

# Ark clams and relatives (Bivalvia: Arcida) show convergent morphological evolution associated with lifestyle transitions in the marine benthos

JORGE A. AUDINO<sup>1\*</sup>, JEANNE M. SERB<sup>2</sup> and JOSÉ EDUARDO A. R. MARIAN<sup>1</sup>

<sup>1</sup>Department of Zoology, University of São Paulo, Rua do Matão, Travessa 14, n. 101, 05508-090 São Paulo, São Paulo, Brazil

<sup>2</sup>Department of Ecology, Evolution, and Organismal Biology, Iowa State University, 2200 Osborn Drive, Ames, IA 50011, USA

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One of the most intriguing puzzles in macroevolutionary studies is to understand how distantly related taxa can evolve towards similar phenotypes in response to similar ecological conditions. Ark clams and their relatives (Arcida) display two main ecologies represented by epifaunal and infaunal lifestyles. Their mantle margin includes features, such as photosensory and muscular organs, that may coincide with each habit, making these bivalves a suitable model to explore evolutionary convergence in the marine benthos. To test for the evolutionary association between lifestyles and morphology, we gathered data on the mantle margin for 64 species across all six extant arcidan families. A molecular phylogeny of Arcida was inferred based on four gene sequences from 54 species and used to study trait evolution. Our results support the hypothesis that photoreceptor organs had a single origin and that infaunal lineages lost these structures in independent events, suggesting a correlated pattern of evolution. In addition, the enlargement of the posterior inner fold, which acts as a functional siphon, favoured the occurrence of convergent transitions to infaunal habits during the Mesozoic. We provide evidence of ecomorphological associations and putative adaptations in a bivalve clade that sheds light on the underlying factors driving evolution of the marine benthos.

ADDITIONAL KEYWORDS: adaptation – correlation – lifestyle – mantle – phenotype – phylogeny.

## INTRODUCTION

Macroevolutionary questions compose the core of evolutionary biology and focus on the association of phenotypical diversity with adaptive landscapes (Simpson, 1953; Schluter, 2000). Understanding whether and how similar ecological factors can drive independent taxa towards the same phenotype may help us to understand the factors that drive evolution better (Losos, 2011; Serb *et al.*, 2017). In this context, the repeated evolution of traits across independent lineages, i.e. evolutionary convergence (Agrawal, 2017), in association with similar environmental factors suggests putative adaptations and predictable responses to similar selective regimes (Harvey & Pagel, 1991; Losos, 2011; Mahler *et al.*, 2017). Numerous

vertebrate taxa are used as models for studies on evolutionary processes and convergence (Losos & Mahler, 2010), whereas invertebrates are proportionally less studied, and supposed ecomorphological patterns in invertebrate taxa remain largely obscure.

Ark clams and their relatives (Bivalvia, Arcida) are marine pteriomorphian bivalves and are a suitable model to gain insights into convergent evolution owing to their morphological and ecological diversity. Two main lifestyles are observed in the group (Oliver & Holmes, 2006). Epifaunal animals are attached to hard substrate (e.g. rocks and coral fragments) by a strong byssus (i.e. filaments secreted to attach the animal to solid surfaces). Alternatively, infaunal and semi-infaunal animals bury into soft sediment, leaving the posterior region exposed above the surface. Previous anatomical studies have identified apparent associations between both modes of life with putative

\*Corresponding author. E-mail: [jorgeaudino@ib.usp.br](mailto:jorgeaudino@ib.usp.br)

adaptations of shell shape, muscle organization and photoreceptor organs (Stanley, 1972; Oliver & Holmes, 2006; Audino & Marian, 2018); however, these hypotheses were not tested directly using comparative methods.

The Arcida Gray, 1854 have a comprehensive fossil record dating back to the lower Ordovician (~450 Mya; Morton *et al.*, 1998; Cope, 2000). The Order currently encompasses the superfamilies Arcoidea and Limopsoidea, with an estimated diversity of > 300 extant species (Oliver & Holmes, 2006; Carter *et al.*, 2011). The Arcoidea traditionally includes the families Arcidae, Cucullaeidae, Noetiidae and Glycymerididae, whereas Limopsidae and Philobryidae are assigned to Limopsoidea (Oliver & Holmes, 2006; Carter *et al.*, 2011). Nevertheless, taxonomic classifications are controversial, with numerous morphological features that are likely to represent homoplasies in response to similar ecologies (Oliver & Holmes, 2006).

One of these features is the mantle margin, a narrow region of soft tissues organized as lobe-like extensions lining the shell margin (Fig. 1A, B). This region is expected to evolve in response to shifts in lifestyle given that the mantle margin plays primary roles of interaction with the surrounding environment, including sensory, protective and muscular functions (Yonge, 1983; Audino & Marian, 2016). The siphons are a classical example of a key morphological innovation in infaunal bivalves as a result of enlargement and fusion of the mantle folds. Siphons create channels for water circulation through the mantle cavity, where the gills are located, in animals that live constantly buried within the sediment (Yonge, 1983; Stanley, 1968). Other mantle structures, such as eyes and tentacles, have also been linked to ecological transitions. For instance, in scallops, depth was suggested to be an important driving force in the evolution of mantle eye components associated with light sensitivity (Malkowsky & Götze, 2014). Light-guided behaviours, e.g. related to predator detection and posture control (Nilsson, 1994), could also be associated with transitions to the epifaunal habit, i.e. when the animal lives on top of the substrate. Consequently, the mantle margin in Arcida represents a promising source of information to identify convergent traits and test correlated evolution. Phenotypic diversity in the number and length of mantle folds and in the presence and complexity of photoreceptor organs are among key traits of this region (Waller, 1980; Morton, 1982; Morton & Peharda, 2008; Audino & Marian, 2018). Nevertheless, the structure of the mantle margin in the ancestor of ark clams and its subsequent morphological diversification have never been inferred, rendering several interesting questions. For example, did photoreceptor organs of the mantle margin evolve as adaptive traits in epifaunal groups? Are changes in mantle morphology related to shifts

to the infaunal lifestyle? For instance, the enlarged posterior mantle fold of infaunal lineages may act as a functional siphon (e.g. Morton, 1982); did this attribute evolve convergently as an adaptation (or exaptation; Gould & Vrba, 1982) to the infaunal lifestyle?

A phylogenetic framework is crucial to provide initial steps towards these answers and elucidate the number of ecological transitions in the clade. Although the Arcida has been recovered monophyletic in many analyses (Steiner & Hammer, 2000; Giribet & Wheeler, 2002; Matsumoto, 2003; Bieler *et al.*, 2014), relationships among families and superfamilies remain under debate (Oliver & Holmes, 2006; Bieler *et al.*, 2014; Feng *et al.*, 2015; Combosch & Giribet, 2016). The placement of some groups, such as the Glycymerididae and the Limopsoidea, is particularly challenging (Combosch & Giribet, 2016). Consequently, a more robust phylogeny is needed to enable further evolutionary studies on the radiation of the group.

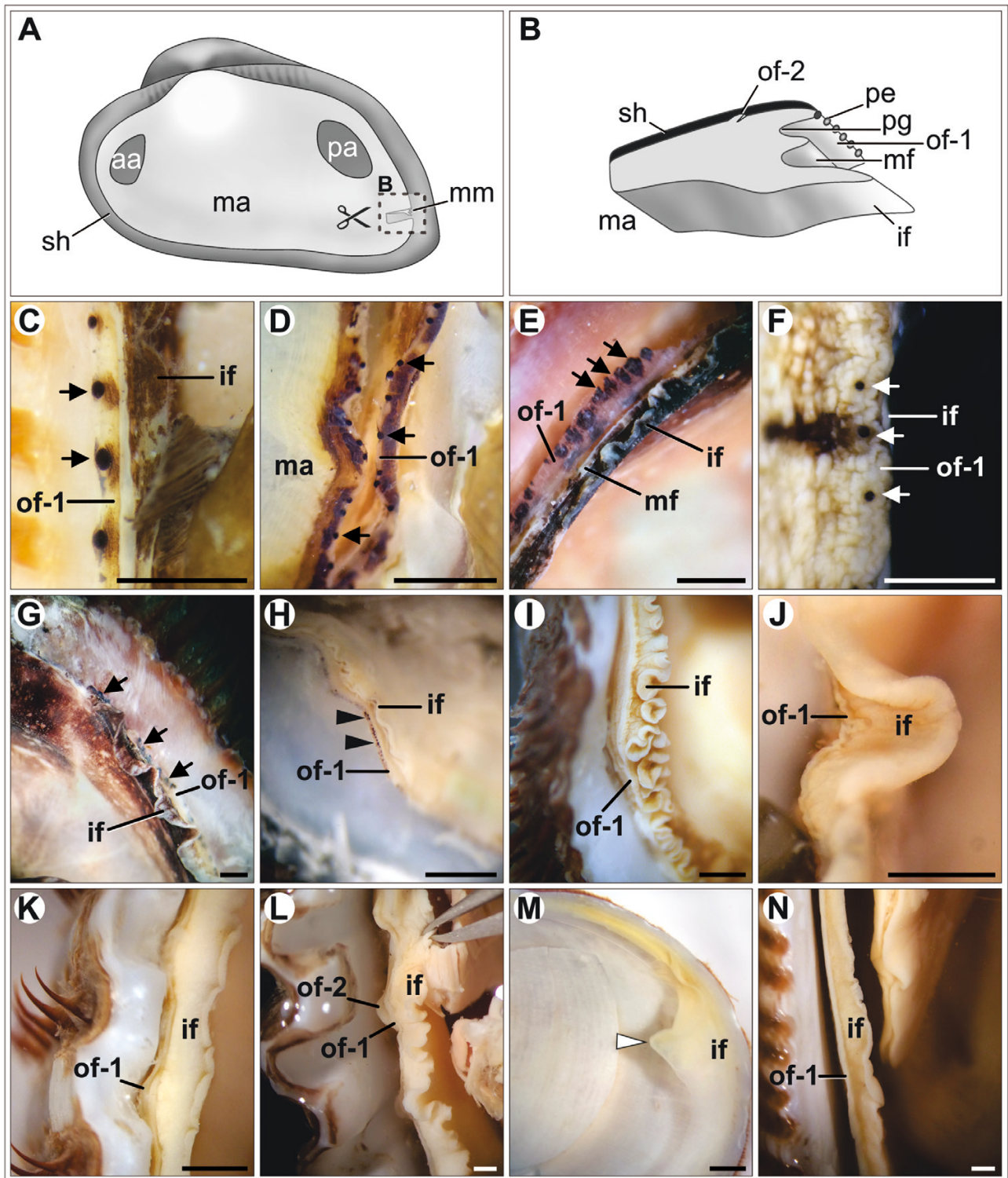
The present study provides the most comprehensive phylogenetic analysis of Arcida to investigate morphological evolution in the clade and cast light on presumed adaptive features. Particularly, mantle margin morphology and lifestyles were studied in 64 species under a phylogenetic framework to test for correlation between lifestyle and morphology. The inferred molecular phylogenies, combined with the extensive morphological survey, provided a robust basis for discussion of evolutionary patterns in the clade.

## MATERIAL AND METHODS

### TAXA SAMPLING

Nucleotide sequences for four genes (18S rRNA, 28S rRNA, *COI* mtDNA and histone *H3*) were obtained from GenBank for 54 species of Arcida, covering both superfamilies, all six families and 20 genera (Table 1). The outgroup comprised seven species from other pteriomorphian orders and five species from the remaining major bivalve clades (Protobranchia and Heteroconchia) (Table 1). Missing data corresponded to 12% of the dataset for nucleotide sequences (Table 1). When possible, sampling effort was proportional to the diversity of each family, i.e. relatively more samples were analysed in groups that were comparatively more diverse (Table 1).

Morphological investigation of the mantle margin included data from 64 species obtained from preserved specimens of the following collections: Museum of Comparative Zoology (MCZ), Museum of Zoology 'Prof. Adão José Cardoso' of the University of Campinas (ZUECBIV), Museum of Zoology of the University of São Paulo (MZSP), Smithsonian National Museum



**Figure 1.** General organization of the mantle (A) and mantle margin (B) in Arcida represented by simplified schemes. Mantle margin morphology in Arcidae (C–E, G–N) and Cucullaeidae (F). Posterior mantle region, ventral view. Scale bars: 1 mm. The first outer fold can be pigmented (C–G), bearing multiple compound eyes (arrows) and pigmented eyespots (arrowheads). The middle fold is reduced (E) or absent (J). The inner fold is much longer than the other folds, forming a large curtain (I–N) or a posterior flap (M). C, *Acar plicata* (USNM 886349). D, *Arca noae* (USNM 1086014). E, *Barbatia fusca* (SBMNH 349329). F, *Cucullaea labiata* (USNM 746883). G, *Barbatia barbata* (MCZ 378867). H, *Barbatia virescens* (MCZ



of Natural History (USNM) and Santa Barbara Museum of Natural History (SBMNH). Respective catalogue numbers are listed in Table 1. From the 64 species studied for morphology, 38 species have available sequences used for phylogenetic inference, and 26 species either belong to genera that include the remaining sequenced species or correspond to taxa included to complement the observations (Table 1). One to five specimens per species were dissected depending on the availability of preserved material.

#### PHYLOGENETIC ANALYSIS AND DIVERGENCE TIMES

Sequence alignments were generated with MAFFT v.7.311 under the L-INS-i option (accurate strategy) (Katoh & Standley, 2013). ModelFinder (Kalyaanamoorthy *et al.*, 2017) was used to obtain the best-fitting model of sequence evolution under the corrected Akaike information criterion (AICc), returning GTR+I+G for the concatenated dataset, which was applied in subsequent analyses. Maximum likelihood (ML) analysis was conducted in IQ-TREE (Nguyen *et al.*, 2014), and node support was estimated by standard non-parametric bootstrap (100 replicates) (Felsenstein, 1985). Divergence times of clades were estimated by Bayesian inference (BI) in RevBayes v.1.0.9 under the fossilized birth–death model (Heath *et al.*, 2014; Höhna *et al.*, 2016). This model imposes a time structure on the tree by marginalizing over all possible attachment points for the fossils on the extant tree. In addition, instead of treating the calibration density as an additional prior distribution on the tree, the model treats it as the likelihood of the fossil data given the tree parameter (Heath *et al.*, 2014).

Following Bieler *et al.* (2014), the root age for Bivalvia was constrained, applying a uniform distribution prior between 520.5 and 530 Mya based on the fossil *Fordilla troyensis* (Pojeta *et al.*, 1973). Four additional fossils were used to calibrate internal node ages, three of them previously adopted elsewhere (Combosch & Giribet, 2016). The age of Arcida was constrained around  $478.6 \pm 5$  Mya, based on *Glyptarca serrata* (Cope, 1997). Glycymerididae was constrained around  $167.7 \pm 5$  Mya, based on *Trigonarca tumida* (Imlay, 1962). The fossil of *Anadara ferruginea* was used to constrain the age of the subfamily Anadarinae around  $138.3 \pm 5$  Mya (Huber, 2010). Finally, the age of Philobryidae was constrained around  $45 \pm 11$  Mya based on the oldest fossil records for the family (Moore & Teichert, 1969).

All priors for fossil ages were drawn from uniform distributions. An uncorrelated exponential model on molecular branch rates was assumed for the relaxed molecular clock. Posterior probabilities were sampled using the Markov chain Monte Carlo method with four independent chains running for 500 000 iterations, each one containing 534 moves (changes of values in stochastic parameters). Convergence of the posteriors were observed in TRACER v.1.6 (Rambaut *et al.*, 2018). Fossil taxa were then pruned from trees because they were used solely to calibrate node ages, rather than to infer phylogenetic placements. Subsequently, phylogenetic trees were summarized as a maximum clade credibility tree, with a burn-in of 10% removed. A lineages-through-time plot was generated in IcyTree (<https://icytree.org/>).

#### CHARACTER EVOLUTION

Mantle margin evolution in Arcida was studied based on morphological data for 64 species from museum collections (Table 1). Specimens were dissected in ethanol and observed under the stereomicroscope for anatomical investigation. Characters were coded, and states were assigned to terminals based on observations of the corresponding species. In the absence of data from the literature, unobserved species had their states assigned as equivalent to closest relatives (i.e. congeneric species) obtained from collections (Supporting Information, Tables S1 and S2). Characters are related to the number and relative size of mantle folds, pigmentation, the presence and type of photoreceptor organs, and presence of the mantle nerve (Supporting Information, Table S1). Given that ethanol often shrinks/distorts tissues during preservation, mantle fold length is a character defined by the relative length of a fold in comparison to another fold, rather than the absolute length. Some multistate characters were also coded as binary (see Supporting Information, Table S1), as required by the correlation test (Pagel, 1994).

Information on habits of life was compiled from the literature for all species included in the phylogenetic analysis (Supporting Information, Table S3). Modes of life include: epifaunal (above the substrate, frequently attached to the surface), semi-infaunal (partly buried in soft sediment) and infaunal (buried in soft sediment), with respective modes of byssal attachment, i.e. epibyssate, endobyssate and abyssate.

378874). I, *Barbatia candida* (MZSP 105572). J, *Anadara broughtonii* (USNM 802331). K, *Anadara ferruginea* (SBMNH 81002). L, *Tegillarca granosa* (MCZ 378820). M, *Batharca corpulenta* (SBMNH 349320). N, *Trisidos kiyonoi* (SBMNH 97422). Abbreviations: aa, anterior adductor; if, inner fold; ma, mantle; mf, middle fold; mm, mantle margin; of, outer fold; of-1, first outer fold; of-2, second outer fold; pa, posterior adductor; pe, pigmented eyespots; pg, periostracal groove; sh, shell.

**Table 1.** Taxa included in the phylogenetic and morphological analyses

| Taxa                            | Reference                       | 18S rRNA | 28S rRNA | COI<br>mtDNA | Histone H3 | Collections                          |
|---------------------------------|---------------------------------|----------|----------|--------------|------------|--------------------------------------|
| Arcidae                         |                                 |          |          |              |            |                                      |
| <i>Acar dominguensis</i>        | (Lamarck, 1819)                 |          |          | FJ480593     | KT757861   | MZSP118292                           |
| <i>Acar gradata</i>             | (Broderip &<br>Sowerby I, 1829) |          |          |              |            | USNM796185                           |
| <i>Acar plicata</i>             | (Dillwyn, 1817)                 | AJ389630 | AJ307533 | FJ480453     | AF416856   | MZSP115322                           |
| <i>Anadara antiquata</i>        | (Linnaeus, 1758)                | JN974491 | JN974542 | HQ258850     | JN974592   | MZSP99848                            |
| <i>Anadara baughmani</i>        | Hertlein, 1951                  |          |          |              |            | USNM803522                           |
| <i>Anadara broughtonii</i>      | (Schrenck, 1867)                | JN974489 | JN974541 | HQ258847     | JN974590   | USNM802331                           |
| <i>Anadara chemnitzii</i>       | (Philippi, 1851)                |          |          |              |            | MZSP43259,<br>ZUECBIV4870            |
| <i>Anadara cornea</i>           | (Reeve, 1844)                   | JN974499 | DQ343860 | HQ258856     | JN974600   |                                      |
| <i>Anadara crebricostata</i>    | (Reeve, 1844)                   | JN974495 | JN974547 | HQ258859     | JN974596   |                                      |
| <i>Anadara ferruginea</i>       | (Reeve, 1944)                   |          |          |              |            | SBMNH81002                           |
| <i>Anadara floridana</i>        | (Conrad, 1869)                  |          |          |              |            | USNM847847                           |
| <i>Anadara globosa</i>          | (Reeve, 1844)                   | JN974484 | JN974535 | HQ258861     | JN974584   |                                      |
| <i>Anadara grandis</i>          | (Broderip &<br>Sowerby I, 1829) |          |          |              |            | USNM803487                           |
| <i>Anadara gubernaculum</i>     | (Reeve, 1844)                   | JN974493 | JN974544 | HQ258857     | JN974594   |                                      |
| <i>Anadara inaequalis</i>       | (Bruguière, 1789)               | JN974497 | JN974548 | AB076937     | JN974598   | MZSP55060                            |
| <i>Anadara notabilis</i>        | (Röding, 1798)                  | KT757768 | KT757816 | AF416828     | KT757863   | MZSP84987,<br>MZSP84886<br>MCZ337676 |
| <i>Anadara obesa</i>            | (G. B. Sowerby I,<br>1833)      |          |          |              |            |                                      |
| <i>Anadara pilula</i>           | (Reeve, 1843)                   | JN974507 | JN974558 | HQ258862     | JN974608   |                                      |
| <i>Anadara subcrenata</i>       | (Lischle, 1869)                 | JN974501 | DQ343861 | HQ258851     | JN974602   |                                      |
| <i>Anadara transversa</i>       | (Say, 1822)                     |          |          |              |            | USNM801135,<br>MCZ359001             |
| <i>Anadara trapezia</i>         | (Deshayes, 1839)                | KT757770 | KT757817 | KX713443     | KT757865   | SBMNH10187                           |
| <i>Anadara vellicata</i>        | (Reeve, 1844)                   | JN974487 | JN974539 | HQ258848     | JN974588   |                                      |
| <i>Arca imbricata</i>           | Bruguière, 1789                 | AY654986 | KT757820 | AF253494     | AY654989   | MZSP95208,<br>MZSP109869             |
| <i>Arca navicularis</i>         | Bruguière, 1789                 | JN974517 | KT757821 | HQ258822     | JN974618   | USNM719071,<br>MCZ378833             |
| <i>Arca noae</i>                | Linnaeus, 1758                  | KC429325 | KT757822 | KC429090     | KC429160   | USNM1086014                          |
| <i>Arca patriarchalis</i>       | Röding, 1798                    | JN974527 | JN974576 |              | JN974627   | MZSP99765                            |
| <i>Arca ventricosa</i>          | (Lamarck, 1819)                 |          |          | AB076935     | AF416854   | MZSP55027                            |
| <i>Arca zebra</i>               | (Swainson, 1833)                | KT757776 | KT757824 |              | AF416864   | MZSP101688                           |
| <i>Barbatia</i>                 | (Röding, 1798)                  | JN974526 | JN974575 |              | JN974626   | SBMNH349329,<br>USNM847011           |
| <i>Barbatia amygdalumtostum</i> |                                 |          |          |              |            |                                      |
| <i>Barbatia barbata</i>         | (Linnaeus, 1758)                | KC429326 | KT757825 | KC429091     | KC429161   | MCZ378867                            |
| <i>Barbatia cancellaria</i>     | (Lamarck, 1819)                 | KT757779 | KT757827 |              |            | MZSP32336,<br>MZSP48857              |
| <i>Barbatia candida</i>         | (Helbling, 1779)                | KT757784 | KT757831 | AF253487     | AF416849   | MZSP105572,<br>ZUECBIV1407           |
| <i>Barbatia lacerata</i>        | (Bruguière, 1789)               | JN974509 | JN974560 | HQ258826     | JN974610   |                                      |
| <i>Barbatia lima</i>            | (Reeve, 1844)                   | JN974511 | JN974563 | HQ258837     | JN974612   | MZSP71135                            |
| <i>Barbatia virescens</i>       | (Reeve, 1844)                   | JN974524 | KT757835 | HQ258840     | JN974624   | MZSP71367,<br>MCZ378874              |
| <i>Batharca corpulenta</i>      | (E. A. Smith, 1885)             |          |          |              |            | SBMNH349320                          |
| <i>Batharca glomerula</i>       | (Dall, 1881)                    | KT757790 | KT757837 |              | KT757880   |                                      |

Table 1. Continued

| Taxa                              | Reference             | 18S rRNA | 28S rRNA | COI<br>mtDNA | Histone H3 | Collections                |
|-----------------------------------|-----------------------|----------|----------|--------------|------------|----------------------------|
| <i>Batharca</i>                   | (Scacchi, 1835)       |          |          |              |            | MCZ348402                  |
| <i>pectunculoides</i>             |                       |          |          |              |            |                            |
| <i>Bentharca asperula</i>         | (Dall, 1881)          |          |          |              |            | MCZ348399                  |
| <i>Lunarca ovalis</i>             | (Bruguère, 1789)      |          |          | GQ166571     | AF416844   | MZSP84823,<br>USNM803532   |
| <i>Tegillarca granosa</i>         | (Linnaeus, 1758)      | JN974505 | KT757857 | HQ258867     | JN974606   | MZSP55596,<br>MCZ378820    |
| <i>Tegillarca nodifera</i>        | (Martens, 1860)       | JN974503 | JN974554 | HQ258869     | JN974604   |                            |
| <i>Trisidos kiyonoi</i>           | (Makiyama, 1931)      | JN974522 | JN974571 | HQ258846     | JN974622   | SBMNH97422,<br>SBMNH97423  |
| <i>Trisidos tortuosa</i>          | (Linnaeus, 1758)      | KT757811 | KT757858 |              | KT757899   |                            |
| Cucullaeidae                      |                       |          |          |              |            |                            |
| <i>Cucullaea labiata</i>          | (Lightfoot, 1786)     | JN974513 | JN974565 | KJ774477     | JN974614   | USNM746883                 |
| Noetiidae                         |                       |          |          |              |            |                            |
| <i>Arcopsis adamsi</i>            | (Dall, 1886)          | KC429327 | KC429419 | KC429092     | KC429162   | MZSP19724,<br>ZUECBIV1153  |
| <i>Didimacar tenebrica</i>        | (Reeve, 1844)         | JN974515 | JN974566 | HQ258870     | JN974616   | SBMNH80722                 |
| <i>Eontia ponderosa</i>           | (Say, 1822)           | KT757793 | KT757840 | AF416834     | AF416860   | SBMNH235066,<br>USNM803530 |
| <i>Sheldonella bisulcata</i>      | (Lamarck, 1819)       |          |          |              |            | MZSP26911                  |
| <i>Striarca lactea</i>            | (Linnaeus, 1758)      | AF120531 | KT757855 | AF120646     |            | USNM857645,<br>MCZ379156   |
| <i>Striarca symmetrica</i>        | (Reeve, 1844)         |          |          |              |            | MZSP55574                  |
| Glycymerididae                    |                       |          |          |              |            |                            |
| <i>Glycymeris decussata</i>       | (Linnaeus, 1758)      |          |          |              |            | MZSP91966                  |
| <i>Glycymeris gigantea</i>        | (Reeve, 1843)         | KT757794 | KT757841 |              | KT757883   | MCZ 378989                 |
| <i>Glycymeris glycymeris</i>      | (Linnaeus, 1758)      | KC429328 | KC429421 | KC429093     | KC429163   | USNM794960                 |
| <i>Glycymeris holoserica</i>      | (Reeve, 1843)         | KT757796 | KT757843 |              | KT757885   | MCZ378984                  |
| <i>Glycymeris longior</i>         | (G. B. Sowerby, 1833) |          |          |              |            | MZSP91201,<br>ZUECBIV78    |
| <i>Glycymeris nummaria</i>        | (Linnaeus, 1758)      | KT757798 | KT757845 | KX785178     | KT757887   | MCZ378985                  |
| <i>Glycymeris septentrionalis</i> | (Middendorff, 1849)   | KT757799 | KT757846 | KF643645     | KT757888   |                            |
| <i>Glycymeris tenuicostata</i>    | (Reeve, 1843)         | KT757800 | KT757847 |              | KT757889   | MCZ378982                  |
| <i>Glycymeris undata</i>          | (Linnaeus, 1758)      |          |          |              |            | MZSP91983                  |
| <i>Tucetona pectinata</i>         | (Gmelin, 1791)        | KT757812 | KT757859 | KX713507     | KT757900   | MZSP91971,<br>ZUECBIV2198  |
| Limopsidae                        |                       |          |          |              |            |                            |
| <i>Limopsis aurita</i>            | (Brocchi, 1814)       |          |          |              |            | ZUECBIV2248,<br>MCZ348438  |
| <i>Limopsis cristata</i>          | Jeffreys, 1876        |          |          |              |            | MZSP104154,<br>MCZ348410   |
| <i>Limopsis cumingi</i>           | Adams, 1863           | KT757802 |          | AB076930     |            |                            |
| <i>Limopsis enderbyensis</i>      | Powell, 1958          | AJ422057 | AY321301 |              |            |                            |
| <i>Limopsis galathea</i>          | Knudsen, 1970         |          |          |              |            | MCZ348437                  |
| <i>Limopsis lilliei</i>           | E. A. Smith, 1915     |          |          |              |            | MZSP90647,<br>USNM904585   |
| <i>Limopsis marionensis</i>       | E. A. Smith, 1885     | AJ422058 | AY321303 |              |            | USNM760835,<br>USNM886526  |
| <i>Limopsis</i> sp.               | Sassi, 1827           | KC429329 | KC429422 |              | KC429164   |                            |
| <i>Limopsis sulcata</i>           | Verrill & Bush, 1898  |          |          |              |            | USNM832925                 |

**Table 1.** Continued

| Taxa                               | Reference                   | 18S rRNA | 28S rRNA | COI<br>mtDNA | Histone H3 | Collections              |
|------------------------------------|-----------------------------|----------|----------|--------------|------------|--------------------------|
| <i>Limopsis tenella</i>            | Jeffreys, 1876              |          |          |              |            | USNM807040               |
| Philobryidae                       |                             |          |          |              |            |                          |
| <i>Adacnarca nitens</i>            | Pelseneer, 1903             | KP340836 | KT757815 |              | KT757862   | MZSP90616,<br>USNM886551 |
| <i>Lissarca notorcardensis</i>     | Melville & Standen,<br>1907 |          | EF192520 | KF612434     |            | MZSP87826,<br>USNM899485 |
| <i>Neocardia</i> sp.               | G. B. Sowerby III,<br>1892  | KT757804 | KT757850 | KX713486     | KT757891   | USNM881121,<br>MCZ378927 |
| <i>Philobrya magellanica</i>       | (Stempell, 1899)            | KP340845 | KT757853 |              | KT757895   |                          |
| <i>Philobrya sublaevis</i>         | Pelseneer, 1903             | KP340835 | KP340812 |              |            | MZSP90645,<br>USNM882353 |
| Outgroup:                          |                             |          |          |              |            |                          |
| Pteriomorpha                       |                             |          |          |              |            |                          |
| <i>Lima lima</i>                   | (Linnaeus, 1758)            | KC429339 | KC429434 | KC429101     | KC429174   | USNM 754383              |
| <i>Malleus albus</i>               | Lamarck, 1819               | KC429334 | HQ329464 | KC429097     | KC429169   | MZSP55595                |
| <i>Mytilus edulis</i>              | Linnaeus, 1758              | KC429331 | KC429424 | KF644190     | KC429166   | MZSP120321               |
| <i>Ostrea edulis</i>               | Linnaeus, 1758              | L49052   | AF137047 | AF120651     | AY070151   | USNM836256               |
| <i>Pecten maximus</i>              | (Linnaeus, 1758)            | L49053   | HM630545 | KC429102     | EU379508   |                          |
| <i>Pinctada margaritifera</i>      | (Linnaeus, 1758)            | AB214451 | AB214466 | AB259166     | HQ329296   | USNM836493               |
| <i>Pinna carnea</i>                | Gmelin, 1791                | HQ329375 | KJ366067 | KJ366325     | KC429172   | MZSP29040                |
| Outgroup: Bivalvia                 |                             |          |          |              |            |                          |
| <i>Chione elevata</i>              | (Say, 1822)                 | KC429387 | KC429495 | KC429136     | KC429219   |                          |
| <i>Macoma balthica</i>             | (Linnaeus, 1758)            | KC429393 | KC429501 | KC429141     | KC429224   |                          |
| <i>Margaritifera margaritifera</i> | (Linnaeus, 1758)            | AF229612 | KC429443 | AF303316     | KC429185   |                          |
| <i>Neotrigonia lamarchii</i>       | (Gray, 1838)                | KC429345 | KC429443 | KC429105     | KC429182   |                          |
| <i>Nucula sulcata</i>              | Bronn, 1831                 | AF207642 | KC984815 | KC984746     | KC984777   |                          |

Nucleotide sequences were obtained in the GenBank database; accession numbers are listed. Morphological investigation was conducted with taxa included in the phylogenetic study (when possible) and additional species; catalogue numbers are indicated. Abbreviations: MCZ, Museum of Comparative Zoology; MZSP, Museum of Zoology of the University of São Paulo; SBMNH, Santa Barbara Museum of Natural History; USNM, Smithsonian National Museum of Natural History; ZUECBIV, Museum of Zoology 'Prof. Adão José Cardoso' of the University of Campinas.

Additional information was also recovered, such as the type of substrate and occurrence relative to depth, varying from shallow (< 200 m) to deep waters (> 200 m). Subsequently, lifestyles were coded (Supporting Information, Tables S1 and S2) and studied for character evolution as detailed below.

Ancestral state reconstructions (ASRs) were conducted under maximum likelihood in MESQUITE (Maddison & Maddison, 2018). Two possible models for trait evolution were applied, i.e. the Markov  $k$ -state one-parameter model (MK1), which assumes equal transition rates, and the asymmetrical Markov  $k$ -state two-parameter model (AsymmMK), in which transition rates can be different. In contrast to the MK1 model, the AsymmMK model allows different rates for 'forward' (0→1) and 'backward' (1→0) transitions. A likelihood ratio (LR) test was used to verify which model fitted the data better (Pagel, 1999; Maddison & Maddison, 2018). Given that the two models are nested, the LR test follows a  $\chi^2$  distribution, with d.f. = 1 (because the

AsymmMK model has only one additional parameter compared with the MK1 model). The reconstructions presented herein follow the statistical decision to reject the null hypothesis (MK1 model) whenever  $LR > 3.84$  (critical value for  $\alpha = 0.05$ , d.f. = 1). To evaluate the possible effects of branch supports and alternative topologies in the reconstruction, bootstrap trees were also investigated to inspect the consistency of the reconstructed evolutionary patterns (see Maddison & Maddison, 2018).

Pagel's correlation test was applied in MESQUITE (Pagel, 1994; Maddison & Maddison, 2018) to compare the evolution of modes of life and morphological traits, such as photoreceptor organs, mantle folds and pigmentation. Although the method has some shortcomings (Maddison & Fitzjohn, 2015), it provides a helpful approach to analyse the evolution of traits statistically by incorporating phylogenetic information. Additionally, tests were conducted considering models representing evolutionary dependence among traits,



i.e. when the shift of state in one character is likely to depend on the state of the second character. Searches were carried out (iterations,  $N = 10$ ) with the  $P$ -value being estimated from 10 000 repeated simulations. Hypotheses of character correlations were accepted whenever a model with eight parameters (correlated hypothesis) presented a better fit ( $P < 0.05$ ) than a model of evolution with four parameters (uncorrelated hypothesis) (Pagel, 1994; Maddison & Fitzjohn, 2015).

## RESULTS

### MANTLE MARGIN DIVERSITY IN ARCIDA

Mantle margin in arcids may comprise four marginal extensions, named mantle folds, identified according to the position relative to the periostracal groove (Fig. 1A, B). They are named, from the outside to the inside: second outer fold, first outer fold, middle fold and inner fold (Fig. 1B). The second outer fold is a short and delicate projection in a proximal position, present in most Arcida representatives. This structure is usually unpigmented and located close to the valve. Although this fold is apparent in ark clams and blood cockles, such as *Anadara* and *Tegillarca* (Fig. 1L), it seems to be extremely reduced or even absent in smaller species. This is the case for some Philobryidae species (e.g. *Adacnarca*, *Lissarca* and *Neocardia*), in which the second outer fold was not observed.

The first outer fold is usually well developed in most species, frequently being pigmented and bearing photoreceptor organs. Strong pigmentation is common in epifaunal species, such as *Arca* (Fig. 1D), although pigmentation is also present in some semi-infaunal (e.g. Glycymerididae; Fig. 2E, F) and infaunal species (e.g. some *Anadara* spp.). Photoreceptor organs vary from small eyespots to large compound eyes (Figs 1, 2). Pigmented eyespots are present in epifaunal Noetiidae, such as *Arcopsis* (Fig. 2B), *Didimacar* and *Stryarca*, most Arcidae taxa (Fig. 1H), except *Trisidos* (infaunal) and *Bathyarca* (infaunal; deep sea), and some Philobryidae, including *Lissarca notorcardensis* and *Neocardia* sp. (Fig. 2K). These eyespots are frequently restricted to the anterodorsal region. Compound eyes are larger, multifaceted structures, occurring on the posterior region of *Acar*, *Arca*, *Cucullaea* (Fig. 1C, D, F), Glycymerididae (Fig. 2E, F) and some *Barbatia* species (Fig. 1E, G).

The middle mantle fold, when present, represents a reduced projection, shorter than the first outer fold (Fig. 1E). No photoreceptor or tentacular structures are associated with this projection. The middle fold is absent in the genera *Arca*, *Cucullaea* and *Trisidos* (Fig. 1C, D, F, N). The mantle margin also lacks a middle

fold in Glycymerididae, Philobryidae and infaunal Noetiidae (e.g. *Eontia* and *Noetia*; Fig. 2).

The inner mantle fold is an enlarged, muscular projection in most arcid taxa, usually longer and robust posteriorly. In the Cucullaeidae, Glycymerididae, Limopsidae, Philobryidae and the genus *Arca*, the inner mantle fold is about the length of the first outer fold or slightly longer (Figs 1D, 2G–M). In contrast, the inner fold is about twice the length of the first outer fold in Noetiidae, *Barbatia* and *Acar* (Fig. 1E, G). A massive enlargement of the inner fold is observed in some *Barbatia* species and in numerous infaunal species, such as *Trisidos*, *Anadara*, *Tegillarca*, *Eontia* and *Noetia* (Fig. 1I–N). A posterior flap, formed by the inner fold, is a long projection found in *Bathyarca* species (Fig. 1M).

The mantle margin in arcids exhibits different levels of variation among taxa. For example, the number of folds and relative lengths are very uniform within the Anadarinae (Fig. 1J–L), but highly variable within *Barbatia* (Fig. 1E, G–I). Within Noetiidae, mantle organization is also variable (Fig. 2A–D), whereas in Glycymerididae it is more uniform (Fig. 2E, F). In contrast, the Limopsidae (Fig. 2G–I) and Philobryidae (Fig. 2J–M) have a less complex and miniaturized mantle margin, usually devoid of photoreceptor organs, pigmentation or enlarged folds.

### PHYLOGENETIC HYPOTHESES

The maximum likelihood tree of the Arcida corroborates the monophyly of the clade and the monophyly of all families, except for Arcidae, which is split into five branches (Fig. 3). Although some internal nodes show low bootstrap values, higher support was obtained for some relationships among families and genera (e.g. *Arca*, Anadarinae, Glycymerididae and Limopsidae). The remaining Pteriomorphia were recovered as the sister group of Arcida.

Arcidae is polyphyletic in our analysis, with *Arca* and *Acar* descending from an early branch of the order. All Anadarinae species are nested together, being sister group to a pair of *Barbatia* species (*Barbatia candida* and *Barbatia lacerata*). Interestingly, *Barbatia* species are scattered across the phylogeny, suggesting separate lineages taxonomically included under the same name. Noetiidae is a monophyletic family, although *Adacnarca nitens*, formally a philobryid, seems also to be included in this clade. A close relationship between Limopsidae and Philobryidae was recovered, with Glycymerididae as the sister group. The three former families were recovered as the sister group of (*Cucullaea* + *Bathyarca*).

A similar topology was recovered for the time-calibrated phylogeny (Fig. 4). Diversification times were estimated for the major lineages with the 95% highest



posterior density interval (HPD): Arcida, 341.3 Mya (95% HPD 261.2–424.1 Mya); Glycymerididae, 194.6 Mya (95% HPD 112.1–278.3 Mya); Anadarinae, 190.5 Mya (95% HPD 124–256.7 Mya); Limopsoidea, 187.7 Mya (95% HPD 113.4–259.3 Mya); Noetiidae, 175.5 Mya (95% HPD 96.6–248.1 Mya); Philobryidae, 143 Mya (95% HPD 77.1–215.9 Mya); and Limopsidae, 110.4 Mya (95% HPD 37.8–195.1 Mya). A lineage-through-time plot also shows a major diversification of Arcida lineages during the Mesozoic (Fig. 4).

#### MANTLE MARGIN EVOLUTION

The history of changes in the mantle margin was reconstructed based on key traits. A second outer fold has arisen in the origin of the Arcida clade, and probably lost in Limopsidae and Philobryidae lineages (data not shown). Intense mantle pigmentation was acquired multiple times, i.e. in the origin of Glycymerididae, *Arca* + *Acar*, *Barbatia barbata* + *Barbatia cancellaria* + *Barbatia fusca*, and some lineages within Anadarinae (Supporting Information, Fig. S1). The ancestor of Arcida had a reduced middle fold, i.e. shorter than the first outer fold (Fig. 5), which is a striking contrast to the remaining Pteriomorphia, in which the middle fold is long and usually bears tentacles and photoreceptor organs. Although most arcids share a reduced middle fold, the complete loss of this projection occurred at least ten times (Fig. 5). Photoreceptor organs were reconstructed to be present in the mantle margin of the Arcida's ancestor. More specifically, the presence of pigmented eyespots represents a plesiomorphy for all arcid taxa, with secondary losses for many infaunal lineages, such as *Eontia*, *Limopsis* and *Trisidos* (Fig. 6A). Likewise, compound eyes were probably present in the Arcida's ancestor, which were subsequently lost in four separate lineages: Limopsoidea, *Bathyarca*, Anadarinae + (*B. candida* + *B. lacerata*), and a clade formed by Noetiidae with some *Barbatia* and *Trisidos* species (Supporting Information, Fig. S2).

The inner fold is commonly longer than the other mantle folds in most bivalves, but in Arcida this trait displays significant variation. The inner fold is reconstructed to be about the length of the first outer fold, or only slightly longer, in the origin of the Order (Fig. 7A). The enlargement of this fold, forming a long projection about twice the length of the first outer fold, occurred in the *Acar*'s ancestor and in the ancestor of a large clade including Noetiidae, Anadarinae and *Barbatia* species (Fig. 7A). Another change in state is represented by a very enlarged inner fold, much longer than first outer fold, forming extensible curtains and flaps. This transition occurred in different clades, e.g. *Trisidos*, *Eontia*, *Bathyarca* and Anadarinae + *Barbatia*, most of them including infaunal bivalves (Fig. 7B).

#### ASSOCIATION BETWEEN MANTLE MARGIN MORPHOLOGY AND LIFESTYLES

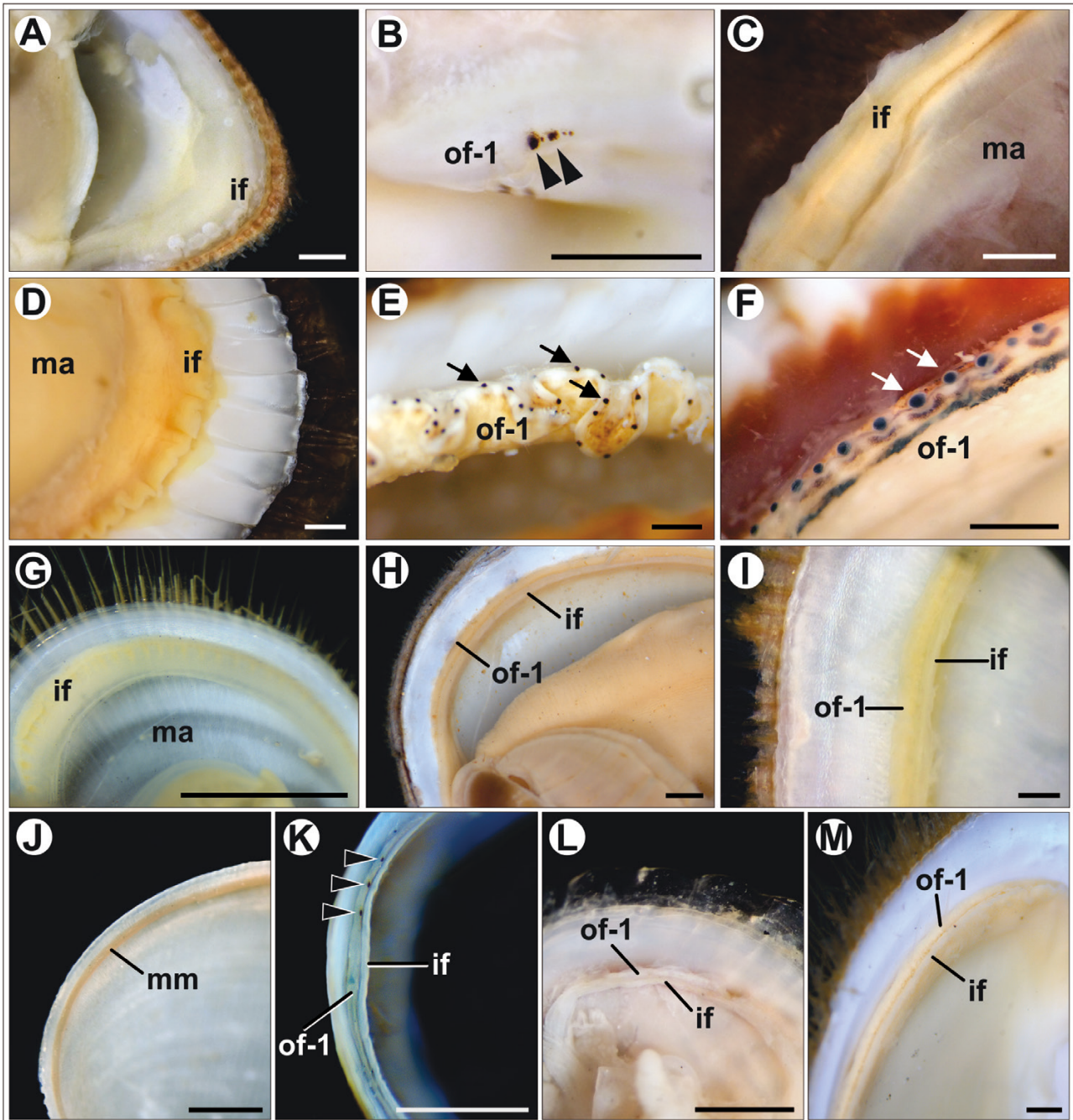
The reconstruction of modes of life suggests that the ancestor of Arcida was likely to be an epifaunal bivalve, possibly attached to rocks and hard substrate by a byssus (Fig. 6B; Supporting Information, Table S3). Soft sediments, such as mud and sand, were later occupied independently by different groups. The semi-infaunal/infaunal lifestyle was secondarily adopted four times during Arcida evolution during the Mesozoic (Figs 4, 6B), by lineages originating Anadarinae, *Trisidos*, *Eontia* (infaunal noetiids) and the ancestor of all Limopsoidea + Glycymerididae + (*Bathyarca* + Cucullaeidae). Among infaunal lineages, a shift to epifaunal lifestyle has occurred in the origin of Philobryidae (Fig. 6B), animals that are frequently byssate on other organisms, such as algae.

Correlation tests were applied when mantle traits seemed to be associated with particular lifestyles. For instance, pigmentation on the first outer fold is common in epifaunal bivalves. The tested hypotheses of evolutionary correlation are shown in Table 2. Pigmentation, which is typical for epifaunal bivalves, was not statistically correlated with lifestyle (Table 2). Pigmented eyespots, however, had a statistically significant correlation with lifestyle (Table 2). Ancestral state reconstructions of eyespots and lifestyles suggested that this correlation was associated with the adoption of infaunal habits and loss of pigmented eyespots (Fig. 6). Inner fold enlargement was also correlated with mode of life, with the results suggesting that the evolutionary shift to infaunal habit was more likely when the inner fold became much longer than the first outer fold (Table 2).

## DISCUSSION

#### PHYLOGENETIC RELATIONSHIPS AND DIVERGENCE TIMES

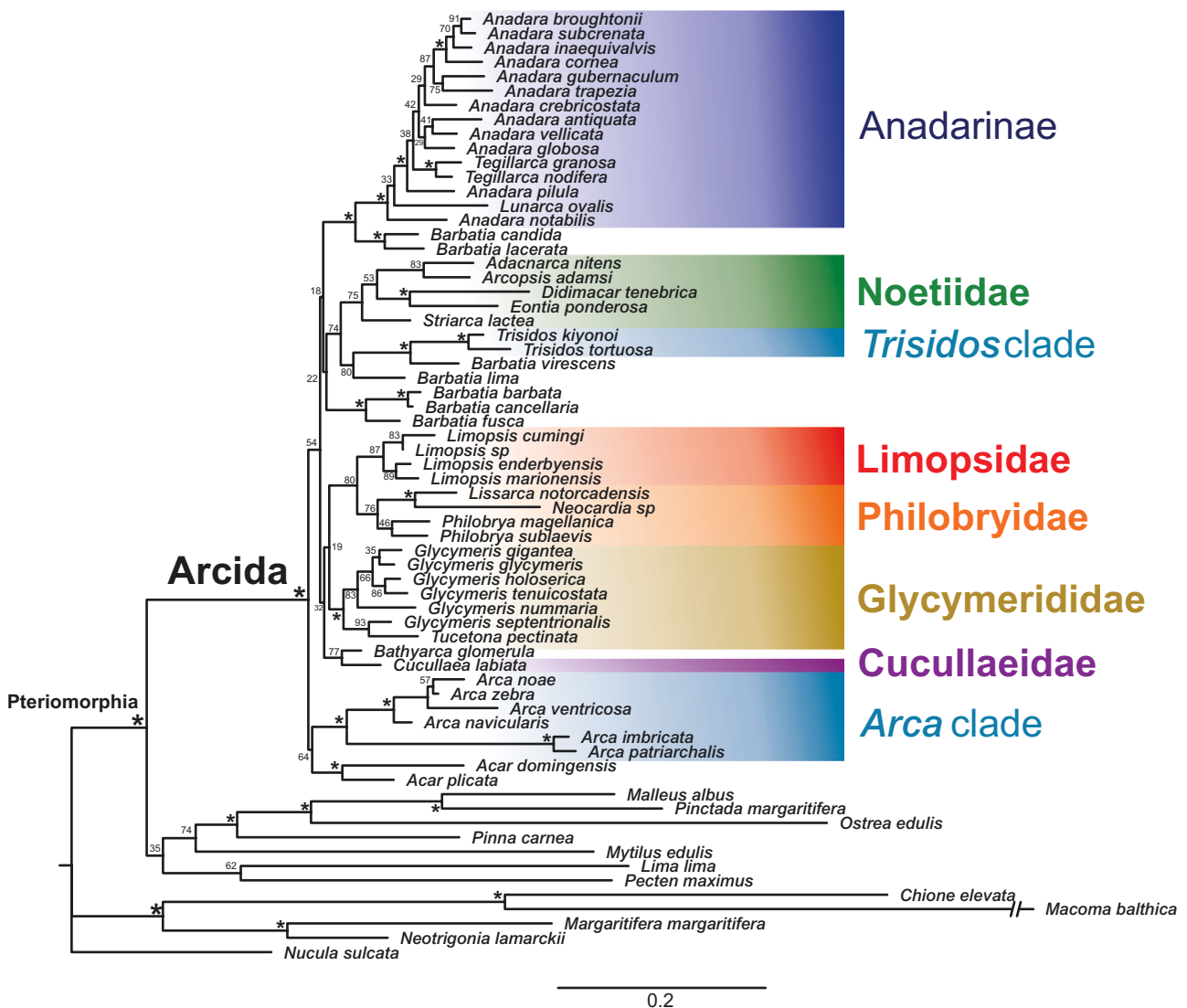
Arcida is a well-supported, monophyletic group (see also Bieler *et al.*, 2014; Feng *et al.*, 2015; Combosch & Giribet, 2016). All families were recovered as monophyletic, with the exceptions of a polyphyletic Arcidae and the placement of the philobryid *Adacnarca nitens* within Noetiidae. Although a previous analysis found support to separate Arcoidea from Limopsoidea (Combosch & Giribet, 2016), our results indicate Arcoidea as non-monophyletic. This is the consequence of an early branch giving rise to *Acar* and *Arca*, whereas Limopsoidea is nested within the remaining Arcoidea. Therefore, the Limopsoidea would have an origin from within the Arcoidea, a hypothesis not supported by previous topologies (Combosch & Giribet, 2016), but



**Figure 2.** Mantle margin morphology in Noetiidae (A–D), Glycymerididae (E, F), Limopsidae (G–I) and Philobryidae (J–M). Posterior mantle region. Scale bars: 1 mm. The first outer fold can bear compound eyes (arrows) and pigmented eyespots (arrowheads). A, *Striarca lactea* (USNM 857645). B, *Arcopsis solida* (USNM 733218). C, *Didimacra tenebrica* (SBMNH 80722). D, *Noetia ponderosa* (USNM 803530). E, *Tucetona pectinata* (MZSP 91971). F, *Glycymeris tenuicostata* (378982). G, *Limopsis aurita* (ZUEC-BIV 2248). H, *Limopsis lilliei* (MZSP 90647). I, *Limopsis marionensis* (USNM 760835). J, *Adacnarcia nitens* (USNM 886551). K, *Lissarca notorcadensis* (MZSP 87826). L, *Neocardia* sp. (MCZ 378927). M, *Philobrya sublaevis* (MZSP 90645). Abbreviations: if, inner fold; ma, mantle; mm, mantle margin; of-1, first outer fold.

already suggested elsewhere (Jackson *et al.*, 2015). Our topology is consistent with the view that Limopsidae and Philobryidae share an exclusive, common history

based on similar development of hinge and alivincular ligament type (Malchus & Warén, 2005; Oliver & Holmes, 2006).



**Figure 3.** Phylogenetic relationships within Arcida based on maximum likelihood analysis of four genes (18S rRNA, 28S rRNA, *COI* mtDNA and *H3*). Asterisks on nodes indicate bootstrap values > 95%. Selected clades are indicated by colour groups. Arcidae is the only non-monophyletic family.

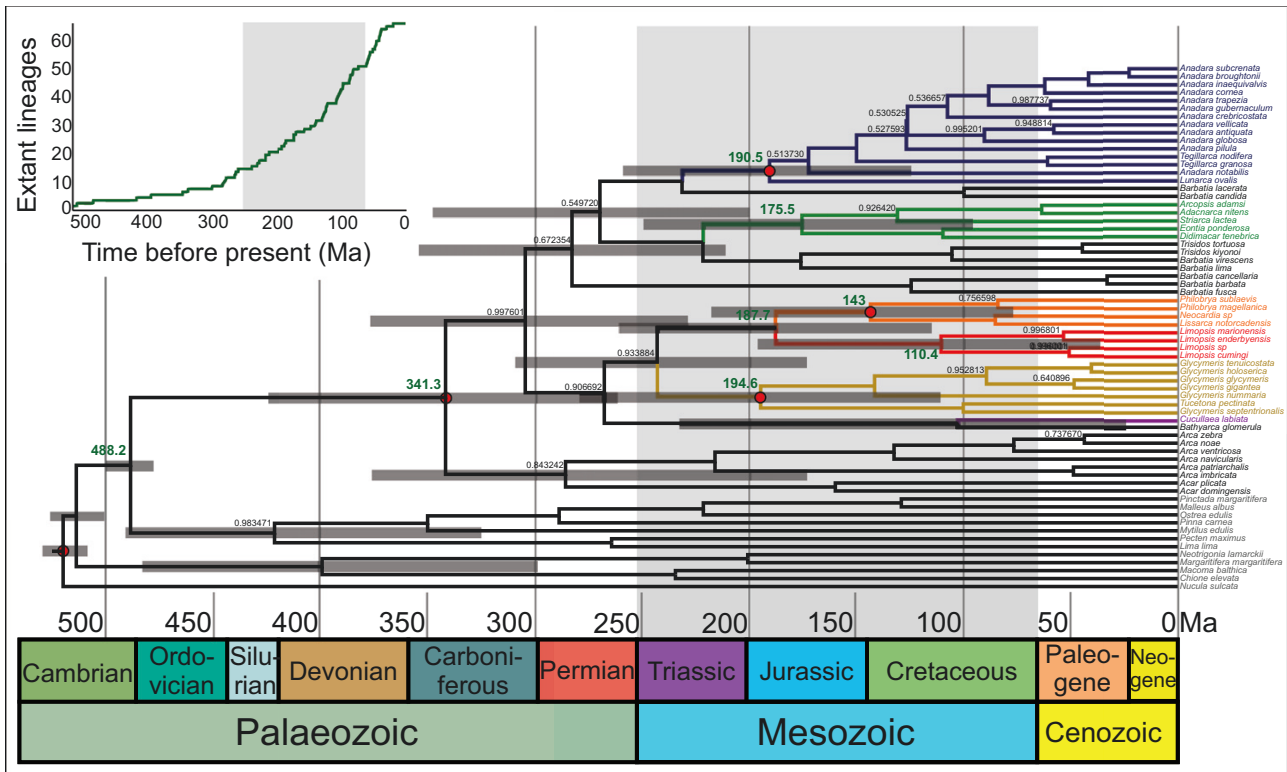
The taxonomic position of Glycymerididae has always been controversial, and our data support this family within Arcoidea, as also suggested by [Combosch & Giribet \(2016\)](#). In contrast to their results, however, the Glycymerididae is the sister group of Limopsoidea in our analysis, forming a clade closely related to *Cucullaea* and *Bathyarca*. The Glycymerididae was previously thought to have originated from the Cucullaeidae based on the duplivincular ligament and other shell characters observed in fossil species ([Nicol, 1950](#)). Our results do not corroborate this view, but their morphological similarity is supported by the close relationship between these families.

Arcidae is not monophyletic in our analyses, which is consistent with previous studies ([Marko, 2002](#);

[Matsumoto, 2003](#); [Feng et al., 2015](#); [Combosch & Giribet, 2016](#)). For instance, the genus *Barbatia* is polyphyletic, and thus in great need of taxonomic revisions. Similar to previous findings ([Combosch & Giribet, 2016](#)), some *Barbatia* species, such as *B. candida* and *B. lacerata*, form the sister group of Anadarinae, whereas others, such as *Barbatia virescens*, are close to *Trisidos* and Noetiidae.

The oldest fossils of Arcida, i.e. *Glyptarca serrata*, date back to the Ordovician (~480 Mya; [Cope, 1997](#)). According to our analysis, the arcid divergence occurred in the late Cambrian (~488 Mya), and the crown group of Arcida had a Carboniferous origin, ~341 Mya. Our time-calibrated phylogeny agrees with the fossil record ([Thomas, 1978a](#); [Oliver & Holmes, 2006](#)), suggesting





**Figure 4.** Time-calibrated phylogeny of Arcida under Bayesian inference based on four genes (18S rRNA, 28S rRNA, *COI* mtDNA and *H3*) and five fossils used to calibrate internal nodes (red circles). Green values indicate median ages on selected nodes. Grey bars indicate 95% highest posterior density intervals (HPD) for nodes of interest. Posterior probabilities different from 1.0 are indicated on nodes. Colour code for clades and taxa is the same as the one used in Figure 3. A lineages-through-time plot is shown at the upper left. After a Cambrian divergence, the crown group of Arcida had an origin ~341 Mya (Carboniferous) and a major diversification during the Mesozoic.

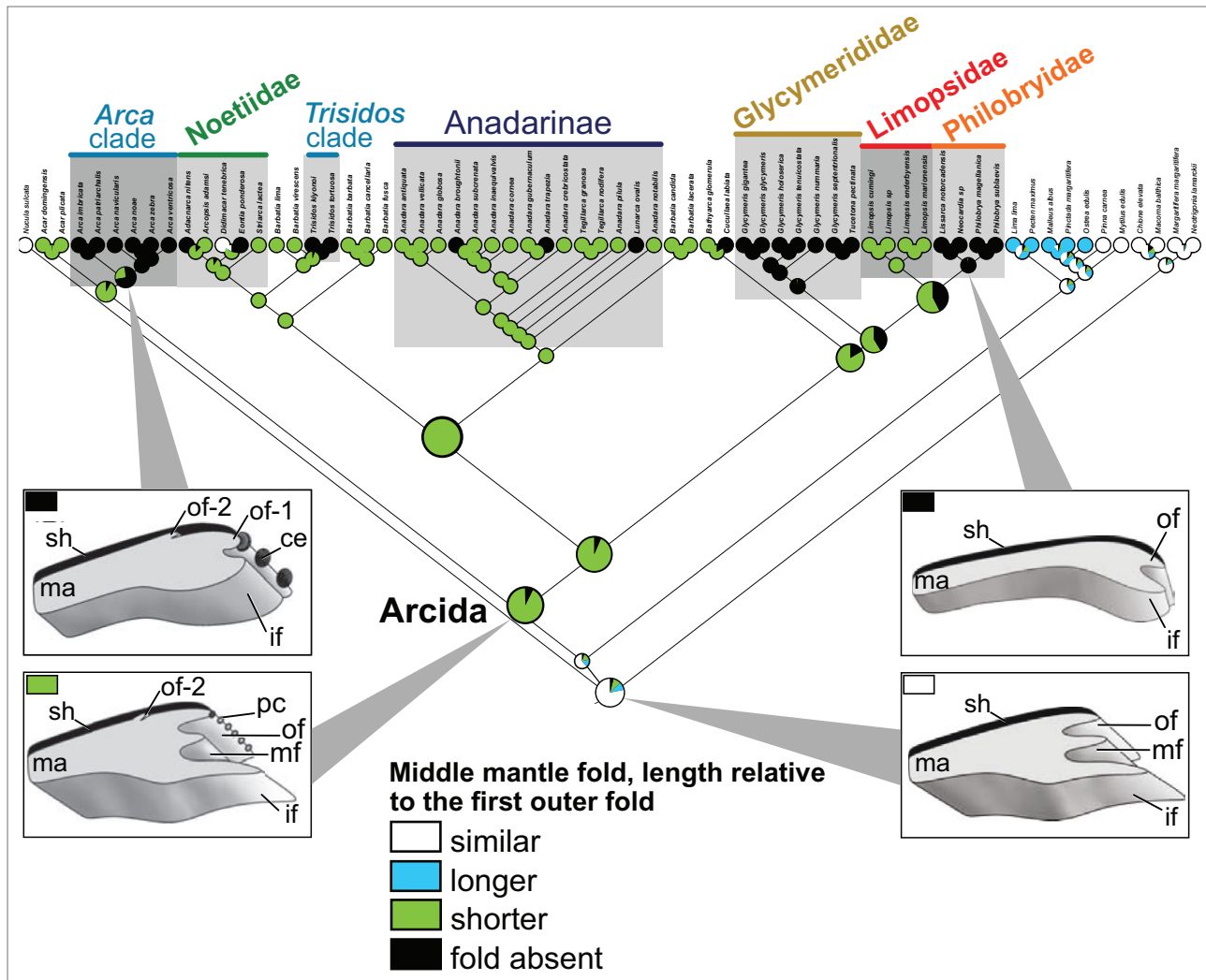
that most diversification of Arcida occurred during the Mesozoic, including the origin of most modern families, i.e. Cucullaeidae, Glycymerididae, Limopsidae and Philobryidae. The convergent transitions to semi-infaunal or infaunal habits by different lineages, such as noetiids, Anadarinae, Cucullaeidae, Glycymerididae and Limopsidae, may have contributed to the diversification of Arcida, which is consistent with the Cretaceous fossil record (Thomas, 1978b; Thomas *et al.*, 2000; Oliver & Holmes, 2006; Combosch & Giribet, 2016). The adoption of an infaunal lifestyle in bivalves is regarded as one of the most important strategies to avoid predation by a diversity of durophagous predators during the long-lasting ecological arms race of the so-called Mesozoic marine revolution (Stanley, 1968; Vermeij, 1977). Our results, therefore, provide further evidence for the Mesozoic infaunalization of bivalves.

#### EVOLUTION OF MANTLE TRAITS AND LIFESTYLE

The second outer mantle fold is an exclusive feature of Arcida, shared by most of its descendants (see also

Waller, 1980). Photoreceptor organs on the first outer fold are also distinctive traits of Arcida, and they are present mainly in epifaunal species inhabiting shallow waters (Waller, 1980; Morton & Peharda, 2008; Morton & Puljas, 2015; Audino & Marian, 2018; present study). Our data support the correlated evolution of photoreceptor organs and mode of life, as previously suggested based on morphological studies alone (Audino & Marian, 2018).

The Arcida's ancestor had pigmented eyespots and posterior compound eyes that were lost in numerous lineages (Fig. 6; Supporting Information, Fig. S2). These findings suggest an important role of light-guided behaviours in ancestral ark clams living on the substrate, possibly related to predator detection and posture control (Nilsson, 1994). A single origin of compound eyes is in accordance with the anatomical similarity of these organs in the distinct arcidan lineages that have been studied so far, such as Glycymerididae, the genera *Arca* and *Acar*, and some *Barbatia* species (Waller, 1980; Morton & Puljas, 2015; Audino & Marian, 2018). Additionally, the loss of

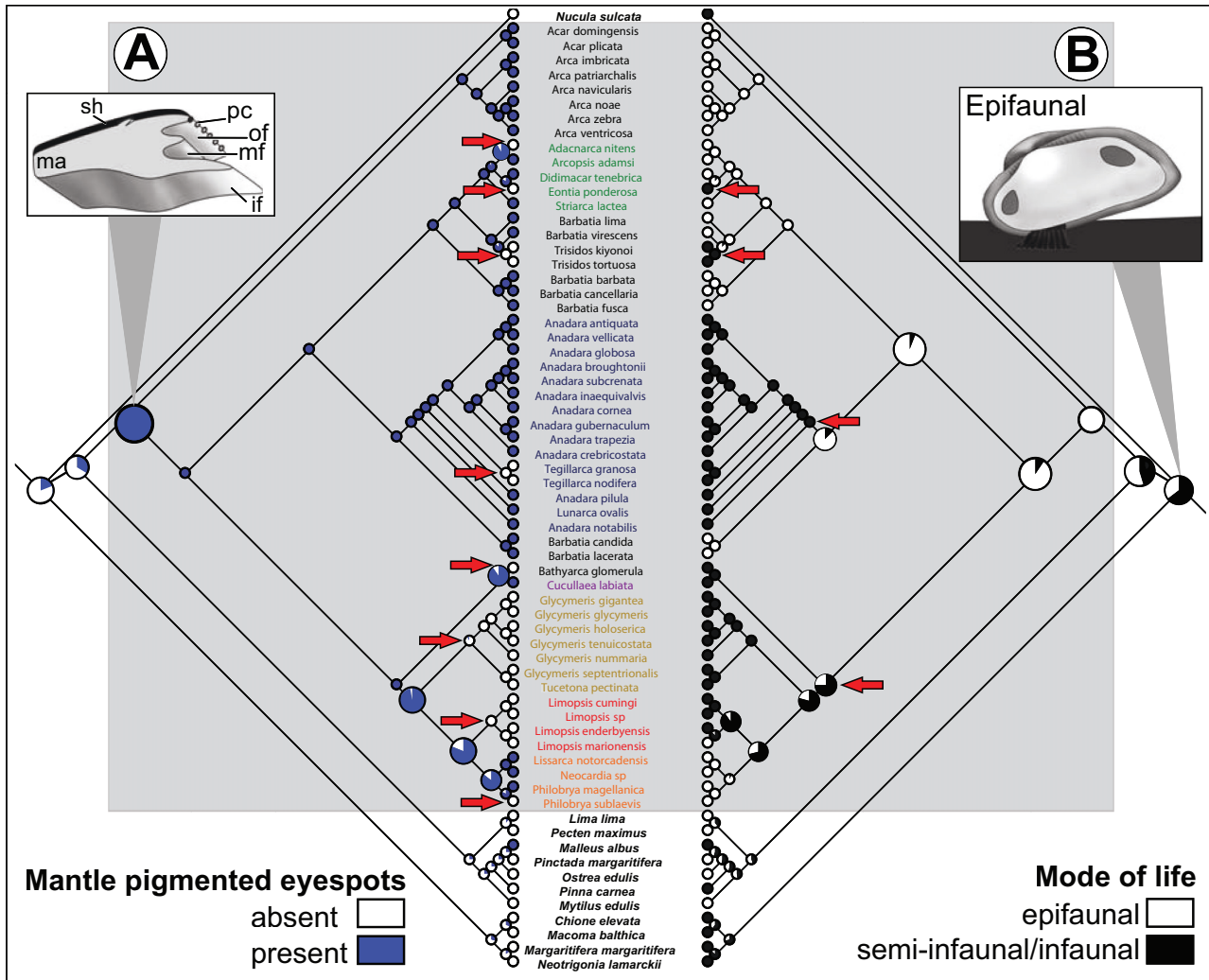


**Figure 5.** Ancestral state reconstruction of the middle mantle fold in Arcida under maximum likelihood, assuming a single rate for all possible transitions (MK1 model). Pie charts represent the likelihood proportions of reconstructed states; nodes of interest have their charts enlarged. Mantle margin schemes indicate mantle morphology as reconstructed by the analysis. Abbreviations: ce, compound eyes; if, inner fold; ma, mantle; mf, middle fold; of, outer fold; of-1, first outer fold; of-2, second outer fold; pc, pigmented eyespots; sh, shell.

photoreceptor organs also provides important insights into the evolution of ark clams. Infaunal lineages frequently lost photoreceptor organs present in their epifaunal ancestor (Fig. 6; Supporting Information, Fig. S2), which can be explained either by a condition of relaxed selection under the infaunal condition or a positive selective pressure for eye reduction.

Relaxed selection can be defined as the elimination or reduction, by means of environmental changes, of a selective force that was important for the maintenance of a particular trait (Lahti *et al.*, 2009). This is an evolutionary process frequently evoked to explain eye and pigment reduction in several groups, including numerous lineages of cave animals (Porter & Crandall, 2003; Wilkens, 2010). Alternatively, other processes

can also produce similar patterns. For example, variability in eye size and pigmentation in cave fishes occurs through multiple mechanisms, suggesting different evolutionary forces synergistically driving eye regression via pleiotropy (Protas *et al.*, 2008). Studies of both vertebrate and invertebrate cave lineages have also demonstrated the high energetic costs of maintaining sensory systems, such as eyes, even in dark conditions (Niven & Laughlin, 2008). For example, eye loss in cavefishes may have been driven by selection for regression of neural tissue, which is associated with high metabolic costs (Moran *et al.*, 2015). In cave crabs, eye reduction seems most likely to be driven by strong directional selective regimes in the subterranean environment (Klaus *et al.*, 2013). In



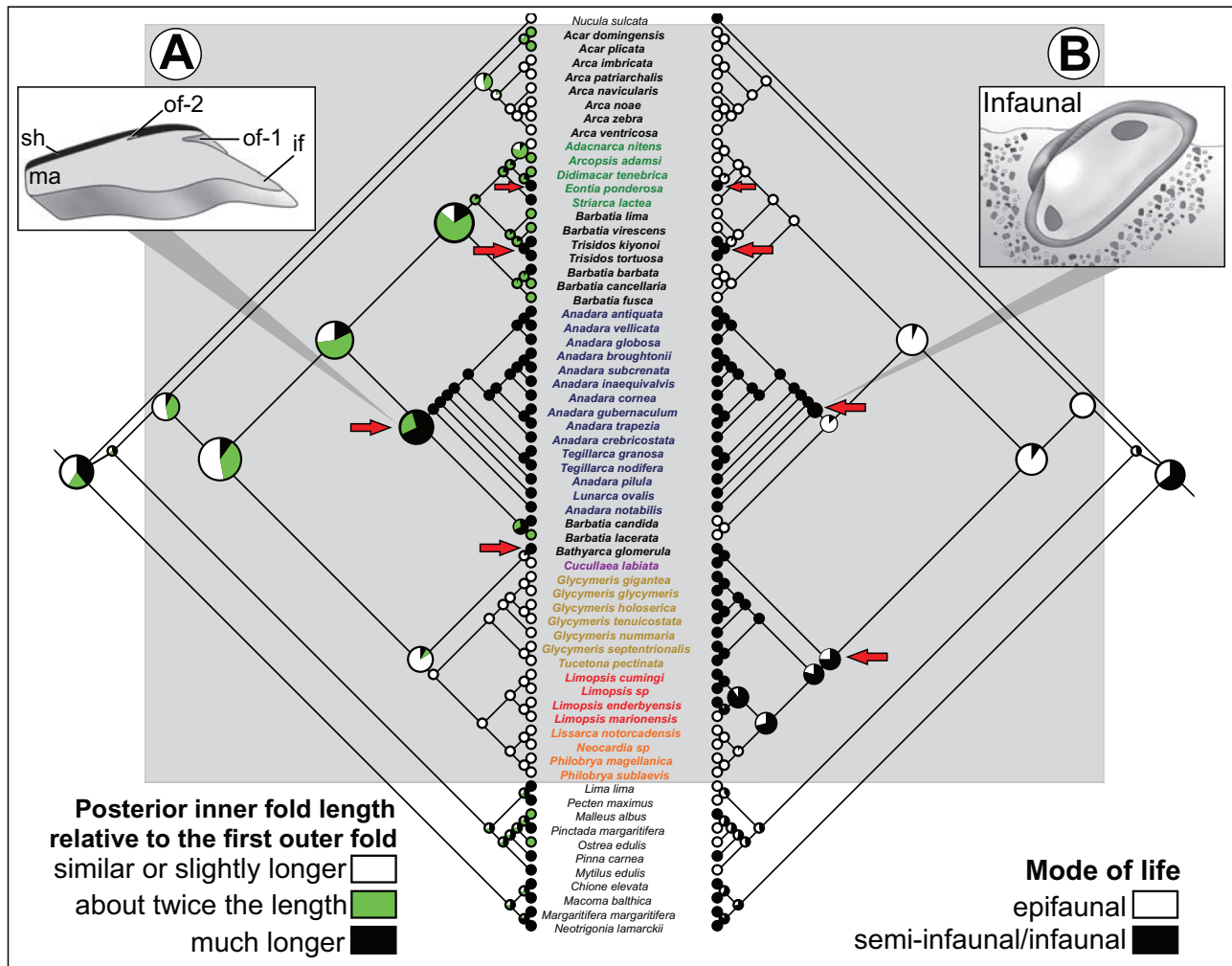
**Figure 6.** Ancestral state reconstruction of mantle photoreceptor organs (left; AsymmMK model) and mode of life (right; MK1 model) in Arcida under maximum likelihood. Ingroup is indicated by the grey boxes. Pie charts represent the likelihood proportions of reconstructed states; nodes of interest have their charts enlarged. The ancestor of Arcida is recovered as an epifaunal animal with simple eyespots on the mantle. Most subsequent losses of eyespots (red arrows on the left) are apparently associated with transitions to semi-infaunal/infaunal habits (red arrows on the right). Abbreviations: if, inner fold; ma, mantle; mf, middle fold; of, outer fold; of-1, first outer fold; pc, pigmented eyespots; sh, shell.

the marine infaunal context, our results provide the initial steps to understand the evolutionary trajectory of photoreceptor organs in ark clams. Similar to many intriguing cases of cave lineages (Niven and Laughlin, 2008), further studies are still necessary to clarify whether eye loss in infaunal bivalves is produced by selective pressure or by genetic drift when selective pressures for eye maintenance are absent.

The middle fold is a mantle margin projection that is well developed in most bivalves, frequently bearing associated structures and playing sensorial roles (Yonge, 1983). An opposite condition was observed in most specimens studied herein, in which the middle fold was shorter than the outer and inner

folds, corresponding to only a slight projection, when present. A shorter middle fold was also noted in *Limopsis cristata* (Morton, 2013) and *Barbatia* species (Simone & Chichvarkhin, 2004). Our results suggest that this fold was already reduced in the ancestor of Arcida, which is a remarkable difference from other pteriomorphians, which frequently display a long and complex projection (Audino & Marian, 2016). The reduction of the middle fold seems to have been a common phenomenon during Arcida diversification, resulting in the complete loss of this structure in several lineages (Fig. 5). This evolutionary pattern is unique among bivalves and leaves many unsolved functional questions. One possible explanation was provided by





**Figure 7.** Ancestral state reconstruction of inner fold length (left) and mode of life (right) in Arcida under maximum likelihood, assuming a single rate for all possible transitions (MK1 model). Ingroup is indicated by the grey boxes. Pie charts represent the likelihood proportions of reconstructed states; nodes of interest have their charts enlarged. The inner mantle fold becomes much longer than the others in numerous lineages (red arrows on the left), which is apparently associated with transitions to semi-infaunal/infaunal habits (red arrows on the right). Abbreviations: if, inner fold; ma, mantle; of-1, first outer fold; of-2, second outer fold; sh, shell.

Morton (1982), who suggested that sensorial roles, such as photoreception, were transferred to the first outer fold. In addition, recent anatomical evidence from different arcid species also corroborated this view, indicating that chemo-/mechanosensorial roles were possibly transferred to the enlarged inner fold (Audino & Marian, 2018).

The hypertrophy of the inner fold in a very extensible organ is observed in many lineages of Arcida (Fig. 7). For example, most semi-infaunal or infaunal arcids, such as some Noetiidae, Anadarinae, *Trisidos* and *Bathylarca*, have very long inner folds (see also Morton, 1982; Audino & Marian, 2018). In infaunal bivalves of other clades (e.g. Heterodonta), siphons (i.e. long, fused inner folds) are present and allow them to

inhabit soft sediments and maintain water circulation through the pallial cavity (Yonge, 1983). In the case of the infaunal *Bathylarca pectunculoides*, the posterior flaps formed by the inner fold are thought to act as functional siphons (Morton, 1982). Accordingly, our phylogenetic and morphological data strongly support the evolution of the inner fold as a functional siphon in arcid lineages, which has possibly facilitated the transition to infaunal lifestyles.

#### EVOLUTIONARY CONVERGENCE AND MACROEVOLUTION

Ecological shifts shaping morphological evolution are known for many vertebrate groups [e.g. lizards

**Table 2.** Evolutionary correlation tests between mantle margin traits and lifestyles in Arcida

| Morphological traits ( <i>y</i> ) and hypotheses ( <i>h</i> ) | Mode of life: epifaunal vs. infaunal ( <i>x</i> ) |                 |                                      |
|---------------------------------------------------------------|---------------------------------------------------|-----------------|--------------------------------------|
|                                                               | Difference in $-\log L$ between models            | <i>P</i> -value | Conclusion                           |
| First outer fold pigmentation                                 |                                                   |                 |                                      |
| $h_1$ correlation                                             | 2.8321                                            | 0.138           | Independent traits                   |
| Compound eyes                                                 |                                                   |                 |                                      |
| $h_1$ correlation                                             | 0.9473                                            | 0.268           | Independent traits                   |
| Pigmented eyespots                                            |                                                   |                 |                                      |
| $h_1$ correlation                                             | 5.12                                              | 0.0223          | Correlated traits                    |
| $h_2$ <i>x</i> depends on <i>y</i>                            | 0.4789                                            | 0.3884          | <i>x</i> does not depend on <i>y</i> |
| $h_3$ <i>y</i> depends on <i>x</i>                            | 2.0402                                            | 0.13            | <i>y</i> does not depend on <i>x</i> |
| Inner mantle fold development                                 |                                                   |                 |                                      |
| $h_1$ correlation                                             | 6.4797                                            | 0.002           | Correlated traits                    |
| $h_2$ <i>x</i> depends on <i>y</i>                            | 2.3043                                            | 0.0112          | <i>x</i> depends on <i>y</i>         |
| $h_3$ <i>y</i> depends on <i>x</i>                            | 3.1362                                            | 0.0569          | <i>y</i> does not depend on <i>x</i> |

The test compares the four-parameter model (independent evolution;  $h_0$ ) and the eight-parameter models (correlated evolution;  $h_1$ ,  $h_2$  and  $h_3$ ) between two binary traits, returning the differences in log-likelihood ( $-\log L$ ) with *P*-values calculated by 10 000 simulations. Significant differences, i.e.  $P$ -value  $< \alpha = 0.05$ , indicate a better fit to the model of correlated evolution. Characters and respective states: first outer fold pigmentation, absent (0) or present (1); compound eyes, absent (0) or present (1); pigmented eyespots, absent (0) or present (1); inner mantle fold development, up to twice the length (0) or much longer (1) than the first outer fold; mode of life, epifaunal (0) or semi-infaunal/infaunal (1).

(Mahler *et al.*, 2013), fishes (Davis & Betancur-R, 2017) and snakes (Esquerré & Scott Keogh, 2016)]. Although marine invertebrates still lack detailed information about ecomorphological evolution, recent progress has been achieved using different clades as models. In cephalopods, for example, several morphological traits represent evolutionary convergences and possible adaptive features associated with benthic or pelagic environments (Lindgren *et al.*, 2012). Although bivalves have traditionally been considered classic examples of convergent evolution associated with lifestyles in the marine benthos (e.g. Stanley, 1972), even in invertebrate zoology textbooks (e.g. Ruppert *et al.*, 2004), these adaptive hypotheses have rarely been tested under an explicit phylogenetic approach. In this context, important progress was recently obtained for Pectinidae (Alejandrino *et al.*, 2011; Serb *et al.*, 2017) and Galeommatoidea (Li *et al.*, 2016).

The Arcida have been consistently regarded as an example of adaptive radiation, with their homoplastic shell characters adapted to infaunal and epifaunal modes of life (Stanley, 1968, 1972; Thomas, 1976, 1978a). Our study provides, for the first time, phylogenetic-based evidence for correlated evolution between the morphology of soft parts and lifestyle transitions in arcids. In addition, evolutionary convergence seems to be a recurrent pattern, including independent losses of eyespots, compound eyes, pigmentation and the middle fold, in addition to independent enlargements of the inner fold. Our results suggest that predation pressure was important in the evolution of Arcida, mainly during the Mesozoic. Pigmented eyespots and compound eyes may aid in predator recognition in

epifaunal bivalves (Nilsson, 1994), and the infaunal habit itself, facilitated by enlarged mantle curtains, might have been a response to predation pressure (Bush & Bambach, 2011). The dramatic increase of infaunal lineages in the marine benthos suggests a successful trend to survive the intensification of predation during the Mesozoic marine revolution (Stanley, 1968, 1972; Vermeij, 1977). In addition to the extensive fossil information for hard parts, we were able to contribute to this hypothesis based on the soft parts of extant lineages of arcids in an integrative approach. Altogether, our results demonstrate evolutionary associations between ecology and morphology during the diversification of bivalve lineages across different benthic lifestyles.

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## REFERENCES

- Agrawal AA. 2017.** Toward a predictive framework for convergent evolution: integrating natural history, genetic mechanisms, and consequences for the diversity of life. *The American Naturalist* **190**: S1–S12.
- Alejandrino A, Puslednik L, Serb JM. 2011.** Convergent and parallel evolution in life habit of the scallops (Bivalvia: Pectinidae). *BMC Evolutionary Biology* **11**: 164.
- Audino JA, Marian JEAR. 2016.** On the evolutionary significance of the mantle margin in pteriomorphian bivalves. *American Malacological Bulletin* **34**: 148–159.
- Audino JA, Marian JEAR. 2018.** Comparative and functional anatomy of the mantle margin in ark clams and their relatives (Bivalvia: Arcoidea) supports association between morphology and life habits. *Journal of Zoology* **305**: 149–162.
- Bielser R, Mikkelsen PM, Collins TM, Glover EA, González VL, Graf DL, Harper EM, Healy J, Kawauchi GY, Sharma PP, Staubach S, Strong EE, Taylor JD, Tëmkin I, Zardus JD, Clark S, Gusmán A, McIntyre E, Sharp P, Giribet G. 2014.** Investigating the Bivalve Tree of Life – an exemplar-based approach combining molecular and novel morphological characters. *Invertebrate Systematics* **28**: 32–115.
- Bush AM, Bambach RK. 2011.** Paleoeologic megatrends in marine metazoa. *Annual Review of Earth and Planetary Sciences* **39**: 241–269.
- Carter JG, Altaba CR, Anderson LC, Araujo R, Biakov AS, Bogan AE, Campbell DC, Campbell M, Jin-hua C, Cope JCW, Dijkstra HH, Zong-jie F, Gardner RN, Gavrilova VA, Goncharova IA, Harries PJ, Hartman JH, Hautmann M, Hoeh WR, Hylleberg J, Johnston P, Kirkendale L, Kleemann K, Koppka J, Machado D, Malchus N, Márquez-aliaga A, Masse J pierre, McRoberts CA, Peter U, Mitchell S, Nevesskaja LA, Özer S, Pojeta J, Polubotko IV, Pons M, Popov S, Sánchez T, Sartori AF, Scott RW, Sey II, Signorelli JH, Silantiev VV, Skelton PW, Steuber T, Waterhouse JB, Lynn G, Yancey T. 2011.** A synoptical classification of the Bivalvia (Mollusca). *Paleontological Contributions* **2011**: 1–48.
- Combosch DJ, Giribet G. 2016.** Clarifying phylogenetic relationships and the evolutionary history of the bivalve order Arcida (Mollusca: Bivalvia: Pteriomorphia). *Molecular Phylogenetics and Evolution* **94**: 298–312.
- Cope JCW. 1997.** The early phylogeny of the class Bivalvia. *Palaeontology* **40**: 713–746.
- Cope JCW. 2000.** A new look at early bivalve phylogeny. *Geological Society, London, Special Publications* **177**: 81–95.
- Davis AM, Betancur-RR. 2017.** Widespread ecomorphological convergence in multiple fish families spanning the marine–freshwater interface. *Proceedings of the Royal Society B: Biological Sciences* **284**: 20170565.
- Esquerré D, Scott Keogh J. 2016.** Parallel selective pressures drive convergent diversification of phenotypes in pythons and boas. *Ecology Letters* **19**: 800–809.
- Felsenstein J. 1985.** Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**: 783–791.
- Feng Y, Li Q, Kong L. 2015.** Molecular phylogeny of Arcoidea with emphasis on Arcidae species (Bivalvia: Pteriomorphia) along the coast of China: challenges to current classification of arcoids. *Molecular Phylogenetics and Evolution* **85**: 189–196.
- Giribet G, Wheeler W. 2002.** On bivalve phylogeny: a high-level analysis of the Bivalvia (Mollusca) based on combined morphology and DNA sequence data. *Invertebrate Biology* **121**: 271–324.
- Gould SJ, Vrba ES. 1982.** Exaptation—a missing term in the science of form. *Paleobiology* **8**: 4–15.
- Harvey PH, Pagel MD. 1991.** *The comparative method in evolutionary biology*. Oxford: Oxford University Press.
- Heath TA, Huelsenbeck JP, Stadler T. 2014.** The fossilized birth–death process for coherent calibration of divergence-time estimates. *Proceedings of the National Academy of Sciences of the United States of America* **111**: E2957–E2966.
- Höhna S, Landis MJ, Heath TA, Boussau B, Lartillot N, Moore BR, Huelsenbeck JP, Ronquist F. 2016.** RevBayes: Bayesian phylogenetic inference using graphical models and an interactive model-specification language. *Systematic Biology* **65**: 726–736.
- Huber M. 2010.** *Compendium of bivalves*. Hackenheim: ConchBooks.
- Imlay RW. 1962.** *Jurassic (Bathonian or early Callovian) ammonites from Alaska and Montana*. US Government Printing Office, Washington.
- Jackson JA, Linse K, Whittle R, Griffiths HJ. 2015.** The evolutionary origins of the Southern Ocean philobryid bivalves: hidden biodiversity, ancient persistence. *PLoS ONE* **10**: e0121198.
- Kalyanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermin LS. 2017.** ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods* **14**: 587.
- Katoh K, Standley DM. 2013.** MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* **30**: 772–780.
- Klaus S, Mendoza JCE, Liew JH, Plath M, Meier R, Yeo DCJ. 2013.** Rapid evolution of troglomorphic characters suggests selection rather than neutral mutation as a driver of eye reduction in cave crabs. *Biology Letters* **9**: 20121098.
- Lahti DC, Johnson NA, Ajie BC, Otto SP, Hendry AP, Blumstein DT, Coss RG, Donohue K, Foster SA. 2009.** Relaxed selection in the wild. *Trends in Ecology & Evolution* **24**: 487–496.
- Li J, Ó Foighil D, Strong EE. 2016.** Commensal associations and benthic habitats shape macroevolution of the bivalve clade Galeommatoidea. *Proceedings of the Royal Society B: Biological Sciences* **283**: 20161006.



- Lindgren AR, Pankey MS, Hochberg FG, Oakley TH. 2012.** A multi-gene phylogeny of Cephalopoda supports convergent morphological evolution in association with multiple habitat shifts in the marine environment. *BMC Evolutionary Biology* **12**: 129.
- Losos JB. 2011.** Convergence, adaptation, and constraint. *Evolution* **65**: 1827–1840.
- Losos JB, Mahler DL. 2010.** Adaptive radiation: the interaction of ecological opportunity, adaptation, and speciation. In: Bell MA, Futuyma DJ, Eanes WF, Levinton JS, eds. *Evolution since Darwin: the first 150 years*. Sunderland: Sinauer Associates, 381–420.
- Maddison WPM, Fitzjohn RG. 2015.** The unsolved challenge to phylogenetic correlation tests for categorical characters. *Systematic Biology* **64**: 127–136.
- Maddison WP, Maddison DR. 2018.** *Mesquite: a modular system for evolutionary analysis, Version 3.51*. Available at: <http://www.mesquiteproject.org>
- Mahler DL, Ingram T, Revell LJ, Losos JB. 2013.** Exceptional convergence on the macroevolutionary landscape in island lizard radiations. *Science* **341**: 292–295.
- Mahler DL, Weber MG, Wagner CE, Ingram T. 2017.** Pattern and process in the comparative study of convergent evolution. *The American Naturalist* **190**: S13–S28.
- Malchus N, Warén A. 2005.** Shell and hinge morphology of juvenile *Limopsis* (Bivalvia: Arcoida) – implications for limopsid evolution. *Marine Biology Research* **1**: 350–364.
- Malkowsky Y, Götze MC. 2014.** Impact of habitat and life trait on character evolution of pallial eyes in Pectinidae (Mollusca: Bivalvia). *Organisms Diversity & Evolution* **14**: 173–185.
- Marko PB. 2002.** Fossil calibration of molecular clocks and the divergence times of geminate species pairs separated by the Isthmus of Panama. *Molecular Biology and Evolution* **19**: 2005–2021.
- Matsumoto M. 2003.** Phylogenetic analysis of the subclass Pteriomorpha (Bivalvia) from mtDNA COI sequences. *Molecular Phylogenetics and Evolution* **27**: 429–440.
- Moore RC, Teichert C. 1969.** *Treatise on invertebrate paleontology: Pt. N Mollusca 6, Bivalvia*. Geological Society of America and University of Kansas Press, Lawrence.
- Moran D, Softley R, Warrant EJ. 2015.** The energetic cost of vision and the evolution of eyeless Mexican cavefish. *Science advances* **1**: e1500363.
- Morton B. 1982.** Functional morphology of *Bathyarca pectunculoides* (Bivalvia: Arcacea) from a deep Norwegian fjord with a discussion of the mantle margin in the Arcoida. *Sarsia* **67**: 269–282.
- Morton B. 2013.** The functional morphology of the abyssal *Limopsis cristata* (Arcoida: Limopsidae) with a discussion on the evolution of the more advanced bivalve foot. *Acta Zoologica* **94**: 74–85.
- Morton B, Peharda M. 2008.** The biology and functional morphology of *Arca noae* (Bivalvia: Arcidae) from the Adriatic Sea, Croatia, with a discussion on the evolution of the bivalve mantle margin. *Acta Zoologica* **89**: 19–28.
- Morton B, Prezant RS, Wilson B. 1998.** Class Bivalvia. In: Beesley PL, Ross GJB, Wells A, eds. *Mollusca: the Southern synthesis. Fauna of Australia, Vol. 5*. Melbourne: CSIRO Publishing, 195–234.
- Morton B, Puljas S. 2015.** The ectopic compound ommatidium-like pallial eyes of three species of Mediterranean (Adriatic Sea) *Glycymeris* (Bivalvia: Arcoida). Decreasing visual acuity with increasing depth? *Acta Zoologica* **474**: 464–474.
- Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ. 2014.** IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution* **32**: 268–274.
- Nicol D. 1950.** Origin of the pelecypod family Glycymeridae. *Journal of Paleontology* **24**: 89–98.
- Nilsson DE. 1994.** Eyes as optical alarm systems in fan worms and ark clams. *Philosophical Transactions of the Royal Society B: Biological Sciences* **346**: 195–212.
- Niven JE, Laughlin SB. 2008.** Energy limitation as a selective pressure on the evolution of sensory systems. *Journal of Experimental Biology* **211**: 1792–1804.
- Oliver PG, Holmes AM. 2006.** The Arcoidea (Mollusca: Bivalvia): a review of the current phenetic-based systematics. *Zoological Journal of the Linnean Society* **148**: 237–251.
- Pagel M. 1994.** Detecting correlated evolution on phylogenies: a general method for the comparative analysis of discrete characters. *Proceedings of the Royal Society B: Biological Sciences* **255**: 37–45.
- Pagel M. 1999.** The maximum likelihood approach to reconstructing ancestral character states of discrete characters on phylogenies. *Systematic Biology* **48**: 612–622.
- Pojeta J, Runnegar B, Kriz J. 1973.** *Fordilla troyensis* Barrande: the oldest known pelecypod. *Science* **180**: 866–868.
- Porter ML, Crandall KA. 2003.** Lost along the way: the significance of evolution in reverse. *Trends in Ecology & Evolution* **18**: 541–547.
- Protas M, Tabansky I, Conrad M, Gross JB, Vidal O, Tabin CJ, Borowsky R. 2008.** Multi-trait evolution in a cave fish, *Astyanax mexicanus*. *Evolution & Development* **10**: 196–209.
- Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA. 2018.** Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology* **67**: 901–904.
- Ruppert EE, Fox RS, Barnes RD. 2004.** *Invertebrate Zoology, a Functional Evolutionary Approach*. Belmont, CA: Brooks/Cole-Thomson Learn. 7th ed.
- Schluter D. 2000.** *The ecology of adaptive radiation*. Oxford: Oxford University Press.
- Serb JM, Sherratt E, Alejandrino A, Adams DC. 2017.** Phylogenetic convergence and multiple shell shape optima for gliding scallops (Bivalvia: Pectinidae). *Journal of Evolutionary Biology* **30**: 1736–1747.
- Simone LRL, Chichvarkhin AY. 2004.** Comparative morphological study of four species of *Barbatia* occurring on the southern Florida coast (Arcoidea, Arcidae). *Malacologia* **46**: 355–379.
- Simpson GG. 1953.** *The major features of evolution*. New York: Columbia University Press.
- Stanley SM. 1968.** Post-Paleozoic adaptive radiation of infaunal bivalve molluscs: a consequence of mantle fusion and siphon formation. *Journal of Paleontology* **42**: 214–229.

- Stanley SM. 1972.** Functional morphology and evolution of byssally attached bivalve mollusks. *Journal of Paleontology* **46**: 165–212.
- Steiner G, Hammer S. 2000.** Molecular phylogeny of the Bivalvia inferred from 18S rDNA sequences with particular reference to the Pteriomorpha. *Geological Society, London, Special Publications* **177**: 11–29.
- Thomas RDK. 1976.** Constraints of ligament growth, form and function on evolution in the Arcoida (Mollusca: Bivalvia). *Paleobiology* **2**: 64–83.
- Thomas RDK. 1978a.** Shell form and the ecological range of living and extinct Arcoida. *Paleobiology* **4**: 181–194.
- Thomas RDK. 1978b.** Limits to opportunism in the evolution of the Arcoida (Bivalvia). *Philosophical Transactions of the Royal Society B: Biological Sciences* **284**: 335–344.
- Thomas RDK, Madzvamuse A, Maini PK, Wathen AJ. 2000.** Growth patterns of noetiid ligaments: implications of developmental models for the origin of an evolutionary novelty among arcoid bivalves. *Geological Society, London, Special Publications* **177**: 279–289.
- Vermeij GJ. 1977.** The Mesozoic marine revolution: evidence from snails, predators and grazers. *Journal of Paleontology* **3**: 245–258.
- Waller TR. 1980.** Scanning electron microscopy of shell and mantle in the order Arcoida. *Smithsonian Contributions to Zoology* **313**: 1–58.
- Wilkens H. 2010.** Genes, modules and the evolution of cave fish. *Heredity* **105**: 413–422.
- Yonge CM. 1983.** Symmetries and the role of the mantle margins in the bivalve Mollusca. *National Wildlife* **16**: 1–10.

### SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

**Table S1.** Characters and states used to study mantle margin traits and lifestyles in Arcida.

**Table S2.** Matrix of mantle margin traits and taxa used in the analyses.

**Table S3.** Lifestyle compilation of ark clams and relatives (Arcida) according to mode of attachment on the substrate and position.

**Figure S1.** Ancestral state reconstruction of pigmentation on the first outer mantle fold in Arcida under maximum likelihood, assuming a single rate for all possible transitions (MK1 model). Pie charts represent the likelihood proportions of reconstructed states. Pigmentation has evolved multiple times in different lineages of epifaunal and infaunal arcids.

**Figure S2.** Ancestral state reconstruction of compound eyes on the first outer mantle fold in Arcida under maximum likelihood, allowing for a different rate for transitions (AsymmMK model). Pie charts represent the likelihood proportions of reconstructed states. Compound eyes have a single origin in the ancestor of Arcida, with subsequent losses in at least four lineages. Abbreviations: ce, compound eyes; if, inner fold; ma, mantle; mf, middle fold; of-1, first outer fold; of-2, second outer fold; sh, shell.