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Hard to get, easy to lose: Evolution of mantle photoreceptor organs in bivalves (Bivalvia, Pteriomorpha)

Running Title

Evolution of photoreceptor organs in bivalves

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**Author contributions**

JAA generated and analyzed data, assembled the figures and tables, and drafted the manuscript.

JMS and JEARM contributed to the acquisition, analysis, and interpretation of data, as well as to manuscript writing. All authors read and approved the final version of the manuscript.

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#### **Data Accessibility**

Summarized ecological and morphological data can be found in supporting information (see Table S2 and S3). Data supporting our results, such as files containing sequence alignments and phylogenetic tree, are archived on the public repository Dryad under the DOI:

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## Abstract

Morphologically diverse eyes have evolved numerous times, yet little is known about how eye gain and loss is related to photic environment. The pteriomorphian bivalves (*e.g.*, oysters, scallops, and ark clams), with a remarkable range of photoreceptor organs and ecologies, are a suitable system to investigate the association between eye evolution and ecological shifts. The present phylogenetic framework was based on amino acid sequences from transcriptome datasets and nucleotide sequences of five additional genes. In total, 197 species comprising 22 families from all five pteriomorphian orders were examined, representing the greatest taxonomic sampling to date. Morphological data were acquired for 162 species and lifestyles were compiled from the literature for all 197 species. Photoreceptor organs occur in 11 families and have arisen exclusively in epifaunal lineages, *i.e.*, living above the substrate, at least five times independently. Models for trait evolution consistently recovered higher rates of loss over gain. Transitions to crevice-dwelling habit appear associated with convergent gains of eyespots in epifaunal lineages. Once photoreceptor organs have arisen, multiple losses occurred in lineages that shift to burrowing lifestyles and deep-sea habitats. The observed patterns suggest that eye evolution in pteriomorphians might have evolved in association with light-guided behaviors, such as phototaxis, body posture, and alarm responses.

**Keywords:** ancestral state estimation, eye, macroevolution, mollusk, morphology, phylogeny

## Introduction

The ability for animals to sense light is critical to perform a wide range of tasks, such as navigation, interaction with other individuals, and detection of environmental cues (Nilsson 2009; Nilsson 2013). The organs that fulfill these functions exhibit a variety of morphologies (Serb and Eernisse 2008; Land and Nilsson 2012) and complicated evolutionary histories (*e.g.*, Picciani et al. 2018; Vopalensky and Kozmik 2009; Henze and Oakley 2015). For example, bivalve mollusks have light-sensitive tissues along the edge of shells that provide nondirectional photoreception (Kennedy 1960; Wiederhold et al. 1973). These dispersed photoreceptors constantly monitor light intensity. Reduction or cessation of light elicit a shadow response, a set of protective behaviors including the retraction of tissues and closure of the shell (Wilkins 2008).

In addition to general photosensitivity, many other animals have evolved eyespots to not only detect intensity, but also direction, of light. Directional photoreception is accomplished when two or more photoreceptors are shielded by screening pigment such that they do not detect light from the exact same direction (Land and Nilsson 2012). These eyespots can produce a rudimentary image of the animal's environment, and consequently, new sensory tasks can be performed based on phototaxis, such as finding suitable habitats and monitoring body posture relative to light (Nilsson 2013). Further elaborations to the optical system, like lenses and mirrors, focus light onto banks of photoreceptor cells to create images of objects. It is this reconstruction of an organism's environment, or spatial vision, which distinguishes an eye from other light sensing organs (*sensu* Land and Nilsson 2012). Image-forming eyes have evolved more than 40 times in animals (Salvini-Plawen and Mayr 1977) and are present in six of the most diverse metazoan phyla (Land and Fernald 1992).

However, eyes, and their associated neural tissues, are energetically costly to use and maintain (Niven and Laughlin 2008; Moran et al. 2015). As a result, eyes are frequently regressed in anatomical complexity or lost in environments with reduced or no light (reviewed in Syme and Oakley 2011; Krishnan and Rohner 2017; Porter and Sumner-Rooney 2018). In aquatic systems, animals occur in light-limited environments through their location at depth, diel activity, or life habit, such as burrowing, boring, and nestling *sensu* Stanley (1970). Eye loss is a frequent phenotype in animals living in low light or aphotic habitats; however, this is not the only outcome, and in many cases remnants of the visual system remain (Pérez-Moreno et al. 2018). In light-limited environments, the visual system is presumably subjected to a variety of evolutionary forces, including direct selective pressure, genetic drift, and pleiotropic effects during development (Porter and Sumner-Rooney 2018). Some cases of eye reduction and/or loss in invertebrates do coincide with transitions to deep-water environments, e.g., in gastropods (Sumner-Rooney et al. 2016), isopods (Menzies et al. 1968), and ostracods (Symes & Oakley 2011). Similar phenotypic outcomes are also found in the inhabitants of submarine caves (Protas and Jeffery 2012; Moran et al. 2015; Re et al. 2018). Analogous to terrestrial animals that live primarily underground, many marine invertebrates live buried within sandy or silty substrates (i.e., infaunal habit), which also have low light conditions. Nevertheless, it is not well understood whether photosensory organs are affected by shifts to sediment-dwelling. Bivalved mollusks can help to elucidate this topic for having different photosensory organs and well-documented transitions to infaunal habits (Stanley 1968, 1975; Yonge 1983; Oliver and Holmes 2006; Sherratt et al. 2016). For example, eye loss is apparently correlated to shifts into burrowing lifestyles in ark clams and their relatives (Audino et al. 2019).

Pteriomorphian bivalved mollusks, which include approximately 20% of all bivalve species (Combosch and Giribet 2016), are a suitable group for testing hypotheses about photoreceptor organ evolution and the relationship between eyes and photic environment (Morton 2008, Audino

et al. 2019). This is a diverse subclass that encompasses many epifaunal bivalves (*i.e.*, living above the substrate), such as scallops, oysters, ark clams, and mussels. Photoreceptor organs occur along the mantle margin and vary from simple eyespots, for directional photosensitivity, to complex camera-type eyes, capable of low-resolution vision (Nilsson 1994; Morton 2008; Wilkens 2008; Nilsson 2013). The most familiar example are the concave mirror eyes, located along the mantle margin of scallops (Pectinidae), which have been extensively investigated through anatomical, developmental, and opsin characterization studies (Dakin 1910; Land 1965; Speiser and Johnsen 2008; Serb et al. 2013; Audino et al. 2015; Porath-Krause et al. 2016, Palmer et al. 2017; Miller et al. 2019).

Other eye types include the invaginated eyes spread along the mantle margin of file clams (Limidae), bivalves that live above the sediment, frequently nested among debris (Bell and Mpitsos 1968; Mpitsos 1973, Morton 2000a). Compound eyes occur in some epifaunal genera of ark clams (Arcidae), as well as in the shallow burrowers Glycymerididae (Nilsson 1994; Waller 1980; Morton and Peharda 2008; Audino and Marian 2018). Simple directional photoreception is performed by pigmented cups and pigmented caps, both examples of eyespots described for Arcidae and Isognomonidae (oysters), respectively (Morton 1987; Nilsson 1994; Tëmkin 2006; Audino and Marian 2018). In addition to the diversity of photoreceptor organs, pteriomorphian species occur in two different reduced-light conditions: the infaunal habit, *i.e.*, buried in the sediment, as seen in many Mytilidae (mussels) and Arcidae taxa (Stanley 1975), and the occupancy of deep-waters by, for example, representatives of Limopsidae, Mytilidae, and “Propeamussiidae” (frequently called glass scallops) (Allen 1983).

One effective way to understand how many times photoreceptor organs have evolved and were lost and whether lifestyles are associated with these transitions is to test these hypotheses in a phylogenetic context. Here, we assemble previously published data from transcriptomic and single-

gene sequence for nearly 200 species to create the most comprehensive molecular analysis of pteriomorphian phylogeny to date. Taxa sampling included 22 out of 26 families *sensu* MolluscaBase (2020), based on Bieler et al. (2010). We collected morphological data on photoreceptor organs and curated ecological data on taxa, and then, we placed these data within a phylogenetic framework of Pteriomorphia to test the following hypotheses: 1) photoreceptor organs had multiple origins in this clade with subsequent, convergent losses; 2) photoreceptor organs are likely easier to be lost than gained; and 3) eye loss is correlated with habitat shifts into low light habitats.

## Material and Methods

### *Phylogenetic analyses*

To estimate ancestral states for eye morphology and ecological transitions under a robust evolutionary framework, we first generated a phylogeny for the Pteriomorphia. We used the transcriptomic data generated by Lemer et al. (2016) for 40 pteriomorphian species in combination with *GenBank* nucleotide sequences for additional 157 species, to comprehensively sample the taxonomic diversity within the clade. In total, the phylogenetic analysis had 205 taxa, including 197 pteriomorphian species and eight other bivalve species from Protobranchia, Paleoheterodonta, and Heterodonta as outgroups (Table S1). We used the amino acid sequences from 277 orthologs in Matrix 2 (Lemer et al. 2016), which had a greater than 75% matrix occupancy per taxon (64,318 amino acids). In addition, we included nucleotide sequences from mitochondrial (16S rRNA and COI) and nuclear (18S rRNA, 28S rRNA and Histone H3) genes available in *GenBank*, for a total character matrix of 6,677 bp. All molecular data with respective accession numbers are listed in Table S1. Alignments were performed in MAFFT v7.311 under the L-INS-i method (Katoh and Standley 2013). ModelFinder (Kalyaanamoorthy et al. 2017) was used to determine the best-fit model of sequence

evolution for each partition: GTR+I+G (nucleotide sequences) and LG+F+I+G (amino acid sequences from the transcriptome dataset). A partitioned analysis was performed in IQ-TREE v1.6.12 (Nguyen et al. 2014) under maximum likelihood and branch support was assessed with ultrafast bootstrap approximation with 10,000 replicates (Hoang et al. 2017).

#### *Ecological and morphological investigation*

Mantle photoreceptor organs were investigated for presence, type, location, and general morphology in 162 species from 22 families of Pteriomorphia (Table S2). Museum specimens were examined in ethanol under the stereomicroscope (Table S2). Histological sections were obtained for six species from different pteriomorphian clades to provide a general anatomical characterization of each eye type, except for the limid invaginated eyes (samples were not available). Sample collection, tissue fixation and additional procedures are detailed in Supporting Information S5.

Ecological data were compiled from literature for 197 species included in the phylogenetic analysis (Table S3). Bivalves frequently display quite different ecologies during their life cycle, from larva to adult stages. Although a variety of ecological factors and selective pressures affect larval phases, we opted for limiting the lifestyle assessment to the adults as only adult animals have photoreceptor organs along the mantle. Major lifestyle categories of adult animals include epifaunal (above the sediment), semi-infaunal (partially buried in the sediment), and infaunal (buried in the sediment). Subsets of epifaunal lifestyles were also discriminated, such as crevice-dwelling.

### *Trait evolution*

To investigate photoreceptor organ evolution and lifestyle transitions, traits were coded, and states were assigned to terminals (Table S4). Not all taxa with molecular data could be represented by morphological museum specimens. Thus, we designed our collection of morphological data to cover most of pteriomorphian diversity across all taxonomic levels. Morphological states for 59 species with sequence data (30% of all pteriomorphian taxa used for phylogenetics) were assigned based on the literature or as equivalent to the closest relative, *i.e.*, examining species within the same genus (taxa indicated in bold in Table S4). We used a maximum likelihood (ML) approach in Mesquite v3.51 (Maddison and Maddison 2018) to perform independent ancestral state estimations (ASE) across the phylogeny to determine if photoreceptor organs had multiple origins (hypothesis 1). Whether transition rates to and from eyed and eyeless states varied were examined for each organ type (hypothesis 2). We compared the empirical fit of the discrete morphological and ecological data to the ML tree under two evolutionary models for transitions: equal rates (Symm, *i.e.*, symmetrical, with  $q_{1 \rightarrow 0} = q_{0 \rightarrow 1}$ ) and different rates (Asymm, *i.e.*, asymmetrical,  $q_{1 \rightarrow 0} \neq q_{0 \rightarrow 1}$ ). Then we identified the best-fit model of character evolution using a likelihood ratio test (LRT) (Pagel 1999; Maddison and Maddison 2018).

In order to test the evolutionary association between photoreceptor organs and lifestyle changes (hypothesis 3), we performed Pagel's correlation tests in Mesquite based on model comparisons. The  $p$  value was estimated from 10,000 repeated simulations with 10 iterations. The correlation hypothesis was accepted when  $p < 0.05$ , indicating a better fit of the model with correlation between traits (eight parameters model) over the model with independent traits (four parameters model) (Pagel 1994; Maddison and Maddison 2018). Additional tests were performed considering the six parameters model representing evolutionary dependence among traits, *i.e.*,

when state transition in one trait is likely to depend on the state of the second trait (Pagel 1994; Maddison and Maddison 2018).

## Results

### *Phylogenetic hypothesis*

Our phylogenetic analysis includes the greatest taxonomic sampling of Pteriomorphia to date (22 out of 26 families). The Pteriomorphia was recovered as monophyletic, with high support for internal nodes (Fig. 1). The ML tree recovers five monophyletic orders, *i.e.*, Arcida, Limida, Mytilida, Ostreida, and Pectinida (Fig. 1). The Mytilida is comprised of a single family, Mytilidae, while the Ostreida embraces Ostreoidea (Ostreidae+Gryphaeidae) sister to the Pterioidea (Isognomonidae, Malleidae, Margaritidae, Pteriidae, Vulsellidae), and Pinnidae as the sister group to the remaining Ostreida. Within Arcida, all families were recovered monophyletic but “Arcidae”, which is polyphyletic. The Limopsoidea (Limopsidae+Philobryidae) is recovered monophyletic, sister to the Glycymerididae. The Limida, comprised of a single family Limidae, is sister to the Pectinida, which consists of [(Anomiidae+Dimyidae)+Plicatulidae] sister to the Pectinoidea (Pectinidae, “Propeamussiidae”, and Spondylidae). The “Propeamussiidae” is not monophyletic in our analysis, as *Propeamussium dalli* was recovered as sister to the Spondylidae, and a grade of “propeamussiid” species with *Cyclopecten sp.* as the sister group to the Pectinidae.

### *Evolution and diversity of photoreceptor organs*

Based on ancestral state estimation, the pteriomorphian ancestor likely bore no elaborated photoreceptor organs (Fig. 2). Subsequently, photoreceptor organs have arisen at least five times

across 11 families in the orders Arcida, Limida, Ostreida, and Pectinida (Fig. 2) (Table S2). These photoreceptor organs can be categorized in five types based on morphology and each type is associated with a different pteriomorphian clade: 1) cap eyespots, 2) pigmented cups, 3) compound eyes, 4) concave mirror eyes, and 5) invaginated eyes (Fig. 2). These data support the hypothesis (1) that pteriomorphian eyes had multiple origins.

We found that the rate of photoreceptor organ gain differed greatly from the rate of loss. In all cases, the asymmetrical model (Asymm), which allows different transition rates, represents the best-fit model for photoreceptor organ data to the phylogeny (Table 1). Interestingly, transition rates for photoreceptor loss were always much higher than those for gain, supporting our hypothesis (2). For example, the rate of transition for loss of mirror eyes is 20 times higher than the converse ( $q_{0 \rightarrow 1} = 0.0738$ ;  $q_{1 \rightarrow 0} = 1.4804$ ). In the case of pigmented eyespots, the rate of loss is almost 84 times higher ( $q_{0 \rightarrow 1} = 0.1058$ ;  $q_{1 \rightarrow 0} = 8.8771$ ). This pattern of lower rates for gain and higher rates for loss are summarized in Table 1. Results from ancestral state estimations were consistent with these observations, suggesting single events for eye origin and multiple losses within lineages (Figure 2).

In the Ostreida (oysters and relatives), photoreceptor organs are restricted to Malleidae (hammer oysters) and Isognomonidae, in which pigmented, photoreceptor cells are arranged in small clusters (Fig. 3C), repeatedly distributed on the outer mantle fold (Fig. 3, Table S2). These pigmented cap eyespots were likely present (proportional likelihood: 0.95) in the ancestor of both families (*i.e.*, Pterioidea) (Fig. 2, red). Subsequent losses of cap eyespots have occurred broadly in epifaunal genera, such as *Pteria* (Pteriidae) and *Pinctada* (Margaritidae), as well as individual species within genera like *Isognomon ehippium* and in the infaunal *Malleus malleus* (Fig. 2).

In the Arcida (ark clams and relatives), pigmented cups on the outer mantle fold were present in the ancestor of the clade (proportional likelihood: 0.98), representing a single, ancient origin (Fig. 2, dark blue). In most extant taxa, these eyespots are repeated cup-like structures (Fig. 3D-F) present in the anterior region or restricted to the anterodorsal region, as in *Anadara* species (Table S2). Interestingly, pigmented cups were lost in some lineages, such as the semi-infaunal *Bathyarca*, *Limopsis*, and *Trisidos* (Fig. 2). Compound eyes are a second type of photoreceptor organ present in the clade. These eyes are formed by multiple units of photoreceptor and pigmented cells in a globular arrangement (Fig. 3G-I), posteriorly located on the outer mantle fold. In our analysis, compound eyes also have a single origin (proportional likelihood: 0.94), but arose after eyespots. Subsequently, compound eyes were lost in three clades, *i.e.*, Limopsidae+Philobryidae (= Limopsoidea) (proportional likelihood: 0.88), *Anadara*+(*Barbatia candida*+*B. lacerata*) (proportional likelihood: 0.84), and *Bathyarca glomerula* (proportional likelihood: 1) (Fig. 2, light blue, Table S2).

Invaginated eyes occur in the Limidae (file clams) as small chambers beneath the mantle surface and among the tentacles of the middle fold along its entire extension (Fig. 4A, B). A single origin was reconstructed for the ancestor of *Lima* and *Ctenoides* (proportional likelihood: 0.96) with a loss in *Acesta* (Fig. 2, purple). These organs were not observed in morphological specimens from the limid genera *Acesta* (3 spp.), *Limaria* (4 spp.), and *Limatula* (5 spp.) (Table S2).

Concave mirror eyes are complex visual organs located at the tip of eyestalks, among tentacles in the middle mantle fold (Fig. 4C-K), and formed by a system of lens, double retina, and reflector (mirror) layer (Fig. 4E, H, K). In our analysis, the concave mirror eyes have a single origin in the ancestor of Pectinoidea (Pectinidae, "Propeamussiidae," and Spondylidae; proportional likelihood: 0.96) (Fig. 2, green), with two convergent losses, in a deep-sea clade formed by *Propeamussium* species, and in *Propeamussium dalli* (Fig. 2). In the glass scallops ("Propeamussiidae" *sensu lato*) investigated herein, mirror eyes were only observed in *Cyclopecten*

*subimbrifer*, *Similipecten nanus*, and *Parvamussium pourtalesianum* (Fig. 4C-E, Table S2), and eyes are restricted to the left side of the body, being absent on the right mantle margin (Table S2). The remaining propeamussiid species do not bear eyes in their mantle margin. In contrast, all scallops (Pectinidae) and thorny oysters (Spondylidae) have numerous mirror eyes. These organs are very similar in morphology and distribution along the mantle margin, although pigmentation varies among blue, brown, and black (Fig. 4F-K). In some scallop species from the genera *Euvola*, *Pecten*, and *Argopecten*, eyes are much more numerous on the left mantle lobe, with few units on the right side (Table S2), which usually faces the substrate and supports the body.

#### *Context-dependent association between eyes and ecological shifts*

We found that eyes have evolved exclusively in epifaunal lineages of pteriomorphian bivalves, but did not find a correlation with eye loss and low-light environments. According to ancestral state estimations (Fig. 5A), all pteriomorphian bivalves descend from an epifaunal ancestor (proportional likelihood: 0.98). Thus, the epifaunal lifestyle is a synapomorphy for the clade, considering the plesiomorphic infaunal condition (Fig. 5A). Subsequently, shifts between lifestyles best fit a symmetrical model with equal transition rates (epifaunal vs. semi-infaunal/infaunal;  $q=1.0662$ ; Table 1). Please see supporting information Fig. S1 for details, including ASE of ecological traits in trees containing all terminal species. Photoreceptor organs have exclusively evolved in lineages with epifaunal ancestors (Fig. 2, 5A). For example, concave mirror eyes are present in the cemented thorny oysters (Spondylidae) and in the scallops (Pectinidae), which includes byssally attached, recessing, swimming, and free (unattached) living scallops (Alejandrino et al. 2011). Invaginated eyes had their origin in epifaunal limids living byssally attached in crevices and byssal nests. Pigmented cups and caps had their origin in byssate, epifaunal ancestors of different clades,

such as Arcida and Pterioidea, respectively. Compound eyes were also gained in an epifaunal ancestor within Arcida.

The epifaunal occupation of exposed surfaces was likely replaced by convergent adoption of the crevice-dwelling habit in two different pteriomorphian lineages: Limidae and Pterioidea (proportional likelihood: 0.91 and 0.95, respectively) (Fig. 5B). This lifestyle is characterized by occupation of crevices in hard substrate, such as boulders and corals, by means of byssus attachment, which limits body exposure. When comparing rates of transitions between crevice-dwelling versus non-crevice dwelling habits, the asymmetrical model (Asymm) is the best-fit to the data and the rate of loss for this particular type of epifaunal ecology is almost 25 times higher than the converse ( $q_{0 \rightarrow 1} = 0.2242$ ;  $q_{1 \rightarrow 0} = 5.6561$ ) (Table 1). Two independent gains of mantle photoreceptor organs, respectively in Limidae and Pterioidea, appear associated with the crevice-dwelling habit (Fig. 5B). Our correlation test supports this link between transitions to crevice-dwelling lifestyle and eyespot acquisitions ( $P=0.036 < \alpha=0.05$ , difference in log-likelihoods is 6.8915, Table 2). In fact, the adoption of such ecology may have contributed to the evolution of photoreceptor organs, as suggested by significant evolutionary dependence ( $P=0.012$ , Table 2).

Pigmented cups and pigmented caps have been lost in lineages that shifted to the semi-infaunal/infaunal habit. This is the case of *Malleus malleus* and semi-infaunal lineages within Arcida, such as *Trisidos*, *Eontia*, *Limopsis*, and *Tegillarca* (Fig. 2, 5A). In addition, compound eyes have been lost in lineages that shifted to semi-infaunal habits, such as *Anadara* and *Limopsis* (Fig. 2, 5A). Nevertheless, our analysis does not support a statistical correlation between eye loss and transitions to semi-infaunal/infaunal habits in Pteriomorphia as a whole ( $P=0.1828 > \alpha=0.05$ , difference in log-likelihoods is 2.7126, Table 2), which tends to refute our hypothesis 3.

## Discussion

How eye gain and loss is related to photic environment is a major question in evolutionary biology. In this study, we characterized the evolutionary history of eyes among pteriomorphian bivalves to elucidate how gain and loss of these organs are associated with habitat type and lifestyle. Our expanded phylogeny with broader taxonomic sampling supports the monophyly of Pteriomorphia, which is organized in five orders. Our tree is in accordance with the previous phylogenomic approach by Lemer et al. (2016), which provided the backbone for this study. Using ancestral state estimation methods, we inferred that photoreceptor organs in pteriomorphians have evolved independently at least five times, thus supporting our hypothesis (1). The vastly different morphologies and lineage specificity of these photoreceptor organs strongly suggest that the structures are not homologous. Furthermore, each photoreceptor organ type has been lost multiple times within its pteriomorphian lineage. As will be discussed, these findings have important implications for understanding the evolution of photoreception in marine benthic invertebrates.

### *Photoreceptor organs are hard to gain and easy to lose*

Because complex traits often involve genome-wide connectivity of *cis*-regulatory elements and pleiotropic genes (Albalat and Cañestro 2016; Roscito et al. 2018), it is expected that complex traits have a higher probability of reduction or loss over gain (Maddison 1994; Miller et al. 2005, Sumner-Rooney et al. 2016; Kobayashi et al. 2018). Our study supports this hypothesis, which corresponds to our hypothesis (2), as pteriomorphian photoreceptor organs have much higher rates of loss over gain (20 to over 80 times greater for loss), a pattern observed in other animal groups (e.g., Omland 1997; Cunningham et al 1998; Oakley 2003; Oakley and Cunningham 2002; Niemiller et al. 2012). This high rate of eye loss is likely a conservative estimate as we used models for discrete

trait evolution that are best suited to estimate evolutionary patterns of simple features (Cunningham et al. 1998; Pagel 1999). Scoring eyes as simple binary characters (present vs. absent) assumes that all components of an eye (*e.g.*, phototransduction genes, lens, retina, etc.) are gained and lost as a single unit (reviewed in Oakley and Speiser 2015). Photoreceptor systems, however, are complex, containing multiple biological components, and each of these components can have a different evolutionary history (Serb and Oakley 2005; Plachetzki and Oakley 2007). Indeed, there are many studies demonstrating that not all eye components are lost together, and also that they may be lost in a different sequential order in different lineages (Sumner-Rooney et al. 2016; Vopalensky and Kozmik 2009; Strausfeld et al. 2016; Emerling 2018). Likely, the converse is also true, *i.e.*, light-sensing ability could be acquired before an organ is formed (Nilsson 2009). The understanding of photoreceptor organ evolution in a group of closely-related bivalves provides a foundation for future investigations to examine individual eye components in a holistic manner.

#### *Evolution of photoreceptor organs in association with ecological changes*

Eyes have evolved exclusively in epifaunal lineages of pteriomorphian bivalves, suggesting some level of adaptive innovation in lighted benthic environments. The converse is also true: eyes are usually absent in lineages occurring in low light environments, such as species with semi-infaunal and infaunal habits. When eyes are present in these lineages (*e.g.*, *Anadara* spp.), photoreceptor organs are always plesiomorphic, *i.e.*, have originated in an epifaunal ancestor. However, correlation between eye loss and infaunality was not statistically supported (Table 2).

Some pteriomorphian bivalves use rock and coral crevices as shelters to reduce their accessibility to potential predators (Harper and Skelton 1993; Harper and Morton 1994). Although our analysis was restricted to a limited set of crevice-dwelling taxa, potentially introducing sampling

bias, we found a significant correlation between a lifestyle transition to crevice-dwelling and eye acquisition. In the Pterioidea (oysters and relatives), the crevice-dwelling families Malleidae and Isognomonidae have simple cap eyespots. A second correlation occurs in the file clams (Limidae), which possess more complex invaginated eyes that can detect movements and shadows (Mpitsos 1973) and likely orientate the animal during bouts of swimming (Donovan et al. 2002). In both cases, cap eyespots and invaginated eyes may be related to shelter-seeking behaviors, which can be enhanced by even moderate visual abilities (Sumner-Rooney et al. 2020; Yerramilli and Johnsen 2010; Kirwan et al. 2018; Blevin and Johnsen 2004).

Loss of eyes is often associated with low light environments. Although our results do not support hypothesis (3), loss of photoreceptor organs in pteriomorphians convergently occurred in semi-infaunal/infaunal lineages. The adoption of infaunal lifestyles can be compared to other reduced light conditions, such as in subterranean environments, which can be relevant to eye reduction through, for example, natural selection (Porter and Sumner-Rooney 2018; Sumner-Rooney et al. 2018; Stern and Crandall 2018). Although we did not obtain statistical support, previous work suggests correlations between eye loss and infaunalization in ark clams and relatives (Arcida) (Audino et al. 2019) and other invertebrate groups, such as trilobites (Schoenemann 2018). We speculate that the broad taxonomic sampling of Pteriomorphia, which included the semi-infaunal representatives of Pinnidae and Mytilidae, whose ancestors likely never had eyes, might have contributed to mask a possible correlation between loss of eyes and infaunality. Future studies comparing the diversity of opsins and eye regulatory genes of eyeless infaunal species and eyed species should cast further light into the association between ecological shifts and eye evolution.

The occupation of deep-waters also reflects conditions that may be analogous to low light habitats. In a previous attempt to explore the evolutionary connection between lifestyle and mirror eyes in scallops (Pectinidae), differences in retina morphology were revealed to be associated with

different depth ranges (Malkowsky and Götze 2014). In addition, a tendency in eye reduction is exhibited from shallow to deep-water species (Malkowsky and Götze 2014), and depth was previously suggested to act as a selective force driving mirror eye evolution (Morton 2001; Malkowsky and Götze 2014). Moreover, for Glycymerididae (Arcida), visual acuity has been suggested to decrease with depth (Morton and Puljas 2015). In the case of the glass scallops (“Propeamussiidae”), bathymetric range is from shallow subtidal depths to approximately 5,000 m with most species occurring in deep-waters, over fine- to coarse-grained sediments (Waller 2006). Interestingly, we have identified loss of mirror eyes in the ancestor of a deep-sea *Propeamussium* spp. clade, which we hypothesize that could be related with invasion of deep waters. To test this hypothesis, however, future work will require detailed morphological study and denser taxonomic sampling of “Propeamussiidae” to develop a more comprehensive phylogenetic framework.

Future work should examine pteriomorphian eye evolution in association with visually-mediated behaviors. It has been argued that behavioral tasks may be the driving evolutionary force from general photosensitivity to low-resolution vision (Nilsson and Bok 2017). The ecological features presented here provide insights to explore the link between bivalve eye evolution and corresponding behaviors, from the perspective of a byssally attached, epifaunal ancestor. For example, morphological and ecological features indicate that the pigmented cups of ark clams (Arcida) may detect local changes in light intensity, possibly related to simple phototaxis, enabling selective positioning and attachment on hard substrate (Morton 1987; Audino and Marian 2018). While in the same species, movement and directional information required to alert the animal to potential predators may have driven refinement of the arcidan’s compound eyes (Nilsson 1994). The crevice-dwelling habit in *Malleus* and *Isognomon* (Pterioidea) may be associated with simple phototaxis behavior and body orientation by directional photoreception provided by the cap eyespots. A convergent lifestyle in the file clams (Limidae) may also have been associated with body

orientation using low-resolution vision and alarm responses by optical inputs in the invaginated eyes (Dougherty et al. 2017). These eyes may also play an important role in swimming behavior of file clams (Donovan et al 2004). Mirror eyes, by their turn, are potentially associated with body orientation and positioning behaviors using low resolution vision in scallops, as well as alarm responses (Hamilton and Koch 1996; Morton 2000b; Speiser and Johnsen 2008). However, the adaptive roles of concave mirror eyes are still debatable, and the investigation of the evolution of such a complex structure is far from being exhausted.

Altogether, our findings indicate association between bivalve eyes and light-exposed environments, given that: (1) eyes have evolved exclusively in epifaunal lineages; (2) there is statistical correlation between transitions to crevice-dwelling habit and gains of eyespots; and (3) eyes were convergently lost during transitions to infaunal habits (although lacking statistical support). Combining these findings with the above morphological and behavioral information on bivalve eyes, we hypothesize that phototaxis (or even area taxis), posture control, and alarm responses might have played major roles during eye evolution in pteriomorphian bivalves, which were likely related to epifaunal habit diversification in this clade.

#### *Comparative morphology of bivalve photoreceptor organs*

Photoreceptor organs have only been examined in a few species for each pteriomorphian family. In our morphological investigation, we documented and described new cases of each eye type. Cap eyespots, *i.e.* pigmented, photoreceptor cells organized in clusters along the outer mantle fold, were known from Pterioidea. Here, the presence of these structures in hammer oyster (Malleidae) is described for the first time, revealing numerous pigmented caps in *Malleus albus*, *M. candeanus*, *M. regula*, but not in *M. malleus*. These organs are also present in Isognomonidae

(except for *Isognomon ehippium*), and were previously reported for *Isognomon spathulatus* (Tëmkin and Printrakoon 2016), *I. legumen* (Harper and Morton 1994), and *I. radiatus* (Tëmkin 2006). In *Pteria brevialeta*, putative mantle photoreceptors were described as eosinophilic cells (possibly photosensory) scattered along the outer mantle fold, based on histological sections (Morton 1995). We did not observe pigmented eyespots in the *Pteria* species and, in accordance with Tëmkin (2006), we argue that although those eosinophilic cells in *P. brevialeta* may have a role in photoreception, their interpretation as ocelli lacks evidence. Considering the ancestral state estimations (Fig. 2), cap eyespots may have been present in the ancestor of Pterioidea, with a subsequent loss in *Pteria*. Therefore, the eosinophilic cells described in *P. brevialeta* may represent a vestigial or reduced tissue of a plesiomorphic photoreceptor organ.

In ark clams and relatives (Arcida), the presence of pigmented cups is relatively well known for *Barbatia virescens* (Morton 1987) and *B. candida* (Audino and Marian 2018). The histological organization of pigmented cups was described for *Philobrya munita* (Morton 1978), although further details were not available for the remaining Philobryidae. Compound eyes are common organs among arcidan species, with anatomical details available for the genera *Arca*, *Barbatia*, and *Glycymeris* (Waller 1980; Nilsson 1994; Morton and Peharda 2008; Morton and Puljas 2015; Audino and Marian 2018). As described herein, compound eyes are restricted to the posterior region of the mantle, while pigmented cups are more frequent in the anterior and anterodorsal region (Table S2). Considering the body orientation of live animals on the substrate, the posterior region of the mantle is more exposed to the surrounding environment in both infaunal and epifaunal bivalves, while the anterior region is usually closer to the substrate (Oliver and Holmes 2006). Such a pattern of distribution may be related to different light-guided behaviors for each type of photoreceptor organ, as hypothesized above.

Mirror eyes are present in all scallops (Pectinidae) and thorny oysters (Spondylidae) examined to date. Detailed studies are available for selected species, including comparative anatomy and visual properties relative to image formation (Dakin 1928; Land 2000; Speiser and Johnsen 2008; Speiser et al. 2011; Malkowsky and Jochum 2014; Audino et al. 2015; Palmer et al. 2017), and even pupilar response (Miller et al. 2019). Interestingly, mirror eyes do not occur in all “Propeamussiidae” *sensu lato*, as indicated by our survey (Table S2) and by the description of the eyeless *Propeamussium lucidum* (Morton and Thurston 1989). A more comprehensive analysis of “Propeamussiidae” is still required to understand the anatomy of these animals and the taxonomic distribution of eyes. Unfortunately, these small and delicate bivalves occur in deep waters and their soft parts are rarely preserved in museum collections, making anatomical investigation a difficult task.

Mantle eyes in file clams (Limidae) are invaginated structures located on the middle mantle fold and embedded within connective tissue beneath the epithelium (Mpitsos 1973). Electrophysiological properties were investigated in the photosensory cells of *Ctenoides scaber* (Mpitsos 1973; Nasi 1991), while functional insights related to visual capabilities were obtained for *C. ales* (Dougherty et al. 2017). In *C. mitis*, eye capsules have cornea, lens, and an organized retina (Morton 2000a); a similar morphology is present in *C. ales* (Dougherty et al. 2017). Invaginated eyes were also reported for *C. miamiensis*, *C. obliquus*, *C. planulatus* (Mikkelsen and Bieler 2003), as well as for *C. mitis* and *C. scaber*, both included in this study. This type of eye is also present in the genus *Lima*, such as in *Lima caribaea* (Mikkelsen and Bieler 2003), but previous descriptions also include lensless cup-shaped organs in *Lima squamosa* and *L. lima* (Hesse 1900; Dakin 1928; Waller 1975). Based on possible anatomical differences, such as lens presence, it has been previously suggested that invaginated eyes in *Ctenoides* and *Lima* actually comprise two different types (Morton 2000a). Our phylogenetic data suggests that photoreceptor organs have evolved just once in the clade, but

we have treated all cases as having the same state, as it was not possible to obtain histological sections from the Limidae. Therefore, future histological investigation is required to elucidate the organization of invaginated eyes in different limid genera, and to verify the uniqueness of this eye type. In addition, eyes were not observed in *Limaria inflata* and *Limaria hians* (Hesse 1900), or in any studied species from the genera *Acesta*, *Limaria*, and *Limatula* (Table S2). Additional investigation should confirm if they represent losses of invaginated eyes.

## Conclusions

Using a clade of benthic, sessile marine organisms, our analyses add to the increasing body of evidence showing that:

- (1) Evolution exploited a myriad of ways of using light as a source of information: during the evolutionary history of the Pteriomorphia, five morphologically distinct types of photoreceptor organs arose independently;
- (2) Eyes are complex traits: photoreceptor organs consistently show higher rates of loss over gain during the evolution of Pteriomorphia;
- (3) Eyes are likely costly: these organs were convergently lost in pteriomorphian lineages that transitioned to environments with reduced-light conditions, such as the infaunal habit, which is a similar scenario of eye regression in aphotic habitats (*e.g.*, deep-sea, caves) seen in other animal taxa;
- (4) Eyes evolve in association with light-guided behaviors: in Pteriomorphia, photoreceptor organs arose only in epifaunal lineages, and independent transitions to the “crevice-dwelling” habit were associated with convergent gains of eyespots in two clades (Limidae and Pterioidea), suggesting that

the photoreceptor organs evolved in association with a combination of light-guided behaviors – such as phototaxis, body orientation, and alarm responses.

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## Tables

**Table 1.** Likelihood ratio test (LRT) between the symmetrical model (Symm) with equal transition rates ( $q_{1\rightarrow 0} = q_{0\rightarrow 1}$ ) and the asymmetrical model (Asymm) that allows different transition rates ( $q_{1\rightarrow 0} \neq q_{0\rightarrow 1}$ ). Note that the former is a simple model nested within the later. The likelihood ratio test is calculated as  $2[\ln L(\text{Symm}) - \ln L(\text{Asymm})]$ , which follows a chi-square distribution with  $df=1$ . Significant P-values are marked in bold. States for photoreceptor organs and for crevice-dwelling habit include absence (0) and presence (1). Main lifestyle states are epifaunal (0) and semi-infaunal/infaunal (1).

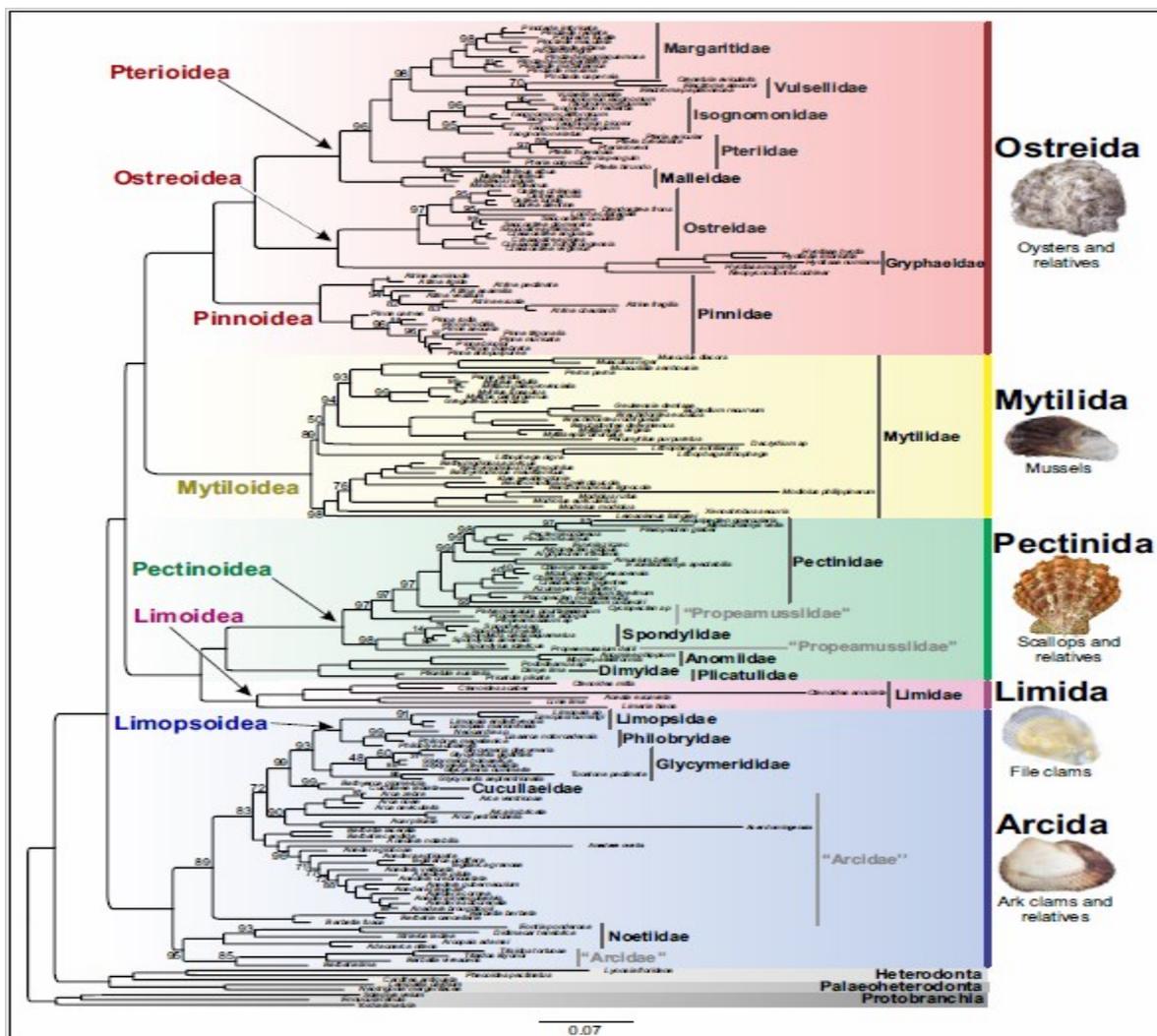
Models	Photoreceptor organs					Lifestyles	
	Cap eyespots	Pigmented cups	Compound eyes	Mirror eyes	Invaginated eyes	Main lifestyle	Crevice-dwelling habit
<b>Symm</b>							
-ln L	-43.2571	-26.2914	-25.0455	-14.629	-11.4488	-62.084	-39.8066
$q$	0.6168	0.2633	0.2623	0.1391	0.13	1.0662	0.5557
<b>Asymm</b>							
-ln L	-38.1407	-18.1502	-21.9679	-12.57	-9.36532	-61.902	-35.7669
$q_{0\rightarrow 1}$	0.1471	0.1058	0.09011	0.0738	0.072	1.1605	0.2242
$q_{1\rightarrow 0}$	4.2347	8.8771	4.6173	1.4804	1.6187	0.7692	5.6561
<b>Likelihood ratio</b>	10.2327	16.2822	6.1551	4.1177	4.167	0.3636	8.0794
<b>P-value</b>	<b>0.0014</b>	<b>5.4572e-5</b>	<b>0.0131</b>	<b>0.0424</b>	<b>0.0412</b>	0.5465	<b>0.0045</b>

**Table 2.** Evolutionary correlation tests between photoreceptor organs ( $x$ ) and lifestyles ( $y$ ) in pteriomorphian bivalves. The test compares the four-parameter model for independent evolution ( $h_0$ ) and the eight-parameter models for correlated evolution ( $h_1, h_2, h_3$ ). In addition, the six-parameter model ( $x$ 's evolution is independent of  $y$ ) was compared to the eight-parameter model ( $x$ 's evolution is dependent of  $y$ ) to test possible evolutionary dependence between trait transitions. The differences in log-likelihood ( $-\log L$ ) are presented with P-values calculated by 10,000 simulations. The model of correlated evolution has a better fit to the data when differences between models are significant, *i.e.*, P-value  $< \alpha=0.05$  (marked in bold). Characters and respective states: *mantle photoreceptor organs*, absent (0) or present (1); *benthic mode of life*, epifaunal (0) or semi-infaunal/infaunal (1); *epifaunal crevice-dwelling habit*, absent (0) or present (1).

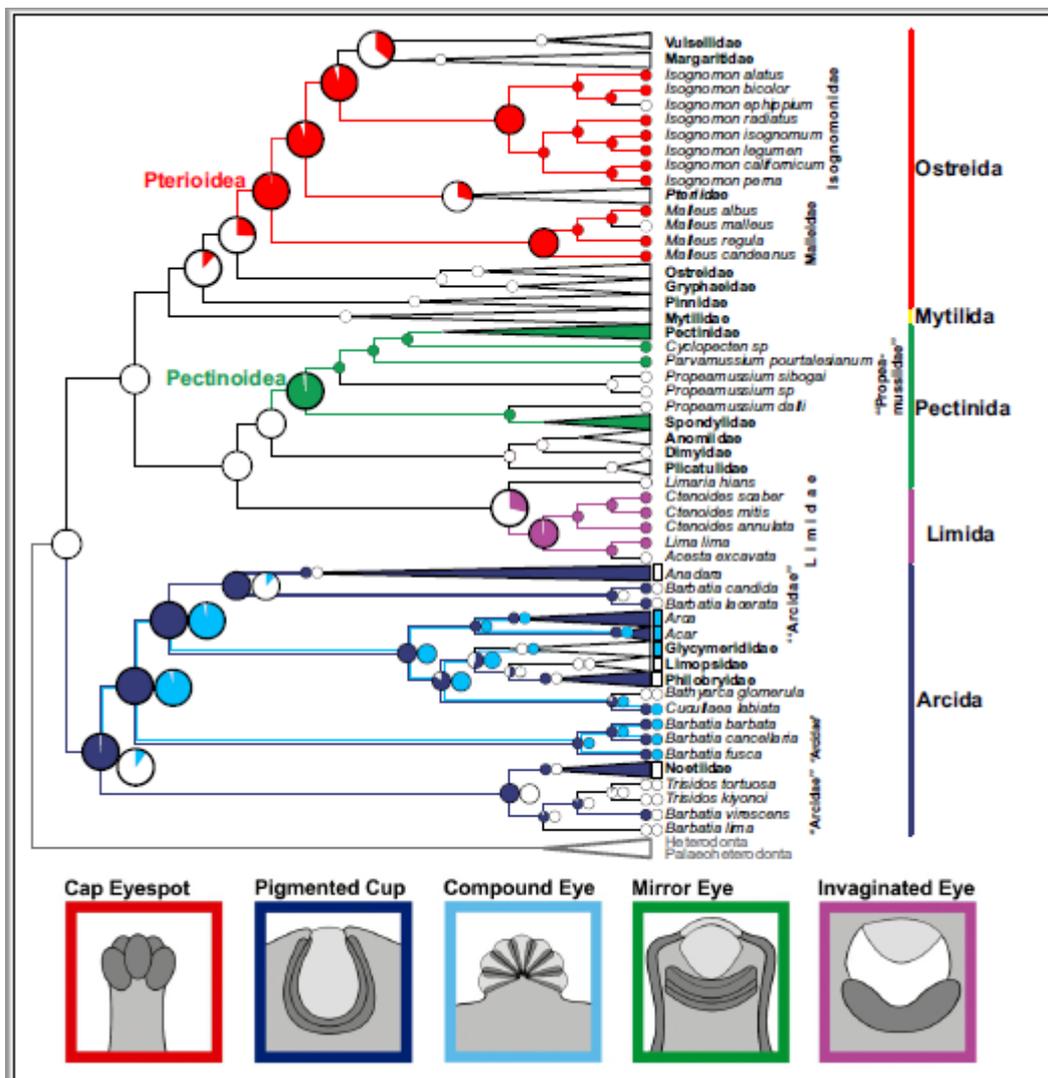
Traits and hypotheses ( $h$ )	Mantle photoreceptor organs ( $x$ )		
	Difference in $-\log L$ between models	P-value	Conclusion
Ecologies ( $y$ )			
<b>Benthic mode of life</b>			
$h_1$ $x$ and $y$ are correlated	2.7126	0.1828	independent traits
$h_2$ $x$ depends on $y$	2.2917	0.1889	$x$ does not depend on $y$
$h_3$ $y$ depends on $x$	1.8294	0.173	$y$ does not depend on $x$
<b>Crevice-dwelling habit</b>			
$h_1$ $x$ and $y$ are correlated	6.8915	<b>0.036</b>	correlated traits
$h_2$ $x$ depends on $y$	1.3714	<b>0.012</b>	$x$ depends on $y$
$h_3$ $y$ depends on $x$	3.2855	0.104	$y$ does not depend on $x$

## Figure legends

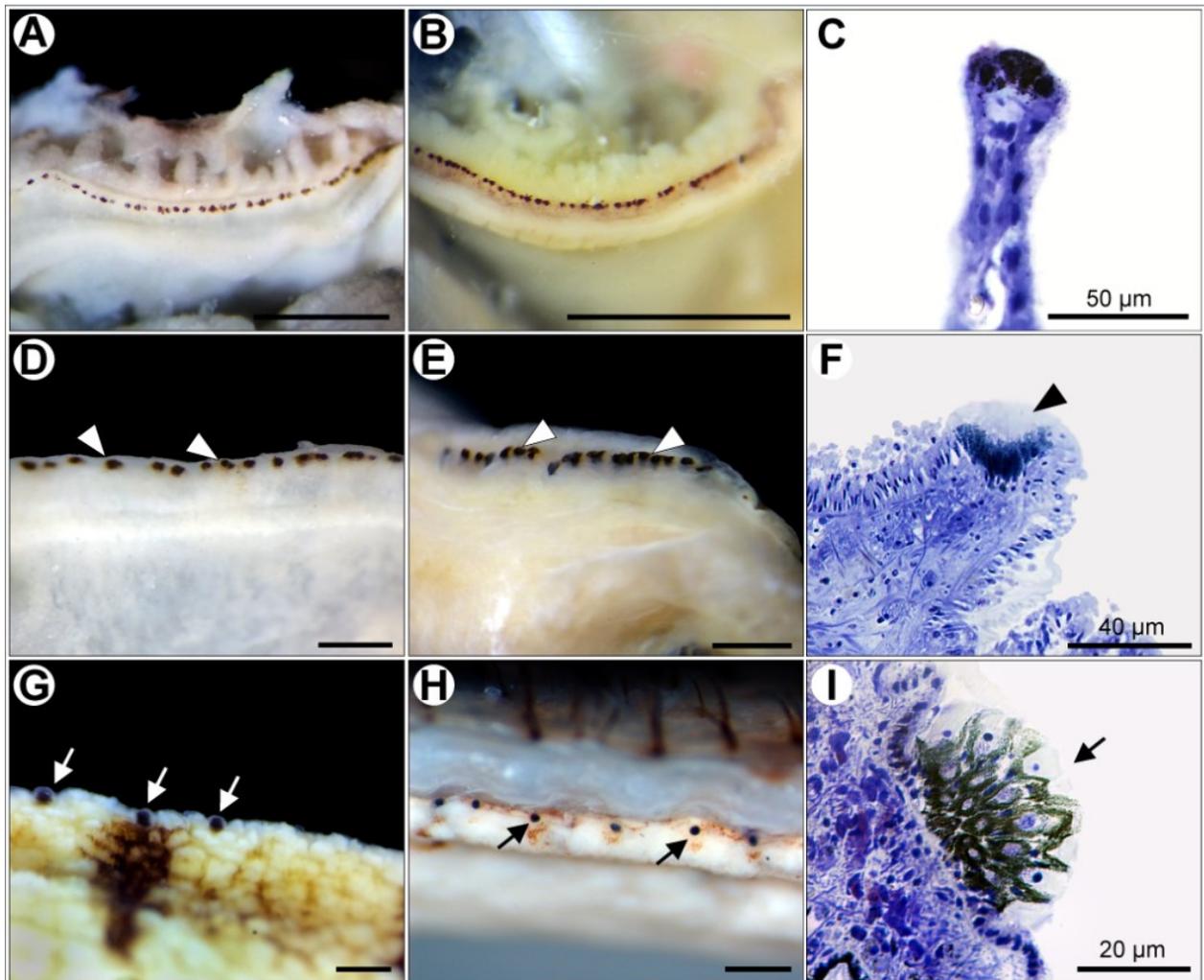
**Figure 1. Pteriomorphia phylogeny.** Maximum likelihood phylogeny of Pteriomorphia based on 277 ortholog genes (dataset from Lemer et al. 2016) and five nucleotide sequences (16S rRNA, 18S rRNA, 28S rRNA, COI, and H3) from *GenBank*. Internal nodes are indicated with respective bootstrap values; nodes with no labels represent bootstrap = 100. Most families were recovered monophyletic (names in black). Non-monophyletic families (grey) are indicated with quotation marks. The five monophyletic orders are depicted by color (Arcida, Limida, Mytilida, Ostreida, and Pectinida) and superfamilies within each order reflect the same color code.



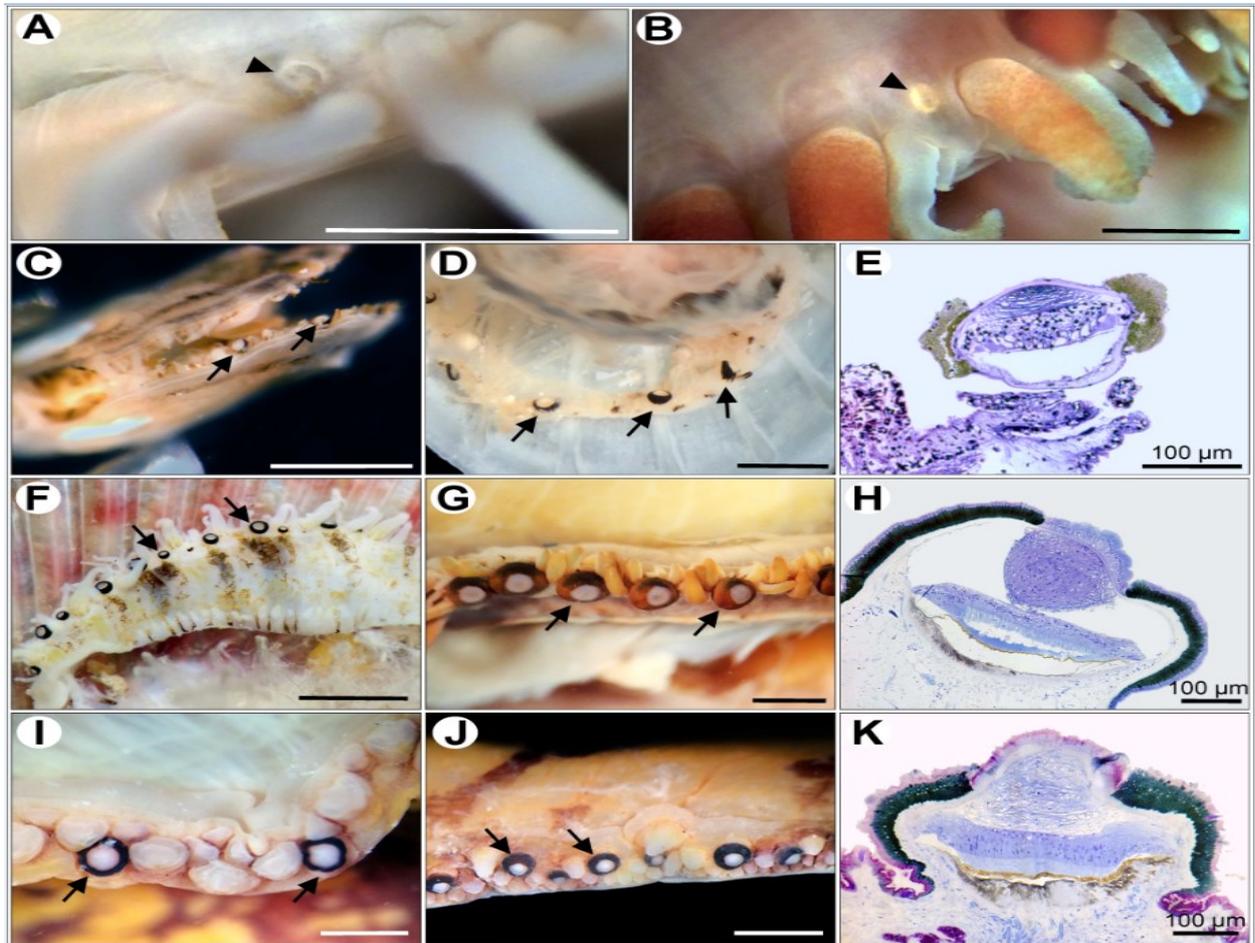
**Figure 2. Evolution of photoreceptor organs.** Summary of five independent ancestral state estimations for photoreceptor organs within Pteriomorpha under maximum likelihood. Pie charts indicate the proportional likelihood scores for presence and absence states. Chart sizes are differently shown for visual purposes. The five types of photoreceptor organs are depicted and color coded. All traits were estimated under the asymmetrical model (best-fit model) allowing for different transition rates. Compound eyes (light blue) had their estimations indicated at the right side of respective nodes and terminals to prevent overlaying the reconstruction of pigmented cups, a different type of photoreceptor organ also present in most arcidans. Eyeless clades were intentionally collapsed to facilitate data presentation.



**Figure 3. Photoreceptor organs in Ostreida and Arcida.** Pigmented cap eyespots (A-C), pigmented cups (D-F; arrowheads), and compound eyes (G-I; arrows). **A.** *Malleus candeanus* (MCZ340681; Malleidae). **B.** *Isognomon radiatus* (USNM803357; Isognomonidae). **C.** *Isognomon bicolor* (collected specimen; Isognomonidae). **D.** *Acar gradata* (USNM796185; Arcidae). **E.** *Anadara antiquata* (USNM802329; Arcidae). **F.** *Barbatia cancellaria* (MZSP48857; Arcidae). **G.** *Cucullaea labiata* (USNM746883; Cucullaeidae). **H.** *Glycymeris undata* (MZSP91983; Glycymerididae). **I.** *Acar dominguensis* (MZSP118292; Arcidae). Scale bars = 0.5 mm. Longitudinal sections in C, F, and I.



**Figure 4. Photoreceptor organs in Limida and Pectinida.** Invaginated eyes (A-B; arrowheads) and concave mirror eyes (C-K; arrows) . **A.** *Ctenoides floridanus* (USNM664306; Limidae). **B.** *Lima lima* (USNM754383; Limidae). **C.** *Parvamussium pourtalesianum* (USNM856965; “Propeamussiidae”). **D.** *Similipecten nanus* (USNM803327; “Propeamussiidae”). **E.** *Parvamussium pourtalesianum* (USNM856965; “Propeamussiidae”. **F.** *Chlamys varia* (MCZ378918; Pectinidae). **G.** *Pecten jacobaeus* (USNM1086023; Pectinidae). **H.** *Nodipecten nodosus* (collected specimen; Pectinidae). **I.** *Spondylus americanus* (USNM833744; Spondylidae). **J.** *Spondylus senegalensis* (USNM1086035; Spondylidae). **K.** *Spondylus ictericus* (collected specimen; Spondylidae). Scale bars = 1 mm. Longitudinal sections in E, H, and K.



**Figure 5. Lifestyle evolution in Pteriomorphia.** Ancestral state estimations (ASE) of mantle photoreceptor organs (left) and lifestyles (right) under maximum likelihood to fit the symmetrical model with equal transition rates for benthic mode of life and the asymmetrical model with different rates for mantle photoreceptor organs and crevice-dwelling habit. Schematic drawings on the right represent a generalized position of the animal body (black) relative to the substrate (grey). Epifaunal habit: animals living above the sediment; crevice-dwelling habit: particular epifaunal lifestyle in which animals live in crevices among corals, boulders, or debris; semi-infaunal/infaunal habit: animals live partially or completely buried within the sediment, respectively. **A.** Benthic modes of life in Pteriomorphia, indicating an epifaunal ancestor (grey) for the clade and secondary shifts to semi-infaunal/infaunal lifestyles across different lineages (orange). Yellow boxes highlight apparent associations between eye loss (red) and infaunalization. **B.** ASE for the crevice-dwelling habit, showing two independent transitions (green) to this habit in epifaunal groups. Yellow boxes highlight apparent associations between gain of eyespots (blue) and transitions to crevice-dwelling lifestyle. For evolutionary correlation tests, please see Table 2. Some clades were intentionally collapsed to facilitate data presentation.

