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Morphology of ctenostome bryozoans: 7. *Hislopia*, *Echinella* and *Timwoodiellina*

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Abstract

Ctenostome bryozoans are a small group of gymnolaemates comprising less than 400 recent species. They are paraphyletic and ctenostome-grade ancestors gave rise to Cheilostomata, the most dominant and speciose taxon of Bryozoa in the present day. Investigations into ctenostomes are important for reconstructing character evolution among Gymnolaemata. As a continuation of studies on a morphological series of ctenostome bryozoans, we herein investigate six species of hislopiids, a small clade of three genera occurring in freshwater habitats. The general morphology of all species is similar in having primarily uniserial chains of encrusting zooids, which are mostly oval to ellipsoid and have a flattened frontobasal axis. *Hislopia prolixa* and *Echinella placoides* often have more slender zooids with a higher frontobasal axis. Apertures of hislopiids are quadrangular, lined by a thickened cuticle. Apertural spines are present in various lengths in *E. placoides*, *Hislopia lacustris* and *Hislopia corderoi*. The remaining cuticle is rather thin except at lateral areas, close to the pore-plates which are prominent in hislopiids because of abundant special and limiting cells. All species except *H. corderoi* and *Timwoodiellina natans* have a prominent collar obstructing the vestibulum, whereas the latter two species instead have an 'external collar' as cuticular, outer folds projecting over the aperture. Hislopiid lophophores carry eight, or more commonly 12−18 tentacles. The digestive tract is distinguished by an often highly elongated esophagus and/or cardia, with the latter always having a prominent bulbous part in the form of a proventriculus—or gizzard in *E. placoides*. The caecum is extensive in all species. In *Hislopia* the intestine is characteristically two-chambered with a proximal and distal part before entering an anal tube of various length. The latter is present in all species except *T. natans* and terminates in mid-lophophoral area. Oocytes in *E. placoides* are large and macrolecithal indicating brooding and the production of lecithotrophic larvae. *Hislopia* species produce small, oligolecithal ones, which suggests zygote spawning and planktotrophy. In general, the morphology is similar among the different hislopiids with characters of the gut aiding in delineating the genera *Echinella* and *Timwoodiellina*.

KEYWORDS
ctenostome evolution, freshwater Bryozoa, gizzard, Gymnolaemata, Hislopioidea

1 | INTRODUCTION

Bryozoa is a lophotrochozoan phylum of colonial suspension-feeders that occur globally in marine and freshwater environments. Colonies are comprised of iterated, asexually produced modules, termed zooids. Each zooid consists of the main feeding organ, the ciliated tentacle crown or lophophore, and a u-shaped digestive tract (together summarized as the polypide) and the protective body wall, or cystid, into which the polypide can be retracted (Mukai et al., 1997). Upon retraction the body wall narrows at the site where the polypide has retracted into the body cavity to form the orifice or aperture.

Most of the 6000+ recent species of bryozoans belong to the clade Myolaemata, which is predominantly marine, whereas its sister-group, Phylactolaemata, is a small taxon of less than 100 described species that live in freshwater habitats. Myolaemates are divided into the sister-groups Stenolaemata and Gymnolaemata. The former is a solely marine group of bryozoans with only one recent clade, Cyclostomata. Gymnolaemata comprises the paraphyletic ctenostomes and the monophyletic Cheilostomata (Schwaha, Ostrovsky, et al., 2020).

Hislopiid ctenostomes (Superfamily Hislopioidea) are a small group of solely freshwater inhabitants consisting of three genera and up to nine currently recognized species, seven of them belonging to the genus *Hislopia*. The genera *Echinella* and *Timwoodiellina* are monotypic (Schwaha, 2020a). Colonies are generally creeping with flattened, oval zooids, which have a characteristic quadrangular aperture often surrounded by large cuticular spines or a cuticular distal rim (Mane-Garzon, 1959, d'Hondt, 1983). Besides differences in the aperture, bud formation

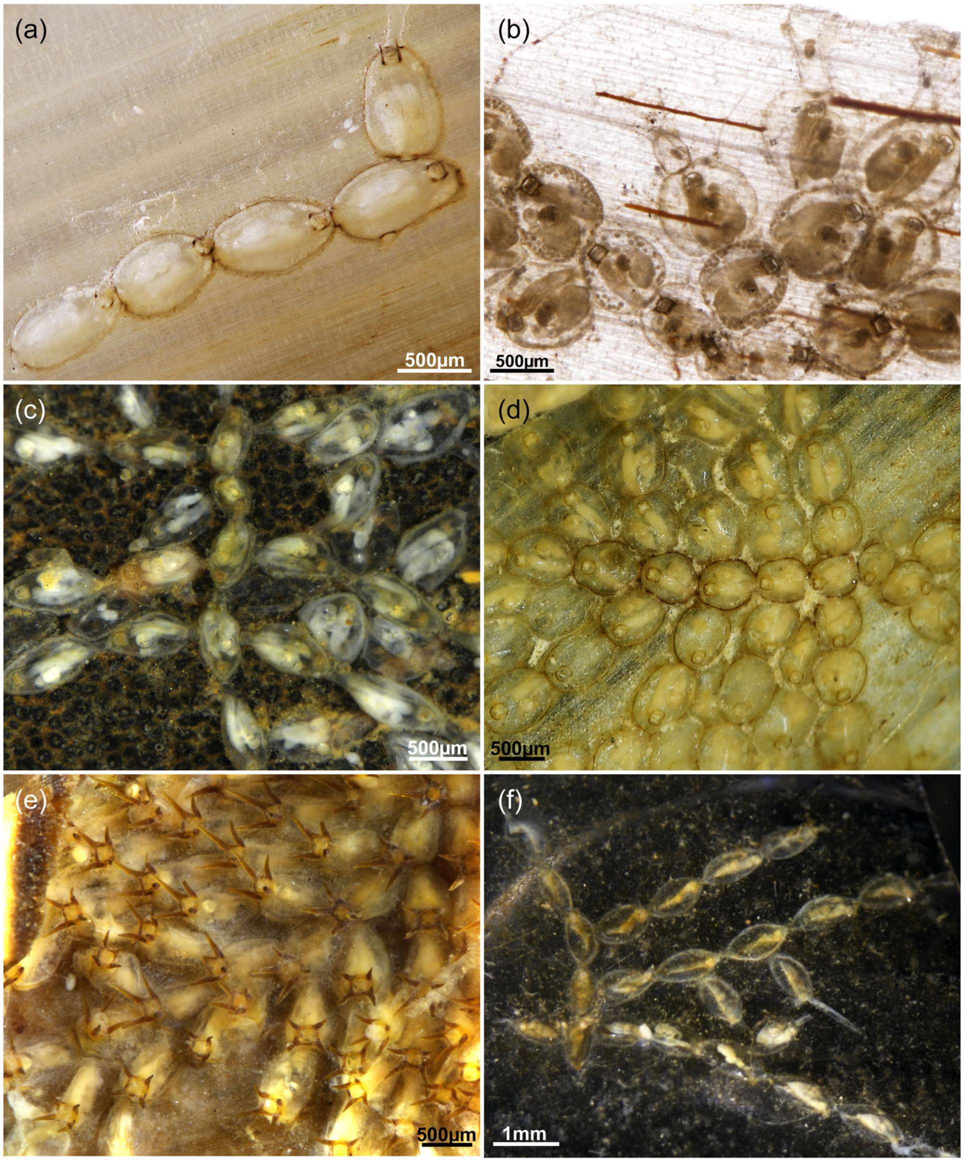

FIGURE 1 General overview of hislopiid ctenostome colonies. (a) *Hislopia corderoi*. (b) *H. lacustris* (c) *H. prolixa* (d) *H. malayensis*. (e) *Echinella placoides* (f) *Timwoodiellina natans*.

is also specific in some species (Hirose & Mawatari, 2007; Hirose & Mawatari, 2011; Wood et al., 2006a). *Echinella placoides* is a species endemic to Lake Baikal and characterized by possession of a true gizzard with teeth (Korotneff, 1901; Wiebach, 1966). Other hislopiids usually only have a cuticular lining to form a proventriculus in the cardiac area of the midgut (Annandale 1911a, 1911b; Schwaha & Wood, 2011). *Timwoodiellina natans* has a unique feature of sexual reproduction leading to the formation of so-called nautizooids (Wood et al., 2006b; Wood et al., 2010), which essentially are free-swimming zooids that after a certain period of time attach to substrates to form new colonies. Morphological studies of hislopiid zooids are limited to characterizing the muscular system and budding of *Hislopia malayensis* (Schwaha & Wood, 2011; Schwaha et al., 2011), or details of the digestive tract of *Hislopia prolixa* (Hirose & Mawatari, 2011).

Since morphological details have not been studied in a comparative context, we studied six hislopiid species across all three genera. Details on apomorphic and plesiomorphic characters might, in turn, provide insights on the still unclear sister-group of hislopiids.

2 | MATERIAL AND METHODS

Hislopia corderoi was collected from Brazil in 2016, *H. malayensis* from Thailand in 2009 and 2020, *H. prolixa* from Korea in 2016 and Japan in 2020, *Timwoodiellina natans* from Thailand in 2010. Samples of *Hislopia lacustris* and *Echinella placoides* were taken from collections of the Zoological Museum of Hamburg (CENAK, B0117 and B0503, respectively).

All samples were preserved in 90–96% ethanol except *H. malayensis*, which was fixed in 2% glutaraldehyde in 0.01 mol L^{-1} cacodylate buffer followed by postfixation with osmiumtetroxide. Fixed samples were dehydrated with acidified dimethoxypropane followed by infiltration into Agar Low Viscosity resin (Agar Scientific, Stansted) via acetone as intermediate. After polymerization of resin blocks, serial sections were conducted for each species (see also Ruthensteiner, 2008).

Serial sections of each species were photographed with a Nikon NiU compound microscope equipped with a DsRi2 camera (Nikon). Image stacks were converted to greyscales and contrast enhanced in FIJI (Schindelin et al., 2012) before importing into the reconstruction software

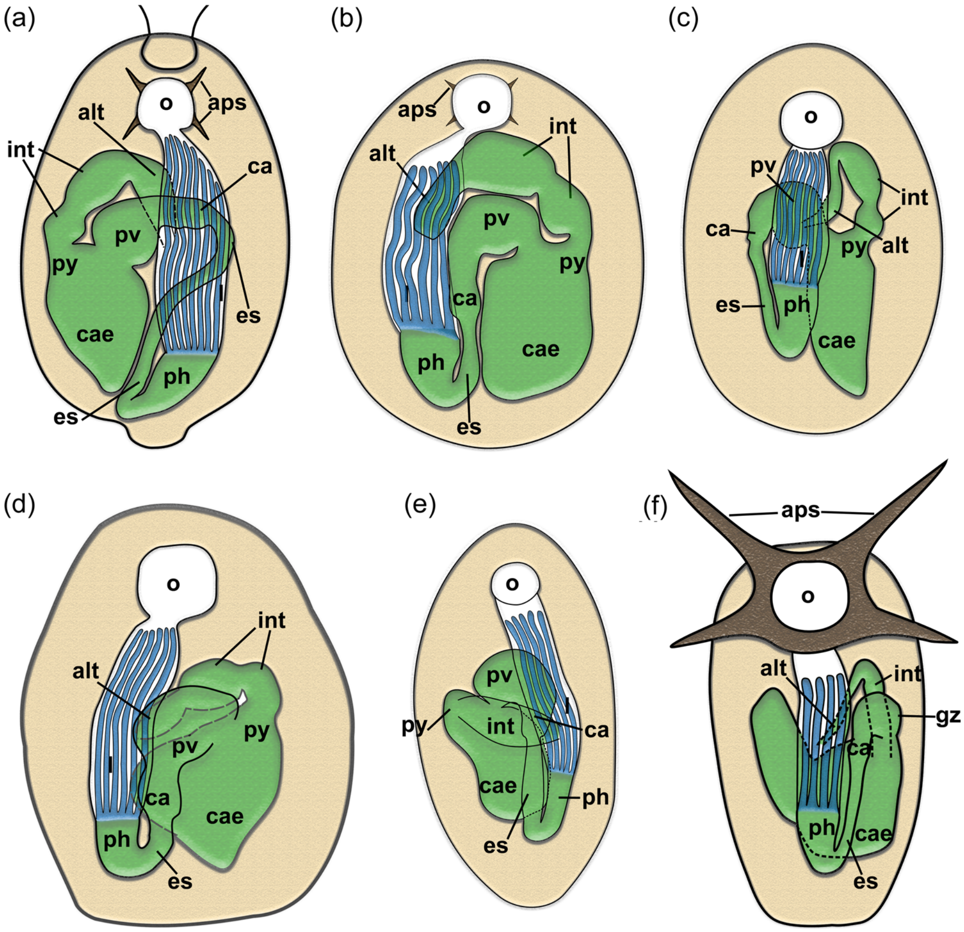

FIGURE 2 Schematic drawings of hislopiid ctenostome zooids. (a) *Hislopia corderoi* (b) *H. lacustris* (c) *H. prolixa* (d) *H. malayensis* (e) *Timwoodiellina natans* (f) *Echinella placoides*. alt, anal tube; aps, apertural spines; ca, cardia; cae, caecum; es, esophagus; gz, gizzard; int, intestine; l, lophophore; o, orifice; ph, pharynx; pv, proventriculus; py, pylorus.

Amira (2020.1, ThermoFisher). After section alignment, structures of interest were semi-manually segmented and rendered as surface models. Surrounding tissues were visualized as volume renderings.

3 | RESULTS

3.1 | Colony structure, general zooidal form and body cavity

In general, colonies of hislopiids consist of uniserial colonies of oval to slightly elongated zooids in proximo-distal direction (Figures 1 and 2). Young colonies consist mostly of elongated chains of zooids with some lateral chain of buds on each side (Figure 1). Zooids are most oval in *H. lacustris* and *H. malayensis*, more elongated in *H. corderoi* and *H. prolixa* and most elongated in *T. natans* and *E. placoides* (Figure 2). Older colonies form dense sheets encrusting the entire substrate, sometimes even overgrowing other parts of the colony. Usually, zooids are transparent allowing observation of internal polypide structures, with the zooidal rim being more strongly cuticularized and often brown or dark in color (Figures 1 and 3a). In all species, the aperture is located at the distal end of each zooid, usually slightly elevated. In *H. prolixa* and *E. placoides* zooids are relatively thicker (having a longer frontobasal axis). A cuticular almost circular rim surrounds the apertural area and the orifice is quadrangular. Four apertural spines are present in *H. lacustris* (Figures 1b and 2b), *H. corderoi* (Figures 1a, 2a, and 4a) and *E. placoides* (Figures 1e, 2f, and 5a,b,d), most prominent in the latter.

Pairs of parietal muscles laterally traverse the body cavity (Figure 5a,b). The proximal zooidal cavity is largely filled with prominent retractor muscles, which attach to the lophophoral base. In *E. placoides* a second bundle also originates from a more lateral position of the zooid (Figure 5a).

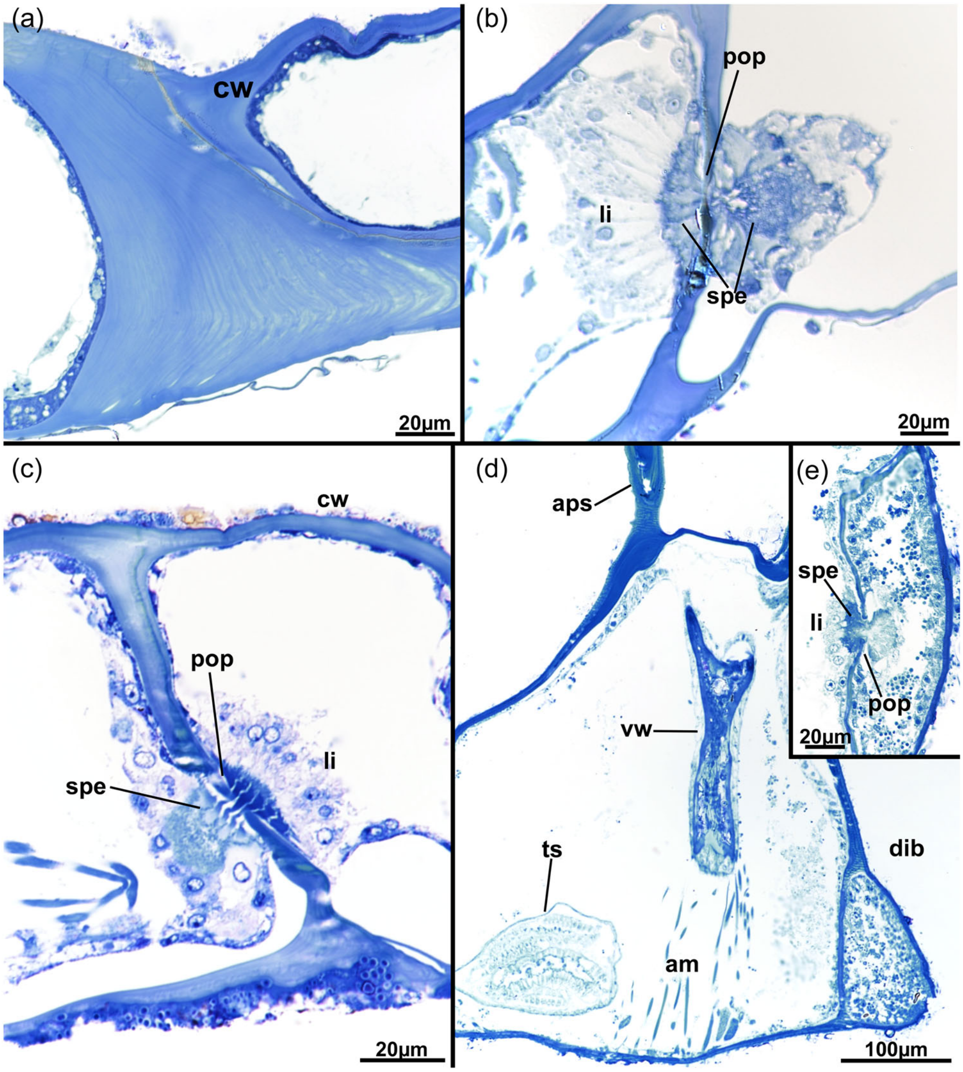

FIGURE 3 Body wall and pore-plates in hislopiids. (a) *Hislopia malayensis* showing thick multilayered cuticle between zooids. (b) Detail of pore-plate of *H. malayensis*. (c) Detail of pore-plate of *H. prolixa*. (d) Overview of internal, distal budding area of *Echinella placoides*. (e) Detail of pore-plate to distal budding compartment of *E. placoides*. am, apertural muscles; aps, apertural spines; cw, cystid wall; dib, distal bud; li, limiting cells; pop, pore plate; spe, special cells; ts, tentacle sheath; vw, vestibular wall.

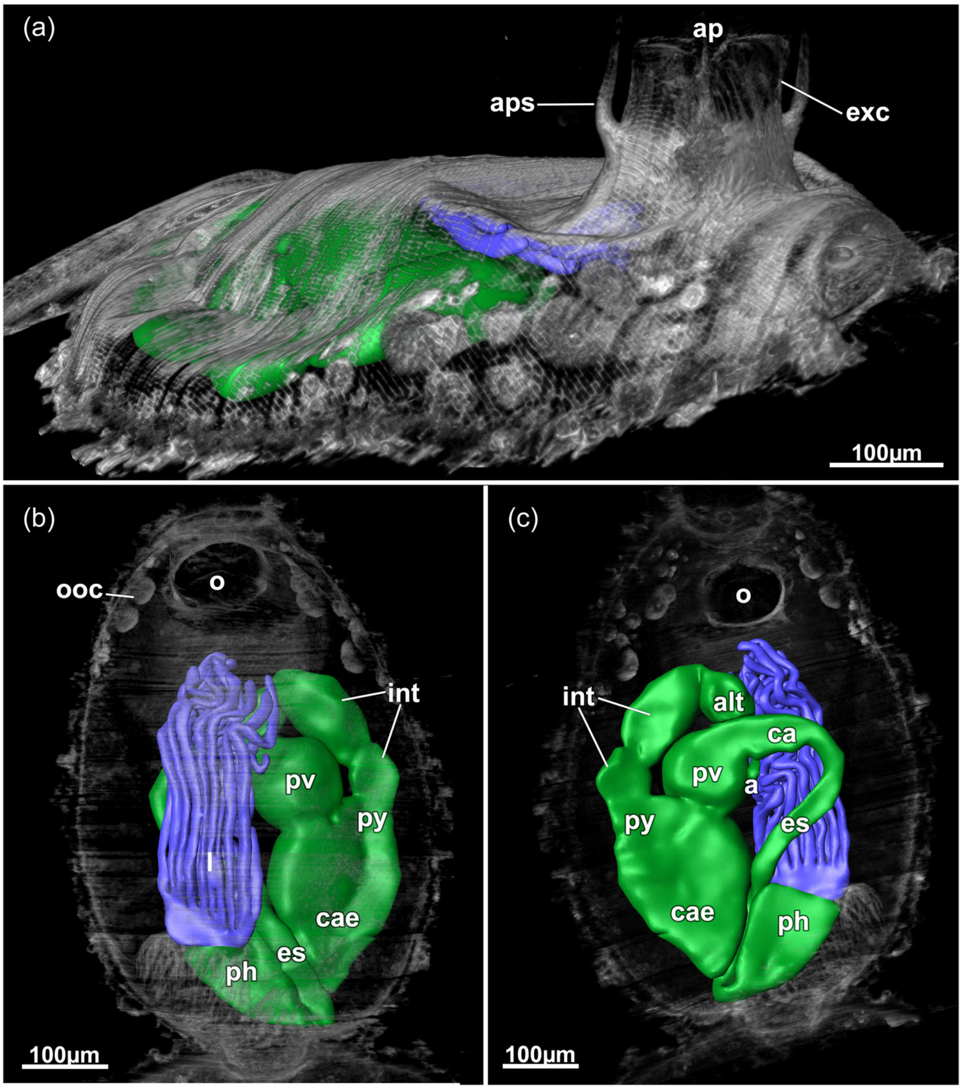

FIGURE 4 *Hislopia corderoi*, 3D reconstruction of polypide features. (a) Lateral view of zooid showing apertural papilla and short apertural spines. (b) Frontal view showing digestive tract and lophophore. (c) Basal view of zooid showing digestive tract. a, anus; alt, anal tube; ap, aperture; aps, apertural spines; ca, cardia; cae, caecum; exc, external collar; es, esophagus; int, intestine; l, lophophore; o, orifice; ooc, oocytes; ph, pharynx; pv, proventriculus; py, pylorus.

3.2 | Lophophore and digestive tract

The lophophore is similar among most hislopiids with a range of 12 to 18 tentacles in the genus *Hislopia* and *T. natans*. *E. placoides* carries only eight tentacles on its lophophore. The digestive tract starts with a mouth opening at the lophophoral base and shows the general compartments of all bryozoans with a foregut consisting of a pharynx and esophagus, a midgut comprising the cardia, caecum and pylorus and a hindgut with the intestine terminating with the anus into the tentacle sheath (Figure 2). Adjoining the mouth opening, the pharynx is a simple tube of prismatic, vacuolated cells (Figure 6b) extending to the proximal border of zooids before bending distally as esophagus (Figure 2). The latter is comparatively short in *H. lacustris* (Figure 7c) and *H. malayensis* (Figure 8), but highly elongated in *H. prolixa* (Figures 2c and 9), *H. corderoi* (Figures 2a and 4b,c) *T. natans* (Figures 2e and 10c) and *E. placoides* (Figures 2f and 5c,d). Ciliary, paired domes are present on the esophagus of *H. malayensis* (Figure 6b), *H. prolixa* (Figure 6c) and H. *corderoi*. The esophagus enters the midgut, the cardia, via a cardiac valve, which is also marked by a general widening of the general gut diameter before entering the bulbous proventriculus or gizzard. The proventriculus is an enlarged cardiac area (Figures 2, 4b,c, 5c,d, 7c, 8a, and 9b) lined by a regular, internal thick cuticle (Figure 11a-d,f). In *E. placoides* this area is differentiated as a massive gizzard with large, cuticular teeth pointing proximally (Figures 11e and 12). The teeth are arranged spirally with larger ones more centrally and smaller more peripherally (Figure 12). Both types, proventriculus and gizzard, are surrounded by massive musculature (Figure 11). The midgut continues into the voluminous caecum, which typically shows highly vacuolated cells (Figure 6a). It is more elongated and slender in *H. prolixa* (Figures 2c and 9b), of

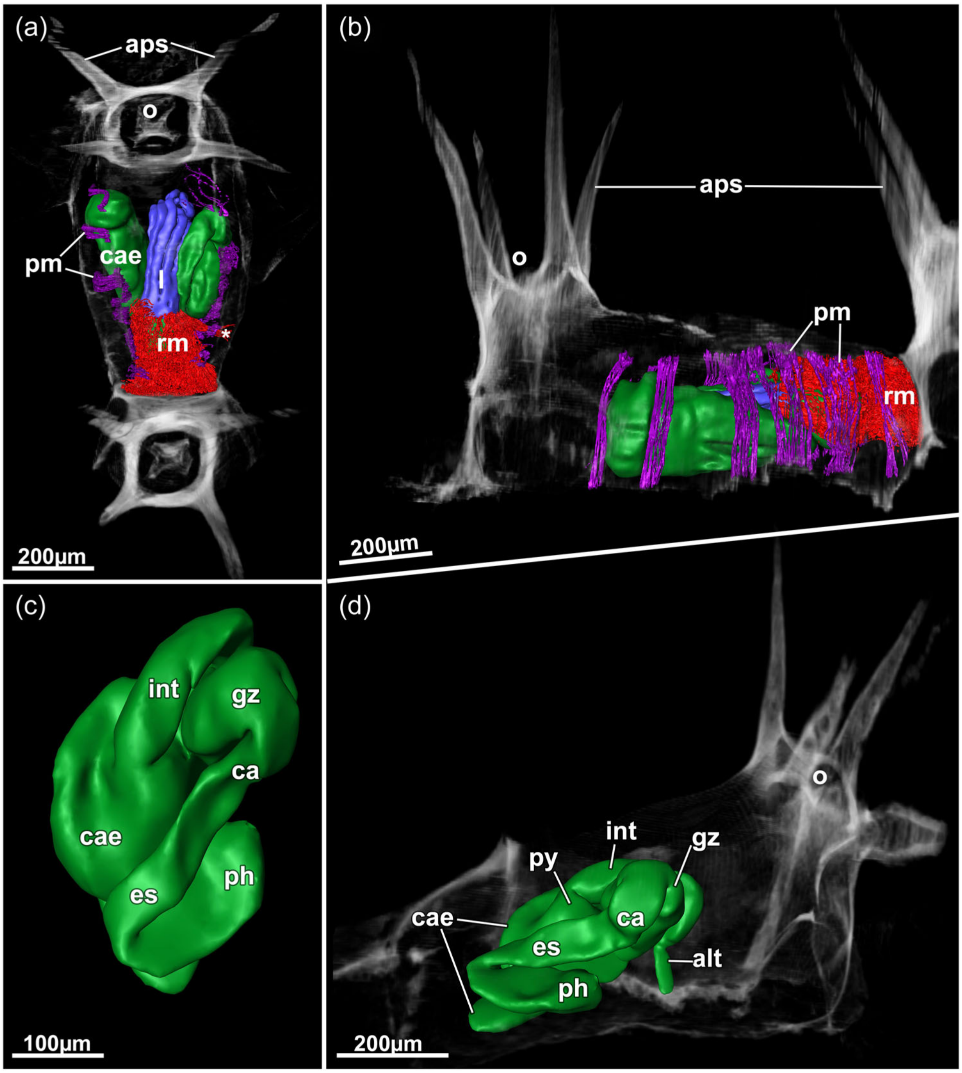

FIGURE 5 *Echinella placoides*, 3D-reconstruction of polypide features. (a) Frontal view of a zooid showing large apertural spines, also from proximal zooid. Asterisk marks lateral retractor muscle bundle. (b) Lateral view showing long apertural spines. (c) Frontal view of the digestive tract. (d) Oblique view of the digestive tract within the zooid. a, anus; alt, anal tube; aps, apertural spines; ca, cardia; cae, caecum; es, esophagus; gz, gizzard; int, intestine; l, lophophore; o, orifice; ph, pharynx; pm, parietal muscles; rm, retractor muscles.

medium size in *T. natans* (Figures 2e and 10a,c) and largest in *H. corderoi* (Figures 2a and 4c), H. *malayensis* (Figures 2d and 8) and *H. lacustris* (Figures 2b and 7c). *E. placoides* frequently shows a lobed caecum (Figures 2f and 5a). Distally, towards the hindgut, the caecum enters a ciliated area, the pylorus, before continuing into the intestine. The intestine has two distinct partitions, a proximal and distal one, in the genus *Hislopia* (Figures 2a–d, 4b,c, 7c, 8a,b and 9b), but has only a single compartment in *T. natans* and *E. placoides* (Figures 2e,f, 5c and 10c). The terminal part of the intestine enters the tentacle sheath via an elongated anal tube at a mid-lophophoral area (Figures 2, 4c, 7c, 8b and 9b). Both intestinal parts of *Hislopia* extend distally into the body cavity with the anal tube reversing proximally in its course to the tentacle sheath (Figure 2a–d), which is less pronounced in *H. malayensis*. In *T. natans* an anal tube is missing and the intestine is simple and directly bends from the pylorus to terminate via an anus into the tentacle sheath, orthogonal to the lophophore (Figures 2e and 10c). The intestinal tube of *E. placoides* is directed similarly to *Hislopia*, exiting distally before turning proximally into the slender anal tube (Figures 2f and 5c,d).

3.3 | Cuticle and apertural area

The cuticle, the outer layer of the cystid wall, is similar in all hislopiids and generally forms a thin, smooth layer (Figures 3a–d, 6d and 13a–e). Local thickenings of cuticle are present on lateral sides of zooids and are commonly multilamellar (Figure 3a). In older zooids these lateral rims often show a brown coloration. The cuticle is thickened in the distal apertural area (Figure 13), particularly when apertural spines are present. Spines are continuous structures of the main body cavity and not separated via pore-plates (Figure 3d). At the aperture the cuticle continues as thick layer on the vestibular wall towards the diaphragm.

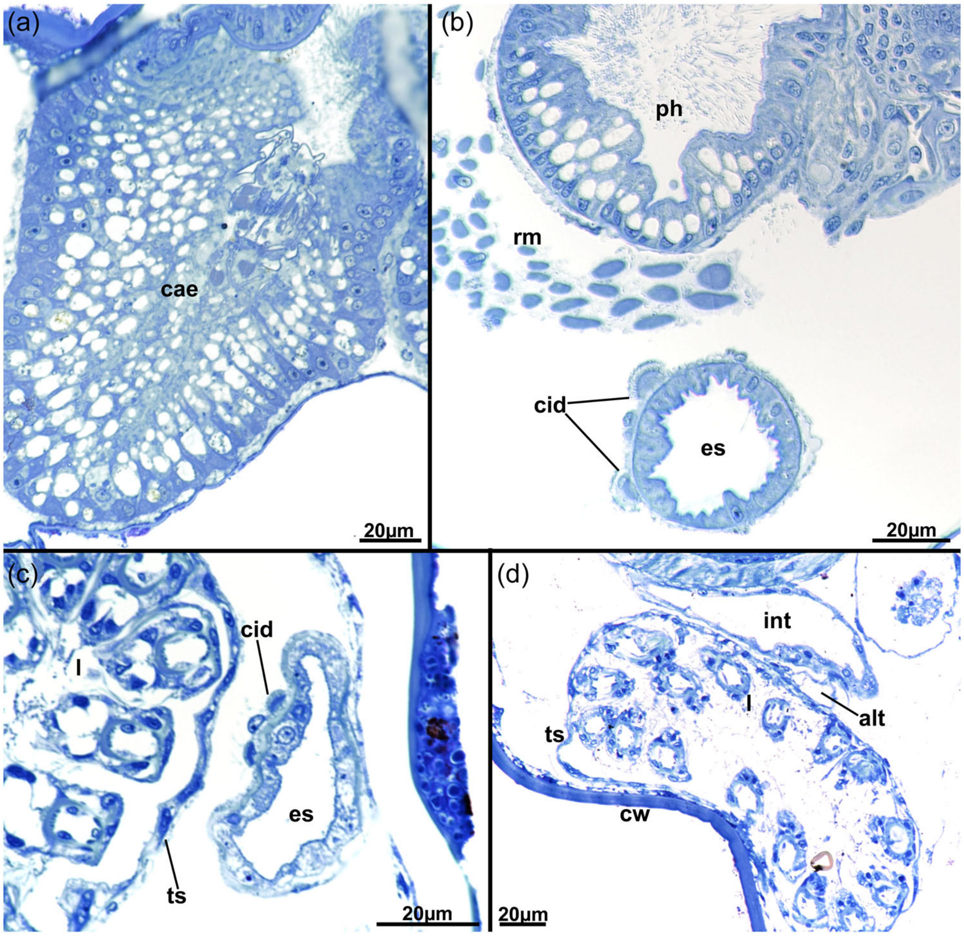

FIGURE 6 Gut histology in hislopiids. (a) *Hislopia malayensis*, caecum structure. (b) *H. malayensis*. Cross-section of foregut: pharynx and esophagus with ciliary domes. (c) Cross-section of the esophagus of *H. prolixa* showing ciliary domes. (d) Cross-section of the intestinal area and anal tube of *H. prolixa*. alt, anal tube; cae, caecum; cid, ciliary domes; cw, cystid wall; es, esophagus; int, intestine; l, lophophore; lb, lophophoral base; ph, pharynx; rm, retractor muscles; t, tentacle; ts, tentacle sheath.

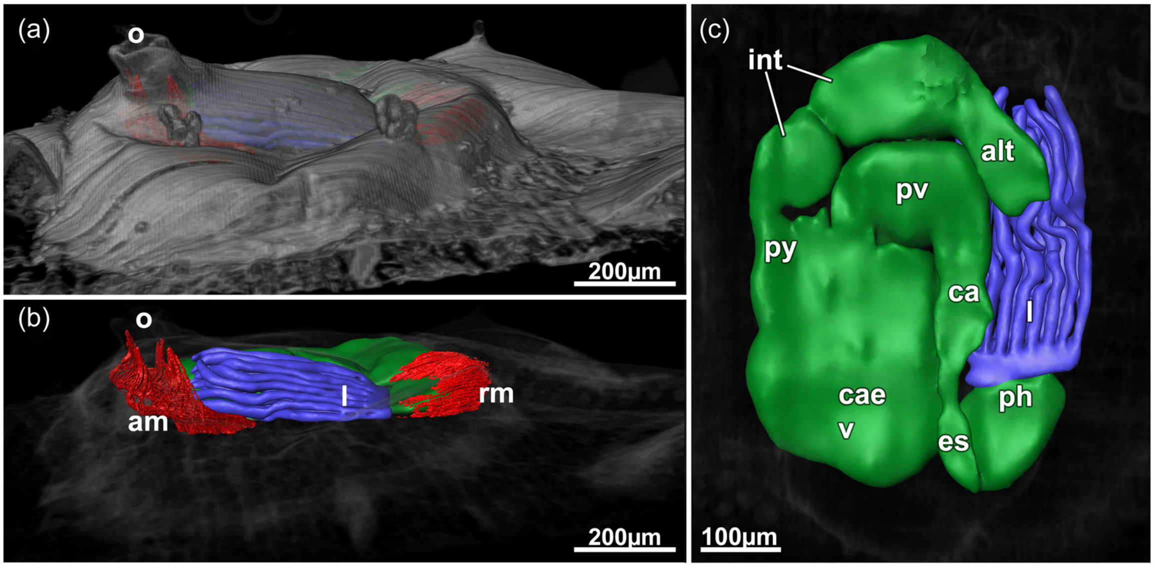

FIGURE 7 *Hislopia lacustris*, 3D reconstruction of polypide features. (a) Lateral view of zooid showing apertural papilla. (b) Lateral view showing digestive tract, lophophore retractor and apertural muscles. (c) Basal view of zooid showing digestive tract features. a, anus; alt, anal tube; am, apertural muscles; ca, cardia; cae, caecum; es, esophagus; int, intestine; l, lophophore; o, orifice; ooc, oocytes; ph, pharynx; pv, proventriculus, py, pylorus; rm, retractor muscles.

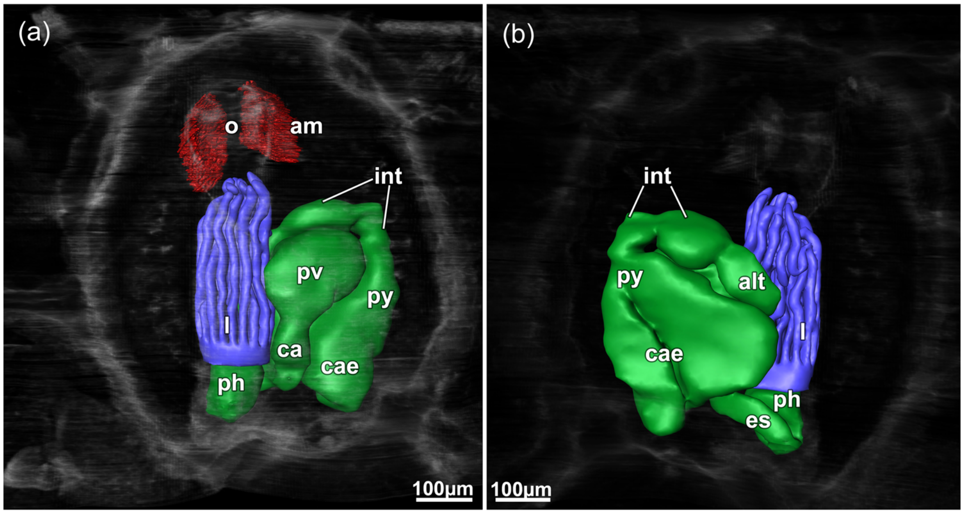

FIGURE 8 *Hislopia malayensis*, 3D reconstruction of polypide features. (a) Frontal view showing lophophore and digestive tract properties within the zooid. (b) Basal view of digestive tract and lophophore. alt, anal tube; am, apertural muscles; ca, cardia; cae, caecum; es, esophagus; int, intestine; l, lophophore; o, orifice; ph, pharynx; pv, proventriculus; py, pylorus.

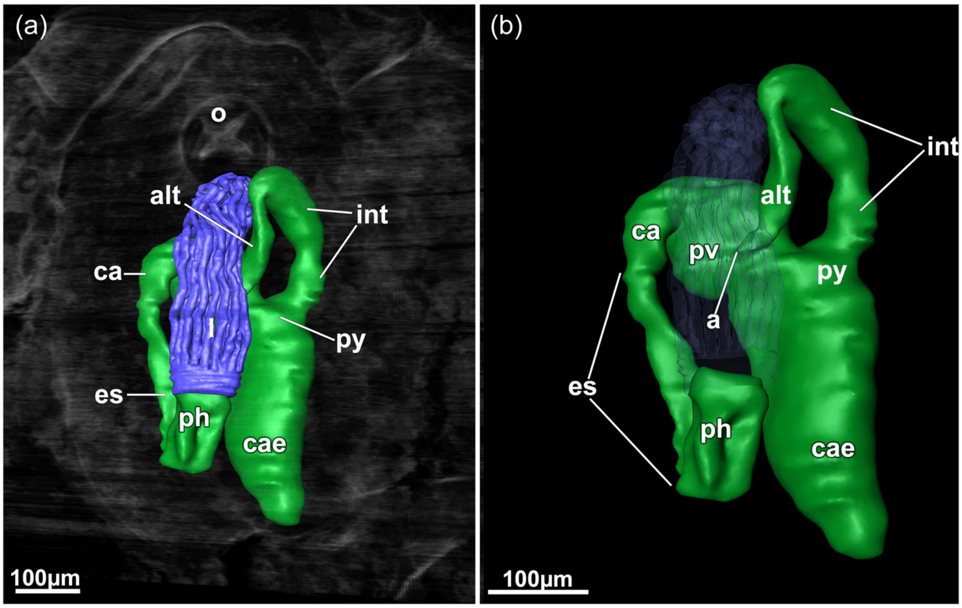

FIGURE 9 *Hislopia prolixa*, 3D reconstruction of polypide features. (a) Frontal view showing lophophore and digestive tract properties within the zooid. (b) Close-up of digestive system with transparent lophophore. a, anus; alt, anal tube; ca, cardia; cae, caecum; es, esophagus; int, intestine; l, lophophore; o, orifice; ph, pharynx; pv, proventriculus; py, pylorus.

On the outer lateral side of the vestibular wall prominent apertural muscles are inserted at the proximal margin (Figures 7b, 8a and 13a,c–f).

At the diaphragm a collar of different proportions extends as a highly folded cuticular structure into the vestibulum in *H. lacustris* (Figure 13b), H. *prolixa* (Figure 13c), *H. malayensis* (Figure 13e) and *E. placoides* (Figure 13f). The collar in the genus *Hislopia* is similar in size and extent, whereas *E. placoides* has a highly folded collar obstructing most of the vestibulum (Figure 13f). *H. corderoi* and *T. natans* lack a collar, but instead possess a pair of outer double cuticle that projects from the distal vestibular wall in *H. corderoi* (Figure 13a) or the lateral outer wall of the apertural papilla in *T. natans* (Figure 13d). These projections are thin and at least in *H. corderoi* join medially in the apertural area.

3.4 | Ovary and oocytes

Oocytes encountered in the studied material usually consist of multiple small ovarian clusters associated with the body wall (Figures 4b

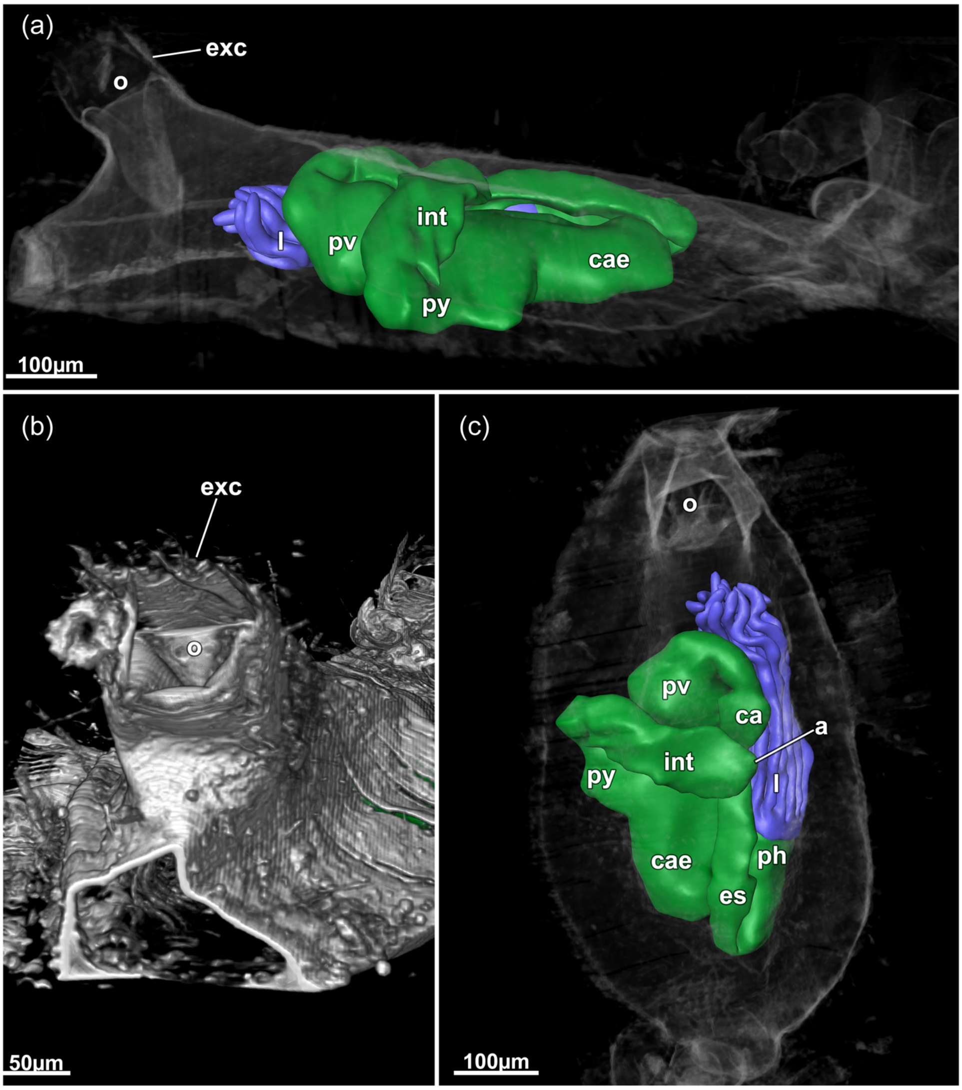

FIGURE 10 *Timwoodiellina natans*, 3D reconstruction of polypide features. (a) Lateral view of zooid showing apertural papilla and double cuticle. (b) Distal view showing quadrangular orifice and double cuticle partially cut to show internal aperture. (c) Basal view of zooid showing digestive tract features. a, anus; ca, cardia; cae, caecum; exc, double cuticle/external collar; es, esophagus; int, intestine; l, lophophore; o, orifice; ooc, oocytes; ph, pharynx; pv, proventriculus; py, pylorus.

and 14a–c). They appear randomly distributed within the body cavity and can be located distally or proximally in the zooid. Oocytes in the genus *Hislopia* are numerous and usually of ~25 µm diameter (Figure 14a–c). Reproductive structures were not observed in *T. natans*. *E. placoides* has a single large, macrolecithal oocyte in association with a second smaller cell (Figure 14d). A peculiar cuticular shield is attached to the frontal side of the oocyte (Figure 14d).

4 | DISCUSSION

4.1 | General zooidal and colony morphology

The general zooidal and colony morphology are similar for most species of hislopiids with uniserial chains of mostly, flattened ellipsoid zooids (Annandale, 1911a, 1911b, 1916, Hirose & Mawatari, 2007; Mane-Garzon, 1959; Wood et al., 2010; d'Hondt, 1983). *E. placoides* shows different zooidal forms that are more slender and higher along the frontobasal axis (Abricossoff, 1924; Wiebach, 1966), whereas *H. prolixa* has truly dimorphic zooids, with one being identical to other species of *Hislopia* and the other formed by erect branches that appear more *Paludicella*-like (Hirose & Mawatari, 2011).

4.2 | Lophophore and digestive tract

The lophophore of hislopiids shows little differences among its species, with the number of tentacles ranging from 8 to 18 (see also Hirose & Mawatari, 2011), although zooids of *H. prolixa* with 22 tentacles were recently reported (Grabow et al., 2022). Eight

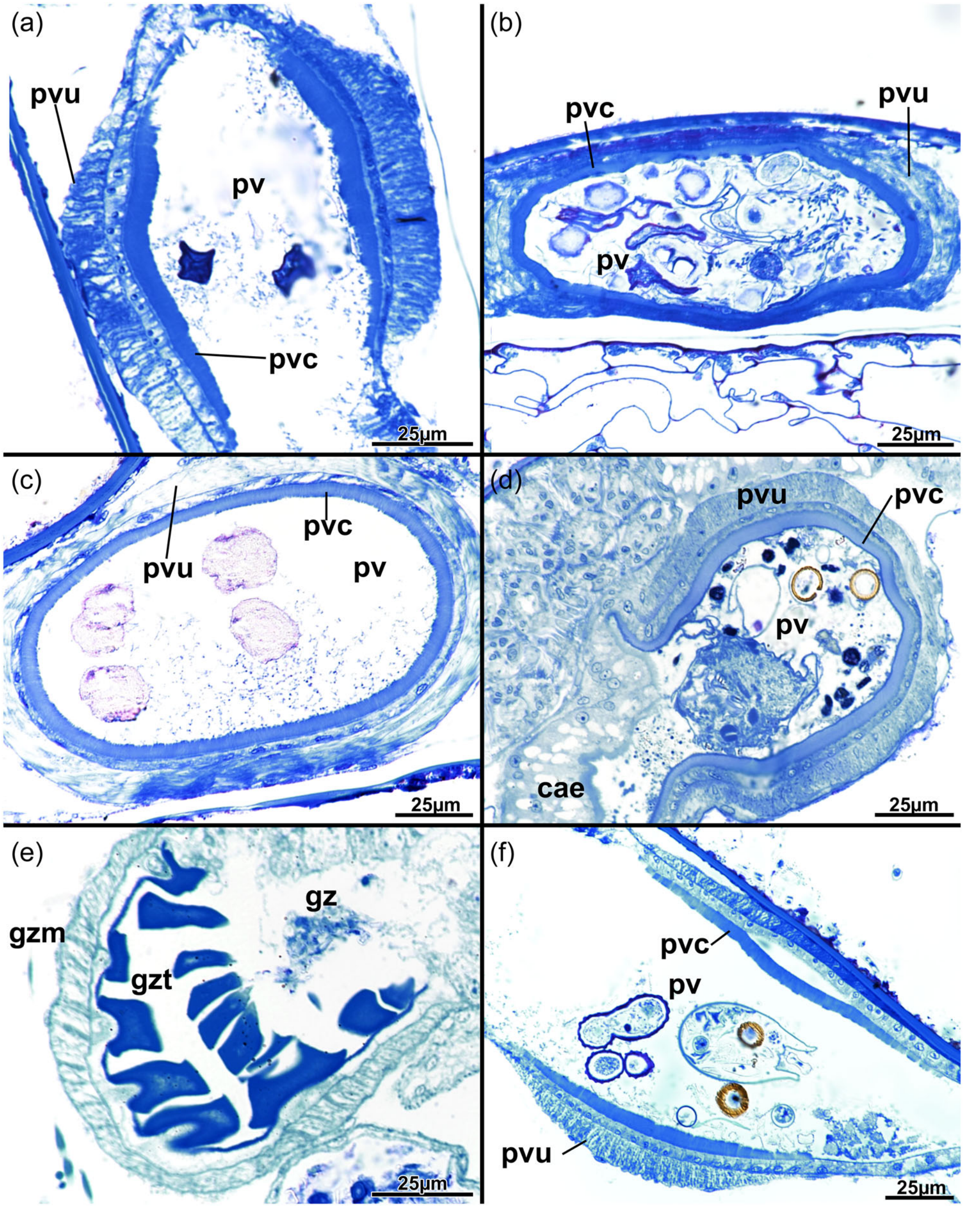

FIGURE 11 Proventriculus and gizzard of hislopiids, histological details. (a) *Hislopia corderoi*. (b) *H. lacustris*. (c) *H. prolixa* (d) *H. malayensis*. (e) *Echinella placoides*. (f) *Timwoodiellina natans*. ca, cardia; cae, caecum; gz, gizzard; gzm, gizzard musculature; gzt, gizzard teeth; pv, proventriculus; pvc, proventriculus cuticle; pvu, proventriculus muscles.

tentacles are typical for many other ctenostome families such as victorellids, most walkerioideans and vesicularioideans (d'Hondt, 1983; Schwaha, 2020a), but also other bryozoans such as cyclostomes (e.g., Jebram, 1986a).

The digestive tract shows several peculiar features. Most obvious is the large proventriculus in all hislopiids except the genus *Echinella*, which has a prominent gizzard. The distinction between proventriculus and gizzard lies in the presence of a simple lining cuticle for the former and distinct food-crushing gizzard teeth for grinding food particles (Markham & Ryland, 1987; Schwaha, 2020a, 2020b). Proventriculi are more associated with mixing and kneading food particles in the cardiac area of the midgut rather than crushing testate organisms (Annandale 1911a; 1911b; Wiebach, 1966). Gizzards are common in many clades of ctenostomes such as walkerioideans, vesicularioideans and *Bulbella* (Markham & Ryland, 1987). The special type of gizzard of *Echinella* with its crooked teeth and their spiral arrangement was previously recognized (Wiebach, 1966), and is confirmed by our observations. *E. placoides* is the only freshwater ctenostome with a true gizzard. Lack of additional data, material and observations, however, fail to explain the functional significance of this massive structure. Proventriculi are scarcer in other ctenostomes and are for example, found in penetrantiids (Decker et al., 2023). A cardia showing a simple cardiac constrictor, i.e. a more prominent circular musculature in this gut region, is more common (Schwaha & Wanninger, 2018; Schwaha, 2020a). Since a phylogeny incorporating most ctenostome clades is still missing, it is not entirely clear how the

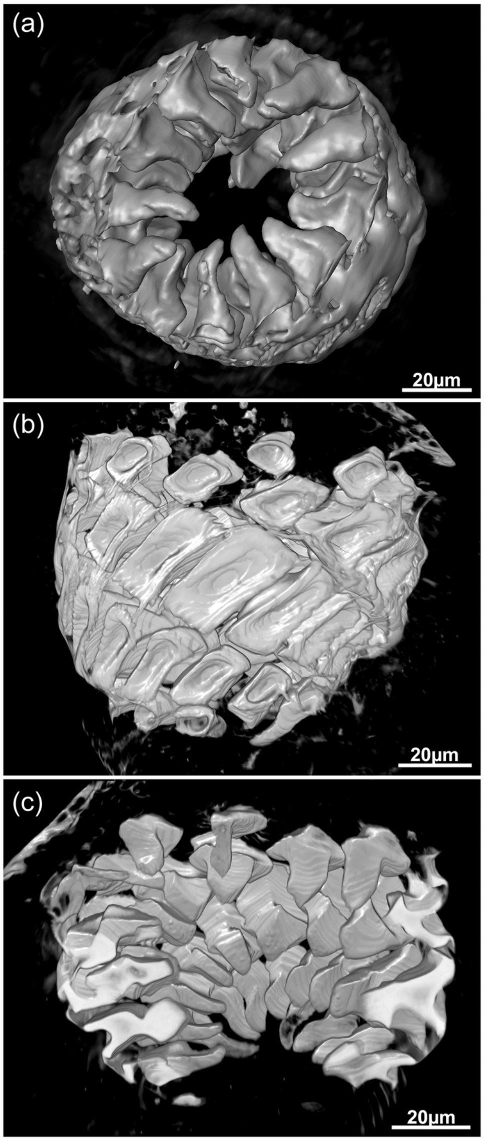

FIGURE 12 *Echinella placoides*, 3D-reconstruction of gizzard. (a) Top view into the gut lumen showing spirally arranged gizzard teeth projecting into the lumen. (b) Lateral view of inner teeth surfaces showing spiral arrangement of teeth. (c) Lateral view into gut lumen showing individual teeth projecting inward. gzt, gizzard teeth.

different types of cardia modifications (gizzard, proventriculus, constrictor) have evolved. It would be interesting to unravel the sister group of hislopiids and also the internal hislopiid phylogeny to assess possible evolutionary transitions from constrictors to proventriculi to gizzards or vice versa.

Besides the obvious large proventriculus or gizzard of hislopiids, the relatively long esophageal tube is also a common feature of hislopiids. *H. lacustris* and *H. malayensis* show the shortest esophagi, but in comparison to other ctenostomes are still much longer. In other taxa such as victorellids (Braem, 1951), alcyonidioideans (Decker et al., 2021; Le Brozec, 1955; Prouho, 1892, Schwaha, 2021; Schwaha, Winston, et al., 2022), walkerioideans (Pröts et al., 2019), *Aethozooides* (Schwaha, Bernhard, et al., 2019), *Haywardozoon* (Schwaha, Grischenko, et al., 2020), *Pierrella* (Schwaha et al., 2021), *and Monobryozoon* (Remane, 1936) the esophagus is always short. Equally long esophagi are rare, but have been reported in penetrantiids (Decker et al., 2023), *Amphibiobeania epiphylla* (Schwaha, Waeschenbach, et al., 2022), and may also characterize the vesicularioidean *Amathia verticillata* (Zirpolo, 1933). The relevance of a long esophagus remains unclear.

Ciliary dome-shaped patches on the esophagus were previously reported in *Hislopia malayensis* (Schwaha, Ostrovsky, et al., 2020), but so far not for any other hislopiid or other ctenostomes. This study reports such patches at least for two additional species, although fixation is also a crucial issue since these domes are easier to identify on glutar-fixed samples than on simple ethanol-fixed samples. Pharyngeal ciliation in form of ciliary streets or a ciliated gutter can be found on the anal side of the pharynx in a species of *Alcyonidium* (Hassall, 1841), *Bowerbankia gracilis* (Reed, 1988) and *Elzerina binderi* (Schwaha, 2021). Similar ciliation is present in some cheilostomes, often in association with an intertentacular organ used for expelling zygotes and entry of alien sperm (see Ostrovsky & Porter, 2011), which is probably also the function of pharyngeal ciliation in ctenostomes. The hislopiid esophageal ciliation might also act in a similar way. Since this ciliation is usually transitional, it would be possible to test this in hislopiids occurring in temperate areas such as *H. prolixa*, which has only a short reproductive period, contrary to tropical/subtropical species (Hirose & Mawatari, 2011; Wood et al., 2021).

Caecum size is large in hislopiids compared to many other taxa. Alcyonidioidean ctenostomes were previously considered more closely related to hislopiids, based mostly on colony morphological characters (see Jebram, 1986b; Todd, 2000). However, their caecum is usually vestigial or small compared to hislopiids (Prouho, 1892, Le Brozec, 1955, d'Hondt, 1983). It is not clear which factors affect caecum size. Possibly the presence of a gizzard/proventriculus, which is always missing in alcyonidioideans, could play a role. Alternatively, the flattened zooidal shape of hislopiids has some effect for increasing surface area. In this regard, it would be interesting to study the erect morph of *H. prolixa*, which does not have the typical flattened zooidal shape (Hirose & Mawatari, 2011). The large, often lobed caecum of *E. placoides* observed in the current study was also noticed by Wiebach (1966).

Unique for ctenostomes is the presence of a two-chambered intestine in the genus *Hislopia*. In other bryozoans, the intestine always forms a single tube (see Schwaha, Ostrovsky, et al., 2020; Silen, 1944). Comparison to phylactolaemate and cyclostome guts, which also have a single tube, indicates that this hindgut type is the ancestral character state and the two-chambered form might be apomorphic for the genus *Hislopia*. Interestingly, the other two hislopiid genera show a single intestinal tube. The function of two distinct intestinal chambers is unknown.

An elongated anal tube entering the tentacle sheath is with the exception of *T. natans* found in all hislopiids. It is also at least present in the ctenostome *Paludicella articulata* (Allmann, 1856;

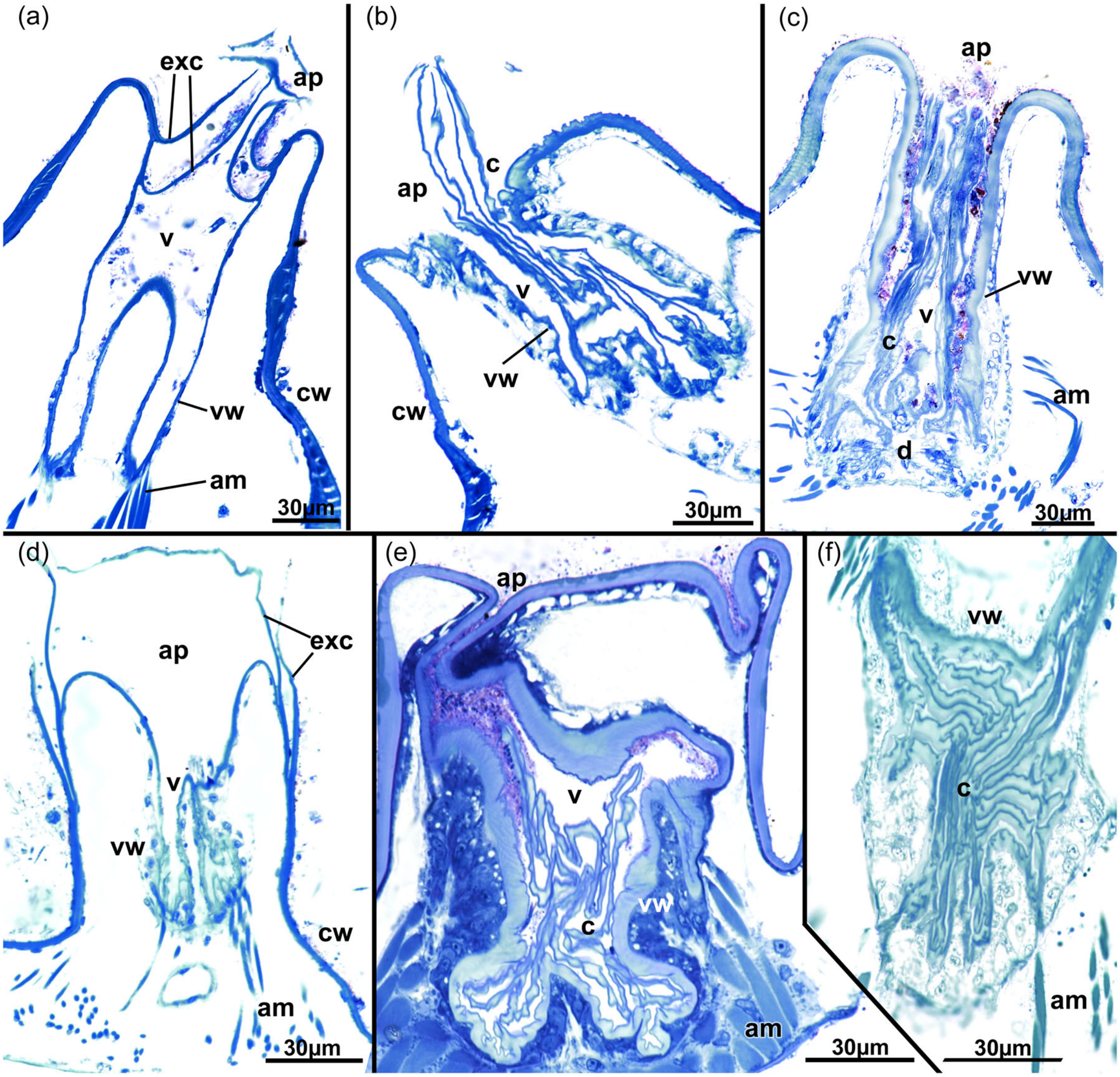

FIGURE 13 Apertural area of hislopiids, histological details. (a) *Hislopia corderoi*. (b) *Hislopia lacustris*. (c) *Hislopia prolixa* (d) *Timwoodiellina natans*. (e) *Hislopia malayensis*. (f) *Echinella placoides*. am, apertural muscles; ap, aperture; c, collar; cw, cystid wall; d, diaphragm; exc, external collar; v, vestibulum; vw, vestibular wall.

Hancock, 1850). In most other analyzed ctenostomes such a tube is missing. Future investigations of cheilostome gut morphology are required to enable comparative insights on digestive tract anatomy.

4.3 | Cuticle and aperture

The cuticle is generally thin in hislopiids and many other ctenostomes (e.g., Victorellidae: Braem, 1951, *Paludicella*: Weber et al., 2014, *Aethozooides*: Schwaha, Bernhard, et al., 2019, *Arachnidium*: Schwaha & De Blauwe, 2020, *Pierrella*: Schwaha et al., 2021). Generally thicker cuticles characterize alcyonidioideans (Decker et al., 2021; Schwaha, 2021; Schwaha, Grischenko, et al., 2020; Schwaha, Winston, et al., 2022).

Collars as protective structures obstructing the vestibulum of retracted zooids are a plesiomorphic character of gymnolaemates (McKinney & Dewel, 2002; Schwaha, Ostrovsky, et al., 2020). Among ctenostomes collars can vary from small to highly elongated forms. Also, the ctenostome collar originates either from the diaphragm or from the proximal vestibular wall, and is accordingly termed diaphragmatic and vestibular collar (Schwaha, 2021). The external collar of *H. corderoi* and *T. natans* is unique and has not been observed in any other species. Only penetrantiids show a double cuticle, particularly in the apertural area (Decker et al., 2023; Silen, 1947). It shows some resemblances to the external collar, but they often retain a diaphragmatic collar. The phylogenetic placement of penetrantiids requires clarification to assess whether such external cuticular structures evolved independently. The external collar in

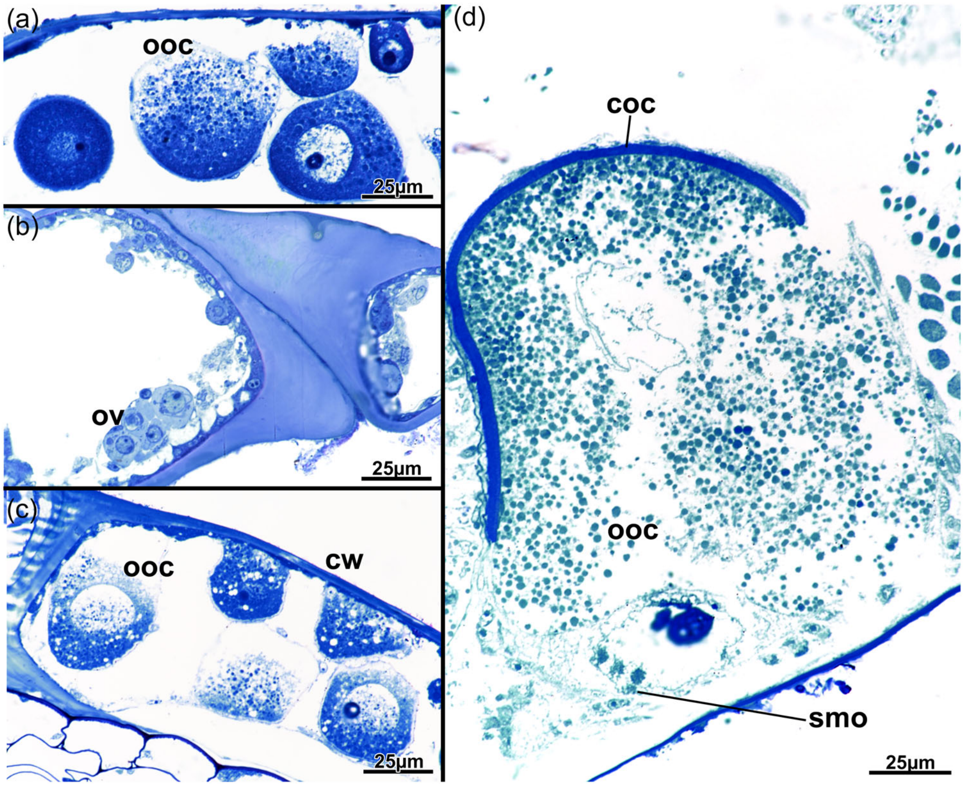

FIGURE 14 Oogenesis in hislopiids, histological details. (a) *Hislopia corderoi*. (b) *H. malayensis*. (c) *H. lacustris*. (d) *Echinella placoides*. A second smaller cell, potentially a nurse cell, is located next to the large oocyte. coc, cuticular oocyte cover; cw, cystid wall; ooc, oocyte; ov, ovary; smo, small oocyte.

hislopiids is associated with the loss of the more common, and most likely ancestral, internal collar. A loss of collar has been reported in other ctenostomes and may be associated with substitute defensive structures (Schwaha, Moosbrugger, et al., 2019). The function of the external collar in *H. corderoi* and *T. natans* is unknown.

4.4 | Reproduction

Data on hislopiid reproduction is mostly based on on *H. malayensis*, which was studied from early development through larval development and metamorphosis (Wood, 2008). *Hislopia malayensis* forms planktotrophic larvae. Such larvae have since been found in *H. prolixa* and *H. corderoi* (Wood et al., 2021). The generally similar structure and small size of oocytes in the genus *Hislopia* indicate that cyphonautes are probably present in all species. *T. natans* has a special mode of development with fertilized oocytes being brooded to form special swimming zooids, nautizooids (Wood et al., 2006b, 2010). Little is known about its oogenesis and development into nautizooids. The lack of any reproductive structure in the current study also gives little insight on reproductive development of this species. Most remarkable is the presence of a large oocyte in *E. placoides*. In cheilostome gymnolaemates involving lecithotrophic development, oogenesis occurs in doublets with one cell remaining small during development and acting as nurse cell for the main oocyte (Ostrovsky, 2013). This could also be the case in *E. placoides* where a small adjacent cell was observed. Such a reproductive pattern is usually associated with various incubation methods for brooding. In cheilostome bryozoans, external brood chambers, ovicells, are the most common incubation mode, but internal brooding also occurs in some clades (Ostrovsky, 2013, 2020). Ctenostome bryozoans lack mineralized skeletons and lack external brood chambers such as ovicells. Instead, brooding can occur most frequently in the tentacle sheath of degenerated zooids, in pouches of the vestibular wall, or in invaginations of the body wall (e.g., Ostrovsky, 2020; Reed, 1991; Schwaha, Bernhard, et al., 2019; Ström, 1977). The finding of a macrolecithal oocyte in *E. placoides* also indicates brooding, but it is not entirely clear, which structure is used for incubating embryos.

4.5 | The validity of the genus *Echinella*

The genus status of *Echinella* has frequently been challenged by previous authors (e.g., Annandale, 1916; d'Hondt, 1983). However, as previously stated by Wiebach (1966), the unique structure of the gizzard with spirally arranged teeth and its comparatively low number of tentacles (8 vs. 11–19, usually around 16) warrants a separate genus. Additional characters found in the current study are its simple intestinal tube versus the double-chambered intestine of *Hislopia* and brooding of embryos contrary to the broadcasting strategy of *Hislopia* (see also Wood et al., 2021; Wood, 2008). As also noted by Hirose and Mawatari (2011), *E. placoides* has more slender zooids of much shorter length of about 460–630 µm compared to species of *Hislopia* that most frequently have 800 µm as minimum of zooidal length.

5 | CONCLUSIONS

The current study analyzed all three hislopiid genera and represents the most complete morphological analysis of the clade. The general morphology is similar, but differences are found in details of gut morphology. Most striking is the external collar of *H. corderoi* and *T. natans*, which is unknown for any other bryozoan. Also, reproductive characters of *E. placoides* support the definition of the genus. All these characters will be helpful in future plotting of characters once a proper ctenostome phylogeny has been established.

AUTHOR CONTRIBUTIONS

Thomas Schwaha: Conceptualization; investigation; visualization; writing—original draft; methodology. **Masato Hirose**: Conceptualization; methodology; resources; writing—review and editing. **Timothy S. Wood**: Conceptualization; methodology; writing—review and editing; resources.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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