Comparative anatomy of siphons in tellinoidean clams (Bivalvia, Tellinoidea)

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Abstract

Siphons are tubular organs formed by fusion and posterior extension of the marginal mantle folds. They are supposed to have performed key roles in the evolution of bivalves by enabling

these animals to occupy several ecological niches. However, anatomical details of these organs are scarce for one of the most diverse lineages of tropical bivalves, the superfamily Tellinoidea. We investigated the siphonal morphology of 15 species, sampling five tellinoidean families, by integrating scanning electron microscopy, confocal microscopy, and histology. The siphons revealed variation in length, pigmentation, tentacles, papillae, and number of nerve cords. Due to the presence of sensorial structures, such as papillae and tentacles, we reclassify the siphons of Tellinoidea from type A to A+. Additional anatomical patterns were identified at family and genus levels. For example, the incurrent siphon shorter than the excurrent and 24 tentacles are putative synapomorphies of Donacidae. We also highlight shared siphonal traits between Donacidae and Solecurtidae as well as between Semelidae and Tellinidae. In addition, our data support the idea of Psammobiidae as a paraphyletic lineage. Overall, we provide an extensive comparative dataset on siphonal traits with significant relevance for bivalve taxonomy, functional anatomy, and evolutionary investigations.

KEYWORDS

Donacidae, Psammobiidae, Semelidae, Solecurtidae, Tellinidae

1 | INTRODUCTION

In infaunal bivalves, siphons are tubular organs formed by fusion and posterior extension of the marginal mantle folds (Carter et al., 2012). These organs channel the water while the animal is protected in the substrate, maintaining the incurrent and excurrent waterflows circulating through the mantle cavity. Consequently, filtering, respiration, waste removal, and gamete release occur while the animal is buried (Vitonis, Zaniratto, Machado & Passos, 2012). The siphons may also perform sensory roles, such as photoreception and mechanoreception

(Fishelson, 2000), as well as defensive mechanisms, including autotomy against predators (e.g., Amouroux, 1980).

Siphons are supposed to have performed key roles in the evolution of bivalves by enabling these animals to occupy several ecological niches during the Mesozoic Era. For example, 14 of the 18 new Mesozoic superfamilies are siphonate bivalves that occupied soft (e.g., Myoidea) or hard substrates, such as rock and wood (e.g., Pholadoidea) (Stanley, 1968). Currently, among the 9,855 extant species of bivalves (MolluscaBase, 2024), at least 5,588 have siphons, which represents more than half of the species of Bivalvia. Consequently, siphonal traits, such as relative size and tentacles, may be relevant to explore bivalve diversification and their adaptations to different habitats. Moreover, siphonal traits can provide morphological data to test hypotheses of key innovation, homology, and evolutionary convergence.

Siphonal morphology has been investigated by distinct studies with a focus on specific morphological features. The degree of fusion of the mantle folds was extensively studied by Yonge (1948, 1957, 1982, 1983). Musculature, innervation, and distribution of haemocoelic spaces were explored by Chapman & Newell (1956), Duval (1963), Vitonis et al. (2012), and Batistão, Audino & Passos (2023). Epithelial ultrastructure, including ciliated and non-ciliated sensory cells, was examined by Frenkiel (1980), Hodgson, Fielden & Cross (1982), Hodgdon & Fielden (1984, 1986), Pekkarinen (1986), and Fishelson (2000). Lastly, the relative size, pigmentation, and presence and form of tentacles were investigated by Amouroux (1980) and Sartori, Printrakoon, Mikkelsen & Bieler (2008).

The superfamily Tellinoidea is one of the most diverse groups of siphonate bivalves (Prezant, 1998). There are 948 valid extant species of tellinoideans (MolluscaBase, 2024), found mainly in shallow waters from tropical regions where they are an economically important resource as food for many human populations (Vitonis et al., 2012). The taxon is composed of

five extinct families (Icanotiidae, Quenstedtiidae, Sowerbyidae, Tancrediidae, and Unicardiopsidae) and five living families (Donacidae, Psammobiidae, Semelidae, Solecurtidae, and Tellinidae) (Carter et al., 2011). The clade Tellinoidea was recovered monophyletic in several phylogenetic analyses based on morphological and molecular data (Taylor, Williams, Glover & Dyal, 2007; Yuan, Li, Yo & Kong, 2012; Bieler et al., 2014; Combosch et al., 2017; Lemer, Bieler & Giribet, 2019; Sun, Jiang, Kong & Li, 2020). However, the phylogenetic relationships within the superfamily are still contentious and largely unexplored (e.g., Combosch et al., 2017; Sun et al., 2020).

The diversity of the Tellinoidea is reflected in the variety of lifestyles and feeding habits. For example, some donacid species can actively migrate across the beach with the movement of the surf (e.g., Ansell 1983) while others are restricted to specific environments, such as West African rivers (Purchon, 1963). Tellinoideans can be deep (e.g., Domaneschi, 1985) or shallow burrowers (e.g., Narchi & Domaneschi, 1993), with species assuming a vertical or horizontal position in the substrate (e.g., Holland & Dean, 1977; Domaneschi, 1995). Relative to feeding habits, tellinoideans can be suspension or deposit feeders, in this later case by using their long, extensible incurrent siphon to sweep superficial detritus. Alternatively, depending on the environmental conditions, some deposit feeder species may also act as opportunistic suspension feeders (e.g., Pohlo, 1969, 1982).

Even though siphons have evolutionary and ecological importance for bivalve diversification, morphological variation is still poorly examined in many clades, including Tellinoidea. Therefore, this study provides a detailed comparative anatomy of siphons in the diverse clade Tellinoidea, sampling 15 species from five families. We applied combined microscopy techniques to examine morphological variation, infer possible functions, and elucidate patterns of siphonal architecture.

2 | MATERIAL AND METHODS

2.1 | Taxa sampling, localities, and sample fixation

Siphons were obtained from 15 tellinoideans species collected in the coast of Bertioga, Caraguatatuba, Ilhabela, and São Sebastião (State of São Paulo, Brazil): *Donax gemmula* J. P. E. Morrison, 1971, *D. hanleyanus* Philippi, 1847, and *Iphigenia brasiliensis* (Lamarck, 1818) (Donacidae), *Sanguinolaria sanguinolenta* (Gmelin, 1791) (Psammobiidae), *Semele proficua* (Pulteney, 1799) and *S. purpurascens* (Gmelin, 1791) (Semelidae), *Solecurtus sanctaemarthae* d'Orbigny, 1853, *Tagelus divisus* (Spengler, 1794), and *T. plebeius* ([Lightfoot], 1786) (Solecurtidae), *Ameritella versicolor* (De Kay, 1843), *Austromacoma constricta* (Bruguière, 1792), *Eurytellina lineata* (W. Turton, 1819), *Psammotreta brevifrons* (Say, 1834), *Strigilla carnaria* (Linnaeus, 1758), and *S. pisiformis* (Linnaeus, 1758) (Tellinidae). Five to ten individuals were collected per species, except for *S. sanguinolenta*, *S. purpurascens*, and *S. sanctaemarthae*, which include one specimen each.

Specimens were collected during low tide by hand or by shoveling and sieving the sediment at nine sites, including estuaries, exposed sandy beaches, mangrove, sheltered sandy beaches, and stony beach, see Table 1.

Siphons were obtained after dissecting animals previously anesthetized in a 7.5 % solution of $MgCl_2$ for 3 hours. For histology and scanning electron microscopy (SEM), fragments of the siphons were fixed in a modified Karnovsky solution (2.5 % glutaraldehyde and 2 % paraformaldehyde in 0.1 mol L^{-1} sodium cacodylate buffer with osmolarity adjusted to 1 Osm with sucrose) for 3 hours at 4 °C, and stored in cacodylate buffer (Audino & Marian, 2019). For confocal laser scanning microscopy (CLSM), samples were fixed in 4 % paraformaldehyde for 3 hours and stored in phosphate buffer (Audino & Marian, 2019).

2.2 | Microscopy techniques

External siphonal morphology, including apertures, epithelia, papillae, and cilia, were investigated for all species by SEM, following the protocol described by Audino et al. (2015) with few modifications. The samples were postfixed in 1 % OsO_4 in sodium cacodylate buffer for 30 min at 4 °C. Subsequently, they were dehydrated in a graded ethanol series and submitted to a CPD-030 critical point dryer (Balzers, USA) using CO_2 as intermediate fluid. Finally, the samples were mounted on aluminum stubs, coated with gold in an SCD 050 sputter coater (Balzers, USA), and analyzed using a JSM 5800 LV (Jeol, Japan).

Papillae on the siphons of Donacidae and Solecurtidae were studied using CLSM, applying the protocol described by Audino & Marian (2019). As the papillae of Semelidae and Tellinidae were only observed in SEM and not by stereomicroscope, samples were not initially prepared for CLSM. Samples were permeabilized in phosphate buffer containing 2 % Triton-X 100 (PBT) overnight. For muscular investigation, samples were incubated in a 1:40 dilution of Alexa Fluor 488 phalloidin (Molecular Probes, USA) in PBT for 24 hours at room temperature in the dark. For cilia and neurite immunohistochemistry, samples were incubated in a 1:400 dilution of anti-alpha-tubulin antibody (monoclonal antibody, B-5-1-2) with Alexa Fluor 488 conjugate (Molecular Probes, USA) in the same conditions as described for phalloidin. After three rinses of 15 minutes in PBT, samples were mounted on microscope slides with Fluoromount-G mounting medium with DAPI (Molecular Probes, USA). Analyses were performed on an Upright LSM780 NLO (Zeiss, Germany) and an LSM 880 with Airyscan (Zeiss, Germany). Maximum projections were obtained in the Fiji platform (Schindelin et al., 2012).

Finally, histology was used to examine tissue organization, mainly siphonal muscle layers and nerve cords, except in *D. gemmula* and *S. purpurascens*. Samples were completely dehydrated in ethanol and embedded in methyl methacrylate (Historesin). Transversal sections (5 to 8 µm thick) were produced in an RM2245 microtome (Leica, Germany) and stained with

toluidine blue and basic fuchsin for general histological investigation, and Gomori's and Mallory's trichrome for musculature investigation (Bancroft and Stevens, 1982). Photographs were captured by a camera coupled to a DM4B microscope (Leica, Germany).

2.3 | Data storage and terminology

Voucher specimens, stubs (SEM), and histological slides were deposited in the Bivalvia collection (ZUEC-BIV) of the Museum of Biological Diversity of the State University of Campinas (MDBio, UNICAMP), see Table 2. Confocal stacks, photos, and SEM images supporting our results but not presented in the paper are available as supplementary material. The classification criteria for ciliary receptors follows Hodgson & Fielden (1984). General terms and definitions used here for bivalve anatomy are in accordance with Carter et al. (2012). The terminology used for the siphonal muscle layers follows Vitonis et al. (2012).

3 | RESULTS

The siphons of tellinoidean bivalves are formed entirely by a tissue-grade union of the inner mantle folds. They are separate, independently mobile, with musculature organized in seven layers, six nerve cords in the incurrent siphon and six or eight nerve cords in the excurrent. Variation and patterns of siphonal tentacles and papillae were determined at family and genus levels. These variations highlight a broad distinction between the siphons of Donacidae and Solecurtidae compared to the siphons of Semelidae and Tellinidae. All morphological features are described in detail below for each species, grouped by family, and the anatomical patterns are summarized in Table 3.

3.1 | Donacidae

The incurrent siphon is shorter than the excurrent (Figure 1a,c). In *Donax gemmula* and *Iphigenia brasiliensis*, the incurrent siphon reaches the length of the shell when fully extended (~5 mm and 4.5 cm, respectively), while the excurrent siphon reaches twice the length of the shell (~10 mm and 9 cm, respectively). In *D. hanleyanus* the siphons are relatively shorter (Figure 1b), with the incurrent siphon reaching 1/6 of the shell length (~0.5 cm) and the excurrent one reaching 1/3 of the shell length (~1 cm). All siphons are whitish and pigmentation was observed only in the incurrent siphon of *I. brasiliensis*, with six longitudinal brownish lines following the papillae (Figure 1d).

The incurrent aperture of the three species has 24 tentacles, six larger interspersed with six intermediate and 12 smaller tentacles. While tentacles in *D. gemmula* are bipinnate (Figure 2a), except for the tertiary row, those in *D. hanleyanus* are highly branched (Figure 2b). In contrast, all tentacles in the incurrent aperture of *I. brasiliensis* are simple (Figure 2c). Herein, simple tentacles are regarded as digitiform, non-branched tentacles. In the excurrent aperture of *D. gemmula* there are six flap-like tentacles (Figure 2d). In *D. hanleyanus* there are six branched tentacles (Figure 2e). In *I. brasiliensis*, the excurrent aperture has eight simple tentacles (Figure 2f).

Longitudinal rows of papillae were observed on the outer epithelium of the siphons of all specimens analyzed (Figure 1d, 2a,c,d,e,f). The rows start at the siphonal base and end at the base of the primary row of tentacles, following the distribution of the nerve cords. In *D. gemmula* and *D. hanleyanus,* each siphon has six rows of papillae. They are small, with ~20 µm length in *D. gemmula* and ~28 µm in *D. hanleyanus* (Figure 2g). In *I. brasiliensis*, there are six and eight rows of papillae in the incurrent and excurrent siphons, respectively, with an average length of 120 µm (Figure 2h). The musculature of each papilla is composed of longitudinal fibers derived from the longitudinal muscles of the siphonal wall and intrinsic circular muscles (Figure 2i).

Type I ciliated receptors (Figure 3a) were observed scattered on the outer and inner epithelia of the siphons (± 40 cilia, 2.5–2.9 µm long in *D. gemmula*; 1.6–3.2 µm in *D. hanleyanus*; 1.9–2.7 µm in *I. brasiliensis*). Type II ciliated receptors (Figure 3b) were found only on the inner epithelium (2 to 6 cilia, 4.2–5.1 µm long in *D. gemmula*; 3.8–5.8 µm in *D. hanleyanus*; 4.5–4.9 µm in *I. brasiliensis*). Type I ciliated receptors were also observed on the edge of each papilla, surrounding a type III ciliated receptor (Figure 3c, d) located in the central region (6 to 13 cilia, 5.7–8.4 µm long in *D. gemmula*; 12 to 16 cilia, 8.3–12 µm in *D. hanleyanus*; 12–14.8 µm in *I. brasiliensis*). In the papillae, innervation is highly branched, as suggested by branching neurites that directly innervate each ciliary tuft in likely sensory receptors (Figure 3e, f). A similar innervation pattern was observed for type I ciliated receptors on the remaining epithelium (Figure 3g).

The siphonal musculature comprises radial, circular, and longitudinal muscles (Figure 3h, i). The radial fibers are attached to the inner and outer epithelia, crossing the circular and longitudinal musculature. In the siphons of donacids, seven muscle layers have the following topology: below the outer epithelium, there is the outer part of the outer circular layer (coo), followed by the outer longitudinal layer (lo), and the inner part of the outer circular layer (coi). The median longitudinal layer is divided into outer (lmo) and inner (lmi) components, separated by the circular median layer (cm). Then, a thin inner circular layer (ci) is below the inner epithelium. In all species, the nerve cords are localized in the lmi layer (Figure 3h, i). In *I. brasiliensis* there are six and eight nerve cords in the incurrent and excurrent siphons, respectively, while there are six nerve cords in each siphon of *D. gemmula* and *D. hanleyanus*.

3.2 | Psammobiidae

In *Sanguinolaria sanguinolenta* (Figure 4a), the incurrent siphon is longer than the excurrent. When fully extended, the incurrent siphon reaches four times the length of the shell

(~24 cm), while the excurrent one reaches three times (~18 cm). The siphons are whitish, with six nerve cords seen by transparency (Figure 4b). The incurrent aperture has six small tentacles (Figure 4c), and the excurrent aperture is devoid of tentacles (Figure 4d). Rows of papillae were not observed.

Type I ciliated receptors (± 40 cilia, 1.6–3 μm long) occur on the outer and inner epithelia of the siphons, and type II ciliated receptors (2 to 4 cilia, 4.1–5.6 μm long) were observed only on the inner epithelium (Figure 4e). Type III ciliated receptors were not observed. Following the pattern observed in Donacidae, the siphonal musculature comprises radial, circular, and longitudinal muscles (Figure 4f,g). There are seven muscle layers, i.e., coo, lo, coi, lmo, cm, lmi, and ci, and six nerve cords inserted in the lmi layer of each siphon (Figure 4f,g).

3.3 | Semelidae

The incurrent siphon is longer than the excurrent. When fully extended, the incurrent siphon reaches four and a half times the length of the shell in *Semele proficua* (~16 cm) and four times in *Semele purpurascens* (~14 cm), while the excurrent one reaches three times the length of the shell in both species (~11 cm). The siphons are whitish (Figure 5a,b), with six nerve cords seen by transparency (Figure 5c). In *S. proficua*, both siphons have six tentacles and those on the incurrent aperture are longer (~0.54 mm, Figure 5d; ~0.31 mm, Figure 5e). In *S. purpurascens*, there are 12 tentacles in the incurrent aperture, six of which are longer and interspersed by six shorter tentacles (~0.35 mm and ~0.21 mm, respectively, Figure 5f), and six short tentacles in the excurrent aperture (~0.17 mm, Figure 5g). Both species have six longitudinal rows of papillae following the distribution of the nerve cords. The papillae are goblet-shaped and short (Figure 5h), with ~13 μm in *S. proficua* and ~16 μm in *S.*

purpurascens. In the center of each papilla, there are type III ciliated receptors (12–16 µm long in *S. proficua*; 11.5–12.9 µm long in *S. purpurascens,* Figure 5h).

Type I ciliated receptors (± 40 cilia, 1.5–2.1 µm long in *S. proficua* and 0.9–1.1 µm long in *S. purpurascens*) are distributed on the outer and inner epithelia of the siphons (Figure 5i). Type II ciliated receptors (3 to 5 cilia, 3.9–6.4 µm long in *S. proficua* and 6.8–13 µm long in *S. purpurascens*) were observed only on the inner epithelia (Figure 5i). The siphonal musculature of *S. proficua* comprises radial, circular, and longitudinal muscles (Figure 5j, k), as in Donacidae and Psammobiidae. There are seven muscle layers, i.e., coo, lo, coi, lmo, cm, lmi, and ci, and six nerve cords inserted in the lmi layer of each siphon (Figure 5j, k).

3.4 | Solecurtidae

The incurrent siphon is slightly longer than the excurrent (Figure 6a,b, contracted in c). When the animals are burrowed into the substratum, they assume a vertical position, and the siphons, when fully extended, form a V shape, reaching about two to three times the length of the shell (~15 cm long in *Solecurtus sanctaemarthae*; ~9 cm long in *Tagelus divisus*; ~30 cm long in *Tagelus plebeius*). Pigmentation was observed in all species analyzed. In *S. sanctaemarthae*, the siphons are white with brownish stripes and spots (Figure 6a). In *T. divisus,* the siphons are pigmented with beige spots on the outer surface, forming discontinuous bands along the entire length (Figure 6b). In *T. plebeius*, the siphons are whitish, with orange bands visible in the distal region of the incurrent siphon (Figure 6c) and pigmented spots concentrated mostly around the nerve cords (Figure 6d).

The three species have the incurrent aperture with six simple tentacles (Figure 6e) and the excurrent one with eight shorter tentacles (Figure 6f). Six longitudinal rows of papillae were observed in the incurrent siphon of all species analyzed, and eight rows of papillae are present in the excurrent siphon (Figure 6e,f). Similar to Donacidae and Semelidae, the rows

follow the nerve cords. Additionally, in *S. sanctaemarthae*, many papillae are scattered on the epithelium besides the longitudinal rows (Figure 6g). The papillae have ± 36 µm length in *S. sanctaemarthae*, ± 30 µm length in *T. divisus*, and ± 45 µm length in *T. plebeius*. The musculature of the papillae in all three species has longitudinal muscle fibers derived from the longitudinal muscles of the siphonal wall and intrinsic circular muscles (Figure 6h).

Type I ciliated receptors (± 40 cilia, 1.8–4.1 µm long in *S. sanctaemarthae*; 1.4–4.1 µm long in *T. divisus*; 1.1–2 µm long in *T. plebeius*) are distributed on the outer and inner epithelia (Figure 7a,b). Type II ciliated receptors (2 to 6 cilia, 5–7.4 µm long in *S. sanctaemarthae*; 5.3–7.1 µm long in *T. divisus*; 4.7–9.1 µm long in *T. plebeius*) occur only on the inner epithelium (Figure 7b). Type I receptors are also on the edge of each papilla, surrounding a type III receptor (11 to 16 cilia, 9–14 µm long in *S. sanctaemarthae*; 10.6–16.1 µm long in *T. divisus*; 8.1–10.3 µm long in *T. plebeius*) located in the central region (Figure 7c,d,e). In the papillae, innervation is similar to donacids, highly branched, with branching neurites that directly innervate each ciliary tuft in likely sensory receptors (Figure 7f,g,h). As previously described, the siphonal musculature of Solecurtidae comprises radial, circular, and longitudinal muscles (Figure 7i, j). There are seven muscle layers, i.e., coo, lo, coi, lmo, cm, lmi, and ci, with nerve cords inserted in the lmi layer (Figure 7i, j).

3.5 | Tellinidae

The incurrent siphon is longer than the excurrent. When fully extended, the incurrent siphon reaches four times the length of the shell (~8 cm long in *Ameritella versicolor*; ~30 cm long in *Austromacoma constricta*; ~20 cm long in *Eurytellina lineata*; ~12 cm long in *Psammotreta brevifrons*), or more than five times (~10 cm long in *Strigilla carnaria* and ~5 cm long in *Strigilla pisiformis*). The excurrent siphon reaches about 3/4 of the length of the incurrent siphon of each species. In *A. versicolor*, the incurrent siphon is white, and the

excurrent siphon is translucent (Figure 8a). In *A. constricta*, the incurrent siphon is orange, and the excurrent siphon is whitish (Figure 8b). Whitish siphons were observed in *E. lineata* and *S. pisiformis* (Figure 8c,f). Finally, *P. brevifrons* has brownish siphons (Figure 8d), and *S. carnaria*'s siphons are completely translucent (Figure 8e). The nerve cords can be seen by transparency.

The incurrent aperture has six simple tentacles in *E. lineata*, *S. carnaria* (Figure 9a), and *S. pisiformis*. On the other hand, the incurrent aperture lacks tentacles in *A. versicolor, A. constricta* (Figure 9b), and *P. brevifrons*. The excurrent aperture has six tentacles in *A. constricta* (Figure 9c), while tentacles are missing in the excurrent aperture of the other species (Figure 9d). Exclusively in *S. carnaria*, the excurrent aperture has ciliary tufts 6.1 to 10.5 µm long (Figure 9e). These ciliary tufts are similar in length to type III ciliated receptors, but they are not located on papillae.

There are six longitudinal rows of papilla following the distribution of the nerve cords in the siphons of *A. constricta*, *E. lineata*, *S. carnaria*, and *S. pisiformis*. As in Semelidae, the papillae have a goblet form and are small, with ~7 µm length in *A. constricta*, ~4.5 µm length in *E. lineata* (Figure 9f), ~4.2 µm length in *S. carnaria*, and ~3.7 µm length in *S. pisiformis*. Papillae were not observed in *A. versicolor* and *P. brevifrons*.

Type I ciliated receptors (± 40 cilia, 0.9–1.1 µm long in *A. versicolor*; 1.4–2.1 µm long in *A. constricta*; 1.6–2.2 µm long in *E. lineata*; 1.8–2.5 µm long in *P. brevifrons*; 1.7–2.3 µm long in *S. carnaria*; 1.9–3.8 µm long in *S. pisiformis*) are distributed on the outer and inner epithelia (Figure 9g). Type II ciliated receptors (2 to 5 cilia, 3–5.8 µm long in *A. versicolor*; 4.9–8.1 µm long in *A. constricta*; 6.1–6.3 µm long in *E. lineata*; 3.8–4.9 µm long in *P. brevifrons*; 5.9–8.8 µm long in *S. carnaria*; 5.4–6.6 µm long in *S. pisiformis*) occur only on the inner epithelium (Figure 9h). Type III ciliated receptors (10 to 13 cilia, 9.8–11.7 µm long in *A. constricta*; 8.5–13.7 µm long in *E. lineata*; 8.7–12.5 µm long in *S. carnaria*; 9–13.3 µm long

in *S. pisiformis*) are restricted to the center of the papilla. Type I ciliated receptors surrounding the type III receptor are absent (Figure 9f).

The siphonal musculature is not different from other tellinoid families. There are radial, circular, and longitudinal muscles, the last two being organized in seven layers: coo, lo, coi, lmo, cm, lmi, and ci (Figure 9i, j). All species have six nerve cords inserted in the lmi layer (Figure 9i, j), including the siphons with no tentacles.

4 | DISCUSSION

4.1 | Siphon morphology

The siphons of Tellinoidea are traditionally classified as type A, i.e., formed by tissue-grade fusion of the inner mantle fold and without tentacles and eyes at the tips of the siphons (Yonge, 1948, 1957, 1982, 1983; Carter et al., 2012). However, putative sensorial structures are present in most species studied, as illustrated by tentacles on the siphonal apertures and numerous papillae on the siphonal epithelium. Therefore, the siphons of the Tellinoidea are better defined as type A+, i.e., siphons formed entirely by tissue-grade union of the inner mantle folds, with tentacles and/or eyes only on the inner mantle fold, apically and in some instances also near the shell margins (Carter et al., 2012). Siphons type A+ are also found in Cardioidea, the sister-group of Tellinoidea (e.g., Combosch et al., 2017; Sun et al., 2020; Wang, Yang, Kong, Sasaki & Li; 2023). Nevertheless, there are three significant differences between these groups. First, the siphons of Cardioidea are very short, separated distally but fused at the base (e.g., Morton, 2000; Vidal, 2001; Signorelli, da Fonseca, Scarabino & Passos, 2019), while the siphons of Tellinoidea are long and completely separated. Second, some species of Cardioidea have siphonal photoreceptor organs (e.g., Vidal, 2001), while similar structures are lacking in Tellinoidea. Finally, all tellinoideans have siphonal retractor muscles, which are absent in most lineages of Cardioidea, except in Tridacninae (Mikkelsen & Bieler, 2007).

Siphonal pigmentation is a topic poorly explored in bivalve literature, mainly because it is necessary to collect live animals to observe the color of these organs. More complete data are available only for the family Veneridae (Sartori et al., 2008), and a brief mention about the brownish lines in the incurrent siphon of *I. brasiliensis* (see Narchi, 1972), also observed here. Our results reveal that intense pigmentation is not common in tellinoideans, with most species exhibiting whitish to translucent siphons. Remarkable exceptions include the tellinid *A. constricta* with an orangish incurrent siphon and solecurtids with brightly pigmented siphons. The role of siphonal pigmentation is an open question. In many coastal invertebrates, such as ascidians, cnidarians, and sponges, the presence of intense pigmentation may be related to the protection against insolation and ultraviolet radiation (e.g., Bandaranayake, 2006; Núñez-Pons, Avila, Romano, Verde & Giordano, 2018). In bivalves from intertidal and infralittoral zones, such as mussels, intense pigmentation is observed in the mantle margins (Audino, Serb & Marian, 2020). In the case of the tellinid *A. constricta*, an infaunal species living in the intertidal zone, most of the incurrent siphon is often exposed above the sediment (Arruda, Domaneschi & Amaral, 2003). In contrast, the excurrent siphon is rarely exposed, which might explain the difference in pigmentation between the siphons. Curiously, solecurtids are infaunal bivalves with pigmented siphons that remain buried, i.e., with the aperture at the sediment level (e.g., Pohlo, 1966). Consequently, the lack of constant exposure to sunlight possibly challenges the view of pigmentation solely as a protective feature. More comparative data for more bivalve species would be important to explore the roles of pigmentation in these animals.

4.2 | Siphonal tentacles

The presence and morphology of tentacles in the siphonal aperture are supposed to be associated with the feeding habit and the type of environment because tentacles constitute the first barrier to the incurrent waterflow (Amouroux, 1980). For example, bivalvesliving where

suspended sediment is heavy, e.g., Veneridae, have branched tentacles in the incurrent aperture (Ansell, 1961), while simple tentacles are observed in those living in cleaner habitats (Sartori et al., 2008). Among tellinoideans, species of *Donax* Linnaeus, 1758 and *Latona* Schumacher, 1817 inhabit the surf zone and have highly branched siphonal tentacles (e.g., Pohlo, 1967; Wade, 1967, 1969; Mouëza & Frenkiel, 1974). This morphology acts as a fine strainer over the aperture, preventing larger and possibly indigestible particles from entering the mantle cavity and occluding the gills. Consequently, the branched tentacles might be an adaptation to the surf zone, an environment with high turbulence and sediment transportation. One exception is *D. gemmula* with bipinnate tentacles. The shell of these bivalves reaches a maximum of 7 mm (Passos & Domaneschi, 2004), representing the smallest species of *Donax* in the world. Therefore, compared to highly branched tentacles, the bipinnate tentacles could represent a structural simplification likely caused by species miniaturization (see Hanken & Wake, 1993).

Based on the most recent phylogeny of Donacidae (Moncada et al., 2022) and our comparative results (Table 3), the incurrent siphon is shorter than the excurrent and the incurrent aperture with 24 branched tentacles possibly represent synapomorphies for the family. In addition, the excurrent aperture with six tentacles is exclusive of the genus *Donax*, while the excurrent aperture of other Donacidae, such as *I. brasiliensis, G. paradoxa* (see Purchon, 1963), and *Latona deltoides* (Lamarck, 1818) (Duval, 1963), shows eight tentacles.

Anatomical studies on Solecurtidae are scarce, particularly those focusing on siphons. We observed tentacles in all suspension-feeding species, with six and eight tentacles in the incurrent and excurrent apertures, respectively. While *S. sanctaemarthae* has short siphonal tentacles, like those observed in *S. strigilatus* (Linnaeus, 1758) (see Amouroux, 1980), the siphonal tentacles of *T. divisus* and *T. plebeius* are longer.

Despite being classified as deposit feeders (Mikkelsen & Bieler, 2007), detailed anatomical studies suggest that psammobiids feed on suspended material (e.g., Pohlo, 1972;

Domaneschi, 1992; Narchi & Domaneschi, 1993). This confusion is because most psammobiids show a mosaic of morphological features of suspension and specialized deposit-feeding tellinoideans (see Pohlo, 1982; Domaneschi & Shea, 2004). Our data for *S. sanguinolenta*, i.e., six tentacles in the incurrent aperture and the siphonal behavior in which the incurrent siphon is not exposed out of the sediment, suggest a suspension feeding habit.

Most species of Psammobiidae have siphonal apertures similar to those of solecurtids. For example, *Gari depressa* (Pennant, 1777), *G. solida* (Gray, 1828), and *Nuttallia obscurata* (Reeve, 1857) have six simple tentacles in the incurrent siphon and eight in the excurrent (Amouroux, 1980; Domaneschi, 1992; Sasaki, Kudo & Ito, 1999). The incurrent aperture of *Asaphis deflorata* (Linnaeus, 1758), *A. violascens* (Forsskål, 1775), and *Heterodonax bimaculatus* (Linnaeus, 1758) include six long tentacles interspersed by six shorter, and eight tentacles in the excurrent aperture (Narchi, 1980; Narchi & Domaneschi, 1993; Domaneschi & Shea, 2004). A remarkable exception is *S. sanguinolenta,* which the excurrent aperture is devoid of tentacles. Such condition, added to others that will be discussed later, makes the siphons of *S. sanguinolenta* more similar to those of some tellinids.

Semelids are classified as a combination of suspension and deposit feeders (Mikkelsen & Bieler, 2007). Previous studies and our data describe the incurrent aperture with six simple tentacles in *S. casali* Doello-Jurado, 1949 and *S. proficua*, six long tentacles interspersed by six short ones in *S. purpurascens*, and 24 tentacles (referred to as "papillae"), being 12 large interspersed by 12 smaller in *Ervilia castanea* (see Narchi & Domaneschi, 1977; Domaneschi, 1982, 1995; Morton, 1990). In the excurrent aperture, six simple tentacles were observed in all species, which are suspension feeders. Alternatively, feeding records of other semelid species, such as *Scrobicularia plana* (da Costa, 1778) and *Cumingia sinuosa* A. Adams, 1850, suggest that they primarily feed on deposited material (Hughes, 1969; Stanley, 1970). The incurrent

aperture appears to be devoid of tentacles in *S. plana*, based on original drawings (Hughes, 1969; Stanley, 1970), and siphonal data for *C. sinuosa* are lacking.

Tellinids encompass a great diversity of feeding habits and siphonal apertures. For example, *S. carnaria and S. pisiformis* are suspension feeders (Piffer, 2011) with six large tentacles in the incurrent aperture. In the case of deposit feeders, *E. lineata* has an incurrent aperture with six flap-like tentacles, while *A. versicolor*, *A. constricta*, and *P. brevifrons* have the incurrent aperture devoid of tentacles. Other examples of deposit feeders are *A. biota* (Arruda & Domaneschi, 2005), with six flap-like tentacles in the incurrent aperture (Piffer, Arruda & Passos, 2011), and *Ardeamya petitiana* (d'Orbigny, 1845) and *Moerella donacina* (Linnaeus, 1758), with the incurrent aperture lacking tentacles (Amouroux, 1980; Barón & Ciocco, 1997). The excurrent aperture of all species is devoid of tentacles, the exceptions being *A. constricta*, with six flap-like tentacles, and *A. biota*, with six digitiform tentacles (Piffer et al., 2011). Finally, the deposit feeding habit seems to be accompanied by an evolutionary trend of reduction or loss of siphonal tentacles (see Pohlo, 1982), however, additional anatomical data and a robust phylogenetic framework for Tellinoidea are necessary to endorse this hypothesis.

4.3 | Siphonal papillae and ciliated receptors

Rows of papillae were observed in all species, except in *S. sanguinolenta*, *A. versicolor*, and *P. brevifrons*. The incurrent siphon has six rows of papillae while the excurrent has six (*Donax* spp., Semelidae, and Tellinidae) or eight (*I. brasiliensis* and Solecurtidae). Siphonal papillae are similarly distributed, following the nerve cords, and in *S. sanctaemarthae*, additional papillae are scattered on the siphon surface.

The papillae of Semelidae (*S. proficua* and *S. purpurascens*) and Tellinidae (*A. constricta*, *E. lineata*, *S. carnaria*, and *S. pisiformis*) can only be viewed through SEM due the

less abundance and small size. They are characterized by a goblet form and type III ciliated receptors located at the central portion. Not coincidentally, these papillae were observed in the siphons of *Macoma balthica* (Linnaeus, 1758) and named "goblet organs" (Pekkarinen, 1984, 1986), posteriorly related to the presence of type III receptors (Fishelson, 2000).

For the first time, we provide detailed evidence of siphonal papillae in Donacidae and Solecurtidae, including morphology, innervation, and type of ciliated receptors. The papillae are tentacular-like in *I. brasiliensis* and solecurtids, but extremely short in the genus *Donax*. Type I ciliated receptors are also present, surrounding the type III ciliated receptor. Furthermore, in both families, the innervation of type I and III receptors is similar, with neurites from the siphonal nerve cords reaching the ciliary tufts.

Although papillae were not observed in *S. sanguinolenta*, other psammobiids, such as *A. violascens* and *A. deflorata* (see Narchi, 1980; Domaneschi & Shea, 2004), *G. solida* (Domaneschi, 1992), and *H. bimaculatus* (Narchi & Domaneschi, 1993), have papillae on the siphonal wall. These papillae were noticed under stereomicroscope and are distributed in six and eight rows in the incurrent and excurrent siphons, respectively.

Type I and II ciliated receptors were observed in all specimens investigated in this study. Type I receptors are present on both inner and outer epithelia, but type II receptors are restricted to the inner epithelium. Previous studies illustrating the details of the inner epithelium, for example in *Donax serra* Röding, 1798 and *Latona sordida* (Hanley, 1845) (Hodgson & Fielden, 1984), *M. balthica* (Pekkarinen, 1986), *D. trunculus* Linnaeus, 1758 and *D. semistriatus* Poli, 1795 (Fishelson, 2000), reinforce our conclusion that type I and II ciliated receptors have a common pattern of distribution across Tellinoidea.

The function of these ciliated receptors is unknown. They are considered the primary receptors for environmental stimuli, notably those of mechanical (i.e., vibrations from the surrounding water and/or direct contact) and chemical origin (Hodgson & Fielden, 1984).

However, sensory functions were inferred just based on form and position, lacking experimental, molecular, and behavioral evidences. Therefore, future studies may elucidate the roles of these ciliated receptors.

4.4 | Musculature and innervation

In Tellinoidea, the siphonal musculature is characterized by the presence of seven muscle layers: three longitudinal, associated with siphonal retraction; and four circular, associated with constriction (Yonge, 1949; Duval, 1963; Vitonis et al., 2012). Our data corroborate this pattern by expanding the taxonomic observations for the group. The only exceptions known are the incurrent siphon of *S. proficua*, with the median longitudinal layer of the incurrent siphon subdivided into three parts instead of two, and the excurrent siphon of *S. proficua* and *S. purpurascens,* with the inner circular layer absent (Domaneschi, 1982, 1995). However, we think that the muscular variation observed in *S. proficua* in previous studies (Domaneschi, 1982, 1995) is an artifact, because we found the same pattern of muscular organization of other Tellinoidea.

Based on the comparative anatomy of Tellinoidea, we determined that (1) nerve cords are present in all siphons, even in species without siphonal tentacles; (2) the nerve cords are always located within the inner part of the median longitudinal layer; (3) the number of nerve cords in the incurrent siphon is six; (4) the number of nerve cords in the excurrent siphon varies with the family, including six in Semelidae and Tellinidae, and eight in and Solecurtidae.

Interestingly, the psammobiid *S. sanguinolenta* has an excurrent siphon with six nerve cords. This feature, added to others, such as length, color, six reduced tentacles in the incurrent aperture, and lack of tentacles in the excurrent siphon, make the siphons of this species much more similar to those observed in semelids and tellinids. The diagnostic characters of Psammobiidae are entirely conchological (Willan, 1993), and recent phylogenetic studies have

no support for the monophyly of this family (Combosch et al., 2017; Sun et al., 2020; Wang et al., 2023). Therefore, the current taxonomic position of *S. sanguinolenta* is arguable, and our anatomical data reinforce the need for a systematic review of the psammobiids.

5 | CONCLUSIONS

We provide the first comparative, detailed study of siphons in Tellinoidea by means of combined microscopy techniques. Superficially similar organs revealed a broad variation in pigmentation, length, and associated structures. In addition, we reclassified the siphons of Tellinoidea from type A to A+ based on the presence of likely sensorial structures, such as siphonal papillae and tentacles. Although the phylogenetic relationships among the families of Tellinoidea are still contentious, our results are in accordance with recent evolutionary hypotheses. For example, the morphology of the papillae and the number of nerve cords are key traits with different states shared between the clades Donacidae and Solecurtidae and between Semelidae and Tellinidae, supporting these phylogenetic relationships (Combosch et al., 2017). In addition, the incurrent siphon shorter than the excurrent and the incurrent aperture with 24 tentacles are exclusive of donacids, suggesting these as synapomorphies of the taxon. Finally, our results demonstrate the importance of siphonal traits in investigating functional morphology and body architecture in bivalves, also highlighting the siphons as a source of data for evolutionary investigations and taxonomy.

AUTHOR CONTRIBUTIONS

All authors designed the project. Alan R. Batistão and Jorge A. Audino prepared the samples for scanning electron and confocal microscopy. Alan R. Batistão and Flávio D. Passos prepared the samples for histology. Alan R. Batistão performed SEM and histology. Alan R. Batistão and Jorge A. Audino performed CSLM. All authors have analyzed the data. Alan R. Batistão

prepared the figures. Alan R. Batistão wrote the first draft of the manuscript. All authors have edited and approved the final version.

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Figure Legends

Figure 1. Extended siphons of (a) *Donax gemmula*, (b) *Donax hanleyanus*, and (c, d) *Iphigenia brasiliensis* photographed under stereomicroscope. In (d) the longitudinal pigmented lines of the incurrent siphon are shown following the rows of papillae. Abbreviations: es: excurrent siphon; is: incurrent siphon; p: papilla; pl: pigmented line.

Figure 2 Siphonal apertures and papillae of Donacidae. Incurrent apertures of (a) *Donax gemmula*, (b) *Donax hanleyanus*, and (c) *Iphigenia brasiliensis*. The incurrent apertures have 24 tentacles: bipinnate in (a), branched in (b), and simple in (c); scanning electron micrographs (SEM). Excurrent apertures of (d) *D. gemmula*, (e) *D. hanleyanus*, and (f) *I. brasiliensis*, with six simple, six branched, and eight simple tentacles, respectively; SEM. Papillae of (g) *D. hanleyanus* and (h) *I. brasiliensis;* SEM. The type I and III ciliated receptors are indicated in (g). (i) Musculature of a papilla in the incurrent siphon of *I. brasiliensis*. The white arrow indicates the siphonal longitudinal muscles originating the papillary longitudinal muscles; confocal micrograph (CLSM). Abbreviations: bt: bipinnate tentacle; brt: branched tentacle; cm: circular muscle; lm: longitudinal muscle; p: papilla; pt: primary tentacle; st: secondary tentacle; tt: tertiary tentacle; t1: type I ciliated receptor; t3: type III ciliated receptor.

Figure 3. Ciliated receptors and siphonal musculature of Donacidae. (a) Type I and (b) type II ciliated receptors in the inner epithelium of *Donax hanleyanus*, incurrent siphon; scanning electron micrograph (SEM). Papillae of the incurrent siphon of (c) *D. hanleyanus* and (d) *Iphigenia brasiliensis*, showing type I and III ciliated receptors; SEM. Innervation of ciliated receptors in a papilla of (e) *D. hanleyanus* and (f) *I. brasiliensis*. Each ciliated receptor is innervated directly by branched neurites derived from the nerve cord; confocal micrograph (CLSM). (g) Ciliary root of a type I receptor in the outer epithelium of the incurrent siphon of *D. hanleyanus*; CLSM. Muscle layers of the (h) incurrent and (i) excurrent siphon of *D. hanleyanus*; Histological sections, Gomori's trichrome staining. Abbreviations: ci: inner circular layer; cm: median circular layer; coi: inner part of the outer circular layer; coo: outer part of the outer circular layer; ie: inner epithelium; lo: outer longitudinal layer; lmi: inner part of the longitudinal median layer; lmo: outer part of the longitudinal median layer; n: nerve

cord; oe: outer epithelium; rm: radial muscle; t1: type I ciliated receptor; t3: type III ciliated receptor.

Figure 4. Siphonal morphology of Psammobiidae. (a) *Sanguinolaria sanguinolenta* with (b) siphons partially exposed. (c) Incurrent siphon with six short tentacles; scanning electron micrograph (SEM). (d) Excurrent siphon devoid of tentacles; SEM. (e) Inner epithelium of the incurrent siphon, with type I and II ciliated receptors; SEM. Muscle layers of the (f) incurrent and (g) excurrent siphons; Histological sections, Gomori's trichrome staining. Abbreviations: ci: inner circular layer; cm: median circular layer; coi: inner part of the outer circular layer; coo: outer part of the outer circular layer; es: excurrent siphon; ie: inner epithelium; is: incurrent siphon; lo: outer longitudinal layer; lmi: inner part of the longitudinal median layer; lmo: outer part of the longitudinal median layer; n: nerve cord; oe: outer epithelium; rm: radial muscle; t: tentacle; t1: type I ciliated receptor; t2: type II ciliated receptor.

Figure 5. Siphonal morphology of Semelidae. (a) *Semele proficua*. (b) *Semele purpurascens*. (c) Incurrent siphon of (a), showing one nerve cord by transparency. (d) Incurrent and (e) excurrent aperture of (a), both with six tentacles. Those in the incurrent siphon are longer; scanning electron micrograph (SEM). (f) Incurrent and (g) excurrent aperture of (b), with twelve and six tentacles, respectively; SEM. (h) Papilla of (b), showing type III ciliated receptor; SEM. (i) Inner epithelium of the excurrent siphon of (b), showing type I and II ciliated receptors; SEM. Muscle layers of the (j) incurrent and (k) excurrent siphon of (a); Histological sections, toluidine blue staining. Abbreviations: ci: inner circular layer; cm: median circular layer; coi: inner part of the outer circular layer; coo: outer part of the outer circular layer; es: excurrent siphon; ie: inner epithelium; is: incurrent siphon; lo: outer longitudinal layer; lmi: inner part of the longitudinal median layer; lmo: outer part of the longitudinal median layer; n:

nerve cord; oe: outer epithelium; p: papilla; pt: primary tentacle; st: secondary tentacle; t: tentacle; t1: type I ciliated receptor; t2: type II ciliated receptor, t3: type III ciliated receptor.

Figure 6. Siphonal morphology of Solecurtidae. (a) *Solecurtus sanctaemarthae*. (b) *Tagelus divisus*. (c) *Tagelus plebeius*. (d) Dissected incurrent siphon of (c), showing the pigmented spots and lines following the nerve cords. (e) Incurrent aperture of (b), with six tentacles and rows of papillae; scanning electron micrograph (SEM). (f) Excurrent aperture of (c), with eight tentacles and rows of papillae; SEM. (g) Excurrent siphon of (a), showing scattered papillae. (h) Musculature in a papilla in the excurrent siphon of *T. plebeius*; confocal micrograph (CLSM). Abbreviations: cm: circular muscle; es: excurrent siphon; is: incurrent siphon; lm: longitudinal muscle; n: nerve cord; p: papilla; t: tentacle.

Figure 7. Ciliated receptors and siphonal musculature of Solecurtidae. (a) Type I ciliated receptors of *Tagelus divisus*, outer epithelium of the incurrent siphon; scanning electron micrograph (SEM). (b) Type I and II ciliated receptors of *Tagelus plebeius*, inner epithelium of the incurrent siphon; SEM. Papillae with type III ciliated receptor surrounded by type I ciliated receptors in the excurrent siphons of (c) *Solecurtus sanctaemarthae*, (d) *T. divisus*, and (e) *T. plebeius*; SEM. Innervation of ciliated receptors in a papilla of (f) *S. sanctaemarthae*, (g) *T. divisus*, and (h) *T. plebeius*; confocal micrograph (CLSM). Muscle layers of the (i) incurrent and (j) excurrent siphon of *T. plebeius*; Histological sections, Gomori's trichrome staining in (i) and Mallory's trichrome staining in (j). Abbreviations: ci: inner circular layer; cm: median circular layer; coi: inner part of the outer circular layer; coo: outer part of the outer circular layer; ie: inner epithelium; lo: outer longitudinal layer; lmi: inner part of the longitudinal median layer; lmo: outer part of the longitudinal median layer; n: nerve cord; oe: outer

epithelium; rm: radial muscle; t1: type I ciliated receptor; t2: type II ciliated receptor; t3: type III ciliated receptor.

Figure 8. Extended siphons of Tellinidae. (a) *Ameritella versicolor*, (b) *Austromacoma constricta*, (c) *Eurytellina lineata*, (d) *Psammotreta brevifrons*, (e) *Strigilla carnaria*, and (f) *Strigilla pisiformis*. Abbreviations: es: excurrent siphon; is: incurrent siphon.

Figure 9 Siphonal morphology of Tellinidae. Incurrent aperture of (a) *Strigilla carnaria*, with six tentacles, and (b) *Austromacoma constricta*, devoid of tentacles; scanning electron micrograph (SEM). Excurrent aperture of (c) *A. constricta*, with six tentacles, (d) *Strigilla pisiformis*, and (e) *S. carnaria*, both devoid of tentacles. Long ciliary tufts are present in (e); SEM. (f) Papilla of *Eurytellina lineata* in the excurrent siphon. Type III ciliated receptor is emerging in the central portion; SEM. (g) Type I ciliated receptor in the outer epithelium of the excurrent siphon of *A. constricta*; SEM. (h) Type II ciliated receptor in the inner epithelium of the incurrent siphon of *Psammotreta brevifrons*; SEM. Muscle layers of the (i) incurrent and (j) excurrent siphon of *A. versicolor*; Histological sections, Gomori's trichrome staining. Abbreviations: ci: inner circular layer; cm: median circular layer; coi: inner part of the outer circular layer; coo: outer part of the outer circular layer; ct: ciliary tuft; ie: inner epithelium; lo: outer longitudinal layer; lmi: inner part of the longitudinal median layer; lmo: outer part of the longitudinal median layer; n: nerve cord; oe: outer epithelium; p: papilla; rm: radial muscle; t: tentacle; t3: type III ciliated receptor.