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A new species of *Temnocephala* (Platyhelminthes, Temnocephalida) commensal of *Pomella megastoma* (Mollusca, Ampullariidae) from Misiones, Argentina

Una especie nueva de *Temnocephala* (Platyhelminthes, Temnocephalida) comensal de *Pomella megastoma* (Mollusca, Ampullariidae) de Misiones, Argentina

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Abstract. *Temnocephala lamothei* n. sp., a commensal of *Pomella megastoma* (Sowerby, 1825), is described herein from specimens collected at Arroyo Yabotí-Miní (Misiones province, Argentina). Juveniles and adults were removed from the mantle cavity by host relaxation. Distinctive characters of the new species are: non-partitioned intestine; conical cirrus with 1 face flat and another concave; distal area with spines, as evidenced by a strong, oblique sclerotized ring, and 2 rows of long spines, an internal one with long spines arising from base of introvert and an external one arising from distal end of the introvert. The closest species are *T. iheringi, T. rochensis* and *T. haswelli*, which are also commensals of mollusc species. The presence of this new species of *Temnocephala*, and its similarity to the other species that are commensals of molluscan species, suggest the existence of a morphologically homogeneous group.

Key words: Turbellaria, commensal, Neotropical region, taxonomy, South America.

Resumen. *Temnocephala lamothei* n. sp., comensal de *Pomella megastoma* (Sowerby, 1825), se describe para el arroyo Yabotí-Miní, provincia de Misiones, Argentina. Se extrajeron ejemplares juveniles y adultos de la cavidad paleal, por relajación de los hospederos. Las características distintivas de la nueva especie son: intestino no septado, cirro de forma cónica, con una cara plana y otra cóncava, zona distal con espinas evidente por un fuerte anillo oblicuo esclerosado. Dos hileras de espinas se reconocen en el extremo distal, 1 interna de espinas largas, que surge desde la base del introverso, y 1 externa, que surge del extremo distal del mismo. Las especies más semejantes son *T. iheringi, T. rochensis* y *T. haswelli,* especies comensales de moluscos con las que es comparada. El hallazgo de esta nueva especie de *Temnocephala* y sus características semejantes a las restantes especies del género comensales de moluscos, sugieren que las especies conocidas hasta la fecha formen un grupo morfológicamente homogéneo.

Palabras clave: Turbellaria, comensal, región neotropical, taxonomía, América del Sur.

Introduction

The family Temnocephalidae (Platyhelminthes, Temnocephalida) includes 12 genera, of which only *Temnocephala* Blanchard, 1849 is represented in South and Central America. Twenty-three species of *Temnocephala* are currently recognized (Damborenea and Cannon, 2001; Amato et al., 2003, 2006; Amato and Amato, 2005; Volonterio, 2007), and they are associated with a wide range of hosts (Mollusca Ampullariidae, Crustacea Decapoda, Insecta Hemiptera, and Chelonia).

Gastropods of the family Ampullariidae are common inhabitants of freshwater bodies in the Neotropical region. Five genera, comprising a large number of widely distributed species, are recognized in the area (Cowie and Thiengo, 2003). In spite of this, studies on their commensals and parasites are relatively few (see Damborenea et al., 2006 and references therein). Only 3 species of commensal temnocephalans have been described from these molluscs. *Temnocephala iheringi* Haswell, 1893, found in the mantle cavity of *Pomacea canaliculata* Lamarck, 1822, *Pomacea lineata* (Spix *in* Wagner, 1827), *Asolene platae* (Maton, 1811) and *Pomella megastoma* (Sowerby, 1825), is the most frequent and widely distributed species (Brazil, Uruguay and Argentina) (Damborenea and Cannon, 2001). *Temnocephala rochensis* Ponce de León, 1980 and *Temnocephala haswelli* Ponce de León, 1989 are known

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only for Uruguay and associated exclusively with *P. canaliculata* (Ponce de León, 1980, 1989).

In this contribution we describe a new species of *Temnocephala* that is a commensal of *Pomella megastoma,* which were collected in Misiones province, Argentina.

Material and methods

Hosts were collected at Arroyo Yabotí-Miní (26º57´39.87´´ S, 53º49´23.07´´ W) in Misiones province, Argentina, in January 2005. The temnocephalans emerged when the hosts were relaxed using menthol. Whole mounts were stained with carmine chloride and mounted in synthetic Canada balsam. Serial sections for histology were made in order to study and interpret the morphology and location of organs, particularly the genital system, and the arrangement and development of muscles. Worms were embedded in Paraplast, cut at 4 µm thick, stained with Mayer's Haematoxylin and Eosin and mounted in synthetic Canada balsam.

Two specimens were dissected for extraction of the cirrus. One was mounted in Polyvinyl-Lactophenol for study under optical microscope (OM) and the other was dehydrated, dried, and metalized for study under scanning electron microscope (SEM).

For SEM observation, whole individuals and egg capsules were dehydrated in a graded ethanol series and critical-point dried, coated with gold and examined using a JEOL 6360 SEM.

Photomicrographs were taken with a Zeiss Axioplan 2 Microscope. Nomarski's interference contrast filters were used for cirrus photomicrographs. Measurements were obtained with the aid of an OM; ranges and number of specimens measured are listed in parentheses following the means.

The terminology used for description of reproductive structures follows Cannon (1993). The materials are deposited in the Invertebrate Collection at Museo de La Plata (MLP), Argentina.

Description

Temnocephala lamothei n. sp. (Figs. 1-16).

Based on 17 specimens: 2 whole-mounted adult specimens; 8 fixed adult and juvenile specimens; 2 specimens and 1 cirrus mounted on stubs for SEM; 1 cirrus mounted in polyvinyl-lactophenol; 2 specimens in sagittal sections and 1 in transversal sections; 9 specimens were measured.

External characteristics. Body elliptic, about 2.03 mm (1.10-2.9 mm, 9) long without tentacles, and about 1.10 mm (0.8-1.7 mm, 9) wide (Fig. 1). Posterior adhesive disk subterminal, pedunculate: disk diameter 0.74 mm at rim (0.75-1.15mm, 9). Epidermis syncytial, thin and unciliated. Mosaic of epidermal syncytia not evident.

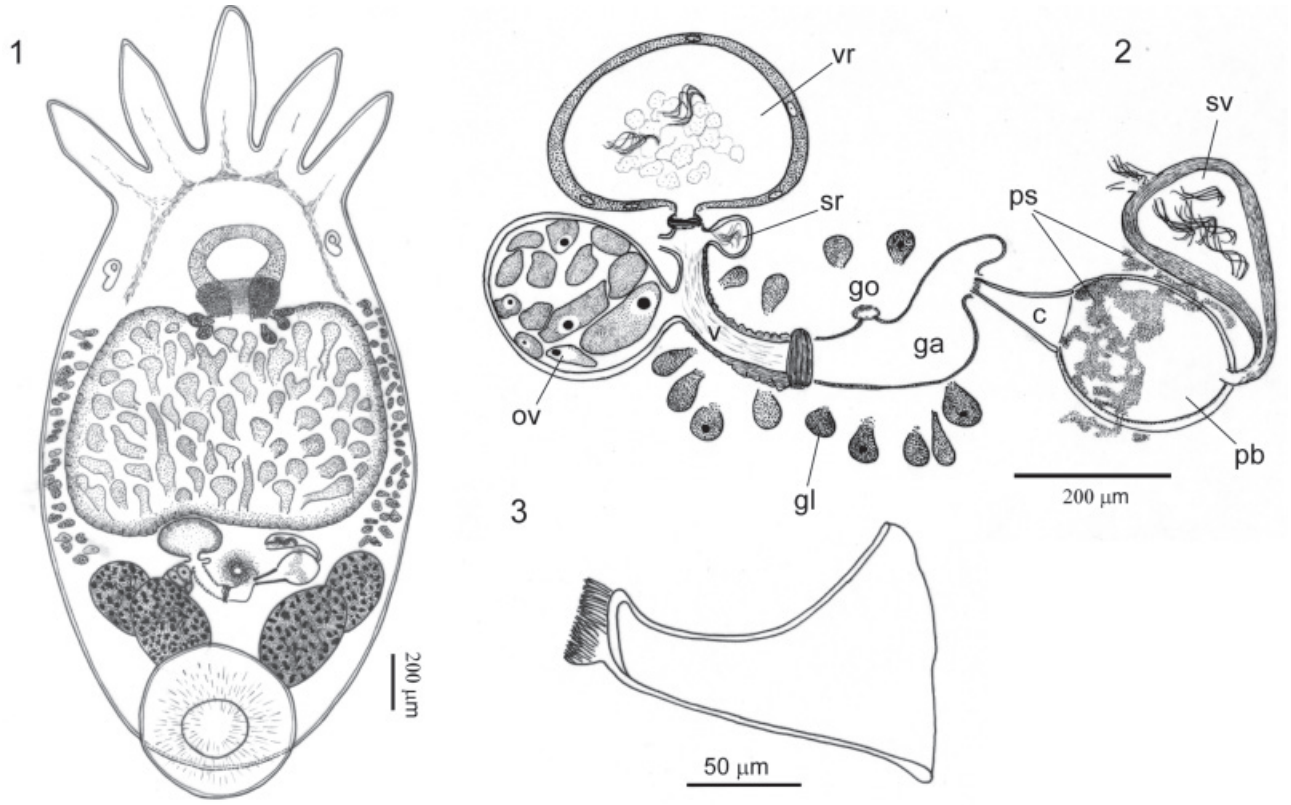
Alimentary system. Mouth mid-ventral, between first and second quarters of body. Pharynx longer than wide, 590 µm long, 363 µm wide, esophageal glands at its base (Fig. 1). In all specimens studied, the pharynx shows a layer similar to a cuticle, that becomes loose in histological samples and can be seen free within the pharyngeal lumen. Intestine saccular, without septa; intestinal walls thick. Paranephrocites not evident.

Excretory system. Excretory pores lateral to mouth, major excretory ducts inconspicuous.

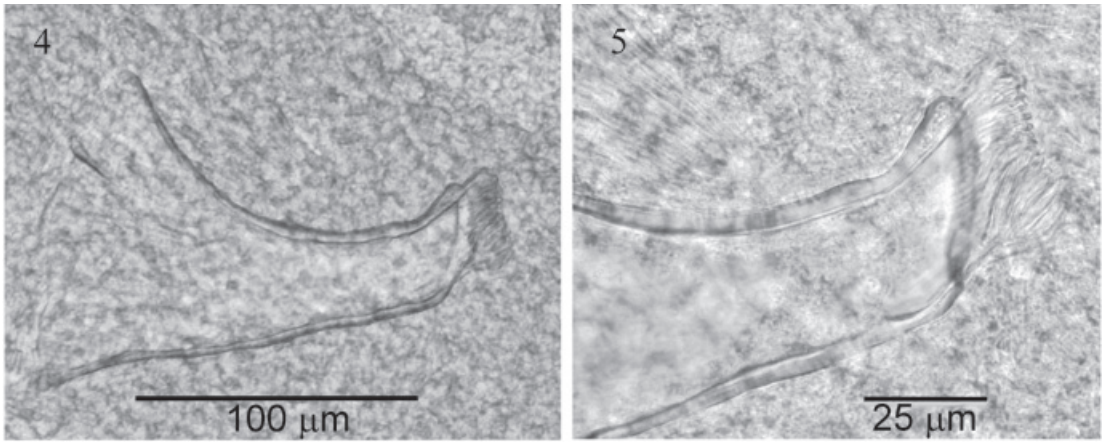
Glands. Rhabdite glands large, numerous, in lateral fields on body, extending onto sides of intestinal sac, with conspicuous rhabdite tracts. Cyanophilus glands inconspicuous, evident only in sectioned specimens, separated from each other in parenchyma, and located among rhabdite glands. Adhesive disks glands scarce and scattered, posterior to posterior testis. Haswell´s cells absent. Shell gland very prominent, near gonopore and opening onto epidermis surrounding gonopore (Fig. 2).

Muscles. Dorsal and ventral circular muscles of body wall similar. Ventral longitudinal muscles of body wall stronger than dorsal ones. Dorso-ventral muscles and attachment muscles of pharynx weak. Muscles controlling male organ strong. Attachment muscles of adhesive disk weak.

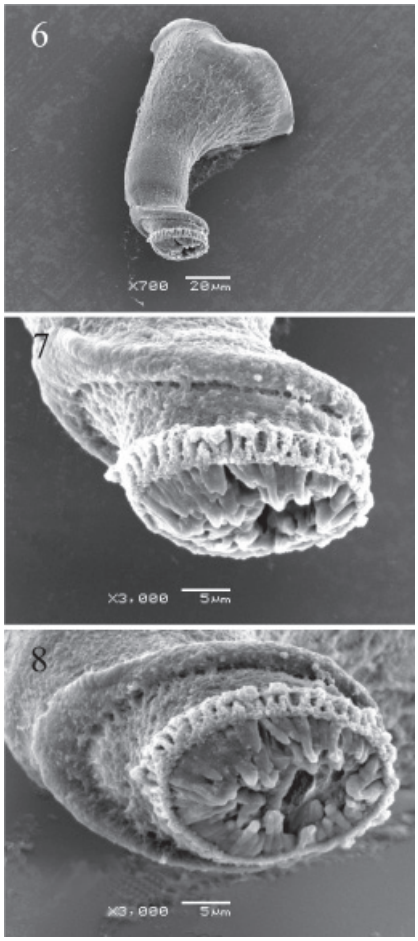
Reproductive system. Male. Four ovoid testes, 2 on each side of body, just behind intestine. Posterior pair oblique, elliptical, larger than anterior testes (Fig. 1). Vasa deferentia extending from inner wall of posterior testes, separately joining a large pyriform thick, seminal vesicle with muscular walls. Seminal vesicle opening into large oval prostatic bulb with muscular walls. Abundant prostatic secretion observed near seminal vesicle and prostatic bulb, entering the latter through its walls. Prostatic bulb prolonged into base of cirrus (Fig. 2). Cirrus curved in lateral view, 167 µm total length; shaft cone-shaped, 146 µm long, 115.5 µm wide at proximal shaft base (Figs. 3-8). Introvert not swollen, proximal margin slightly oblique, marked with a conspicuous, thickened oblique ring, evident under SEM and OM; introvert portion 21.5 µm long, 40 µm wide at its proximal base (Figs. 3-8). Ratio between total length of cirrus and maximum width of shaft at base 5.45; ratio between total length of cirrus and total length of introvert 7.95. Shaft with 1 side straight and the other curved (Fig. 3). Two rows of spines: an inner 1 arising from shaft base, from thickened ring, approximately 11-12 µm long; and an outer row arising from distal margin of introvert, with approximately 45-50 spines, 5-7 µm long (Figs. 6-8).



Figures 1-3. *Temnocephala lamothei* n.sp. 1, diagram of a specimen in dorsal view. 2, reconstruction of the male and female genital systems, showing cirrus (c), genital atrium (ga), glands cells around ootype, genital atrium, and vagina (gl), gonopore (go), ovary (ov), prostatic bulb (pb), prostatic secretion (ps), seminal receptacle (sr), seminal vesicle (sv), vagina (v) and vesicula resorbens (vr). 3, cirrus.



Figures 4-5. *Temnocephala lamothei* n.sp. Photomicrographs of the cirrus seen with Nomarski interference. 4, total view. 5, detail of the introvert showing the spines.



Figures 6-8. *Temnocephala lamothei* n. sp. Cirrus seen with SEM. 6, general view. 7, detail of the introvert in lateral view, showing the thickened oblique ring. 8, frontal view of the introvert showing the 2 rows of spines and their insertion points.

Female. Gonopore mid-ventral, in posterior third of body, surrounded by a muscular sphincter, genital atrium large, elongate (Fig. 2). Most female organs difficult to observe and measure in whole mounts. Ovary small, round; 1 seminal receptacle present, with spermatozoids inside. Vesicula resorbens thick-walled, slightly insinuated into intestinal sac. A short oviduct opening into the ootype, posterior to seminal receptacle. Abundant gland cells around ootype, genital atrium, and vagina, with ducts opening into them. Vagina large and muscular, opening in front of cirrus introvert, with 1 weak sphincter (Fig. 2). Vitellaria dendrite covering dorsal and ventral sides of intestinal sac, never surpassing its limits.

Eggs clavate, 625-800 µm long and 75-350 µm wide (Fig. 9). Polar filament long (115 µm). Opercular plates large, arranged almost perpendicularly to great axis of eggs, so that fracture plane of opercula shows a straight angle respect to great axis of egg (Figs. 10-12). Eggs deposited on external surface of host, on umbilical area, operculum and at contact zone of peristome and suture at opening (Figs. 13-14). Some eggs covered by callus (Figs. 15-16).

Taxonomic summary

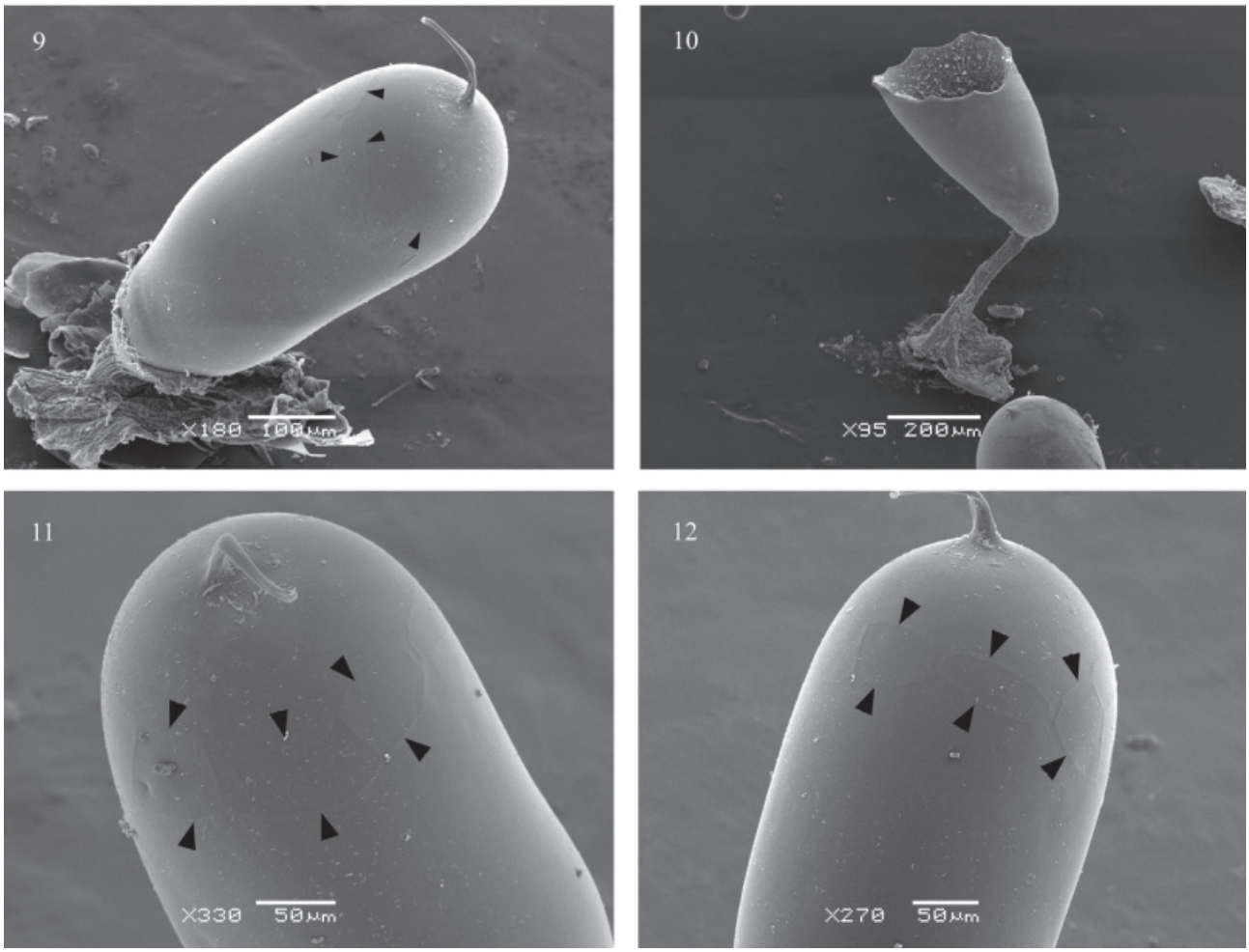
Type host: Pomella megastoma (Sowerby, 1825). Two parasitized snails.
Site: mantle cavity of snail. Numerous eggs fixed over umbilicus and operculum and some eggs within spire.
Type locality: Arroyo Yabotí-Miní (26º57´39.87´´S, 53º49´23.07´´W), Misiones province, Argentina. January 2005.
Helminth specimens deposited: holotype: sagittally sectioned specimen, MLP5718. Paratypes: 2 whole-mounted specimens, MLP5719; 1 dissected cirrus in polyvinyl-lactophenol, MLP5720; 1 sagittally sectioned specimen, 1 transversely sectioned specimen, MLP5721. Other material: 8 specimens preserved in alcohol, unhatched eggs, MLP5722.
Host specimens deposited: 2 specimens, MLP.
Etymology: species named in honor of Dr. Rafael Lamothe Argumedo for his important contribution to the knowledge of helminth diversity.

Remarks

Temnocephala lamothei n. sp. is the fourth species described from mollusc hosts (Gastropoda, Ampullariidae). Despite the great diversity of potential hosts of the family occurring in the Neotropical region, few commensal species of temnocephalans are known. Among them, only *T. iheringi* has been recorded in association with *Pomella megastoma* (=*Asolene megastoma*) (Damborenea et al., 1997) in Argentina.

The mosaic pattern of the epidermal syncytia is constant within *Temnocephala* species. These have only 4 plates: 1 body syncytium, 2 “excretory” syncytia and 1 adhesive syncytium (Damborenea and Cannon, 2001). The shape and size of these plates vary slightly between the species of the genus. Nevertheless, of the 3 known species of temnocephalans from molluscs, only the plate pattern of *T. iheringi* has been described. Unfortunately the specimens described herein were relaxed before fixation, and the plate pattern was not evident.

The 3 known species of *Temnocephala* described as commensals of ampullariids are the most similar to the new species from a morphological point of view. In addition to sharing the same host, they have common morphological



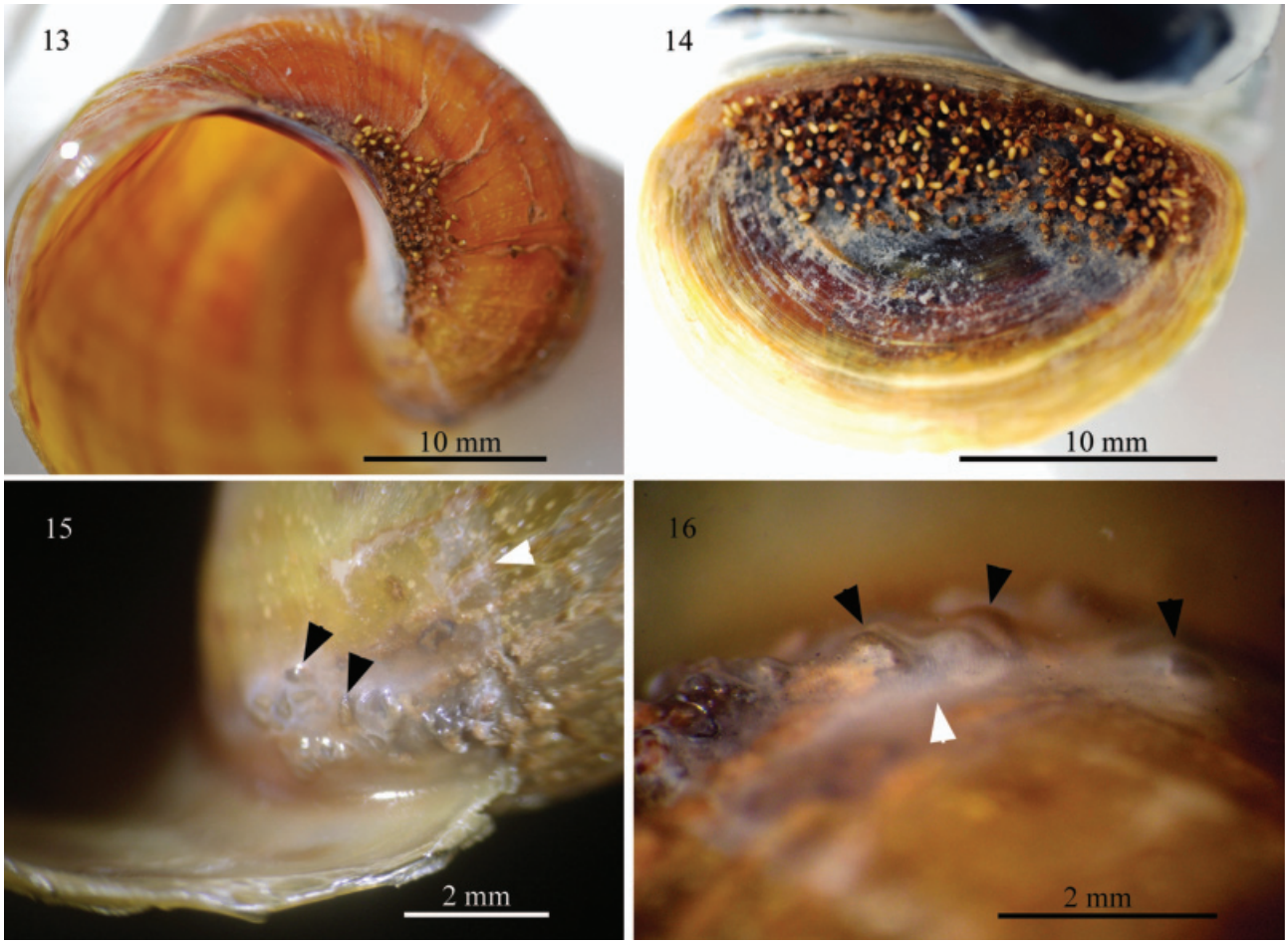
Figures 9-12. *Temnocephala lamothei* n. sp. Egg capsules seen with SEM. 9, general view of an egg capsule and polar filament. 10, open egg capsule. 11, 12, details of the opercular plates in lateral view. Arrows indicate opercular plate limits.

features such as a large sucker (compared to those of other species that are commensals of crustaceans) a non-partitioned intestine, and the absence of paranephrocytes. However, the presence of a strongly sclerotized, oblique ring at the base of the cirrus introvert in *T. lamothei*, as well as the possession of a row of spines at the base of the introvert and another row at its distal end, are characteristic of the new species.

This introvert structure of the cirrus of the new species is unique; unlike the other species, its proximal end is slightly oblique, marked with a conspicuous thickened ring.

In comparison, *T. iheringi* is the species with the cirrus structure more similar to the new species; it is similarly shaped, with 1 flat and 1 concave side. The cirri of these 2 species are also similar in length, although the base of this structure is longer in the new species (the cirrus of *T. iheringi* is approximately 157 µm in total length and approximately 70 µm in basal width (Damborenea, 1992), *vs*. 167 µm and 115 µm respectively in *T. lamothei*). *T. iheringi* bears several rows of spines at the distal end of the introvert.

With respect to the morphology of the distal end of the cirrus, the new species resembles *T. haswelli*. The description of the latter species only mentions a *single crown with digitiform spines* (Ponce de León, 1989). However, a detailed drawing of the distal end of the cirrus shows an arrangement similar to that observed in the new species, *i.e*., with a row of small spines inserted along the distal edge and a row of larger spines. The shape of the cirrus in *T. haswelli* – as in *T. rochensis* – is conical, with both sides curved, differing from the condition observed in the new species, and even longer (200 µm in *T. haswelli* and 186 µm in *T. rochensis*).



Figures 13-16. *Pomella megastoma* with *Temnocephala lamothei* n. sp. Egg capsules. 13, umbilical zone. 14, operculum. 15, callus in the contact zone of the peristome with the suture, showing egg capsules covered by the callus. 16, detail of the peristome zone showing egg capsules covered by the callus. Black arrows indicate egg capsules and white arrows the limits of the callus.

The site of attachment of the eggs of *T. lamothei* n. sp. on the host mollusc is very peculiar. The egg capsules of *T. iheringi* are always laid over the periostracum, especially at the contact zone between the peristome and the suture at the opening, and in the umbilicus. This pattern is repeated with no changes in different populations studied (Damborenea, 1992; 1996; Martín et al., 2005). The site of egg attachment for *T. haswelli* and *T. rochensis* has not been described.

The new species attaches most of its eggs onto the host's umbilicus and over the basal region of the operculum, a feature never recorded in *T. iheringi*. In addition, some eggs are attached onto the contact zone between the peristome and the suture at the opening, so that they are covered by the mantle. Because of this unique placement of eggs, many of them (both hatched and unhatched) were found to be covered by the callus of the host.

The presence of this new species of *Temnocephala*, and its features similar to those of the other species of this genus that are commensals of molluscs, suggests the existence of a morphologically homogeneous group. More detailed studies of all known species, as well as the search for other temnocephalan species in ampullariids present in the Neotropical region, will provide valuable information in the future on the relationships of these species to each other, and to other temnocephalans that are commensals of crustaceans and chelonians. Finally, studies on the relationships among commensal species will contribute new information and a better understanding of the relationships among host species.

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