New species of *Temnocephala* Blanchard (Platyhelminthes, Temnocephalida) ectosymbiont on giant water bugs, *Belostoma* spp. (Hemiptera, Belostomatidae) from southern Brazil  

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ABSTRACT. A new species of *Temnocephala* Blanchard, 1849 is described from southern Brazil, ectosymbiont on giant water bugs (aquatic hemipterans), *Belostoma* spp. from the greater Porto Alegre, State of Rio Grande do Sul. Some hemipterans examined were positive for this species of *Temnocephala* and carried bunches of eggs between the first two pairs of legs and between the first pair of legs and the rostrum, as well as adult and young specimens. The adults were darkly pigmented (greenish-brown), mimetizing the color of the hemipteran hosts. The new species is similar to *Temnocephala decarloi* Moretto, 1978, from Argentina, the only other species of *Temnocephala* from belostomatid hemipterans known to date, by having an extra long and curved cirrus, but differs by having the distal portion of the introvert not bent as a ‘full-bent’ smoking pipe-like, as the author depicted originally for *T. decarloi*.

KEY WORDS. Ectocommensals, Neotropical, Rio Grande do Sul, South America, taxonomy.
The present report describes a new species of Temnocephala from three species of Belostoma, as well as documents photographically and through illustrations, for the first time the: 1) areas of egg deposition on the host, shape and color of the eggs; 2) morphology of the young, and live body pigmentation; 3) cirrus structure as revealed by de Faure's mounting medium preparations, Nomarski's differential interference contrast and phase contrast microscopy; and 4) paired dorso-lateral, post-tentacular 'excretory' syncytial plates as revealed by silver nitrate staining and scanning electron microscopy.

MATERIAL AND METHODS

Live temnocephalans were obtained from belostomatid hemipterans collected from a canal located 5 km West of Interstate Road BR-290, locality of Arrozeira, Municipality of Eldorado do Sul (30°01’36”S, 051°22’42”W) and from Lago Tarumã, Municipality of Viamão (30°04’14”S, 051°01’20”W), State of Rio Grande do Sul. Collections of hemipterans extended from 1998 to 2003. Insects were collected with dip nets or large sand sieves, and transported live to the laboratory. Some helminths from live hosts were fixed in cold A.F.A. (70ºGL ethanol – 93 parts; formalin 37º – 5 parts, glacial acetic acid – 2 parts), under slight cover slip pressure, stained in Delafield's hematoxylin (HUMASON 1972), cleared in cedar oil and mounted in Canada balsam. Photomicrographs were taken with a Zeiss Axiolab photographic images and scanned line drawings were prepared using Adobe's Photoshop®. Measurements are in micrometers (µm) unless otherwise indicated; ranges are followed (between parentheses) by the mean, the number of specimens measured for a given character (when different than 11) and the standard deviation values. Measurements of cirrus length were made along the outside of the shaft wall inside the introvert swelling; measurements of total cirrus length were made along its longest side and include the introvert; measurements of width of the introvert's base include the thickness of the shaft walls but exclude the introvert swelling; measurement of the width of the introvert swelling were taken at the widest point; all cirrus measurements were taken from cirri extracted from the specimens by micro dissection and mounted individually, laying flat, in deF. The prostatic bulb measurements include the muscular walls. 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 Helminthológica do Instituto Oswaldo Cruz (CHIOC), Rio de Janeiro, RJ, Brazil.

RESULTS

Temnocephala curvicirri sp. nov.

Figs 4-35

Description (based on: 167 specimens collected; 11 adult whole-mounted specimens; 49 juveniles; four specimens mounted on stubs for SEM; 11 cirri mounted in deF; nine mounted specimens, fixed in SN, two specimens embedded in paraffin for sectioning); 11 specimens killed in cold A.F.A. under slight cover slip pressure, measured:

External characteristics. Body length (without tentacles) 1.73-3.14 mm (2.16 mm, 459); 1.02-2.26 mm (1.43 mm, 355); adhesive disk sub-ventral, pedunculate, 329-696 (492, n = 9, 108) in diameter, much narrower than total body width; live body pigmentation present (Fig. 5), greenish-brown, well pronounced in adult specimens. Recently hatched and juvenile specimens still not pigmented. Eyespots round, with red pigmentation in live specimens (Fig. 7); pigment dissolving if specimens are fixed in ethanol, but not in formalin.

Epidermal mosaic (demonstrated through staining with SN) with two dorso-lateral, post tentacular ‘excretory’ syncytial plates (Figs 9, 15-17 – head arrows), ‘excretory’ syncytial plates, extending from the base of first and fifth tentacles, respectively; left plate 422-566 (492, n = 4, 76) long, right plate 444-555 (519, n = 4, 52) long; length of ‘excretory’ syncytial plates/total body length, without tentacles, relationship 3.33:1. Excretory pore (nephridiopore) inside the area of each ‘excretory’ syncytial plate, at the widest point, displaced toward the inner side of the plate (Figs 9, 15-17).

Alimentary system. Mouth surrounded by a small muscular sphincter (Fig. 7), between first and second thirds of body; pharynx wider than long (Figs 10 and 11), 137-322 (203, n = 7, 64) long, 282-661 (445, n = 7, 133) wide, with a large sphincter; esophageal glands surrounding it at base (Fig. 33 eg); in-
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Figures 1-6. (1-3 and 5) Belostomatid hemipteran hosts of *Temnocephala curvicirri* sp. nov.: (1) *Belostoma dentatum*, ventral view, showing darkly pigmented *temnocephalans* on the ventral surface of the abdomen and areas of egg deposition, bar = 10 mm; (2-3) *Belostoma dilatatum*, ventral view, showing the egg bunches of *temnocephalans*, bars = 2 mm; (4) unhatched eggs, showing the smooth anti-peduncular pole and the sub-terminal filament (arrow), bar = 250 µm; (5) *B. dilatatum*, live adult specimen showing the greenish-brown body pigment and red pigment of eyespots, bar = 2 mm; (6) adult specimen of *T. curvicirri* sp. nov. killed in hot formalin, without compression, seen under partial phase contrast illumination, bar = 500 µm.

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testine saccular, with conspicuous septations in young and in adults; intestinal walls thick (Fig. 33 is).

Excretory system. Excretory ampullae, round, at level of mouth (Figs 10-11), generally directed outwards while the nephridiopore is off-center in the anterior, wider portion of the ‘excretory’ syncytial plates.
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Figures 10-14. *Temnocephala curvicirri* sp. nov.: (10) very young specimen showing the rhabdite producing glands (rg), extending along the sides of intestinal sac, still uncovered by vitellaria, and its ducts entering tentacles, bar = 100 µm; (11) incomplete diagram of an adult specimen (holotype) killed with cold A.F.A and slight cover slip pressure, bar = 500µm; (12) adhesive disc glands, including the pair of larger glands between testes (arrows), bar = 200 µm; (13) female reproductive complex, inside genital capsule (gc), showing vesicula resorbens (vr), ovary (ov), vagina (va), cirrus (ci) entering the vagina, ‘muscular plug’ (mp) of the cirrus sheath and genital atrium (ga) leading to the genital pore (gp), bar = 200 µm; (14) prostatic bulb receiving, laterally, the ejaculatory duct: prostatic cells (pc), prostatic secretion (ps), prostatic bulb muscular wall (pw), seminal vesicle (sv), ejaculatory duct (ej), proximal portion of cirrus shaft (pcs), bar = 100 µm.
Glands. Rhabdite producing glands large, numerous, forming bunches of 19-26 (24) glands, in lateral fields; extending along the length of the intestinal sac, best observed in very young specimens (Figs 8 and 10); rhabdite tracts clearly visible, uniting just before entering tentacles (Fig. 10).

Cyanophilous glands inconspicuous, probably separated from each other, not forming two, lateral, 'grape-like' bunches. Two Haswell's cells irregular shaped, showing little affinity with hematoxylin, in front of the eyespots and the brain transverse band (Figs 11 and 29 hc, arrows); left cell in pair 82-128 (104,
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n = 9, 16) across, right cell 55-125 (90, n = 9, 19) across. Esophageal glands inconspicuous; staining reddish-purple; forming a collar between pharynx and intestinal sac (Fig. 33 eg). Ootype glands conspicuous. Disc glands between adhesive disc and genital complex, forming two, lateral bunches extending from mid-level of posterior testes to anterior border of adhesive disc uniting to two, large, more central cells (Fig. 12 arrows). Shell glands between testes of the same side, difficult to see.

Reproductive system. Female. Gonopore between middle and posterior thirds of body (Fig. 7 arrow); ovary pyriform, 103-210 (140, n = 11, 26) long, 80-137 (96, n = 11, 18) wide (Figs 13 and 31); seminal receptacles inconspicuous; vitellaria, extending laterally beyond the limits of intestinal sac and touching sides of body (even in unflattened specimens), superimposing the rhabdite producing glands, and having a unique shape which varies from individual to individual and from dorsal to ventral sides of the body (Figs 6, 7 and 28); genital atrium spacious, elongate (Figs 13 and 31), vagina long (Figs 13 and 32 va), with a well developed sphincter at the proximal end, opening in front of cirrus introvert; vesicula resorbens (Figs 13, 31 and 33 vr), with thick wall, 183-375 (277, n = 6, 71) long, 229-403 (299, n = 6, 65) wide, indenting intestinal sac and vitellaria, posteriorly. Eggs with long peduncles, deposited in groups under the rostrum, between the coxopodites of the first and the second pair of legs (Figs 1, 2 and 3), always deposited on the ventral side of body, anti-peduncular pole smooth, but with filament sub-terminal (Fig. 4 - arrow), 524-638 (569, n = 9, 34) long, 273-319 (296, n = 9, 19) wide, peduncles 34-55 (47, n = 10, 8) long, 2.7 wide.

Male. Testes four, usually rounded, slightly oblique; right anterior testis round, 183-458 (283, n = 10, 82) long; 92-302 (232, n = 10, 57) wide; right posterior testis round to oval, 256-549 (357, n = 10, 88) long, 211-376 (291, n = 10, 53) wide; left anterior testis round, 192-376 (272, n = 10, 65) long, 183-366 (249, n = 10, 56) wide; left posterior testis irregularly oval, 274-550 (360, n = 10, 85) long, 210-366 (269, n = 10, 54) wide; both deferent vessels unite in large, pyriform, thick-walled, seminal vesicle, 394-421 (407, n = 2, 20) long, 62-110 (88, n = 10, 17) wide; genital capsule present, observed in sagittal section (Fig. 33 gc); prostatic bulb (Figs 14 and 30) with thick, muscular walls, 211-284 (238, n = 5, 27) long, 101-128 (112, n = 5, 10) wide, enclosed as well as the cirrus, in a muscular ‘sleeve-like’ tissue, sometimes showing folds; proximal portion with few, large cells and prostate secretion in the distal portion; prostate bulb receives the ejaculatory duct on either side (Fig. 14); cirrus when viewed from top, apparently straight, curved in lateral view, 953-1062 (1003, n = 4, 54) long, shaft 659-889 (799, n = 4, 98) long, shaft base 61-84 (72, n = 4; 10) wide (Fig. 18); introvert never observed more everted than is shown in figures 22-27, 132-164 (155, n = 4, 20) wide at base; maximum introvert width at level of swelling, 25-32 (27, n = 4, 3) long, observed in progressive focusing planes with the Nomarski’s DIC microscopy (Figs 22-27). In transversal section, the introvert’s swelling shows approximately 55 longitudinal rows of fine spines and approximately 55 spines per row; each row can be seen as teeth of a comb, with the ‘handle’ positioned backwards (Fig. 20). Each spine in the perimeter of the swelling portion is approximately 7 µm long. Bottom of the introvert’s swelling portion receives a group of larger spines which are placed circularly on the inner wall. Among these are four still wider, ‘blade-like’ spines placed in the cardinal positions (Figs 19, 20 and 24-26 arrows) and resting on the wall of the introvert. Proximal limit of introvert marked with a narrowing of the lumen’s diameter, and is seen from the side as two fine spines (Figs 19 and 20 arrows). Ratio between total length of cirrus and maximum width of shaft’s base 12.6-15.4; ratio between total length of cirrus and total length of introvert 6.5-7.2.

Synonym. T. decarloi of Vianna & de Mello (2002).

Type host. Belostoma dilatatum (Dufour, 1863).

Other hosts. B. denatum (Mayr, 1863), B. elegans (Mayr, 1871), B. testaceopallidum Latreille, 1807 (State of Minas Gerais).

Figures 20-21. (20) Temnocephala curvicirri sp. nov.: line drawing of the cirrus’ introvert, (arrows indicate proximal limit of introvert), bar = 30 µm; (21) cirrus and prostatic bulb of T. decarloi, redrawn from Moretto’s original description: (21a) cirrus and prostatic bulb, bar = 100 µm; (21b) the ‘full-bent’ smoking pipe-like distal portion of the introvert, bar = 50 µm.
Site. Ventral side of body; eggs located under the rostrum and between the coxopodites of the first and second pairs of legs.

Type locality. Lago Tarumã, Viamão, Rio Grande do Sul (30°04′14″S, 051°01′20″W).

Other localities. Stream and canal (30°01′36″S, 051°22′42″W), on the road to the town of Arrozeira, Municipality of Eldorado do Sul, Rio Grande do Sul (2 km to the North of Interstate Road BR-290), Brazil; Retiro das Pedras, Brumadinho, Minas Gerais.

Deposited specimens. CHIOC # 36461 – holotype fixed in AFA, CHIOC # 36462a - paratype fixed in AFA, CHIOC # 36462b-c - paratypes fixed in HF, # 36462d - paratype fixed in SN, CHIOC # 36462e – cirrus from paratype, in deF; # 36462f – hatched eggs, # 36462g – unhatched eggs (all from type host specimen SBA-1727); # 36463 – cirrus from paratype, in deF, from host SBA-1630; # 36464 – paratype fixed in AFA, from host SBA-1120; # 36465 - paratype fixed in AFA, from host SBA-1150.

Figures 22-27. *Temnocephala curvicirri* sp. nov. optical frontal sections of the cirrus’ introvert swelling portion, as seen in different focusing planes, from the external surface to the mid-section, with the Nomarski’s DIC microscopy. Arrows in figures 24-26 indicate one of the four large, cardinal spines (arrows). Bar = 10 µm, valid to all figures.
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Figures 28-32. *Temnocephala curvicirri* sp. nov.: (28) photomicrograph of the holotype, ventral view, killed by cold A.F.A. under slight cover slip pressure, bar = 1 mm; (29) anterior region of a specimen killed by HF, showing the rhabdite tracts and the two Haswell’s cells (arrows - hc), bar = 250 µm; (30) entire cirrus inside the ‘sleeve-like’ sheath (cs) entering the vagina (va), cirrus introvert (civ), prostatic bulb (pb) showing the large prostatic cells, prostatic secretion (ps), seminal vesicle (sv), genital atrium (ga), vitellaria (v), and anterior testis (at), bar = 250 µm; (31) female reproductive complex and the distal portion of the cirrus entering the vagina. Vagina with large sphincter (cv) in the direction of the ootype, ovary (ov), vitellaria (v), vesicula resorbens (vr), genital atrium (ga), ‘muscular plug’ (mp) entering the vagina (va), cirrus introvert (civ), seminal receptacles? (sr?), and egg (e), bar = 250 µm; (32) higher magnification of the introvert’s swelling portion (ci), ‘muscular plug’ (mp) entering the vagina (va), and the genital atrium (ga), bar = 50 µm.
Etymology. (L. curvus, m. curved + cirrus – the copulatory structure). Compound name in the genitive. *Temnocephala curvicirri* sp. nov. having the long cirrus in the shape of a bow. The specific epithet *curvicirri* was given because the tip of the cirrus’ introvert is not bent (as in a full-bent smoking pipe), as is the cirrus in *T. decarloi* from Argentina (Figs 21a and 21b).

Remarks. *Temnocephala curvicirri* sp. nov. is described from southern Brazil, epizoic on several species of giant water bugs of the genus *Belostoma*. Many species of this genus occur together in the same localities. Within the genus *Belostoma*, some species are large: *B. dentatum* (30 specimens examined – two specimens positive); *B. dilatatum* (10 specimens examined – six specimens positive), and *B. elongatum* (30 specimens examined – all negative) while other species are small: *B. elegans* (93 specimens examined – one specimen positive) for *T. curvicirri* sp. nov.

Although Vianna & de Melo (2002) described in detail the hemipterans and the characteristics of the streams they surveyed, did not describe the only adult and the two young temnocephalan specimens found, nor presented illustrations (drawings and/or photomicrographs), which could ensure the correct species identification of both species of temnocephalans. These authors must have used their own work (Nieser & de Melo 1997) to identify the hemipterans. Vianna & de Melo (2002) wrote: “the species diagnose is based on the penis morphology which
is a long and sigmoid structure”. But the cirrus (not penis) is not “sigmoid”. Figure 21a-b show that the morphology of the cirrus of *T. decarloi*, described by MORETTO (1978) and the cirrus in the Brazilian specimens (Fig. 20) have different shapes, thus not warranting using the name *T. decarloi* for the specimens reported by VIANNA & DE MELO (2002) from the State of Minas Gerais. After seen pictures of the adult specimen from *B. testaceopallidum*, identified as *T. decarloi*, we saw that the cirrus was exactly like the one being now described for *T. curvicirri* sp. nov., i.e., it is not bent as it is shown in figure 21a-b.

The cirrus in *T. curvicirri* sp. nov., as well as the prostatic bulb, are enclosed in a sleeve-like tissue which is highly muscular at the distal end. This ‘muscular plug’ covers the cirrus’ extremity as it enters the vagina. Figures 31 and 32 show it advancing in front of the spiny, swollen section of the cirrus, as if it was ‘devised’ to ‘protect’ the vagina. In some specimens with the cirrus still not penetrating the vagina this ‘muscular plug’ is found displaced to the side. These peculiarities might end up being common to *T. decarloi*.

DAMBORENEA & CANNON (2001) mentioned that the prostatic bulb, in the Neotropical temnocephalans, always receives the ejaculatory duct on the proximal end while in the Australian species the prostatic bulb receives the ejaculatory duct laterally. In this respect (Fig. 14) the new species and, probably *T. decarloi*, are more like the Australian species.

At present, with the impossibility of examining specimens from Argentina (being used by Dr M. C. Damborenea to redescribe *T. decarloi* – Pers. Comm.) and with the impossibility of examining the only adult specimen collected in the State of Minas Gerais (sent to Dr Walter Dioni, in México – Pers. Comm. by Dr A. L. De Melo), one has only to wait.

The present work with the temnocephalans of *Belostoma* spp. has shown us along the years that temnocephalans, in general, should always be collected from live hosts and should be killed/fixated preferentially by HF. A number of specimens pressed between slide and cover slip should be immediately separated for internal morphometry. The specimens killed with HF but not pressed (Fig. 6) will not allow the observation of various female characters. Besides, it is also necessary to kill live specimens in SN to determine the shape of the ‘excretory’ syncytial plates. For histology and SEM the specimens killed in HF are fine. Another aspect that is necessary to document is the shape of the unhatched eggs, to show if the anti-peduncular pole has any filament or if the filament is lateral or sub-terminal. The eggs of some species we are currently studying are symmetrical, having the filament in the anti-peduncular pole or displaced to the side while in others they are asymmetrical having the filament displaced to the side. *Temnocephala curvicirri* sp. nov. has symmetrical eggs, with the filament sub-terminal, displaced to the side (Fig. 4 arrow).

The body pigmentation displayed by some temnocephalan species is becoming critical to be observed in live specimens; our figure 5 shows that the specimens from the different species of *Belostoma* show the highest degree of body pigmentation among the several species of *Temnocephala* we have observed alive. They tend to be mimetic with the hemipterans’ exoskeleton. Figure 1 shows a specimen of *B. dentatum*, which is lighter in color, where is possible to see several live, darkly pigmented temnocephalans.

The ‘excretory’ syncytial plates of *T. curvicirri* sp. nov. (Figs 9, 15-17) are similar to those drawn by DAMBORENEA & CANNON (2001) for *T. decarloi*. The tentacles observed, photographed, and filmed while our specimens were alive, are held in the same manner described by these authors, with the three central tentacles extended showing that they do share a common base. The eyespots are red in live specimens and remain visible after fixation and preservation in formalin (Fig. 7).

The rhabdite producing glands can only be observed in young specimens where the vitellaria, dorsal and ventral, are not yet developed to conceal them. Figure 10 shows the rhabdite ducts from the two groups of cells run forward on the two sides of the intestinal sac. The disc secretion glands are large forming two groups that run latero-posteriorly from the posterior testes (Fig. 12 arrows) and appear whitish under partial phase contrast illumination (Fig. 6). Joining these groups of disc gland cells there is a pair of large and also whitish cells located between the two posterior testes. These cells stain well with carmine and have been observed in other species of the genus *Temnocephala*.

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