

## ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

A NEW SPECIES OF *TEMNOCEPHALA* (PLATYHELMINTHES, TEMNOCEPHALIDA)  
ECTOSYMBIONT ON *NERITINA ZEBRA* (MOLLUSCA, NERITIDAE)  
FROM THE BRAZILIAN AMAZONIAUMA NOVA ESPÉCIE DE *TEMNOCEPHALA* (PLATYHELMINTHES,  
TEMNOCEPHALIDA) ECTOSIMBIONTE SOBRE *NERITINA ZEBRA* (MOLLUSCA,  
NERITIDAE) DA AMAZÔNIA BRASILEIRASamantha A. Seixas<sup>1</sup>, José F. R. Amato<sup>2</sup> & Suzana B. Amato<sup>3</sup>

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## ABSTRACT

*Temnocephala euryhalina* sp. n., an ectosymbiont of the mollusk *Neritina zebra* (Bruguière, 1792), is described from the State of Pará, Brazilian Amazonia. Representatives of Neritidae Lamarck, 1809, usually euryhaline, are adapted to habitats with a certain degree of salinity. Up to now, all species of *Temnocephala* epibiont on mollusks were described/recorded on Ampullariidae Gray, 1824, hosts strictly from freshwater, as all previous hosts of *Temnocephala* spp. Twenty-one mollusks were collected from Rio Tocantins, Municipality of Cameté. The new species differs from all the epibiont temnocephalan species of mollusks described so far, by having the following characters: 1. small body size; 2. cirrus simple and short, different from the cirrus of other epibiont species of mollusks that, unless specific variations, has a 'brush' format, where the boundary between the shaft and the introvert is well marked and the introvert has a swelling portion; 3. feminine reproductive system simple and inconspicuous; 4. vagina not muscular, without differentiation between the proximal and distal portions; 5. vesicula intermedia small, replacing the seminal receptacles; and 6. vaginal sphincter single and symmetric. The eggs of the temnocephalans were not found, inside or outside the shells and on the opercula. This is the first record of a species of *Temnocephala* epibiont on Neritid mollusks and the first record of an euryhaline host for any *Temnocephalida*.

**Keywords:** mollusks - Neotropic Region - Taxonomy - temnocephalids.

## RESUMO

*Temnocephala euryhalina* sp. n., ectosimbionte de moluscos da espécie *Neritina zebra* (Bruguière, 1792) é descrita do Estado do Pará, Amazônia Brasileira. Representantes da família Neritidae Lamarck, 1809, geralmente eurialinos, são adaptados aos habitats que apresentam um certo grau de salinidade. Até o momento, todas as espécies de *Temnocephala* epibiontes em moluscos foram descritas/registradas em hospedeiros da família Ampullariidae Gray, 1824, estritamente dulciaquícola como todos os hospedeiros conhecidos das espécies de *Temnocephala*. Vinte e um moluscos foram coletados no Rio Tocantins, Município de Cametá. A nova espécie é diferente de todas as espécies descritas sobre moluscos até o momento, e possui os seguintes caracteres: 1. tamanho corporal diminuto; 2. cirro simples e curto, diferente dos cirros das espécies epibiontes de moluscos que, salvo variações específicas, apresentam o formato de pincel, onde o limite entre o *shaft* e o *introvert* é bem marcado e o último apresenta uma inflação; 3. sistema reprodutor feminino simples e inconspícuo; 4. vagina pouco muscular e sem diferenciação entre as porções proximal e distal; 5. vesícula intermédia pequena, substituindo os receptáculos seminais; e 6. esfíncter vaginal único e simétrico. Ovos de temnocefalídeos não foram encontrados dentro ou fora da concha dos hospedeiros, assim como sobre os opérculos. Este é o primeiro registro de uma espécie de *Temnocephala* epibionte em moluscos neritídeos e o primeiro registro sobre um hospedeiro de hábito eurialino.

**Palavras-chave:** moluscos - Região Neotropical – Taxonomia – temnocefalídeos.

## INTRODUCTION

Up to the present, all known mollusk hosts of species of *Temnocephala* Blanchard, 1849 are ampullariid gastropods. This family has a large number of species distributed in five genera, and it is quite common in freshwaters of the Neotropic Region (Cowie & Thiengo, 2003).

Five species were described as epibionts of ampullariid mollusks (Haswell, 1893; Ponce de León, 1979; 1989; Damborenea & Brusa, 2008). *Temnocephala iheringi* Haswell, 1893 was the first of them, and has several records in several genera/species of ampullariid mollusks in Brazil and in Argentina (Pereira & Cuocolo, 1941; Hyman, 1955; Damborenea, 1992; Damborenea & Cannon, 2001; Damborenea *et al.*, 1997; Seixas *et al.*, 2010a). *Temnocephala rochensis* Ponce de León, 1979 and *Temnocephala haswelli* Ponce de León, 1989

were described from Uruguay on *Pomacea canaliculata* (Lamarck, 1822) (Ponce de León, 1979; 1989); both had their descriptions updated when recorded from Brazil by Seixas *et al.* (2010b; 2010c). *Temnocephala lamothei* Damborenea & Brusa, 2008 has been described from *Pomella megastoma* (Sowerby, 1825) of Argentina (Damborenea & Brusa, 2008), and, recently, *Temnocephala colombiensis* Garcés, Puerta, Tabares, Lenis & Velásquez, 2013, has been described from an undetermined species of *Pomacea* Perry, 1810, from Antioquia, Colombia (Garcés *et al.*, 2013).

Representatives of Neritidae Lamarck, 1809, usually are euryhaline, some of them more associated to the marine environment, while other species are associated to brackish waters (Tan & Clements, 2008). Species of *Neritina* Rafinesque, 1815, inhabit sandy and muddy environments, living over pieces of wood and

other plant remains near the shore. They are found on submerged areas, never exposed, always on roots of trees of the river (Barroso & Matthews-Cascon, 2009). *Neritina zebra* (Bruguière, 1792) occurs in the Brazilian seashore regions (State of Pará to State of Rio de Janeiro), and in Suriname.

The present work records a new species of *Temnocephala*, which is found for the first time on Neritid mollusks, and is the first record of a host with euryhaline habitat.

## MATERIAL AND METHODS

Twenty-one specimens of *N. zebra* (Figs 1 and 2) were collected in February of 2010 in Rio Tocantins (2°14'35.39"S, 49°29'50.29"W), Municipality of Cametá, Pará, Brazil, distant 144 km, in a straight line to Belém, PA. The mollusks were collected 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)", Belém, PA, to be examined for temnocephalans. Temnocephalans were fixed with 10% phosphate-buffered formalin 90°C or with AFA, under slight cover slip pressure, following the protocols established by Amato *et al.* (2007) and Seixas *et al.* (2010a). Some specimens were stained in Delafield's hematoxylin or aceto-carminé /fast green, cleared in cedar oil, and mounted as permanent slides in Canada balsam. Other specimens were prepared for Scanning Electron Microscopy (SEM) at the 'Centro de Microscopia Eletrônica da UFRGS (CME)'. The temnocephalans from *N. zebra* were studied through a series of techniques 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. the shape of the 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 (DIC) prisms. The photographic images and line drawings were scanned and prepared using CorelDraw X5 and Adobe's Photoshop CC, 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 8). 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.* (2010a). The distribution of rhabditogenic and disc glands was studied by clearing juvenile specimens in lactophenol (Amato & Amato, 2005). The whole mounts of adult and juvenile specimens, as well as slides containing individual cirri mounted in (F) were deposited in the following scientific collections: 1. 'Coleção Helminológica 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; 3. 'Coleção de Invertebrados do Instituto Nacional de Pesquisas da Amazônia (INPA)', Manaus, AM, Brazil; and 4. 'Coleção Helminológica do Laboratório de Helminologia, Departamento de Zoologia, Universidade Federal do Rio Grande do Sul', Porto Alegre, RS."

## RESULTS

### *Temnocephala euryhalina* n. sp. (Figs 3 - 24)

Description. Based on 14 specimens collected: 8 whole mounted adults; 2 specimens mounted

on stubs for SEM; 6 dissected cirri mounted in (F); 8 specimens measured.

External characteristics. Body (without tentacles) (Figs 3 and 5) 869–1.36 mm (1.10 mm  $\pm$  164) long, 494–691 mm (612 mm  $\pm$  64) wide; adhesive disk ventral, subterminal, partially covered by body (Figs 5, 10, and 13 - ad) 197–336 (247  $\pm$  57) long, 237–454 (355  $\pm$  82) wide; disc peduncle 190–310 (226  $\pm$  39) wide. Eyespots with red pigmentation present (observations made on live specimens). Two rounded DLSPs, slightly wider than long (Figs

9 and 11-12), external margin reaching ventrolateral margin of body (Fig. 12), left plate 99–147 (123  $\pm$  34, 2) long, 109.2–109.85 (109  $\pm$  0.45, 2) wide; right plate 107-154 (131  $\pm$  33, 2) long, 100–112 (106  $\pm$  8, 2) wide; length ratio of DLSPs /total body length, without tentacles, 8: 1. Excretory pore in the anterior portion of the plate, near the internal limit (Figs 11-12 - ep).

Glands. Rhabditogenic glands forming bunches (average 13 cells) extending from



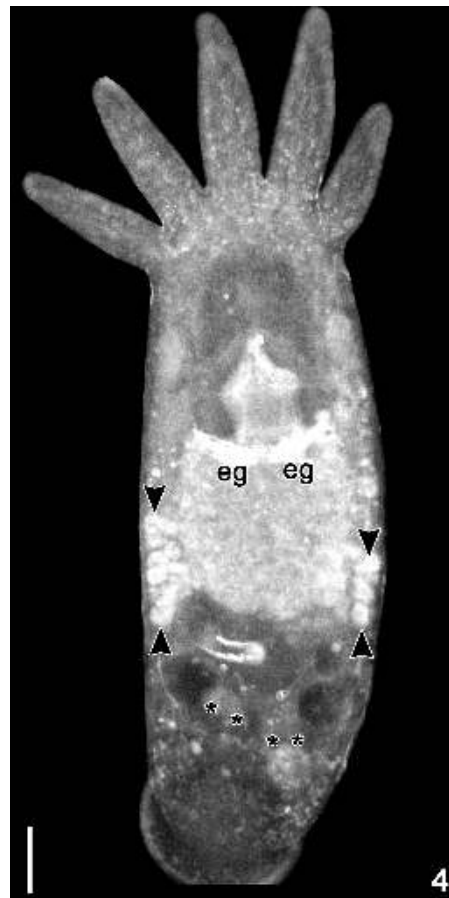
**Figures 1-2.** *Neritina zebra*. (1) Shell, dorsal view. Bar = 2.5 mm. (2) Shell, ventral view, showing the operculum (asterisk) and the mollusk foot (arrow), still fixed inside the shell. Bar = 2.5 mm.

**Figure 3.** Adult of *Temnocephala euryhalina* n. sp., stained with acetic carmine/fast-green and mounted *in toto*. Bar = 100  $\mu$ m.

mid-level of intestine to anterior level of posterior testes, in lateral fields of body, 25–42.5 ( $35 \pm 7$ ) in diameter (Fig. 4 – black head arrow), ducts inconspicuous. Two groups of two Haswell glands (Fig. 5 - hg), showing little affinity with hematoxylin, in front of the cerebral transverse band (Fig. 5 - b), diameter of largest cell 37.5–52.5 ( $45 \pm 5$ ; 6). Esophageal glands between pharynx and the intestine (Fig. 4 - eg). Disc glands between adhesive disc and genital complex, 27.5–55 ( $39 \pm 8$ ) in diameter, forming two, lateral bunches extending from mid-level of posterior testes to anterior margin of adhesive disc, including two pairs of large, round, more central paranephrocytes, 50–62.5 ( $56 \pm 4$ ; 5) long (Figs 4 and 24 - asterisk).

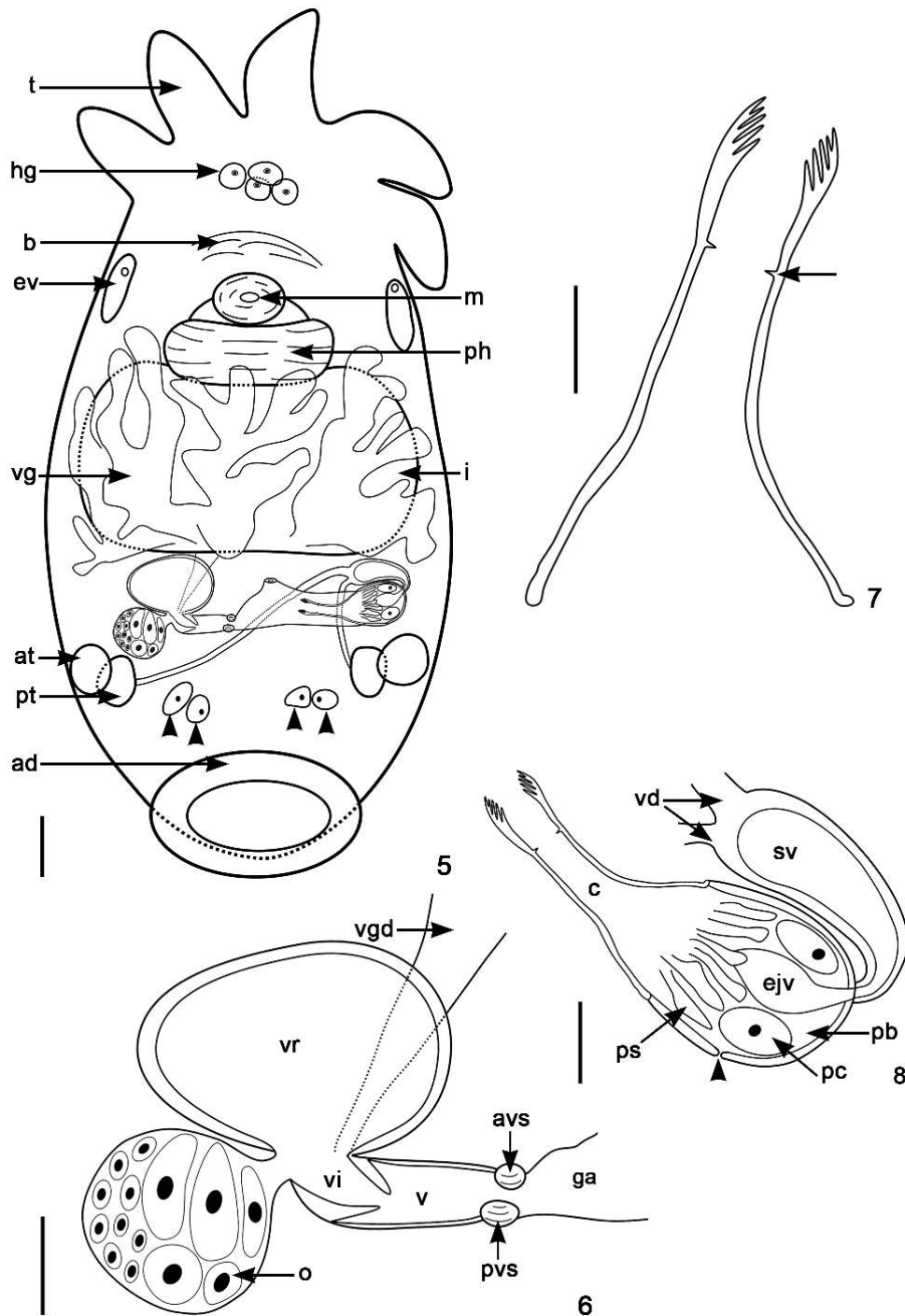
#### Reproductive system.

Female. Ovary 57.5–117.5 ( $85 \pm 18$ ) long, 57.5–100 ( $76 \pm 15$ ) wide, located between the vagina and the vesicula resorbens (Figs 6 and 20-21 - o). Vitellarium arborescent and dense (Figs 3 and 5 - vg); vagina with thick muscular walls 47.5–97.5 ( $75 \pm 18$ ) long, 22.5–40 ( $28 \pm 6$ ) wide (Figs 6, 20-22 - v); single vaginal sphincter symmetrical, 25–37.5 ( $28 \pm 5$ ) in total diameter (Figs 6 and 21-22 – avs/pvs); vesicula intermedia small, 7.5–15 ( $11 \pm 2$ ) long (Figs 6 and 21 - vi); vesicula resorbens usually full of sperm, 37.5–100 ( $60 \pm 22$ ) long, 67.5–150 ( $100 \pm 26$ ) wide (Figs 6 and 20-22 – vr).

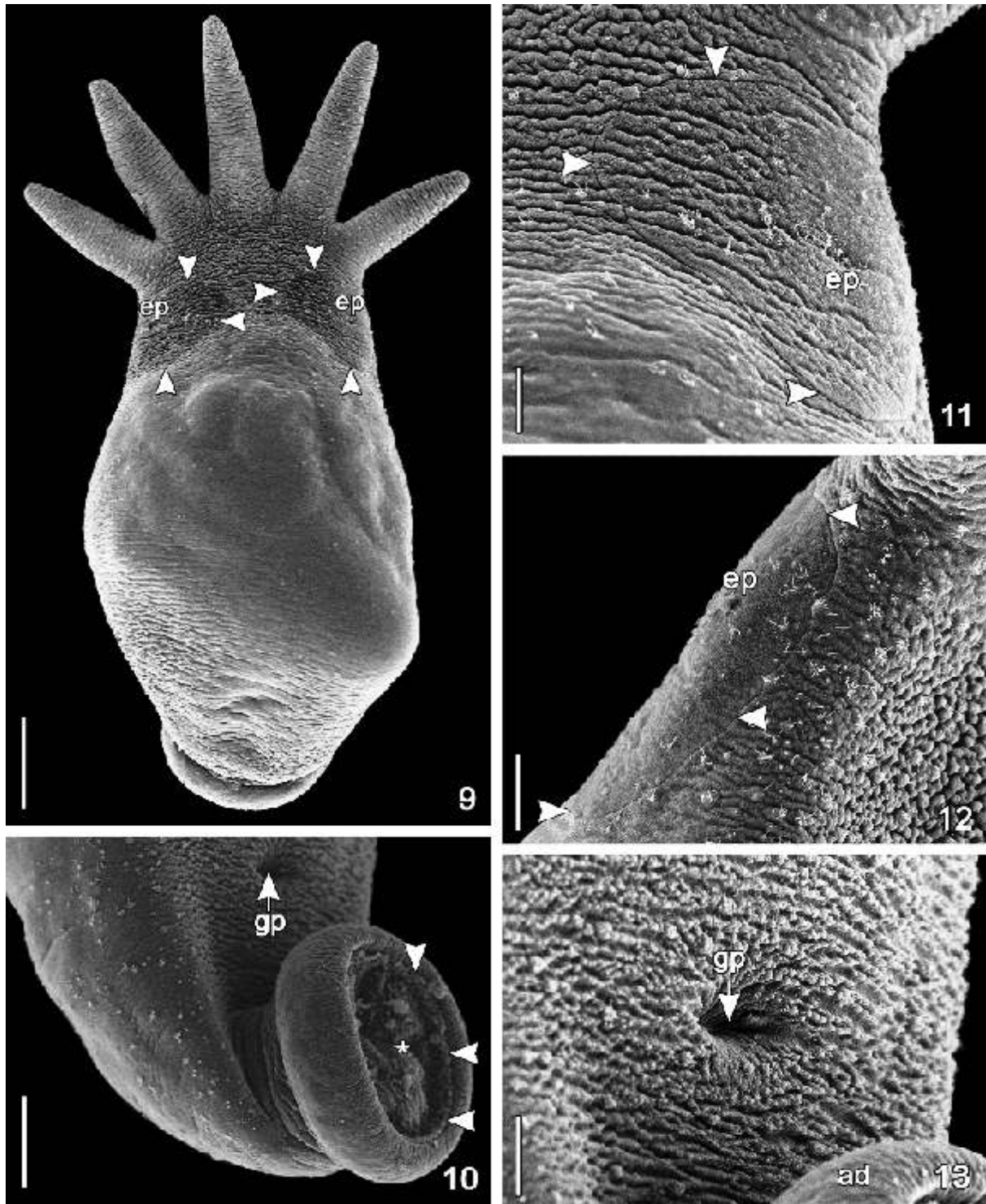


**Figure 4.** Juvenile of *Temnocephala euryhalina* n. sp., cleared in lactophenol and mounted *in toto*, showing esophageal glands (eg), paranephrocytes (asterisks) and the limit of the rhabditogenic glands (black head arrows). Bar = 50  $\mu$ m.





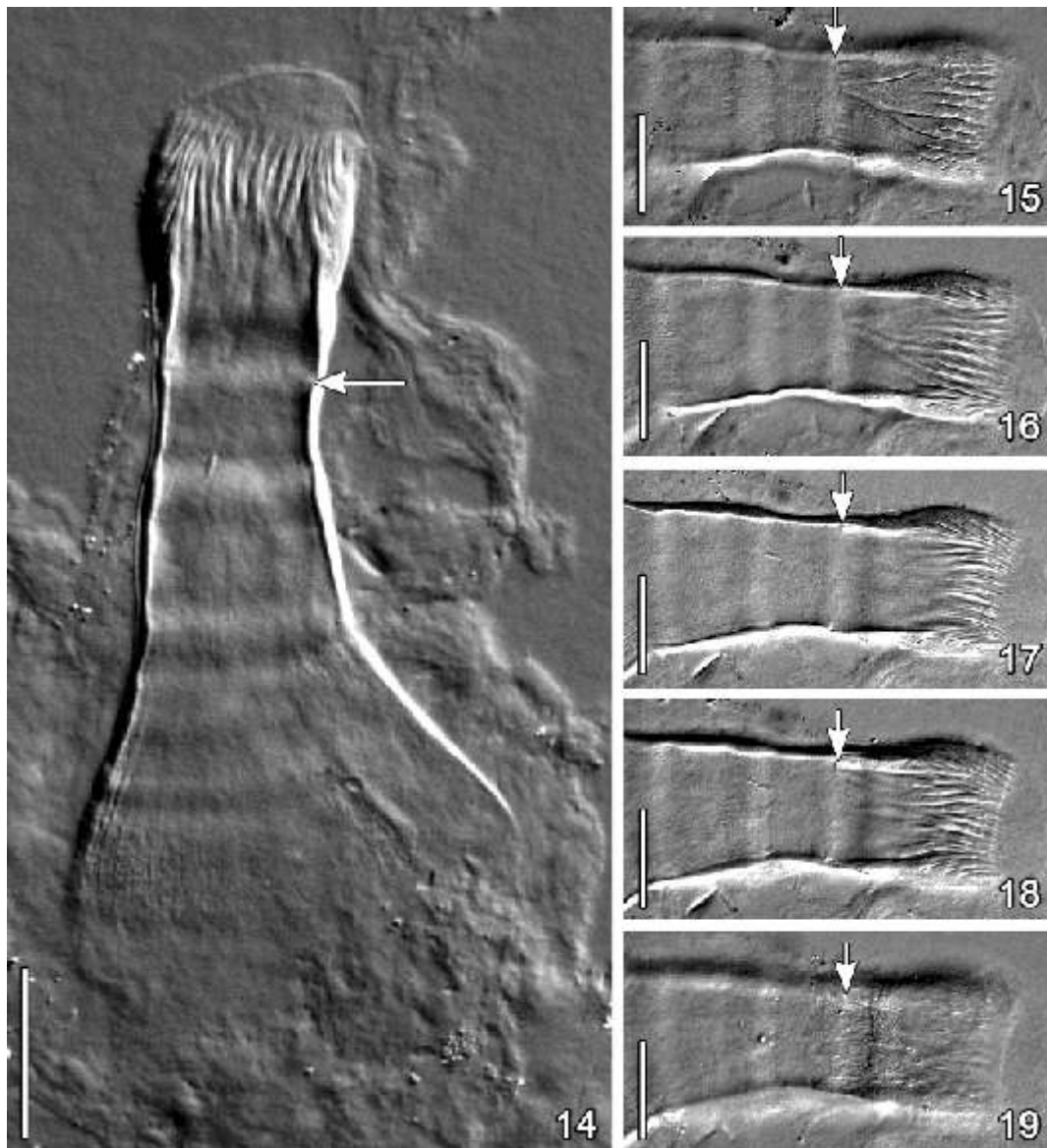
**Figures 5-8.** Partial diagrams of *Temnocephala euryhalina* n. sp. (5) Adult, ventral view. Mouth (m), adhesive disc (ad), pharynx (ph), cerebral band (b), Haswell glands (hg), vitelline glands (vg), intestine (i), paranephrocytes (head arrows), tentacles (t), anterior testes (at), posterior testes (pt), excretory vesicles (ev). Bar = 100  $\mu$ m. (6) Feminine organs of the reproductive system. Genital atrium (ga), vitelline gland duct (vgd), anterior vaginal sphincter (avs), posterior vaginal sphincter (pvs), ovary (o), vesicula intermedia (vi), vagina (v), and vesicula resorbens (vr). Bar = 50  $\mu$ m. (7) Cirrus, showing the limit between introvert and shaft (arrow). Bar = 25  $\mu$ m. (8) Masculine organs of the reproductive system. Prostatic bulb (pb), showing an opening of the wall (head arrow), cirrus (c), prostatic cells (pc), vasa deferentia (vd), prostatic secretions (ps), ejaculatory vesicle (ejv), and seminal vesicle (sv). Bar = 50  $\mu$ m.



**Figures 9-13.** *Temnocephala euryhalina* n. sp., observed with SEM. **(9)** Adult specimen, showing the limits of the dorsolateral 'excretory' syncytial plates (DLSPs) (head arrows) and the excretory pores (ep). Barra = 100  $\mu$ m. **(10)** Posterior region showing the adhesive disc syncytial plate limits (head arrow), the genital pore (gp), and the secretions produced by the disc glands (asterisk). Bar = 50  $\mu$ m. **(11)** Limits (internal, superior, and inferior) of the right DLSP (head arrows) and the excretory pore (ep). Bar = 20  $\mu$ m. **(12)** Limits (external, superior, and inferior) of the right DLSP (head arrows) and the excretory pore (ep). Bar = 20  $\mu$ m. **(13)** Posterior region, detail of the genital pore (gp) and the adhesive disk (ad). Bar = 20  $\mu$ m.

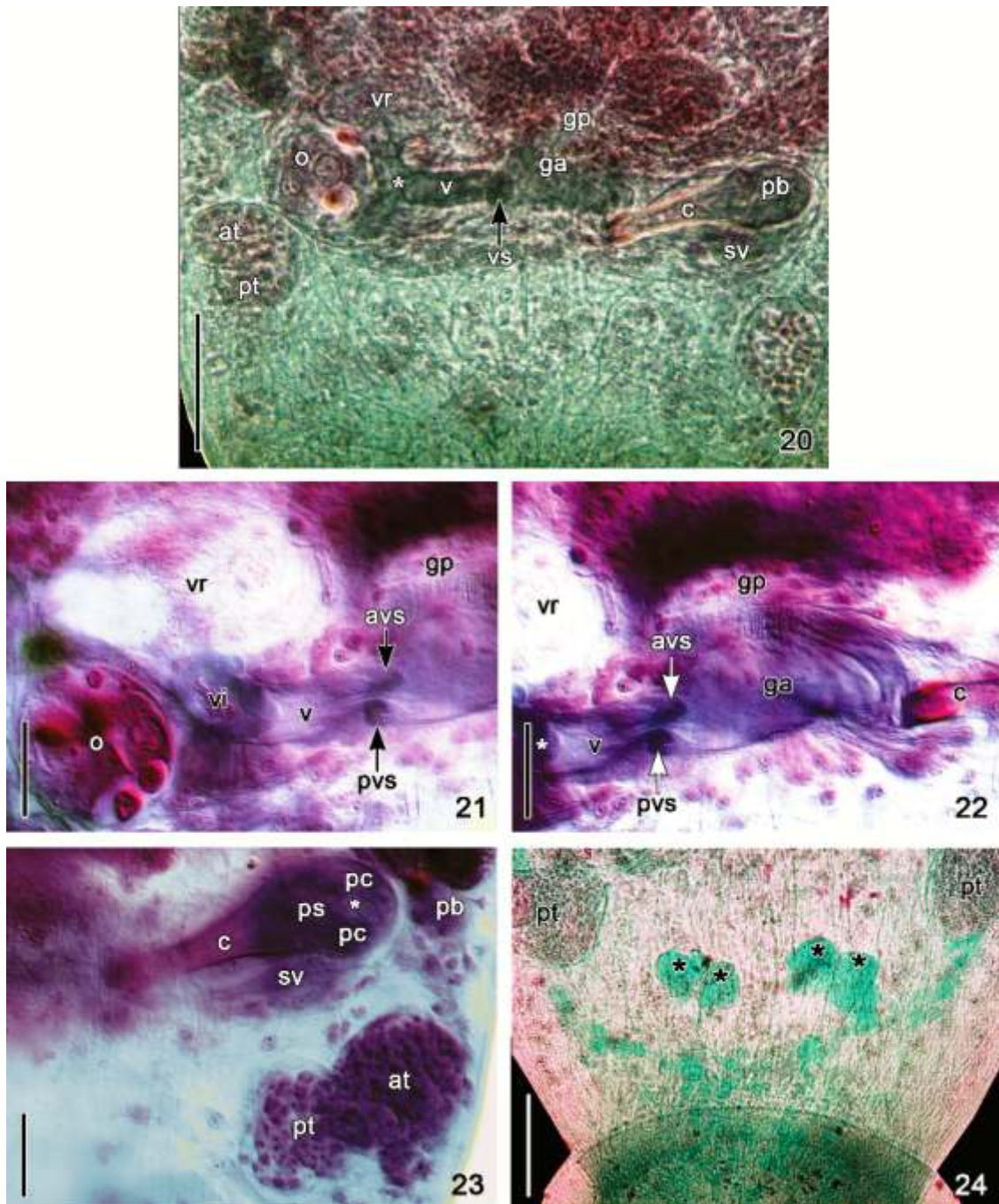
Male. Four testes usually rounded, slightly oblique; anterior testes always superposed to posterior testes (Figs 5 and 20 - at/pt), deferent vessels unite in small, pyriform seminal vesicle, 70–117.5 ( $87 \pm 17$ ; 6) long, 35–60 ( $40 \pm 10$ ; 6) wide (Figs 8, 20, and 23 - sv); prostatic bulb round and short, 42.5–92.5 ( $62 \pm 19$ ; 7)

long, 42.5–65 ( $52 \pm 79$ ; 7) wide (Figs 8, 20, and 23 - pb). Cirrus short, wide 112.5–140 ( $124 \pm 10$ ; 5) long (Figs 7-8, 14, 20 and 23), introvert distal portion with spines from tip to mid-level, followed by smooth region, extending to the introvert proximal limit (Figs 7 and 15-19). Shaft 77.5–115 ( $90 \pm 14$ ; 5) long, shaft



**Figures 14-19.** Cirri of *Temnocephala euryhalina* n. sp., photomicrographed with differential interference contrast (DIC) with Nomarski's prisms. (14) Entire cirrus, showing the limit between the shaft and the introvert (arrow). Bar = 25  $\mu$ m. (15-19) Cirrus introvert observed in a sequence of several focusing planes, showing the limit between the shaft and the introvert (arrow). Bar = 20  $\mu$ m.





**Figures 20-24.** *Temnocephala euryhalina* n. sp. **(20)** Organs of the reproductive system. Genital atrium (ga), prostatic bulb (pb), cirrus (c), vaginal sphincter (vs), ovary (o), genital pore (gp), anterior testes (at) slightly superposed to the posterior testes (pt), vesicula intermedia (asterisk), vagina (v), seminal vesicle (sv) and vesicula resorbens (vr). Bar = 100  $\mu$ m. **(21)** Organs of the feminine reproductive system. Anterior vaginal sphincter (avs) and posterior vaginal sphincter (pvs), ovary (o), genital pore (gp), vagina (v), vesicula intermedia (vi), and vesicula resorbens (vr). Bar = 50  $\mu$ m. **(22)** Organs of the feminine and part of the masculine reproductive systems. Genital atrium (ga), cirrus (c), anterior vaginal sphincter (avs) and posterior vaginal sphincter (pvs), genital pore (gp), vagina (v), vesicula intermedia (asterisk), and vesicula resorbens (vr). Bar = 50  $\mu$ m. **(23)** Organs of the masculine reproductive system. Prostatic bulb (pb), cirrus (c), prostatic cells (pc), prostatic secretions (ps), anterior testes (at) slightly superposed to the posterior testes (pt), ejaculatory vesicle (asterisk), and seminal vesicle (sv). Bar = 50  $\mu$ m. **(24)** Posterior region showing the posterior testis (pt) and the paranephrocytes (asterisks). Bar = 100  $\mu$ m.

maximum width at base 57.5–75 ( $64 \pm 7$ ; 5); introvert 25–37.5 ( $33 \pm 5$ ; 5) long; introvert width at base 22.5–27.5 ( $25 \pm 2$ , 5); maximum introvert width at level of swelling 30–40 ( $32 \pm 4$ , 5). Introvert's swelling with approximately 28 rows of spines, and 5 spines long and thin in each row (Figs 15–19). Ratio between total body length (without tentacles)/ total length of cirrus 8.8: 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: *Neritina zebra* (Bruguière, 1792) (Gastropoda, Neritaeomorphi, Neritidae).

Type locality: Rio Tocantins ( $2^{\circ}14'35.39''S$ ,  $49^{\circ}29'50.29''W$ ), Cametá, Pará, Brazil.

Site of infestation: Adults and juveniles in mantle cavity, eggs not found in the body, on the shell, and neither on the operculum.

Prevalence: 71.42%.

Helminth specimens deposited: 'Coleção Helmintológica do Instituto Oswaldo Cruz': CHIOC 38046 (SBA 3199-1-1 - Holotype); CHIOC 38047 (SBA 3216-1-1 - cirrus). 'Coleção de Invertebrados do Instituto Nacional de Pesquisas da Amazônia': INPA 661 (SBA 3199-1-4 - Paratype); INPA 662 (SBA 3214-1-3 - cirrus). 'Colección de Invertebrados, División Zoología Invertebrados, Museo de La Plata': MLP-He 6842 (SBA 3199-1-3 - Paratype; SBA 3214-1-4 - cirrus).

Other helminth specimens examined: *Temnocephala iheringi*, *Temnocephala haswelli*, and *Temnocephala rochensis* from *P. canaliculata* – voucher specimens deposited in the 'Coleção Helmintológica do Laboratório de Helmintologia, Departamento de Zoologia, Universidade Federal do Rio Grande do Sul'; *Temnocephala iheringi* - MLP 3118, 3119, 3121, and 3120 deposited in the 'Colección de Invertebrados, División Zoología Invertebrados, Museo de La Plata (MLP)'.

## DISCUSSION

*Temnocephala euryhalina* n. sp. is the smallest among the five species from mollusks known to date, having an average body length of 1.10 mm. *Temnocephala iheringi*, *T. rochensis*, *T. haswelli*, and *T. lamothei* are, in average, 2.03–2.80 mm long (Damborenea & Brusa, 2008; Seixas *et al.* 2010a; 2010b; 2010c), while *T. colombiensis*, the smallest species described to date, has, in average a body length of 1.62 mm (Garcés *et al.*, 2013).

*Temnocephala lamothei* is the most similar species to the epibionts from *N. zebra*, presenting a reproductive system less complex than those of the three other species described from mollusks, notwithstanding, the body size, the female reproductive system, and the cirrus are larger than in *T. euryhalina* n. sp. Another important aspect of *T. lamothei* is the introvert which does not show an inflation area, but shows a widening similar to a ring (Damborenea & Brusa, 2008).

The specimens from *N. zebra* have unique characteristic among the epibiont species from mollusks; the cirrus of *T. euryhalina* n. sp. is simple and short, while in epibionts of other species from mollusks, except for intraspecific variations, it has the shape of a brush (Seixas *et al.* 2010a; 2010b), where the limit between the shaft and the introvert is well marked and the later has an inflation area. The female reproductive system is simple and inconspicuous, different from the feminine organs in the other species from ampulariideans (Seixas *et al.* 2010a; 2010b; 2010c). The vagina of *T. euryhalina* n. sp. is not very muscular (Figs 6 and 20–22 - v) and has no differentiation between the proximal and distal portions, besides having a single and symmetrical vaginal sphincter (Fig. 6 - avs/pvs).

*Temnocephala euryhalina* n. sp. is the only

species epibiont on mollusks with vesicula intermedia (Figs 6 and 21 - vi). *Temnocephala lamothei* has only one, instead of four seminal receptacles (Damborenea & Brusa, 2008). The vesicula intermedia is only a widening of the wall while seminal receptacle is a single vesicle located just after the ootype. All the other species epibiont on mollusks have four, conspicuous seminal receptacles. Seixas *et al.* (2010c) when describing specimens of *T. rochensis*, from Rio Grande do Sul, mistakenly called vesicula intermedia the proximal portion of the vagina, although the species has four seminal receptacles and the distal portion has a very strong musculature, which differentiates it from the proximal portion.

The testes, usually large and oblique in the epibionts from mollusks, are small, rounded, of similar size, and always superposed in the specimens of *T. euryhalina* n. sp. (Figs 5, 20, and 23 - at/pt).

The shells of ampulariideans infested with temnocephalans usually show a large number of eggs in the spire and in the body whorl of the shell (Seixas *et al.*, 2010a; 2010b; 2010c), as well as the operculum (Damborenea & Brusa, 2008). Shells and opercula of *N. zebra* were painstakingly examined for eggs, however, not a single temnocephalan egg was found in any of the collected mollusks. The euryhaline habit of the host, promotes the contact of the temnocephalans with a certain degree of salinity, which opens the possibility that this species lays the eggs in the interior portion of the shell. However, more than 90% of the collected shells (some shells were kept as testimonials) were broken in the search for internal postures, and not a single egg has been found.

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