

ORIGINAL ARTICLE / ARTÍCULO ORIGINAL**A NEW SPECIES OF *TEMNOCEPHALA* (PLATYHELMINTHES, TEMNOCEPHALIDA) IN MOUNTAIN CRABS FROM TOLIMA, COLOMBIA****UNA NUEVA ESPECIE DE *TEMNOCEPHALA* (PLATYHELMINTHES, TEMNOCEPHALIDA) EN CANGREJOS MONTANOS DE TOLIMA, COLOMBIA**Julian Yessid Arias-Pineda^{1-2*}; Cristina Damborenea³ & José Joaquín Castro Avellaneda⁴¹Laboratorio de Zoología y Ecología Acuática LAZOE, Universidad de los Andes, Bogotá, Colombia.
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ABSTRACT

Temnocephala icononcensis n.sp. an ectosymbiont on several species of montane Pseudothelphusidae crabs such as, *Hypolobocera bouvieri*, *Phallangothelphusa dispar* and *Strengeriana cajenensis*, is described from brooks and small streams in the mountains of Tolima, Colombia. Eggs and adult specimens were found on the carapace and orbital cavities of the hosts. The morphology of the species was studied with whole mounted specimens. The most distinctive character of the new species includes the size, shape and introvert morphology of the cirrus and the absence of sphincter in the vagina. The finding of this species in Pseudothelphusidae of Colombia demonstrates the potential diversity of temnocephalans in this country.

Keywords: Colombia - Ectosymbiont - Neotropical region - South America - Taxonomy - *Temnocephala icononcensis* n.sp.**RESUMEN**

Una nueva especie de *Temnocephala* Blanchard, 1849, *Temnocephala icononcensis* n.sp. ectosimbionte de varias especies de cangrejos montanos de la familia Pseudothelphusidae, *Hypolobocera bouvieri*, *Phallangothelphusa dispar* y *Strengeriana cajenensis*, encontrado en quebradas y pequeños arroyos en las montañas del Tolima, Colombia. Huevos y adultos de los especímenes fueron encontrados en el caparazón y cavidades orbitales de los hospederos. La morfología de la especie fue estudiada con ejemplares enteros montados. El carácter más distintivo de la nueva especie incluye el tamaño, la forma y la morfología del introvertido de los cirros y la ausencia de esfínter en la vagina. El hallazgo de esta especie en cangrejos de la familia Pseudothelphusidae para Colombia demuestra la diversidad potencial de temnocéfalos para este país.

Palabras clave: Colombia - Ectosimbiontes - Región Neotropical - América del sur - Taxonomía.

INTRODUCTION

Temnocephala Blanchard, 1849 is a genus of commensal turbellarians, endemic to the Neotropical region (Pereira & Cuocolo, 1941; Cannon, 1993; Damborenea & Cannon, 2001; Garcés *et al.*, 2013). The first species described was *T. chilensis* (Moquin-Tandon, 1846) from the anomuran crab *Aegla laevis* (Latreille, 1818) from Chile. Since then around 33 species have been described, associated to freshwater invertebrates (*i.e.* Crustacea: Brachyura, Anomura, Caridea and Astacidea; Mollusca: Ampullariidae and Neritidae; Insecta: Belostomatidae, Naucoridae and Odontoceridae) and turtles (*i.e.* Chelidae and Emydidae) (Garcés *et al.*, 2013; Martínez-Aquino *et al.*, 2014; Seixas *et al.*, 2015). Brazil, Argentina and Uruguay are the countries where these species have been studied more in depth.

The freshwater crab fauna encountered in the neotropics includes two families (Trichodactylidae and Pseudothelphusidae) with approximately 311 species (Cumberlidge *et al.*, 2014). Despite the great species diversity, only eight temnocephalan species have been described associated with seven species of Trichodactylidae (*i.e.* *Dilocarcinus pagei* Stimpson, 1861, *D. septemdentatus* (Herbst, 1783), *Sylviocarcinus australis* Magalhães & Türkay, 1996, *S. pictus* (H. Milne Edwards, 1853), *Trichodactylus fluviatilis* Latreille, 1828, *T. panoplus* (von Martens, 1869), and *T. petropolitanus* (Göldi, 1886), and only five with Pseudothelphusidae (*Hypolobocera henrici* Pretzmann, 1978, *Kingsleya ytupora* Magalhães, 1986, *Pseudothelphusa tristani* Rathbun, 1896, *P. jouyi* Rathbun, 1893, and *Telphusa* sp.) (Martínez-Aquino *et al.*, 2014).

Pseudothelphusidae has the highest species diversity in the rivers and streams of the mountains on Colombia and Ecuador.

Colombia, considered as a biodiversity hotspot area, has 88 Pseudothelphusidae described species, being 78 of them endemic (Cumberlidge *et al.* 2009, 2014). However, no temnocephalan species associated with crustaceans are recorded for this country. Only *Temnocephala colombiensis* Garcés *et al.*, 2013, has been described in association with the snail *Pomacea* sp. from the Department of Antioquia, Colombia (Garcés *et al.*, 2013).

During a research work done in Tolima, Colombia, between 2009 and 2012, several temnocephalans specimens were found associated with three pseudothelphusid species. These individuals were identified as a new species, which is described in this article. The new species was compared with the morphology of other temnocephalan related to Pseudothelphusidae.

This finding is very important since it is the first *Temnocephala* species found living in association with crabs from Colombia. Considering the unique characteristics of the crab fauna in Colombia, this discovery indicates the high potential diversity of *Temnocephala* waiting to be studied.

MATERIALS AND METHODS

Eighty eight crabs belonging to *Phallangotelphusa dispar* (Zimmer, 1912), *Strengeriana cajaensis* Campos & Rodriguez, 1993 and *Hypolobocera bouvieri* (Rathbun, 1898) were collected on brooks and streams from Caferria, Hoya Grande, Pie de Cuesta and Valparaiso, Icononzo Municipality in Tolima Department (the samples were taken between 4°06.515 N - 74°35.395 W and 4°06.669 N - 74°35.215 W) (Table 1). The crabs were manually collected from March 2009 to September 2012. Females and juveniles crabs were released alive in the sample site. Crabs were examined in the field searching for temnocephalans.

Approximately 50 commensals were recovered from the coxae and pereopods of seventeen crabs. The crabs were determined by specialized key Campos 2005 and deposited in the collection of arthropods and other invertebrates CAUD-216. The turbellarians were fixed at the moment in 70% ethanol and were transported to the laboratory for posterior processing. The worms were flattened between cover and slide, transferred to AFA and kept in this solution overnight. The AFA solution was replaced by ethanol 50%, followed by 70%, and stained overnight with acetocarmine. Then the *Temnocephala* were clarified in acidified ethanol (0.5 ml HCl in ethanol 70%), dehydrated in ascending series of ethanol (80%, 90%, and 100%) for 15 min in each, cleared with a mixture of 1:1 ethanol methyl salicylate, and later in 100% methyl salicylate. The specimens were transferred to xylene and mounted in permount mounted medium (Fisher Scientific Co., N. J.).

Measurements were taken from fixed and mounted specimens and expressed in microns (μm), unless specified otherwise. The mean is shown, followed by the number of specimens measured and the size range. The terminology used for the description of reproductive structures, were recognized using Cannon's (1993) diagnosis. The specimens were photographed and drawn using the programs Corel Draw X5 and Photoshop.

The studied material was deposited in the Colección Artrópodos y otros Invertebrados, Universidad Distrital Francisco José de Caldas (CAUD), Bogotá, Colombia and in the Museo de Historia Natural, Colección de Invertebrados, Universidad de los Andes (ANDES-IN), Colombia.

RESULTADOS

Family Temnocephalidae Monticelli, 1899
***Temnocephala* Blanchard, 1849**

***Temnocephala icononcensis* n. sp.**
(Fig. 1-4)

Specimens examined: 30 specimens of *Temnocephala icononcensis* n. sp, collected in 17 montain crabs: *Hypolobocera bouvieri*, n=3, *Phallangothelphusa dispar*, n=8 and *Strengeriana cajaensis*, n=6.

Type locality: Colombia, Tolima, Icononzo, Cafreterias, Las Lajas brook, 4° 06' 51" N 74° 35' 39" W, 1,029 meters above sea level; July 2th, 2010; collected by J. Y. Arias-Pineda and R. Barbosa Cornelio.

Etimology: The species has been named after the locality were they were collected, Icononzo, Tolima, Colombia.

Holotype: Specimen stained and mounted *in toto*, deposited in CAUD 216-TEM-0001.

Paratypes: Ten specimens stained and mounted *in toto*, deposited in ANDES-IN (3125), ANDES-IN (3126), ANDES-IN (3127), ANDES-IN (3128), ANDES-IN (3129), ANDES-IN (3130), CAUD 216-TEM-0002, CAUD 216- TEM-0003, CAUD 216- TEM-0004, CAUD 216- TEM-0005, CAUD 216-TEM-0006.

Host species: *Hypolobocera bouvieri* (Rathbun, 1898) CAUD 216 -DEC-0004, CAUD 216-DEC-0006; *Phalangothelphusa dispar* (Zimmer, 1912) CAUD 216-DEC-0001, CAUD 216-DEC-0002, CAUD 216-DEC-0003; and *Strengeriana cajenensis* Campos & Rodriguez, 1993, CAUD 216-DEC-0005, CAUD 216-DEC-0007.

Site of infection: Coxopodite of ambulatory legs, around the eyes and on the ventrolateral sides of the carapace.

Type Host: *Phallangothelphusa dispar* (Zimmer, 1912), Colombia, Tolima, Icononzo, Cafreterias, Quebrada La Fría, 1,029 a 1,258 m.a.s.l., 1 ♂ (ancho del caparazón 30.7 mm, largo del caparazón 28.5 mm). Juny 2th, 2009, collected by J. Y. Arias-Pineda and R. Barbosa Cornelio (Fig. 1A-B).

Description

External features. Body ovoid, adult length

without tentacles 1,403.40 μm (n=30; 1000 μm - 1840 μm), maximum width 804.49 μm (n: 30; 560 μm - 1040 μm). Adhesive disk circular, diameter 320 μm (n: 30, 220 μm - 400 μm), occupying the third posterior of the body, with a small peduncle (Figs. 1C-D, 2A and 3A). Eyespots red. The excretory syncytial plates were not observed. Genital pore located in the middle portion of the body.

Digestive system. A large muscular ovoid pharynx, width 296.56 μm (n: 30, 200 μm - 450 μm), length 227.58 μm (n: 30; 160 μm - 320 μm), was found in the first third of the body (Fig. 3C). The intestinal sac is wide and ends at the level of the middle of the body, not surpassing it.

Excretory system. A pair of excretory vesicles very conspicuous, located at the same level of

the mouth. The excretory pores are very evident (Figs. 2A and 3F). Glands. Rhabditogen glands formed by numerous globular, elongated glands, creating clusters of cells on the lateral sides of the body, extending from the caudal region of the intestine to the level of the excretory pore, nor surpassing it (Figs. 2A and 3A). In the caudal region, posterior to the intestine sac, adhesive glands are present. Their ducts are very evident and converge in the adhesive disk (Fig. 3E). In this caudal region, in a more central position, two big cells, paranephrocytes, are observed.

Female reproductive system. Ovary circular, small, 73.5 μm (n: 30; 56.8 μm - 91.3 μm) long, 54 μm (n: 30; 37 μm - 67 μm) wide. Vesicular resorbens rounded, 82.3 μm (n: 30; 40.3 μm - 80.2 μm); its anterior wall contact the intestinal wall. Seminal receptacles are not observed. A

Table 1. Samples sites at Icononzo (Tolima, Colombia) and host species. The samples were taken between 4°06.515 N - 74°35.395 W and 4°06.669 N - 74°35.215 W.

Host	Localities	Altitude	Date
<i>Hypolobocera bouvieri</i> 1 ♂ MUD 046 -DEC-0006	Icononzo, Pie de Cuesta, Quebrada Mataburros	1150	March 28th 2009
<i>Phallangothelphusa dispar</i> 1 ♂ MUD 046-DEC-0003	Icononzo, Cafreterias, Quebrada La Fría,	1029	June 2nd 2009
<i>Phallangothelphusa dispar</i> 3 ♂, 3 ♀ MUD 046 -DEC-0001	Icononzo, Hoya Grande, Quebrada Mataburros	1119	August 25th 2009
<i>Phallangothelphusa dispar</i> 3 ♂, 1 ♀ MUD 046 -DEC-0002	Icononzo, Cafreterias, Quebrada Las Lajas	850	October 2009
<i>Strengeriana cajaensis</i> 3 ♂, 2 ♀ MUD 046 -DEC-0007	Icononzo, Cafreterias, Quebrada Las Lajas	1450	April 2nd 2010
<i>Strengeriana cajaensis</i> 5 ♂, 2 ♀ MUD 046 -DEC-0005	Icononzo, Pie de Cuesta, Quebrada Las Lajas	1320	June 2nd 2010
<i>Hypolobocera bouvieri</i> 1 ♂ MUD 046 -DEC-0004	Icononzo, Cafreterias Quebrada La Lajas	1150	September 14th 2012

wide vagina runs to the genital atrium. No conspicuous vaginal sphincter is observed. Vitelline glands branched, covering the dorsal and ventral surface of the intestine. Genital atrium large and elongated. Genital pore encircled by abundant small cement glands

(Figs. 2B, 3A-B and D). Eggs claviform, fixed by a peduncle, 220 y 330 μm long by 30 y 63 μm wide, subpolar filament (Figs. 1E-G). Opercular plates at small angle respect to longitudinal axis, the fracture plane is oblique (Fig. 2D). Male reproductive system. Four

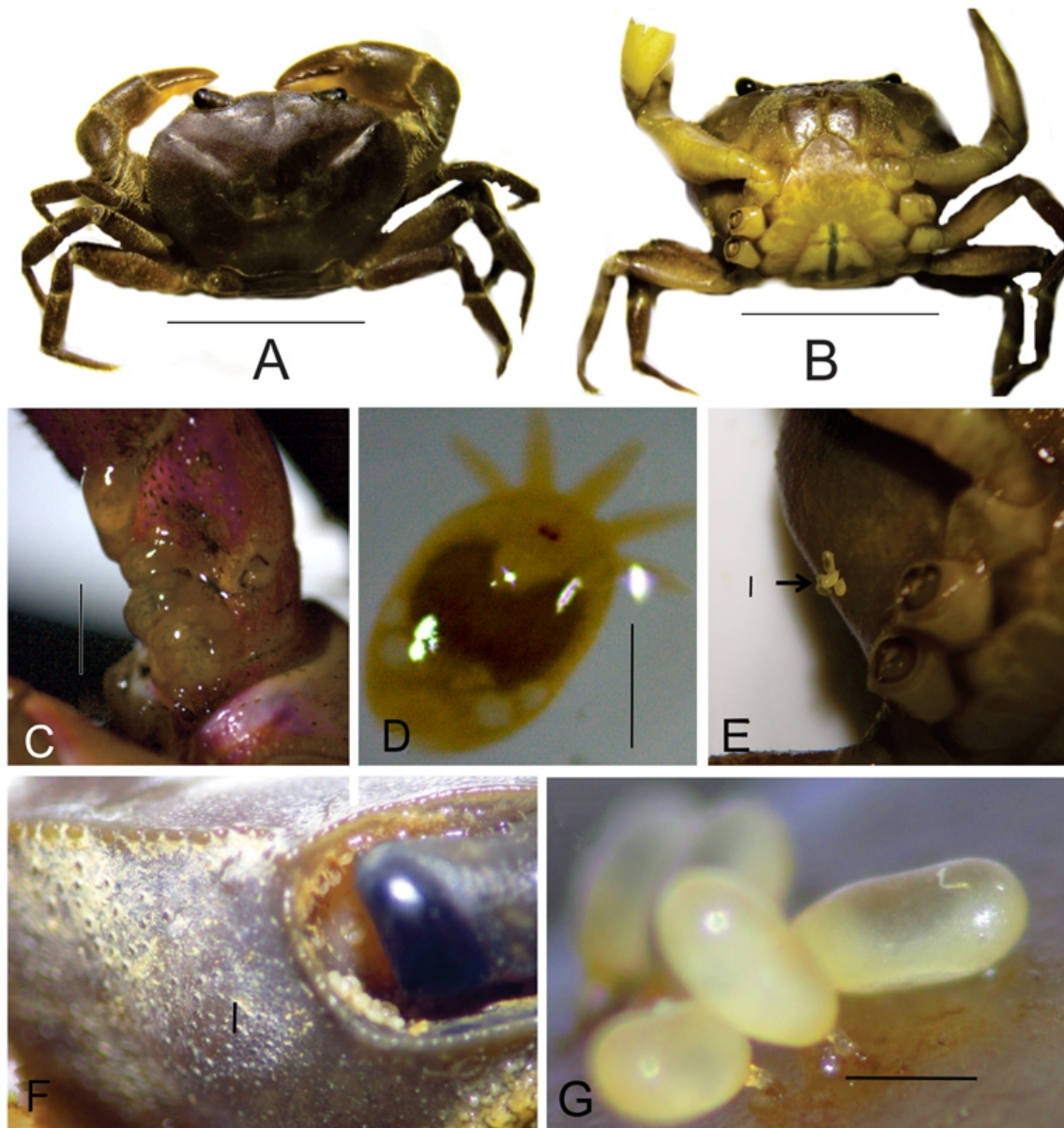


Figure 1. *Phallangothelphusa dispar* and *Temnocephala icononcensis* n. sp. A-B, dorsal (A) and ventral (B) view of *P. dispar*, (scale bars: 25 mm); C-D, specimens of *T. icononcensis* n. sp. on a pereiopod of a crab (C, scale bar: 1,500 μm) and detail of an adult (D, scale bar: 1,000 μm); E-F, eggs capsules of *T. icononcensis* n. sp. deposited on the carapace (E) and on the orbital angle of *P. dispar* (F) (scale bars: 350 μm); G, detail of the egg capsules (scale bar: 200 μm).

testes, anterior testes 74.02 μm (n = 30, 61.7 μm - 83.98 μm) long, 75.7 μm (n: 30, 61.8 μm - 86.45 μm) wide; posterior testis 96 μm (n: 30, 70 μm - 113 μm) long, 94 μm (n: 30, 80 μm - 113.6 μm) wide. The anterior pair of testicles

lateral to the posterior region of the intestine, at the level of the ovary, posterior testis posterior to the anterior ones, slightly overlapping. Seminal vesicle small and elongated (Figs. 2B-C, 3B-C and 4). Cirrus curved in the third distal

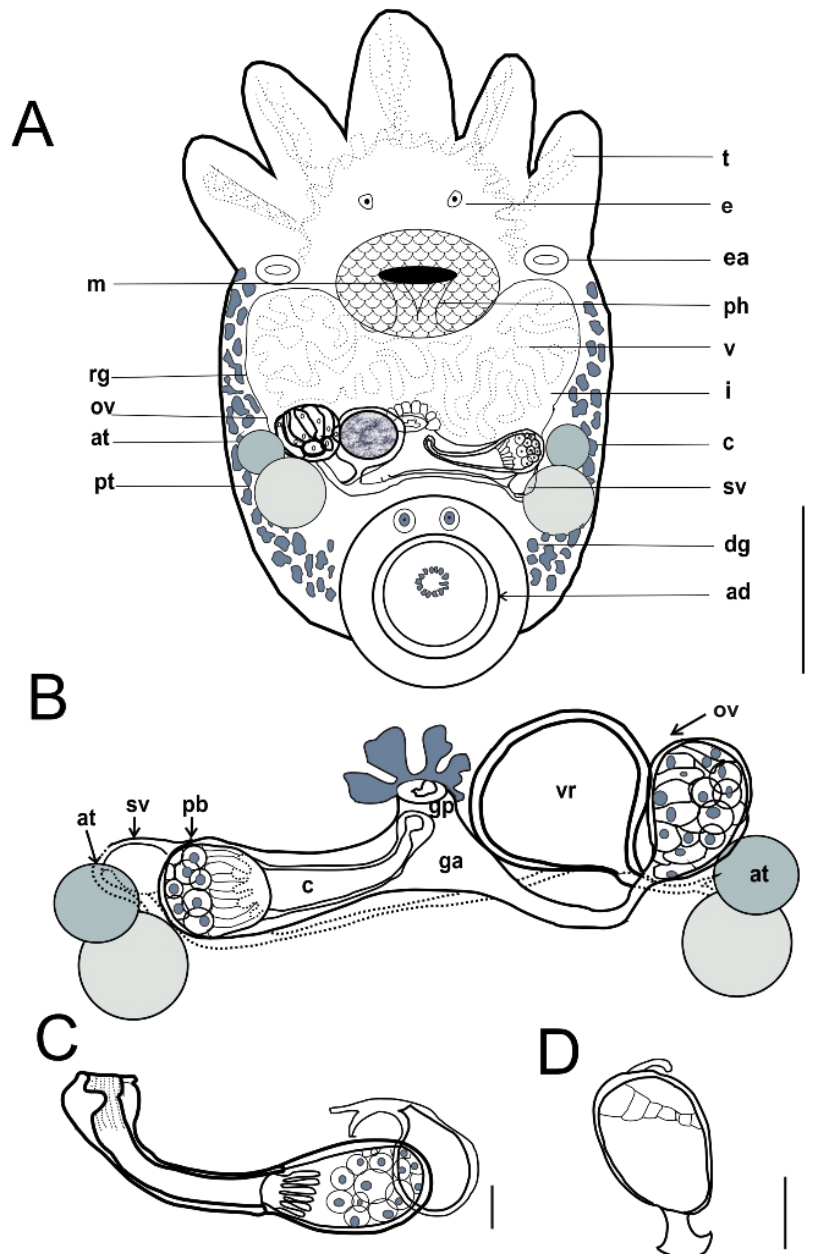


Figure 2. *Temnocephala icononcensis* sp., holotype. A, adult specimen (scale bar: 250 μm); B, detail of the reproductive system (scale bar: 100 μm); C, detail of the distal male reproductive system; D, egg and plane of fracture of the operculum, filament, peduncle (scale bar: 100 μm). ad: adhesive disk, at: anterior testes, c: cirrus, dg: adhesive disk glands, ea: excretory ampullae, ga: genital atrium, gp: genital pore, i: intestinal sac, m: mouth, ov: ovary, pb: prostatic bulb, ph: pharynx, pt: posterior testis, rg: rhabditogenic glands extending along sides of intestinal sac, sv: seminal vesicle, t: tentacles, v: vitellarium, va: vagina, vr: vesicula resorbens, arrow: paranephrocyte.

part, with the concave curvature towards the intestine; 242.45 μm (n=30; 120 μm - 300 μm) long, basal wide 75.42 (n: 30, 50 μm - 110 μm), cirrus introvert 26.25 μm (n: 30, 25 μm - 30 μm) long, with 30-40 rows of spines, each with approximately 30 spines. The introvert

expands slightly. Some specimens has the distal part curved (Fig. 4 D) almost at right angle, while others are almost straight (Fig. 4E). The number of rows of spines can vary between individuals.

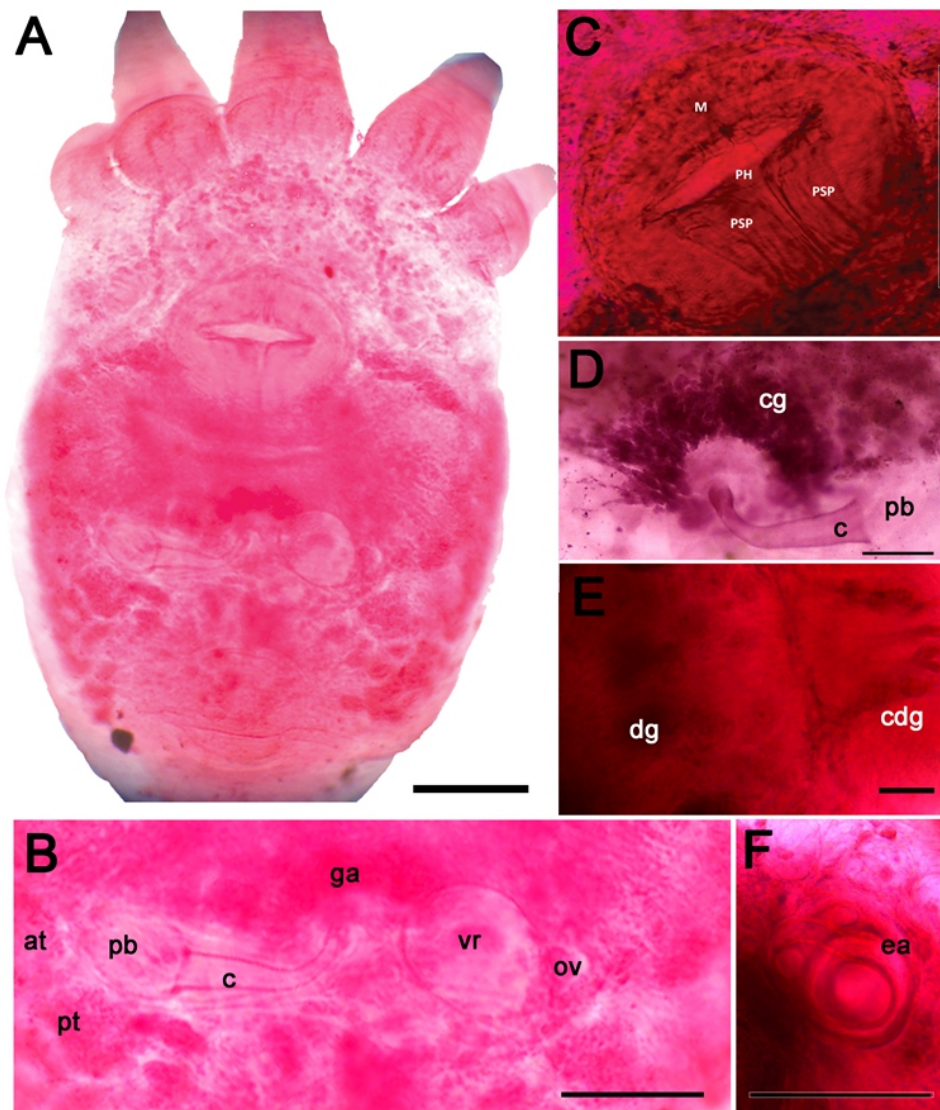


Figure 3. *Temnocephala icononcensis* n. sp., A, general view of the holotype (scale bar 200: μm); B, detail of the genital system (scale bar 200: μm); C, detail of the pharynx (scale bar 200: μm); D, cirrus and the gonopore showing the cement glands (scale bar: 200 μm); E, detail of the posterior region, the conducts of the adhesive glands are evident (posterior end to the right) (scale bar: 50 μm); F, excretory ampullae (scale bar: 50 μm).at: anterior testes, c: cirrus, dg: adhesive disk glands, ea: excretory ampullae, m: mouth, ov: ovary, pb: prostatic bulb, ph: pharynx, pt: posterior testis, vr: vesicula resorbens.

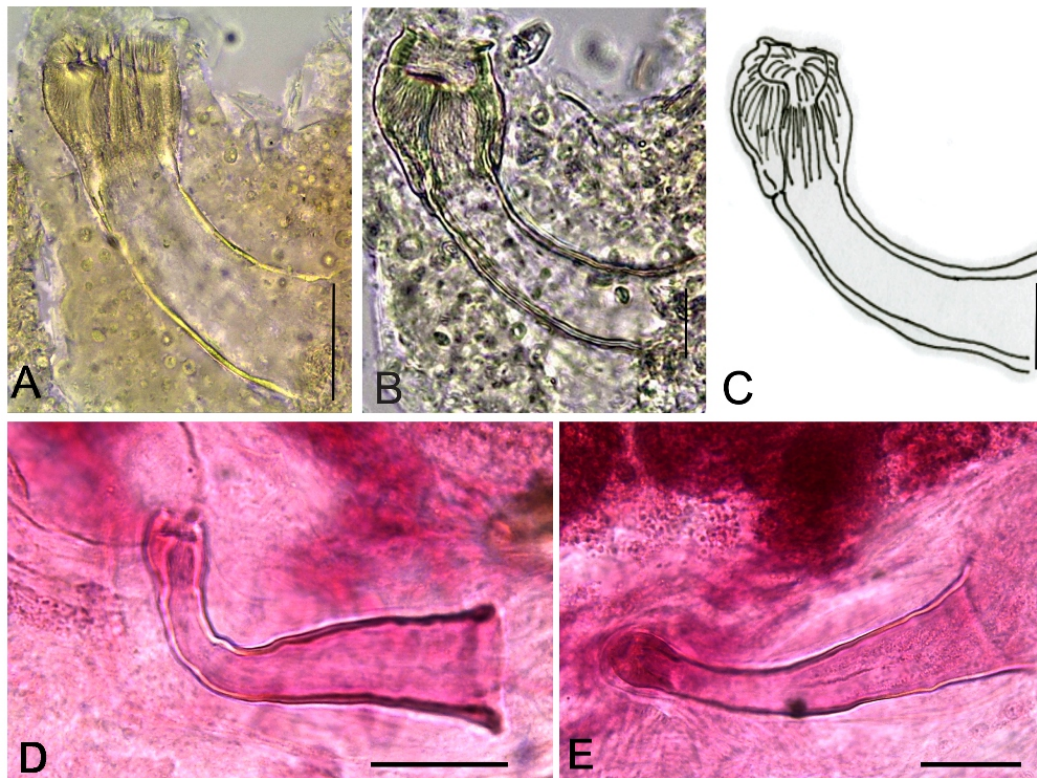


Figure 4. Cirrus structure of *Temnocephala icononcensis* n. sp. A-B, Photography from a dissection of a juvenile specimen, cirrus showing fine spines (scale bar= 50 µm); C, line drawing of cirrus (scale bar= 10 µm); D-E, cirrus of two specimens showing variation in the curvature (scale bar: 100 µm).

DISCUSSION

Only one species of temnocephalan, *Temnocephala colombiensis*, is known for Colombia, and lives associated with *Pomacea* sp. (Mollusca, Ampullariidae) (Garcés *et al.*, 2013). *Temnocephala icononcensis* n. sp. is the first temnocephalan species living on crustaceans for Colombia.

Five temnocephalan species associated with Pseudothelphusidae are known: *Temnocephala costarricensis* Lamothe-Argumedo, 1974 from Costa Rica (Lamothe-Argumedo 1974); *T. kingsleyae* Damborenea, 1994 and *T. lutzi* Monticelli, 1913 from Brazil (Damborenea 1994, Pereira and Cuocolo,

1941); *T. mexicana* Vayssiere, 1898 from Mexico (Lamothe-Argumedo 1968) and *T. peruensis* Ibáñez Herrera & Jara, 2003 from Peru (Ibáñez & Jara 2003). *T. lutzi* was originally described in association with *Telphusa* sp. (Monticelli 1913). Posteriorly, this specie was frequently found associated with trichodactylids crabs in Brazil, Peru and Uruguay (Pereira & Cuocolo 1941, Damborenea 1994, Amato *et al.* 2005, Volonterio 2007) and it is discussed by Amato *et al.* (2005), that the original host of *T. lutzi* might be misidentified. Also, different concerns about this species have been mentioned by Volonterio (2007), due to different morphology interpretations. On the other hand, the description of *T. peruensis*, associated with *Hypolobocera henrici*, is

insufficient for an accurate identification of the species.

The cirrus morphology of *Temnocephala icononcensis* n. sp. is unique, and it could not be compared with other *Temnocephala* species from Pseudothelphusidae. The temnocephalan species with a comparable introvert with one of the new species is *Temnocephala chilensis*, species associated with several species of *Aegla* sp. (Crustacea, Aegliidae) and *Samatacus* sp. and *Parastacus* sp. (Crustacea, Parastacidae). *T. chilensis* has an introvert more expanded than the new species, and the spines are stronger than the ones in the new species; however, the size and the shape of the cirrus in both species are different (Dioni, 1967, 1972; Damborenea, 1991, 1992). The cirrus of *T. chilensis* is shorter (149.59 µm long and 62.91 µm basal width), after Damborenea (1992) and the cirrus never has a curvature so pronounced as observed in the new species. Other feature shared by both species is the lack of a vaginal sphincter.

Among the limitations of this study, the first was the difficult access to the study area, and difficult collection of specimens by the topography of the area. The host and temnocephalan populations vary with respect to the season, being in some months more than others.

The cirrus morphology is the most relevant feature for the identification of temnocephalid species. Other relevant structures for species diagnosis are the presence and development of vaginal sphincter, the general morphology of the genital organs, and the pharynx. All these characters could be studied and described. Size and morphology of the excretory syncytial plates are features that allow comparison between the temnocephalan species. Unfortunately, the syncytial plates are not known for temnocephalan species from Pseudothelphusidae, and they could not be studied for the new species. Future studies of

the excretory plates of *Temnocephala icononcensis* n. sp. and of other known species from Pseudothelphusidae will be relevant to analyze the relationships between the species that share the host family.

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