 | MCZ 378874 | KT757789 | KT757836 | KT757879 | Japan |
| *Bathyarca glomerula* | Dall, 1881 | DNA 100650 | KT757790 | KT757837 | KT757880 | Colombia (Caribbean) |
| *Trisidos kiyonoi* | Makiyama, 1931 | GenBank/Feng et al. (2015) | JN974522 | JN974571 | JN974622 | Hainan, China |
| *Trisidos tortuosa* | Linnaeus, 1758 | BivAToL-91 | KT757811 | KT757858 | KT757899 | Queensland, Australia |
| *ANADARINAE* | | | | | | |
| *Anadara antiquata* | Linnaeus, 1758 | GenBank/Feng et al. (2015) | JN974491 | JN974542 | JN974592 | Hainan, China |
| *Anadara broughtonii*[6] *(1)* | Schrenck, 1867 | GenBank/Feng et al. (2015) | JN974499 | JN974550 | JN974600 | Jiangsu, China |
| *Anadara broughtonii*[6] *(2)* | Schrenck, 1867 | GenBank/Feng et al. (2015) | JN974500 | JN974551 | JN974601 | Liaoning, China |
| *Anadara cornea*[7] *(1)* | Reeve, 1844 | GenBank/Feng et al. (2015) | JN974495 | JN974546 | JN974596 | Hainan, China |
| *Anadara cornea*[7] *(2)* | Reeve, 1844 | GenBank/Feng et al. (2015) | JN974496 | JN974547 | JN974597 | Hainan, China |
| *Anadara crebricostata (1)* | Reeve, 1844 | GenBank/Feng et al. (2015) | JN974489 | JN974540 | JN974590 | Guangxi, China |
| *Anadara crebricostata (2)* | Reeve, 1844 | GenBank/Feng et al. (2015) | JN974490 | JN974541 | JN974591 | Guangxi, China |
| *Anadara globosa*[8] | Reeve, 1844 | GenBank/Feng et al. (2015) | JN974484 | JN974534 | JN974584 | Hainan, China |
| *Anadara gubernaculum*[9] *(1)* | Reeve, 1844 | GenBank/Feng et al. (2015) | JN974493 | JN974544 | JN974594 | Hainan, China |
| *Anadara gubernaculum*[9] *(2)* | Reeve, 1844 | GenBank/Feng et al. (2015) | JN974494 | JN974545 | JN974595 | Hainan, China |
| *Anadara inaequivalvis*[10] *(1)* | Bruguiere, 1789 | GenBank/Feng et al. (2015) | JN974497 | JN974548 | JN974598 | Hainan, China |
| *Anadara inaequivalvis*[10] *(2)* | Bruguiere, 1789 | GenBank/Feng et al. (2015) | JN974498 | JN974549 | JN974599 | Guangxi, China |
| *Anadara notabilis* | Röding, 1798 | MCZ 378821 | KT757768 | KT757816 | KT757863 | Florida, USA |
| *Anadara pilula*[11] | Reeve, 1843 | GenBank/Feng et al. (2015) | JN974507 | JN974558 | JN974608 | Hainan, China |
| *Anadara sativa*[12] *(1)* | Bernard, Cai & Morton,1993 | GenBank/Feng et al. (2015) | JN974501 | JN974552 | JN974602 | Guangxi, China |
| *Anadara sativa*[12] *(2)* | Bernard, Cai & Morton,1993 | GenBank/Feng et al. (2015) | JN974502 | JN974553 | JN974603 | Jiangsu, China |
| *Anadara transversa* | Say, 1822 | MCZ 378822 | KT757769 | | KT757864 | Massachusetts, USA |
| *Anadara trapezia* | Deshayes, 1839 | BivAToL-76 | KT757770 | KT757817 | KT757865 | Queensland, Australia |
| *Anadara vellicata (1)* | Reeve, 1844 | GenBank/Feng et al. (2015) | JN974487 | JN974538 | JN974588 | Guangxi, China |
| *Anadara vellicata (2)* | Reeve, 1844 | GenBank/Feng et al. (2015) | JN974488 | JN974539 | JN974589 | Guangxi, China |
| *Anadara* sp.[13] *(1)* | | GenBank/Feng et al. (2015) | JN974485 | JN974536 | JN974586 | Hainan, China |
| *Anadara* sp.[13] *(2)* | | GenBank/Feng et al. (2015) | JN974486 | JN974537 | JN974587 | Guangxi, China |
| *Tegillarca granosa (1)* | Linnaeus, 1758 | GenBank/Feng et al. (2015) | JN974505 | JN974556 | JN974606 | Hainan, China |
| *Tegillarca granosa (2)* | Linnaeus, 1758 | GenBank/Feng et al. (2015) | JN974506 | JN974557 | JN974607 | Wenzhou, China |
| *Tegillarca granosa (3)* | Linnaeus, 1758 | MCZ 378820 | KT757810 | KT757857 | KT757898 | Rayong, Thailand |
| *Tegillarca nodifera (1)* | v. Martens, 1860 | GenBank/Feng et al. (2015) | JN974503 | JN974554 | JN974604 | Jiangsu, China |
| *Tegillarca nodifera (2)* | v. Martens, 1860 | GenBank/Feng et al. (2015) | JN974504 | JN974555 | JN974605 | Jiangsu, China |

**Table 1** (*continued*)

| Sample | Reference | Source | 18S rRNA | 28S rRNA | Histone H3 | Sampling location |
|---|---|---|---|---|---|---|
| ***CUCULLAEIDAE*** | | | | | | |
| *Cucullaea labiata (1)* | Lightfoot, 1786 | GenBank/Feng et al. (2015) | JN974513 | JN974564 | JN974614 | Guangxi, China |
| *Cucullaea labiata (2)* | Lightfoot, 1786 | GenBank/Feng et al. (2015) | JN974514 | JN974565 | JN974615 | Hainan, China |
| ***GLYCYMERIDIDAE*** | | | | | | |
| *Glycymeris tenuicostata* | Reeve, 1843 | MCZ 378982 | KT757800 | KT757847 | KT757889 | Queensland, Australia |
| *Glycymeris gigantea* | Reeve, 1843 | MCZ 378989 | KT757794 | KT757841 | KT757883 | Sea of Cortez, Mexico |
| *Glycymeris glycymeris (1)* | Linnaeus, 1758 | MCZ 378987 | KT757795 | KT757842 | KT757884 | North Atlantic |
| *Glycymeris glycymeris (2)* | Linnaeus, 1758 | BivAToL-133 | KC429328 | KC429421 | KC429163 | Uncertain (fish market) |
| *Glycymeris holoserica* | Reeve, 1843 | MCZ 378984 | KT757796 | KT757843 | KT757885 | Queensland, Australia |
| *Glycymeris nummaria (1)* | Linnaeus, 1758 | MCZ 378985 | KT757797 | KT757844 | KT757886 | Blanes, Spain |
| *Glycymeris nummaria (2)* | Linnaeus, 1758 | MCZ 378985 | KT757798 | KT757845 | KT757887 | Blanes, Spain |
| *Glycymeris* sp. *(1)* | | GenBank/Feng et al. (2015) | JN974530 | JN974578 | JN974632 | Guangxi, China |
| *Glycymeris* sp. *(2)* | | GenBank/Feng et al. (2015) | JN974531 | JN974579 | JN974633 | Guangxi, China |
| *Glycymeris septentrionalis* | Middendorff, 1849 | MCZ 377780 | KT757799 | KT757846 | KT757888 | Washington, USA |
| *Tucetona pectinata* | Gmelin, 1791 | BivAToL-32 | KT757812 | KT757859 | KT757900 | Florida, USA |
| ***NOETIIDAE*** | | | | | | |
| *Arcopsis adamsi* | Dall, 1886 | BivAToL-3/37 | KC429327 | KC429419 | KC429162 | Florida, USA |
| *Arcopsis* sp. | | GenBank/Feng et al. (2015) | JN974519 | JN974568 | JN974619 | Guangxi, China |
| *Didimacar tenebrica* | Reeve, 1844 | GenBank/Feng et al. (2015) | JN974516 | JN974566 | JN974617 | Zhejiang, China |
| *Eontia ponderosa* | Say, 1822 | BivAToL-210 | KT757793 | KT757840 | KT757882 | South Carolina, USA |
| *Striarca lactea (1)* | Linnaeus, 1758 | BivAToL-115 | AF120531 | KT757855 | KT757897 | Blanes, Spain |
| *Striarca lactea (2)* | Linnaeus, 1758 | MCZ 379156 | KT757809 | KT757856 | | Roses, Spain |
| *Verilarca interplicata*[14] *(1)* | Grabau & King, 1928 | GenBank/Feng et al. (2015) | JN974520 | JN974569 | JN974620 | Shandong, China |
| *Verilarca interplicata*[14] *(2)* | Grabau & King, 1928 | GenBank/Feng et al. (2015) | JN974521 | JN974570 | JN974621 | Shandong, China |
| **LIMOPSOIDEA** | | | | | | |
| ***LIMOPSIDAE*** | | | | | | |
| *Limopsis cumingi*[15] | Adams, 1863 | | KT757802 | AB101610 | | Japan |
| *Limopsis* sp. | | BivAToL-213 | KC429329 | KC429422 | KC429164 | Philippines |
| **PHILOBRYIDAE** | | | | | | |
| *Adacnarca nitens* | Pelseneer, 1903 | MCZ 376663 | KT757767 | KT757815 | KT757862 | South Shetland Islands |
| *Neocardia* sp. *(1)* | | MCZ 378927 | KT757803 | KT757849 | KT757890 | Port Elizabeth, South Africa |
| *Neocardia* sp. *(2)* | | MCZ 378927 | KT757804 | KT757850 | KT757891 | Port Elizabeth, South Africa |
| *Neocardia* sp. *(3)* | | MCZ 378927 | KT757805 | KT757851 | KT757892 | Port Elizabeth, South Africa |
| *Philobrya sublaevis* | Pelseneer, 1903 | BivAToL-399 | KT757807 | KT757853 | KT757895 | Adelaide Island, Antarctica |
| **OUTGROUPS** | | | | | | |
| **PTERIOMORPHIA** | | | | | | |
| *Brachidontes exustus*[16] | Linnaeus, 1758 | BivAToL-243 | KT757791 | KT757838 | | Florida, USA |
| *Ctenoides mitis*[17] | Lamarck, 1807 | BivAToL-28 | KT757792 | KT757839 | KT757881 | Florida, USA |
| *Isognomon legumen*[18] | Gmelin, 1791 | BivAToL-425 | KT757801 | KT757848 | KT757894 | Hong Kong |
| *Spondylus gaederopus*[19] | Linnaeus, 1758 | MCZ 376622 | KT757808 | KT757854 | KT757896 | Balearic Islands, Spain |
| **PROTOBRANCHIA** | | | | | | |
| *Nucula atacellana*[20] | Schenck, 1939 | BivAToL-215 | KT757806 | KT757852 | KT757893 | Bermuda |

Numbers in parenthesis are specimen identifiers.
Superscript: 1 = *Arca* sp.1 on GenBank, *A. avellana* in Feng et al. (2015); 2 = *Arca* sp.1 on GenBank; 3 = *Barbatia fusca* in Feng et al. (2015) and on GenBank; 4 = *Barbatia lima on GenBank, B. trapeziana* in Feng et al. (2015); 5 = *Barbatia lacerate* on GenBank, *B. decussata* in Feng et al. (2015); 6 = *Scapharca broughtonii* in Feng et al. (2015) and on GenBank; 7 = *Scapharca cornea* in Feng et al. (2015) and on GenBank; 8 = *Scapharca globosa* in Feng et al. (2015) and on GenBank; 9 = *Scapharca gubernaculum* in Feng et al. (2015) and on GenBank; 10 = *Scapharca inaequivalis* in Feng et al. (2015) and on GenBank; 11 = *Potiarca pilula* in Feng et al. (2015) and on GenBank; 12 = *Scapharca subcrenata* in Feng et al. (2015) and on GenBank; 13 = *Scapharca* sp. in Feng et al. (2015) and on GenBank; 14 = *Arcopsis interplicata* in Feng et al. (2015) and on GenBank; 15 = *Empleconia cumingi* in Feng et al. (2015) and on GenBank = only sample with sequences from different specimen; 16 = Mytiloida, Mytilidae; 17 = Limoida, Limidae; 18 = Pterioida, Pteriidae; 19 = Pectinoida, Spondylidae; 20 = Nuculida, Nuculidae.

### 2.2. Molecular methods

Total genomic DNA was extracted from muscle or mantle tissue using Qiagen's DNeasy tissue kit (Valencia, CA, USA), following manufacturer's instructions, and used as PCR templates. Three molecular markers were analyzed: Two nuclear ribosomal genes (18S rRNA and 28S rRNA) and one nuclear protein-coding gene (histone H3). Primer sequences are listed in Supplementary Table S1. PCR amplifications (25 μl) were conducted on Eppendorf® Mastercycler Pro (Hamburg, Germany). Each PCR mix contained 1 μl of template DNA, 1 μl of each primer (100nM), 0.5 μl of dNTP's (100 nM, Invitrogen®, Carlsbad, CA), 0.1 μl of GoTaq DNA Polymerase (Promega®, Madison, WI, USA), 2.5 μl of 10x PCR buffer (Promega) and molecular grade DI water up to 25 μl. PCR conditions consisted of an initial denaturation step at 95 °C for 2 min, followed by 35 cycles of 30 s at 95 °C, 30 s at 50 °C (18S rRNA & 28S rRNA) or 45 °C (histone H3), and 60 s at 72 °C, and a final 5 min extension step at 72 °C. PCR products were visualized on 1% agarose gels and purified using ExoSAP-IT (Affymetrix, Santa Clara, CA, USA). Clean PCR products were labeled using BigDye terminator 3.1 (Applied Biosystems, Carlsbad, CA, USA), repurified using Sephadex (Amersham Biosciences, Amersham, UK) and sequenced on ABI Prism 3730 Genetic Analyzer (Applied Biosystems). Sequence data was visualized and edited in GENEIOUS Pro 7.1.7 (Biomatters Limited, Auckland, New Zealand) and Sequencher 5.1 (Gene Codes Corporation, Ann Arbor, MI, USA). Sequences were screened for contamination using BLAST searches (ncbi.nlm.nih.gov) and new sequences were deposited on GenBank under the accession codes listed in Table 1.

### 2.3. Parsimony analyses under dynamic homology

Parsimony analyses were conducted with POY 5.1.1 (Varón et al., 2012; Wheeler et al., 2015) using a direct optimization

(DO) approach (Wheeler, 1996) with six different analytical parameter sets (111, 121, 211, 221, 3211 & 3221). Timed searches of 1 h were run for the combined analyses of sequence data under each parameter set. Four rounds of sensitivity analysis tree fusing (SAFT) (Giribet, 2007) were conducted, using all output trees from previous round of analyses. Resulting tree lengths were checked for heuristic stability and once all parameter sets stabilized and the optimal tree was found multiple times for each parameter set, we stopped the analyses.

Parameter set *3221* (indel opening cost = 3; transversions = transitions = 2; indel extension cost = 1) was chosen based on the Wheeler incongruence length difference metric (wILD; Sharma et al., 2011; Wheeler, 1995; Table 3). General advantages of this parameter set are discussed elsewhere (De Laet, 2005; Sharma et al., 2011; Bieler et al., 2014). Nodal support was estimated using a jackknife resampling analysis (Farris et al., 1996; Farris, 1997) with 100 replicates and a deletion probability of each character of $e^{-1}$ (see Giribet et al., 2012; Bieler et al., 2014). Nodes recovered in the parsimony analyses with other explored parameter sets are indicated as Navajo rugs.

### 2.4. Probabilistic analyses under static homology

Bayesian inference and maximum likelihood analyses were conducted on static alignments generated with MUSCLE 3.6 (Edgar, 2004) with default parameters as implemented in GENEIOUS. Amplicon sequence alignments were subsequently treated with GBlocks 0.91b (Castresana, 2000), using default parameters, to remove position of ambiguous homology and concatenated using GENEIOUS. Protein-coding histone H3 sequences were additionally confirmed using protein translations prior to GBlocks treatment. Alignment sizes pre- and post-GBlocks are listed in Table 2. jModelTest (Darriba et al., 2012) was used to obtain the best-fit model of sequence evolution under the Akaike information criterion (AIC); this was GTR + I + G for all three data partitions.

Maximum likelihood analyses were conducted using RAxML on XSEDE (Stamatakis, 2014) as implemented on the CIPRES web portal (Miller et al., 2010). A unique GTR model of sequence evolution was specified for each molecular marker with corrections for a discrete gamma distribution for site-rate heterogeneity (GTRGAMMA). Nodal support was estimated via the rapid bootstrap algorithm (1000 replicates) using the GTRCAT model (Stamatakis et al., 2008).

Bayesian inference analyses were carried out with MrBayes 3.2 (Ronquist et al., 2012) as implemented on the CIPRES web portal (Miller et al., 2010). Analyses were conducted with the same nucleotide substitution model inferred by jModelTest, a unique GTR model for each partition with gamma corrections and a proportion of invariables sites (GTR + I + G). Convergence diagnostics were analyzed using Tracer 1.6 (Rambaut et al., 2014). MrBayes analyses started with random trees, default priors and five runs, each with 3 hot and 1 cold Markov chains, until the average deviation of split frequencies reached <0.01 (~5–12 M generations). Log and tree files were combined with LogCombiner (see the BEAST documentation; Drummond et al., 2012). Phylogenetic trees were summarized with TreeAnnotator 1.8.1 (see the BEAST documentation; Drummond et al., 2012) as a maximum clade credibility tree with a burn-in of 10% removed.

**Table 2**
Alignment sizes prior to (pre-) and after (post-) treatment with Gblocks 0.91b (GB).

| | 18S | 28S | H3 | Total |
|---|---|---|---|---|
| Pre-GB | 1827 | 2250 | 328 | 4405 |
| Post-GB | 1668 | 1778 | 327 | 3773 |
| Retained | 91% | 79% | 100% | 86% |

**Table 3**
Number of steps and incongruent length difference values (wILD) for the six parameter sets used in parsimonious dynamic optimization analyses. The optimal parameter set (3221) is indicated in bold.

| Parameter | Tree cost | | | | |
|---|---|---|---|---|---|
| Set | 18S | 28S | H3 | Final | wILD |
| 111 | 691 | 2152 | 851 | 3860 | 0.043 |
| 121 | 1040 | 3213 | 1155 | 5640 | 0.041 |
| 211 | 786 | 2589 | 852 | 4414 | 0.042 |
| 221 | 1218 | 4075 | 1152 | 6690 | 0.037 |
| 3211 | 1082 | 3436 | 1155 | 5884 | 0.036 |
| **3221** | **1443** | **4600** | **1704** | **7987** | **0.030** |

### 2.5. Estimation of divergence times and diversification through time

Divergence times of major clades were inferred using BEAST 1.8.1 (Drummond et al., 2012) as implemented on the CIPRES web portal. A starting tree was generated by running 10 independent analyses for 50,000,000 MCMC generations, sampling every 5000 generations with random starting trees, relaxed uncorrelated lognormal clocks for each partition, and the Yule speciation model. Results were analyzed with Tracer and TreeAnnotator as described above and the maximum clade credibility tree from the run with the highest likelihood was used as starting tree in subsequent dating analyses. Dating analyses were conducted as 4 independent analyses for 500,000,000 generations. Convergence of analytical statistics was confirmed after 150,000,000 generations and the maximum clade credibility tree was obtained from TreeAnnotator.

Four fossil taxa were used to calibrate divergence times. The root age of Bivalvia was constrained with a uniform distribution prior between 520.5 and 530 Ma (sensu Bieler et al., 2014). The age of Arcida was constrained using a normal distribution prior with a mean of 478.6 Ma and a standard deviation of 5 Ma, based on *Glyptarca serrata* (Cope, 1997).

The age of Glycymerididae was constrained at around 167.7 Ma based on *Trigonarca tumida* (Imlay, 1962) and the age of Anadarinae was constrained at around 138.3 Ma, based on *Anadara ferruginea* (Jaccard, 1869; Huber, 2010). To account for uncertainty, we applied a normal distribution prior to both nodes with the means around their mean ages and standard deviations of 5 Ma.

To assess whether Arcida speciation rates have remained constant over time the $\gamma$-statistic (Pybus and Harvey, 2000) was calculated using the R package phytools 0.4 (Revell, 2012). Variation in speciation and extinction rates were explored using likelihood analyses in the R package LASER 2.4 (Rabosky, 2006a). Six different rate-variable and rate-constant birth–death models were fitted to the diversification chronogram: pure-birth, birth–death, logistic density dependent, exponential density dependent, 2-rate Yule and 3-rate Yule. The test statistic dAICrc was calculated as the difference in AIC scores between the best rate-constant and rate-variable models (Rabosky, 2006b).

## 3. Results

### 3.1. Direct optimization

Parsimony tree searches with direct optimization using POY 5.1.1 resulted in a single most parsimonious tree of 7987 weighted steps under the optimal parameter set (Fig. 1). This tree is very similar to the topologies of ML and BI trees based on static alignments. It is also broadly similar to the most parsimonious trees based on other parameter sets as indicated in Fig. 1. The monophyly of Arcida is fully supported in all POY trees as it is on the ML and both BI trees, with a jackknife frequency (JF hereafter) of 98% under the optimal parameter set (Figs. 1–4). At the

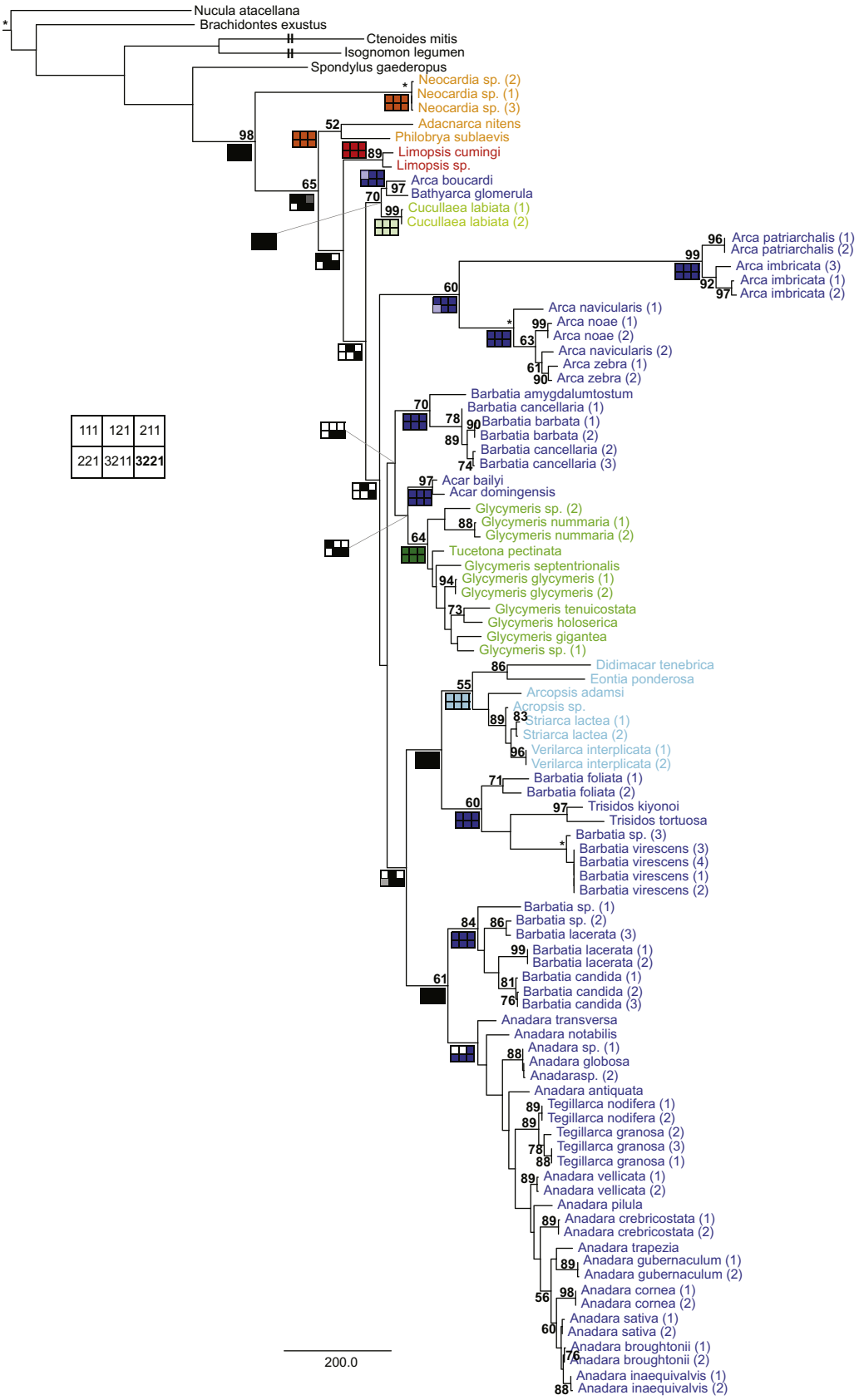


**Fig. 1.** Phylogenetic tree of Arcida relationships based on parsimony under direct optimization with POY of three genes (7987 weighted steps). Numbers on nodes indicate jackknife resampling frequencies (asterisks indicate a jackknife support of 100). Navajo rugs on selected nodes and after clades indicate recovery in a parameter set. If more than one most parsimonious tree was identified and major nodes were not recovered in every single tree, ambiguous nodal support is indicated by light colors (i.e. gray and light blue instead of black and blue, respectively). Colors correspond to the 6 currently accepted families. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

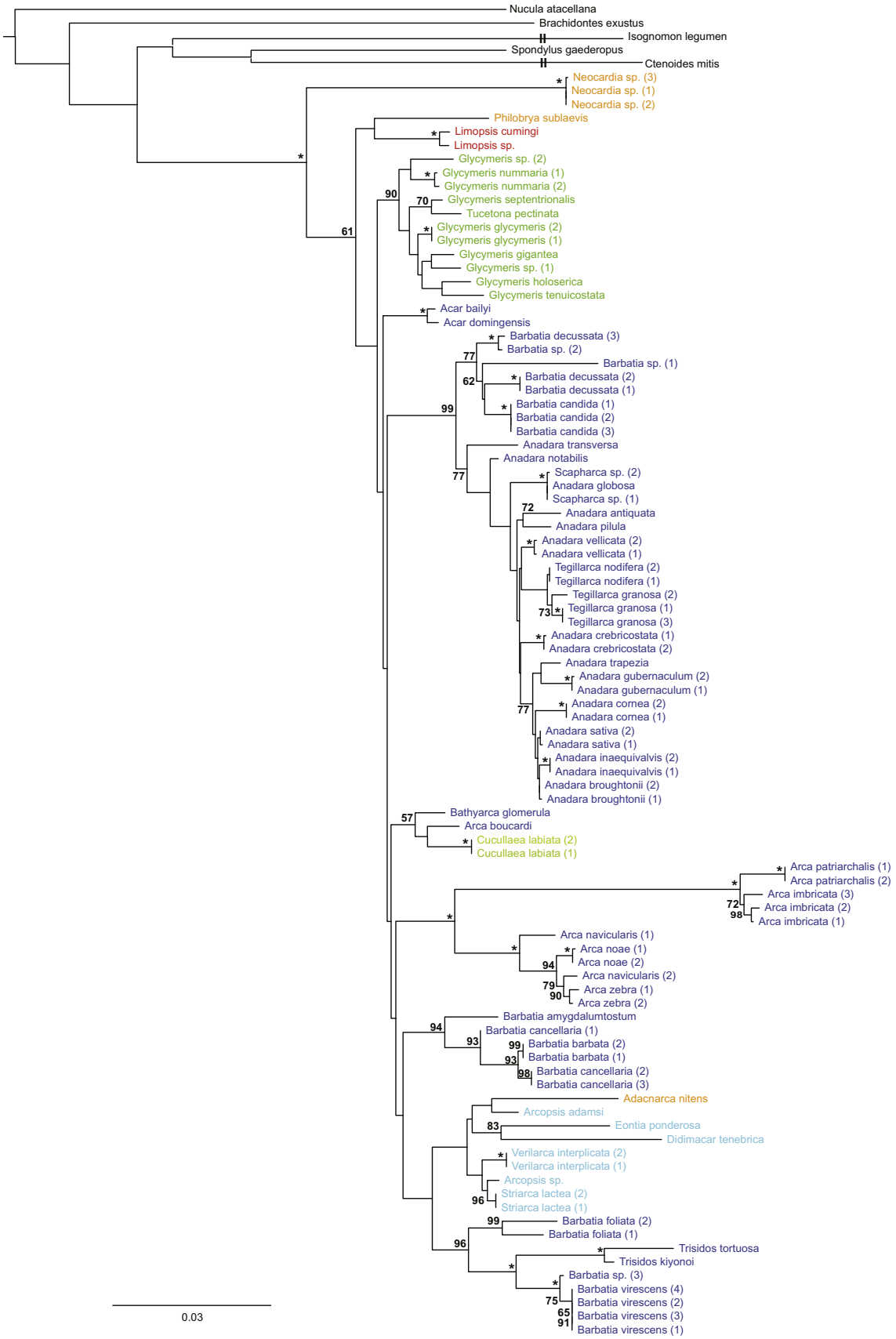


**Fig. 2.** Phylogenetic tree of Arcida relationships based on maximum likelihood analysis with RAxML of three genes (ln*L* −18375.99). Numbers on nodes indicate bootstrap resampling frequencies (asterisks indicate a bootstrap support of 100). Colors correspond to the 6 currently accepted families. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

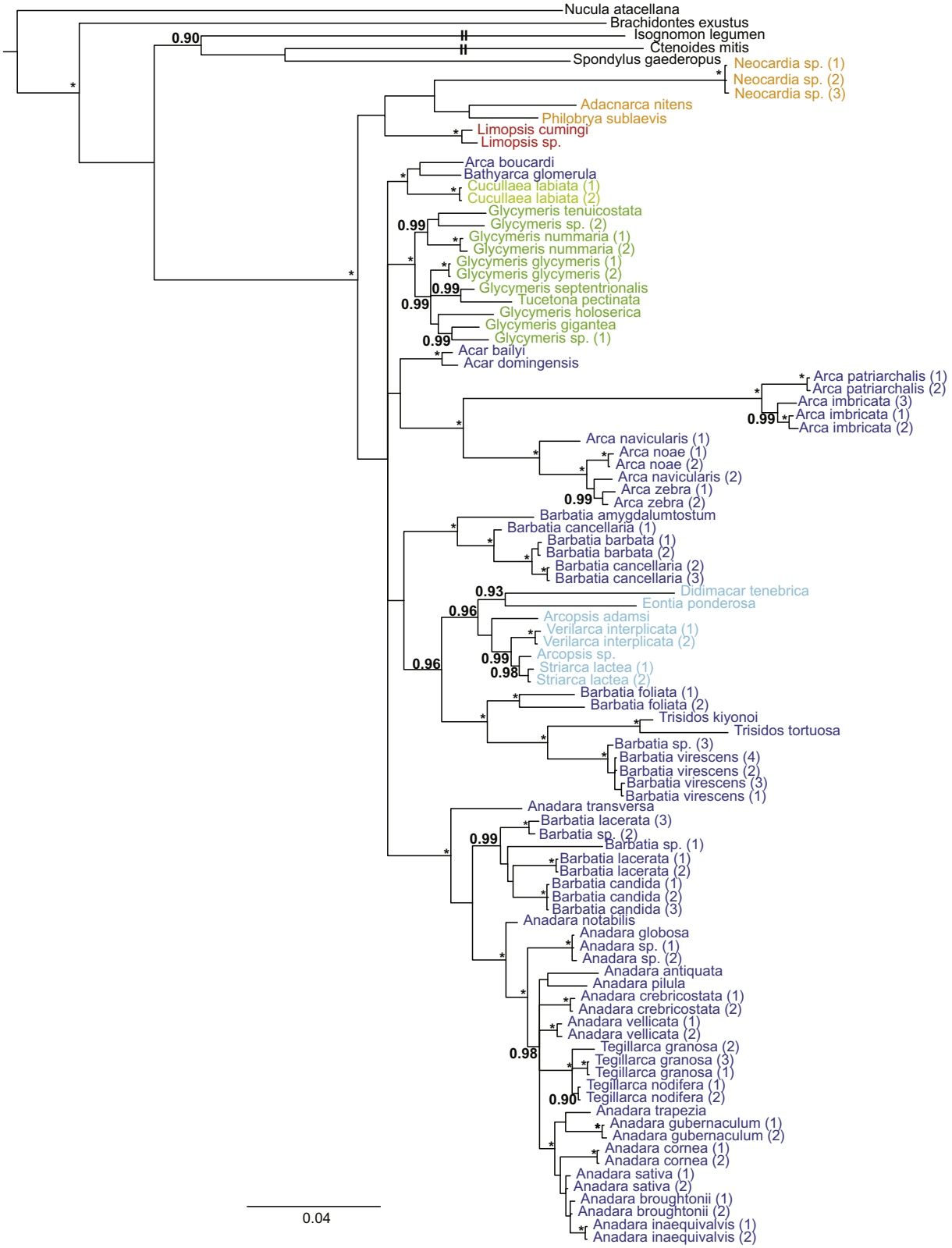


**Fig. 3.** Phylogenetic tree of Arcida relationships based on Bayesian inference with MrBayes of three genes. Numbers on nodes indicate posterior probabilities (asterisks indicate a posterior probability of 1.0). Colors correspond to the 6 currently accepted families. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

superfamily level, Limopsoidea (Limopsidae and Philobryidae) was recovered as a grade, forming three clades (*Neocardia*, *Phylobrya* + *Adacnarca* and *Limopsis*), leading to a monophyletic Arcoidea (sensu WoRMS), which includes Glycymeridae. However, parameter sets *211* and *221* find alternative resolution for the Limopsoidea, with *Phylobrya*, *Adacnarca* and *Limopsis* forming a clade nested within Arcoidea, and *Neocardia* constituting the sister group of all other Arcida under parameter set *211* or nested within

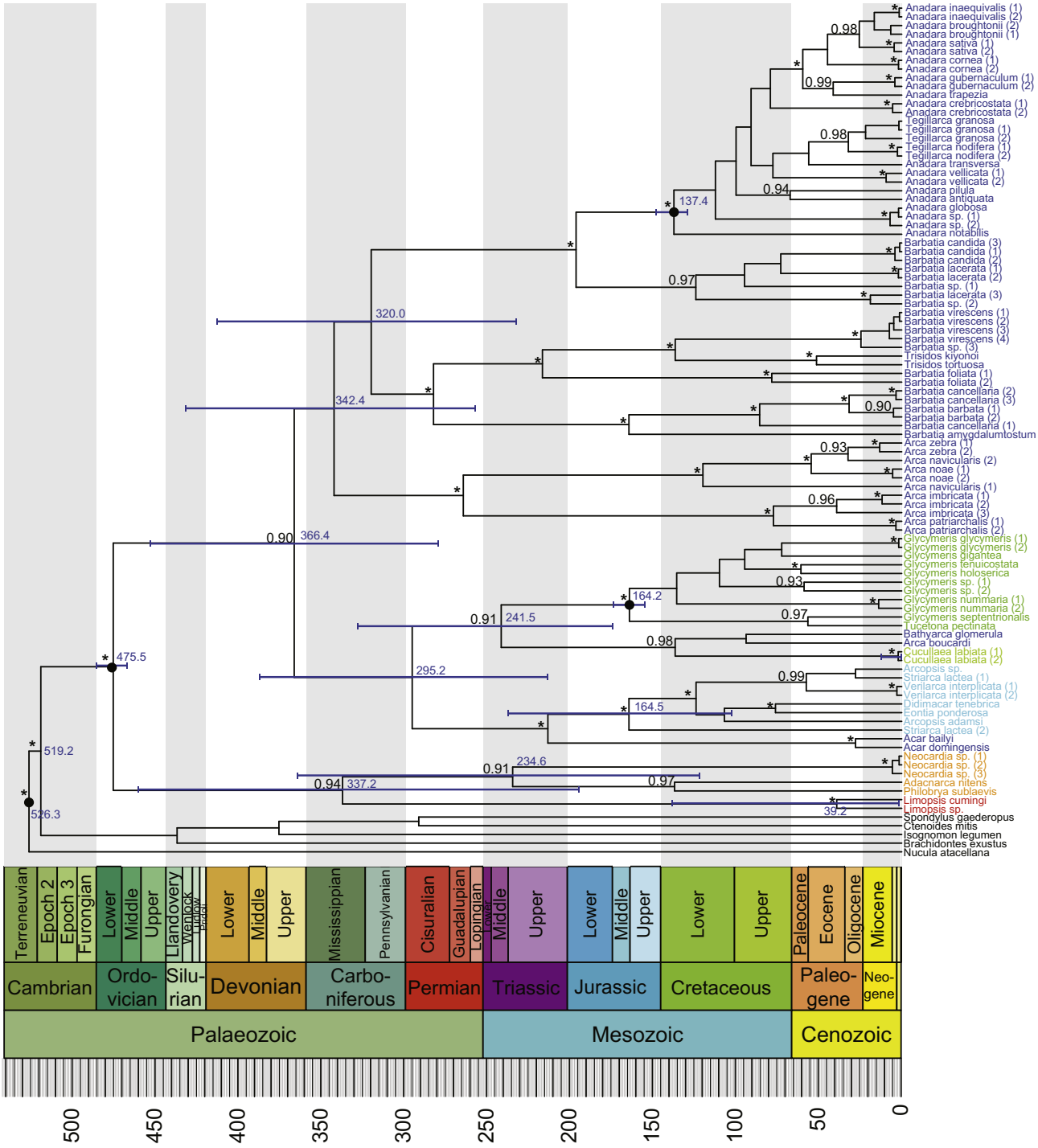


**Fig. 4.** Evolutionary timetree of Arcida relationships inferred from Bayesian inference analyses with BEAST of three genes. Blue text adjacent to selected nodes indicates median ages. Blue bars indicate 95% highest posterior density intervals for nodes of interest. Black text below selected nodes indicates posterior probabilities (asterisks indicate a posterior probability of 1.0). Colors correspond to the 6 currently accepted families. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

a clade of *Arca* under parameter set *221*. However, most of these relationships appear generally poorly supported, and only the clade of Arcida excluding *Neocardia* receives a JF of 65%, most other deep splits receiving less than 50% JF.

Major aspects of the POY analyses at the family level (for those families represented by more than one species) include the monophyly of Glycymerididae (JF = 64%), Noetiidae (JF = 55%), Limopsidae (JF = 89%) and the subfamily Anadarinae (without significant JF). The monophyly of these three families was recovered under all six parameter sets while Anadarinae was recovered under 4 parameter sets only. The family Arcidae was found to be paraphyletic with respect to Glycymerididae, Noetiidae and Cucullaeidae, but with several well-supported clades. Philobryidae was also found to be paraphyletic. The phylogenetic relationship among families was generally poorly supported (JF < 50%) with the exception of a clade containing Cucullaeidae with *Bathyarca glomerula* and *Arca boucardi* (JF = 70%), which was recovered under all parameter sets.

All four genera with more than 4 species/specimen were found to be non-monophyletic (in all phylogenetic analyses). For example, *Glycymeris* was paraphyletic due to the inclusion of *Tucetona* and *Anadara* was paraphyletic due to *Tegillarca*. *Arca* was monophyletic with the exception of *Arca boucardi*, which was sister group to *Bathyarca glomerula*, both constituting the sister group of Cucullaeidae. Finally *Barbatia* was recovered as a polyphyletic assemblage of at least three lineages, one related to *Acar* and Glycymerididae, another possibly related to Noetiidae and paraphyletic with respect to *Trisidos*, and a third clade sister group to the *Anadara*/*Tegillarca* clade. Other genera represented by 2+ species and/or specimens were generally found to be monophyletic with the exception of the paraphyletic *Arcopsis*, which included *Striarca* and *Verilarca*.

### 3.2. Maximum likelihood

Maximum likelihood analysis of the 3 locus dataset resulted in an optimal tree topology with $\ln L = -18375.99$ (Fig. 2). Most significantly, this ML tree did not recover either superfamily as monophyletic due to the inclusion of *Adacnarca nitens*, a philobryid, within Noetiidae. This placement is unique to the ML tree, and was not recovered with any other phylogenetic analyses. Other aspects of topology and support of the ML tree remained consistent with the direct optimization analyses, for example the monophyly of Glycymerididae (bootstrap support [BS] = 90%), Limopsidae (BS = 100%) and Anadarinae (BS = 77%). Likewise, the ML analysis failed to recover monophyly of the larger genera *Arca*, *Anadara*, *Barbatia* and *Glycymeris*. *Arca* also formed a clade with the exception of *A. boucardi*; *Anadara*, paraphyletic with respect to *Tegillarca*, formed the sister clade of a *Barbatia* clade; another *Barbatia* clade included *Trisidos*; and the third *Barbatia* clade appears related, but without support, to a clade including Noetiidae, *Adacnarca*, and the *Barbatia*/*Trisidos* group.

Again, phylogenetic relationship among these clades remained poorly supported (generally BS < 60%) with the exception of a clade containing Anadarinae and one of the three *Barbatia* clades (BS = 99%). A particularity of this analysis is that with the exception of the philobryid *Adacnarca nitens*, this analysis finds Limopsoidea (*sensu* Beesley et al., 1998) to be outside Arcoidea; i.e., Glycymerididae nest outside Arcoidea – as opposed to most current classification systems that include Glycymerididae in Arcoidea. This tree, however, does not find monophyly of Limopsoidea, and support for the basal relationships is low.

### 3.3. Bayesian inference

Bayesian inference analyses with MrBayes reached stationarity after $5–12 * 10^6$ generations (Fig. 3). The resulting tree topology is more congruent with the parsimony tree than with the ML tree. For example, Limopsoidea and Arcoidea (including Glycymerididae) were recovered as sister taxa, this time both being monophyletic (albeit unsupported, posterior probability [pp] = 0.5 & 0.75, respectively). In addition, Noetiidae (pp = 0.96) are recovered as monophyletic again and the monophyly of Glycymerididae (pp = 1.0), and Limopsidae (pp = 1.0) is confirmed. Philobryidae were recovered as monophyletic for the first time, but without support (pp = 0.57). Anadarinae appeared paraphyletic here, due to the inclusion of one of the three *Barbatia* clades (monophyly of this *Barbatia* clade and Anadarinae is supported pp = 1.00; but the paraphyly of Anadarinae is not).

Interestingly, MrBayes resolves fewer relationships among families than the previous trees with the exception of significant support (pp = 0.96) for a clade containing Noetiidae and a *Barbatia-foliata* clade, which had been recovered but not well supported in the previous analyses. Again, phylogenetic relationships among families remained poorly supported (pp < 0.60) with the exception of a clade including Anadarinae and a *Barbatia-decussata* clade (pp = 1.00), which had been recovered in all previous analyses although with lower support.

### 3.4. Diversification times & rates

Diversification times of major lineages with BEAST is estimated as follows: Arcida, 475.5 Ma (95% highest posterior density interval [HPD] 465.8–485.1 Ma); Arcoidea, 366.4 Ma (95% HPD 278.9–453.2 Ma); Limopsoidea, 337.2 Ma (95% HPD 191.4–458.4 Ma); Philobryidae, 234.6 Ma (95% HPD 121.9–365.6 Ma); Noetiidae, 164.5 Ma (95% HPD 102.3–237.7 Ma); Glycymerididae, 164.2 Ma (95% HPD 154.3–173.8 Ma); Limopsidae, 39.2 Ma (95% HPD 1.9–139.3 Ma); Anadarinae, 137.4 Ma (95% HPD 127.7–147.3 Ma). Most aspects of the dated topology (Fig. 4) are comparable to the POY, ML and BI results, particularly the monophyly of Arcoidea and Noetiidae; and in the case of the Bayesian tree the monophyly of Limopsoidea and Philobryidae, which receive marginal support in this analysis ($pp^{BEAST} = 0.94$ and 0.91, respectively).

The $\gamma$-statistic for Arcida was 3.42, which indicates that the internal nodes are closer to the tips than expected under a pure-birth process, i.e. that the speciation rate increased over time (Pybus and Harvey, 2000). The deviation from a constant speciation rate ($\gamma = 0$) was highly significant ($p = 0.0006$). Of the six tested diversification models, the optimal model was the 3-rate Yule model ($\ln L = -156.6$; AIC = 323.3) (Table 4). The 2-rate Yule model also fitted well (dAIC = 0.4) but all other models had significantly higher AIC scores (dAIC $\geqslant$ 17.0). The dAICrc statistic was 18.5, which testifies to the significant deviation from a rate-constant diversification. The best-fit 3-rate Yule model indicated an initial diversification rate of 0.006, which increased first to 0.01 at 137.4 Ma and to 0.042 at 7.0 Ma.

## 4. Discussion

This study represents the first comprehensive multi-locus phylogenetic analysis of the bivalve order Arcida, one of the most

**Table 4**
Model fit to the Arcida log-lineage through time plot.

| Model | Param. | ln*L* | AIC | dAIC | Rate1 | Rate2 | Rate3 | ST1 | ST2 |
|---|---|---|---|---|---|---|---|---|---|
| PureBirth | 1 | −174.6 | 351.3 | 28.0 | 0.011 | | | | |
| BirthDeath | 2 | −168.1 | 340.3 | 17.0 | 0.005 | | | | |
| DDX | 2 | −171.0 | 345.9 | 22.6 | 0.003 | | | | |
| DDL | 2 | −182.0 | 368.0 | 44.7 | 0.014 | | | | |
| Yule2rate | 3 | −158.8 | 323.7 | 0.4 | 0.011 | 0.014 | | 1.5 | |
| **Yule3rate** | **5** | **−156.6** | **323.3** | **0.0** | **0.006** | **0.010** | **0.042** | **137.4** | **7.0** |

Param. = Number of parameters; ln*L* = log Likelihood; AIC = Akaike Information Criterion; dAIC = delta AIC compared to the best-fit model; ST = Shift time of rate change [Ma]; DDX = density dependent exponential; DDL = density dependent logistic.
Bold values indicate the optimal model 'Yule3rate'.

recalcitrant clades in bivalve taxonomy (Giribet and Distel, 2003; Giribet, 2008; Bieler et al., 2014). Previous molecular phylogenies were limited either by the use of a single molecular marker (Steiner and Hammer, 2000; Matsumoto, 2003; Xue et al., 2012; some of Jackson et al., 2015 analyses) or by including only a few Arcida taxa in studies with much broader focus (Giribet and Wheeler, 2002; Sharma et al., 2012; Bieler et al., 2014). In contrast, a recent study of the superfamily Arcoidea by Feng et al. (2015) was nominally based on five molecular markers, but ~25% of their dataset consisted of single-locus sequence data from previous studies, which might have contributed to some of their results. Likewise, the study of Jackson et al. (2015) mostly focused on Limopsoidea, missing key Arcida taxa such as Noetiidae. The challenging nature of Arcida taxonomy and the controversial results of some of the previous studies prompted us to use a broad range of taxa with at least two molecular markers per specimen and a wide range of algorithmic approaches. This broad but stringent approach led to a number of results that differ significantly from previous studies. For example, although not entirely conclusive, we provide the first molecular evidence that support the separation of Arcoidea from Limopsoidea, as in some of the analyses of Jackson et al. (2015), although the precise position of Glycymerididae remains unresolved, and the monophyly of Limopsoidea is algorithm-dependent, unlike in Jackson et al. (2015), which focused sampling within this superfamily. In addition, we present a time-calibrated evolutionary tree of Arcida relationships, indicating a significant increase in the diversification of lineages of Arcida at the beginning of the Cretaceous, around 140 Ma. The monophyly of Arcida, which has been reported by several previous phylogenetic studies was further corroborated and strongly supported in all our analyses.

### 4.1. Arcida superfamilies

Most classification systems of bivalves divide Arcida into two superfamilies, Limopsoidea and Arcoidea, with Glycymerididae sometimes nested within Limopsoidea (e.g., Beesley et al., 1998; Okutani, 2000), sometimes nested within Arcoidea (e.g., Bieler et al., 2010; Coan and Valentich-Scott, 2012), yet sometimes constituting its own superfamily Glycymeridoidea (Coan et al., 2000). With the exception of the ML analysis, which places the philobryid *Adacnarca nitens* within Noetiidae (Fig. 2), most parameter sets under direct optimization (Fig. 1) and the Bayesian analyses (Figs. 3 and 4) place Glycymerididae within a monophyletic Arcoidea, and find paraphyly (Fig. 1) or monophyly (Figs. 3 and 4) of Limopsoidea. Nonetheless, the deep separation of Arcoidea and mono- or paraphyletic Limopsoidea was a fundamental characteristic of virtually all our phylogenetic analyses. Consequently, we did not find any support for Waller's hypothesis that Limopsoidea derived from Arcoidea, based on their ligament structure (Waller, 1978), a result found in some recent analyses (Jackson et al., 2015) with poor taxon sampling within Arcoidea.

The family Glycymerididae was placed firmly inside Arcoidea in all but one analyses: the ML analyses indicated Glycymerididae might be sister group to all other Arcoidea. The inclusion of Glycymerididae into Limopsoidea (*sensu* e.g., Beesley et al., 1998) was not supported by any analysis, which testifies to the synapomorphic value of their duplivincular ligaments of all arcoideans, including Glycymerididae, supporting their convergence with Limopsoidea (Oliver and Holmes, 2006)—although a topology like the one presented in the ML analysis (Fig. 2) would render some of the "convergences" in body shape as plesiomorphies for Arcida.

The separation of Arcoidea and Limopsidae had so far not received support in previous phylogenetic studies, mostly due to a lack of limopsoidean samples, especially Philobryidae (e.g., Bieler et al., 2014). For example, the only Limopsoidea (*Limopsis cumingii*; as *Empleconia cumingii*) in Matsumoto (2003) was recovered as nested within Arcoidea, as was the only Limopsoidea in Bieler et al. (2014; *Limopsis* sp.), the two Limopsoidea from Feng et al. (2015) and the four Limopsoidea from Xue et al. (2012). This was also the case of the limopsoid-dense analyses of Jackson et al. (2015).

### 4.2. Arcida families

With the exception of the larger family Arcidae, all sampled Arcida families were recovered as monophyletic in most phylogenetic analyses (e.g. Figs 1–4). Limopsidae, however, were only represented by two closely related species, and Cucullaeidae was represented by two conspecific samples, so their monophyly is not really tested.

Glycymerididdae was monophyletic in all analyses with high nodal support. It is recovered as sister group to Arcidae in both the RAxML and the MrBayes analyses but nested with *Acar* in the POY analysis (Fig. 1) and with Cucullaeidae (+ *Arca boucardi* and *Bathyarca glomerula*) in the BEAST analysis (Fig. 4). Previous studies included only 2–4 Glycymerididdae, all *Glycymeris* sp., and obtained mixed results: Matsumoto (2003) and Feng et al. (2015) found Glycymerididdae to be non-monophyletic but Steiner and Hammer (2000), Xue et al. (2012) and Jackson et al. (2015) recovered them as monophyletic. The status of Glycymerididdae as a separate family is firmly supported by our analyses due to its consistent monophyly with generally high nodal support. However, while its placement outside of the Limopsidae is strongly supported, its exact position remains unresolved, and Jackson et al. (2015) found Glycymerididdae to be the sister group of Cucullaeidae + Limopsoidea in their 28S rRNA data analysis.

Noetiidae was monophyletic with mostly high nodal support in all analyses but ML, which placed the philobryid *Adacnarca nitens* within Noetiidae (Fig. 2). Noetiidae monophyly is consistent with the results of Marko (2002; based on 6 specimen/4 species) and Feng et al. (2015; based on 5 specimen/3 species). It was recovered as sister group to a clade of Arcidae species (including *Barbatia* and *Trisidos*) in all parameter sets under direct optimization, the maximum likelihood and the MrBayes analysis (Figs. 1–3). However, the Bayesian analysis with BEAST recovered Noetiidae as sister to *Acar* with a posterior probability of 1.00. This result is particularly interesting since *Acar* has a unique duplivincular ligament pattern that resembles the characteristic synapomorphic noetiid ligament (Malchus, 2004; Thomas et al., 2000). Its consistent monophyly testifies to the synapomorphic value of the noetiid ligament but it might be better described as a subfamily, Noetiinae, as proposed originally by Stewart (1930), but not within Glycymerididdae.

Philobryidae was monophyletic in both Bayesian analyses (Figs. 3 and 4) but not monophyletic in any parameter set under direct optimization (Fig. 1) and neither in the ML analysis, where phylobryids were polyphyletic due to the placement of *Philobryia sublaevis* as sister group to Limopsidae but with *Adacnarca nitens* nested inside Noetiidae (Fig. 2). A recent study including 34 philobryid specimens from 18 species found further support for the monophyly of the family (Jackson et al., 2015); however, that study did not include any noetiid, which is the family that conflicted with limopsoid monophyly in some of our analyses. Its status a distinct family is therefore tentatively supported here but it merits further investigation.

Limopsidae was represented here by two *Limopsis* species that always formed a clade. Most analyses placed *Limopsis* as sister group to Philobryidae (Figs. 2–4), but parsimony analyses frequently recovered *Limopsis* as sister group to Arcoidea (Fig. 1). This is consistent with previous analyses that recovered Limopsidae as monophyletic (e.g., Xue et al., 2012, based on four *Limopsis* species;

and Jackson et al., 2015, based on five species) and as sister group to Philobryidae (Feng et al., 2015).

Cucullaeidae was represented in the present study by two specimens of the only extant species, *Cucullaea labiata*. The two specimens were always recovered together and always as sister group to a clade containing the Arcidae *Bathyarca glomerula* and *Arca boucardi* with high nodal support. Interestingly, Cucullaeidae share a conspicuous morphological character with these species: the massive myophoric flanges that distinguish cucullaeids are also found in *Bathyarca glomerula* and *Arca boucardi* (and *A. tetragona*; see below; Oliver and Holmes, 2006). This is consistent with the study of Feng et al. (2015), which also recovered *A. boucardi* as sister group to Cucullaeidae (*B. glomerula* was not included it that or any other study). The status of Cucullaeidae as a distinct family may be supported by its rich fossil record but our results indicate that the family should be revised and possibly expanded.

Arcidae is the only family that was never recovered as monophyletic in any of our analyses, consistent with previous studies (e.g., Steiner and Hammer, 2000; Marko, 2002; Matsumoto, 2003; Xue et al., 2012; Feng et al., 2015).

The arcid subfamily Anadarinae, in contrast, was recovered as monophyletic in virtually all analyses here, which is again consistent with previous findings (Marko, 2002; Matsumoto, 2003; Feng et al., 2015; but not Xue et al., 2012). It was always recovered as sister group to a clade with *Barbatia* species (incl. *Barbatia candida* and *B. lacerata*) with high nodal support, indicating that the subfamily may be valid if expanded.

The other Arcidae subfamily, Arcinae, was non-monophyletic with at least three (Fig. 4), but most commonly six different lineages (Figs. 1–3). Two small clades were consistently recovered separate from other Arcidae clades in all phylogenetic analyses. One, composed of *Bathyarca glomerula* and *Arca boucardi*, was always recovered as sister to Cucullaeidae (as discussed above). The second clade, composed of the two *Acar* species (*A. bailyi* and *A. domingensis*), was always well supported but its placement was highly unstable. However, the Bayesian analyses with BEAST recovered *Acar* as sister group to Noetiidae with high $pp^{BEAST} = 1.0$, which is interesting since *Acar* has a unique duplivincular ligament pattern that resembles the characteristic synapomorphic noetiid ligament (Thomas et al., 2000). The remaining Arcinae were recovered as one large, albeit unsupported clade with BEAST ($pp^{BEAST} = 0.28$), but split into 4 lineages in other analyses.

All *Arca* species (but *A. boucardi*, see above) were recovered in one clade, which was further subdivided in two subclades with high nodal support, one of which has a long branch. These two subclades match two morphologically distinguishable groups, *A. avellana*/*A. imbricata* and *A. noae*/*A. zebra*, described by Oliver and Holmes (2006) and Vermeij (2013), the latter corresponding to the subgenus *Arca*. Since the two groups are phylogenetically and morphologically clearly distinct they could constitute separate genera (with *A. noae*/*A. zebra* remaining *Arca* Linnaeus, 1759 since *A. noae* is the type species for the genus). Oliver and Holmes (2006) and Vermeij (2013) distinguished a third group of *Arca* species, *A. tetragona* and *A. boucardi*, and Vermeij (2013) speculated that it is well removed from the two previous groups, a result supported by our results as discussed above. This clade could constitute the genus *Tetrarca*, although we did not include the type species of that subgenus here, *Arca tetragona* Poli, 1795.

Beside *Arca*, Arcinae species (mostly *Barbatia*) were split into three distinct clades. Two of these clades have already been discussed above, one as sister group to Anadarinae and the other as sister group to Noetiidae. The third Arcinae clade included the type species of *Barbatia*, *B. barbata* (as well as *B. cancellaria* and *B. amygdalumtostum*). This clade was well supported in all analyses but its placement was unstable. Interestingly, the other *Barbatia* clade includes *Trisidos*, a genus of burrowing torted shells, which has been postulated to evolve from *Barbatia*-like species (Tevesz and Carter, 1979).

Although several relationships among families and groups of genera remain unresolved, we found support for most Arcida families represented by multiple species. However, Arcidae, and particularly the subfamily Arcinae, are a major source of inconsistencies in the current Arcida classification and in dire need of substantial revision. In addition, many Arcida genera remain to be sampled and incorporated into an explicit analysis to test familial and generic relationships.

### 4.3. Arcida diversification through time

Species of the order Arcida have an old fossil record dating back to the lower Ordovician, ~480 Ma, with *Glyptarca serrata* being considered the first Arcida (Cope, 1997). According to the fossil record, most of the Arcida diversification occurred during the Mesozoic with modern families appearing for the first time in the Triassic (e.g., Cucullaeidae, Limopsidae and Philobryidae; Münster, 1841; Stiller and Jinhua, 2004) and Jurassic (e.g., Arcinae and Glycymerididae; Imlay, 1962; Oliver and Holmes, 2006). According to our time-calibrated phylogeny, most deep lineages likely originated earlier, around the Carboniferous (360–300 Ma; Fig. 4), which might be related to a significant sea level decrease (as a consequence of glaciation) and/or other palaeo-ecological events during the formation of Pangaea. By the end of the Triassic all extant families diverged, but major diversification did not occur until the Cretaceous (Fig. 5). This is consistent with the appearance of Limopsidae, Noetiidae and Anadarinae and the diversification of Cucullaeidae, Glycymerididae and Arcinae in the Cretaceous fossil record (Coan et al., 2000; Thomas et al., 2000; Oliver and Holmes, 2006).

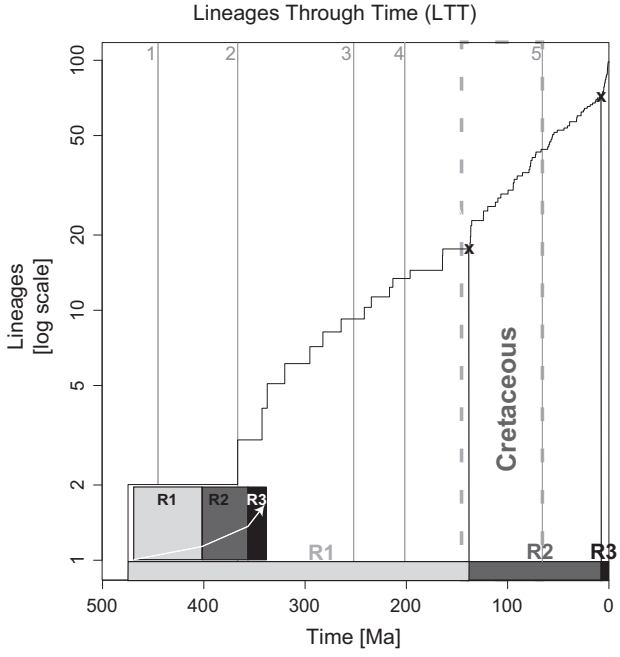


**Fig. 5.** Diversification rates of the Arcida as log-lineage through time plot (LTT, thin black line). Two black **X** indicate the shift times of diversification rate changes (137.4 and 7.0 Ma) for the Yule-3rate model. The three intervals with different diversification rates (R1, R2 and R3) are indicated at the bottom of the diagram in different shades of gray and in the schematic insert. The Cretaceous (145–66 Ma) is outlined by a dashed gray box. Thin gray lines indicate major historic marine extinction events as listed below. 1 Ordovician–Silurian (445 Ma); 2 Late Devonian (367 Ma); 3 = Perm-Triassic (252 Ma); 4 = Triassic–Jurassic (201 Ma); 5 = Cretaceous–Paleogene (66 Ma, identical with the end of the Cretaceous).

Discrepancies between the fossil record and a dated phylogeny are not uncommon (e.g., see Ho and Phillips, 2009). For example, one of the most common problems are gaps in the fossil record and resulting problems for the calibration of molecular methods to estimate divergence times. Since diversification commonly occurs much later than a lineage's first appearance, it is not surprising that the fossil record frequently indicates much younger ages. For example, Philobryidae were long considered to have originated in the Eocene until a Triassic philobryid was found (Stiller and Jinhua, 2004), which is consistent with the beginning of philobryid diversification according to our chronogram (Fig. 4). Another example is the appearance of glycymeridids and noetiids in the Cretaceous fossil record (Thomas et al., 2000; Oliver and Holmes, 2006), i.e., during a time of accelerated divergence according to our chronogram (see below, Fig. 5), but much later than the lineages originated according to our dated phylogeny (Fig. 4). Another common source for discrepancies is underrepresentation of extant taxa. For example, Cucullaeidae has a long and rich fossil record but only one extant species, and Limopsoidea was represented here by species from only 1 out of 11 extant genera. Consequently, both families are biased to appear much later in our dated phylogeny than they are in the fossil record. A third source of discrepancies lies in the difficulty to calibrate molecular clocks. For example, differences in mutation rates between lineages can make tree-wide average mutation rates poor estimators for lineage-specific speciation events. These problems can be reduced by using local or relaxed-clock models if multiple calibration points are available (Ho and Phillips, 2009).

Our time-calibrated phylogeny enabled testing several morphology-based hypotheses about the evolution of Arcida. For example, it does not entirely refute a hypothesis that Glycymerididae evolved from a cucullaeid ancestor (Nicol, 1950), since they are part of the same clade, and despite having an older fossil record, the extant diversity of Cucullaeidae is reduced to a single species. Although the two lineages separated in the Triassic, they did not diversify until the Late Jurassic. In contrast, we did not find any support for the hypothesis that Limopsoidea derived from Arcoidea, as had been hypothesized based on their ligament structure (Waller, 1978).

Bivalve subclasses/lineages show significantly different diversification patterns over the last 500 Ma, as recently demonstrated by Bieler et al. (2014). Imparidentia, for example, diversified fairly constantly and Palaeoheterodonta and Archiheterodonta started to diversify at the beginning of the Triassic. The diversification of Pteriomorphia, including Arcida, was described as either antisigmoidal or with a density-dependent deceleration. The most unusual case, however, is presented by Sharma et al. (2013) who showed that the chronogram of Protobranchia captured the signal of the Permian mass extinction event as a dramatic breakdown of diversification over a 180 Ma period with a subsequent sharp increase in diversification at the beginning of the Triassic. The diversification rate of Arcida does not follow these previously described patterns. No significant effects of previous extinction events on the diversification rate were detected (Fig. 5). Instead, diversification remained virtually constant for 350 Ma after their first appearance (~480 Ma), something that seems unusual for a group including a large number of shallow water species. However, a statistically significant increase in diversification was observed 137 Ma, at the beginning of the Cretaceous (Fig. 5).

The Cretaceous (~145–65 Ma) is considered to be a time of major faunal rearrangements. In the marine realm, this period has been termed Mesozoic Marine Revolution by Vermeij (1977) and was characterized by an ecological arms race between new durophagous predators (e.g., teleost fishes and crustaceans) and corresponding prey adaptations (e.g., thicker exoskeletons in bivalves and gastropods; Vermeij, 1987). Bivalves adapted to this transition, e.g., by growing thicker shells and adopting infaunal habitats (Vermeij, 1977), which is a common feature of arcidans (especially Anadarinae, Noetiidae and *Bathyarca* species). Interestingly, Anadarinae is a major driver of the observed increase in diversification during the Cretaceous (Fig. 4) and the fossil record indicates first diversification of noetiids, limopsids, and glycymeridids during the Cretaceous (Coan et al., 2000; Thomas et al., 2000; Oliver and Holmes, 2006).

The Cretaceous is also considered a time of warm climate and consistently high sea level, which lead to marine transgressions and the creation of numerous extensive shallow inland seas (e.g., the Western Interior Seaway and the Eromanga Sea), providing new habitats that might have further facilitated the diversification of shallow water marine taxa like Arcida. Another major paleoecological event during the Cretaceous was the break-up of Gondwana (150–140 Ma), which lead to the formation of the South Atlantic and Indian Oceans. Speciation was likely facilitated by the separation of species and populations and the copious areas of new habitat.

## 5. Concluding remarks

Here we present a comprehensive analysis of the pteriomorphian order Arcida using dense sampling of extant species and a multi-locus approach. Our results, based on a series of analyses using different phylogenetic methods and assumptions, support the monophyly of Arcida, as well as that of Arcoidea, but are unclear on whether Limopsoidea is monophyletic or paraphyletic, probably due to limited taxon sampling, as evidenced by a recently published analysis (Jackson et al., 2015). Relationships among families and genera suggest that some small families of Arcoidea may indeed nest within the larger family Arcidae, but Glycymerididae is supported as a separate family in most analyses, contrary to earlier molecular phylogenies based on much sparser taxon sampling. Although the systematics at the genus level is still largely unresolved, certain clades appear consistently across analyses, and our dated phylogeny shows that most of the Arcida diversification occurred during the Mesozoic with the modern families appearing for the first time in the Triassic and Jurassic, with most deeper lineages likely originated earlier, around the Carboniferous, during a time of significant sea level decrease. However, further resolution of the shape and timing of the Arcida tree is desirable and will require concerted sampling effort and perhaps a novel approach using larger amount of genetic data.

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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.2015.09.016.

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