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ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

FIRST REPORT OF *TEMNOCEPHALA PEREIRAI* (PLATYHELMINTHES, TEMNOCEPHALIDAE) ON *TRACHEMYS DORBIGNI* (EMYDIDAE) FROM SOUTHERN BRAZIL –A COMPLETE MORPHOLOGICAL STUDY

PRIMER REGISTRO DE *TEMNOCEPHALA PEREIRAI* (PLATYHELMINTHES, TEMNOCEPHALIDA) SOBRE *TRACHEMYS DORBIGNI* (EMYDIDAE) EN EL SUR DE BRASIL – ESTUDIO MORFOLÓGICO COMPLETO

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Abstract

Specimens and eggs of *Temnocephala pereirai* Volonterio, 2010 found on *Trachemys dorbigni* (Duméril & Bibron, 1835), were reported for the first time extending the known geographical distribution of *T. pereirai* from Uruguay to southern Brazil. Sixteen turtles were collected from a dam at 'Centro Agropecuário da Palma', Universidade Federal de Pelotas (UFPel), Municipality of Capão do Leão, State of Rio Grande do Sul, Brazil. The helminthes were fixed according to our standard protocols. Juvenile and adult temnocephalans were translucent and without the red-eye pigmentation. The worms were living in the inner corners of the plastron, near the legs, together with the eggs, and in the center of the plastron. Eggs were elongated, thick-shelled, with a small and subpolar filament. The most distinctive features of this species were: 1. cirrus's introvert proximal portion with spines from top to mid-level, followed by a smooth region, extending to the introvert proximal limit; 2. vagina with strong muscular walls and a single, symmetrical sphincter; 3. vesicula intermedia unusually long; 4. small dorsolateral 'excretory' syncytial epidermal plates larger in the anterior portion; and 5. excretory pore in the anterior portion of the plate, near the internal limit. The specimens in the present work had larger body length and base diameter of the shaft than the Uruguayan specimens.

Keywords: freshwater turtles - Neotropic Region – taxonomy – temnocephalans - *Trachemys dorbigni*.

Resumen

Especimenes de *Temnocephala pereirai* Volonterio, 2010 ectosimbiontes en *Trachemys dorbigni* (Duméril & Bibron, 1835) se describen del Municipio de Capão do Leão, Estado de Rio Grande do Sul, Brasil. Este registro permite la extensión de la distribución geográfica conocida de *T. pereirai* desde Uruguay hacia el sur de Brasil. Dieciséis tortugas se recogieron en una represa del 'Centro Agropecuário da Palma', Universidade Federal de Pelotas (UFPel), Municipio de Capão do Leão, Estado de Rio Grande do Sul, Brasil. La totalidad de la muestra se fijó de acuerdo a nuestros protocolos. Temnocéfalos juveniles y adultos eran translúcidos y la pigmentación de ojos rojos estaba ausente. Se localizan en las esquinas internas del plastrón, cerca de las patas, junto con los huevos, y en el centro del plastrón. Los huevos eran alargados con cáscara espesa y con un pequeño filamento desplazado hacia un lado. Las características más distintivas de esta especie fueron las siguientes: 1. cirro con la porción proximal del introverto con espinas desde la extremidad distal hasta la mitad, seguido por una región desnuda, que se extiende hasta el límite proximal del introverto; 2. vagina con fuertes paredes musculares y esfínter vaginal simétrico; 3. vesícula intermedia inusualmente larga; 4. placas dorsolaterales, sincitiales 'excretoras' pequeñas, más anchas en su parte anterior; y 5. poro excretor en la porción anterior de la placa, cerca del límite interno. Los especímenes del presente trabajo tenían una mayor longitud del cuerpo y diámetro de la base del 'shaft' en comparación con los especímenes uruguayos.

Palabras clave: Región Neotropical- taxonomía – temnocéfalos – tortugas.

INTRODUCTION

Temnocephala brevicornis Monticelli, 1889 was the second species of the genus to be described and for 121 years was the only species recorded from chelonians. Monticelli (1889) studied temnocephalans from *Hydromedusa maximiliani* (Mikan 1820) and *Hydraspis gibba* (= *Mesoclemmys gibba* (Schweigger, 1812) collected in Brazil and deposited in the helminthological collection of the Zoological Museum of Copenhagen.

The description of *T. brevicornis* was made in a short note, limited to the external characters, allegedly because there were not enough specimens in good condition to be studied. Monticelli (1898) published a detailed study of the internal anatomy based on specimens ectosymbiont on *Hydromedusa tectifera* Cope, 1869, collected in 1894 in the South of Brazil. Baer (1931) studied specimens identified as *T. brevicornis* from *H. maximiliani*, *Hydromedusa platanensis* (= *H. tectifera*), and *M. gibba*. The author studied specimens which included the type series deposited by Monticelli in the 'Museum für Naturkunde Zentralinstitut der Humboldt-Universität zu Berlin, Institut für Systematische Zoologie', thus observing that his specimens as well as those studied by Monticelli, did not have body and ocellar pigmentation. Based on this information Baer did not find valid the variety described by Merton in 1922 (*Temnocephala brevicornis* var. *intermedia*) which could be differentiated from *T. brevicornis* by the presence of body and ocellar pigmentation and insignificant details in the disposition of the reproductive system (Cordero, 1946). Pereira & Cuocolo (1940, 1941) conducted an extensive work on the morphology, bionomy, and ecology of specimens identified as *T. brevicornis*. They studied ectosymbiont specimens from *H. tectifera* collected in the State of São Paulo and from *H. maximiliani* collected in the States of Rio de Janeiro and Minas Gerais.

Caballero & Zerecero (1951) recorded specimens they identified as *T. brevicornis* on brachyuran crustaceans of the genus

Pseudothelphusa De Saussure, 1857, collected in Sabana de Piedra, Venezuela. Although these authors have provided a detailed description of the specimens, it is probable that the specimens belong to a different species, as *T. brevicornis* is not known to occur on crustaceans.

The first record of specimens identified as *T. brevicornis* outside Brazil was published by Dioni (1967). This author studied ectosymbionts from *H. tectifera* collected in Tacuarembó, Uruguay, as well as four other species of temnocephalans, publishing a dichotomic key for these species.

Yuki *et al.* (1993) recorded *T. brevicornis* in two new hosts: *Acanthochelys spixii* (Duméril & Bibron, 1835), and *Trachemys dorbigni* (Duméril & Bibron, 1835) collected in the Municipality of Viamão, State of Rio Grande do Sul. The authors found that the cirrus morphology (shape) was similar to that described by Pereira & Cuocolo (1940), although other characters presented significant differences. Novelli *et al.* (2009) recorded specimens they identified as *T. brevicornis* on *H. maximiliani* collected in the State of Minas Gerais.

Brusa & Damborenea (2000) published the first record for Argentina of specimens identified as *T. brevicornis* collected from *H. tectifera* in Magdalena, Buenos Aires. Volonterio (2010) described two new species (*Temnocephala pereirai* Volonterio 2010 and *Temnocephala cuocoloi* Volonterio 2010) ectosymbiont on *H. tectifera* collected from a road-killed turtle in Canelones, Uruguay. The present work is the second record of *T. pereirai* and the first record of the species in Brazil.

MATERIAL AND METHODS

Sixteen turtles, *T. dorbigni*, were collected between July and December of 2010 in a dam (31º48'01.10"S, 52º30'48.60"W) at the 'Centro Agropecuário da Palma', Universidade Federal de Pelotas (UFPel), located in the rural area of the Municipality of Capão do Leão, RS, Brazil. Turtles were captured manually (with the permission of the Ethics and Animal Experimentation Committee of the UFPel (nº 3026 CEEA) and with dip nets, and were taken in plastic boxes to the 'Laboratório de Parasitologia de Animais Silvestres da UFPel', to be examined for temnocephalans. Temnocephalans were fixed with 10% phosphate-buffered formalin 90ºC or with AFA, under slight cover slip pressure, following the protocols established by Amato *et al.* (2007) and Seixas *et al.* (2010). 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. Other specimens were prepared for Scanning Electron Microscopy (SEM) at the 'Centro de Microscopia Eletrônica da UFRGS (CME)'. The temnocephalans from *T. dorbigni* 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. 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 CS6, 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 27). 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 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. 'Coleção de Invertebrados do Instituto

Nacional de Pesquisas da Amazônia (INPA)', Manaus, AM, Brazil; and 3. 'Coleção Helmintológica do Laboratório de Helmintologia, Departamento de Zoologia, Universidade Federal do Rio Grande do Sul', Porto Alegre, RS.

RESULTS

Temnocephala pereirai Volonterio, 2010
(Figs 3 - 27)
Description. Based on 391 specimens collected: 27 whole mounted adults and 1 juvenile; 3 specimens mounted on stubs for SEM; 12 dissected cirri mounted in (F); 27 specimens measured.

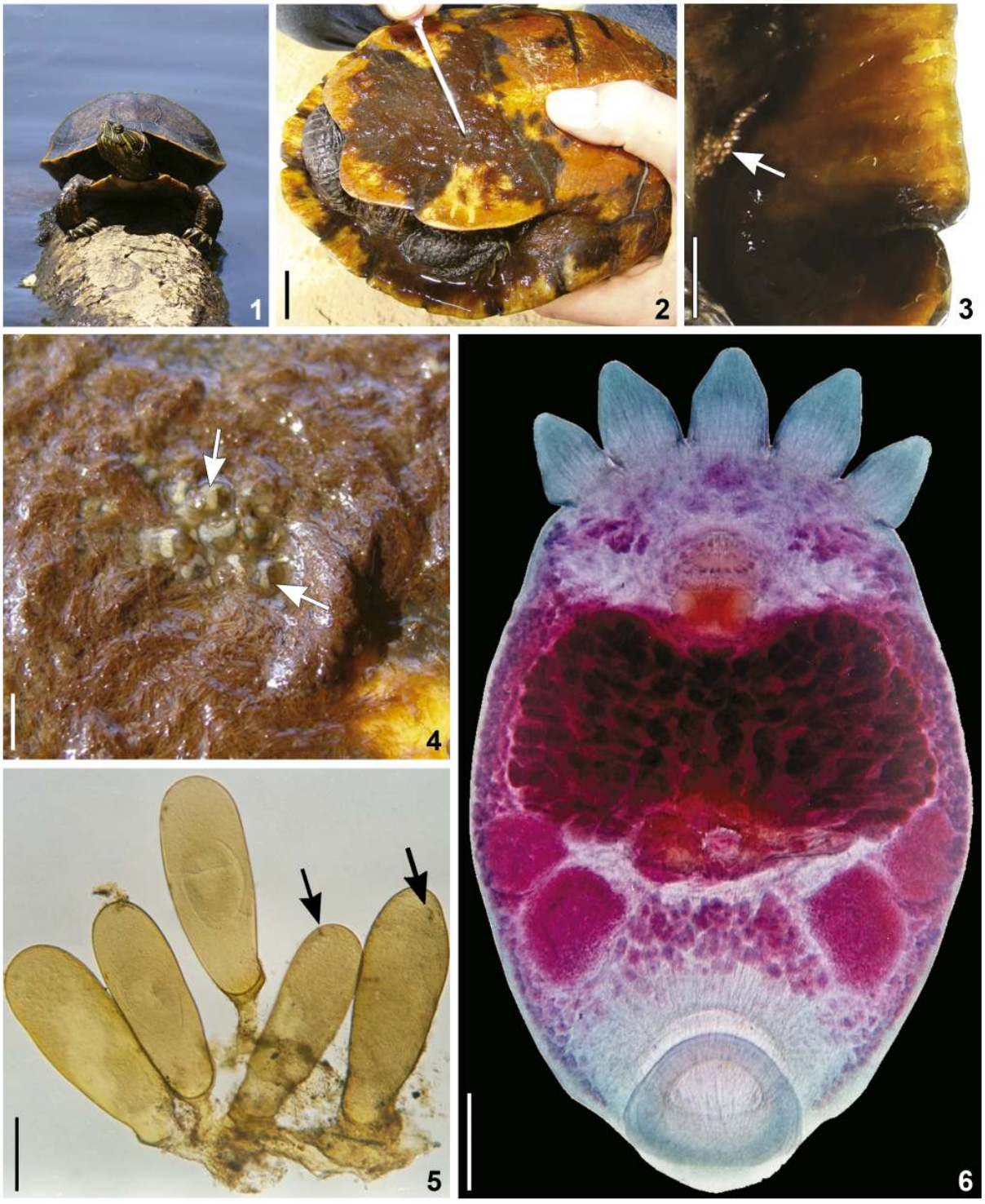
External characteristics. Body (without tentacles) (Figs 6 and 12) 2.11–4.03 mm (2.97 mm ± 451) long, 1.48–2.84 mm (2.23 mm ± 345) wide; adhesive disk ventral, subterminal, completely covered by body (Figs 6 and 7) 434–968 (750 ± 137) long, 632–1027 (860 ± 117) wide; disc peduncle 237–612 (411 ± 91) wide. Red pigmentation of the eyes absent. Two DLSPs, small and kidney-shaped, larger in the anterior portion (Figs 16 and 17), left plate 217.5–253.4 (230.5 ± 20; 3) long, 111.7–130.85 (121.3 ± 13; 3) wide; right plate 213.65–250 (226.6 ± 20; 3) long, 76.9–113.85 (94.5 ± 18; 3) wide; length ratio of DLSPs /total body length, without tentacles, 13: 1. Excretory pore in the anterior portion of the plate, near the internal limit (Fig. 17 - head arrow).

Glands. Rhabditogenic glands forming bunches extending from posterior level of pharynx to mid-level of posterior testes, bunches much wider anteriorly (average 41 cells), in lateral fields of body (Figs 7-8 and 10), 40-87.5 (62 ± 12) in diameter, ducts conspicuous (Figs 7 and 10). Two groups of two Haswell glands (Fig. 24 - asterisks), showing little affinity with hematoxylin, in front of the brain transverse band (Fig. 24), diameter of largest cell 60–140 (93 ± 23; 26). Disc glands between adhesive disc and genital complex (Figs 7-9 and 11), 47.5–105 (71 ± 17) in diameter, forming two, lateral bunches extending from mid-level of posterior testes to anterior margin of adhesive disc, including pair, of large, round, more central cells (paranephrocytes?), 77.5–127.5 (101 ± 17; 7) long (Fig. 11).

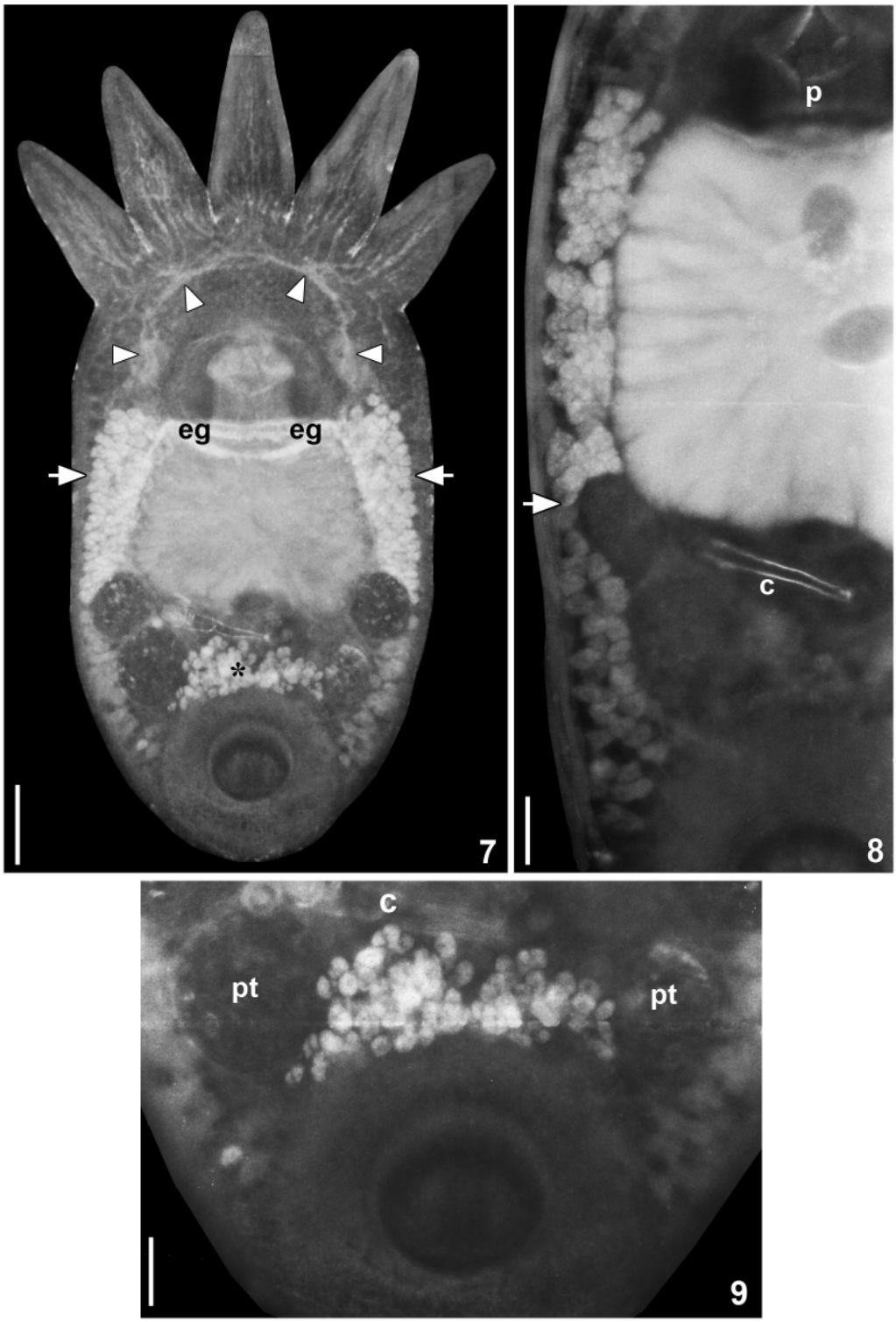
Reproductive system. Female. Ovary 85–287.5 (170 ± 41; 26) long, 60–150 (109 ± 22; 26) wide, located between the vagina and the vesicula resorbens (Figs 15 and 26-27). Vitellarium arborescent, thick, almost covering the entire intestine dorsally (Fig. 6); vagina 112.5–200 (173 ± 24; 26) long, 105-215 (172 ± 33; 27) wide, muscular walls strong, 25-62.5 (42 ± 11; 26) (Figs 15 and 26-27); single vaginal sphincter symmetrical, 90-180 (139 ± 23; 25) in total diameter (Figs 15 and 26-27); vesicula intermedia unusually long, 62.5-127.5 (92 ± 17; 16) long (Figs 15 and 27); vesicula resorbens usually full of sperm (Fig. 15), 80–247.5 (156 ± 47; 13) long, 127.5–275 (203 ± 47; 13) wide.

Male. Four testes usually rounded, slightly oblique; deferent vessels (Figs 14 and 25) unite in large, seminal vesicle pyriform (Fig. 14 and 25), 140–337.5 (240 ± 42) long, 45–100 (74 ± 17) wide; wall thickness 2.5–15 (8 ± 3); prostatic bulb short, muscular walls thick (Figs 14 and 25), 67.5–195 (117 ± 30) long, 65–122.5 (96 ± 16) wide, wall thickness 2.5–5 (4 ± 1). Cirrus 302.5–352.5 (322 ± 17; 12) long, introvert proximal portion with spines from tip to mid-level, followed by a smooth region, extending to the introvert proximal limit (Figs 13, 18, and 21). Shaft 272.5–312.5 (288 ± 14; 12) long, shaft maximum width at base 77.5–110 (91 ± 9; 12) (Figs 13 and 18); introvert 30-42.5 (34 ± 4; 12) long; introvert width at base 15-27.5 (21 ± 4, 12); maximum introvert width at level of swelling 15-20 (17 ± 2, 12). Introvert´s swelling with approximately 21 spines per longitudinal row (Figs 20 and 21). Ratio between total body length (without tentacles)/ total length of cirrus 9.2: 1; ratio between total length of cirrus/ maximum width of shaft´s base 3.5: 1; ratio between total length of cirrus/ total length of introvert 9.5: 1.

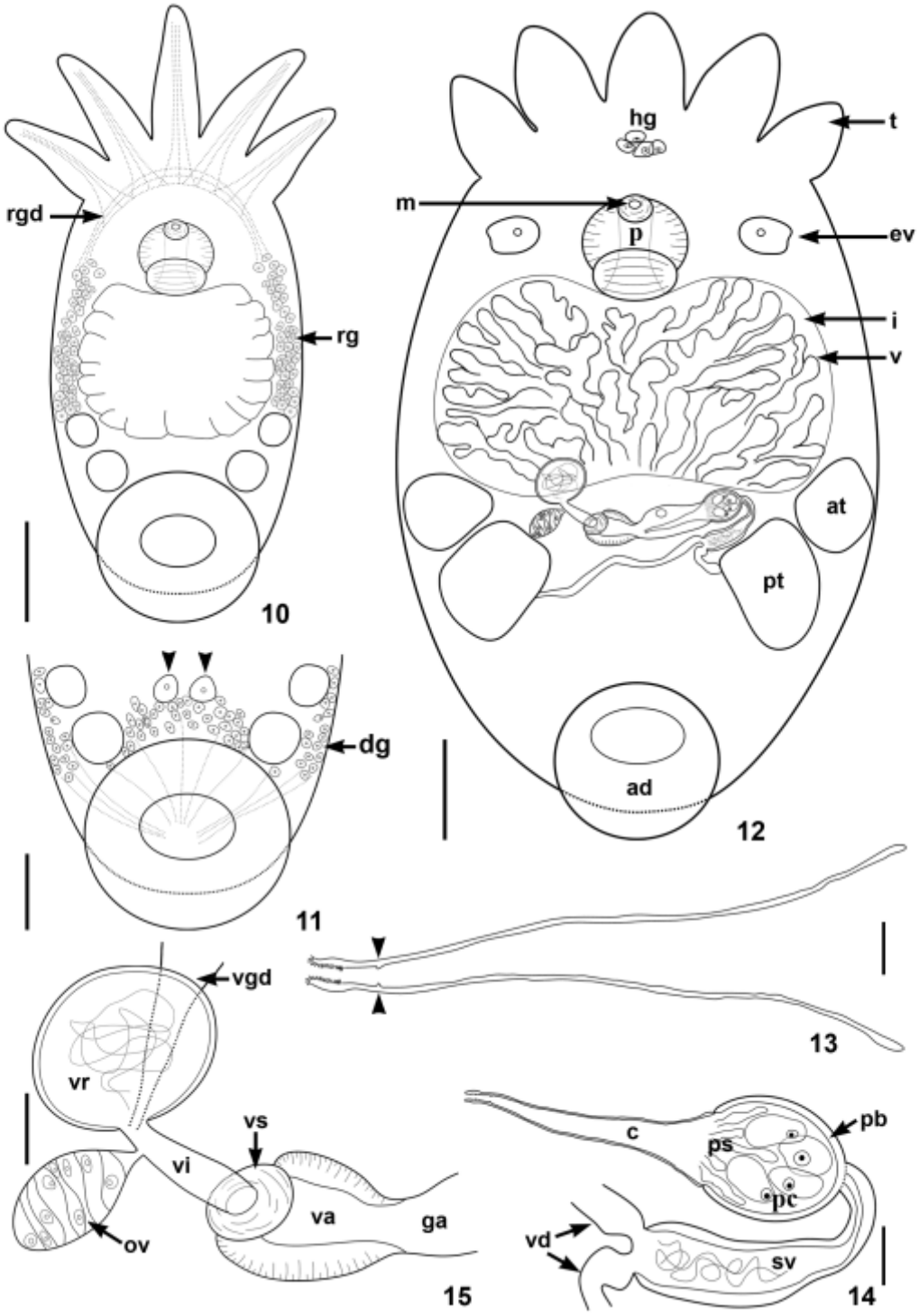
Taxonomic summary.
Type host: *Hydromedusa tectifera* Cope, 1869 (Chelidae).
Type locality: Barra do Carrasco, Ruta 10, km 15



Figures 1–3. *Trachemys dorbigni*. 1. Female specimen of *Trachemys dorbigni*. 2. Ventral view of male specimen of *T. dorbigni* showing the temnocephalans concentrated in the femoral and anal areas of the plastron. Scale bar = 1.5 cm. 3. Live eggs deposited in the inner marginal area of the carapace. Scale bar = 0.5 cm. **Figures 4–6.** *Temnocephala pereirai*. 4. Live specimens in the femoral area of the plastron. Scale bar = 3 mm. 5. Unhatched eggs cleared in cedar oil, showing shape, peduncles, and filament. Scale bar = 250 µm. 6. Adult specimen. Scale bar = 500 µm.



Figures 7–9. *Temnocephala pereirai* juveniles cleared in lactophenol. 7. Juvenile showing rhabditogenic gland ducts entering tentacles (head arrow); rhabditogenic glands (arrow); esophageal glands (eg); and disc glands (asterisk). Scale bar = 250 µm. 8. Magnification of the left side showing the limit between the anterior rhabditogenic glands and the posterior disk glands (arrow); pharynx (p); and cirrus (c). Scale bar = 100 µm. 9. Juvenile; posterior portion showing the disk glands; posterior testes (pt); and cirrus (c). Scale bar = 100 µm.



Figures 10–15. *Temnocephala pereirai.* 10. Incomplete diagram of a juvenile specimen, showing rhabditogenic gland ducts entering tentacles (rgd); and the rhabditogenic glands (rg). Scale bar = 250 µm. 11. Juvenile, posterior portion showing disk glands (dg) and the pair of paranephrocytes (head arrow). Scale bar = 150 µm. 12. Incomplete diagram of adult specimen showing adhesive disk (ad), anterior testis (at), excretory vesicle (ev), Haswell glands (hg), intestine (i), mouth (m), posterior testis (pt), pharynx (p), tentacles (t), and vitellarium (v). Scale bar = 500 µm. 13. Cirrus showing the proximal limit of the introvert (head arrows). Scale bar = 25 µm. 14. Male reproductive complex, showing cirrus (c), prostatic bulb (pb), prostatic cells (pc), prostatic secretion (ps), seminal vesicle (sv), and vasa deferentia (vd). Scale bar = 50µm. 15. Female reproductive complex, showing genital atrium (ga), ovary (ov), vagina (va), vaginal sphincter (vs), vesicula resorbens (vr), vesicula intermedia (vi), and vitelline gland duct (vgd). Scale bar = 100 µm.

(34°52'S, 56°02'W), Departamento de Canelones, Uruguay.
Other host (present work): *Trachemys dorbigni* (Duméril & Bibron, 1835) (Emydidae).
Other locality (present work): Centro Agropecuário da Palma, Universidade Federal de Pelotas (UFPel), Capão do Leão, RS, Brazil.
Site of infestation: inner corners of the plastron, near the legs, together with the eggs, and in the center of the plastron.

Intensity of infestation: 6-169.

Average Intensity of infestation: 43.4.

Prevalence: 56.25%.

Helminth specimens deposited: 'Coleção Helmintológica do Instituto Oswaldo Cruz': CHIOC 37929a - JFA 3222-1-4; CHIOC 37929b - JFA 3222-1-1; CHIOC 37931 – JFA 3223-1-6 (juvenile); CHIOC 37930 - JFA 3137-1-19 (cirrus); CHIOC 37929c - JFA 3222-1-20 (cirrus). 'Coleção de Invertebrados do Instituto Nacional de Pesquisas da Amazônia': INPA 636 - JFA 3137-1-1; INPA 635 - JFA 3223-1-2A (cirrus).

Other helminth specimens examined: 'Colección de Invertebrados, División Zoología Invertebrados, Museo de La Plata' (MLP): MLP 3155 (194; 195) – *Temnocephala brevicornis* from *A. spixii*. MLP 4585 (162; 167; 168) – *Temnocephala brevicornis* from *H. tectifera*.

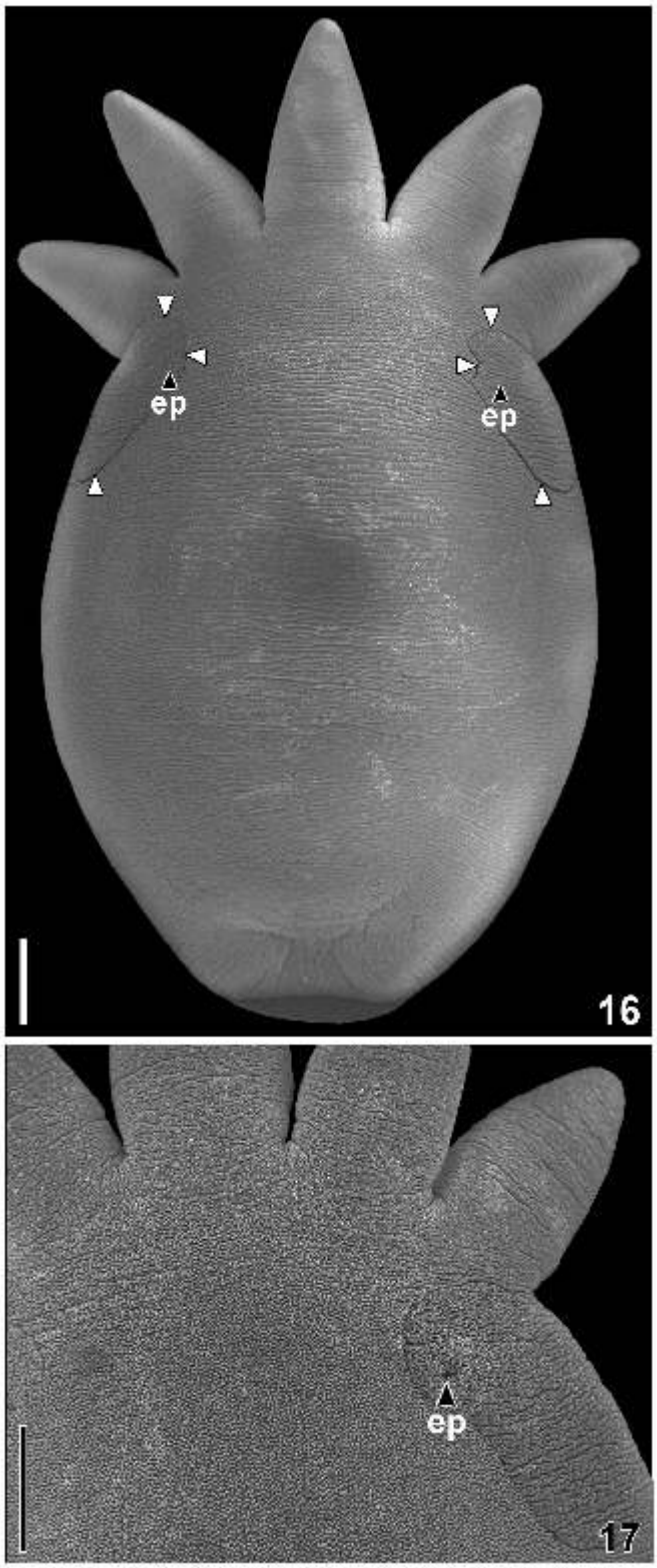
DISCUSSION

Volonterio (2010) when describing *T. pereirai* called attention to some important differences observed between this species and *T. brevicornis*, mainly related to the specimens studied by Pereira & Cuocolo (1940). Yuki *et al.* (1993) also described important differences between their specimens and those of Pereira & Cuocolo (1940) stating that even so they were going to identify their specimens as *T. brevicornis*. These differences were in shape and size of the vagina, the presence of a vesicula intermedia (described as a duct linking the ootype to the vesicula resorbens), smaller body size, and the introvert spines present in larger number although being more delicate. Most of these differences were attributed by Volonterio (2010) to the species *T. pereirai*.

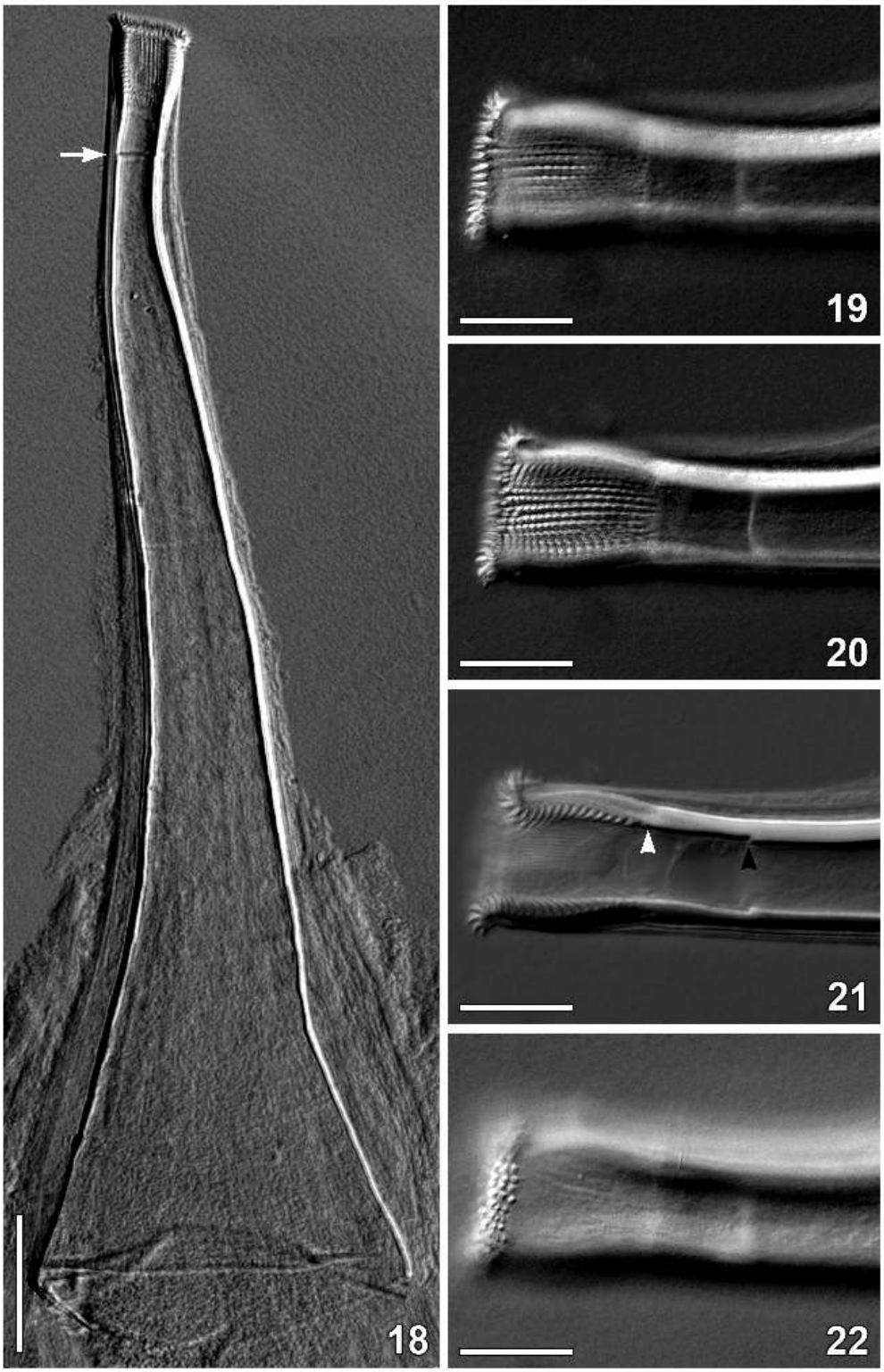
We tried (unsuccessfully) to collect turtles in the same locality [ULNA (Uma Luz No Amanhã), Águas Claras, RS, Brasil] where Yuki *et al.* (1993) collected their specimens, although, the examination of the specimens that these authors deposited at the "Museo de La Plata (MLP)" confirmed that they belonged to *T. pereirai*. Brusa & Damborenea (2000) also reported that the Argentinean specimens showed smaller body size, and smaller and thinner cirri than those described by Pereira & Cuocolo (1940). The examination of Argentinean specimens of *T. brevicornis* deposited at the "Museo de La Plata (MLP)" also showed that those specimens belong to *T. pereirai.*

The specimens of the present work show larger body length (average length of 2.96 mm) when compared to the specimens described by Volonterio (2010) (average length of 2 mm). Although the average body length of the specimens identified as *T. brevicornis* studied by Pereira & Cuocolo (1940) is even larger (average length of 3.76 mm), these authors left a doubt if this measure included or not the tentacles. Even with this discrepancy in body size other important characters are similar to *T. pereirai.* According to Volonterio (2010), the vagina has two sphincters, one proximal, smaller, well developed, and another distal, larger and less developed.

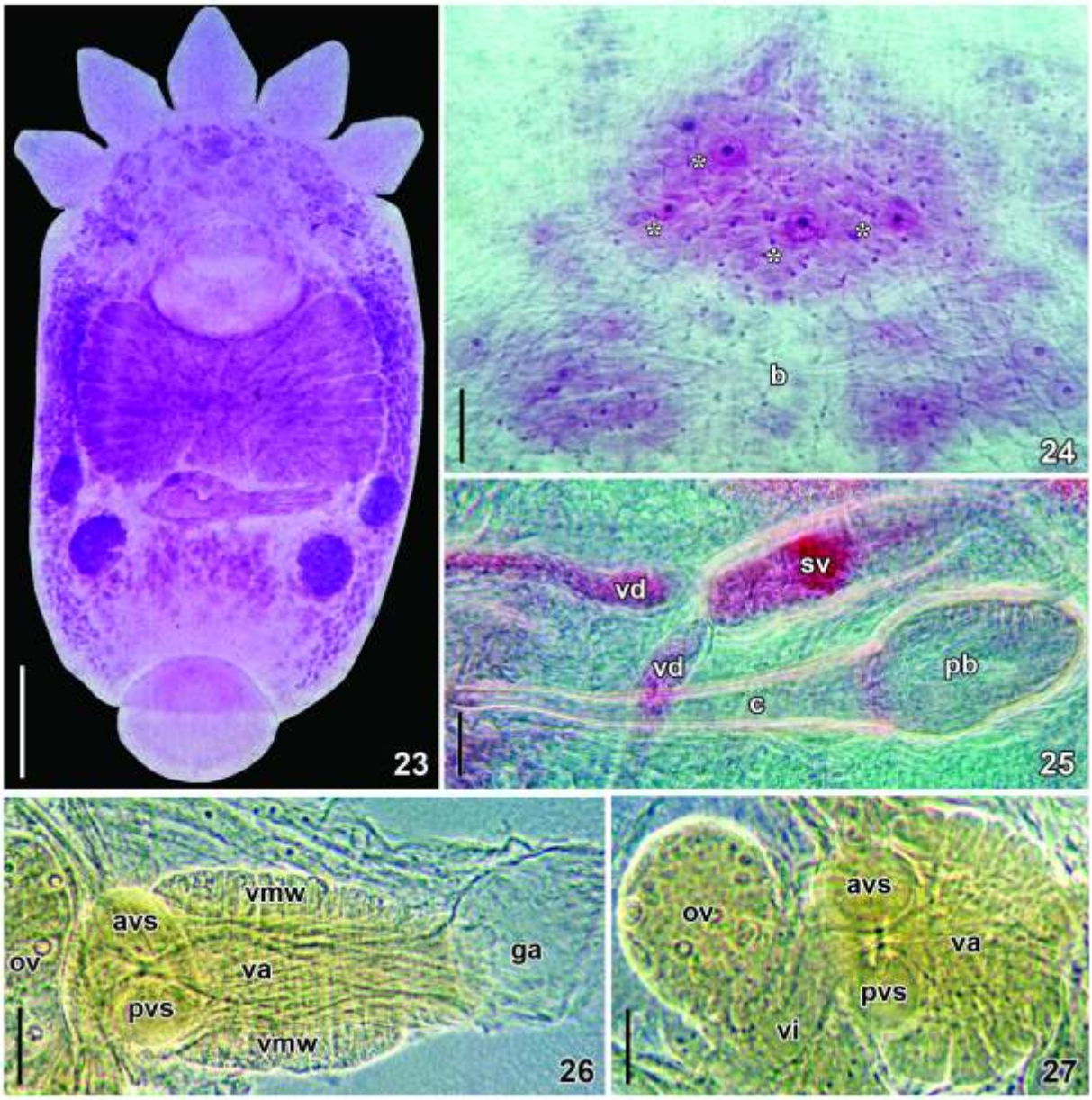
The specimens of the present work have a well-developed proximal sphincter (average diameter of 139 µm – Fig. 26), with almost identical values of the Uruguayan specimens (average diameter of 134 µm), however, what the author called distal sphincter, we believe to be the vagina wall, which in this species is highly muscular (average thickness of 42 µm). When the measurements of the vagina length between the Uruguayan specimens (average length of 177 µm) and the specimens of the present work (average length of 172.6 µm) are compared it is possible to observe that the structure is the same.



Figures 16–17. *Temnocephala pereirai* observed with SEM. 16. Entire specimen, showing the dorsolateral 'excretory' syncytial plates (DLSP) (head arrows) and position of excretory pore (ep). Scale bar = 100 µm. 17. Right DLSP and position of excretory pore (ep). Scale bar = 100 µm.



Figures 18-22. *Temnocephala pereirai* cirrus mounted in (F) and observed with DIC. 18. Cirrus, showing the proximal limit of the introvert (arrow). Scale bar = 25 µm. 19-22. Cirrus introvert observed in different focusing planes. 21. The proximal limit of the introvert (black arrow) and the beginning of the spines row (white arrow). Scale bar = 10 µm.



Figures 23-27. *Temnocephala pereirai.* 23. Juvenile specimen. Scale bar = 500 µm. 24. Brain transverse band (b) and Haswell glands (*). Scale bar = 50 µm. 25. Male reproductive system: prostatic bulb (pb), cirrus (c), vasa deferentia (vd), and seminal vesicle (sv). Scale bar = 50 µm. 26-27. Female reproductive system. 26. Anterior portion of the vaginal sphincter (avs), genital atrium (ga), ovary (ov), posterior portion of the vaginal sphincter (pvs), vagina (va), and vaginal muscular walls (vmw). Scale bar = 50µm. 27. Anterior portion of the vaginal sphincter (avs), ovary (ov), posterior portion of the vaginal sphincter (pvs), vagina (va), and vesicula intermedia (vi). Scale bar = 50 µm.

Volonterio (2010) drew and commented in the species description of *T. pereirai* about the "expansion of duct wall connecting the vesicula resorbens and the ootype". This expansion, the vesicula intermedia, is evident, having an average length of 92 µm, and probably substitutes the function of the seminal receptacles (Damborenea, 1994; Amato *et al.*, 2005). Differentiating the cirrus of *T. brevicornis* described as straight, Volonterio (2010) described the cirrus of *T. pereirai* as: "stylet is quite straight, but the distal portion of the shaft is slightly sinuous", exactly as the cirrus of the specimens studied in the present work (Fig. 18). The number of spines per longitudinal row in the introvert (16 in Uruguayan and 21 in Brazilian specimens), the length of the shaft (average length of 324 µm in Uruguayan and 322 µm in Brazilian specimens), and the length of the introvert (average length of 37 µm in Uruguayan and 34 µm in Brazilian specimens) are very close. The base diameter of the shaft in our specimens is larger (average 91 µm) than the values presented by Volonterio (2010) (average 74 µm). It is known that the base of the shaft gets thicker until it forms a rim in adult individuals. All cirri measured in the present work were micro dissected from adult individuals, with the copulatory organ well formed. Some young adults in the sample measured by Volonterio (2010) could explain the smaller values found in base diameter of the shaft in Uruguayan specimens. Although the proportion shaft length/ introvert length (one of the cirrus characters with less variation) is, practically equal, being 8.8 µm in Volonterio's specimens and 8.5 µm in the specimens of the present work.

The specimens of the present work also have very similar DLSPs when compared to those described by Volonterio (2010). The insertion of the seminal vesicle into the prostatic bulb in the Uruguayan specimens was described as subpolar or equatorial, although in *T. brevicornis*, the insertion is subpolar or polar. In the specimens of the present work, the insertion is always subpolar (Fig. 25).

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BIBLIOGRAPHIC REFERENCES

Amato, JFR, Amato, SB & Seixas, SA. 2005. Temnocephala lutzi *Monticelli (Platyhelminthes, Temnocephalida) ectosymbiont on two species of* Trichodactylus *Latreille (Crustacea, Decapoda, Trichodactylidae) from southern Brazil*. Revista Brasileira de Zoologia, vol. 22, pp. 1085-1094.

Amato, JFR, Seixas, SA & Amato, SB. 2007. *A new species of* Temnocephala *Blanchard (Platyhelminthes, Temnocephalida) ectosymbiont on creeping water bugs,* Cryphocricos granulosus *De Carlo (Hemiptera, Naucoridae) from southern Brazil*. Revista Brasileira de Zoologia, vol. 24, pp. 1043–1051.

Baer, JG. 1931. *Étude monographique du groupe des Temnocephales*. Bulletin Biologique de la France et de la Belgique, vol.1, pp. 1-57.

Brusa, F & Damborenea, MC. 2000. *First report of* Temnocephala brevicornis *Monticelli 1889 (Temnocephalidae: Platyhelminthes) in Argentina*. Memórias do Instituto Oswaldo Cruz, vol. 95, pp. 81-82.

Caballero, E & Zerecero, MC. 1951. *Presencia de* Temnocephala brevicornis *Monticelli, 1889, en crustaceos venezolanos*. Revista de Medicina Veterinaria y Parasitologia Caracas, vol. 10, pp. 111-117.

Cordero, EH. 1946. Ophiotaenia cohospes n. sp., *de la tortuga fluvial* Hydromedusa tectifera *Cope, una larva plerocercoide en el parênquima de* Temnocephala brevicornis *Mont., y su probable metamorphosis*. Comunicaciones Zoológicas del Museo de Historia Natural de Montevideo, vol. 2, pp. 1-14.

Damborenea, MC. 1994. *Temnocefalos neotropicales:* Temnocephala kingsleyae sp. n. *y* T. lutzi *Monticelli, 1913 (Platyhelminthes, Temnocephalidea) comensales de crustáceos de Brasil*. Iheringia, Série Zoologia, vol. 77, pp. 99-105.

Dioni, W. 1967. *Temnocephalas uruguayas II. Descripción de* Temnocephala talicei n. sp. *y notas sobre* T. axenos *Monticelli (Platyhelmintha)*. Physis, vol. 26, pp. 477-484.

Monticelli, FS. 1898. *Sulla* Temnocephala brevicornis *Montc. (1889) e sulle temnocefale in generale*. Bollettino della Societá dei Naturalisti in Napoli, vol. 12, pp. 72-127.

Novelli, IA, Sousa, BM, Carvalho, AR, Bessa, ECA & Souzalima, S. 2009. *Ocorrência de* Temnocephala brevicornis *Monticelli, 1889 (Platyhelminthes, Temnocephalidae) associada a* Hydromedusa maximiliani *(Mikan, 1820) (Testudines, Chelidae) em Juiz de Fora, Minas Gerais, Brasil*. Zoociências, vol. 11, pp. 175-179.

Pereira, C & Cuocolo, R. 1940. *Contribuição para o conhecimento da morfologia, bionomia e ecologia de* "Temnocephala brevicornis *Monticelli, 1889"*. Arquivos do Instituto Biológico, vol. 11, pp. 367-403.

Pereira, C & Cuocolo, R. 1941. *Estudos sobre "Temnocephalidae Monticelli, 1899", com estabelecimento de dois novos gêneros australianos e descrição de duas novas espécies neotrópicas*. Arquivos do Instituto Biológico, vol. 12, pp. 101-127.

Seixas, SA, Amato, JFR & Amato, SB. 2010. *Redescription of* Temnocephala iheringi *(Platyhelminthes: Temnocephalida) based on specimens from* Pomacea canaliculata *(Mollusca: Ampullariidae) of the state of Rio Grande do Sul, Brazil: the possible type host and type locality*. Zoologia, vol. 27, pp. 245-257.

Volonterio, O. 2010. *Two new species of* Temnocephala *(Platyhelminthes, Temnocephalida) from the South American snake-necked turtle* Hydromedusa tectifera *(Testudines, Chelidae)*. Zoological Science, vol. 27, pp. 965-970.

Yuki, VLF, Damborenea, MC & Mallman, MTO. 1993. Acanthochelys spixii *(Duméril & Bibron, 1835) (Chelidae) e* Trachemys dorbigni *(Duméril & Bibron, 1835) (Emydidae) (Testudines) como hospedeiros de* Temnocephala brevicornis *Monticelli, 1889 (Temnocephalidae) (Platyhelminthes)*. Comunicação do Museu de Ciências da PUCRS, vol. 6, pp. 75-83.

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