

# A new species of *Temnocephala* Blanchard (Platyhelminthes, Temnocephalida) ectosymbiont on *Trichodactylus fluviatilis* Latreille (Crustacea, Decapoda, Trichodactylidae) from southern Brazil <sup>1</sup>

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**ABSTRACT.** *Temnocephala trapeziformis* **sp. nov.**, ectosymbiont on *Trichodactylus fluviatilis* Latreille, 1828 is described from the State of Rio Grande do Sul, southern Brazil. Sixty-eight crabs were examined, of which 61 (89.7 %) were positive for this species of the genus *Temnocephala* Blanchard, 1849. Eggs, as well as adult and young specimens, were found on the pleural areas of the carapace, in the orbital cavities, and, usually, on the fourth pair of pereopods. The temnocephalans were always devoid of body pigmentation, although they kept the red eye pigment, undiluted in specimens fixed by hot (90°C) phosphate buffered 10% formalin. The most distinctive characters of the new species are: 1) the shape and size of the cirrus and the characteristics of its introvert section; 2) the trapezoidal shape of the dorsolateral post-tentacular 'excretory' epidermal syncytial plates, with the nephridiopore in the lower inner corner of the plate; and 3) the number, size, and distribution of the rhabdite producing glands, as observed in very young specimens.

**KEY WORDS.** Ectocommensals; Neotropical region; Rio Grande do Sul; South America; taxonomy.

**RESUMO.** Nova espécie de *Temnocephala* Blanchard ectosimbionte sobre *Trichodactylus fluviatilis* Latreille (Crustacea, Decapoda, Trichodactylidae) da região Sul do Brasil. *Temnocephala trapeziformis* **sp. nov.**, ectosimbionte sobre *Trichodactylus fluviatilis* Latreille, 1828 é descrita para o Estado do Rio Grande do Sul, região sul do Brasil. Sessenta e oito caranguejos foram examinados, sendo que 61 (89,7 %) estavam positivos para esta espécie do gênero *Temnocephala* Blanchard, 1849. Posturas estavam localizadas, sobre as pleuras da carapaça, nas cavidades orbitais e no quarto par de pereiópodos, locais onde também foram encontrados espécimes jovens e adultos. Os temnocefalídeos sempre se apresentaram sem pigmento corporal, embora o pigmento vermelho dos olhos tenha sido preservado nos espécimes fixados em formalina 10% fosfato tamponada (90°C). Os caracteres mais distintos da nova espécie são: 1) a forma e o tamanho do cirro e as características do 'introvert', 2) as placas sinciciais 'excretoras' trapeziformes, com o nefrídíoporo sempre deslocado para o canto inferior interno de cada placa, e 3) o número, o tamanho e a distribuição das glândulas produtoras de rabdites, observadas em espécimes muito jovens.

**PALAVRAS-CHAVE.** Ectocomensais; região Neotropical; Rio Grande do Sul; América do Sul; taxonomia.

Six species of the genus *Temnocephala* Blanchard, 1849 were described from hosts in the family Trichodactylidae H. Milne-Edwards, 1853. Four of them were described originally from Brazil: *Temnocephala microdactyla* Monticelli, 1903, *Temnocephala lutzi* Monticelli, 1913, *Temnocephala travassosfilhoi* Pereira & Cuocolo, 1941, and *Temnocephala lanei* Pereira & Cuocolo, 1941. Two more were described from Argentina: *Temnocephala pignalberiae* Dioni, 1967 and *Temnocephala santafesina* Dioni, 1967.

MONTICELLI (1903) described *T. microdactyla* epizoic on *Trichodactylus (Dilocarcinus) orbicularis* Meuschen, 1781, today *Dilocarcinus pagei* Stimpson, 1861, from the State of Mato Grosso,

Brazil, and in 1913, described *T. lutzi* epizoic on "*Telphusa* sp. from São Paulo, Brazil". The validity of the genus *Telphusa* Chambers, 1872 has been discussed by AMATO *et al.* (2005) with the conclusion that, possibly, the type host of *T. lutzi* is one of the species of *Trichodactylus* occurring in the State of São Paulo.

PEREIRA & CUOCOLO (1941) presented one of the most detailed accounts of the Temnocephalidae Monticelli, 1899 and considered meager the descriptions given by MONTICELLI (1903, 1913), as the author did not indicated the specific characters, in particular the male reproductive system, on which he based his brief descriptions. PEREIRA & CUOCOLO (1941) re-described *T.*

*microdactyla* ectocommensal on *Trichodactylus* (*D.*) *pictus* (H. Milne-Edwards, 1853) (currently a junior synonym of *Sylviocarcinus australis* Magalhães & Türkay, 1996) and *T. lutzi* from *Trichodactylus petropolitanus* (Göldi, 1886), both from the same localities indicated by MONTICELLI (1903, 1913). In the same paper, these authors described two new species: *T. travassosfilhoi*, from *T. petropolitanus* collected in Serra da Cantareira and São Bernardo do Campo, greater São Paulo and *T. lanei*, from an unidentified species of *Trichodactylus* collected in the Municipality of Juquiá (Fazenda Poço Grande), State of São Paulo, Brazil.

DIONI (1967) revising specimens of trichodactylids collected in Argentina deposited in the collection of the Instituto Nacional de Limnología, La Plata, Provincia de Buenos Aires, recorded *T. microdactyla* for the first time in Argentina, on *T. pictus* and described two new species: *T. pignalberiae* and *T. santafesina*, from *T. orbicularis*, *T. pictus*, and from an unidentified species of the genus *Trichodactylus*.

DAMBORENEA (1994), referred the presence of *T. lutzi* epibiont on *Sylviocarcinus pictus* (H. Milne-Edwards, 1853) collected in the State of Amazonas, Brazil. Finally, AMATO *et al.* (2005) recorded the presence of *T. lutzi* in southern Brazil, over two species of *Trichodactylus*, *Trichodactylus panoplus* (von Martens, 1869) and *Trichodactylus fluviatilis* Latreille, 1828 (new host records – NHR), being this last species also the host for a new species described in the present paper. The latter authors not only described the specimens but made the first extensive photographic documentation of the species, specially the reproductive system, and the dorsolateral, epidermal ‘excretory’ syncytial plates (DLSPs) using special techniques described in Material and Methods.

DAMBORENEA & CANNON (2001) presented a comprehensive account of the Neotropical temnocephalans, including pictures of the cirrus in de Faure’s mounting medium (deF) for the following species: *Temnocephala chilensis* (Moquin-Tandon, 1846), *T. microdactyla*, *Temnocephala talicei* Dioni, 1967, *Temnocephala decarloi* Moretto, 1978, *Temnocephala iheringi* Haswell, 1893, *Temnocephala digitata* Monticelli, 1902, and *T. pignalberiae*; drawing the DLSPs for the following species: *T. microdactyla*, *T. chilensis*, *T. talicei*, *T. decarloi*, *T. iheringi*, *T. digitata*, and *T. pignalberiae*.

*Temnocephala cyanoglandula* Amato, Amato & Daudt, 2003 epibiont on *Aegla serrana* Buckup & Rossi, 1977; *T. lutzi* recorded for the State of Rio Grande do Sul, epibiont on *T. panoplus* and *T. fluviatilis* (AMATO *et al.* 2005); and *Temnocephala curvicirri* Amato & Amato, 2005 epibiont on two species of *Belostoma*, are the Neotropical species for which full descriptions/illustrations are known. The present paper adds one more epibiont species on trichodactylids, object of a complete documentation study.

## MATERIAL AND METHODS

Collections extended from 2000 and 2004. Sixty-eight crabs were collected with dip nets and/or large sand sieves, and transported live to the Laboratório de Helminologia, Universidade Federal do Rio Grande do Sul (UFRGS). Live temnocephalans were obtained from each host specimens collected

from several localities: Arroio Água Parada (type locality) (29°66’20”S, 050°21’15”W), Arroio Carvão (29°32’29”S, 050°13’49”W), and Arroio Forqueta (29°32’17”S, 050°14’44”W), Maquiné basin, all in the Municipality of Maquiné; Vale das Trutas, head waters of Rio das Antas (28°47’00”S, 049°50’53”W), Taquari-Antas basin, Municipality of São José dos Ausentes. All in the State of Rio Grande do Sul, Brazil.

Some helminths from live hosts were fixed in cold A.F.A. under slight cover slip pressure, stained in Delafield’s hematoxylin, cleared in cedar oil, and mounted in Canada balsam, for internal morphometry (AMATO & AMATO 2005). The morphology of the DLSPs was studied in live specimens fixed with silver nitrate (SN) (ROMEIS 1968, JOFFE *et al.* 1995). Some specimens were flooded with hot formalin (HF) for the preservation of the red eye pigmentation, for SEM, and for the preservation of the body shape (AMATO *et al.* 2005). Cirrus morphology was studied after micro dissection and mounting each individual cirrus in deF (CANNON & SEWELL 1995). The cirrus measurements follow AMATO *et al.* (2005). Groups of unhatched eggs, removed by scrapping the pleural sides of the carapace and the mero and carpo segments of the fourth pair of pereopods, were dehydrated, cleared in cedar oil, and mounted in Canada balsam.

Photomicrographs were taken with a Zeiss Axiolab microscope equipped with phase contrast (or just the phase contrast condenser) and/or with a Leica DMR Hc microscope and Nomarski’s differential interference contrast (DIC) prisms. Measurements are in micrometers (µm) unless otherwise indicated; ranges are followed by the mean, the number of specimens measured for a given character (when different than 15), and the standard deviation values (between parentheses). The terminology to describe the male reproductive structures follows CANNON (1993), CANNON & SEWELL (1995), and SEWELL & CANNON (1998). Drawings were made with a drawing tube on a Leitz Dialux 20-EB microscope.

The holotype and some paratypes fixed in HF and cold A.F.A., as well as slides containing individual cirri in deF and unhatched eggs, was deposited in the Coleção Helminológica do Instituto Oswaldo Cruz (CHIOC), Rio de Janeiro, Brazil; paratypes were also deposited in the Instituto de Pesquisas da Amazônia, Manaus, Amazonas, Brazil. The crab hosts were deposited in the Coleção de Crustáceos, Laboratório de Crustáceos, Departamento de Zoologia, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Brazil.

## RESULTS

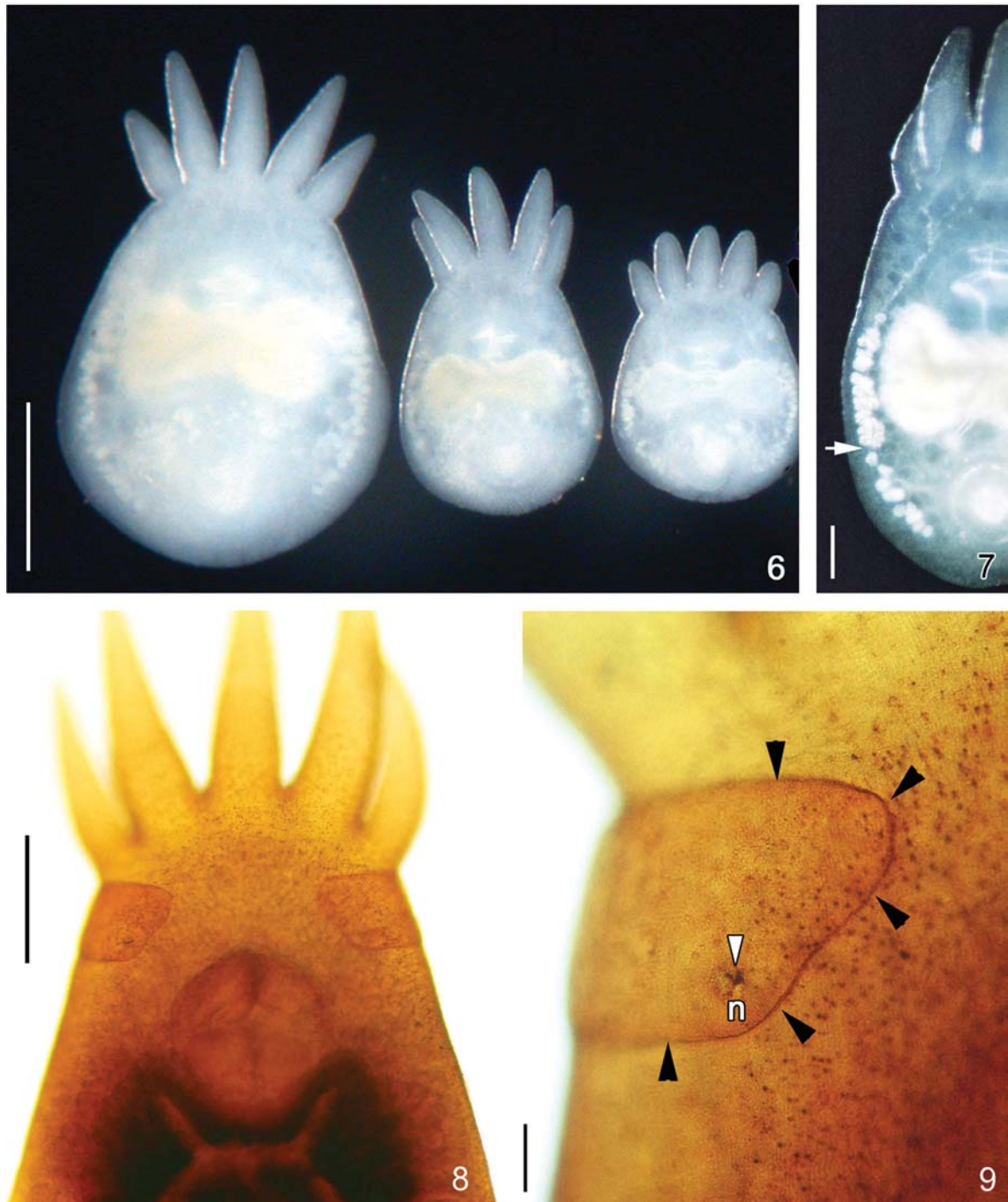
### *Temnocephala trapeziformis* sp. nov.

Figs 4b-29

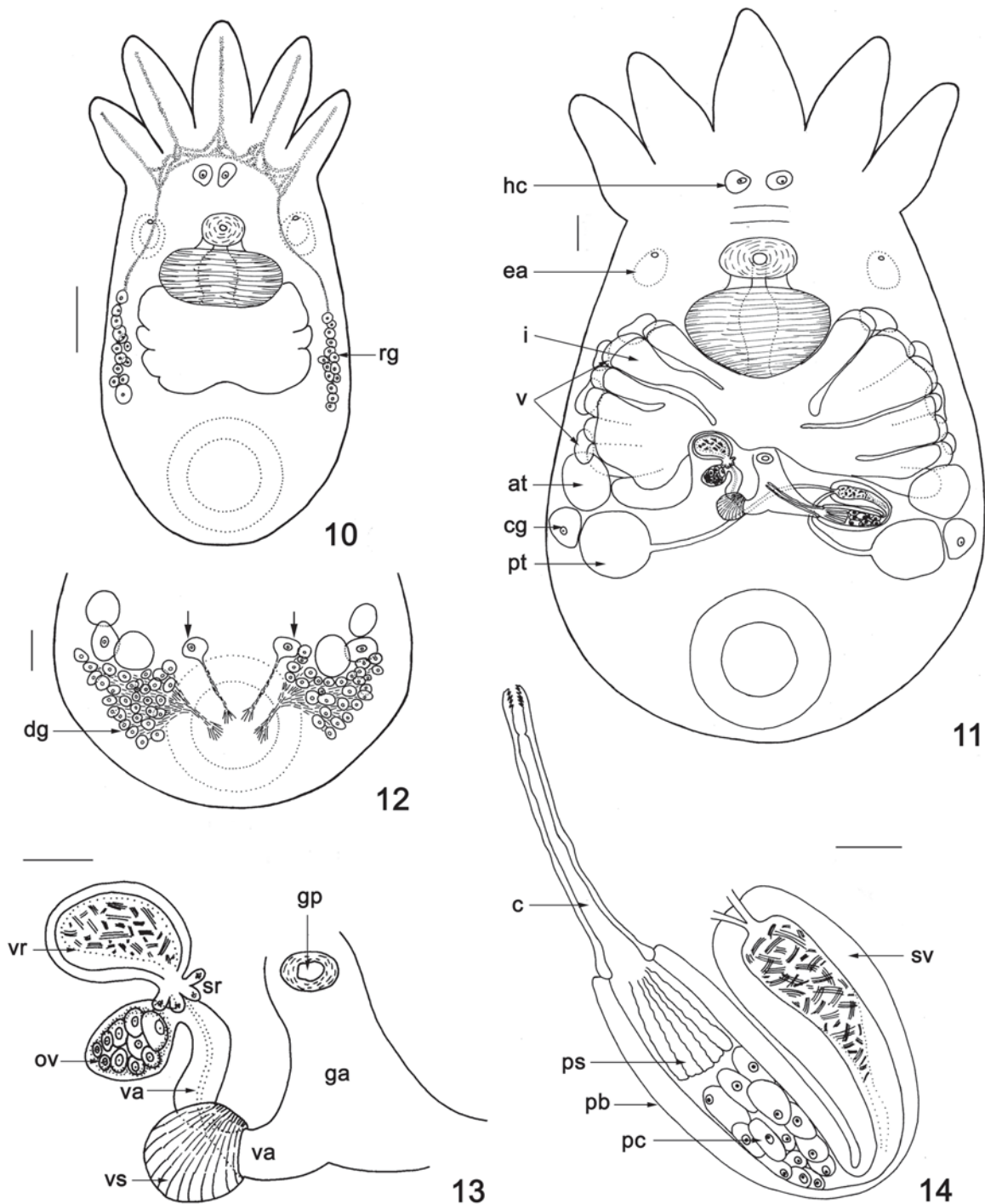
Description (based on: 417 specimens collected; 129 adult whole-mounted specimens (killed in cold A.F.A., under slight cover slip pressure); 135 juveniles; 7 specimens mounted on stubs for SEM; 11 cirri mounted in deF; 4 mounted specimens (fixed with SN); 177 mounted specimens (fixed with HF); 15 measured.



Figures 1-5. (1-4) *Trichodactylus fluviatilis*: (1) specimen in dorsal view, bar = 10 mm; (2) specimen in ventral view showing patches of hatched eggs (arrows), bar = 10 mm; (3) eggs deposited in the orbital cavity, bar = 1 mm; (4a) pleural side of carapace showing mostly unhatched eggs (black arrows) and hatched eggs (white arrow), bar = 3 mm; (4b) unhatched eggs cleared in cedar oil, showing shape, width and length of peduncles, and filament (head arrows), bar = 200  $\mu$ m; (5) *Temnocephala trapeziformis* sp. nov., adult specimen (holotype), bar = 500  $\mu$ m.



Figures 6-9. *Temnocephala trapeziformis* sp. nov.: (6) specimens showing the wide posterior portion of the body, typical shape when fixed with hot formalin, bar = 1 mm; (7) specimen cleared with lactophenol, with arrow showing the limit between the anterior rhabdite secreting glands and the posterior disc glands, bar = 200  $\mu$ m; (8) specimen stained by silver nitrate, dorsal view, showing the bilateral trapezoidal dorsolateral 'excretory' syncytial plates, bar = 200  $\mu$ m; (9) specimen stained by silver nitrate, dorsal view, showing the left trapezoidal dorsolateral 'excretory' epidermal syncytial plate (black head arrows) and nephridiopore in the inner, lower posterior corner of the syncytial plate (n, white head arrow), bar = 125  $\mu$ m.



Figures 10-14. *Temnocephala trapeziformis* sp. nov.: (10) partial diagram of a very young specimen, dorsal view, showing the rhabdite secreting glands (rg), bar = 100  $\mu$ m; (11) partial diagram of an adult specimen, ventral view, showing the Haswell cells (hc), excretory ampullae (ea), vitellaria (v), intestinal sac (i), anterior testis (at), cement gland (cg), and posterior testis (pt), bar = 100  $\mu$ m; (12) specimen in dorsal view showing disc glands (dg) and the pair of large glands (arrows), bar = 100  $\mu$ m; (13) female reproductive organs: ovary (ov), vagina (va), vaginal sphincter (vs), genital atrium (ga), vesicula resorbens (vr), and genital pore (gp), bar = 50  $\mu$ m; (14) male genitalia: cirrus (c), prostatic bulb (pb), prostatic secretion (ps), prostatic cells (pc), seminal vesicle (sv), bar = 50  $\mu$ m.

External characteristics. Body length (without tentacles) 1.42-2.17 mm (1.77 mm, 182); 1.11-2.05 mm (1.43 mm, 273); adhesive disk sub-ventral, 395-750 (517, 96) in diameter.

Epidermal mosaic (demonstrated through staining with SN) with two trapezoidal DLSPs with more or less pronounced angular corners (Figs 8 and 15), DLSPs with slight intraspecific variation (Figs 9 and 16-18), extending from just below the base of first and fifth tentacles, respectively; left plate 120-130 (125, n = 2, 7) long, 140-150 (145, n = 2, 7); right plate 130 long (n = 1), 150 wide (n = 1); length of the DLSPs/total body length, without tentacles, relationship 10: 1. Excretory pore (nephridiopore) inside the area of each DLSP, displaced to the lower inner corner (Figs 9, 16-18).

Alimentary system. Mouth surrounded by a muscular sphincter, between first and second thirds of body; pharynx wider than long (Fig. 28), 276-474 (362, 53) long, 336-790 (532, 120) wide, with a large sphincter; esophageal glands surrounding it at base (Fig. 28); intestine saccular, with conspicuous septations in young and in adults; intestinal walls thick (Fig. 11).

Excretory system. Excretory ampullae, round, at level of mouth (Fig. 11), generally directed outwards while the nephridiopore (Fig. 9) is inner lower corner of the syncytial plates.

Glands. Rhabdite producing glands large, numerous, forming bunches, in lateral fields of the body; extending on the sides of the intestinal sac, with inconspicuous ducts, best observed in very young specimens still without vitellaria (Figs 7 and 10). Two large, irregular shaped Haswell cells (Fig. 11), showing little affinity with hematoxylin, in front of the eyespots and the brain transverse band; left cell in pair 63-113 (91, n = 5, 21) across, right cell 68-130 (97, n = 5, 25) across. Disc glands (Fig. 12) between adhesive disc and genital complex, forming bunches extending from the end of the intestinal sac to anterior border of the adhesive disc, including to two, large, lobed, more central cells (Figs 12 and 29), 78-130 (101, n = 4, 22) long.

Reproductive system. Female. Gonopore between anterior and middle thirds of the body (Fig. 13); ovary pyriform, 50-98 (72, n = 13, 14) long, 65-113 (87, n = 13, 17) wide (Figs 13 and 29); four seminal receptacles present (Fig. 13); vitellaria covering dorsal and ventral sides of the intestinal sac (Figs 11 and 27); genital atrium spacious, elongate (Figs 13 and 26), vagina with large muscular sphincter, (Figs 13, 26 and 29) 88-190 (133, n = 10, 32) long, muscular sphincter 65-137.5 (91, n = 14, 23) long, 57.5-125 (81, n = 14, 18) wide, opening in front of the cirrus' introvert (Figs 13, 26 and 29); vesicula resorbens thin walled (Fig. 13), 48-138 (87, n = 11, 33) long, 88-220 (135, n = 11, 40) wide, indenting intestinal sac and vitellaria, posteriorly. Eggs with medium size peduncles (Fig. 4), deposited over the pleural areas of the carapace (Fig. 4), in the orbital cavities (Fig. 3), and on the ventral side of the fourth pair of pereopods (Fig. 2), 430-460 (453, n = 6, 12) long, 260-280 (270, n = 6, 9) wide, peduncles 110-290 (230, n = 5, 67) long; and a filament displaced to the side (Fig. 4).

Male. Testes four; anterior and posterior testes of different sizes, oblique, oval; anterior testes sometimes slightly lobed;

posterior testes always more voluminous; right anterior testis 120-200 (161, 24) long; 70-170 (128, 27) wide; right posterior testis round, 140-230 (194, 27) long, 130-250 (186, 37) wide; left anterior testis oval, 120-190 (154, 21) long, 60-160 (125, 25) wide; left posterior testis round to oval, 150-290 (198, 34) long, 120-290 (196, 40) wide; vasa deferentia wide, uniting to large, pyriform, thick-walled, seminal vesicle (Figs 14 and 29), 120-190 (147, n = 14, 26) long, 43-100 (73, n = 14, 15) wide; prostatic bulb (Figs 14 and 29) 98-248 (154, 37) long, 50-78 (66, 7) wide, cirrus median, slightly curved, frequently with crooked lines near the shafts base (Figs 14, 19 and 24), 163-195 (182, n = 7, 10) long, shaft 128-168 (149, n = 7, 13) long; 38-53 (46, n = 7, 5) wide at base; maximum introvert width at level of swelling, 13-23 (18, n = 7, 3), introvert's swelling portion 18-23 (20, n = 7, 2) long, observed in four different focusing planes with the Nomarski's (DIC) microscopy (Figs 20-23). Proximal limit of introvert marked with a narrowing of the lumen's diameter, and is seen from the side as two fine spikes (Fig. 19). Base of cirrus shaft with thick borders in adult specimens (as growth of cirrus starts from distal extremity) showing growth is complete (Fig. 19 and 24). Ratio between total length of cirrus and maximum width of shaft's base 4: 1; ratio between total length of cirrus and total length of introvert 5.5: 1. Introvert spines large, in approximately, 14 longitudinal rows of 10 spines each (Figs 20-23).

Type host. *Trichodactylus fluviatilis* Latreille, 1828.

Site. External surface of body, including the orbital cavities; eggs fixed over pleural sides of the carapace, orbital cavities, and mostly on the fourth pair of pereopods.

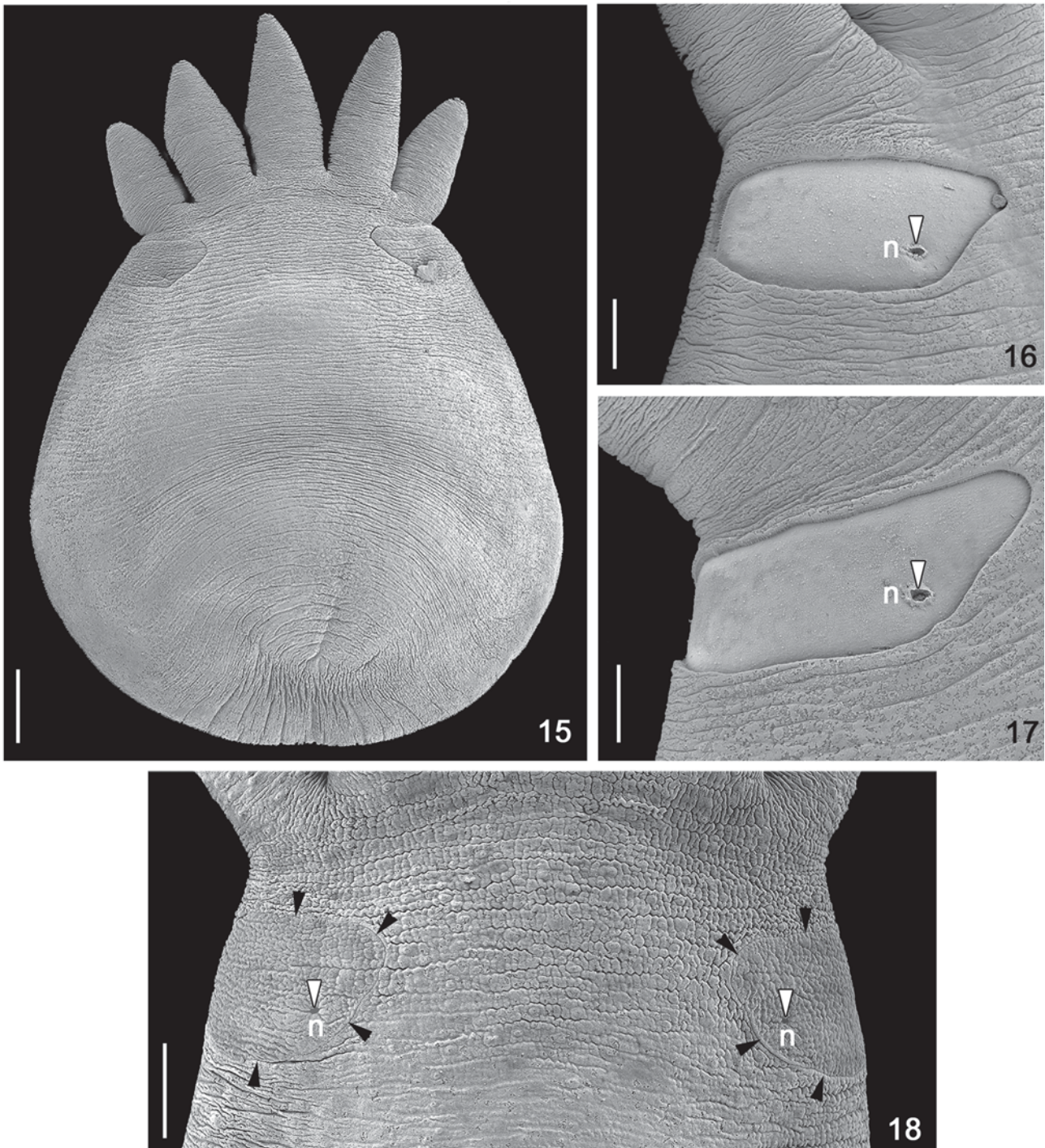
Type locality. Arroio Água Parada (29°66'20"S, 050°21'15"W), Maquiné basin, Municipality of Maquiné, State of Rio Grande do Sul.

Other localities. Arroio Carvão (29°32'29"S, 050°13'49"W) and Arroio Forqueta (29°32'17"S, 050°14'44"W), Maquiné basin, Municipality of Maquiné, State of Rio Grande do Sul; Vale das Trutas, head waters of Rio das Antas (28°47'00"S, 049°50'53"W), Taquari-Antas basin, Municipality of São José dos Ausentes, State of Rio Grande do Sul.

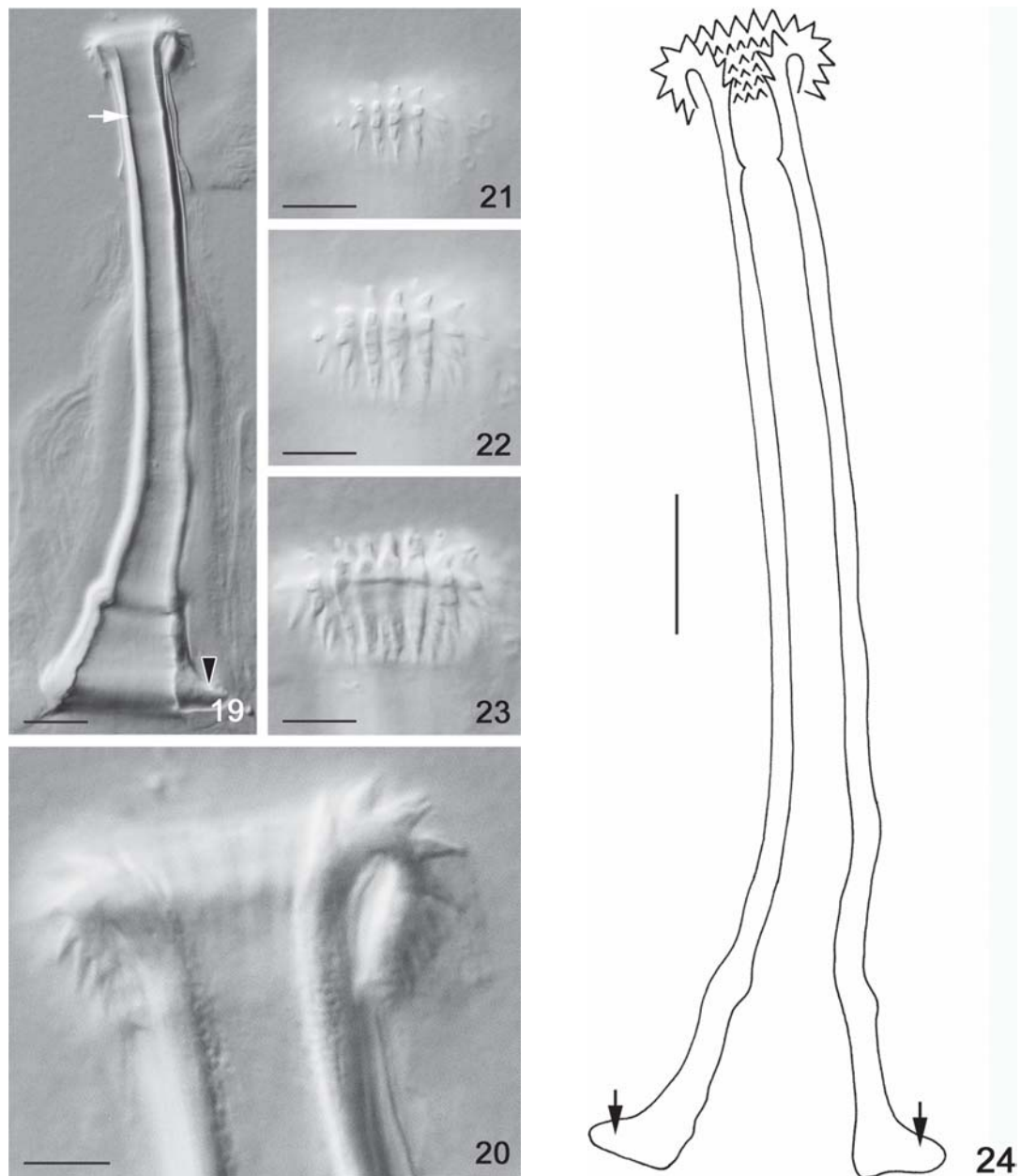
Helminth specimens deposited. CHIOC N° 36.617 – holotype; CHIOC N°s 36.618, 36.619, 36.620, 36.621 – paratypes, CHIOC N° 36.622 – paratype fixed in SN, CHIOC N° 36.623 – cirrus from paratype, in deF, and CHIOC N° 36.624 – unhatched eggs. INPA N°s 451, 471a and b, 486 – paratypes, INPA N° 487 – cirrus from paratype, in deF, and 488 – unhatched eggs.

Host specimens deposited. CCDZ-UFRGS – Arroio Carvão (middle stretch) N°s 3863-3867; Arroio Carvão (lower stretch) N°s 3868-3872; Arroio Carvão N°s 3887-3892; Vale das Trutas, head waters of Rio das Antas N°s 3965-3966; Arroio Água Parada N°s 3967-3974; Arroio Forqueta 3873-3875.

Etymology. (Gr. *trapézion*: small table + *-formis*: Latin suffix – with the shape of a small table – diminutive of *trapéza*). Adjective biform, of the third declension. *Temnocephala trapeziformis* sp. nov. The specific epithet *trapeziformis* was given because the DLSPs have the typical shape of a trapezium.



Figures 15-18. *Temnocephala trapeziformis* sp. nov., seen with SEM: (15) entire specimen showing the trapezoidal dorsolateral 'excretory' syncytial plates and the wide posterior region of the body, bar = 100 µm; (16-17) the DLSPs, seen in different specimens showing an artifact of technique in which the epidermis inside the syncytial plate was lost (sloughed off), bars = 50 µm; (18) the syncytial plates (head arrows) and nephridiopores in the typical location (white head arrow – n) and a slight variation in their shape, bar = 50 µm.



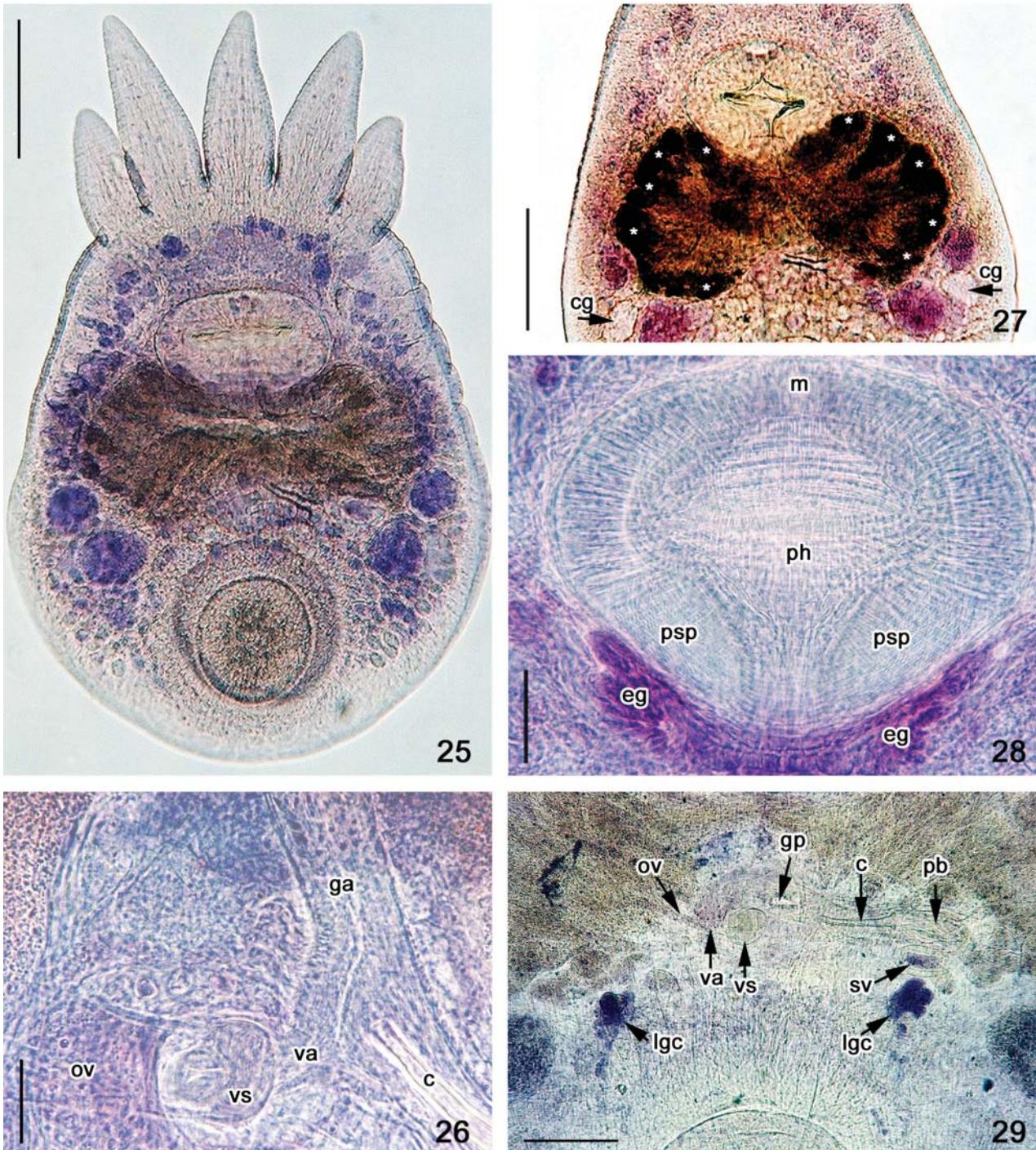
Figures 19-24. *Temnocephala trapeziformis* sp. nov. (19-23) Photomicrographs of a cirrus, seen with Nomarski's DIC microscopy: (19) entire cirrus, showing the proximal limit of the introvert (white arrow), black headed arrow shows the wider base of the shaft, indicating that the cirrus has finished growing, bar = 20  $\mu$ m; (20) cirrus introvert showing how spines are inserted laterally (lateral focus), bar = 10  $\mu$ m; (21-23) cirrus introvert showing the number of spines along a longitudinal row with focus on the lower wall, bar = 10  $\mu$ m; (24) cirrus (arrows indicate the wider base of the shaft), bar = 20  $\mu$ m.

## DISCUSSION

*Temnocephala trapeziformis* sp. nov. is the second species described from trichodactylid hosts in which the special techniques were used to allow a clearer study of the structure of the

cirrus introvert and the shape of the DLSPs. The new species can only be compared, as far as the shape of the DLSPs is concerned, with *T. lutzi* which has elliptical DLSPs. The syncytial plates of *T. trapeziformis* sp. nov., being trapezoidal, are much different than the plates of the seven Neotropical species





Figures 25-29. *Temnocephala trapeziformis* sp. nov., selected characters in the internal anatomy: (25) young specimen, fixed with HF, bar = 200  $\mu$ m; (26) partial female reproductive apparatus (phase contrast), genital atrium (ga), ovary (ov), vaginal sphincter (vs), cirrus (c), bar = 50  $\mu$ m; (27) specimen with the typical vitellaria folding over sides of the intestinal sac (stars) and large, intertesticular cement glands (cg), bar = 200  $\mu$ m; (28) mouth (m), pharynx (ph), pharyngeal sphincter (psp), and esophageal glands (eg), bar = 100  $\mu$ m. (29) reproductive area: cirrus (c), genital pore (gp), lobated, large glandular disc cells (lgc), ovary (ov), prostatic bulb (pb), seminal vesicle (sv), vagina (va), vaginal sphincter (vs), bar = 200  $\mu$ m.

showed by DAMBORENEA & CANNON (2001) and the three species described from aeglid anomuran crustaceans, belostomatid hemipterans, and trichodactylids of Rio Grande do Sul (AMATO *et al.* 2003, 2005, AMATO & AMATO 2005). These records for Rio Grande do Sul are the only papers which can be compared to the present report for having used the same techniques to show the DLSPs and mounting cirri laying flat in deF mounting medium, and only *T. lutzi* has been recorded on trichodactylid crabs but it shows very distinct characters when compared to what is now observed in *T. trapeziformis* **sp. nov.**

Figures 16 and 17, in addition to show the typical trapezoidal shape of the DLSPs also show an accidental loss, occurred during SEM sample preparation, (shlough off) of the plate's epithelium along the "interepithelial groove at the epidermal surface" as pointed out by WILLIAMS (1982) when studying with SEM the epidermal topography of *Temnohaswellia novaezealandiae* (Haswell 1887) Pereira & Cuocolo, 1941 (= *Temnocephala novaezealandiae*) and other Australasian temnocephalans. The loss of the epidermal surface promoted the visualization of the basal membrane.

Among the species of *Temnocephala* so far described from trichodactylids, *T. microdactyla* is the only species comparable to the new species by the cirrus redescription given by PEREIRA & CUOCOLO (1941): "Cirro quitinoso, longo e fino, arqueado, tendo a concavidade olhando para a frente e para dentro; não apresenta estrutura com espículos quitinosos em sua porção distal. Mede de 0,33 a 0,36 mm de comprimento."

MONTICELLI (1903), when describing *T. microdactyla* did not mention spines in the introvert, what made PEREIRA & CUOCOLO (1941) to describe and illustrate the cirrus of this species without such spines. DIONI (1967) and DAMBORENEA (1992) must have followed PEREIRA & CUOCOLO (1941) as they also did not describe nor illustrate spines in the introvert. *Temnocephala trapeziformis* **sp. nov.** has a smaller cirrus than *T. microdactyla*, with a length variation of 16 to 19  $\mu\text{m}$ , and robust spines in the introvert (Figs 19-23), and, although being slightly curved the cirrus does not have a scythe or sickle shape.

Another well distinct character is the voluminous vaginal sphincter present in the new species (Figs 13 and 26) measuring from 65 to 130  $\mu\text{m}$  in diameter. PEREIRA & CUOCOLO (1941), described the sphincter of *T. microdactyla* as: "... apresentando distalmente um poderoso esfíncter terminal, provido internamente de uma estrutura quitinosa e dentilhada.". These authors did not provide measurements for the vaginal sphincter of *T. microdactyla* what does not allow a better comparison, but certainly the new species does not have the 'chitinous tooth-like structures'. A peculiarity of the new species is the size of the cement glands (Figs 11 and 27), located, as usually, between the testes of both sides of the body. In the other species studied by the same methods these glands are not so large and conspicuous as they are in *T. trapeziformis* **sp. nov.**, in which they measure 6 to 17  $\mu\text{m}$  in diameter.

## ACKNOWLEDGEMENTS

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