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A NEW SPECIES OF *TEMNOCEPHALA* BLANCHARD (PLATYHELMINTHES, TEMNOCEPHALIDA) ECTOSYMBIONT ON *DILOCARCINUS SEPTEMDENTATUS* (DECAPODA, TRICHODACTYLIDAE) FROM THE BRAZILIAN AMAZONIA

NUEVA ESPECIE DE *TEMNOCEPHALA* BLANCHARD (PLATYHELMINTHES, TEMNOCEPHALIDA) ECTOSIMBIONTE EN *DILOCARCINUS SEPTEMDENTATUS* (DECAPODA, TRICHODACTYLIDAE) DE LA AMAZONÍA BRASILERA

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Abstract

Temnocephala longivaginata sp. n., an ectosymbiont on *Dilocarcinus septemdentatus* (Herbst, 1783), is described from crabs in the State of Pará, Brazilian Amazonia. Fifty crabs were collected from the Rio Peixe-Boi, Municipality of Peixe-Boi. *Dilocarcinus septemdentatus* is the type host of *Temnocephala microdactyla* Monticelli, 1903, a species with a precarious original description, but redescribed on two other occasions from different hosts: *Sylviocarcinus australis* Magalhães & Türkay, 1996 and *Dilocarcinus pagei* Stimpson, 1861. The new species is most similar to *Temnocephala pignalberiae* Dioni, 1967, but differs by having the following characters: 1. cirrus with a circle of sclerites in the distal portion of the introvert, followed by a smooth portion (without spines or ridges); 2. proximal, inner portion of the introvert with longitudinal ridges; 3. vesicula 'intermedia' long, replacing the seminal receptacles; 4. vagina long, with a widening of its distal portion, near the asymmetrical vaginal sphincter; and 5. small and 'shoe sole' shaped dorsolateral 'excretory' syncytial epidermal plates. Prior to the present study, all species of *Temnocephala* were shown to have the cirrus' introvert either smooth or with spines. Ridges in the inner wall of the introvert and a circle of sclerites are here recorded for the first time in Temnocephalidae.

Key words: crustaceans-Neotropic Region-taxonomy-temnocephalids

Resumen

Temnocephala longivaginata sp. n. ectosimbionte en *Dilocarcinus septemdentatus* (Herbst, 1783), es descrita del Estado de Pará, Amazonia brasilera. Cincuenta cangrejos fueron colectados en Rio Peixe-Boi, Municipio de Peixe-Boi. *Dilocarcinus septemdentatus* es el hospedero-tipo de *Temnocephala microdactyla* Monticelli, 1903, especie con una descripción original pobre, pero que fue re-descrita en otras dos ocasiones en diferentes hospederos: *Sylviocarcinus australis* Magalhães & Türkay, 1996 y *Dilocarcinus pagei* Stimpson, 1861. La nueva especie es más parecida con *Temnocephala pignalberiae* Dioni, 1967, de quien se diferencia por los siguientes caracteres: 1. cirro con un círculo de escleritos en la porción distal del introverto, seguida por una porción lisa (sin espinas o cristas); 2. porción proximal interna del introverto con cristas longitudinales; 3. vesicula 'intermedia' larga, sustituyendo los receptáculos seminales; 4. vagina larga, con una extensión en la porción distal, próximo al esfínter vaginal asimétrico; y 5. placas dorsolaterales, sincitiales 'excretoras' pequeñas y en forma de 'suela de zapato'. Hasta el momento, todas las especies de *Temnocephala* tienen el introverto del cirro liso o con espinas. Cristas en la pared interna del introverto y un círculo de escleritos son registrados por primera vez en Temnocephalidae.

Palabras clave: crustáceos-Región Neotropica-taxonomía-temnocefalideos

INTRODUCTION

Crustacea Brünnich, 1772 is the most common host group for species of *Temnocephala* Blanchard, 1849-of the 27 species described from the Neotropic Region, 17 are ectosymbionts on crustaceans. *Dilocarcinus septemdentatus* (Herbst, 1783) (Trichodactylidae) (Figs 1-2) is the type host of *Temnocephala microdactyla* Monticelli, 1903 collected in Carandazinho, State of Mato Grosso, Brazil (Monticelli, 1903). Pereira & Cuocolo (1941) redescribed the species due to the precariousness of the original description, especially that of the cirrus. These authors based their redescription of *T. microdactyla* on specimens collected in Bodoquena, MS, from *Trichodactylus pictus* (= *Sylviocarcinus australis* Magalhães & Türkay, 1996). Based on the work of Pereira & Cuocolo (1941), Dioni (1967) identified three species (*T. microdactyla*, *Temnocephala santafesina* Dioni, 1967, and *Temnocephala pignalberiae* Dioni, 1967) from trichodactylidean crustaceans in Argentina. Dioni (1967) did not indicate the type host of *T. pignalberiae*, which could be *Dilocarcinus pagei* Stimpson, 1861, *S. australis,* or an unidentified species of *Trichodactylus* Latreille, 1828 from three different localities in Argentina. Damborenea (1992) recorded *T. pignalberiae* on *D. pagei* and *Sylviocarcinus pictus* (Milne-Edwards, 1853) also in Argentina. Recently, *T. pignalberiae* had its description updated by Amato *et al.* (2010) based on ectocommensal specimens found on *D. pagei* collected in the States of São Paulo (SP) and Mato Grosso (MT). This was the first record for the species in Brazil. The present paper adds one more ectocommensal species of *Temnocephala* living on trichodactylids, and presents a complete documentation study.

MATERIAL AND METHODS

Fifty crabs were collected from Rio Peixe-Boi (01°07'17.65"S, 47°18'48.35"W), Municipality of Peixe-Boi, State of Pará (PA), Brazil. Specimens of *D. septemdentatus* were collected manually or with dip nets by Edilson R. Mattos and were taken still alive to the "Laboratório de Pesquisa Carlos Azevedo – LPCA, Universidade Federal Rural da Amazônia (UFRA)", in Belém, PA. Temnocephalans were fixed with 10% phosphate-buffered formalin 90ºC or with AFA (70°GL ethanol; formalin 37%; glacial acetic acid), under slight cover slip pressure, following the protocols established by Amato *et al.* (2007) and Seixas *et al.* (2010). The specimens were sent to the "Laboratório de Helmintologia, Universidade Federal do Rio Grande do Sul (UFRGS)", where some specimens were stained in Delafield's hematoxylin or aceto-carmine /fast green, cleared in cedar oil and mounted as permanent slides in Canada balsam. Some specimens were prepared for the Scanning Electron Microscopy (SEM) at the "Centro de Microscopia Eletrônica da UFRGS (CME)". The temnocephalans from *D. septemdentatus* were studied through a series of techniques fully described by Amato *et al.* (2007) and Seixas *et al.* (2010), focusing especially on the: 1. morphology of the cirrus structure; 2. morphology of the vagina and other female reproductive organs; 3. distribution of the rhabditogenic glands in juveniles; and, 4. paired, dorsolateral, post-tentacular 'excretory' syncytial plates (DLSPs). Photomicrographs were taken with the microscopes Zeiss Axiolab and Leica DMR Hc equipped with Nomarski´s differential interference contrast prisms (DIC). The photographic images and line drawings were scanned and prepared using CorelDraw X5 and Adobe's Photoshop CS2, respectively. Measurements are in micrometers (µm) unless otherwise indicated and were taken from specimens killed under slight cover slip pressure and mounted in Canada balsam; ranges are followed (between parentheses) by the mean, the standard deviation values, and the number of specimens measured for a given character (when different than 11). Cirrus measurements were taken from extracted cirri mounted in Faure´s mounting medium (F), while the terminology used to describe the male reproductive structures followed Seixas *et al.* (2010). The whole mounts of adult and juvenile type specimens, as well as slides containing individual cirri mounted in (F) were deposited in the following scientific collections: 1. "Coleção Helmintológica do Instituto Oswaldo Cruz" (CHIOC), Rio de Janeiro, RJ, Brazil; 2. "Colección de Invertebrados, División Zoología Invertebrados, Museo de La Plata (MLP)", La Plata, Argentina; and 3. "Coleção Helmintológica do Laboratório de Helmintologia, Departamento de Zoologia, Universidade Federal do Rio Grande do Sul", Porto Alegre, RS.

RESULTS

Temnocephala longivaginata n. sp.
(Fig. 3 - 25)
Description**.** Based on 20 specimens collected: 11 whole mounted adults; 1 juvenile; 3 specimens mounted on stubs for SEM; 5 dissected cirri mounted in (F); 11 specimens measured.
External characteristics. Body (without tentacles) (Figs 5 and 22) 2.48–4.54 mm (3.14 mm ± 678) long, 1.61–2.79 mm (2.02 mm ± 419) wide; adhesive disk ventral, subterminal, completely covered by body (Figs 5 and 22) 501–905 (651 ± 124) long, 529–836 (663 ± 102) wide; disc peduncle 390–613 (481 ± 90) wide. Red pigmentation of the eyes not observed. Two dorsolateral, epidermal 'excretory' syncytial plates ($DLSP_S$) small, 'shoe sole-shaped (Figs 11-12), left plate 152.7–313.2 (230 ± 80; 3) long, 53.7–119.5 (83 ± 34; 3) wide; right plate 165.6–310.2 (229 ± 74; 3) long, 65.7–84.6 (76 ± 10; 3) wide; length ratio of DLSPs /total body length, without tentacles, 14: 1. Excretory pore in the equatorial line of the plate, near the internal limit (Fig. 12).

Glands. Rhabditogenic glands forming bunches (average 15 cells), in lateral fields of body (Figs 6-7), 33-100 (64 ± 15) in diameter, ducts conspicuous (Figs 7 and 23). Two groups of five Haswell glands, showing little affinity with hematoxylin, in front of the brain transverse band, diameter of largest cell 17–39 (24 ± 13; 3). Disc glands between adhesive disc and genital complex, 22–61 (40 ± 11) in diameter, forming two, lateral bunches extending from posterior testes to margin of adhesive disc (Figs 6-7), including pair, of large, round, more central cells (paranephrocytes?), 67–155 (111 ± 27) long (Figs 6-7).

Reproductive system. Female. Ovary located between vagina and vesicula resorbens, 88–190 (144 ± 40; 9) long, 75–170 (113 ± 34; 9) wide (Figs 9 and 25). Vitellarium arborescent, wispy, barely covering intestine dorsally (Figs 8 and 22); vagina long, 56–210 (147 ± 49; 8) long, 60-80 (72 ± 8; 8) wide (Figs 9 and 25) with a widening of its distal portion; vaginal sphincter asymmetrical, 75-97.5 (83.5 ± 6; 10) in total diameter (Figs 9 and 25); diameter of anterior portion 30-37.5 (33 ± 4; 10), diameter of posterior portion 32.5-42.5 (38 ± 2; 10); vesicula 'intermedia' 40-80 (60 ± 16; 5) long; vesicula resorbens usually full of sperm (Fig. 25), 102.5–360 (227 ± 101; 6) long; 130–310 (264 ± 97; 6) wide; wall thickness 5–30 (16 ± 9; 6).

Male. Four testes quite small, usually rounded, slightly oblique; deferent vessels unite in large, pyriform, thick-walled seminal vesicle, 215–350 (288 ± 38) long, 40–100 (70 ± 18) wide; wall thickness 5–10 (9 ± 2); prostatic bulb elongated, with thick muscular walls, 100–340 (239 ± 63) long, 87.5–190 (111 ± 24) wide, wall thickness 5–20 (11 ± 5) (Fig. 10). Cirrus 112.5–130 (121.25 ± 12.4; 2) long, with a circle of sclerites (average 10) in the distal portion of the introvert (Figs 13 and 20), followed by a smooth portion (without spines or ridges) (Figs 14 and 21). Introvert proximal portion with longitudinal ridges reaching the proximal limit (Figs 14-19, and 21). Shaft 82.5–95 (88.8 ± 8.9; 2) long, shaft maximum width at base 57.5–62.5 (60 ± 3; 2) (Fig. 21); introvert 30 (n = 2) long; introvert width at base 20 (n = 2); maximum introvert width at level of swelling 22.5 (n = 2). Introvert´s swelling with approximately 10 ridges (Figs 14-19). Ratio between total body length, without tentacles/ total length of cirrus 26: 1; ratio between total length of cirrus/ maximum width of shaft´s base 2: 1; ratio between total length of cirrus/ total length of introvert 4: 1.

Taxonomic summary.
Type host: *Dilocarcinus septemdentatus* (Herbst, 1783) (Trichodactylidae).
Type locality: Rio Peixe-Boi (01º07'17.65"S, 47º18'48.35"W), Municipality of Peixe-Boi, PA, Brazil.

Site of infestation: branchial chambers.

Helminth specimens deposited: "Coleção Helmintológica do Instituto Oswaldo Cruz": CHIOC 37458a - Holotype (SBA 3226-1-3); CHIOC 37458b - juvenile paratype (SBA 3226-1-7); CHIOC 37459 - cirrus in Faure´s mounting medium (SBA 3227-1-6). "Colección de Invertebrados, División Zoología Invertebrados, Museo de La Plata": MLP 6292 - paratype (SBA 3226-1-1); - cirrus in Faure´s mounting medium (SBA 3227-1-7A).

Other helminth specimens examined: *Temnocephala pignalberiae* – Amato *et al.* 2010: CHIOC Nº 37308; CHIOC Nº 37309; CHIOC Nº 37310 a-b; CHIOC Nº 37311; MLP Nº 6091; MLP Nº 6092; MLP Nº 6094; MLP Nº 6095. "Coleção de

Invertebrados do Instituto Nacional de Pesquisas da Amazônia (INPA)": INPA Nº 525; INPA Nº 526; INPA Nº 527; INPA Nº 528. *Temnocephala pignalberiae* – Damborenea (1992): MLP Nº 3126; MLP Nº 3127.

Host specimens deposited: The collector of the crabs from Rio Peixe-Boi for the present work, Edilson R. Matos, did not deposit specimens, but in 1994 deposited two male specimens collected from the same location in the collection of the "Museu de Zoologia da Universidade de São Paulo (MZUSP)" under the Nº 11694, which were identified by Gustavo A. S. de Melo as *D. septemdentatus*. Etymology: the specific epithet is given after the unusually long vagina of this species.

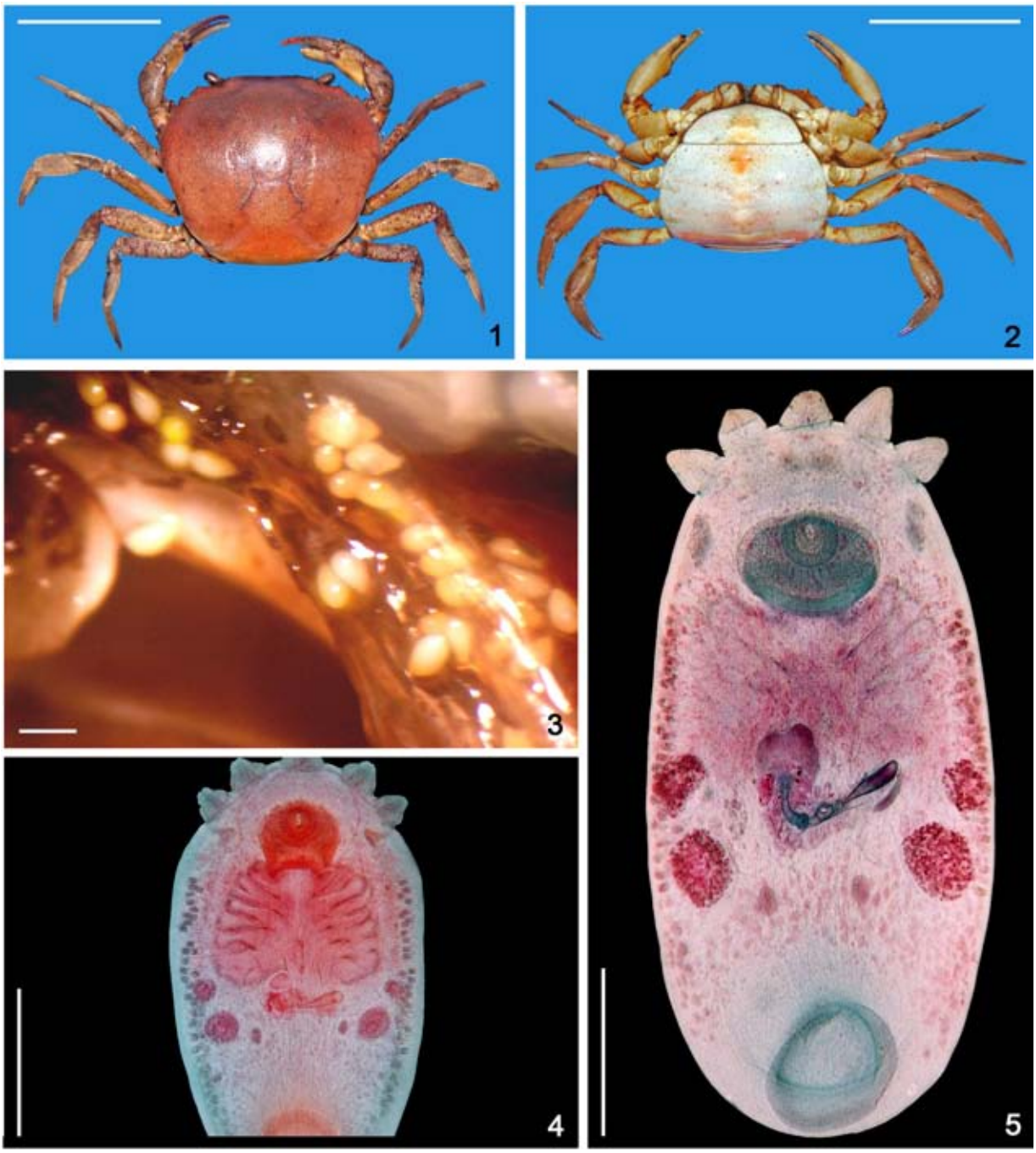
DISCUSSION

Célio Magalhães (pers. comm.) received dorsal and ventral pictures of a female crab used in the present work, but indicated that the taxonomy within the genus *Dilocarcinus* is done exclusively through the male gonopods and recommended the utilization of the name *D. septemdentatus* for these crabs. He also cautioned that *Goyazana castelnaui* (H. Milne Edwards, 1853) may occur in the region, but has not yet been recorded at Rio Peixe-Boi. Magalhães & Türkay (2008) examined the male specimens of *D. septemdentatus* deposited at the MZUSP, and confirmed that they, in fact, were *D. septemdentatus.*

Pereira & Cuocolo (1941) when redescribing *T. microdactyla* assumed that the large specimens with long cirrus without introvert spines collected at Bodoquena, MS belonged to the same species described by Monticelli (1903). The original description mentioned the short size of the tentacles in relation to body size, thus explaining the specific epithet. Since the reproductive system on the original description of *T. microdactyla* was not described, it is possible that this species might be as described by Pereira & Cuocolo (1941), by Dioni (1967), and by Damborenea (1992), but it also could be *T. pignalberiae*, recently collected in Poconé, MT, less than 20 km from the type locality of *T. microdactyla* by Amato *et al.* (2010) or the species being described in the present work found in the type host of *T. microdactyla*.

The ectocommensal temnocephalans found on *D. septemdentatus* are most similar to *T. pignalberiae* in size and shape of the body, but show important differences in the reproductive system (Figs 9-10, and 25). The new species differs from *T. pignalberiae* by having the cirrus internal wall with ridges and unique sclerites in the distal portion of the introvert, followed by a smooth portion (without spines or ridges) (Figs 13-21). To present, all species of *Temnocephala* have the cirrus' introvert either with spines or are smooth, *i.e.*, without spines. Ridges in the inner wall of the introvert were recorded for the first time in Temnocephalidae. The cirrus of *T. pignalberiae* is similar in size (105µm in length), but lacks either spines or ridges. It also differs by having a long vesicula 'intermedia' (average of 60µm in length) (Figs 9 and 25) replacing the seminal receptacles and a long vagina (average 147µm in length) (Figs 9 and 25) with a widening of its distal portion (Fig. 25), near the asymmetrical vaginal sphincter (Figs 9 and 25). The 'excretory' syncytial plates ($DLSP_s$) also differ in shape from that of *T. pignalberiae* which is large and elongate (Amato *et al.* 2010, Figs 19-21, pg. 23). The new species has the 'excretory' syncytial plates ($DLSP_s$) small and 'shoe sole'-shaped (Figs 11-12).

The finding of characters so unusual as a circle of sclerites and ridges in the introvert of *T. longivaginata* n. sp., a species close to *T. pignalberiae* which has the introvert smooth and occurs in another species of *Dilocarcinus* calls attention to the fact that temnocephalans cannot be studied by the techniques used prior to 1994, which used specimens collected from hosts fixed in cold ethanol and studied only through pressure between slide and coverslip. Without the extraction of the cirrus to be mounted in F, from specimens collected alive fixed with hot formalin and observed with DIC and SEM one would not have found the fine details of the introvert shown in the present work. This allowed the best comparison possible between the shape of the DLSPs of the new species and those of *T. pignalberiae*. In our opinion species of *Temnocephala* described prior to the year 2000 should be collected again from live hosts and reexamined with the techniques used in the present work.



Figures 1–3. Female specimen of *Dilocarcinus septemdentatus*. 1. dorsal view. 2. ventral view. Scale bars = 3 cm. 3. live eggs deposited internally in the upper side of the carapace. Scale bar = 500 µm. **Figures 4–5.** *Temnocephala longivaginata* n. sp. 4. young specimen. Scale bar = 1 mm. 5. Adult specimen. Scale bar = 500 µm.

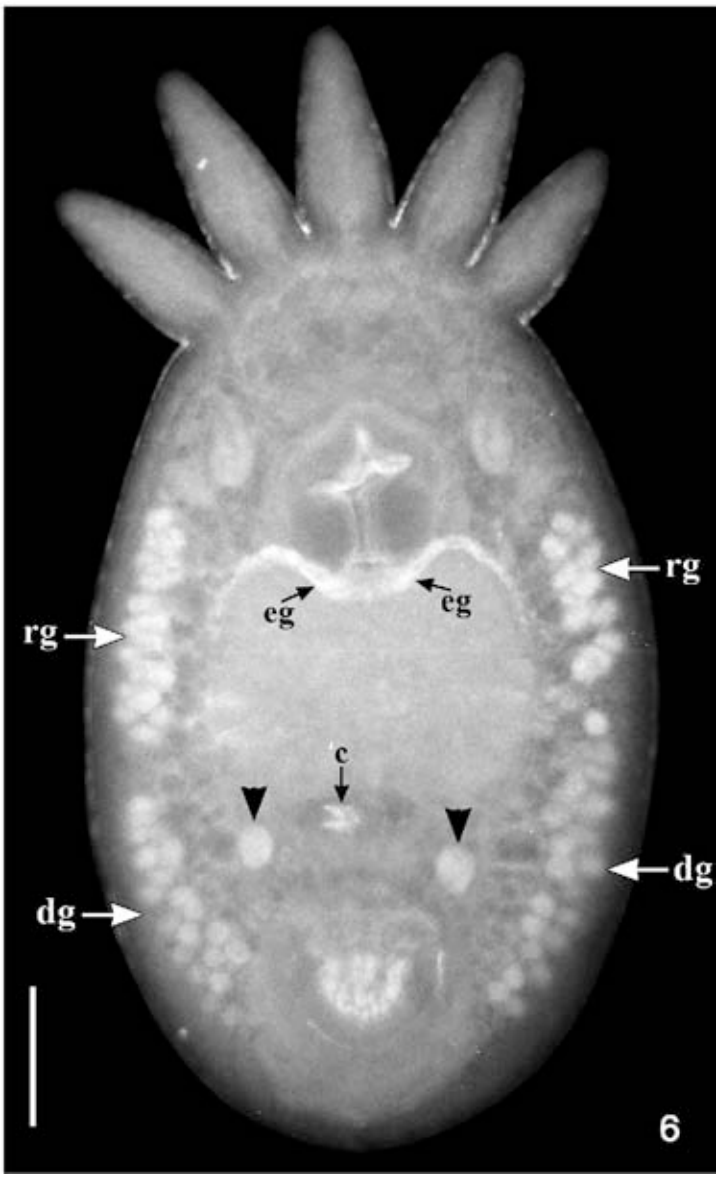
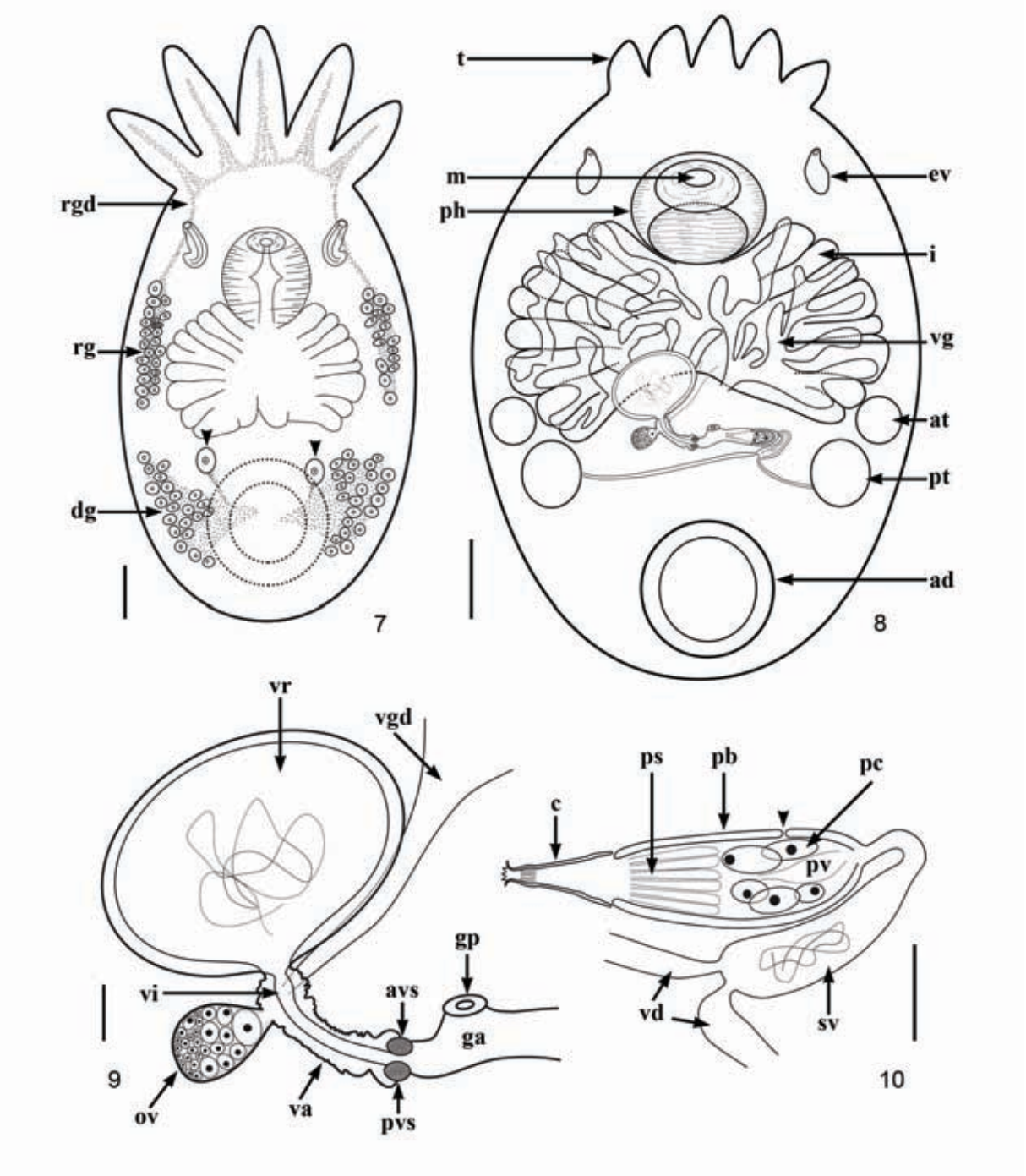
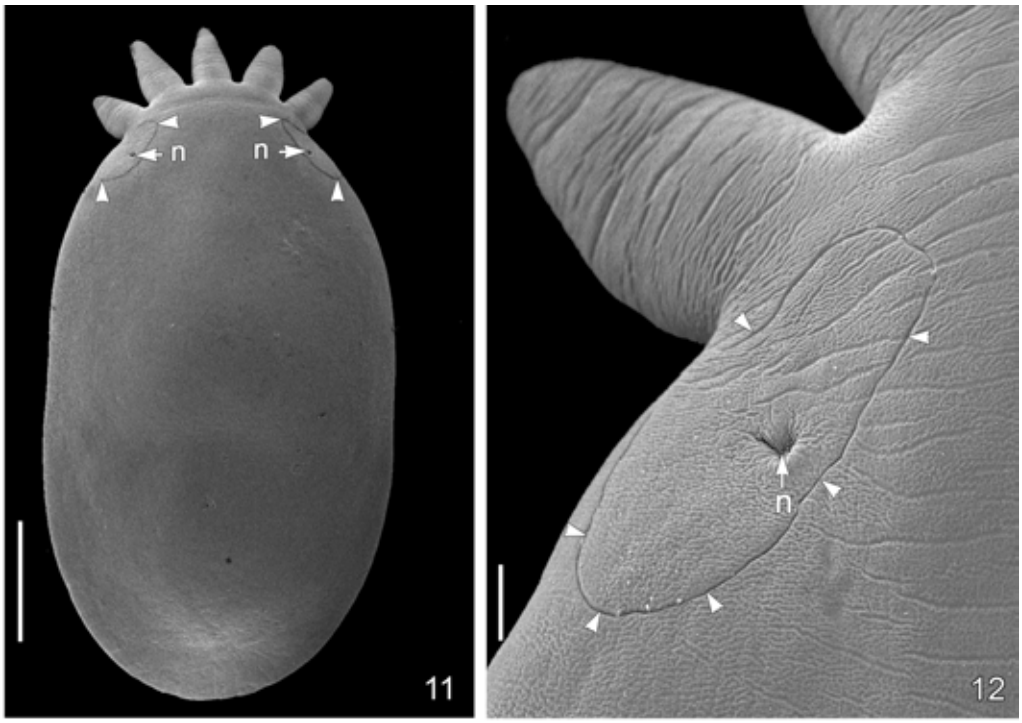


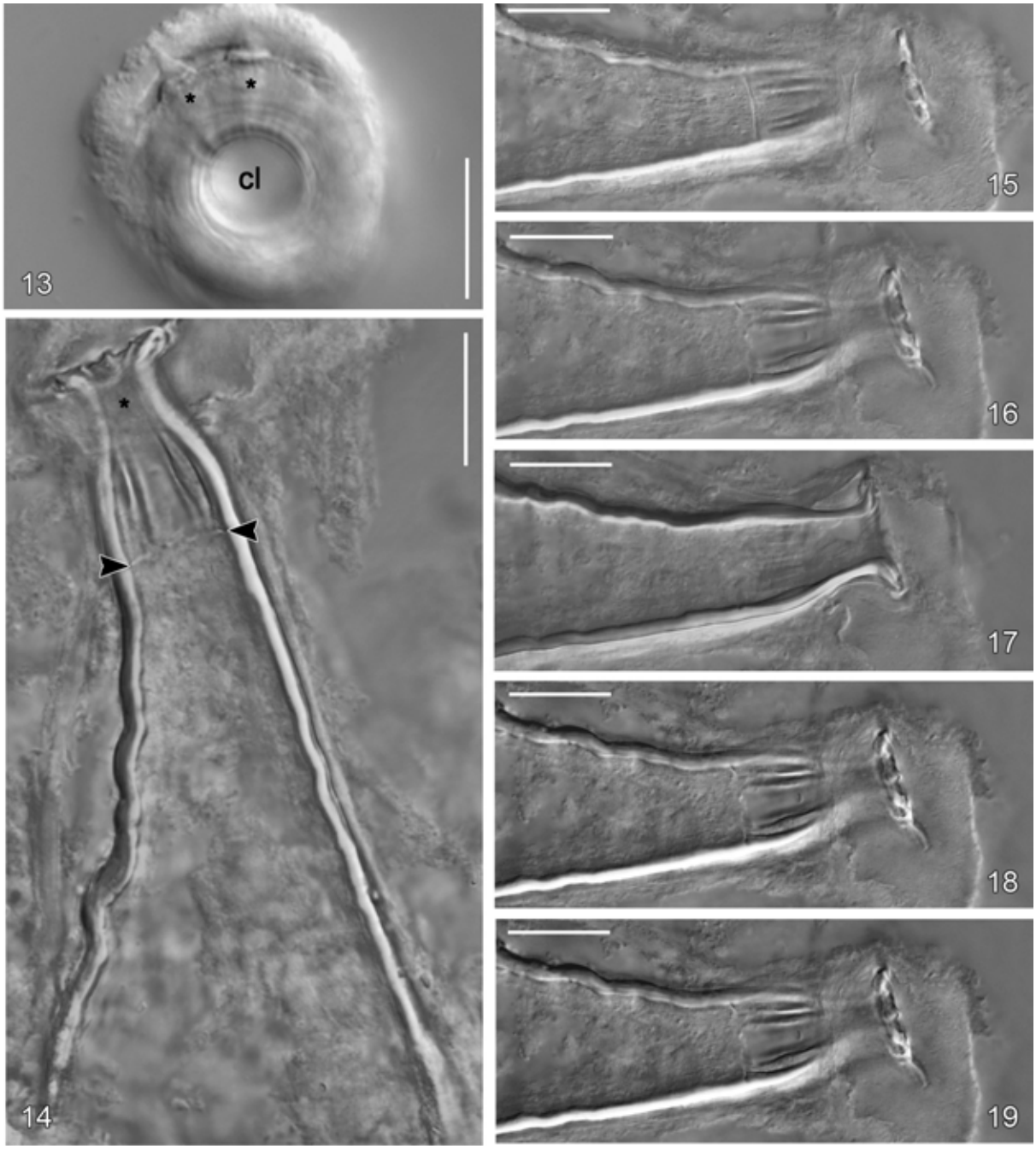
Figure 6. *Temnocephala longivaginata* n. sp. Juvenile, ventral view, showing rhabditogen glands (rg), esophageal glands (eg), posterior disc glands (dg), incipient cirrus (c), and the pair of large disk glands (paranephrocytes?) (head arrows). Scale bar = 150 µm.



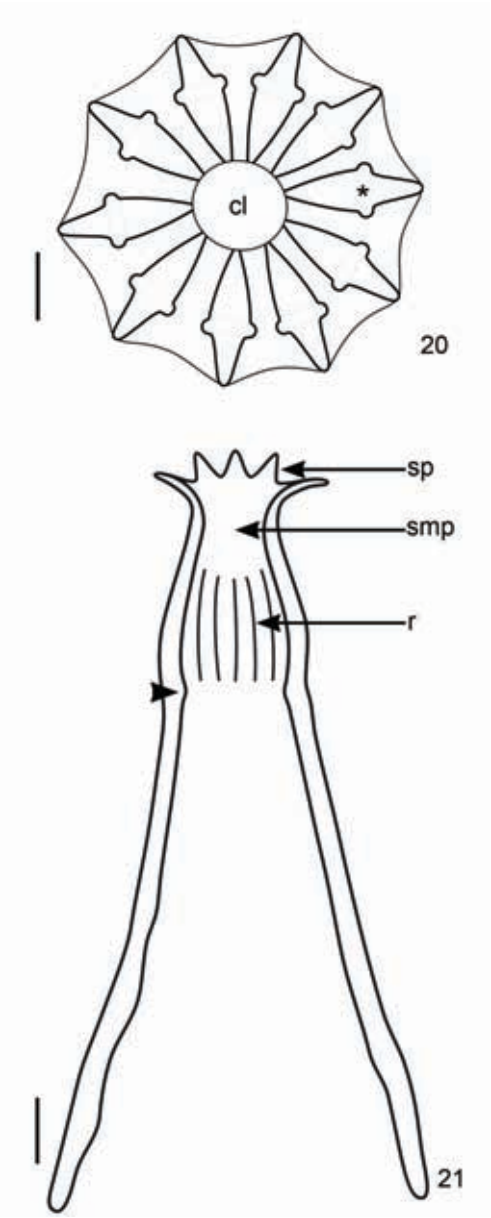
Figures 7-10. *Temnocephala longivaginata* n. sp. 7. Juvenile, ventral view, showing rhabditogenic glands (rg) extending along sides of intestinal sac, and its ducts (rgd) entering tentacles. The posterior disc glands (dg) and the pair of large disk glands (paranephrocytes?) (head arrows). Scale bar = 100 μm. 8. Incomplete diagram of an adult specimen, ventral view, showing: adhesive disk (ad), anterior testis (at), excretory vesicles (ev), intestinal sac (i), mouth (m), pharynx (ph), posterior testis (pt), tentacles (t), and vitelline glands (vg). Scale bar = 500 μm. 9. Female reproductive organs: anterior portion of the vaginal sphincter (avs), genital atrium (ga), genital pore (gp), ovary (ov), vagina (va), vitelline glands duct (vgd), vesicula 'intermedia' (vi), vesicula resorbens (vr), and posterior portion of the vaginal sphincter (pvs). Scale bar = 100 μm. 10. Male reproductive system: cirrus (c), opening in the prostatic bulb wall (head arrow), prostatic bulb (pb), prostatic cells (pc), prostatic secretion (ps), prostatic vesicle (pv), seminal vesicle (sv), and vasa deferentia (vd). Scale bar = 100 μm.



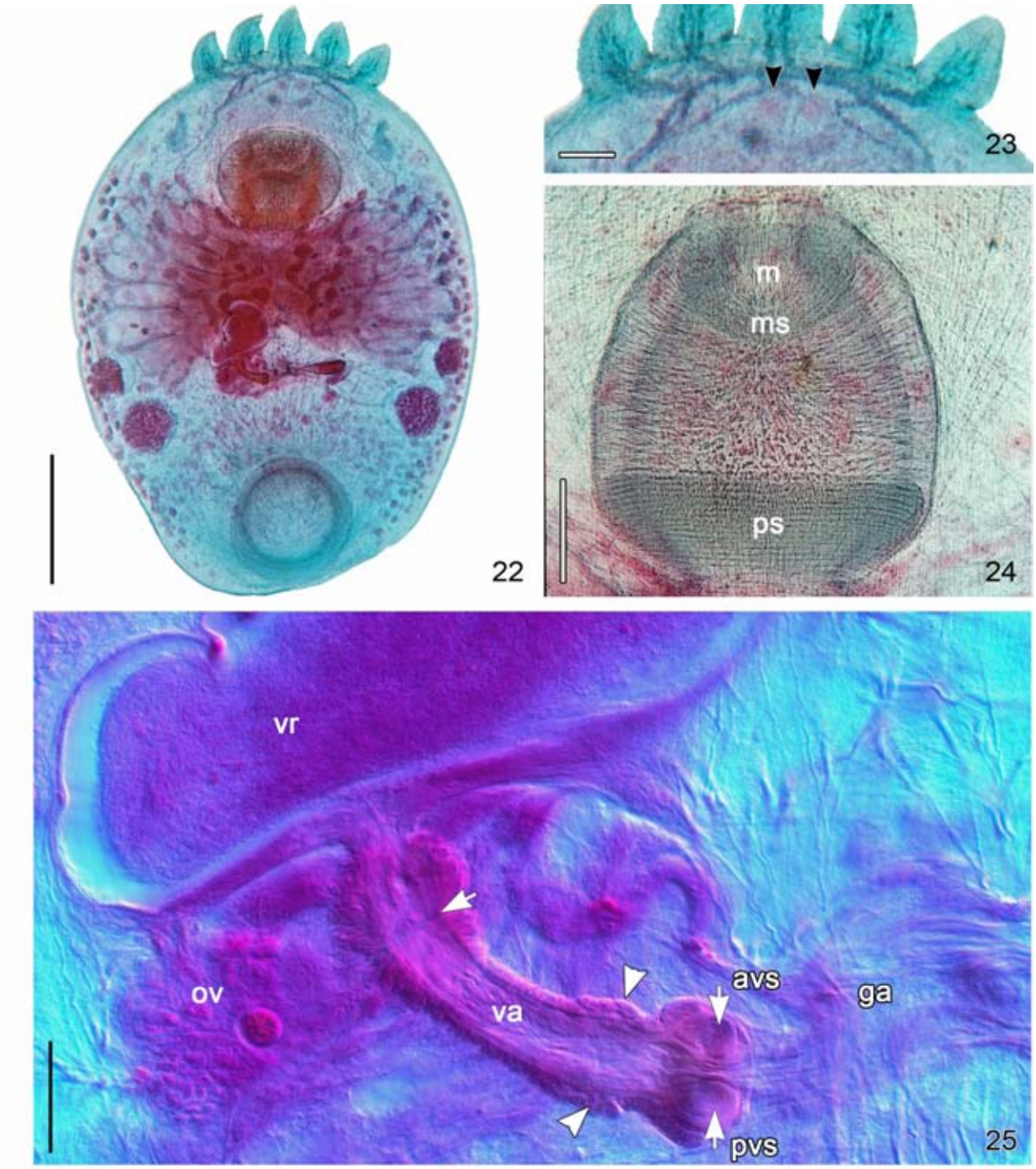
Figures 11–12. *Temnocephala longivaginata* n. sp. observed with SEM. 11. Entire specimen, showing the dorsolateral 'excretory' syncytial plates (head arrows) and position of excretory pores (n – white arrows). Scale bar = 500 μm. 12. Left DLSP (head arrows)



Figures 13–19. *Temnocephala longivaginata* n. sp. cirrus mounted in (F) and observed with DIC. 13. *En face* view of the distal portion of the introvert indicating the circle of sclerites (*) and the cirrus lumen (cl). Scale bar = 10 µm. 14. Cirrus, showing the proximal limit of the introvert (black head arrows) and the smooth portion (*) between the ridges and the sclerites. 15-19. Cirrus introvert observed in different focusing planes. Scale bar = 20 µm.



Figures 20–21. *Temnocephala longivaginata* n. sp. 20. *En face* line drawing of distal portion of the introvert indicating the circle of sclerites (*) and the cirrus lumen (cl). Scale bar = 5 μm. 21. Line drawing of cirrus, showing the sclerites portion of the introvert (sp), the smooth portion (smp), and the ridges (r) of the inner wall of the introvert, just after the limit between the introvert and the shaft (head arrow). Scale bar = 10 μm.



Figures 22–25. *Temnocephala longivaginata* n. sp. 22. Adult specimen pressed between slide and cover slip, showing that too much pressure may be good to allow all characters to be observed in the same focusing plane, but alters the body shape. Scale bar = 250 μm. 23. Pair of largest cells of Haswell glands (head arrows) and the ducts of rhabditogenic glands entering tentacles. Scale bar = 200 μm. 24. Mouth (m), mouth sphincter (ms), and pharyngeal sphincter (ps). Scale bar = 200 μm. 25. Female reproductive system: anterior portion of the vaginal sphincter (avs), genital atrium (ga), ovary (ov), posterior portion of the vaginal sphincter (pvs), vagina (va), vesicula 'intermedia' (arrow); widening of the vagina (head arrows), and vesicula resorbens (vr). Scale bar = 100 μm.

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