Zoologischer Anzeiger 285 (2020) 27–36

Contents lists available at ScienceDirect

Zoologischer Anzeiger

journal homepage: www.elsevier.com/locate/jcz

Research paper

Detailed comparative anatomy of the Pinnidae (Mollusca, Bivalvia) reveals further unusual mantle specializations

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A R T I C L E I N F O

Article history:
Received 22 August 2019
Received in revised form
31 October 2019
Accepted 23 January 2020
Available online 6 February 2020

Corresponding Editor: C. Lueter

Keywords:
Bivalve
Discoid gland
Pinna
Secretion
Semi-infaunal

A B S T R A C T

Pinnids are semi-infaunal bivalves with several unusual morphological features, some of them regarded as adaptations to environments with high concentration of suspended particles. The presence of a waste canal on the mantle wall, for example, helps rejecting surplus particles from the mantle cavity. Despite being such anatomically intriguing bivalves, detailed comparative morphology studies on pinnids are still scarce. To gain further insights into the functional anatomy of pinnids, we have thoroughly investigated the mantle of *Pinna carnea* Gmelin, 1791 by means of scanning electron and light microscopy, including the application of histochemical staining methods. Additionally, the mantle was analyzed in ten pinnids from the genera *Atrina*, *Pinna*, and *Streptopinna* obtained in museum collections. Comparative morphology revealed a uniform structural organization of the mantle in Pinnidae, but a pronounced variation in pigmentation, presence of commarginal folds, and papillae. Our results also revealed intense secretory activity in the inner mantle fold, inner mantle epithelium, and waste canal, including at least three secretory cell types. Secretions are diverse and comprise mainly acidic mucosubstances on the mantle margin, while the waste canal secretes abundant acidophilic material apparently composed of basic proteins, and also possibly lipid content. Based on these results, we hypothesize that efficient mucociliary transportation is achieved in a hydrophobic environment provided by the waste canal, rapidly agglutinating and removing large amounts of particles from the mantle. Discoid glands secreting protein-rich content were found on the middle mantle fold of *Pinna carnea* and *Streptopinna saccata* (Linnaeus, 1758). Surprisingly, these mantle glands have hitherto not been recorded in the Bivalvia, representing, therefore, a new morphological feature in the Pinnidae.

1. Introduction

The Pinnidae Leach, 1819 comprises subtidal and coastal bivalves often called pen shells, fan shells, or even fan mussels. Pinnids are semi-infaunal bivalves living in sandy and muddy sediments, mainly seagrass beds, but also embedded in coral reefs (Rosewater 1961). The body is vertically positioned in the sediment with the anterior region deeply buried and anchored by byssus threads attached to surrounding materials, leaving the enlarged posterior region exposed above the substrate (Grave 1911; Yonge 1953).

The fossil record indicates the Pinnidae dates back to the Mississippian Carboniferous (358.9–323.2 Ma), having an abundant fossil record (Turner & Rosewater 1958; Cox & Hertlein 1969). Comparative morphology of shell and muscle scars among fossil and living species suggests an epifaunal origin for the family, with a subsequent transition to the infaunal habit (Stanley 1972). The extant diversity of pinnids encompasses around 55 species in three genera, i.e., *Atrina*, *Pinna*, and *Streptopinna* (Schultz & Huber, 2013). Based on a comprehensive taxonomic sampling, a phylogenic study of Pinnidae supported the monophyly of the genera *Atrina* and *Pinna*; however, *Streptopinna* is nested within *Pinna* (Lemer et al., 2014).

Pinnids are cosmopolitan, occupying shallow waters in temperate to tropical environments (Turner & Rosewater 1958). Recent investigations have suggested that features related to shell size and shape, as well as burying depth, are habitat adaptations possibly related to bathymetry and sediment composition (Printrakoon et al. 2019). Pinnids are also known to host many commensal organisms, particularly small crustaceans, that find both refuge in the mantle cavity and food supplies in the rejected

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https://doi.org/10.1016/j.jcz.2020.01.006
0044-5231/

particles trapped by the bivalve mucus (Turner & Rosewater 1958; Sastry & Menzel 1962; Richardson et al. 1997; Rabaoui et al. 2008; Aucoin & Himmelman 2010).

The biology and anatomy of Pinnidae were thoroughly investigated in a few species, such as *Atrina fragilis* (Pennant, 1777), *Atrina rigida* (Lightfoot, 1786), *Pinna carnea* Gmelin, 1791, and *Pinna nobilis* Linnaeus, 1758, including studies focused on the structure of the shell and the functional anatomy of ctenidia and stomach (Grave 1911; Atkins 1938; Yonge 1953; Purchon 1957; Turner & Rosewater 1958; Rosewater 1961). In addition, the mantle of pinnids has attracted great attention of morphologists because of two specialized structures uniquely found in this family: the pallial organ and the waste canal.

The pallial organ is an elongated structure located in the suprabranchial chamber, dorsal to the anus and extending posteriorly (Grave 1911). The organ comprises a proximal stalk and a distal, swollen head, which was initially assumed to clean the suprabranchial cavity and remove shell fragments (Yonge 1953). Later, detailed investigations of *Atrina pectinata* (Linnaeus, 1767) and *P. nobilis* revealed that the pallial organ head secretes sulphuric acid, and that these bivalves feed on microcrustaceans (Liang & Morton 1988; Morton & Puljas 2018). The pallial organ is thus thought to be involved in opportunistic prey capture, using acidic secretion to stun or kill their prey, which would then be transported by the strong incurrent stream to the gill ciliary tracts and mouth (Morton & Puljas 2018).

The waste canal was originally described for *A. rigida* and *Pinna nobilis* as a specialization of the mantle that continuously removes pseudofaeces from the mantle cavity (Stenta 1903; Grave 1911). In combination with the posterior extension of the mantle, the waste canal is regarded as a major adaptation for cleansing, possibly associated with the evolution of the pinnid semi-infaunal habit (Yonge 1953). The canal was described as having a ciliated epithelium with mucous glands in *Pinna carnea* (Yonge, 1953). Nevertheless, the nature of their secretions and the distribution of secretory cells along the mantle are still unknown. Similarly, comparative information on mantle morphology is still lacking for the family.

Pinnids are generally found in environments with a high concentration of suspended particles (Grave 1911; Yonge 1953). Given that they are suspension-feeders that use their ciliated gills (i.e., ctenidia) to obtain food particles, they are often faced with the challenge of keeping their mantle cavity clean of surplus sediment particles that would otherwise obstruct their ctenidial filaments. Further information on mantle anatomy and secretory activity should provide additional evidence to understand pinnid adaptations to live in environments with such high concentration of suspended particles.

The present study investigated in detail the mantle margin of *Pinna carnea* to gain insights into its anatomy and inferred functions. We focused on the secretory activity of the mantle margin and waste canal to elucidate possible roles associated with the infaunal lifestyle. In addition, we comparatively analyzed the anatomy of preserved individuals of selected pinnid species to check for patterns in mantle morphology.

2. Material and methods

A specimen of *Pinna carnea* was found partially buried in the sand and among debris in Ilhabela (São Sebastião Island, State of São Paulo, Brazil; 23°45′57.2″S 45°21′00.2″W). Prior to fixation, anesthesia was performed with a 7.5% solution of $MgCl_2$, which was slowly added into the seawater container holding the specimen; complete anesthesia was achieved after 3 h. This procedure was adopted to prevent undesired reflexes, such as shell closure, and to allow a gradual anesthesia, thus reducing the chances of muscular contraction. Subsequently, mantle samples from one individual were dissected for microscopy procedures, fixed for 3 h at 4 °C in a modified Karnovsky solution, and stored in cacodylate buffer (see Audino & Marian 2018).

Mantle morphology was also examined in other nine pinnid species, based on museum specimens (Table 1). These specimens have originally been fixed in formalin and preserved in 70% ethanol, and during our survey they were dissected and analyzed under a stereomicroscope.

Table 1
Species of Pinnidae included in the morphological investigation of the mantle, with respective collection and catalog numbers for observed specimens. Presence and absence of the following structures are indicated by + and −, respectively: (1) waste canal, (2) marginal tentacles on the inner mantle fold, (3) commarginal folds on the mantle epithelium of the suprabranchial chamber, and (4) discoid glands on the middle fold.

Species	Authority	Catalog number	1	2	3	4
Atrina inflata	(Dillwyn, 1817)	MZSP 55029	+	+	−	−
Atrina maura	(G. B. Sowerby I, 1835)	USNM 828614	+	+	−	−
Atrina rigida	(Lightfoot, 1786)	USNM 847971	+	+	−	−
Atrina seminuda	(Lamarck, 1819)	ZUEC-BIV 2135	+	+	−	−
Atrina serrata	(G. B. Sowerby I, 1825)	USNM 801651	+	+	−	−
Atrina vexillum	(Born, 1778)	USNM 793718	+	+	−	−
Pinna carnea	Gmelin, 1791	MZSP 29040 USNM 804284	+	+	+	+
Pinna muricata	Linnaeus, 1758	USNM 836526 MCZ 238056	+	+	+	−
Pinna rudis	Linnaeus, 1758	MZSP 114038	+	+	+	−
Streptopinna saccata	(Linnaeus, 1758)	USNM 793744 USNM 780031	+	+	+	+

Abbreviation of museum collections: MCZ, Museum of Comparative Zoology; MZSP, Museum of Zoology of the University of São Paulo; USNM, Smithsonian National Museum of Natural History; ZUEC-BIV, Museum of Zoology "Prof. Adão José Cardoso" of the University of Campinas.

2.1. Histological investigation

Mantle samples of *P. carnea* were completely dehydrated in a graded ethanol series, embedded in resin (Leica Historesin Kit, Germany) and serial sectioned into 4-μm-thick sections. The following staining methods were applied to describe the nature of secretions from different mantle structures (Humason 1962; Behmer et al. 1976; Bancroft & Stevens 1982; Pearse 1985; Junqueira 1995): hematoxylin and eosin (HE) and toluidine blue and basic fuchsin (TF) were applied for general histological study; Gomori trichrome (GO) and Mallory's trichrome (MA) were used for general investigation and for detection of secretory cells; mercury-bromophenol blue (BB) and naphthol yellow (NY) were used to evidence protein aggregates; Sudan black B (SB) was applied to identify neutral lipids; alcian blue (AB) and periodic acid-Schiff stain (PAS) were used to detect mucosubstances in secretory cells, including glycoconjugates with acidic or neutral carbohydrates, respectively. The histological slides and electron microscopy stubs produced during this study are deposited at the Museum of Zoology 'Prof. Adão José Cardoso' of the State University of Campinas (ZUEC, UNICAMP; catalog numbers: ZUEC-BIV 8174-8180).

2.2. Scanning electron microscopy

Mantle samples were post-fixed, dehydrated, critical point dried, mounted on stubs, coated with gold, and analyzed as described in Audino et al. (2015).

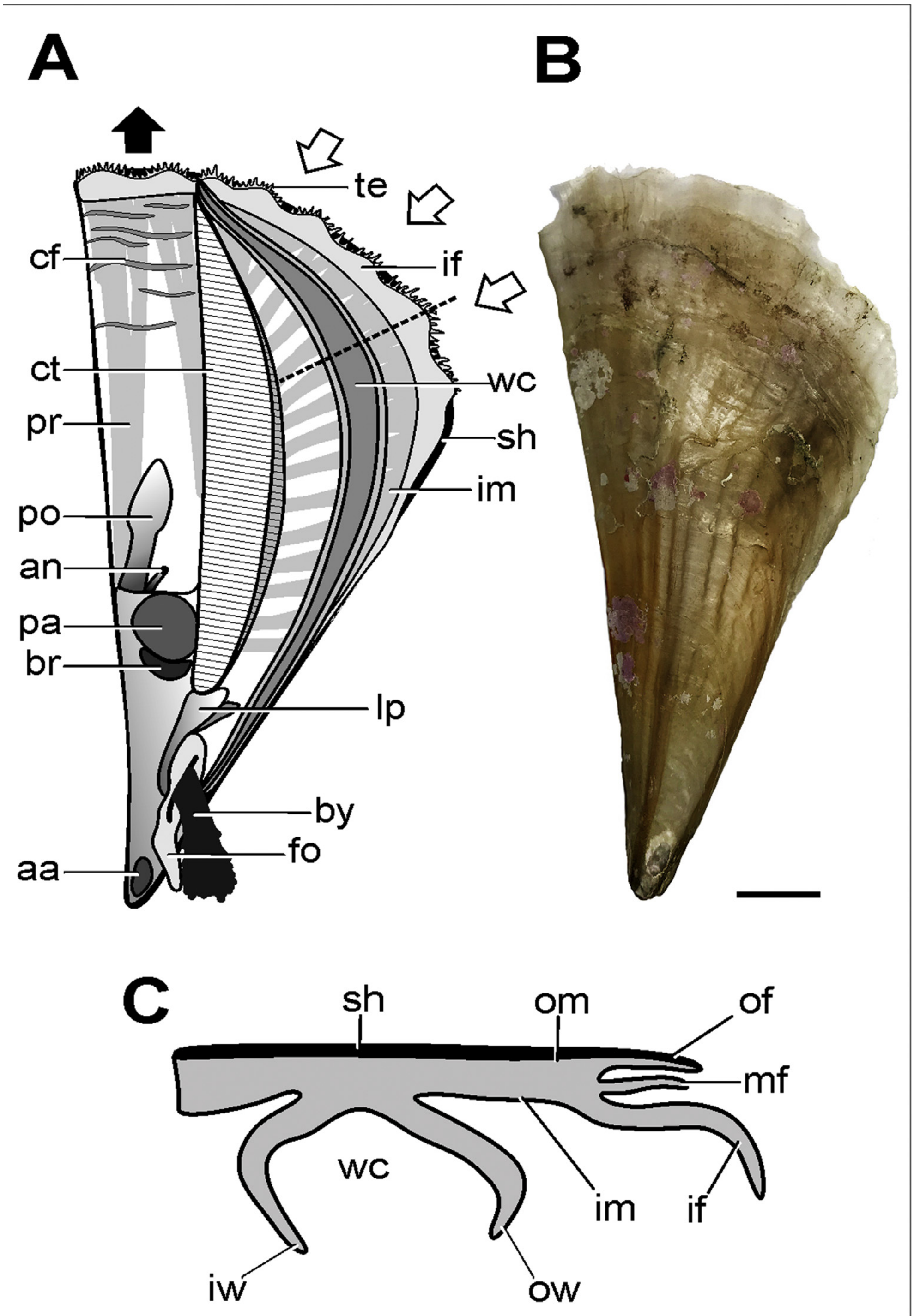

Fig. 1. Mantle and pallial cavity anatomy of *Pinna carnea*. (A) Schematic representation of an individual with the left mantle lobe removed to evidence the waste canal; lateral view, ventral to the right and posterior to the top. Water flow is indicated by incurrent streams (white arrows) to the infrabranchial chamber and excurrent stream (black arrow) out of the suprabranchial chamber. The dashed line represents the region of the section illustrated in C. (B) Left valve of *P. carnea*. (C) Schematic section of the mantle (dashed line in A) showing the mantle margin, mantle folds, and the waste canal; distal to the right. Abbreviations: aa, anterior adductor muscle; an, anus; br, byssal retractor muscle; by, byssus; cf, commarginal mantle folds; ct, ctenidium; fo, foot; if, inner mantle fold; im, inner mantle epithelium; iw, inner waste canal fold; lp, labial palps; mf, middle mantle fold; of, outer mantle fold; om, outer mantle epithelium; ow, outer waste canal fold; pa, posterior adductor muscle; po, pallial organ; pr, mantle retractor muscle; sh, shell; te, tentacle; wc, waste canal.

3. Results

3.1. Functional pallial anatomy in *Pinna carnea*

The mantle is an extensible organ in *Pinna carnea*, being greatly enlarged posteriorly (Fig. 1A–C). Within the infrabranchial (incurrent) chamber, the inner surface of each mantle lobe bears a waste canal (Fig. 1A). Each waste canal is formed by two parallel folds that run from the anterior to the dorso-posterior region where the ctenidia attach to the mantle margin (Fig. 1A). The mantle margin is comprised by outer, middle, and inner folds (Fig. 1C). While all marginal folds have similar size along the antero-ventral region, the inner mantle fold becomes much longer and enlarged posteriorly, where it also bears marginal tentacles (Fig. 1A). The middle and outer folds remain similar along the whole mantle margin extension, although in the posterior region brown pigmentation is present on the outer fold. The inner mantle surface varies from whitish to translucent, containing large muscle fibers that form the mantle retractor system (Fig. 1A). In the suprabranchial (excurrent) chamber, numerous commarginal folds are distributed on both mantle lobes (Fig. 1A).

Scanning electron microscopy showed that the whole mantle epithelium is covered by abundant cilia. The middle fold is covered by long and densely distributed cilia on both inner and outer surfaces; putative sensory structures were not found on this fold (Fig. 2A). Longer and more densely distributed cilia are present on the inner mantle fold (Fig. 2B), as well as numerous droplets of possible mucosubstances interspersed with cilia (Fig. 2C). The commarginal folds are also ciliated (Fig. 2D), with cilia organized in bands and patches (Fig. 2E). In general, the inner mantle epithelium is also densely ciliated, particularly between the inner mantle fold and the waste canal (Fig. 2F). Similar to the inner mantle fold, the outer and inner folds of the waste canal have dense, long cilia sometimes interspersed with abundant droplets of possible mucosubstances (Fig. 2G–I).

Three secretory cell types were identified in the mantle epithelium based on their morphology and staining affinities (Table 2). Type I corresponds to secretory cells containing vesicles with large granules that stained strongly with AB and slightly with MA (in light blue), PAS, SB, and TF (in pink) (Fig. 3A–E, arrows). These cells are abundant on the epithelium of the middle and inner mantle folds (Fig. 3A–C), being also present in the inner mantle epithelium (Fig. 3D and E) and waste canal. Type II secretory cells (Fig. 3, black arrowheads) have medium-sized granules with affinity for eosin (in pink; Fig. 3F, I), MA (in orange; Fig. 3E, L), GO (in red; Fig. 3H), BB

Table 2
Histochemical affinities of the three secretory cell types found in the mantle epithelium of *Pinna carnea*. Positive reactions to different staining methods with weak affinity and strong affinity are indicated by (+) and (++), respectively, while lack of affinity is denoted by (−).

Staining method	Type I	Type II	Type III
Alcian Blue	++	−	+
Bromophenol blue	−	++	−
Gomori trichrome (red staining)	−	++	−
Hematoxylin and eosin (pink staining)	−	++	−
Mallory's trichrome (blue staining)	+	−	+
Mallory's trichrome (orange staining)	−	++	−
Naphthol yellow	−	++	−
PAS	+	+	+
Sudan black B	+	−	++
Toluidine blue and fuchsin (pink staining)	+	−	++
Regions of higher abundance	IF, MF	IM, WC	IM, WC

Mantle regions: IM, inner mantle epithelium; IF, inner mantle fold; MF, middle mantle fold; WC, waste canal. Please see Fig. 1B for location of mantle regions.

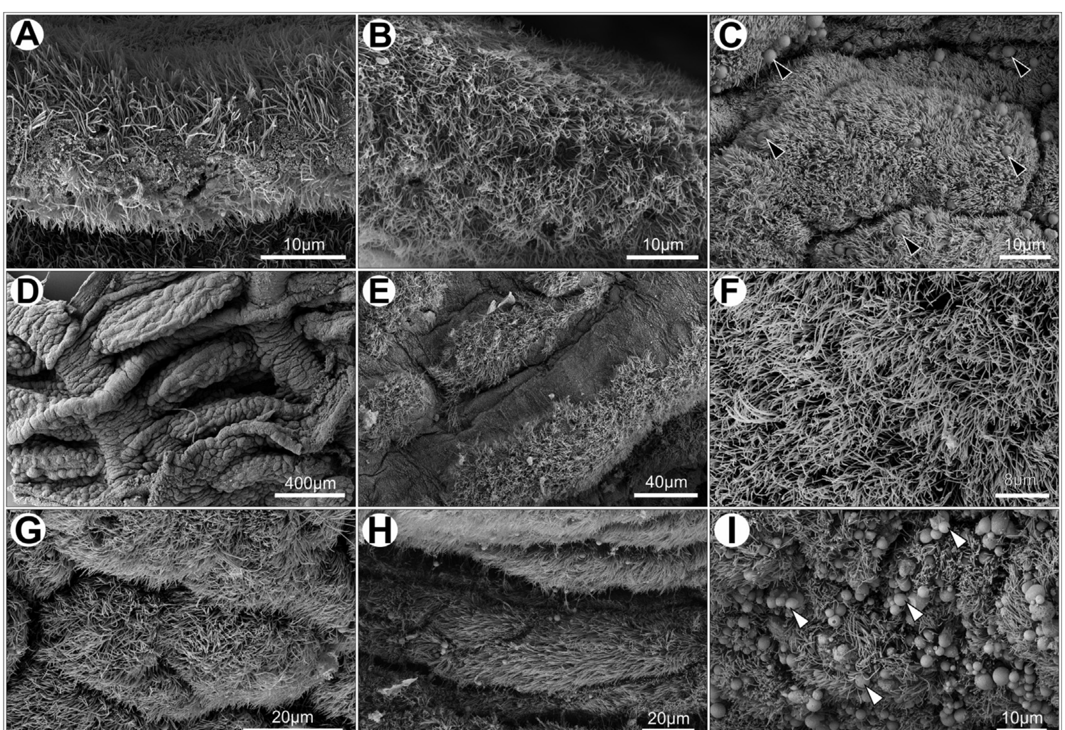

Fig. 2. Mantle epithelium of *Pinna carnea*. Scanning electron micrographs. (A) Cilia distribution on the middle mantle fold. (B) Cilia distribution on the inner mantle fold. (C) Ciliated epithelium of the inner fold, including abundant droplets of secretion (black arrowheads). (D) Commarginal folds in the suprabranchial chamber. (E) Cilia distribution on the commarginal folds. (F) Cilia on the inner mantle epithelium. (G) Ciliated epithelium of the inner fold of the waste canal. (H) Ciliated epithelium of the outer fold of the waste canal. (I) Detail of dense ciliary distribution on the outer fold of the waste canal, with abundant droplets of possible mucosubstances (white arrowheads).

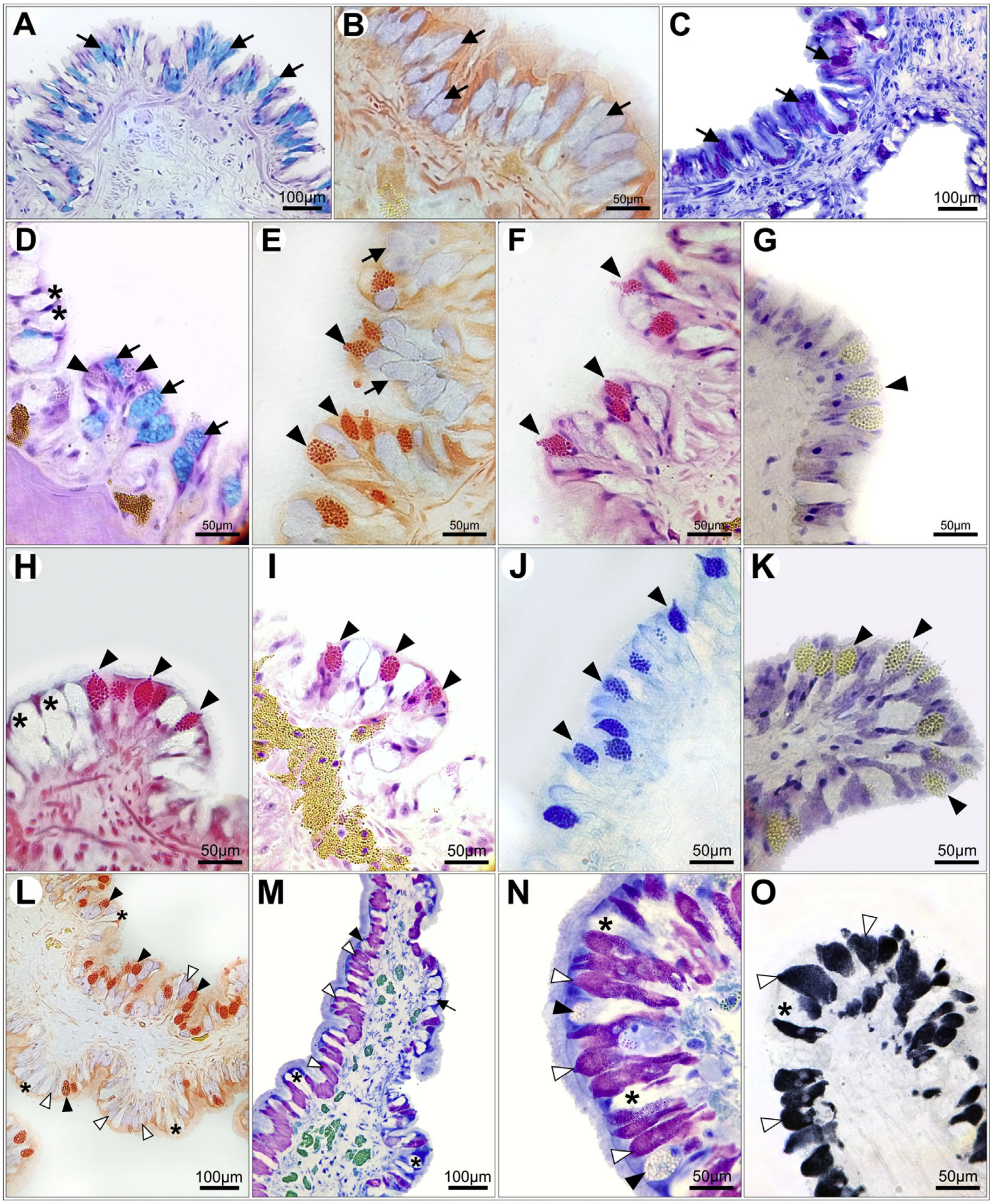

Fig. 3. Mantle secretory activity of *Pinna carnea*. Histological sections of the inner mantle fold in A–C, inner mantle epithelium in D–G, and waste canal in H–O. Three types of secretory cells are evident: type I (arrows), type II (black arrowheads), and type III (white arrowheads). Another possible secretory cell type (asterisks) had no affinity for any of the applied stains. (A) Type I secretory cells, with acidic mucosubstances stained with alcian blue (AB). (B) Same as A, but stained in light blue with Mallory's trichrome (MA). (C) Granular content stained in dark purple with toluidine blue and fuchsin (TF). (D) Periodic acid-Schiff (PAS) and alcian blue (AB); type I secretory cells have strong affinity for AB. (E) Type I secretory cells are slightly stained in light blue (MA), while type II cells in orange (MA). (F–K). Type II secretory cells showing strong affinity for HE (pink staining; F and I), naphthol yellow (G and K), Gomori's Trichrome (red staining; H), and bromophenol blue (J). (L) Type II (orange staining) and type III (light blue staining) secretory cells; MA. (M) TF staining showing most type III secretory cells distributed on the surface of the canal. (N) Detail of type III cells stained with TF (in pink). (O) Type III secretory cells showing affinity for Sudan black B. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

(Fig. 3J) and NY (Fig. 3G, K), and weak affinity for PAS (Fig. 3D). They are distributed in all mantle regions, but mostly in the inner mantle epithelium (Fig. 3D–G) and waste canal (Fig. 3H–L). Type III secretory cells (Fig. 3, white arrowheads) have fine granular content that stained slightly with AB, MA (in blue, Fig. 3L) and PAS, and strongly with TF (in pink; Fig. 3M, N) and SB (Fig. 3O). Type III secretory cells are abundant in the inner mantle epithelium and in both folds of the waste canal (Fig. 3L–O). In the mantle and waste canal epithelia, some secretory cells had no affinity for any of the applied stains (Fig. 3D, H, I, L–O, asterisks), which suggests that at least another secretory cell type is present in these regions.

In the posterior region, the middle mantle fold bears numerous discoid structures distributed on its inner surface, at the base of the fold (Fig. 4). Histological investigation revealed them to be a concentration of secretory cells, therefore justifying the term "discoid gland". Each organ has a cap-like morphology in sagittal section and is attached to the middle fold through a short connection (Fig. 4B). Their secretory cells are elongate; the nuclei are basal and the

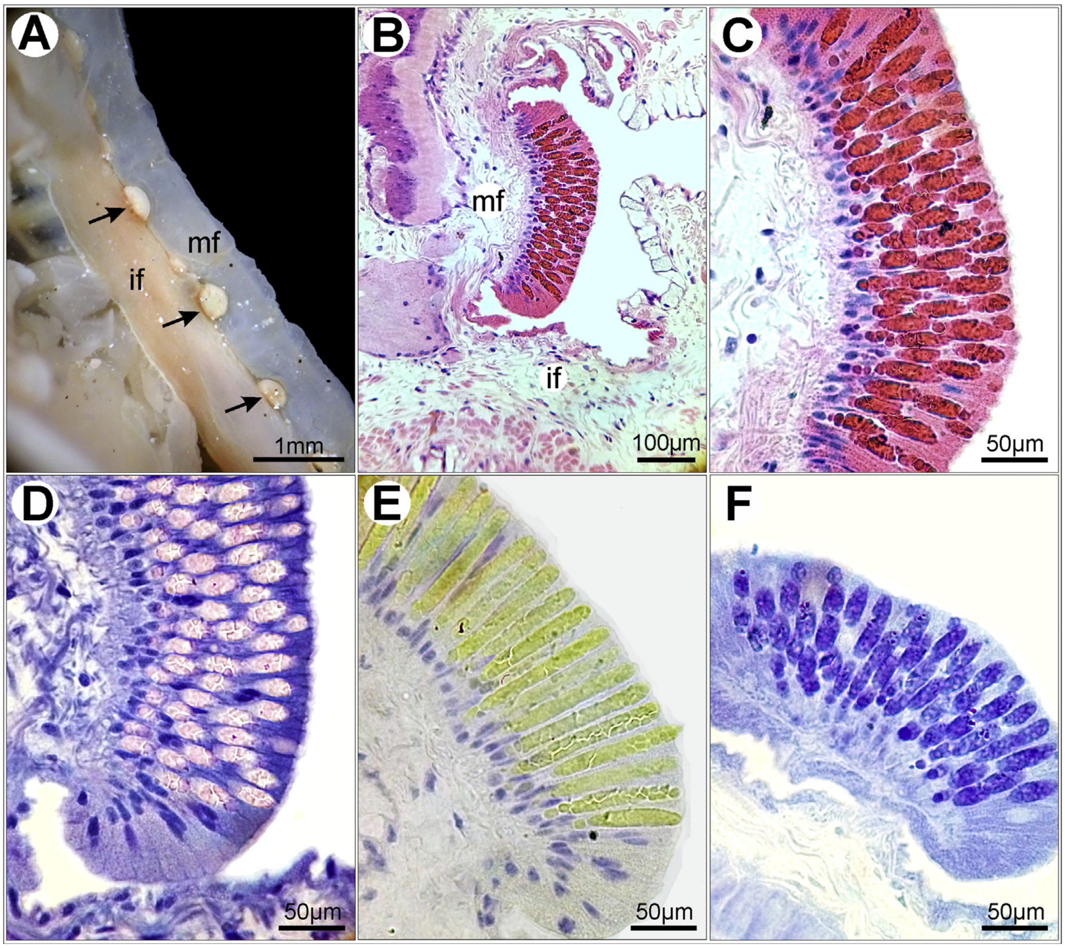

Fig. 4. Discoid glands on the middle fold of *Streptopinna saccata* (A, stereomicrograph, USNM 793744) and *Pinna carnea* (B–F, histological sections, collected specimen). (A) Glands (arrows) regularly distributed on the inner surface of the middle mantle fold. (B) Section of the discoid gland showing its attachment position at the base of the middle mantle fold. (C) Secretory content organized in vesicles and strongly stained by eosin. (D) Secretory cells slightly stained by toluidine blue and fuchsin (light pink). (E) Positive reaction to naphthol yellow. (F) Positive reaction to mercury-bromophenol blue. Abbreviations: if, inner mantle fold; mf, middle mantle fold. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

vesiculated content runs from the base to the apical region, where it is supposedly released. The content had no affinity for AB and PAS, weak affinity for TF (in pink; Fig. 4D), and strong affinity for eosin (Fig. 4C), NY (Fig. 4E), BB (Fig. 4F), MA (in orange) and GO (in red).

3.2. Comparative mantle morphology in Pinnidae

The three genera of Pinnidae, i.e., *Atrina* (Fig. 5A–H), *Pinna* (Fig. 5I–O), and *Streptopinna* (Fig. 5P), have a similar mantle organization (Table 1; Fig. 5). The waste canal is present in all studied species as an enlarged structure in the suprabranchial region (Fig. 5, arrows), frequently exhibiting agglutinated particles from the sediment along its extension. Their mantle margin has an elongated inner fold in the posterior region, also bearing abundant marginal tentacles of similar sizes (Fig. 5B, D, I). Pigmentation varies greatly among species, being frequent on the outer and inner folds, with different patterns of spots and bands, ranging from light yellow to dark brown. In *Atrina*, white papillae may occur on the inner mantle epithelium, as observed in *Atrina seminuda* (Lamarck, 1819) (Fig. 5A). Commarginal folds are only present in *Pinna* and *Streptopinna* (Fig. 5J, M), located in the suprabranchial chamber of both mantle lobes. Discoid glands were found only in *Pinna carnea* and *Streptopinna saccata* (Linnaeus, 1758) (Table 1 and Fig. 4), on the middle fold of the posterior region.

4. Discussion

4.1. The functional pallial anatomy of Pinna carnea

Our results with *Pinna carnea* contribute to expand the knowledge of the pinnid mantle structure and function, previously settled by classical anatomical investigations conducted with *A. rigida, P. carnea,* and *P. nobilis* (Stenta, 1903; Grave, 1911). The waste canal is a remarkable feature involved in mantle cleansing, bearing cilia and numerous mucous glands (Yonge 1953). Cilia type and distribution match the conditions required for mucociliary transportation (Sleigh 1989). Also, the ciliated inner mantle epithelium and waste canal surface of *P. carnea* possibly play an analogous role of mantle ciliary tracts involved in rejecting particles in other bivalves (Yonge 1953; Turner & Rosewater 1958). For instance, particles can be bounded in sticky mucus secreted by mantle glands to be subsequently carried by ciliated rejecting tracts and expelled from the mantle cavity (e.g., Morton 1977; Sartori & Domaneschi 2005). In Pinnidae, a string of rejected material is formed within the waste canal and carried vertically upward until elimination (Turner & Rosewater 1958). Dense distribution of long cilia in a large canal possibly facilitates the rapid agglutination of undesirable particles of various sizes for continuous transport out of the mantle cavity. Correspondingly, the

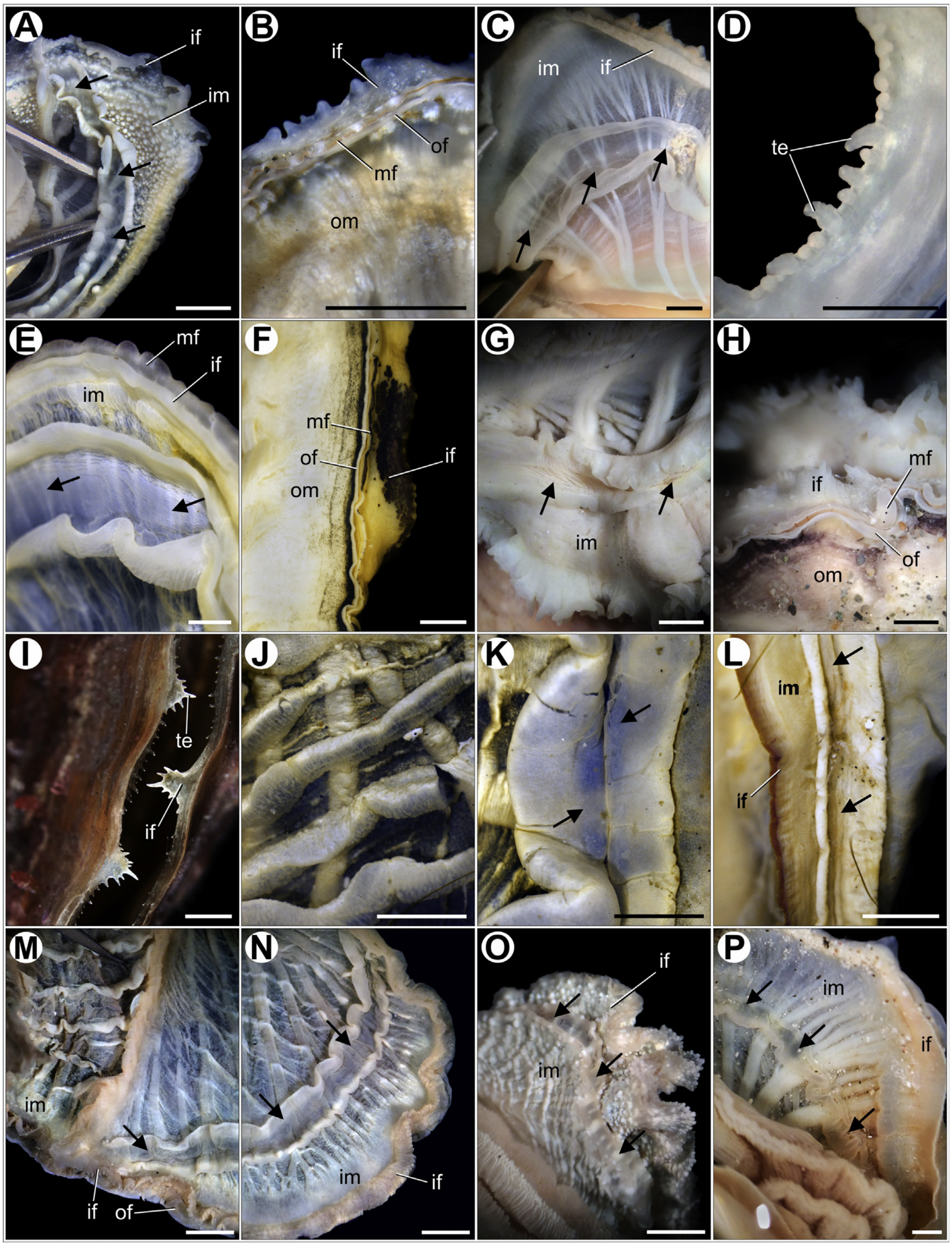

Fig. 5. Mantle morphology in *Atrina* (A–H), *Pinna* (I–O), and *Streptopinna* (P). Detail of the posterior mantle margin in B, D, F, H, and I; lateral view of the inner mantle surface in the remaining. The waste canal is indicated by arrows. (A) *Atrina seminuda* (ZUEC-BIV 2135). B. *Atrina seminuda* (ZUEC-BIV 2135). (C) *Atrina serrata* (USNM 801651). (D) *Atrina serrata* (USNM 801651). (E) *Atrina inflata* (MZSP 55029). (F) *Atrina inflata* (MZSP 55029). (G) *Atrina rigida* (USNM 847971). (H) *Atrina rigida* (USNM 847971). (I) *Pinna carnea* (collected specimen), inner mantle fold. (J) *Pinna carnea* (MZSP 29040), commarginal folds. (K) *Pinna carnea* (MZSP 29040), waste canal. (L) *Pinna rudis* (MZSP 114038). (M) *Pinna muricata* (MCZ 238056); posterodorsal region. Commarginal folds are located in the suprabranchial chamber (at left). (N) *Pinna muricata* (MCZ 238056); posteroventral region. (O) *Pinna muricata* (USNM 836526). (P) *Streptopinna saccata* (USNM 793744). Abbreviations: if, inner mantle fold; im, inner mantle epithelium; mf, middle mantle fold; of, outer mantle fold; om, outer mantle epithelium; te, tentacle. Scale bars: 2 mm.

diversity of mantle secretions in *P. carnea* should also play a major role in the efficiency of the cleaning mechanism.

Secretory activity has been extensively studied for the bivalve mantle, mainly focused on mucin producing cells that contain different combinations of polysaccharides and glycoproteins, producing a viscous secretion (Denny 1983; Prezant 1990; Beninger et al. 1999). A previous study conducted with different bivalve species demonstrated the abundance of viscous acid or acid-dominant mucopolysaccharides associated with particles transport on mantle surfaces (Beninger & St-Jean 1997). Mucus secreting cells are abundant on the mantle epithelium of scallops (Pectinidae), oysters (Ostreidae), and pearl oysters (Pteriidae), as well as in the mantle margin of ark clams (Arcidae) (Richardson et al. 1981; Jabbour-Zahab et al. 1992; Beninger & St-Jean 1997; Audino &

Marian 2018). A remarkable difference was noted herein for *P. carnea*, in which mantle secretion is performed exclusively by epithelial cells, in contrast to numerous subepithelial secretory cells present in most studied bivalves (Jabbour-Zahab et al. 1992; Beninger & St-Jean 1997; Audino & Marian 2018).

Four types of secretory cells were found, although one of them had no affinity for the applied stains. Type I secretory cells are probably composed of acidic mucosubstances (AB-positive; Bancroft & Stevens 1982), which were previously detected in the waste canal epithelium of *Pinna carnea* (Yonge, 1953). Type II secretory cells are highly acidophilic and probably largely composed of basic proteins (BB and NF positive; Pearse 1985). At higher mucin concentration, cross-links between proteins and polysaccharides molecules cause the formation of a gel network with enhanced viscoelastic properties (Denny 1983). For instance, in marine snails, biomechanical differences between adhesive and trail mucus (i.e., used for locomotion) include much larger amounts of glycoproteins in the former, suggesting that the interactions of these macromolecules have an important role in adhesion (Smith & Morin 2002). In addition, the snail mucus has its adhesive force enhanced when associated with glycoprotein complexes (Zhong et al. 2018). The fact that abundant secretory cells of types I and II were found in the inner mantle surface may indicate that, in addition to lubricating the epithelia, the resulting mucus of *P. carnea* may have its viscosity and adhesive properties increased, which would explain the rapid and continuous rejection of sediment pellets (Grave 1911; Yonge 1953).

Type III secretory cells probably correspond to neutral to acidic mucins (stained slightly with PAS, AB, and MA in light blue; Behmer et al. 1976; Bancroft & Stevens 1982). The fact that type III cells also stained with SB in the waste canal of *P. carnea* is certainly striking, because this result suggests the secretion of lipid molecules. Lipid droplets were detected in mucin-secreting glands on the sensory tentacles of a terrestrial snail, but their functions are unknown (Chase & Tolloczko 1985). Different types of mucin glycoproteins secreted by animal epithelial cells were demonstrated to be extremely hydrophobic and associated with a variety of lipids, which could also increase the gel-forming capability and viscosity of the mucus by enhancing the intermolecular hydrophobic interactions (Kim & Singh 1990; Rogunova et al. 1997). Therefore, lipid secretion in *P. carnea* could provide a hydrophobic environment along the waste canal, contributing to bound sediment particles. However, the lack of specificity of SB for lipids has already been pointed out in previous histochemical studies (e.g., Pfüller et al. 1977), so the presence of lipids in *P. carnea* should be confirmed in the future with more detailed protocols.

4.2. Pinnid mantle margin and discoid glands

The mantle margin is similar among the studied pinnid species, including middle and outer folds relatively small in comparison to the enlarged inner fold. A major difference between them is the presence of commarginal folds in the suprabranchial chamber of *Pinna* and *Streptopinna*, which are absent in *Atrina*. These structures were previously observed in the posterior mantle lobes of *P. carnea* (Simone et al., 2015). The presence of commarginal folds in *Pinna* and *Streptopinna* suggests a shared morphological feature, which is in accordance with the phylogenetic history inferred for Pinnidae, i.e., composed of two main clades: *Atrina* and *Pinna* (the latter including *Streptopinna*) (Lemer et al., 2014).

In contrast to many pteriomorphian bivalves (Yonge 1983; Audino & Marian 2016), sensory structures were not detected on the middle fold of Pinnidae. Another unusual finding was the presence of discoid glands on the middle fold of *S. saccata* and *P. carnea*. Surprisingly, these structures have hitherto not been recorded in the Bivalvia. While most studied bivalve species exhibit abundant epithelial and subepithelial secretory cells spread on the mantle, the discoid glands of *S. saccata* and *P. carnea* are concentrated in patches along the posterior mantle margin. The secretion of discoid glands is highly acidophilic and likely composed of basic proteins. It is difficult to envisage the role of these glands. Although lubrication of the mantle folds could be a function for their secretion, such role is generally performed by mucous cells diffused throughout the epithelium of the mantle, as already reported for many bivalves (Richardson et al. 1981; Beninger & St-Jean 1997; Audino & Marian 2018). Therefore, we hypothesize they are involved with other functions.

Pinnids are known to host many different species of crustaceans, such as Palaemonidae shrimps and Pinnotheridae pea crabs (Turner & Rosewater 1958; Sastry & Menzel 1962; Morton 1987; Aucoin & Himmelman 2010). Commensal crustaceans live within the mantle cavity of pinnid species, including *P. carnea*, where they found refuge from predators, shelter, and food from particles trapped in mucus of the rejection currents of the host (Johnson & Liang 1966; Courtney & Couch 1981; Richardson et al. 1997). By their turn, the behavior of the commensal crustaceans is thought to act as an alarm signal against predators, stimulating shell closure and hence contributing to host protection (Yonge 1953; Rabaoui et al. 2008). In addition, several lines of evidence suggest a long–term association between palaemonid shrimps and pinnids (Morton 1987; Aucoin & Himmelman 2010; Góngora-Gómez et al. 2015). Considering the reported commensalism between pinnids and some crustaceans, and also that crustaceans largely depend on chemical communication (Breithaupt & Thiel 2010), one hypothesis for the function of discoid glands would be attraction of shrimps by chemotaxis. Surface-bound glycoprotein and peptides are known to act as pheromones in crustacean chemical communication and recognition (Rittschof & Cohen 2004; Derby & Weissburg 2014). In addition, experimental studies have demonstrated that pinnotherid crabs are able to chemically recognize their hosts (Sastry & Menzel 1962; Derby & Atema 1980). Therefore, the proteinaceous content observed in the discoid glands could presumptively serve to attract commensal crustaceans to the host's mantle cavity.

Another type of function that could be ascribed to these glands could be protection. Pinnids are prey for a variety of animals, such as sea urchins, crabs, gastropods, and fishes (Wu & Shin 1998; Printrakoon et al. 2019), yet they have thin, fragile shells and keep their posterior region constantly exposed above the substrate (Yonge 1953). Given that discoid glands were found only on the posterior region of the mantle, we also speculate that they could be involved in the production of some sort of defensive secretion, e.g., to repel predators. Other bivalves are also known to produce defensive secretions. For example, the mantle margins of *Limaria hians* (Limidae) and *Galeomma takii* (Galeommatidae) secrete noxious substances possibly associated with avoiding predation (Gilmour 1967; Morton 1973). Regardless of their function, the discovery of such intriguing organs clearly adds to the unusual specializations of pinnids, and should stimulate further studies to elucidate their roles.

Declarations of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by the São Paulo Research Foundation (FAPESP, grants 2015/09519-4 and 2017/01365-3) and in part by

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES, Finance Code 001). This study is part of the first author's Doctorate's thesis through the Graduate Program in Zoology of the Institute of Biosciences (University of São Paulo). The authors thank the following institutions that provided materials for the development of this study: Museum of Comparative Zoology (MCZ), Museum of Zoology "Prof. Adão José Cardoso" of the University of Campinas (ZUEC), Museum of Zoology of the University of São Paulo (MZSP), and Smithsonian National Museum of Natural History (USNM). The authors also thank the Center for Marine Biology (CEBIMar–USP), their technical staff, and Prof. Alvaro Migotto for the invaluable support in the collection and analysis of live specimens used in this study. This is a contribution of NP-BioMar (Research Center for Marine Biodiversity – USP).

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