Temnocephala lutzi Monticelli (Platyhelminthes, Temnocephalida) ectosymbiont on two species of Trichodactylus Latreille (Crustacea, Decapoda, Trichodactylidae) from southern Brazil

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ABSTRACT. Temnocephala lutzi Monticelli, 1913, ectosymbiont on brachyuran freshwater crabs of the genus Trichodactylus Latreille, 1828 is recorded for the first time in the State of Rio Grande do Sul, southern Brazil. Two hundred and seventy-nine crabs of two species were examined: 211 Trichodactylus panoplus (von Martens, 1869) and 68 Trichodactylus fluviatilis Latreille, 1828 of which 51 (24.17%) and 28 (41.17%) were positive for T. lutzi, respectively. Crabs of both species carried eggs fixed, exclusively, on the floor of the branchial chambers and/or on the lower side of the gills. Adult as well as young specimens were found inside and outside the branchial chambers. The temnocephalans were always devoid of body pigmentation, although they kept the red eye pigment undiluted in specimens fixed by formalin. The cirrus of T. lutzi showed the typical shape for the species, while the dorsolateral, post tentacular ‘excretory’ syncytial plates, demonstrated by two special techniques (silver nitrate staining and SEM), appeared with an elliptical shape and a central nephridiopore.

KEY WORDS. Brachyura, ectocommensals, Neotropical region, Rio Grande do Sul, South America, taxonomy.

RESUMO. Temnocephala lutzi Monticelli (Platyhelminthes, Temnocephalida) ectosimbionte sobre duas espécies de Trichodactylus Latreille (Crustacea, Decapoda, Trichodactylidae) da região sul do Brasil. Temnocephala lutzi Monticelli, 1913, ectosimbionte sobre caranguejos braquiúros de água doce do gênero Trichodactylus Latreille, 1828, é assinalada pela primeira vez no Estado do Rio Grande do Sul, região sul do Brasil. Duzentos e setenta e nove caranguejos foram examinados: 211 de Trichodactylus panoplus (von Martens, 1869) e 68 de Trichodactylus fluviatilis Latreille, 1828, dos quais 51 (24.17%) e 28 (41.17%) estavam positivos para T. lutzi, respectivamente. Caranguejos das duas espécies apresentaram posturas de T. lutzi com os ovos fixados, exclusivamente, no interior das câmaras branquiais, sobre o assoalho e/ou na face interna das brânquias. Espécimes juvenis e adultos foram encontrados dentro e fora das câmaras branquiais. Os temnocefalídeos sempre se apresentaram sem pigmento corporal, embora o pigmento vermelho dos olhos tenha sido preservado nos espécimes fixados em formalina. Os cirrós em T. lutzi apresentaram-se com a forma típica da espécie, as placas sinciciais dorsolaterais, pós-tentaculares ‘excretoras’, demonstradas através de duas técnicas especiais (nitrato de prata e MEV), apresentaram a forma elíptica com o nefridioporo central.

PALAVRAS CHAVE. América do Sul, Brachyura, ectocomensais, região Neotropical, Rio Grande do Sul, taxonomia.

The present report records *T. lutzi* from two new hosts of the genus *Trichodactylus* Latreille, 1828, *Trichodactylus panoplus* (von Martens, 1869) and *Trichodactylus fluviatilis* Latreille, 1828, documenting for the first time, photographically and through illustrations, the: 1) areas of egg deposition on the hosts, shape and color of the eggs, and relative position of the egg filament; 2) morphology of the young; 3) cirrus structure as revealed by different techniques; and 4) paired dorsolateral, post-tentacular, ‘excretory’ syncytial plates also as revealed by two different techniques.

**MATERIAL AND METHODS**

Collections of hosts extended from 1998 to 2004. Two hundred seventy-nine crabs were collected with dip nets and/or large sand sieves, and transported alive to the Laboratório de Helmintologia, Universidade Federal do Rio Grande do Sul (UFRGS). Alive temnocephalans were obtained from each host species collected from several locations: *T. panoplus* – canals located 5 km West of Interstate Road BR-290, locality of Arrozeira, Municipality of Eldorado do Sul, (30º01’36”S, 51º22’42”W); Praia Florida (30º15’54”S, 51º32’25”W), and Arroio do Conde (30º05’73”S, 51º31’63”W), both in the Municipality of Guaíba, and Rio Jacuí, at Ilha da Pintada (30º02’23”S, 51º25’49”W), Municipality of Porto Alegre. *T. fluviatilis* – Arroio Carvão (29º32’29”S, 50º13’49”W), Arroio Água Parada (29º66’20”S, 50º21’15”W), and Arroio Forqueta (29º32’17”S, 50º14’44”W), all in the Municipality of Maquiné; locality of Vale das Trutas, head waters of Rio das Antas (28º47’00”S, 49º50’53”W), Municipality of São José dos Ausentes. All municipalities in the State of Rio Grande do Sul.

Some specimens 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 morphology (Amato & Amato 2005).

The morphology of the paired, dorsolateral, post-tentacular ‘excretory’ syncytial plates was studied in live specimens fixed with hot (60ºC) silver nitrate (SN) (Roméis 1968, Joffe et al. 1995). For scanning electron microscopy (SEM), specimens were flooded with hot (90ºC) 10% buffered formalin (HF), without cover slip pressure and washed several times with distilled water, dehydrated in a graded ethanol/acetone series, and critical point dried; coated with gold, and examined with a Jeol (JSM-6060) scanning electron microscope. Cirrus morphology was studied after micro dissection and mounting the cirri in de Faure (deF) medium (Cannon & Sewell 1995). Groups of unhatched eggs, removed by scraping the floor of the branchial chamber were dehydrated, cleared in cedar oil, and mounted in Canada balsam. The description of the specimens follows the Delta System format (Dallwitz & Paine 1986).

Photomicrographs were taken with a Zeiss Axiolab microscope equipped with phase contrast (or just the phase contrast condenser) or with a Leica DMRC differential interference contrast (DIC) microscope with Nomarski’s prisms. 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 16) and the standard deviation values. The terminology used 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.

Voucher specimens fixed in HF and cold A.F.A., as well as slides containing individual cirri mounted in deF, and unhatched eggs, were deposited in the Coleção Helmintológica do Instituto Oswaldo Cruz (CHIOC), Rio de Janeiro, Rio de Janeiro, Brazil and in the Coleção de Invertebrados, Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, Amazonas, Brazil.

**RESULTS**

*Temnocephala lutzi* Monticelli, 1913

Figs 2-24

Description (based on 477 specimens collected; 101 adult whole-mounted specimens killed in cold A.F.A., under slight cover slip pressure); 59 juveniles; two specimens mounted on stubs for SEM; 13 cirri mounted in deF; 4 mounted specimens (fixed with SN); 41 mounted specimens (fixed with HF); 16 specimens measured:

External characteristics. Body shape elliptical (Figs 6 and 16); length (without tentacles) 700-1090 (931, 109), 410-1015 (605, 166); elliptical, wider at mid-body; adhesive disk sub-ventral, pedunculate, 130-350 (244, 55) in diameter; eye spots round to irregular shaped, with red pigment in live specimens (Figs 7 and 9 arrows); pigment dissolving if specimens are fixed in ethanol, but not in formalin.

Epidermal mosaic (demonstrated through staining with SN and observed with SEM) with two dorsolateral, post-tentacular, elliptical-shaped (Figs 9, 10, 16, and 17 head arrows), ‘excretory’ syncytial plates, extending from middle insertion of first and fifth tentacles, respectively; left plate 135-183 (159, n = 2, 34) long, 68-73 (71, n = 2, 3); right plate 130-174 (152, n = 2, 31) long, 59-73 (66, n = 2, 10); length of ‘excretory’ syncytial plates/total body length, without tentacles, relationship of 4: 1. Excretory pore (nephridiopore) well centered inside the area of each ‘excretory’ syncytial plate (Figs 9, 10, 16 and 17 n).

Alimentary system. Mouth surrounded by muscular sphincter (Figs 11 and 12), between first and second thirds of body; pharynx wider than long, 130-250 (205, n = 10, 40) long, 165-275 (223, n = 10, 42) wide, with a large sphincter (Fig. 12); intestine sacular, with conspicuous septations (Figs 11 and 12) in young and in adult specimens.
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Figures 1-6. (1) Trichodactylus panoplus: (1a) specimen without the carapace, showing patches of eggs (arrows), hatched (dark areas, black arrow) and unhatched (white areas, white arrow), bar = 5 mm; (1b) inset with the carapace removed (dorsal view), bar = 7 mm; (2-6) Temnocephala lutzi: (2) eggs (hatched and unhatched) on the floor of the branchial chamber of T. panoplus, bar = 500 µm; (3) two anterior gills removed from the branchial chamber, showing hatched and unhatched eggs (arrow) on their inner surface, bar = 1 mm; (4) higher magnification of eggs, showing filament (arrow), bar = 200 µm; (5) eggs cleared in cedar oil showing their shape, width and length of peduncles, bar = 300 µm; (6) T. lutzi, adult specimen, seen with phase contrast condenser, bar = 100 µm.
Excretory system. Excretory ampullae, round to elongate, at level of mouth (Fig. 12 ea), generally directed outwards with the nephridiopore in the center of the ‘excretory’ syncytial plates.

Glands. Rhabdite producing glands large, numerous, forming bunches, in lateral fields of the body; extending from mid-level of the intestinal sac to the adhesive disc, with inconspicuous ducts, best observed in very young specimens still without vitellaria (Fig. 11 rg). Two irregular shaped Haswell cells (Figs 12 hc and 21 head arrows), showing little affinity with hematoxylin, in front of the eyespots and the brain transverse band; left cell in pair 35-100 (53, n = 11, 22) across, right cell 30-88 (48, n = 10, 18) across. Disc glands (Fig. 13) between adhesive disc and genital complex, forming bunches extending from mid-level of posterior testes to the anterior border of the adhesive disc including to two, large, more central cells (Figs 13 and 23 lgc arrows), 55-78 (63, n = 8, 8) long.

Reproductive system. Female. Gonopore between middle and posterior thirds of body; ovary pyriform, 35-100 (57, n = 14, 18) long, 30-75 (55, n = 14, 12) wide (Figs 22-23 ov); seminal receptacles not observed; vitellaria covering the dorsal and the ventral sides of the intestinal sac, sometimes (in specimens killed with cover slip pressure) exceeding this limit (Figs 12...
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and 22 va); genital atrium spacious, elongate (Fig. 22 ga), vagina short, incospicuous, without observable muscular sphincter (Fig. 22 va), opening in front of the cirrus’ introvert; vesicula resorbens (Figs 22-23 vr), thick walled, 40-113 (72, n = 14, 25) long, 50-138 (88, n = 14, 26) wide, indenting intestinal sac and vitellaria, posteriorly. Eggs with peduncles of medium

Figures 11-15. Temnocephala lutzi: (11) very young specimen showing the distribution of the rhabdite producing glands (rg), bar = 100 µm; (12) partial diagram of an adult specimen, showing the intestinal sac (is), and the distribution of vitellaria (v), the Haswell cells (hc), and the excretory ampullae (ea), bar = 500 µm; (13) disc glands, showing the pair of larger glands (arrows), bar = 150 µm; (14) vasa deferentia (vd arrows), seminal vesicle (sv), ejaculatory duct (ej), prostatic cells (pc), prostatic secretion (ps), and cirrus (c), bar = 50 µm; (15) cirrus, showing the proximal limit of the introvert (arrow) and the shafts proximal end thicker rim (head arrows), bar = 20 µm.
length (Fig. 5), always deposited inside the branchial chambers, on the floor (Fig. 1a unhatched eggs white arrow and hatched eggs black arrow) and on the inner side of the anterior gills (Fig. 3 arrow), 260-490 (343, n = 7, 96) long, 170-270 (201, n = 7, 44) wide, peduncles 30-90 (60, n = 7, 22) long; and a filament displaced to the side (Fig. 4 arrow).

Male. Testes four, anterior and posterior testes of different sizes, oblique; anterior testes round to oval, sometimes more elongate and slightly lobed; posterior testes always more voluminous; right anterior testis round to oval, 75-263 (134, 46) long; 45-128 (87, 25) wide; right posterior testis round, 115-263 (167, 44) long, 55-225 (128, 48) wide; left anterior testis, 75-263 (134, 46) long; 45-128 (87, 25) wide; left posterior testis round to oval, 117-390 (176, 67) long, 68-233 (133, 45) wide; both vasa deferentia wide (Figs 22 and 24 vd) uniting in large, pyriform, thick-walled, seminal vesicle (Figs 22 and 24 sv), 60-123 (88, 16) long, 25-50 (38, n = 9, 10) wide; prostatic bulb (Figs 22 and 23 pb) 40-73 (55, n = 10, 10) long, 35-55 (44, n = 10, 6) wide, cirrus short (Figs 14, 18 and 24 c), slightly bent anteriorly (30% from distal end), 87-109 (96, n = 10, 8) long, shaft 55-82 (68, n = 10; 8) long; 30-46 (37, n = 10; 4) wide at base; maximum introvert width at level of swelling, 9-16 (10, n = 10, 2), introvert’s swelling portion 21-36 (26, n = 10; 5) long, observed in two different focusing planes with the Nomarski’s (DIC) microscopy (Figs 19 and 20). Proximal limit of introvert marked with a narrowing of the lumen’s diameter, and is seen from the side as two fine spikes (Fig. 15 arrow). Base of cirrus shaft with thick borders in adult specimens (as growth of cirrus starts from distal extremity) showing growth is complete (Fig. 15 head arrows). Ratio between total length of cirrus and maximum width of shaft’s base 2.54:1; ratio between total length of cirrus and total length of introvert 3.69:1. Introvert spines displaced, in approximately, 16-20 longitudinal rows of 12-15 spines each (Figs 19 and 20); some longitudinal rows with more spines than others.


Other hosts and localities. *Trichodactylus panoplus* (von Martens, 1869) (new host record) and *Trichodactylus fluviatilis* Latreille, 1828 (new host record); *Trichodactylus petropolitanus* Göldi, 1886, suburb of Pinheiros, São Paulo City and City of Piracicaba, SP, Brazil (Pereira & Cuocolo 1941); *Sylviocarcinus pictus* (Milne-Edwards, 1853), Rio Negro, Lago do Prato, Amazonas State and Rio Amapá, State of Amapá, Brazil (Damborenea 1994).

Other localities (present work). T. panoplus – canals on the road to the locality of Arrozeira, Municipality of Eldorado do Sul (2 km to the North of Interstate Road BR-290 (30°01’36″S, 051°22’42″W); Praia Florida (30°15’54″S, 051°32’25″W) and Arroio do Conde, Municipality of Guiaiba (30°05’73″S, 051°31’63″W); Rio Jacuí, at Ilha da Pintada (30°02’23″S, 051°25’49″W), Municipality of Porto Alegre. T. fluviatilis – Arroio Carvão (29°32’29″S, 050°13’49″W), Arroio Água Parada.
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Figures 18-20. Temnocephala lutzi, photomicrographs of a cirrus, seen with Nomarski’s DIC microscopy: (18) entire cirrus, showing the bent distal region and the ‘sleeve-like’ sheath (white arrow), bar = 25 µm; (19) cirrus introvert showing how spines are inserted laterally (lateral focus), bar = 5 µm; (20) cirrus introvert showing the number of spines along a longitudinal row with focus on the lower wall, bar = 5 µm. Black arrows show point of bending.

(29°66′20″S, 050°21′15″W), and Arroio Forqueta (29°32′17″S, 050°14′44″W), Municipality of Maquiné; Vale das Trutas, head waters of Rio das Antas (28°47′00″S, 049°50′53″W), Municipality of São José dos Ausentes, all in the State of Rio Grande do Sul, Brazil.

Site. Branchial chambers and external surface of body; eggs always located inside the branchial chamber, on the floor and over the lower side of gills, in both *T. panoplus* and *T. fluviatilis*.

Prevalences. 41.17% in *T. fluviatilis* and 24.17% in *T. panoplus*.


Remarks. A number of considerations have been made along the time, because MONTICELLI (1913) described *T. lutzi* from a crab host that he called “Telphusa sp.” and declared “São Paulo”, State of São Paulo (SP), Brazil as the type locality for the new species. BAER (1931: 41) listing the same material of *T. lutzi* used by Monticelli to describe the new species, referred: “Habitat: Cavité branquiale de *Telphusa* sp. Localité: Sao Paolo”, and the following comment in a footnote: “D’après les renseignements qui nous ont été obligeamment communiqués par le Dr. J. Roux de Bâle, le genre *Telphusa* = *Potamon*. Mais ce genre n’ existe pas en Amérique du Sud. Il s’agit donc d’un Crustacé mal determine”. The validity of the generic name *Thelphusa* also has been discussed by PRETZMANN (1982), while surveying the river crabs of the western Mediterranean area. Dr Célio Magalhães stated in a letter to us: “As to the question of *Telephusa* or *Thelphusa*, what I know is that *Telphusa* is a valid generic name of Insecta: Lepidoptera: Gelechiidae, whose author is Chambers, 1872. *Thelphusa* has been proposed by Latreille, 1819 for a freshwater crab, but Ortmann (1896) verified that it was synonym of *Potamon*, proposed by Savigny, 1816.
But I believe that *Thelphusa* is not used for any genus or supra-generic taxon within the Brachyura. It has been, by tradition associated to a freshwater crab and many valid taxa (generic and supra-generic) were named using combinations of this word". Thus, the type host of *T. lutzi* should be a species of *Trichodactylus*, occurring in SP.
**Temnocephala lutzi** is one of the four species of temnocephalans epizoic over trichodactylids recorded in Brazil, and one of the six recorded in the world. Although this species has been described by Monticelli (1913) and re-described by Pereira & Cuocolo (1941), this is the first time that it is studied by SN and MEV techniques, which require live specimens to be killed/fixed by different fixatives. These techniques have shown that *T. lutzi* has elliptical syncytial plates (Figs 9 and 17), with the excretory pores at the center (Fig. 10 n). Another aspect that is necessary to document is the shape of the unhatched eggs, to show the relative position of the filament, which, in the case of *T. lutzi* is not located at the anti-peduncular extremity (Fig. 4 arrow).

Damborenea & Cannon (2001) re-examined 19 Neotropical species of the genus *Temnocephala*, showing the epidermic ‘excretory’ syncitial plates, with differences in the shapes and position of the excretory pore inside the plates. The epidermic, post-tentacular ‘excretory’ syncytial plates of *T. lutzi* are shown for the first time to have an elongated elliptical shape which differs from the plates of the seven species illustrated by Damborenea & Cannon (2001), from those of *T. cyanoglandulata* Amato, Amato & Daudt, 2003, and *T. curvicirri* Amato & Amato, 2005, demonstrated using the SN and MEV techniques in species from the State of Rio Grande do Sul (Amato et al. 2003, Amato & Amato 2005). Because *T. lutzi* has been found to co-occur in *T. fluviatilis* with another species which is being described and illustrated in a separate paper, at first, the separation of the specimens in mixed infestations posed a problem. But, providentially, the shape of the epidermic, post-tentacular ‘excretory’ syncytial plates found in *T. lutzi* are well distinct than those of the co-occurring species.

Previous authors have not considered the distribution and position of the rhabdite producing glands to be of much systematic value. The size, distribution, and position of these glands seen in *T. lutzi* have never been illustrated. They differ from those of other species described previously, the distribution of these glands in *T. lutzi* range from the mid-line of the intestinal sac to the anterior border of the adhesive disc super-imposed to the disc glands.

The absence of seminal receptacles promoted a discussion over a peculiar structure found in the female reproductive system, which is an expansion of the duct that connects the vesicula resorbens to the ootype and the vagina (Fig. 23 vi). Damborenea (1994), describing *T. kingsleyae*, illustrated a similar structure to this reported in *T. lutzi* (present paper), a structure called “vesicula intermedia” would have the same function of seminal receptacles, explaining the absence of the later.

Damborenea (1994) found specimens of *T. lutzi* with smaller measurements than those found by Pereira & Cuocolo (1941). Now we have specimens with measurements still smaller than those given by Damborenea (1994). We consider the smaller measurements of our specimens a case of intraspecific variation.

Pereira & Cuocolo (1941) described the cirrus as “short and bent in an angle near the distal portion, showing thus two parts, one long and straight and the other short and vertical. At the distal extremity it has a crown of small, very delicate, and chitin spines”. This description has been confirmed by Damborenea (1994).

In the present paper, micro dissection of the cirrus mounted in deF medium made possible the observation of the real complexity of the inflated portion of the introvert, as well as to obtain DIC photomicrographs and measurements of the cirri laying flat, to count the number of spines in each longitudinal row, the approximate number of longitudinal rows, and the position of the inner projections which limit the proximal limit of the introvert.

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