A new species of *Temnocephala* (Platyhelminthes, Temnocephalida) and a description of *T. axenos* from Uruguay

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**Abstract**

In Uruguay, two species of *Temnocephala* Blanchard, 1849 have been reported on the anomuran crab *Aegla* Leach, 1920, namely *Temnocephala axenos* Monticelli, 1899 and *Temnocephala talicei* Dioni, 1967. A third species, described here as *Temnocephala mertoni* n. sp., has been found on *Aegla platensis* Schmitt, 1942 from southern Uruguay. The new species resembles *T. talicei*, but differs by having a penial stylet with a sinuous distal portion of the shaft; a small introvert with short, distal spines, and a discrete thickening in its wall. The penial stylet of *T. mertoni* n. sp. is more similar to that of *T. axenos*. In view of the importance of stylet structure and shape in the taxonomy of this group, a description of *T. axenos* is included, and a comprehensive study has been carried out to establish the differences between these species. Based upon this study, new characters are proposed for the taxonomy of the genus *Temnocephala*.

**Keywords:** Neotropical region, new species, Platyhelminthes, systematics, Temnocephala, taxonomy, Uruguay

**Introduction**

Six species of *Temnocephala* Blanchard, 1849 (Platyhelminthes, Temnocephalidae) have been described from freshwater anomuran crabs belonging to the genus *Aegla* (Crustacea, Aeglidae). These are: *Temnocephala chilensis* (Moquin-Tandon, 1846); *Temnocephala axenos* Monticelli, 1899; *Temnocephala brasiliensis* Merton, 1922 (synonymized with *T. axenos*); *Temnocephala bresslauii* Pérez-González, 1949 (synonymized with *T. axenos*); *Temnocephala talicei* Dioni, 1967; and *Temnocephala cyanoglandula* Ribeiro Amato, Amato and Campos Dautd, 2003. Only two of them, namely *T. axenos* and *T. talicei*, have been found in Uruguay so far.

The first study of Uruguayan material of *T. axenos* was made by Dioni (1967a). He found this species on the anomuran crab *Aegla* sp. in Del Sauce Lagoon and Aguas Blancas (Departamento de Maldonado), Santa Lucía River (Departamentos de Lavalleja and Montevideo), and Cebollati River (Departamento de Lavalleja), and on a crayfish,
Parastacus sp., in Negro River (Departamento de Tacuarembó). Subsequently, Ponce de León (1988) also found it on Aegla sp. in Negra Lagoon (Departamento de Rocha). In Argentina, it has been reported on Aegla franca Schmitt, 1942, Aegla humahuaca Schmitt, 1942, Aegla platensis Schmitt, 1942, Aegla uruguayana Schmitt, 1942, and Aegla sp. (Dionis 1967b; Damborenea 1991; Damborenea et al. 1997). In Brazil, Temnocephala axenos has been found on Aegla laevis (Latreille, 1818) (Baer 1931), Aegla castro Schmitt, 1942 (Pérez-González 1949), Aegla sp. and Parastacus sp. (Dionis 1967a).

Temnocephala talicei was described from Uruguayan material of Aegla prado Schmitt, 1942 by Dionis (1967a), from El Prado Botanical Park, Montevideo (Departamento de Montevideo); he also found this species on Aegla sp. in Lunarejo Creek (Departamento de Rivera) and Tres Islas (Departamento de Cerro Largo). In Argentina, Temnocephala talicei has been found on A. platensis and A. uruguayana (Dionis 1968; Damborenea 1991, 1992), and in Paraguay on A. platensis (Dionis 1968).

Unlike T. talicei, the taxonomy of T. axenos has proven difficult because of the poor original description by Monticelli (1899). Merton (1922) attempted to give a better description when he described Temnocephala brasiliensis (now a synonym of T. axenos), but he only had access to a single specimen in bad condition, and therefore he only pointed out a few characters to differentiate it from T. brasiliensis. Baer (1931) studied the type material of both T. axenos and T. brasiliensis, so he described and illustrated T. axenos better, and in the same work synonymized T. brasiliensis with the latter. More recently, Pérez-González (1949) described Temnocephala bresslaui, pointing out a few diagnostic characters in order to separate it from T. axenos, but some of these characters have been proven to have no taxonomic value (Dionis 1967a; Damborenea 1991). Finally, Dionis (1967a) synonymized T. bresslaui with T. axenos in the same work in which he described T. talicei.

While searching for material of T. axenos and T. talicei for a study on Uruguayan temnocephalids, a third species was found. This species does not correspond to any of the known species of Temnocephala, and therefore is here described as T. mertoni n. sp. This species resembles T. talicei, but the penial stylets of both species are clearly different. However, the penial stylet of T. mertoni n. sp. is morphometrically similar to that of T. axenos. Therefore, to avoid introducing more confusion in the taxonomy of the latter, a comprehensive study was carried out with material of both species, and therefore a detailed description of Uruguayan material of T. axenos is also given. Based upon the comparison of these species, new characters are proposed for the taxonomy of the species of Temnocephala.

Materials and methods

Specimens of Aegla platensis Schmitt, 1942 were collected from the Molles Stream along Route 8, Km 238 (33°36′S, 54°35′W, Departamento de Lavalleja, Uruguay) in December 2005, and from Colorado Stream, Route 6, Km 35 (34°41′S, 56°04′W, Departamento de Canelones, Uruguay), in February 2006.

Hosts were brought alive to the laboratory, where all temnocephalids were removed with the aid of a stereomicroscope, examined and identified alive under a microscope, recovered and killed with hot Formalin-Acetic acid-Alcohol (FAA) and, after 24 h, transferred to 70% ethanol. Temnocephalid eggs were removed from the surface of the hosts and preserved in 70% ethanol. After removal of temnocephalids and their eggs, hosts were killed and preserved in 70% ethanol.

On Route 8, Km 238, three species of Temnocephala were found, namely T. talicei, T. axenos, and a previously unknown species. In the Colorado Stream only the latter two were found. Given the low number of specimens found at Colorado Stream, the morphometry of
T. axenos and of the new species was determined with material from the Route 8 location. Temnocephalids were stained with acetic carmine and fast green, and mounted in Canada balsam. Some of the specimens were whole-mounted; others were dissected with the aid of a stereomicroscope, and mounts of the reproductive complex were made.

Eggs were removed from the uterus of previously identified gravid individuals and distinctive, qualitative characters were determined for the eggs of each species using transitory mounts in lactophenol. Eggs of the new species could not be separated qualitatively from the eggs of T. talicii, and therefore egg morphometry had to be carried out with material from Route 6, Km 35. Eggs taken from the surface of hosts from this location were classified using the aforementioned characters, and were measured in water to avoid contraction.

Drawings were made with the aid of a camera lucida. Measurements are given as mean (range, standard deviation, n), in micrometres (μm).

In addition to the Uruguayan material, specimens of T. axenos deposited in the Facultad de Ciencias Naturales y Museo de la Universidad Nacional de La Plata (Argentina) were also examined.

The following abbreviations are used in the figures: ag, acetabulum glands; at, atrium; br, brain; cv, contractile vesicle; ep, excretory pore; es, excretory syncytium; ev, excretory vesicle; hg, Haswell glands; in, intestine; mt, metraterm; og, ootype glands; op, opercular plate; or, ornamentation; ov, ovary; pf, plane of fracture; pg, prostatic glands; ph, pharynx; pn, paranephrocyte; ps, penial stylet; rv, resorbens vesicle; sp, sphincter; sr, expansion of the ootype wall in place of the seminal receptacles; st, stalk; sv, seminal vesicle; te, testis; tg, tentacular glands; vd, vas deferens; vi, vitellaria.

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**TEMNOCEPHALIDA** Bresslau and Reisinger, 1933  
**TEMNOCEPHALIDAE** Monticelli, 1899  
*Temnocephala* Blanchard, 1849  
*Temnocephala mertoni* n. sp.  
(Figures 1–5)

**Taxonomic summary**

**Etymology.** The specific name honours Dr Hugo Merton for his contribution to the study of Neotropical temnocephalids.

**Type specimens.** Holotype: whole mount, deposited in the Helminthological Collection of the Laboratorio de Zoológia de Invertebrados, Facultad de Ciencias (Universidad de la República), Montevideo, Uruguay (AP11567). Paratypes: nine whole mounts and 10 mounts of the reproductive complex, deposited in the same Helminthological Collection (AP11568–AP11586).

**Distribution.** Type locality: Molles Stream, Route 8, Km 238 (33°36′S, 54°35′W, Departamento de Lavalleja, Uruguay). Other localities: Colorado Stream, Route 6, Km 35 (34°41′S, 56°04′W, Departamento de Canelones, Uruguay).

**Type host.** *Aegla platensis* Schmitt, 1942. Deposited in the Invertebrate Collection of the Laboratorio de Zoológia de Invertebrados, Facultad de Ciencias (Universidad de la República), Montevideo, Uruguay (BP11001–BP11010).
Location on host. Ventral surface, coxa of chelae and locomotory legs, and base of antennae and eyes.

Diagnosis. Small temnocephalid; sucker small, peduncle oblique with respect to body surface; elongate excretory syncytia, wider in area surrounding excretory pore; two paranephrocytes; intestine with central constriction not pronounced, and inconspicuous septa; one asymmetrical sphincter in female portion of atrium; eggs with subapical, almost lateral, medium-sized ornamentation, and opercular plates oblique to longitudinal axis of egg; penial stylet about 140 μm long; straight, with distal portion of shaft sinuous; small introvert with about 10 rows of small, distal spines, and a discrete thickening in its wall.

Anatomical description

Temnocephalid of small size; body elliptic with maximum width at equatorial level (Figure 1). Body without tentacles 1260.6 μm (1044.4–1481.5; 142.13; 11) long by 639.1 μm (503.7–733.3; 88.59; 11) wide. Epidermis with elongate excretory syncytia, extending from base of external tentacles to level of anterior portion of intestine (Figure 2). Acetabulum subterminal, 170.4 μm (133.3–200.0; 24.12; 11) long by 185.9 μm (163.0–214.8; 13.02; 11) wide. Peduncle generally oblique with respect to ventral body surface,
with acetabulum directed backwards. Ratio of body length without tentacles to acetabulum length: 1:0.10–0.18 (1:0.14). Ratio of width of same organs: 1:0.26–0.37 (1:0.29). Acetabulum glands anterior to acetabulum, scattered between and posterior to posterior testes (Figure 1). Haswell glands conspicuous, anterior to pharynx (Figure 1). Pharynx 207.4 μm (155.6–244.4; 29.44; 11) long by 238.4 μm (192.6–274.1; 26.67; 11) wide.

Figure 2. Excretory syncytia of *Temnocephala mertoni* n. sp.

Figure 3. Reproductive complex of *Temnocephala mertoni* n. sp., drawn from a dissected animal. Scale bar: 100 μm.
Intestine wider than long, with slight central constriction and inconspicuous septa (Figures 1, 2). Tentacular glands abundant, lateral to intestine (Figure 1). Excretory vesicles small, lateral to pharynx; nephridiopores dorsal, situated in a slightly anterior position within the excretory syncytia, close to their internal border (Figure 2). Two paranephrocytes posterior to posterior testes, or dorsal to their posterior portion (Figure 1).

Ovary ovoid. Short oviduct, opens into ootype just behind seminal receptacle (Figure 3). Abundant glandular cells surrounding ootype, with ducts opening into it (Figure 3). Resorbens vesicle in space left by posterior constriction of intestine (Figure 1); ovoid, with anterior side convex, with thin wall, and posterior side flattened and with thicker wall (Figure 3). Ten per cent of the individuals with four small seminal receptacles. The others with an expansion of the wall of the ootype, 56.5 μm (29.0–126.8; 24.16; 15) long by 48.4 μm (27.2–103.3; 18.89; 15) wide, in the same position (Figure 3). Metraterm 74.6 μm (58.0–92.4; 11.94; 15) long by 48.7 μm (38.0–59.8; 6.49; 15) maximum width; distal portion with an asymmetrical sphincter, 44.8 μm (36.2–56.2; 5.91; 15) diameter, 23.2 μm (14.5–29.0; 4.99; 15) maximum thickness, and 14.3 μm (7.2–19.9; 3.55; 15) minimum thickness (Figure 3). Vitelline glands branched, surrounding intestine completely (Figure 1). Eggs 504.5 μm (451.9–548.1; 30.64; 9) long by 237.9 μm (207.4–281.5; 19.75; 9) wide. Ornamentation subapical, almost lateral, of medium size (Figure 4a). Opercular plates of medium size, at a certain angle with respect to longitudinal axis of egg, giving an oblique plane of fracture of operculum (Figure 4a, b).

Figure 4. Eggs of *Temnocephala mertoni* n. sp. (a) Whole egg, showing ornamentation and opercular plates; (b) hatched egg, showing line of fracture of operculum. Scale bar: 200 μm.

Figure 5. Penial stylet of *Temnocephala mertoni* n. sp. drawn from a dissected animal. Scale bar: 50 μm.
Testes slightly lobed; two posterolateral to intestine and two larger, posterior to same organ and more central (Figure 1). Anterior and posterior testes of each side connected by short spermatic ducts. Vas efferens originate on inner portion of posterior testes; right long, left short, enter seminal vesicle adjacent to each other (Figures 1, 3). Seminal vesicle pear-shaped, 130.3 μm (83.3–175.7; 26.00; 15) long by 78.6 μm (43.5–106.9; 21.29; 15) wide; wall 4.7 μm (3.6–5.4; 0.92; 15) thick. Contractile vesicle 108.3 μm (83.3–135.9; 15.41; 14) long by 82.4 μm (54.3–99.6; 13.54; 15) wide; wall 3.5 μm (1.8–5.4; 0.86; 14) thick. Prostatic glands external to contractile vesicle not conspicuous. Penial stylet straight but with distal portion of shaft sinuous in all specimens studied (Figure 5); 138.0 μm (123.2–157.6; 10.09; 15) long by 46.0 μm (38.0–56.2; 5.55; 15) wide. Introvert 27.2 μm (23.6–30.8; 2.74; 15) long, 14.1 μm (12.7–16.3; 1.02; 15) maximum width, and 12.3 μm (10.9–12.7; 0.75; 15) minimum width; with about 10 rows of spines; wall with thickening between proximal end and base of proximal spines (Figure 5). Ratio of body length without tentacles to distance between gonopore and base of tentacles: 1:0.57–0.64 (1:0.61). Gonopore glands surrounding gonopore and ventral to intestine, with conspicuous ducts (Figure 1).

**Temnocephala axenos** Monticelli, 1899
(Figures 6–10)

**Taxonomic summary**

**Material examined.** Ten whole mounts and 10 mounts of the reproductive complex deposited in the Helminthological Collection of the Laboratorio de Zoología de Invertebrados, Facultad de Ciencias (Universidad de la República), Montevideo, Uruguay (AP11587–AP11606).

**Host.** Aegla platensis Schmitt, 1942. Deposited in the Invertebrate Collection of the Laboratorio de Zoología de Invertebrados, Facultad de Ciencias (Universidad de la República), Montevideo, Uruguay (BP11002).

**Location on host.** Ventral surface, coxa of chelae and locomotory legs, and base of antennae and eyes.

**Distribution.** Route 8, Km 238 (33°36′S, 56°35′W, Departamento de Lavalleja, Uruguay); Colorado Stream, Route 6, Km 35 (34°39′S, 56°04′W, Departamento de Canelones, Uruguay).

**Diagnosis.** Large temnocephalid, body rounded; sucker large, with peduncle perpendicular with respect to body surface; elliptical excretory syncytia, small, extends from base of external tentacles to level of anterior portion of intestine; four paranephrocytes; intestine with central constriction pronounced, and conspicuous septa; two sphincters in the distal region of the metraterm, one more proximal, asymmetrical, and one more distal, symmetrical; eggs with subapical, medium-sized ornamentation, and opercular plates perpendicular to longitudinal axis of egg; penial stylet about 140 μm long; curved; small introvert with about 10 rows of small, distal spines.

**Anatomical description**

Body relatively large; contour rounded, with maximum width at level of gonopore (Figure 6). Length without tentacles 1754.4 μm (1163.0–2666.7; 445.52; 13); width
1439.6 μm (829.6–1833.3; 353.08; 13). Epidermis with excretory syncytia small, elliptical, from base of external tentacles to level of anterior portion of intestine (Figure 7). Acetabulum subterminal, 479.3 μm (251.9–629.6; 115.23; 12) long by 509.6 μm (288.9–

Figure 6. *Temnocephala axenos* in ventral view. Scale bar: 300 μm.

Figure 7. Excretory syncytia of *Temnocephala axenos*. 

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Figure 8. Reproductive complex of *Temnocephala axenos*, drawn from a dissected animal. Scale bar: 100 μm.

Figure 9. Eggs of *Temnocephala axenos*. (a) Whole egg, showing ornamentation and opercular plates; (b) hatched egg, showing line of fracture of operculum. Scale bar: 200 μm.
629.6; 108.30; 12) wide. Peduncle perpendicular to body surface. Ratio of body length without tentacles to acetabulum length: 1:0.22–0.35 (1:0.28). Ratio of width of same organs: 1:0.31–0.43 (1:0.36). Acetabulum glands scattered between posterior testes and lateral to acetabulum (Figure 6). Haswell glands conspicuous, anterior to pharynx (Figure 6). Pharynx 315.1 µm (192.6–407.4; 66.38; 13) long by 447.9 µm (281.5–629.6; 105.70; 13) wide. Intestine wider than long, with pronounced posterior, central constriction, and with conspicuous septa (Figures 6, 7). Abundant tentacular glands, lateral to intestine (Figure 6). Excretory vesicles small, lateral to pharynx (Figure 6). Pharynx 315.1 µm (192.6–407.4; 66.38; 13) long by 447.9 µm (281.5–629.6; 105.70; 13) wide. Intestine wider than long, with pronounced posterior, central constriction, and with conspicuous septa (Figures 6, 7). Abundant tentacular glands, lateral to intestine (Figure 6). Excretory vesicles small, lateral to pharynx; nephridiopores dorsal, situated in a slightly anterior position within the excretory syncytia, close to their internal border (Figures 6, 7). Four paranephrocytes; two in intertesticular zone, external to testes, and two in the zone of posterior testes, central to or dorsal to the inner surface of the latter (Figure 6).

Ovary ovoid. Oviduct short, opens into ootype just behind seminal receptacle (Figure 8). Gland cells surrounding ootype, with ducts opening into it (Figure 8). Resorbs vesicle in space left by the posterior constriction of intestine, or slightly posterior and to the right of the latter (Figure 6); ovoid, with anterior side convex, with thin wall, and posterior side flattened to concave, with thicker wall (Figure 8). Ten per cent of the individuals with four small seminal receptacles. The rest with an expansion of the wall of the ootype, 49.9 µm (32.6–90.6; 17.36; 14) long by 41.8 µm (23.6–88.8; 19.63; 14) wide, in the same position (Figure 8). Metraterm 70.4 µm (38.0–106.9; 21.99; 15) long by 49.3 µm (32.6–70.7; 11.68; 15) maximum width; distal portion with two sphincters (Figure 8). Proximal sphincter asymmetrical, 43.4 µm (30.8–59.8; 9.10; 15) diameter, 23.6 µm (12.7–36.2; 7.93; 15) maximum thickness, and 9.7 µm (3.6–18.1; 3.91; 15) minimum thickness. Distal sphincter symmetrical; 45.5 µm (27.2–61.6; 11.68; 15) diameter, 11.4 µm (7.2–16.3; 3.17; 15) thickness. Vitelline glands branched, surrounding intestine completely (Figure 6). Eggs 428.1 µm (392.6–466.7; 20.76; 20) long by 255.6 µm (229.6–281.5; 15.10; 20) wide. Ornamentation subapical, short (Figure 9a). Opercular plates large, perpendicular to longitudinal axis of egg, giving a horizontal plane of fracture (Figure 9a, b).

Testes slightly lobed; two anterior, posterolateral to intestine, and two posterior to same organ and more central, larger (Figure 6). Anterior and posterior testes of each side connected by short spermatic ducts. Vas efferens originate on inner side of posterior testes; right long, left short (Figure 6). Seminal vesicle pear-shaped, 146.0 µm (81.5–212.0; 42.73; 15) long by 89.7 µm (58.0–154.0; 27.23; 14) wide; wall 6.4 µm (3.6–9.1; 1.35; 15) thick. Contractile vesicle 107.9 µm (68.8–161.2; 33.21; 15) long by 67.9 µm (47.1–106.9; 19.02; 14) wide; wall 5.2 µm (3.6–9.1; 1.72; 14) thick. Prostatic glands external to contractile vesicle inconspicuous, only evident in dissected animals (Figure 8). Penial stylet slightly curved (Figure 10), 141.4 µm (128.6–163.0; 10.66; 15) long by 42.0 µm (30.8–54.3; 6.25; 15) base; introvert 23.9 µm (18.1–29.0; 2.95; 14) long, 11.9 µm (10.9–12.7; 0.93; 14) maximum width, and 10.4 µm (9.1–10.9; 0.85; 14) minimum width, with about 10 rows of
spines (Figure 10). Ratio of body length without tentacles to distance between gonopore and base of tentacles: 1:0.41–0.50 (1:0.47). Gonopore glands conspicuous, abundant around gonopore, and some ventral to intestine, with short ducts (Figure 6).

**Taxonomic affinities**

*Temnocephala mertoni* n. sp. is most similar to *T. talicei*. Based upon the characters used to distinguish species of *Temnocephala*, the features shared by both species separating them from *T. axenos* are the following. Firstly, both species are smaller than *T. axenos* despite some overlap in length. The length range reported here for *T. mertoni* n. sp. is 1.0–1.5 mm. Dioni (1967a) reported a range of body length of 1.3–1.5 mm for *T. talicei* from Uruguay, and, subsequently, Damborenea (1991, 1992) reported a mean body length of 1.5 mm for Argentinian populations. Body length in *T. axenos* specimens has been reported to range from 1.1 to 5.0 mm, with most reports ranging from about 1.5 to 3.0 mm (Merton 1922; Baer 1931; Pérez-González 1949; Dioni 1967a; Damborenea 1991, 1992); our sample, with an observed range of 1.2–2.7 mm, is in agreement with the latter. Secondly, in both *T. talicei* and *T. mertoni* n. sp., the acetabulum is small. The relation of total body length to acetabulum diameter is 6.7 for *T. mertoni* n. sp. Dioni (1967a) reported a value of 5 for *T. talicei*, which is in agreement with the measurements provided for Argentinian populations of *T. talicei* by Damborenea (1991, 1992). In *T. axenos*, Dioni (1967a) reported this relation to be 3–4, and Damborenea (1992) reported it to be 3.9; in our sample, it has a value of 3.7. As for qualitative differences, in both *T. talicei* and *T. mertoni* n. sp. the intestine has a central constriction that is not as pronounced as in *T. axenos*, and the penial stylet is straight, whereas in *T. axenos* the central constriction of the intestine is quite pronounced and the stylet is generally curved. The eggs of *T. mertoni* n. sp. and *T. talicei* are qualitatively identical, both having subapical, almost lateral, medium-sized ornamentation, and opercular plates oblique to the longitudinal axis of the egg. Other characters could not be compared, because *T. talicei* was defined mainly by its distinctive penial stylet, and the original description by Dioni (1967a) lacks further details of the species’ anatomy.

Despite the similarities observed, *T. mertoni* n. sp. can be clearly differentiated from *T. talicei* by the penial stylet. The stylet in *T. mertoni* n. sp., with a mean length of 138 μm and a width of 46 μm at the base, is somewhat similar in size to that of *T. talicei*, with a mean length of about 120 μm, and a width of 50 μm (Dioni 1967a; Damborenea 1992). However, the introverts in both species are completely different. The introvert is larger in *T. talicei* than in *T. mertoni* n. sp.; according to the illustration of the penial stylet by Dioni (1967a) the ratio of the length of the introvert to the penial stylet length would be 1:0.3, whereas in *T. mertoni* n. sp. this ratio is 1:0.2. Furthermore, in *T. talicei*, the spines extend throughout the whole length of the introvert, whereas in *T. mertoni* n. sp. they are only present in the distal portion. Dioni (1967a) reported these spines to measure 5–7 μm in length in *T. talicei*, but in *T. mertoni* n. sp. most spines measure 4–5 μm, and only the basal ones, which are the longest, measure 6 μm.

On the other hand, the stylet in *T. mertoni* n. sp. is morphometrically very similar to that of our sample of *T. axenos*, with a mean length of 141 μm, a width of 42 μm at the base, and a ratio of the length of the introvert to the penial stylet length of 1:0.2. These measurements are in agreement with those of Damborenea (1991, 1992). What is more, in *T. axenos* the spines are also present only in the distal portion of the introvert. The differences between both stylets are merely qualitative: in *T. axenos* the stylet is curved, whereas in *T. mertoni* n. sp. it is always straight but with a sinuous distal portion of the shaft, and in *T. mertoni* n. sp. there is a thickening in the introvert wall which is absent in *T. axenos*. 
Therefore, in order to separate further these two species, and in addition to the characters mentioned above, the following differences should be considered: in *T. axenos* the excretory syncytia are elliptical, whereas in *T. mertoni* n. sp. they are elongate, and wider in the area surrounding the excretory pore; in *T. axenos*, the intestinal septa are very conspicuous, whereas in *T. mertoni* n. sp. they are barely visible; *T. axenos* has four paraneophrocytes, and *T. mertoni* n. sp. has only two; in *T. axenos* there are two sphincters in the female portion of the atrium, but in *T. mertoni* n. sp. there is only one. Differences were also found between the eggs of both species, given that in *T. axenos* the eggs have a short, subterminal ornamentation, and opercular plates perpendicular to the longitudinal axis of the egg.

**Discussion**

Some aspects of the taxonomy of *T. axenos* remain unclear. In particular, the reports of the length of the penial stylet are noteworthy: when Pérez-González (1949) described *T. bresslau**, which she considered different from *T. axenos*, she reported stylet lengths of 280–304 µm. Subsequently, Dioni (1967a), considering that *T. bresslau** was a synonym of *T. axenos*, reported stylet lengths of 125–250 µm for Uruguayan material of *T. axenos*; in a following work (Dioni 1967b), he stated that in populations from Argentina he had only found *T. axenos* with small stylets. Damborenea (1991, 1992) reported stylets of around 125 µm in length for other Argentinian populations. Our results are similar to those of Damborenea, because we did not find any stylet longer than 163 µm.

This discrepancy in the measurements of the penial stylet among populations of *T. axenos* casts doubts on the synonymization of *T. bresslau** and *T. axenos* by Dioni (1967a). According to the report of Pérez-González (1949), *T. bresslau** would be a species very similar to *T. axenos*, but with a penial stylet that is twice as long. Ribeiro Amato et al. (2003) recently described a species with those features, *Temnocephala cyanoglandula* from *Aegla serrana* Buckup and Rossi, 1977. Therefore, taking into account that *T. bresslau** might indeed be a valid species, it would be desirable to make a comparison of material of *T. axenos* with the type material of *T. bresslau** and of *T. cyanoglandula*, in order to establish the taxonomic status of the latter two species.

Another interesting aspect is that in *T. axenos* both Merton (1922) and Baer (1931) reported the presence of four seminal receptacles. We observed the same in only 10% of the individuals studied both alive and in dissected animals, whereas the rest of the specimens showed an expansion in the walls of the ootype, exactly where the seminal receptacles would normally be. This expansion was filled with sperm in some individuals, but completely empty in others, so we can confirm that this state does not result from the accumulation of sperm in this area. Pérez-González (1949) mentioned for *T. bresslau** that the seminal receptacles were transitory; this statement seems therefore to apply to both *T. axenos* and *T. mertoni* n. sp.

The penial stylet remains the main taxonomic character for the identification of species of *Temnocephala*. However, as we demonstrate here, there are species whose stylet morphometry is similar to the extent that the only detectable differences are qualitative. In this context, it has become evident that in taxonomic descriptions of species of *Temnocephala*, authors not only must provide an accurate description of the penial stylet, they also must include a detailed morphological study including soft-part characters with taxonomic weight.
The comparison between *T. mertoni* n. sp., *T. talicei*, and *T. axenos* has shown that there are many characters of taxonomic weight which are not normally used in the taxonomy of *Temnocephala*, and whose inclusion in descriptions would facilitate species identification. We propose that the following characters are taken into account in addition to those already in use: shape and extension of the excretory syncytia, in agreement with Damborenea and Cannon (2001), who were the first to suggest that the excretory syncytia vary among species; presence/absence of conspicuous intestinal septa; number and position of paraneuphocytes; number and shape of the sphincters in the female reproductive system; size and position of egg ornamentation, and disposition of opercular plates of the eggs; shape of the penial stylet; number, size, and position of the spines in the introvert; and presence/absence of thickenings in the wall of the introvert. We are confident that the use of these characters will facilitate the identification of species within this genus, in particular those which share their host, as is the case with *T. mertoni* n. sp., *T. axenos*, and *T. talicei*.

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