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The dorsal plate is a critical feature in the reassessment of the rotiferan genus *Ptygura* (Monogononta; Gnesiotrocha; Flosculariidae)

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Abstract

All species of Flosculariidae (Rotifera; Monogononta; Gnesiotrocha; Flosculariaceae) possess a flexible integument; they are termed illoricate. But species of four genera (*Beauchampia*, *Floscularia*, *Limnias*, and *Ptygura*) possess a distinctive hardened region on the dorsal side of their anterior end, which unfortunately has received little attention beyond the species level. In our work we study the ultrastructure of these hardened regions. In *Beauchampia*, *Limnias,* and some species of *Ptygura* the hardened zone takes the form of a dorsal plate (hereafter DP) with or without additional protruding processes. The DP has the shape of a ~50 µm diameter disc enfolding the anterior dorsal region of the animal, with a perimetral inward fold, smooth surface, and pores. The DP of *Limnias ceratophylli* shows three main ultrastructural differences from the rest of the body: (1) type of glycocalyx, (2) absence of small mounds on its surface, and (3) presence of broad cisternae at the base of the pores. In *Floscularia* and some *Ptygura* the hardened structures are reduced to a pair of dorsal projections protecting the dorsal antenna: i.e., lacking a full DP. These structures deserve attention because their adaptive importance has not been studied. Here we augment those observations with other characters: i.e., presence/absence of a birefringent body in late-stage embryos and morphology of their trophi and corona. Our goal was to provide information to help develop better taxonomy, as well as advance our understanding of the evolution of sessile taxa in general and of the potentially polyphyletic *Ptygura* genus in particular.

Key words: Anatomy, loricate, morphology, *Ptygura*, sessile rotifers, taxonomy

Conflicts of interest/Competing interests

The authors have no conflicts of interest/competing interests. The sponsors had no role in the design, execution, interpretation, or writing of the study.

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Ethics Approval

Not applicable

Authors' contributions

Conceptualization, V.F., P.M., R.L.W.; validation, V.F., P.M., R.L.W.; formal analysis, V.F., P.M.; data curation, V.F., P.M.; writing, review, editing, V.F., P.M., R.L.W.; project administration, V.F., P.M., R.L.W. All authors have read and agreed to the submitted version of the manuscript.

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Introduction

Within the Syndermata (Hagemann *et al.* 2023; Sielaff *et al.* 2015), phylum Rotifera (*sensu stricto*) comprises a diverse group of micrometazoans that reside in diverse habitats and exhibit a rich array of morphologies and life histories. Within this diversity rotifers do possess common features including a ciliated anterior end, trophi (jaws), an intracytoplasmic lamina (ICL) (Fontaneto *et al.* 2015; Wallace *et al.* 2015).

While all rotifers are mobile for a portion of their life, the adults of many species may attach, but do not permanently affix themselves to a surface. Examples of these facultatively sessile species are seen in Seisonidae (Sørensen *et al.* 2005), Bdelloidea (Fontaneto & De Smet 2015; Hollowday 1948), and Monogononta (Ejsmont-Karabin & Karpowicz 2019; Gilbert 2019, 2023). Obligatory sessile species are all grouped in the monogonont superorder Gnesiotrocha (de Beauchamp 1965; Kutikova 1970; Segers 2002); however, of the seven families in this taxon, only three contain species that are sessile as adults. These are Atrochidae and Collothecidae (order Collothecaceae) and Flosculariidae (order Flosculariaceae). Species in the remaining four gnesiotrochan families are obligatorily planktonic as adults: i.e., Conochilidae, Hexarthridae, Testudinellidae, and Trochosphaeridae. However, Conochilidae is traditionally grouped with truly sessile taxa (Edmondson 1944; Segers & Wallace 2001; Wallace 1980). Indeed, the life history of the sessile gnesiotrochans is diverse and in some taxa puzzling. For instance, in the genus *Collotheca* both obligatorily planktonic and obligatorily sessile species occur, while in *Floscularia* both solitary and colonial species are found (Wallace *et al.* 2015). Larvae that hatch from subitaneous embryos and diapausing embryos (resting eggs) of the sessile species swim for a day or so, but they eventually attach to a surface, form a stable bond with that substratum, metamorphose into adults, and reproduce; unless dislodged by a severe abrasion they remain permanently bound to their substratum (Butler 1983; Edmondson 1945; Wallace 1980).

Although less attention has been given to sessile rotifers than their planktonic counterparts, research on these species has a rich tradition. Investigations have focused on their taxonomy and diversity (Meksuwan *et al.* 2011; Sarma *et al.* 2020; Segers & Shiel 2008), genetics (Brown & Walsh 2019; Kordbacheh *et al.* 2018; Meksuwan *et al.* 2015), larval development (Hochberg & Hochberg 2015), life history (Kutikova 1995; Tiefenbacher 1972), substratum selection (Edmondson 1944; Wallace 1977; Wallace & Edmondson 1986; Young *et al.* 2019), anatomy

(Franch 2021; Hochberg 2014; Hochberg *et al.* 2019), and ecology (Jiménez-Santos *et al.* 2019a; Jiménez-Santos *et al.* 2019b; Wallace 1980; Wallace & Edmondson 1986). But besides difficulties in collecting and processing live specimens for study (Wallace *et al.* 2006), research on sessile rotifers is hampered by the lack of comprehensive understanding of their taxonomy and phylogeny.

While sessile Collothecaceae are all raptorial macrophages with uncinate trophi, Flosculariaceae are microphages filter feeders with malleoramate trophi (Obertegger & Wallace 2023). Currently nine genera of Flosculariidae are recognized: *Beauchampia*, *Floscularia*, *Lacinularia*, *Lacinularoides*, *Limnias*, *Octotrocha*, *Pentatrocha*, *Ptygura*, and *Sinantherina* (Meksuwan *et al.* 2011). These are diagnosed based on several characteristics including (1) coronal morphology, (2) size and position of their antennae, (3) morphology of their malleoramate trophi, (4) composition of tubes that they may construct, and (5) presence or absence of an egg-bearing organ, the oviferon (Koste 1978; Wallace & Snell 2010). One curious feature present in several species of *Beauchampia*, *Floscularia*, *Limnias*, and *Ptygura* that has been generally overlook is the presence of hardened regions on the dorsal side of their anterior end. In *Beauchampia* the hardened region is a long, slender tube surrounding the dorsal antenna (Meksuwan 2015). In *Floscularia* (Segers 1997) and *Ptygura* (Edmondson 1949; Koste 1975) the hardened regions are seen as pointed or rounded projections. In *Limnias* the hardened area is a distinct shield-like, bumpy structure, termed the dorsal plate (hereafter DP) (Meksuwan *et al.* 2018). DPs warrant attention as they provide a character used in identification of species and because their adaptive significance has not been well studied. We posited that analyzing the DP and similar hardened regions may lead to greater insight into the evolution of these sessile taxa. To aid future investigations regarding evolution of sessile rotifers, we expanded our study to consider other characters: i.e., presence of a birefringent body in late-stage embryos and morphology of their trophi and corona. Thus, the overall goal of our study was to provide this information to advance understanding of the evolution of the sessile rotifers.

Materials and methods

We began our study by compiling data on sessile species that appear to exhibit a DP. Thus, we reviewed the original descriptions of species of *Beauchampia, Floscularia, Limnias*, and *Ptygura* and added our unpublished information on these species. Additionally, we examined other literature that reviewed these species whether the authors specifically discussed the DP or not, and expanded our review to include those studies that provided information on presence of a birefringent body in late-stage embryos and the morphology of the corona and trophi (Banik 1996; Bielańska-Grajner *et al.* 2015; Dartnall & Hollowday 1985b; Edmondson 1940, 1944, 1945, 1949, 1959; Franch 2021; Kordbacheh *et al.* 2018; Koste 1972, 1974, 1978; Koste & Böttger 1989; Koste *et al.* 1995; Koste & Poltz 1987; Koste & Shiel 1986; Koste & Tobias 1990; Kutikova 1995, 2007; Leutbecher & Koste 1998; Meksuwan *et al.* 2018; Meksuwan *et al.* 2011; Meksuwan *et al.* 2015; Ruttner-Kolisko 1953; Segers 1997; Segers *et al.* 2010; Segers & Shiel 2008; Wallace *et al.* 2018; Wallace *et al.* 2006; Wei *et al.* 2019; Wright 1957; Wulfert 1966; Yang *et al.* 2019).

Light microscopy (LM)

In our LM study we reexamined the dorsal plate of *Limnias ceratophylli* Schrank, 1803, *Limnias melicerta* Weisse, 1848, *Ptygura ctenoida* Koste & Tobias, 1990, *Ptygura furcillata* (Kellicott, 1889), *Ptygura innominata* Franch, 2021, and *Ptygura mucicola* (Kellicott, 1888). Recognizing that the DP can be seen by LM in lateral view, we achieved a better view by digesting the rotifer's soft parts with a 5% solution of sodium hypochlorite (bleach). We also applied the bleach treatment to species that we knew lacked a DP: i.e., *Ptygura beauchampi* Edmondson, 1940, *Ptygura longicornis* (Davis, 1867), *Ptygura stygis* (Gosse, 1886), and *Ptygura tridorsicornis* Wright, 1957. Other features not previously reported in the literature for these species have been noted such as the presence/absence of birefringent bodies in late-stage embryos (Wallace 1993; Yang *et al.* 2019).

Preparation and imaging of specimens for Scanning Electron Microscopy (SEM)

We examined the DPs of *Beauchampia crucigere* (Dutrochet, 1812), *L. melicerta, P. ctenoida, P. furcillata*, and *P. innominata*, and the trophi of *P. ctenoida* and *P. innominata.* We have also examined the dorsal hooks of *Ptygura thalenoiensis* Meksuwan, Pholpunthin & Segers, 2011. Preparation of dorsal plates and the trophi were performed by three methods: (1) direct air drying (Meksuwan *et al.* 2018), (2) modified NaOCl (bleach) digestion (De Smet 1998; Segers *et al.* 1993), and (3) hot glutaraldehyde (Örstan 2015) and ethanol fixation. In the method of direct air drying, specimens were fixed in 95% ethyl alcohol; these contracted specimens were extracted from their tubes and placed into a small drop of distilled water on a cover glass. The specimen is oriented dorsally and left until the water evaporated. Modified bleach digestion was used for both the DPs and trophi. In this protocol tissues were digested in 5% bleach on a small cover glass; then the remaining structures were washed in distilled water (5–7 times) and then air-dried for at least two hours. In the glutaraldehyde and ethanol fixation method we followed the following process. We removed a specimen from its tube using a fine entomologic needle (000) and placed it in a drop of water in a depression slide. We then fixed the specimen with a drop of hot glutaraldehyde 2.6% in cacodylate buffer 0.1M at 85º C for 15 minutes and then rinsed it with fresh cacodylate 0.1M twice. After fixation we moved the specimen to a cover glass using a micropipette, ran it through a dehydration series by slowly adding drops of ethanol to achieve a final concentration of 96%, and lastly air-drying the specimen for at least two hours.

Once the specimen was fixed, the cover glass was attached to an Aluminum stub. Some samples processed by methods 1 and 2 were sputter coated with gold; in methods 1 and 3 specimens were sputter coated with platinum. SEM photographs were taken in two electron microscopy centers: from method 1 and 2 in the Scientific Equipment Centre of the Prince of Songkla University (Thailand), using FEI Quanta 400 and JEOL JSM-5800 LV; from method 1 and 3 in the Scientific and Technical Research Area (ACTI) of the University of Murcia (Spain), using FESEM ApreoS.

Transmission Electron Microscopy (TEM)

Using fine-tipped forceps we transferred live specimens into their tubes to a slide and placed them in ~20 µl of deionized water. After a few minutes, the specimens were fixed by adding a drop of 2.5% glutaraldehyde in 0.1 M cacodylate buffer at 85° C. Fixed specimens were then transferred to a 2.5 mL Eppendorf tube and held for 45 minutes; after that time the specimens were washed in 0.855 mg/100 mL saccharose cacodylate, postfixed in 1% osmium tetroxide for two hours, dehydrated in a graded acetone series, and embedded in Epon 812 (Serva, Heidelberg, Germany). Semithin sections (1 µm) were stained with toluidine blue. Ultrathin sections (90–120 nm) were cut with a Reichert-Imy Ultracut ultramicrotome, contrasted with uranyl acetate and lead citrate, and examined with a JEOL 1011 Transmission Electron Microscope with GATAN ORIUS SC200 High Contrast Digital Camera.

Results

Light microscopy

When the corona of an animal is extended, the DP is barely recognizable because it is transparent and often obscured by the corona (Fig. 1A). However, in lateral view when the corona is retracted, they appeared supported by a pedestal-like structure, easily distinguishable from the rest of the trunk; it was wider in frontal region, tapering to the caudal side (Fig. 1 A and B). In *Limnias* various processes were distributed over the surface of the DP, but in other species they emerged from the anterior third of the DP (Figs. 1 A and B). While the long tube of *B. crucigere* contained the dorsal antenna, in the rest of species bearing DP the dorsal antenna was absent or not visible.

Once the soft parts of the animal were digested by bleach (Fig. 1 C), the DP may be observed, but the discoidal shape of the DP in *Limnias* often became distorted during this treatment. Indeed, with a longer exposure the DP eventually dissolved. In the sessile rotifers that lack a DP, no distinguishable integumentary structure was seen after the bleach treatment (Fig. 1 D).

Information on the presence/absence of birefringent body in late-stage embryos was noted for several species. *Limnias ceratophylli*, *P. ctenoida* and *P. innominata* did not possess these objects, while *P. longicornis*, *P. stygis* and *P. tridorsicornis* did (Fig. 2).

Scanning electron microscopy

Under SEM, the DP was clearly distinguished from the rest of the integument. Its surface was smoother, without papillae or ridges that are present on other parts of the body surface (Figs. 3 A, C and E); see also previously published SEM images of *L. melicerta* (Yang & Hochberg 2018). The edge of the DP was folded inwards giving the appearance of a superimposed structure, but in some places, it was continuous with the integument (Figs. 3 A, C and E, arrows). The DP of *P. ctenoida* and *P. innominata* had a gentle surface undulation (Fig. 3 B, C, and E) and possesses pores (Fig. 3 F). The integument also had pores, but its surface showed fine granulation that was not seen in the DP (Fig. 3F).

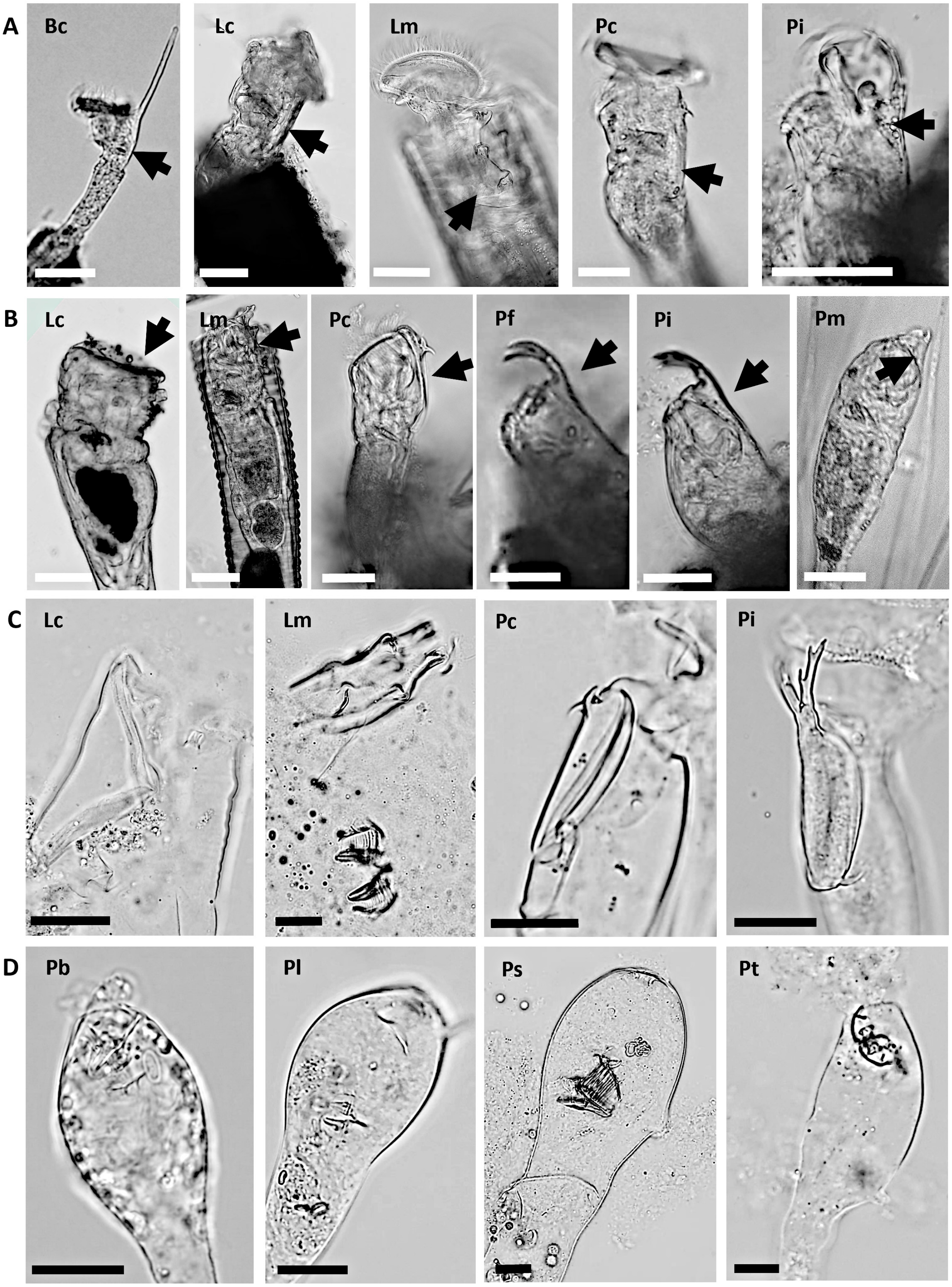

FIGURE 1. Light microscopy images of dorsal plates in selected sessile taxa. A: *in vivo* with corona open; lateral view. B: *in vivo* with corona retracted. C: dorsal plate revealed with bleach treatment. D: species lacking dorsal plate with bleach treatment. A and B: arrows mark the dorsal plates. Bc, *Beauchampia crucigere*; Lc, *Limnias ceratophylli*; Lm, *Limnias melicerta*; Pb, *Ptygura beauchampi*; Pc, *Ptygura ctenoida*; Pf, *Ptygura furcillata*; Pi, *Ptygura innominata*; Pl, *Ptygura longicornis*; Pm, *Ptygura mucicola*; Ps, *Ptygura stygis*; Pt, *Ptygura tridorsicornis*. Bars: 50 µm for A and B, 20 µm for C and D .

FIGURE 2. Presence and absence of birefringent bodies in late-stage embryos that have not been previously reported in some sessile rotifers. Birefringent body absent: A) *Ptygura ctenoida*, late-stage embryo (fixed with hot glutaraldehyde) and free-swimming larva in lateral and dorsal view. B) *Ptygura innominata*, late-stage embryo, and free-swimming larva in lateral and dorsal view. C) *Limnias ceratophylli*, late-stage embryo, and free-swimming larva in dorsal view (foot contracted). Birefringent body present: D) *Ptygura longicornis*, free swimming larva in dorsal view. E) *Ptygura stygis*, free swimming larva in dorsal view. F) *Ptygura tridorsicornis*, free-swimming larva in dorsal view. Arrows mark birefringent bodies. Bar: 50 µm, same scale for all images.

Comparing images of dorsal region of a DP-bearing species like *P. ctenoida* (Fig. 4 A) with an unidentified *Ptygura* demonstrated that some species in this genus lack a DP on their dorsal side (Fig. 4 B). Some species of *Floscularia* and *Ptygura* have dorsal hooks (Meksuwan *et al.* 2011; Segers 1997), thus, we compared SEM images of these species with those that have DP to determine whether there is a dorsal plate supporting the dorsal hooks. The dorsal hooks of *P. thalenoiensis,* located on both sides of the dorsal antenna (Fig. 4 C and D), was quite like those of the genus *Floscularia* (Fontaneto *et al.* 2003; Segers 1997). In SEM images of *P. thalenoiensis* the DP was absent (Fig. 4 C). In fact, while their processes showed a smooth surface, the integument around them showed ridges and grooves like the rest of the trunk integument (Fig. 4 D). This also was seen in images of *Floscularia bifida* Segers, 1997; for example see figures 35 and 36 from (Segers 1997). The dorsal disc, which was seen in a dorsal view of SEM images of *Floscularia ringens* (Linnaeus, 1758), was not apparent in lateral view: e.g., see figure 4 a, c in (Fontaneto *et al.* 2003). This disc did not resemble the peculiar structure of the DP that we have described above.

Using SEM data, we provide novel information on the trophi of *P. innominata* and *P. ctenoida*. Trophi of *P. innominata* (Fig. 5 A and B) was small (15 µm total width, 7 µm rami length), with symmetrical tricamerate manubria, no right proximal connection; unci plates symmetrical; unci teeth weakly differentiated, left/right proximal teeth = 3/3, distal teeth 7/7; shafts of proximal teeth completely webbed, shafts of distal teeth partially webbed, heads of proximal and distal teeth club-shaped; subuncus teeth absent; rami symmetrical with scleropili all along it, lateral fenestrulae small and shallow, alula absent; fulcrum with inconspicuous basal plate. The trophi of *P. ctenoida* (Fig. 5 C and D) was somewhat larger (19 µm total width, 10 µm rami length), left/right proximal teeth = 3/3, distal teeth 9/9, and small projection on the left manubrium, the rest of the characteristics being like that of *P. innominata*.

Transmission electron microscopy

The superficial plane of the DP is somewhat wavy (Fig. 6A inset), but completely smooth, without papillae or ridges (Fig. 6 A and B). The perimeter of the DP formed an inward facing fold (Fig. 6 A). The limit between the plate and the rest of the integument was delimited by (1) a change in the type of glycocalyx (cuticle), electron dense, wider, and brush-like on the DP, and less electron dense, shorter, and bush-like on the trunk; (2) formation of mounds in the integument of the trunk, as previously reported for *L. melicerta* (Yang & Hochberg 2018), absent in the DP; and (3) by the presence of broad cisternae connected to the vesicles or bulbs at the base of the pores in the DP, absent in the trunk integument (Fig. 6 A and B). Surprisingly, the thickness of the ICL did not show a sudden change, although along the trunk its thickness decreased from the head to the foot. Based on its electron density, the composition of the ICL did not change either.

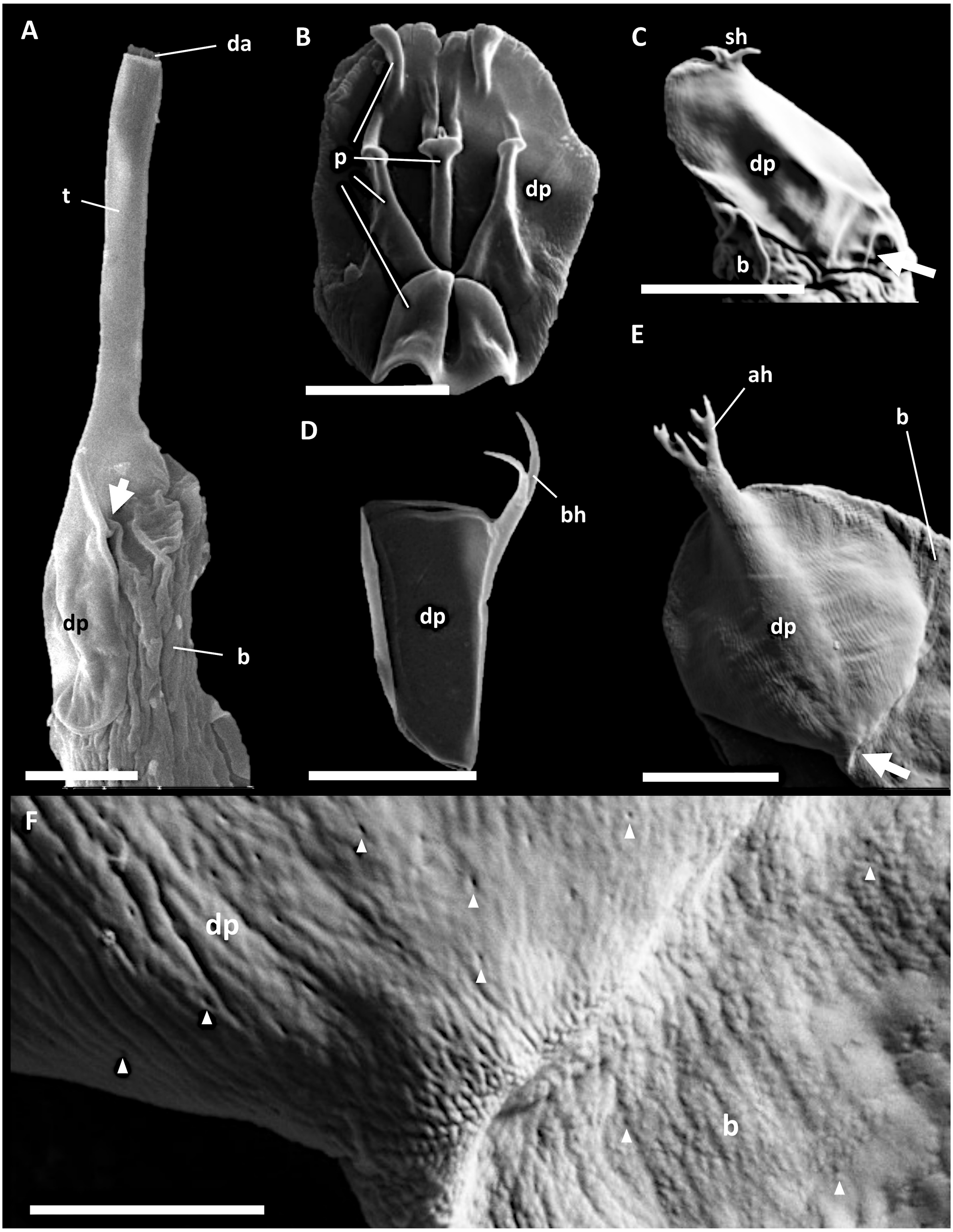

FIGURE 3. SEM images of the dorsal plate. A: antero-ventral region of a dried specimen of *Beauchampia crucigere* showing the body integument (b) and the dorsal plate (dp) with its stiffened tubular process (t) containing the dorsal antenna (da) with the terminal sensory filaments coming out the end. B: dorsal plate of a dried specimen of *Limnias melicerta* showing the dorsal plate (dp) with its species-specific seven protrusions (p). C: antero-dorsal region of a specimen of *Ptygura ctenoida* fixed with hot glutaraldehyde showing the body integument (b) and the dorsal plate (dp) with its aligned short hooks (sh). D: dorsal plate (dp) and long branched hooks (bh) of a dried specimen of *Ptygura furcillata*. E: dried specimen of *Ptygura innominata* showing the body integument (b) and the dorsal plate (dp) with its long-branched, antler-like hooks (ah). F: closeup of figure E, showing pores (arrowhead) and difference of surface ultrastructure between the dorsal plate (dp) and the body integuments (b). Arrows mark continuity between dorsal plate and the rest of the integument. Bars: 20 µm for A to E; 5 µm for F.

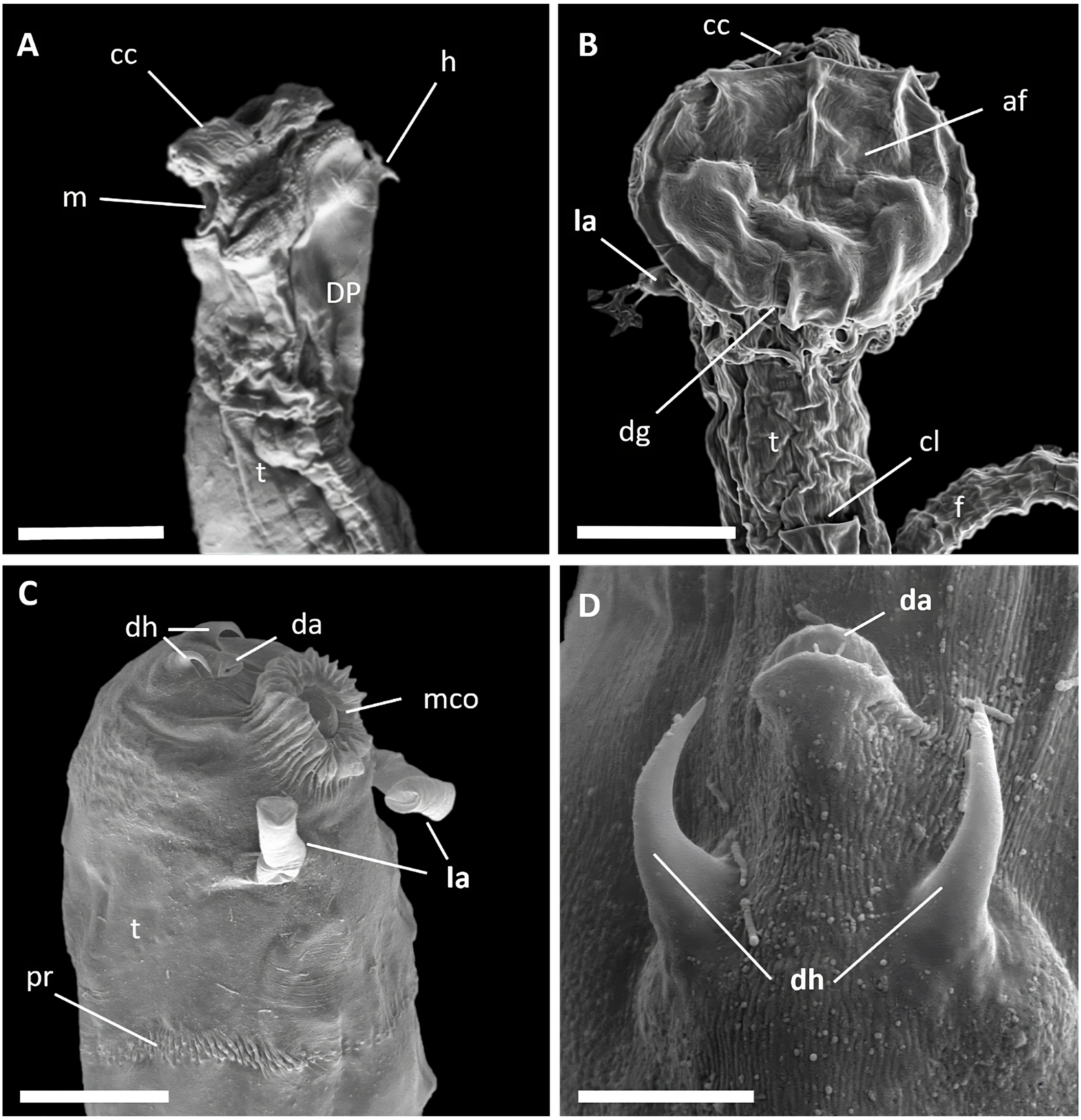

FIGURE 4. Comparison of dorsal plate bearing species with species lacking a dorsal plate. A: Lateral view of the anterior half of *Ptygura ctenoida* showing the corona ciliature (cc), the mouth (m), the dorsal plate (DP), the short hooks (h), and the trunk (t). B: Dorsal view of the anterior half of *Ptygura* sp. showing the corona ciliature (cc), the apical field of the corona (af), the dorsal gap in the corona ciliature (dg), a lateral antenna (la), the trunk (t), the cloaca opening (cl), and part of the foot (f). C: trunk (t) and head of a specimen of *Ptygura thalenoiensis* showing the dorsal antenna opening (da) located in the middle of a pair of dorsal hooks (dh), the lateral antennae (la), the opening of corona and mouth (mco), and the papillate ring on the trunk (pr). D: closeup of the paired dorsal hooks (dh) and dorsal antenna opening (da) of another specimen of *Ptygura thalenoiensis*; note the grooves and ridges of the integument. Bars: 20 µm for A, B, and C; 5 µm for D.

Discussion

Gnesiotrochans are illoricate but some species possess hardened structures located in the antero-dorsal region of the body. These have been described in *Beauchampia*, *Floscularia,* and *Limnias,* as well as a few species of *Ptygura.*

In *Beauchampia* the hardened structure is in the form of a dorsal tube; in *Floscularia* and *Ptygura* it is seen as variously shaped hooks; and in *Limnias* it takes the form of a shield-like structure elaborated with nodules: i.e., the DP (Meksuwan *et al.* 2011). However, except for the genus *Limnias* presence of a DP has not been well documented (Meksuwan *et al.* 2018). The rigid DP was reported by Dartnall and Hollowday (1985) for *Ptygura melicerta* Ehrenberg, 1832, by Segers and Shiel (2008) for *P. ctenoida* (Koste & Tobias 1990), and for *P. innominata* (Franch 2021). It also has been recognized as a differential characteristic for species of the *P. melicerta* and *P. furcillata* groups (Segers & Shiel 2008). As shown in SEM images (Fig. 3), the DP can be described as a thickened curved disc (~ 50 µm diameter) that covers the antero-dorsal side of the trunk that may or may not possess various conspicuous processes. A structure resembling a DP has not been described in those species of *Floscularia* or *Ptygura* that possess dorsal hooks (Segers 1997).

In the genus *Limnias* the DP is flexed backwards when the animal opens its corona for feeding but moves to a frontal position when the corona retracts (Fig. 1 A and B, and 6 A inset). This behavior allows the DP to act as a lid or operculum that closes the tube (Kordbacheh *et al.* 2018; Yang & Hochberg 2018). In *Beauchampia* and DP-bearing *Ptygura* species the DP always maintains its dorsal position, probably because it covers a greater proportion of the trunk than in *Limnias*.

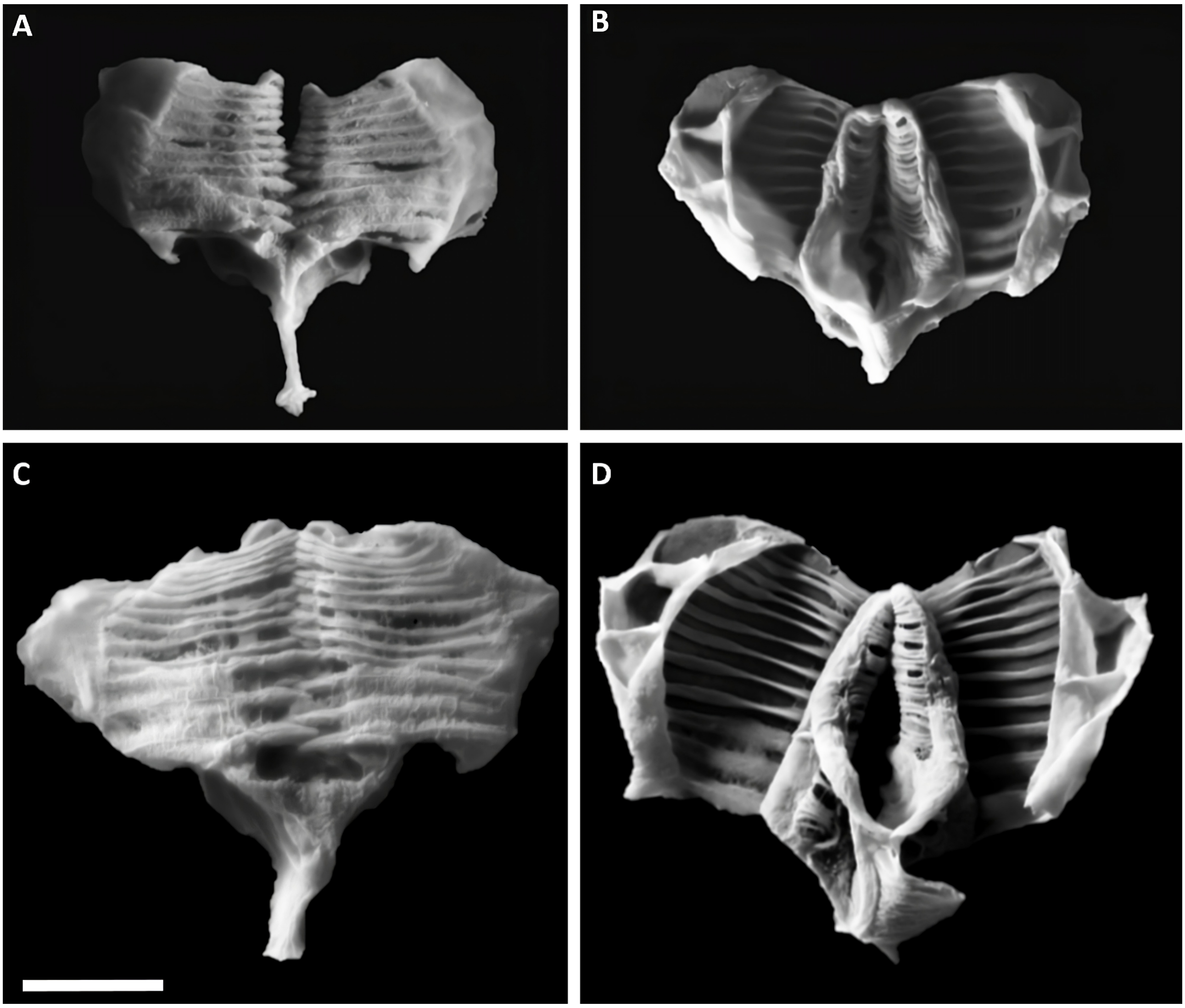

FIGURE 5. Scanning Electron Microscope images of trophi of two *Ptygura* species. Trophi of *Ptygura innominata* in frontal (A) and caudal view (B), and trophi of *Ptygura ctenoida* in frontal (C) and caudal view (D). Bar: 5 µm, same scale for all images.

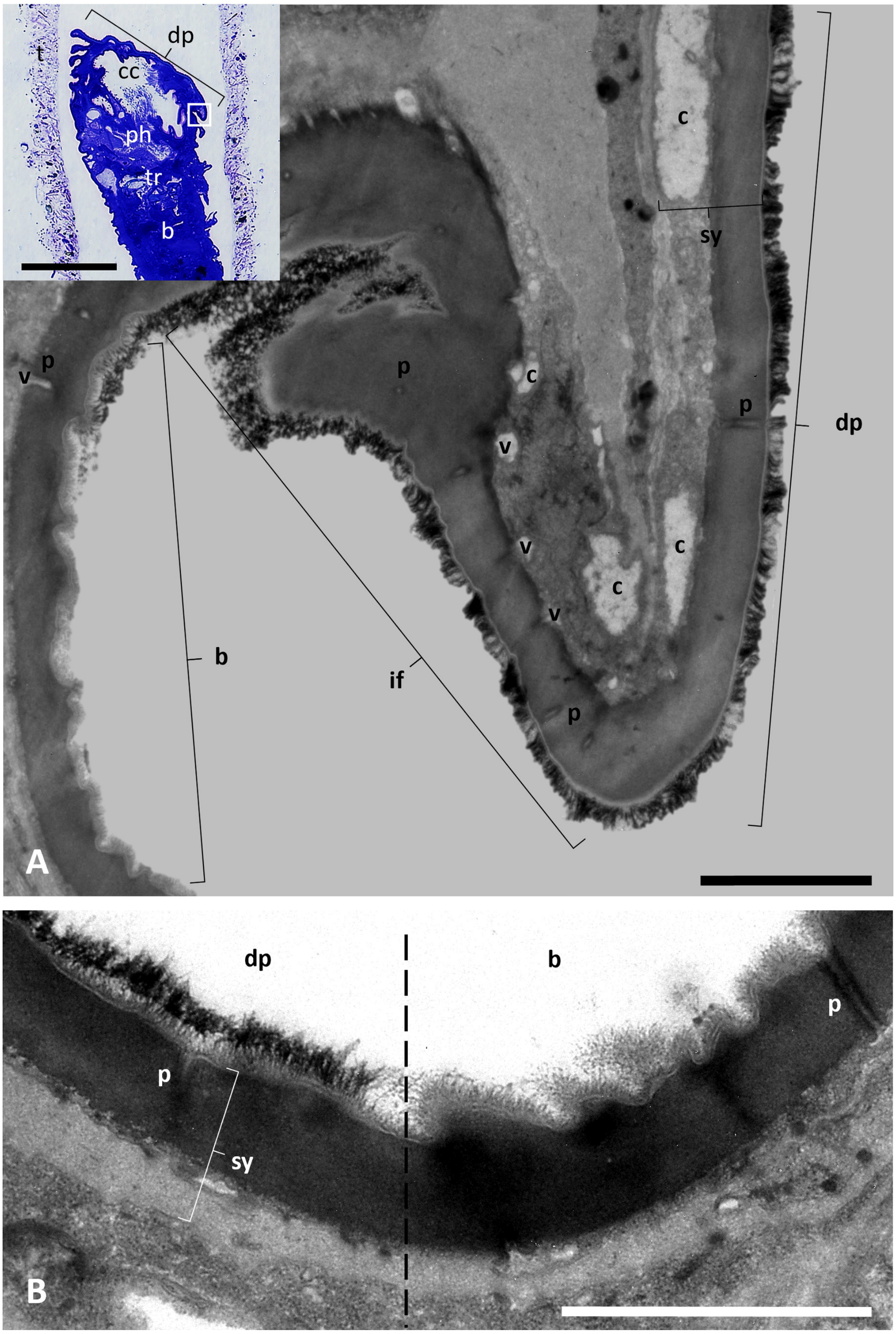

FIGURE 6. Transmission Electron Microscope images of the dorsal plate. Inset: Light photomicrograph of a sub-sagittal section of a specimen of *Limnias ceratophylli* in its tube stained with blue toluidine. A: TEM image of the inset white line square area showing the inwards fold of the dorsal plate and the transition from the dorsal plate integument to the body one. The dark grey perimetral band is the intracellular lamina (ICL) of the integument. In this image the transition zone has been obliquely sectioned (circles in the ICL of this zone are pores), so the ICL thickness appeared enlarged here with the two types of glycocalyx overlapping. B: Transition from the dorsal plate to the body integument in transversal section. The transition is not progressive, but abrupt. See text for details. Symbols: b, body integument; v, vesicules at the base of pores; c, cisterns; cc, corona ciliature; dp, dorsal plate; if, inwards fold of the dorsal plate; p, pores; ph, pharynx; t, tube; tr, trophi; sy, syncytial integument. Bars: 2 µm for A, B, and 50 µm for inset.

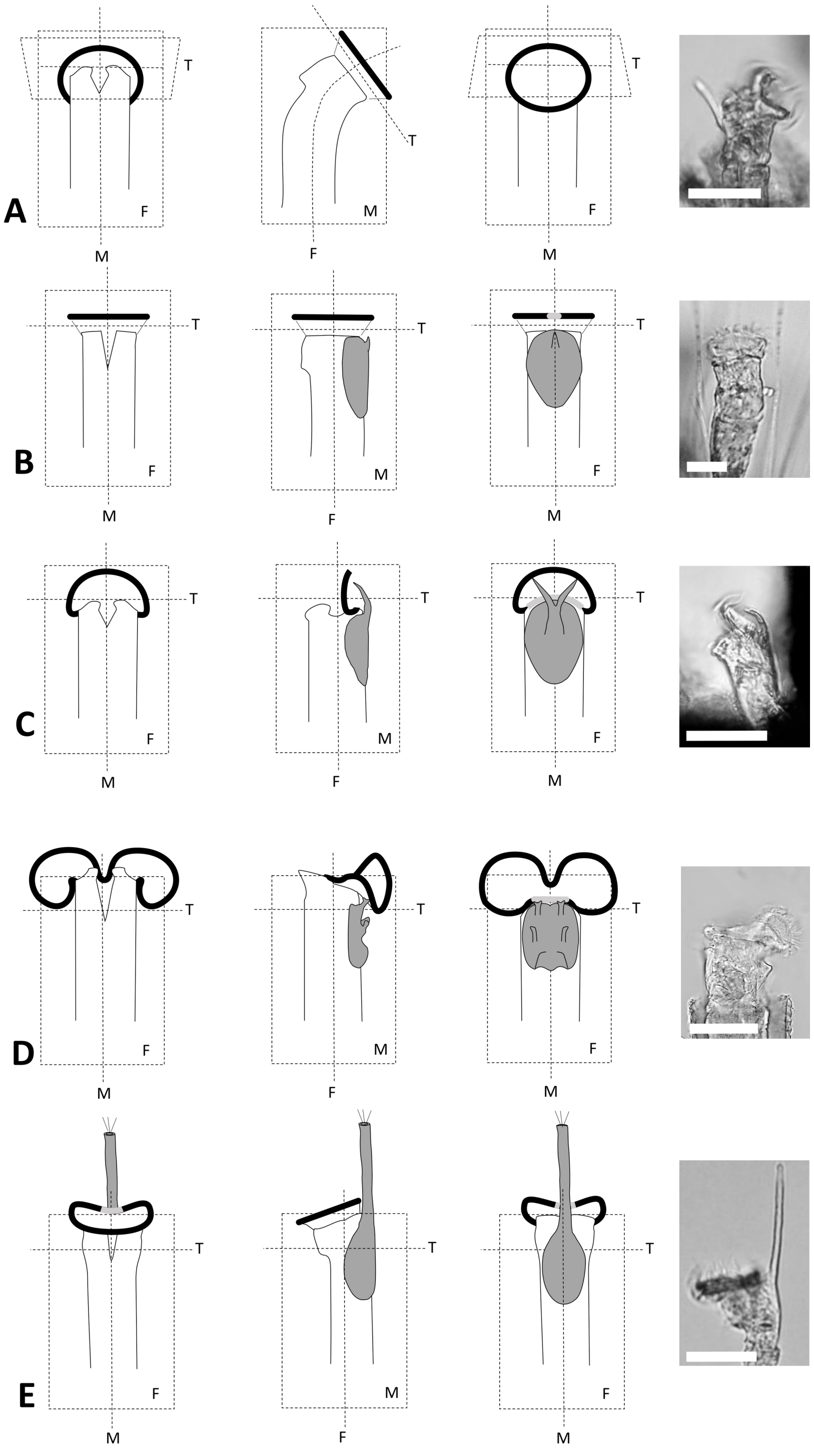

FIGURE 7. Relationship between dorsal plate and corona arrangement. Photographs from top to bottom: A: *Ptygura longicornis* (no dorsal plate). Next, representative species with dorsal plate: B: *P. mucicola*, C: *P. furcillata*, D: *Limnias melicerta*, and E: *Beauchampia crucigere*. Diagrams show the different layout models of the cephalic region: A: no dorsal plate, no dorsal gap ciliature, neck dorsal flexion, corona plane transversal to the body axis, opened dorsally; B: dorsal plate with short hooks, small dorsal gap ciliature, no dorsal flexion, corona plane transversal, opened frontally; C: dorsal plate with long hooks, wide dorsal gap ciliature, no dorsal flexion, corona in the frontal plane, opened dorsally; D: dorsal plate with protrusions, wide dorsal gap ciliature, no dorsal flexion, corona plane from transversal to frontal, opened dorsally; E: dorsal plate with long tube protecting the dorsal antenna, medium size dorsal gap ciliature, slightly ventral flexion, corona plane transversal, opened frontal to ventrally. Diagram columns from left to right: ventral view, lateral view, dorsal view. Symbols: thin black line, trunk, pharynx, and corona basal field; thick black line, corona ciliature; thick light grey line, dorsal gap; dark grey, dorsal plate and processes; dotted lines, body planes frontal (F), transverse (T) and medial (M). Bars: 50 µm, same scale for all images.

There are few references that consider possible functions of the DP in sessile rotifers (Hudson & Gosse 1886; Kellicott 1888; Wallace *et al.* 2018). The most obvious is as a defense against predators, protecting the rest of the body when the animal contracts or covering the entrance of the tube like a lid or operculum (Yang & Hochberg 2018). However, TEM images do not show a significantly different ICL thickness between the DP and the anterior region of the trunk, casting doubt on whether its sole function is protection against predators or parasites from gaining entrance into the tube through the opening. However, both the inward folding and presence of DPs can contribute to the mechanical resistance of the structure as a whole.

The ultrastructure of the glycocalyx of the DP differs from the rest of the integument, suggesting a different composition and resulting function. On the other hand, in other sessile species, e.g., *Stephanoceros fimbriatus* (Goldfuß, 1820), the glycocalyx appears to be the same in all regions of the integument (Yang *et al.* 2021). The glycocalyx of the DP of *L. ceratophylli* is electron dense and fibrous, resembling toothbrush bristles that give the entire smooth surface of the DP a velvety appearance. The function of this type of glycocalyx is unknown but it could contribute to a uniform distribution of the secretions with which the tube is formed.

In addition to the function of the DP, it is necessary to consider the function of the processes associated with it. In *B. crucigere* a protective function of the long dorsal antenna seems evident. In *Limnias* it has been suggested that the nodules of the dorsal plate contribute to the distribution of secretions with which the tube is formed (Hudson & Gosse 1886; Kellicott 1888; Wallace *et al.* 2018). The antler-like hooks in *P. innominata*, do not seem to be used as a defense, but rather as a rake to remove floccular materials around the head and incorporate them into the tube (Franch 2021). On the other hand, the function of the short hooks in the *P. melicerta* complex remains an enigma. They may have originally functioned as protection for the dorsal antenna, but then evolved to encompass other roles.

The dorsal processes are supported by the DP, but which is ancestral is not clear. There are two possibilities. (1) The dorsal plate evolved first and then the processes developed in some species thereby providing additional adaptive value. (2) The dorsal processes appeared first and then the dorsal plate evolved which provided the processes additional strength. The DP is smooth in *L. ceratophylli*, *Limnias lenis* Meksuwan, Jaturapruek & Maiphae, 2018, and some variants of *P. melicerta*, while the DP of the rest of species of that group have stiffened processes of varied morphology (Kordbacheh *et al.* 2018; Koste 1978; Meksuwan *et al.* 2018). The current existence of species with smooth DP suggests that the first option is the most parsimonious, but the relationship that may exist with the dorsal antenna, as in the case of *Beauchampia*, challenges this conclusion. Perhaps the hooks evolved to protect the dorsal antenna, but in those species with longer hooks additional functions were added: general predator defense and as a tool to assist in the construction of the tube.

Comparing SEM images of the trophi of *Ptygura ctenoida* and *P. innominata* (Fig. 5) with those of *B. crucigere* and genus *Limnias* available in the literature (Koste 1978; Meksuwan *et al.* 2018; Meksuwan *et al.* 2015; Yang & Hochberg 2018) we conclude that there is a notable coincidence in the morphology of the trophi in all DP-bearing species: manubria typically tricamerate, symmetrical, no right proximal connection; unci plates symmetrical, supported by the three manubrial camera; unci teeth weakly differentiated; shafts of proximal teeth partly to completely webbed, shafts of distal teeth partially webbed, heads of proximal and distal teeth club-shaped; subuncus teeth absent; left/right proximal teeth = 3/3, number of distal teeth variable, species specific; rami symmetrical; frontal rami scleropili present all along the rami; fulcrum with inconspicuous basal plate.

The morphology of trophi in some *Ptygura* with DPs clearly differs from other species including some in the same genus; this reinforces a previously suggestion that the genus *Ptygura* is paraphyletic (Meksuwan *et al.* 2015) or even polyphyletic. Indeed, while *P. ctenoida* and *P. innominata*, both with DPs, have symmetrical trophi (manubria, uncinate plates, and rami), other species such as *P. longicornis*, *P. beauchampi* and *P. crystallina*, lacking DPs, have asymmetrical trophi like that of the family Conochilidae (Meksuwan *et al.* 2015). Thus, we suggest that detailed morphological studies should be undertaken of the trophi in the genus *Ptygura*.

The polyphyletic origin of the current genus *Ptygura* also is supported by the presence/absence of birefringent bodies in late-stage embryos. All DP-bearing taxa examined in this work lack these objects. In contrast, *Ptygura* species lacking DP do possess it. On the other hand, for *L. ceratophylli* our observations could help to resolve confusion about the birefringent body: e.g., compare (Kutikova 1995; Wallace 1993; Yang *et al.* 2019).

Most of sessile rotifers flex the neck dorsally when feeding, so the corona faces backwards probably to avoid particles from gaining entrance to tube, thereby keeping the tube clean. But, in contrast to *Lacinularia, Lacinularoides, Octotrocha, Pentatrocha, Sinantherina,* and most *Ptygura* species, animals possessing the DP cannot flex their neck backwards, probably because the DP prevents that from happening. In species with paired dorsal hooks, as *Floscularia* and some *Ptygura*, the flexion seems to be limited but not avoided. Thus, in DP-bearing species other adaptations may have arisen to keep the tube clean. These include the following. (1) Three species, *P. ctenoida*, *P. melicerta*, and *P. mucicola,* do not possess a distinct tube — they produce flocculant gelatinous matrix — thus their corona may be oriented transversally to the body axis. (2) In *P. furcillata*, *P. innominata*, and probability in *Ptygura elsteri* Koste, 1972 and *P. kostei* José de Paggi, 1996 the corona is located in a central-frontal plane respect to the body axis. (3) In *Limnias* the corona is located in a rear-frontal plane, with a wide dorsal gap of the ciliature. (4) In *B. crucigere* orientation of the corona to the ventral side. These morphologic relationships between the DP and the corona arrangement are illustrated in Figure 7.

Currently the defining characteristic of *Ptygura* is the circular-elliptic shape of the corona (Koste 1978, 1989). However, our work shows that focusing on this feature alone may be inadequate. Four characteristics demonstrate this problem. (1) We show that some *Ptygura* species possess a DP (others do not), but they share other traits with *Beauchampia* and *Limnias*: e.g., morphology of the trophi, absence of birefringent body in late-stage embryos, and inability to flex the neck backwards. (2) Some *Ptygura* possess dorsal hooks on both sides of their dorsal antenna and a ciliated region under the mouth. Privileging those features one could suggest that *P. thalenoiensis* and/or *Ptygura noodti* (Koste, 1972) are related to some *Floscularia* (Meksuwan *et al.* 2011). (3) Asymmetric trophi are found in *P. beauchampi* and *P. crystallina*, a feature shared with *Conochilus* (Meksuwan *et al.* 2015). (4) Some *Ptygura* have processes located on the dorsal surface of their corona: these are seen in *P. stygis* and *Ptygura wilsonii* (Anderson & Shephard, 1892). These reasons argue for review of the genus *Ptygura* that is based on a larger suite of characters.

Conclusions

Although previously workers have not employed the DP as a diagnostic character, we posit that it is an important trait for several sessile gnesiotrochans including *Beauchampia*, *Limnias*, and some *Ptygura* spp. Indeed it may be a significant synapomorphy, especially when combined with other features: i.e., morphology of their trophi, dorsal gap in the corona, absence of birefringent bodies in late-stage embryos, and inability to flex the neck backwards (Edmondson 1940; Franch 2021; Koste 1972, 1978; Kutikova 2007; Leutbecher & Koste 1998; Meksuwan *et al.* 2018; Wallace *et al.* 2018).

The genera *Conochilus*, *Oecistes*, and *Ptygura* were subsumed into *Ptygura* by Dujardin (1841) based on his conclusion that the following three differences did not warrant separation. (1) Individual *Oecistes* produce a gelatinous envelope (tube) in which particulate material may become embedded. (2) *Conochilus* forms colonies in which the individuals are clustered in a gelatinous mass and possesses two eyespots and "deux appendices charnus en forme de tentacules saillants" (i.e., antennae within the apical field). (3) Dujardin (1841) states that *Ptygura* lacks a gelatinous tube. While *Conochilus* (Conochilidae) Harring, 1913, has been removed from the genus *Ptygura*, no comprehensive study has been published that provides a detailed analysis of the species in the taxa originally assigned to *Ptygura* and *Oecistes*. Because *Ptygura* is such a highly variable genus that we suggest that it warrants a substantial re-analysis and probably a taxonomic revision (Meksuwan 2015).

While we know a good deal about the morphology of rotifers in general, it is clear that more research should be undertaken, particularly on the Gnesiotrocha. Additional work should include comparisons of male anatomy, musculature organization of females (Kotikova 2013; Kotikova *et al.* 2001, 2006), nervous system anatomy (Hochberg & Hochberg 2015), ultrastructural of external features (Fontaneto *et al.* 2003), ultrastructure of the integument (Fontaneto *et al.* 2003; Hochberg *et al.* 2015, 2017; Yang & Hochberg 2018), and SEM analysis of their trophi (Meksuwan 2015). Nevertheless, we are cognizant that a comprehensive phylogeny of these taxa will not be possible without using an integrative approach (i.e., total evidence) by combining morphological and multi-genic molecular data (Funch *et al.* 2005; Sørensen & Giribet 2006; Wilke *et al.* 2020).

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