

First report of *Temnocephala haswelli* (Platyhelminthes: Temnocephalida) in *Pomacea canaliculata* (Mollusca: Ampullariidae) from Brazil: description update based on specimens from the state of Rio Grande do Sul, Brazil

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ABSTRACT. The impact of the discovery of *Temnocephala haswelli* Ponce de León, 1989, described as ectosymbionts of ampullariid apple snails outside of Uruguay, motivated us to collect a large number of specimens of *Pomacea canaliculata* (Lamarck, 1822) from several localities in the southern portion of the state of Rio Grande do Sul. This species was recorded three times after its description: in a study of chromosomes, in a study about the ultrastructure of the collar receptor cells, and in a study of the Haswell glands, all conducted in Uruguay. A total of 301 specimens of *P. canaliculata* were collected from 1999 to 2007. Temnocephalans found in the pallial cavity were identified as *T. haswelli*, which occurred in single infestations or concurrently with *Temnocephala iheringi* Haswell, 1893. Helminths usually showed a light-orange body pigmentation and conspicuous, intense red-eye pigment. Many taxonomic characters evidenced by several techniques were documented photographically for the first time. The typical curved cirrus, approximately 90°, typical of the species, showed some variation in the width of the shaft base, whereas the first longitudinal row of spines of the introvert appeared with shorter spines. The vagina was found to be thick-walled, but not very muscular, and to have a single, large and slightly asymmetrical sphincter, with the posterior portion of slightly larger diameter. Eggs were observed in the umbilicus and along the suture, but predominantly in the body whorl of the shell. Egg peduncles were found to be very short or, most of the time, the eggs were sessile, always with a long apical filament. The rounded shape of the dorsolateral 'excretory' syncytial epidermal plates had external margins reaching the ventrolateral region of the body and eccentric nephridiopores. This is the first record of the species outside Uruguay and in Brazil.

KEY WORDS. Ectocommensals; mollusks; Neotropical Region; South America; taxonomy.

Four species have been described as ectosymbionts of ampullariid apple snails (HASWELL 1893, PONCE DE LEÓN 1979, 1989, DAMBORENEA & BRUSA 2008). *Temnocephala iheringi* Haswell, 1893, the first of these species to be reported, was originally described from Brazil; it is the most studied species of temnocephalans from apple snails and has been recorded from different genera/species of ampullariid snails: *Pomacea lineata* (Spix in Wagner, 1827) (PEREIRA & CUOCOLO 1941), *Asolene platae* (Maton, 1811) (HYMAN 1955), *Pomacea canaliculata* (Lamarck, 1822) (DAMBORENEA 1992, DAMBORENEA & CANNON 2001), and *Pomella megastoma* (Sowerby, 1825) (DAMBORENEA *et al.* 1997), in Brazil and Argentina. Recently, SEIXAS *et al.* (2010) redescribed *T. iheringi* inferring about the type locality and the type host of this species. *Temnocephala rochensis* Ponce de León, 1979 was the second species described as an ectosymbiont of *P. canaliculata*, and, so far, has been recorded only once, from Uruguay. *Temnocephala haswelli* Ponce de León, 1989, is the third species described as ectosymbiont of *P. canaliculata*, also in Uruguay, and was recorded three times after its description in a cytogenetic study along with

T. iheringi (GONZÁLEZ *et al.* 1987), on a study about the ultrastructure of the collar receptor cells (PONCE DE LEÓN & VOLONTERIO 2003), and in a study about the Haswell glands also with *T. iheringi* (VOLONTERIO & PONCE DE LEÓN 2004), also conducted in Uruguay. The fourth species – *Temnocephala lamothei* Damborenea & Brusa, 2008 –, has been described recently as ectosymbiont of *P. megastoma*, from Argentina (DAMBORENEA & BRUSA 2008).

The present work records for the first time *T. haswelli* in Brazil and outside of Uruguay. A complete study (showing several specific characters) of this species has not yet been published. Thus, we present a complete characterization of the species following the methodology of AMATO *et al.* (2007) and SEIXAS *et al.* (2010).

MATERIAL AND METHODS

A total of 301 specimens of *P. canaliculata* were collected between 1999 and 2007, using dip nets and/or large sand sieves, and transported live to the Laboratório de Helmintologia, Universidade Federal do Rio Grande do Sul (UFRGS). Live

temnocephalans were obtained from hosts collected from: an artificial lake in the area of Parque Marinha do Brasil (30°03'34.2"S, 051°13'49.7"W); Sava Clube (30°06'09"S, 051°15'57.5"W), Lago Guaíba, and Rio Jacuí, at Ilha da Pintada (30°02'23"S, 051°25'49"W), all within the municipality of Porto Alegre; Praia Florida (30°15'54"S, 051°32'25"W), municipality of Guaíba; ditches around rice plots 5 km West of Interstate Road BR-290, near the locality of Arrozeira (30°01'36"S, 051°22'42"W), municipality of Eldorado do Sul; and Ponta do Ceroula (30°15'07.51"S, 051°16'49.55"W), municipality of Barra do Ribeiro. All localities are in the Guaíba River Basin, Lago Guaíba Sub-water Basin. Rio Cachoeira 'Barragem do Cerrito' (Tributary of Rio Maquiné), district of Barra do Ouro (29°34'15"S, 050°16'51"W), municipality of Maquiné, in the Coastal River Basin, Tramandaí Sub-water basin. All the above municipalities are in the state of Rio Grande do Sul, Brazil. These areas are included in the Atlantic Rain Forest (Brazilian Atlantic Forest Biome).

Some helminths taken from live hosts were studied and photomicrographed alive or fixed and stained according to SEIXAS *et al.* (2010) for internal morphometry. To preserve the eyespots' red pigmentation, body shape, and for the purpose of scanning electron microscopy studies (SEM), some specimens were flooded with hot, phosphate-buffered 10% formalin (HF) (AMATO *et al.* 2005, 2006). The morphology of the dorsolateral 'excretory' syncytial epidermal plates (DLSPs) and the distribution of rhabditogen and disc glands followed AMATO *et al.* (2007). Photomicrographs of hosts showing egg distribution and live helminth pigmentation were taken with a Zeiss Stemi SV-6 stereomicroscope (Figs 1-2, 4, and 5). Photomicrographs were taken with a Zeiss Axiolab microscope with phase contrast (or just the phase contrast condenser), a Leica DMR Hc microscope with Nomarski's differential interference contrast (DIC) prisms, and a Jeol (JSM-6060) SEM. Drawings were made with a drawing tube on a Nikon E-200 microscope. The photographic images and scanned line drawings were prepared using Adobe's *Photoshop CS2*® and *CorelDRAW X4*®. Measurements are in micrometers (µm) unless otherwise indicated; ranges are followed by the arithmetic mean, the number of specimens measured for a given character (when different than 30), and the standard deviation values (between parentheses). A number of internal organs were no longer measured and the terminology used followed SEIXAS *et al.* (2010). Voucher specimens fixed in AFA or silver nitrate (SN), as well as slides containing individual cirri in Faure's mounting medium (F) were deposited in the Coleção Helmintológica do Instituto Oswaldo Cruz (CHIOC), Rio de Janeiro, RJ, Brazil. Other voucher specimens stained in Delafield's hematoxylin, were deposited in the Colección de la Sección Helmintológica, División Zoología Invertebrados, Museo de La Plata (MLP), La Plata, Buenos Aires, Argentina. Some mollusk hosts were deposited in the Coleção Malacológica do Instituto Oswaldo Cruz (CMIOC). The remaining host specimens and temnocephalans are in the host and helminth collections of the UFRGS Coleção Helmintológica do Laboratório de Helmintologia, Porto Alegre, RS, Brazil.

TAXONOMY

Temnocephala haswelli Ponce de León, 1989

Figs 3-30

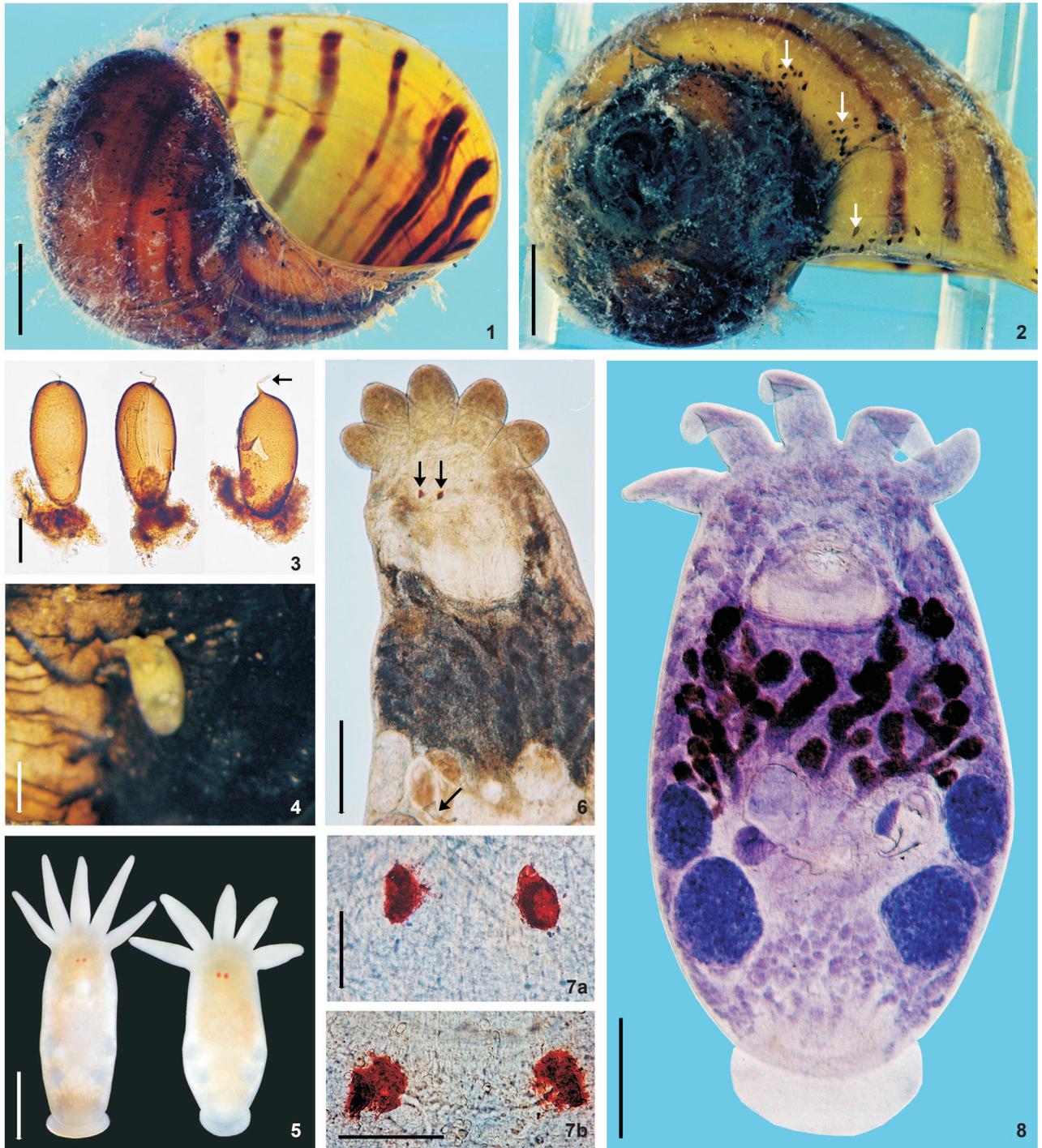
Description. Based on 218 specimens collected; 144 whole mounted adult specimens; 74 juveniles; 6 specimens mounted on stubs for SEM; 15 dissected cirri mounted in F; 12 mounted specimens fixed in SN; 30 specimens fixed in AFA under slight cover slip pressure measured:

External characteristics. Body, without tentacles (Figs 8 and 13) 1461.5-3476 (2173, 462) long, 987.5-1935.5 (1329, 224) wide; adhesive disk ventral, subterminal, partially covered by body (Figs 8 and 11-13) 350-940 (546, 115) long, 470-1150 (677, 153) wide; disc peduncle 100-350 (177, n = 26, 55) long, 190-450 (308, 80) wide; ratio between of maximum cirrus length/adhesive disk diameter 3.15: 1. Body pigmentation absent (Fig. 6) or light-orange (Fig. 4) observed in live adults and juveniles. Eyespots' red pigmentation present (observations made on live specimens and fixed in HF) (Figs 5-7a, and 7b). Two dorsolateral, rounded epidermal 'excretory' syncytial plates, slightly wider than long (Figs 16 and 17); external margin reaching ventrolateral margin of body (Fig. 18), extending from basis of first and fifth tentacles, respectively; left plate 150-340 (263, n = 12, 49) long, 170-300 (254, n = 12, 38) wide; right plate 180-360 (254, n = 12, 58) long, 190-330 (255, n = 12, 48) wide; ratio between length of DLSPs/total body length, without tentacles, 5.35: 1. Nephridiopore (excretory pore) usually central, sometimes appearing off-centered (Figs 16 and 17).

Alimentary system. Mouth surrounded by small sphincter; pharynx wider than long (Fig. 25), 257-573 (382, 156) long, 237-652 (445, 143) wide, with large sphincter; esophageal glands surrounding esophagus at base (Figs 9 and 25); intestine saccular (Figs 11 and 13), inconspicuous septations in juveniles and adults; intestinal walls thick.

Glands. Rhabditogen glands small, numerous, with granular appearance, forming bunches (average 18 cells), in lateral fields of body (Fig. 11), 37.5-80 (51, 10) in diameter, extending from sides of intestinal sac, to the anterior testes, ducts conspicuous (Fig. 11), best observed in juvenile specimens cleared in lactophenol, before complete development of vitellaria. Two groups of three Haswell glands (Fig. 13), showing little affinity with hematoxylin, in front of the brain transverse band (Fig. 13), diameter of the largest cell 50-132.5 (74, n = 29, 17). Disc glands between adhesive disc and genital complex, forming two lateral bunches extending from posterior testes to margin of adhesive disc (Fig. 12), including two pairs, of large, round, more central cells (paranephrocytes?), 37.5-87.5 (55, 12) in diameter (Fig. 12). Prostatic glands observed between anterior and posterior testes of the same side (Fig. 30); prostatic secretion observed entering prostatic bulb through the prostatic bulb wall (Fig. 29).

Reproductive system. Female. Vitellaria arborescent, completely covering intestine dorsally (Figs 8 and 13), ventral region partially covered in lateral margins; vagina short, wide,



Figures 1-9. (1-2) *Pomacea canaliculata*, showing areas of egg deposition, bars = 2 cm. (3-8) *Temnocephala haswelli*: (3) eggs, cleared and mounted in Canada balsam, showing filament (arrow), bar = 150 μ m; (4) live specimen over the mollusk tissue, bar = 500 μ m; (5) white to light-orange, showing the red eye pigmentation, bar = 500 μ m; (6) live specimen showing the red eyes pigmentation, bar = 500 μ m; (7a and 7b) the red eyes pigmentation in two different specimens, bars = 50 and 125 μ m, respectively; (8) adult specimen, pressed between slide and cover slip, stained in Delafield's hematoxylin, bar = 500 μ m.



Figures 9-10. *Temnocephala haswelli*: (9) juvenile, ventral view, showing rhabditogen glands (rg), extending along sides of intestinal sac, and its ducts entering tentacles, esophageal glands (eg), the limit between the anterior rhabditogen glands and the posterior disc glands (white arrows), and cirrus (c), bar = 250 μ m; (10) dorsal view showing posterolateral disk glands (dg), the two pairs of large disk glands (paranephrocytes?) (black head arrows), and cirrus (c), bar = 250 μ m.

thick-walled, not very muscular (Figs 14 and 27), 40-120 (85, 17) long, 40-100 (71, 15) maximum width; sphincter large, slightly asymmetrical (Figs 14 and 27) 65-107.5 (86, 11) in diameter; diameter of anterior portion 25-40 (30, 4), diameter of posterior portion 27.5-55 (39, 7); vesicula resorbens thick-walled, usually full of sperm (Figs 14 and 27), 140-400 (227, 60) long, 150-320 (234, 51) wide; wall thickness 2.5-22.5 (8, 5). Eggs sessile or with tiny peduncles (Fig. 3), fixed in umbilicus, suture, and in spire, sometimes in larger number in the body whorl of the mollusks (Figs 1 and 2), 360-430 (400, n = 10, 21) long, 170-200 (189, n = 10, 10) wide; filament long, sub-apical or apical (Fig. 3) 50-140 (97, n = 10, 32) long; peduncle, when present, 37.5-72.5 (53, n = 3, 18) long; opercular plates inconspicuous.

Male. Seminal vesicle short, wide, thin-walled (Figs 15 and 28) 122-287.5 (204, 25) long, 60-120 (86, 25) wide; wall thickness 5-17.5 (10, 4); prostatic bulb with muscular wall (Figs 15 and 28), 115-267.5 (180, 25) long, 87.5-217.5 (151, 25) wide; wall thickness 5-22.5 (11, 4); prostatic vesicle present (Figs 15 and 28). Cirrus curved (curvature approximately 90°) (Figs 15 and 19-20) 197.5-230 (215, n = 10, 12) long, shaft 140-185 (163, n = 10, 14) long, shaft base 105-145 (122, n = 10, 12) wide; introvert 45-57.5 (52, n = 10, 4) long, 20-22.5 (21, n = 10, 1) wide at base; maximum introvert width at level of swelling, 25-32.5 (27, n = 10, 2). Introvert swelling with approximately 18 longitudinal rows of long, stout spines, approximately 4 spines per row (Figs 21-24), first longitudinal row with shorter spines (Fig. 22). Ratio between total body length, without tentacles/total length of cirrus 10: 1; ratio between total length of cirrus/maximum width of shaft's base 1.7: 1; ratio between total length of cirrus/total length of introvert 4.1: 1.

Taxonomic summary

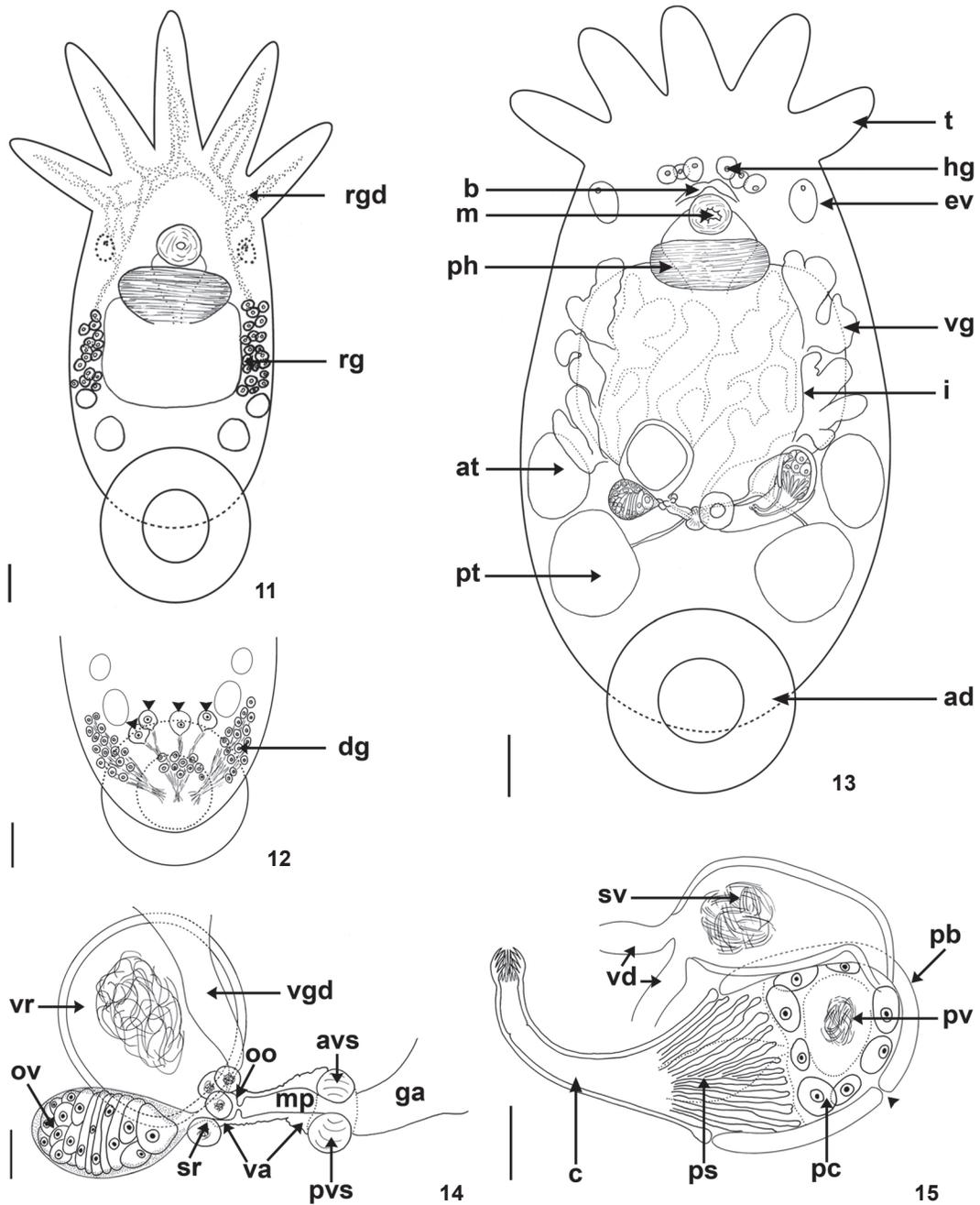
Type host and locality. *Pomacea canaliculata* (Lamarck, 1822) (Gastropoda: Caenogastropoda: Ampullariidae), Río Canelón Chico, Canelones, Uruguay (PONCE DE LEÓN 1989).

Other localities (present work). Parque Marinha do Brasil, Ilha da Pintada, and Sava Clube, Lago Guaíba, Porto Alegre, RS, Brazil; Ponta do Ceroula, Barra do Ribeiro, RS, Brazil; Praia Florida, Lago Guaíba, Guaíba, RS, Brazil; Arrozeira, Eldorado do Sul, RS, Brazil; Barra do Ouro, Maquiné, RS, Brazil.

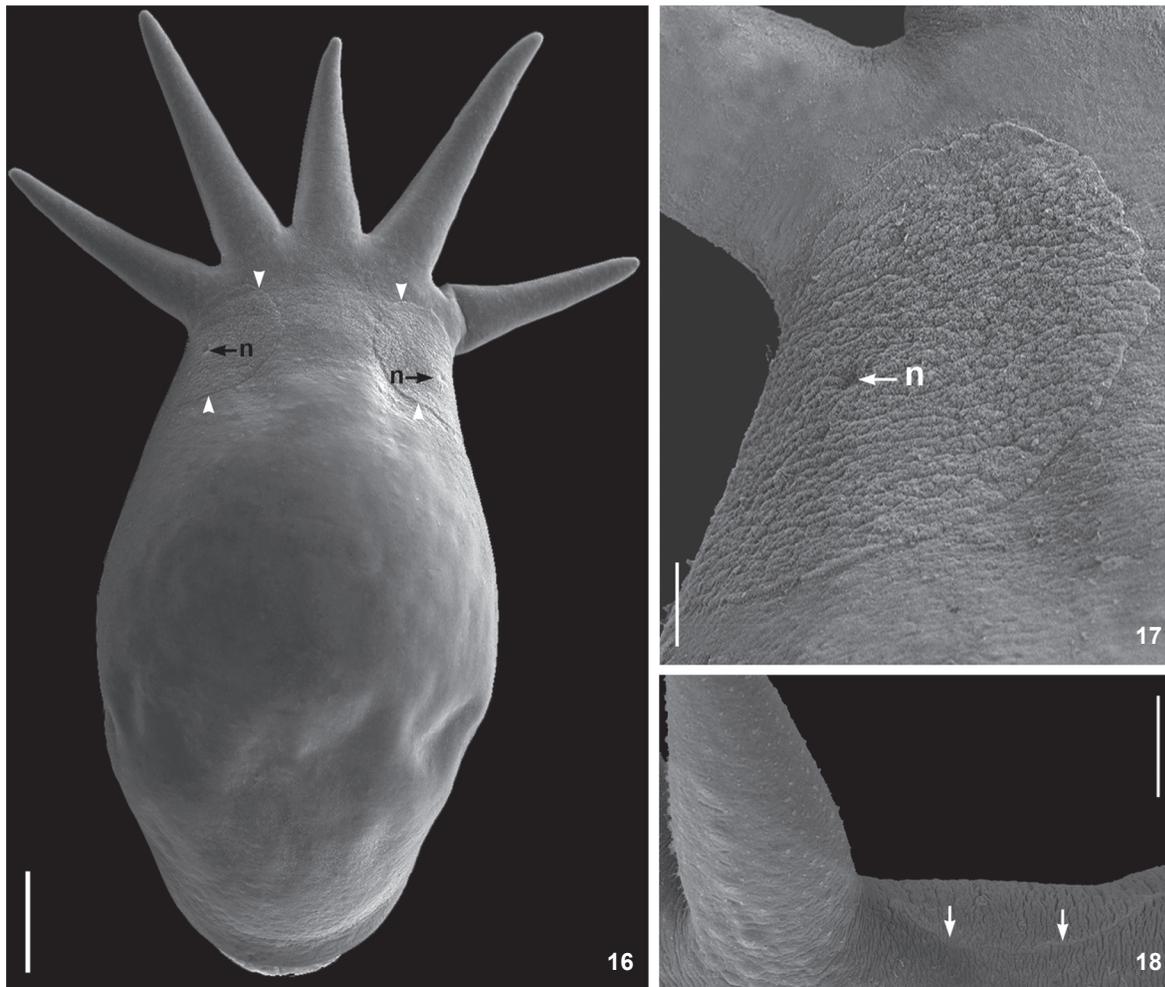
Site of infestation. Adults and juveniles were found in the mantle cavity, eggs in umbilicus, suture, and spire, sometimes in larger numbers in the body whorl of the host shell; never present on operculum.

Overall prevalence. 79.8%.

Helminth specimens deposited. CHIOC N° 37246, 37239a-d – voucher specimens fixed in AFA, stained in Delafield's hematoxylin, CHIOC N° 37243 – voucher specimens (juvenile) fixed in AFA, stained in Delafield's hematoxylin; CHIOC N° 37244a-b, 37245 – voucher specimens fixed in AFA, stained in aceto-carmine/fast green, CHIOC N° 37241 – cirrus from voucher specimen, in F, CHIOC N° 37242 – unhatched eggs. MLP N° 5921 – 5926 – voucher specimens fixed in AFA and stained in Delafield's hematoxylin.



Figures 11-15. *Temnocephala haswelli*: (11) incomplete diagram of a juvenile specimen, rhabditogen gland ducts entering the tentacles (rgd), rhabditogen glands (rg), bar = 100 μ m; (12) juvenile, posterior region showing disk glands (dg) and the two pairs of large disk glands (paranephrocytes?) (black head arrows), bar = 100 μ m; (13) incomplete diagram of adult specimen fixed with cold AFA and slight cover slip pressure showing tentacles (t), Haswell glands (hg), brain transverse band (b), mouth (m), pharynx (ph) excretory vesicles (ev), vitellaria (vg), intestinal sac (i), anterior testes (at), posterior testes (pt), adhesive disk (ad), bar = 250 μ m; (14) female reproductive complex, showing vesicula resorbens (vr), ovary (ov), seminal receptacles (sr), ootype (oo), vagina (va), distal vagina with muscular portion (mp), anterior (avs) and posterior (pvs) portions of the vaginal sphincter, genital atrium (ga), vitelline gland duct (vgd), bar = 50 μ m; (15) male reproductive complex: cirrus (c), vasa deferentia (vd), seminal vesicle (sv), prosthetic bulb (pb), prosthetic vesicle (pv), prosthetic cells (pc), prosthetic secretion (ps), and space in the wall of the prosthetic bulb (head arrow), bar = 50 μ m.

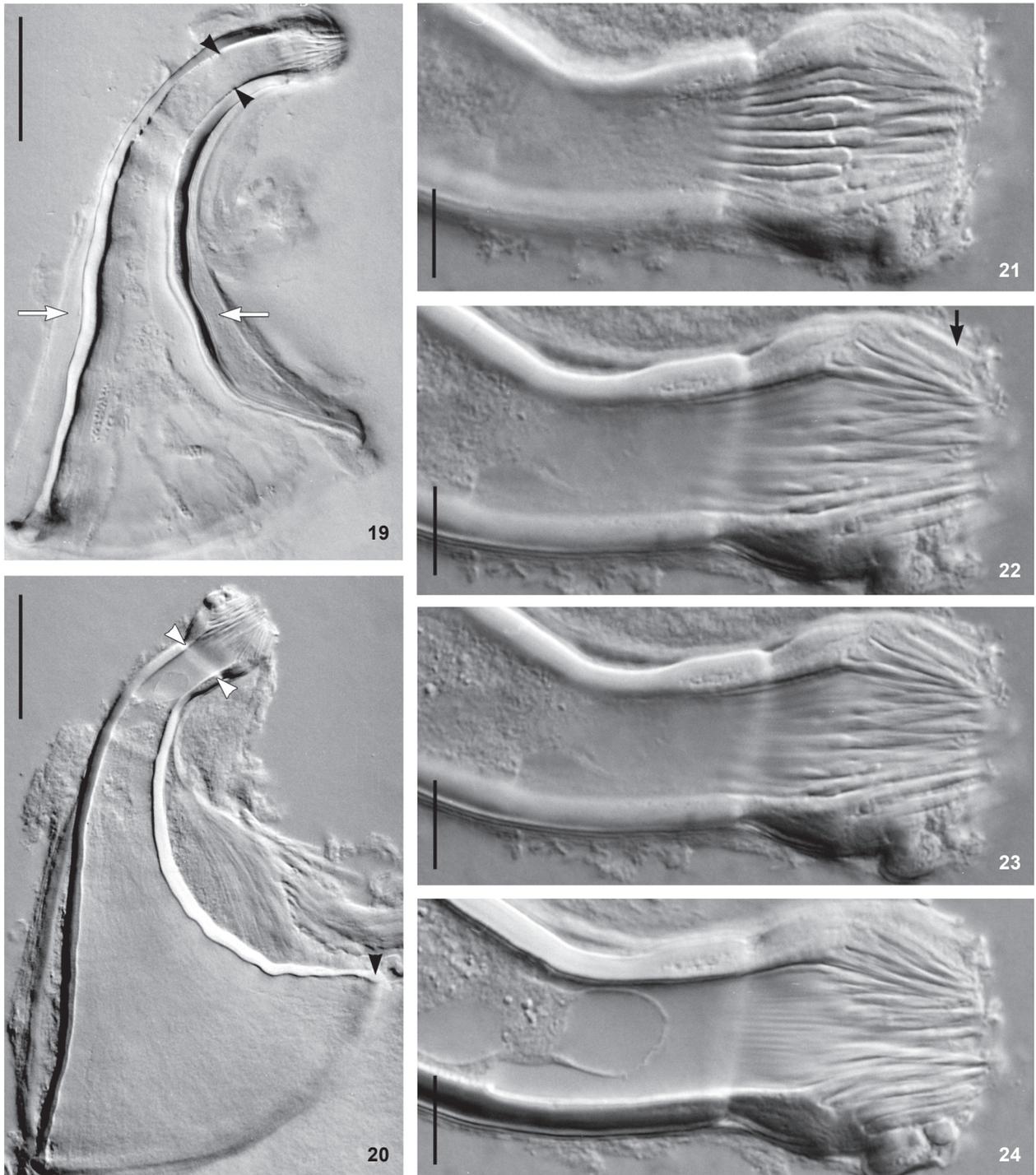


Figures 16-18. *Temnocephala haswelli* (SEM): (16) adult, dorsal view, showing dorsolateral 'excretory' syncytial plates (head arrows) and nephridiopore (n), bar = 250 μ m; (17) higher magnification of the right syncytial plate, showing the off-center nephridiopore (n), and the limits of the plate, bar = 50 μ m; (18) ventrolateral limits of the left plate (arrows), bar = 50 μ m.

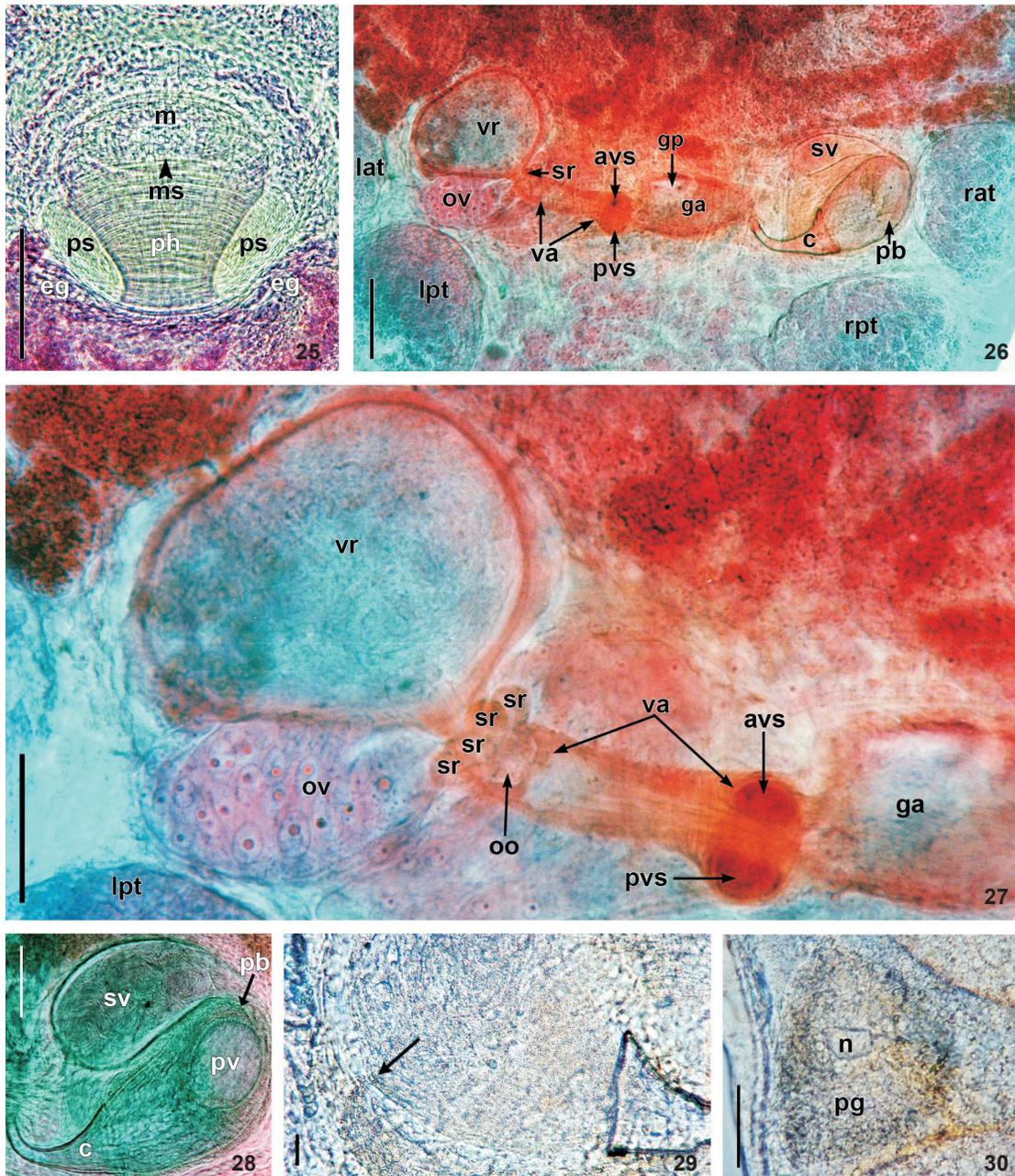
Host specimens deposited. Representative specimens of *P. canaliculata* collected at Sava Clube, Lago Guaíba, and euthanized according to the indicated procedures by malacologists, were identified using molecular methodology, as a species included in the complex *P. canaliculata*, being described by Dr Silvana A. Thiengo (pers. comm.) were deposited in the CMIOC under the number 5820.

Remarks. Like the majority of Neotropical species, *T. haswelli* has red-eye pigment, which in this species is conspicuous and intense (Figs 5-7b). The DLSP's are rounded, with external margins on the ventrolateral region of the body (Figs 16-18). The nephridiopores are eccentric and displaced to the inner region (Figs 16 and 17). As the female parts of the reproductive system of this species do not stain well with hematoxylin, measurements and photomicrographs were taken of

specimens stained with aceto-carmine/fast green (Figs 26 and 27). Ootype and vagina (Figs 14 and 27) with thick walls, not very muscular, being thicker proximally. The vaginal sphincter is more difficult to observe, in some specimens it is symmetrical, but in the majority it is slightly asymmetrical (30 μ m in average in the anterior portion, 39.5 μ m in the posterior portion), with the anterior portion thinner, showing considerable variation within the sample (25-40 μ m). Almost all eggs observed were sessile while others had minute peduncles. *Temnocephala haswelli* was found in single infestations, allowing the documentation of its eggs. The cirrus showed important intra-specific variation, on the curvature as well as on the width of the shaft's base (70-100 μ m). The size of spines in the introvert of *T. haswelli* is not so uniform; first row has smaller spines than all the rest (Figs 22, arrow).



Figures 19-24. *Temnocephala haswelli*, photomicrographs of cirri, seen with Nomarski's DIC microscopy: (19) entire cirrus, showing the proximal limit of introvert (head arrows) and the retractor muscles (arrows), bar = 50 μ m; (20) entire cirrus (another focus), the shaft rim (black head arrow) typical of adult worms, and the proximal limit of the introvert's inflation (white head arrows), bar = 50 μ m; (21-24) photomicrographs selected from a series showing the introvert seen in different focusing planes, from the top of the internal surface to mid-section, bars = 10 μ m.



Figures 25-30. *Temnocephala haswelli*, selected characters in the internal anatomy: (25) mouth (m), mouth sphincter (ms), pharynx (ph), pharyngeal sphincter (ps), esophageal glands (eg), bar = 250 μ m; (26) reproductive zone: left anterior testis (lat), left posterior testis (lpt), right posterior testis (rpt), right anterior testis (rat), genital pore (gp), genital atrium (ga), cirrus (c), prostatic bulb (pb), seminal vesicle (sv), ovary (ov), vagina (va), anterior (avs) and posterior (pvs) portions of the circular vaginal sphincter, seminal receptacles (sr), vesicula resorbens (vr), bar = 125 μ m; (27) partial female reproductive complex: vesicula resorbens (vr), ovary (ov), seminal receptacles (sr), ootype (oo), vagina (va), anterior (avs) and posterior (pvs) portions of the circular vaginal sphincter, genital atrium (ga), and left posterior testis (lpt), bar = 100 μ m; (28) partial male reproductive complex: seminal vesicle (sv), prostatic bulb (pb), prostatic vesicle with spermatozooids (pv), cirrus (c), bar = 50 μ m; (29) prostatic bulb with prostatic secretion flowing in through spaces in the wall of the prostatic bulb (arrow), bar = 50 μ m; (30) prostatic gland (pg) with its nucleus (n), between left anterior testis (lat) and left posterior testis (lpt), bar = 50 μ m.

DISCUSSION

Temnocephala haswelli was described based on 23 specimens collected from Río Canelón Chico, in Uruguay (PONCE DE LEÓN 1989). Three papers published about *T. haswelli* also included information on *T. iheringi* (GONZÁLEZ *et al.* 1987, PONCE DE LEÓN & VOLONTERIO 2003, VOLONTERIO & PONCE DE LEÓN 2004). PONCE DE LEÓN (1989) described the body pigmentation as a variation from white to yellow, similar to that reported in the present work, in which this variation went from white to light-orange. There are differences in body size (without tentacles) in relation to the specimens of the present work when compared to the original description, but despite being of smaller size the specimens from Brazil (2.17 mm in Brazil and 3.82 mm in Uruguay) have the length as well as the shape of the cirri similar to those characteristic of the species (average length of cirri: 215 µm in Brazil and 200 µm in Uruguay). The red-eye pigment is conspicuous and intense in *T. haswelli* (Figs 5-7a, and 7b), whereas the vitellaria are very characteristic, which, according to PONCE DE LEÓN (1989), is arborescent with long branches which do not exceed 4-8 in number in the dorsal side of the body, agreeing with the observations made in the present work (Fig. 8), the vitellaria have, in average, four branches, with two more limited branches to the margins of the ventral region. Staining with aceto-carminé/fast green the cement glandular become evident around the genital pore, as PONCE DE LEÓN (1989), pointed out in the original description.

The eggs of *T. haswelli* were well studied herein (measurements, shape, filament position, and opercular plates) because we were able to collect mono-specific infestations of *P. canaliculata* with this species, although the opercular plates were sometimes difficult to see in all eggs. Measurements showed considerable differences when compared to those of the original description. PONCE DE LEÓN (1989) measured eggs of the interior of the "ootype" (= genital atrium) in his studies, but this method is likely to give inaccurate measurements. The measurement presented as being of the peduncle, is probably a measurement of the sub-apical or apical filament. This becomes clear when the average length of the peduncle (115 µm) is compared to the average of the length of the filament in Brazilian specimens (97.5 µm), which are quite similar. These values contrast with the average length of peduncles (53.3 µm), which, besides being minute, are only present in 30% of the eggs observed in the present study, as they are largely sessile. Although the original description indicates the limits of the rhabditogen glands and disk glands, two pairs of the large disk glands (paranephrocytes?) are shown for the first time in the present work (Fig. 12).

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