



## ***Argulus ventanensis* sp. n. (Crustacea, Branchiura) parasite of *Hypsiboas pulchellus* tadpoles (Anura, Hylidae)**

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**Abstract.** *Argulus ventanensis* is described as a new species of crustacean branchiuran ectoparasitic on *Hypsiboas pulchellus* tadpoles. It was collected from the Belisario stream in Villa Ventana, Buenos Aires province, Argentina (38° 4' S, 61° 55' W). The most striking morphological differences between *A. violaceus*, a sympatric congener, and the new species are the body size, development of the lateral sinus, the extent and armature of antennae, armature of the rods supporting the suckers (first maxillae), the proportional length of the second antennal segment in respect to the third, the length of the flagellae on the first and second legs, the basal plate of maxillae, the number of setae on the caudal rami and the form of the respiratory areas. It also differs from *A. patagonicus* in the development of the carapace which only covers the first two pairs of legs, the general colouring, the form of the post maxillary spines, spatulate versus pointed, the flagellae on the first and the second pair of legs and the number of setae on the caudal rami.

**Keywords:** tadpoles, crustacean parasites, branchiuran, *Argulus*, amphibian

**Resumen.** *Argulus ventanensis* sp. n. (Crustacea, Branchiura) parásito de renacuajos de *Hypsiboas pulchellus* (Anura, Hylidae). Se describe, ilustra y propone una nueva especie de crustáceo branquiuro, *Argulus ventanensis* sp. n. ectoparásito de renacuajos de la rana del zarzal, *Hypsiboas pulchellus*. El material estudiado procede del arroyo El Belisario, perteneciente al sistema hidrográfico de Ventania, en la provincia de Buenos Aires, Argentina (38° 4' S, 61° 55' O). La nueva especie se caracteriza por estar dentro de las más pequeñas del género y se diferencia de sus congéneres simpátricos *A. violaceus* y *A. patagonicus* por el tamaño corporal, el seno lateral levemente marcado, la extensión y armadura de antenas y anténulas, la armadura de los escleritos de sostén de las ventosas (maxilas I), el largo proporcional del segundo segmento antenal respecto del tercero, la longitud del flagelo en los primeros dos pares de patas nadadoras, la placa basal de las maxilas II, el número de setas en los rami caudales y la forma del área respiratoria.

**Palabras clave:** renacuajos, crustáceos parásitos, branchiuros, *Argulus*, anfibios

### **Introduction**

The Branchiuran family Argulidae includes about 149 valid species distributed in four genera, *Argulus* Müller, 1785, *Chonopeltis* Thiele, 1900, *Dipteropeltis* Calman, 1912 and *Dolops* Audouin, 1837, the first being the most abundant and diverse (Neethling & Avenant-Oldewage, 2016). The greatest diversity of genera and species is located in the tropics of Africa and the Neotropical region

(Poly, 2008; Neethling & Avenant-Oldewage, 2016). However, the systematic composition of this group is unresolved as some species are poorly described or recorded under more than one different name, with vague or dubious citations. These semi-permanent, intermittent ectoparasites or micropredators (Lafferty & Kuris, 2002) are characterized by their ubiquity in natural ecosystems, their low host specificity and the

potential damage to their hosts (Ringuelet, 1943, Oprean & Vulpe, 2002, Ziliukiene et al. 2012). The genus *Argulus* affects mainly fresh water fish (85 described species), with some records in marine and estuarine fish (44 spp.), as well as agnathans (Cochran et al. 1992), urodele amphibians (Bower-Shore, 1940; Poly, 2003) and larvae of anurans (Goin & Ogrre, 1956; Clark, 2001; Wolfe et al. 2001). The frequency with which they change their hosts also positions them as potential vectors of infectious agents (Shimura et al. 1983; Ahne, 1985; Landsberg, 1989; Paperna, 1996). They feed greedily on the hosts' plasma and red blood cells (Ringuelet, 1943; Hogans, 1994, Walker et al., 2004). Open lesions made when the branchiuran leaves the attachment sites allow access for colonization by opportunistic fungi, such as *Saprolegnia* (Paperna op. cit.). Molnár & Székely (1998) reported the occurrence of nematode larvae of the Skrjabillanidae on its intermediate host, *Argulus foliaceus* L. Moravec et al. (1999) suggested the role of an intermediate host for an unnamed species of *Argulus* as a potential vector of larvae of the nematode *Mexiconema cichlasomae* Moravec, Vidal-Martínez and Salgado-Maldonado, 1992. Later, May-Tec et al. (2013) evaluated the seasonal variation in *M. cichlasomae* on its intermediate host, *Argulus yucatanus* Poly, 2005, in a coastal lagoon in Southeast Mexico.

The aim of this contribution is to describe a new species of the genus *Argulus* that parasitizes larvae of *Hypsiboas pulchellus* (Duméril & Bibron, 1841) in streams of the hydrographic system of Sierra de la Ventana (Argentina). This is a new association between amphibians and argulids.

## Materials and Methods

The sample comes from the Belisario stream in Villa Ventana, Buenos Aires province, Argentina (38° 4' S, 61° 55' W). Tadpoles of *H. pulchellus* were collected by means of a hand net with 5mm mesh. The specific identification of the tadpoles followed Kehr & Williams (1990), and the ontogenetic criteria of Gosner (1960). The argulids were collected alive, fixed and conserved in 10% formalin. Later they were transferred to 70% ethanol for deposition in the collection at the La Plata Museum (MLP). The specimens were mounted without any previous clearing and they were illustrated with the aid of a camera lucida. All measurements were made with an ocular micrometer.

### *Argulus ventanensis* sp. n.

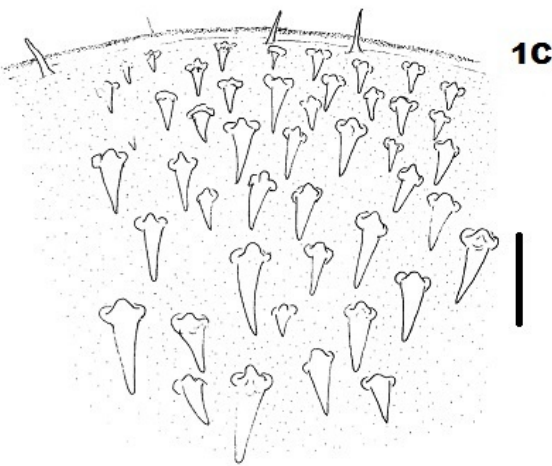
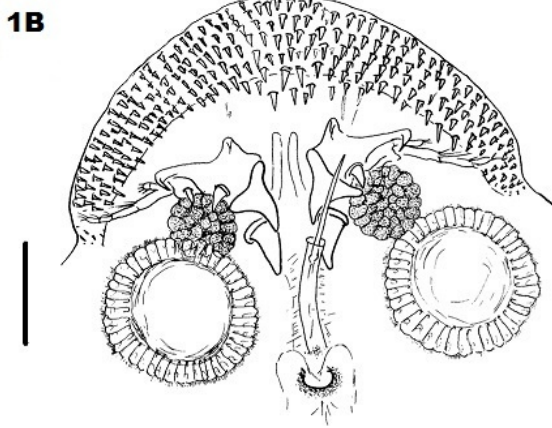
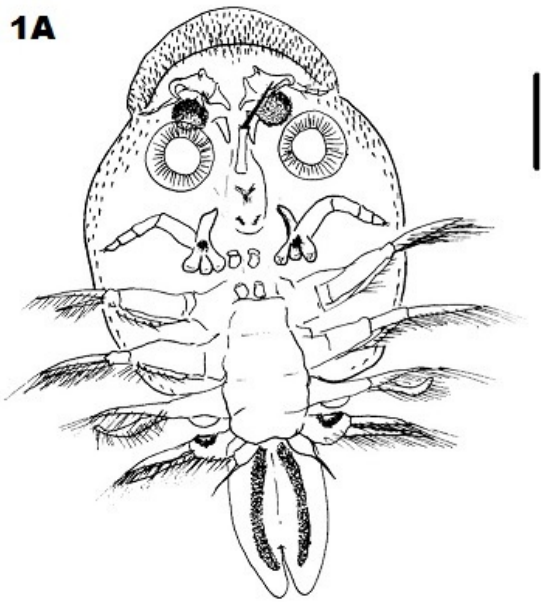
#### Description

**Adult male** (All measurements are given in Table I): General body form slightly orbicular; carapace comprising 63% of total body length. Antero-lateral depressions slightly discernible (Figure 1A). Frontal spiny region of head delineated by thin chitinous suture. Frontal margin of carapace with row of minute sensillae. Dorsal surface smooth, slightly pigmented. Ventral surface of head densely ornamented with pointed spines regularly arranged, median spines (mean length = 32.5 µm) larger than lateral ones (mean length = 15 µm) and extending to margins of carapace, from level of maxillae as single row of minute spines (Figure 1B and 1C).

Lateral lobes of carapace broadly rounded, covering third pair of swimming legs; broad sinus about 15% of length of carapace. Compound eyes (170 µm in diameter, interocular distance= 300 µm) at level of antero-lateral sinuses and basal segments of antennae. Ratio of ocular diameter/carapace width (at level of ocular region) 0.25. Nauplius eye conspicuous with one anterior and two posterior lenses. Interocular "ribs" long with median curvature and short projections to level of nauplius eye. Pair of strong post-antennular spines present in interocular space. Posterior cephalic groove "U-shaped" and well developed. Two pairs of respiratory areas on ventral surface in tandem, anterior subtriangular or ovoid, smaller, about 18% of length of posterior. Posterior respiratory area with deep medial notch in internal margin (Figure 2).

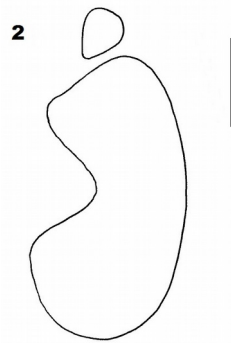
Thorax 4-segmented expanded, about 1/3 ventral surface, and bearing four swimming legs. Abdomen oblong, 27% of body length with ventral surface densely ornamented with minute spines. Dorsal surface smooth. Abdominal sinus short, 37% the length of the abdomen. Testes visible occupying almost total length of abdomen. Two small furcal rami at base of sinus bearing five apical setae, one minute. Abdominal margin covered with spines.

First and second antennae at level of antero-lateral sinuses, well developed, medial, moderately armed with spines, setae and hooks. First antenna divided into two sections, basal robust section and small distal two-segmented section (designated as "antennal palp" by several authors) reaching midpoint of second segment of antenna II. Basal section with first segment triangular, prolonged into large pointed posterior antennular spine. Second segment roughly polygonal bearing anterior spine forming hooklet, medial conical spine at base and large terminal spine ("lateral hook"). Third segment smooth and twice as large as fourth. Terminal segment with group of four setae and small conical

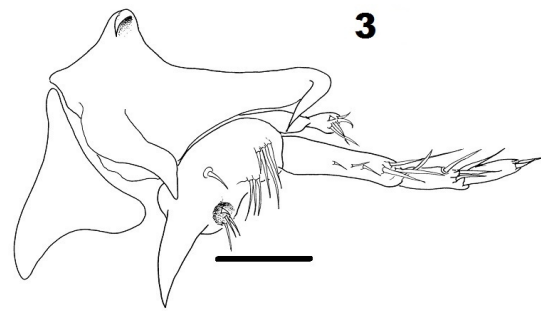


**Figure 1-** *Argulus ventanensis* sp. n. Adult male. A. Total, ventral view. B. Cephalic area. C. Armature of the frontal lobe. Scale bars: A= 0.5 mm, B= 0.25 mm, C= 0.05 mm.

spine. Second antenna 4-segmented not reaching carapace margin. First segment with stout posterior spine (Ratio length posterior spine /length first segment = 0.69), small swelling with four setae; isolated seta in middle of exposed face; group of three setae on posterior margin and four distal setae, one small. Second segment relatively large, 1.87 times length of third, armed with three spines on distal half and four larger distal spines. Third segment bearing four terminal spines. Fourth segment similar in size as third, ending in two apical spines (Figure 3).

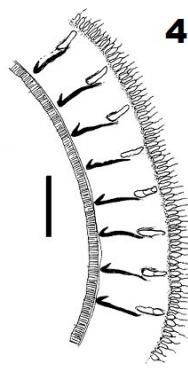


**Figure 2-** *Argulus ventanensis* sp. n. Adult male. Left respiratory area (ventral view). Scale bar= 0.25 mm



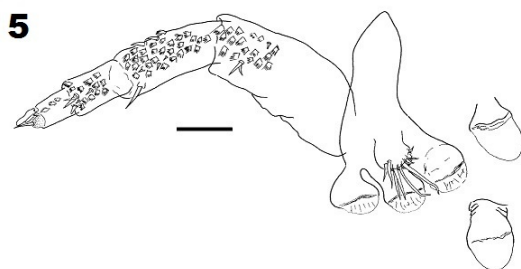
**Figure 3.** *Argulus ventanensis* sp. n. Adult male. Antennae I and II. Scale bar= 0.10 mm

First maxillae (modified as “suckers”) large (350 μm in diameter, separated by 350 μm each other ) supported by 40 rods, most of them composed of “J-shaped” rod and oblong distal sclerite. Some rods bearing two distal elliptical sclerites. Margin covered with minute projections or lappets and sensillae (Figure 4). Mouth tube short, half as broad as long, denticulate mandibles within mouth tube. Retractable pre-oral stylet between maxillary suckers.



**Figure 4-** *Argulus ventanensis* sp. n. Adult male. Detail of sucker armature. Scale bar= 0.05 mm

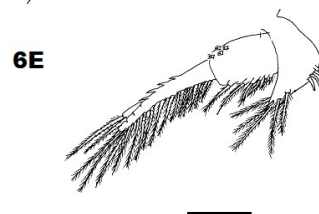
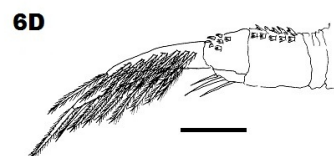
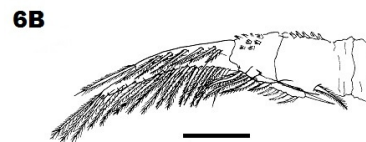
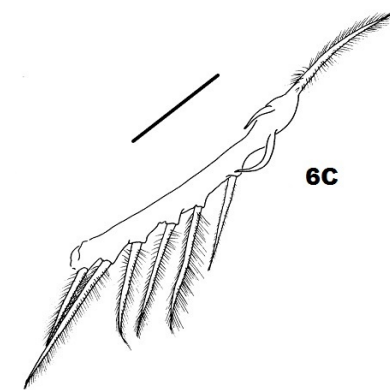
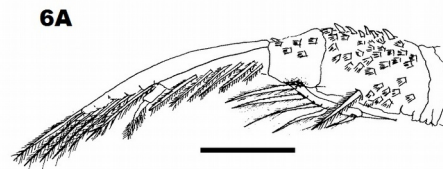
Second maxilla 5-segmented . Basal plate broad, roughly triangular with three stout spatulate coxal spines, pointed anterior projection and pad with 6 large spines and 4 small hooklets. Second to fifth segments rectangular, decreasing progressively in size. Second segment ornamented with about 26 pectinate scales and small setae. Third segment ornamented with 40 pectinate scales and 1 seta. Fourth segment with 11 pectinate scales and 4 setae in terminal portion and fifth segment with few pectinate scales, ending in lobe with minute apical seta surrounded by two hooks. Two pairs of stout post-maxillary spatulate spines, anterior pair at level of maxillary coxae; posterior pair at level of first legs (Figure 5).



**Figure 5-** *Argulus ventanensis* sp. n. Adult male. Maxilla II. Scale bar= 0.10 mm

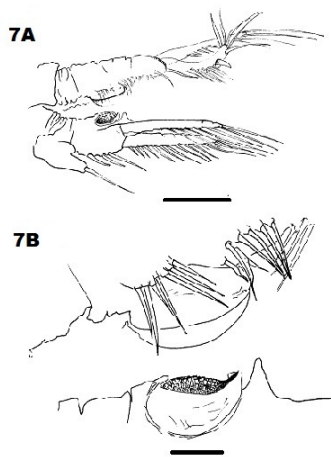
Four pairs of thoracic legs each composed of precoxa, coxa, basis, endopod and exopod (Figures 6 A-E). Two first pairs of legs bearing flagellae extending from origin on dorsal surface of exopod to near basal portion of coxae. Each flagellum with 6 pinnate setae, two smaller naked setae on distal margin and apical pinnate seta. Exopod and endopod with rows of plumose setae. Accessory copulatory structures on posterior margin of third coxae with cup-shaped socket directed to base of fourth leg

(Figures 7A and B). Fourth leg ornamented with several spines and bearing peg covered with minute scales surrounded by spiniform projection. Precoxa and coxa of fourth leg forming triangular natatory lobe covered with fine setae and apical spine.

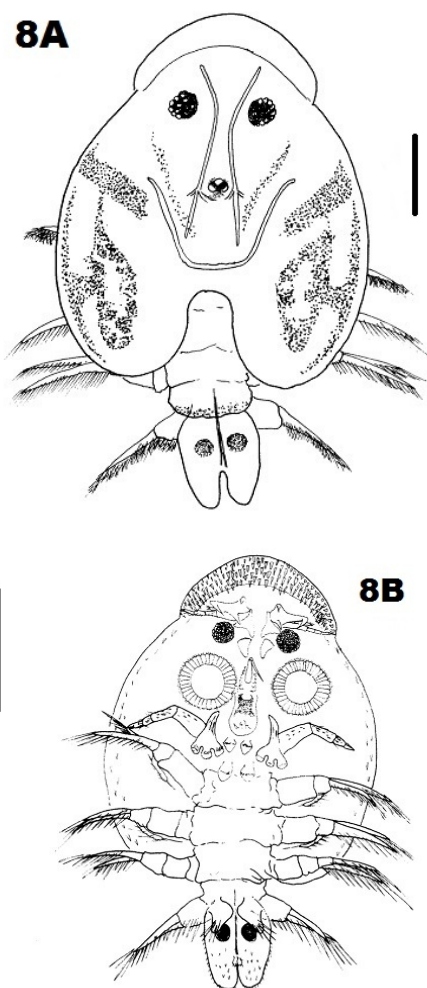


**Figure 6-** *Argulus ventanensis* sp. n. Adult male. Legs (right). A: first ; B: second; C= flagellum of the first leg; D: third; E= fourth leg. Scale bars= 0.25 mm (A,B, D and E), 0.10 mm (C)

*Adult female* (All measurements are given in Table I) General morphological aspect of body similar to male. Females larger than males as in most argulids (Figure 8). Carapace comprising 77.6% of total

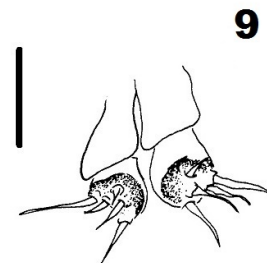


**Figure 7-** *Argulus ventanensis* sp. n. Adult male. A: third and fourth (left) legs showing accessory copulatory structures. B: detail of the copulatory structures. Scale bars= 0.10 mm (A), 0.05 mm (B)



**Figure 8-** *Argulus ventanensis* sp. n. Adult female. A: dorsal view; B: ventral view. Scale bar= 0.5 mm.

length. Respiratory areas similar to male. First maxillae (suckers) median sized with 40 (left cup) – 45 (right cup) rods each composed of basal “J-shaped” and usually one apical ovoid sclerite. Both first maxillae with eight rods bearing two elliptical distal sclerites. Abdomen rectangular, shorter than male with a pair of spermathecae on each side of anterior half, filled with sperm. Abdominal sinus short, 1/3 length of abdomen; ventral surface ornamented with minute spines. Furcal rami similar in size and shape to those of male (Figure 9). First and second antennae, as well as first two pairs of thoracic legs, of same shape as male. Third and fourth pairs of legs lacking accessory copulatory structures. Fourth pair produced into triangular postero-lateral natatory lobe.



**Figure 9.** *Argulus ventanensis* sp. n. Adult female. Furcal rami. Scale bar= 0.05 mm

#### Taxonomic summary

*Argulus ventanensis* n. sp. (Crustacea; Branchiura; Argulidae)

Type host: *Hypsiboas pulchellus* (Duméril & Bibron, 1841) (Amphibia: Hylidae)

Site of infection: Skin

Type locality: Belisario stream, Villa Ventana, Buenos Aires province, Argentina (38° 4' S, 61° 55' W).

Etymology: The specific names *ventanensis* refers to the geographic locality where the hosts were collected (Villa Ventana).

Type material: Holotype male accession number= 27219 (Museo de la Plata, Argentina, Colección de la División Zoología Invertebrados, sección Carcinología).

Allotype female accession number= 27220 (Museo de la Plata, Argentina, Colección de la División Zoología Invertebrados, sección Carcinología).

Paratype female accession number= 27221 (Museo de la Plata, Argentina, Colección de la División Zoología Invertebrados, sección Carcinología).

**Table I.** Comparative measurements \* in *Argulus ventanensis* sp. n., *A. violaceus* and *A. patagonicus*. TL= Total length; CL= Carapace length; CW= Carapace width; CSL= Carapace sinus length; TO= Thorax length; AL= Abdomen length; AW= Abdomen width; ASL= Abdominal sinus length; SD= Suckers diameter.

Morphological features	<i>Argulus ventanensis</i>		<i>Argulus violaceus</i> <sup>1</sup>	<i>Argulus patagonicus</i> <sup>1</sup>		
	Holotype male	Allotype female	Male (n=2) <sup>2</sup>	Female(n=4)		
TL (mm)	3.27	3.57	5.48-6.24	6.49		
CL (mm)	2.07	2.77	3.88-4.46	4.75		
CW (mm)	1.75	2.25	3.36-4.20	4.01		
CSL	450	630	840-940	1200		
TO	850	1330	1200-1310	1970		
AL	875	692	1230-1310	1007		
AW	525	575	780-840	920		
ASL	325	288	420-520	380		
SD	350	475	520-520	760		
Hosts	<i>Hypsiboas pulchellus</i>		<i>Plecostomus Rhamdia Pimelodus Parapimelodus Hoplias Odonthestes</i>	<i>commersoni sapo albicans valenciennesi malabaricus bonariensis</i>		
<b>Ratios:</b>						
TL/CL	1.58	1.29	1.39-1.41	1.36	1.99	1.52
TL/CW	1.88	1.59	1.48-1.63	1.61	1.86	1.70
CL/CW	1.18	1.10	1.06-1.15	1.18	1.09	1.12
Sd/CW	0.17	0.17	0.12-0.15	0.19	0.25	0.26
CL/CSL	4.61	4.40	4.61-4.75	3.96	5.00	7.37
AL/TL	0.27	0.19	0.26-0.28	0.15	0.32	0.20
AL/AW	1.66	1.20	1.46-1.67	1.09	1.92	1.32
AL/ASL	2.50	2.40	2.51-2.92	2.65	4.75	2.55

\*Given in  $\mu\text{m}$  unless otherwise stated<sup>1</sup> Taken as the mean from the measurements given by Ringuélet (1943). <sup>2</sup> Range

## Discussion

Branchiurans are primarily ectoparasites of freshwater, estuarine and marine fish (Poly, 2008). There are very few records of this group as parasites of amphibians. The oldest record we know of is that of Cuvier (1798) who cited *Argulus foliaceus* on tadpoles, designated as "Pou de têtard, *Monoculus gyrini*" (Wilson, 1902). Bower-Shore (1940) cited *A. foliaceus* parasitizing *Triturus vulgaris vulgaris* L tadpoles. Ringuélet (1943) mentioned that argulids attack fish but *A. foliaceus* L. and *Dolops ranarum* Stuhlmann, 1891 have also been found on tadpoles. Goin & Ogren (1956) recorded *Argulus americanus* Wilson, 1902 parasitizing a tadpole of *Rana heckscheri* Wright, 1924 in Florida, USA. Later, Clark (2001) collected 185 *Argulus americanus* and 20 *A. diversus* Wilson, 1944 on the same host from Osceola National Forest, Florida, USA. Wolfe et al. (2001) collected specimens of *Argulus* sp. on the tails and dorsal trunks of many tadpoles of the river frog, *R. heckscheri* from Pointsett State Park, South Carolina, USA. Poly (2003) described *Argulus*

*ambystoma* on the salamander "ajolote" *Ambystoma dumerilii* (Dugès, 1870) from lake Pátzcuaro, Michoacán State, Mexico.

Lemos de Castro & Gomes-Correa (1985) proposed the species *Argulus hylae*, a parasite of *Hyla geographica* Spix, 1824 (actually *Hypsiboas geographicus*) from Lagõa do Campelo, Rio de Janeiro state, Brazil. As this taxon lacks a published description (International Code of Zoological Nomenclature, 4th edition on line, available in <http://www.iczn.org/iczn/index.jsp>) it should be considered as a *nomen nudum* (Neethling & Avenant-Oldewage, 2016).

Ringuélet (1943, 1948) in his reviews of the Argentine argulids recorded six species in the genus *Argulus*, all of them collected on freshwater fish, with *A. violaceus* being the most common species collected on six teleost endemics of the Parano-platense ichthyofauna and from *Oncorhynchus mykiss* Walbaum, 1792 (cited as *Salmo irideus*), an exotic fish. Brian (1947) added a record of two specimens of *A. violaceus* on the Argentine hake

**Table II.** Major points of difference between *Argulus violaceus* and *Argulus ventanensis* sp. n.

<i>Argulus violaceus</i>	<i>Argulus ventanensis</i> sp. n.
Antero-lateral sinuses pronounced	Antero-lateral sinuses slight
Second antenna extending beyond the carapace margin	Second antenna not reaching the carapace margin
Posterior spine of the first segment of antenna II as long as the segment	Posterior spine of the first segment of antenna II only 63% of length of the segment
Second segment of antenna II 2.5 times the third	Second segment of antenna II 1.8 times the third
Third and fourth segments of antenna I long, reaches the 3rd segment	Third and fourth segments of antenna I short, reaches the proximal half of the 2nd segment
A row of 7-10 setae on the second segment of antenna II	Only 3 setae on the distal half of the second segment of antenna II
Third segment of antenna II with 2 single distal setae	3rd segment with 4 single distal setae
Fourth segment of antenna II with 5-6 apical small setae	4th segment with 2 single setae
40-42 (male)/42-45 (female) rods in each sucker	40 (male)/ 40-45 (female) rods in each sucker
Two distal sclerites per rod in the sucker armature	One distal sclerite per rod (about 8-12 rods with 2 distal sclerites)
Flagella on the first pair of legs extend to the midline of coxa and bear 10-12 pinnate setae	Flagella on the first pair of legs reach the precoxa and bear 6 pinnate setae, 2 naked distal setae and an apical pinnate seta
Three spines on the basal plate of 2nd maxillae, the medial slightly blunt or pointed, the marginal spatulate	Three similar spatulate spines on the basal plate of 2nd maxillae

*Merluccius* sp. from Mar del Plata, Argentina. As this marine fish inhabits the southwestern Atlantic Continental Shelf at depths between 100 and 200 m (Froese and Pauly, 2017), and *A. violaceus* is the most common branchiuran collected from freshwater fish in the Neotropics, it is necessary to review this record of Brian.

In possessing an orbicular bilobed carapace that partly covers the third pair of swimming legs, two pairs of antennae armed with spines and hooks, cup-like suckers supported by chitinous sclerites, a pre-oral spine and mandibles reduced into a mouth tube, the new species belongs to the genus *Argulus*.

*Argulus violaceus* has been recorded previously in the present geographic area, Arroyo del Negro, Sierra de la Ventana, on rainbow trout *Oncorhynchus mykiss*, cited as *Salmo irideus* (Ringuet, 1948) and from other hosts sharing this habitat with *H. pulchellus* (*Rhamdia quelen* (Quoy & Gaimard, 1824), *Oligosarcus jenynsii* (Günther, 1864), and *Parapimelodus valenciennis* (Lütken, 1874)). The most striking morphological differences between *A. violaceus* and the new species are body size, the development of the lateral sinuses, the extent and armature of the antennae, the composition of the rods supporting the suckers, the

proportional length of the second antennal segment in respect to the third, the length of the flagellae on the first and second legs, the basal plate of maxillae, the number of setae on the caudal rami and the form of the respiratory areas. Tables II summarises the main morphological differences with *A. violaceus*.

*Percichthys trucha* (Valenciennes, 1833), the type host of *A. patagonicus* Ringuet, 1943, has also been found by the authors in streams in Sierra de la Ventana, sharing the habitats of *H. pulchellus*. The new species differs from *A. patagonicus* (Table I) mainly in the development of the carapace, which only covers the first two pairs of legs, the general coloration, the form of the post maxillary spines (spatulate versus pointed), the flagellae on the first and the second pair of legs, and the number of setae on the caudal rami.

On the basis of these differences, the argulid found on *H. pulchellus* tadpoles is considered to represent a new species, designated as *Argulus ventanensis* sp. n. This constitutes a new biotic association between branchiurans and amphibian hosts.

#### Acknowledgments

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