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ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

ARGULUS YAWEWITUS SP. N. (CRUSTACEA: BRANCHIURA) PARASITE OF *ARAPAIMA GIGAS* (SHINZ, 1822) (OSTEOGLOSSIFORMES: ARAPAIMIDAE) FROM BRAZIL

ARGULUS YAWEWITUS N. SP. (CRUSTACEA: BRANCHIURA) PARÁSITO DE *ARAPAIMA GIGAS* (SHINZ, 1822) (OSTEOGLOSSIFORMES: ARAPAIMATIDAE) DE BRAZIL

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ABSTRACT

Argulus yawewitus sp. n. is described from pirarucu, *Arapaima gigas* (Shinz, 1822) from a fish farm in Northeast Brazil. The characteristics that distinguish it from other species are shape of the carapace, abdomen, respiratory areas, the ornamentation and morphology of the antenna and antennule, the sexual dimorphism that distinguishes female from male, and the flag-like protrusions on the male's second and third pair of legs.

Keywords: fish ectoparasite – freshwater Argulidae – *Argulus yawewitus* sp. n. – South America

RESUMEN

Argulus yawewitus n. sp. se describe de pirarucu, *Arapaima gigas* (Shinz, 1822) de una piscifactoría en el noreste de Brasil. Las características que lo distinguen de otras especies son la forma del caparazón, abdomen, áreas respiratorias, la ornamentación y morfología de la antena, y anténula, el dimorfismo sexual que se acentúa entre hembra y macho, y las protuberancias en forma de bandera en el segundo y tercer par de patas del macho.

Palabras clave: América del Sur – Argulidae de agua dulce – *Argulus yawewitus* n. sp. – ectoparásito de peces – morfología

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INTRODUCTION

The species of Branchiura are ectoparasite crustaceans of fish that live primarily on the outer surface of the body and the base of the fins of their hosts. Their larvae are free-living in at least one of their phases: in the last larval stage they are fixed in a host (Ringuet, 1943). Few species of *Argulus* Müller, 1785 have been described from marine fish and scattered reports of Branchiura on tadpoles, salamanders and even alligators can be found (Ringuet, 1943; Piasecki & Avenant-Oldewage, 2008).

Branchiura species feed on blood, plasma, mucus, or epithelial cells (Ringuet, 1943). In the species of the genus *Argulus* the oral apparatus consists of two distinct parts united in their bases; the anterior part is formed by a fine point stylus. When not in use it is partially retracted into a sheath. Inside the stylet there is a duct that attaches to a poison gland, located on the bottom of this unit; this organ is similar to the buccal apparatus of the hemiptera and is used to pierce the fish epidermis for food purposes. This piece is called a pre-oral spine (Malta & Varela, 2009).

The branchiurans have a significant economic importance due to the damage they cause to natural populations of fishes, debilitating adult hosts and generally killing young fish. These damages are increased in intensive crops given the high concentration of fish and small areas creating optimal conditions for the development of these and other parasites. In these environments, populations of Branchiura species increase rapidly and as the fish cannot escape the attacks, they become true pests (Kabata, 1970; Souza & Malta, 2018ab).

A new species of *Argulus* is described from pirarucu, *Arapaima gigas* (Shinz, 1822) of a fish farm in Northeast Brazil.

MATERIAL AND METHODS

Branchiurans were carefully taken from fish captured with delicate tweezers. Eleven specimens of *Argulus yawewitus* n. sp. were collected on the

surface of the *A. gigas* body raised at the fish farm of the Federal Rural University of Pernambuco, Recife and Pernambuco state, Brazil. The specimens were preserved in 90% ethyl alcohol and studied with the aid of stereo and light microscopes. Two specimens (1 male and 1 female) were cleared in lactic acid + water 1:1

Drawings were made (with the aid of a drawing tube). Measurements were made with a micrometer eyepiece and given as means followed by the range in brackets and in millimeters. Five females and two males were deposited in the non-insect Invertebrate Collection of the National Research Institute of Amazonia (INPA), Manaus, Amazonas, Brazil (INPA 2549 and INPA 2550).

For the external ultrastructural analysis, two specimens, one male and one female, were selected, underwent post-fixation in 1% OsO₄ (Osmium Tetroxide) during 3 h, followed by dehydration in an increasing series of ethanol from 80% ethanol to 100%, and then dried in a CO₂ Critical Point device. then the Branchiura were carefully mounted on “stubs” (metallic aluminum), metallized with gold and analyzed under a Tescan scanning electron microscope VEGA 3 of the Laboratory of Animal Histology and Embryology at the Institute of Health and Animal Production in partnership with the Federal Rural University of the Amazon.

Ethic aspects: This study followed and committed to all ethical aspects to be carried out.

RESULTS

Taxonomy

Phylum Arthropoda

Class Maxillopoda

Subclass Branchiura

Order Argulidea

Family Argulidae

Genus *Argulus* Müller O.F., 1785

Argulus yawewitus sp. n.

Material examined – Holotype adult female (INPA - CR 2549) from the body surface of *Arapaima gigas* in liquid medium from fish farms of the Federal Rural University of Pernambuco, Brazil.

Adult female (Fig. 1 - 4)

Based on ten females the body shape is rounded (Figs 1 - 4) with paired incisions of antero-lateral carapace to form a defined frontal region and comprising cephalon, four thoracic somites, and abdomen. Color in alcohol-fixed is light yellow with drawings formed by spots of light brown pigments in the carapace and dark brown in the abdomen. Total length (tl) 4.08-5.44 (4.69 ± 0.59);

carapace length (cl) 3.45-4.59 (3.95 ± 0.48), carapace width (cw) 3.06-4.89 (3.99 ± 0.81). Relations between total length / carapace length (tl / cl) 1.09-1.33 (1.2 ± 0.07). Cephalon longer than wide, bearing pair of compound eyes and nauplius eye. Compound eye bean-shaped with medial cleft on interior margin. Nauplius eye composed of three ocelli, two ocelli facing anteriorly and one facing posteriorly.

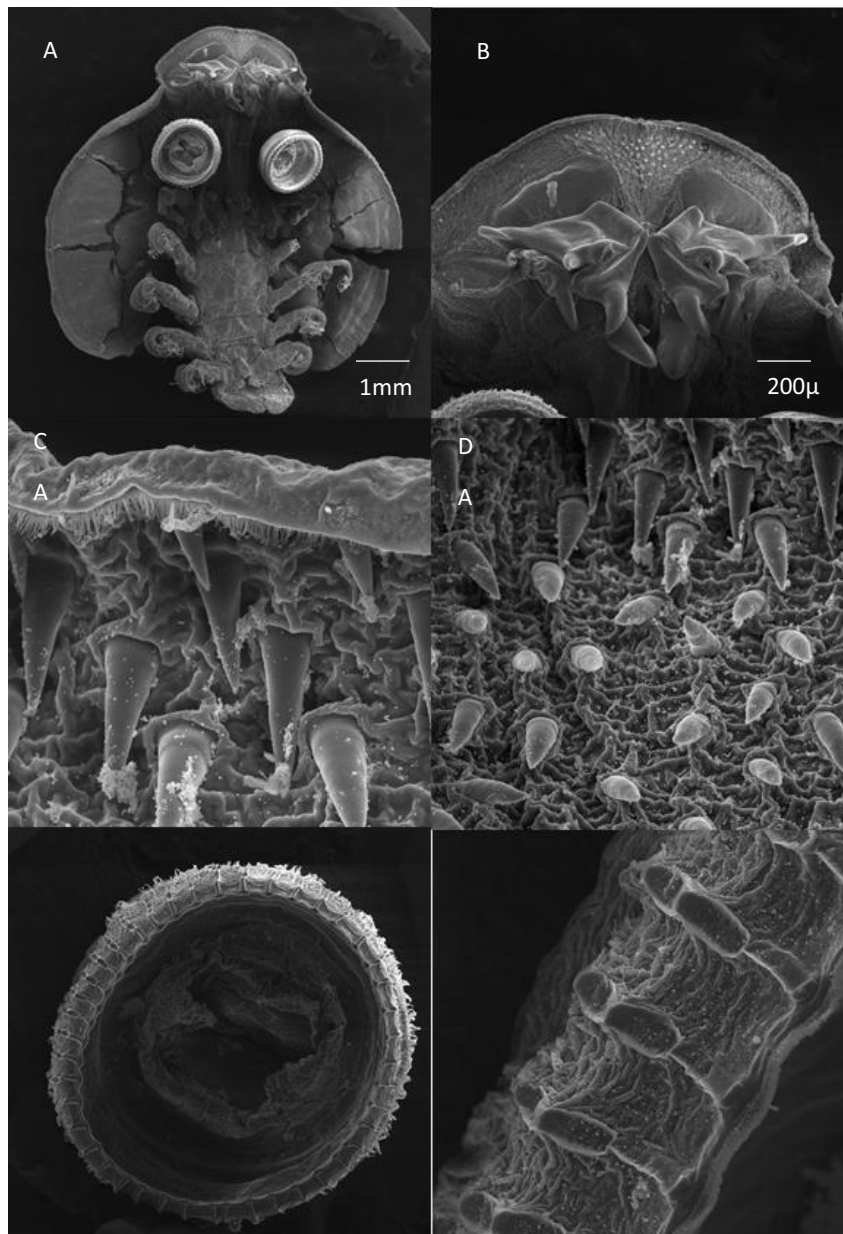


Figure 1. (A–F). A - Female of *Argulus yawewitus* sp. n. B – Antenna and Antennula; C and D – Cephalic Spine; E – Sucker; F – support rods Second Maxilla.

A pair of separate interocular rods originates near the margin anterior of cephalon and extend towards and pass between compound eyes going to the anterior margin of the first somite of the cephalothorax. Carapace longer than wide, posterior margin fully covers all legs and exceeds half of abdomen length. Suckers partially visible in dorsal view. Lateral lobes broadly rounded, posteriorly separated by broad sinus 1/3 length of carapace. Abdomen wider than short, length 0.54-

0.85 (0.71 ± 0.11) and width 0.93-1.14 (0.99 ± 0.09); ratio total length / length of the abdomen (tl/la) 5.75 - 8.96 (6.74 ± 1.34), but somewhat variable between paratypes in length-to-width ratio ranging from 0.54-0.81. Widely rounded abdominal lobes; rami furcal small, located next to the midline. Small and orbicular spermatheca. The anal sinus about a quarter of the length of the abdomen. Light yellow colored dorsal surface with light yellow markings.

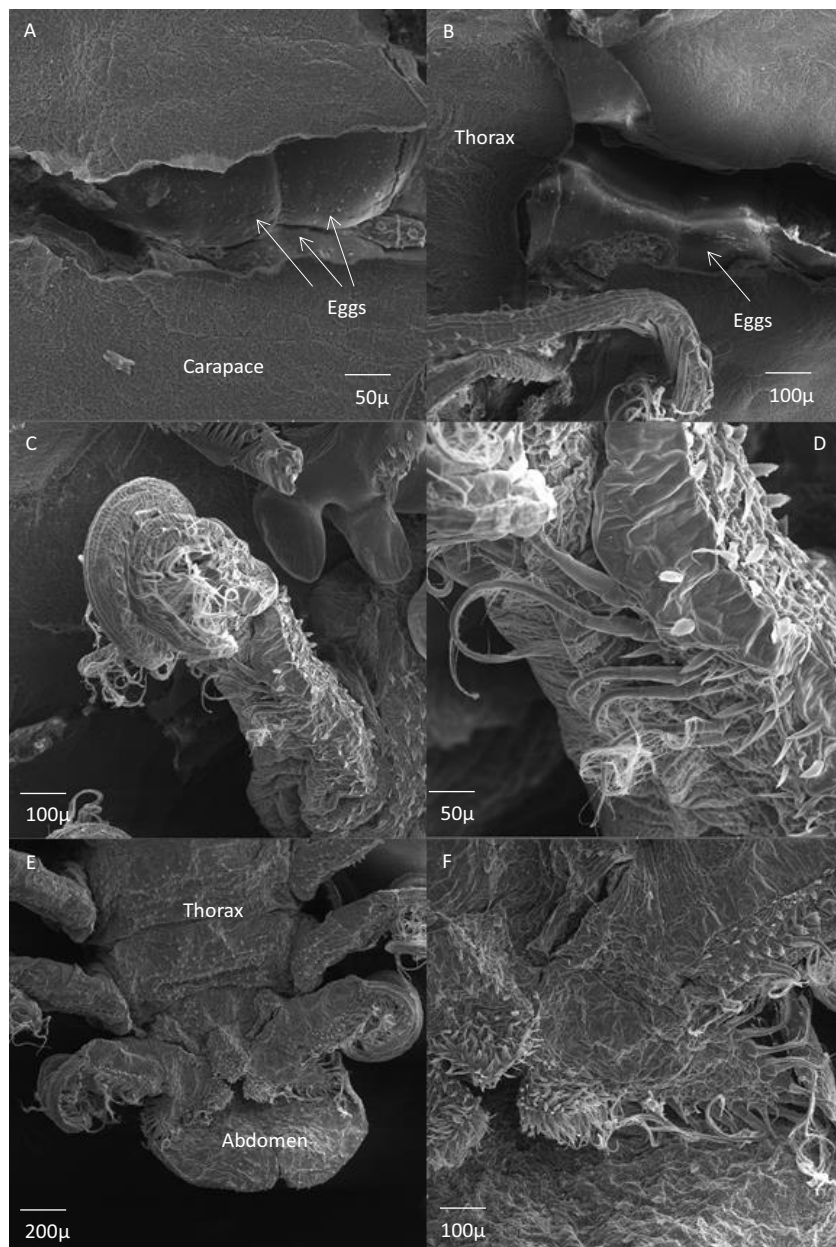


Figure 2. (A – F): A and B - Female of *Argulus yawewitus* sp. n., detail of eggs on the carapace; C and D – First legs; E – Thorax and abdomen; and F – Natatory lobule fourth pair of legs.

Ventral surface of the cephalic region and anterior part of the carapace ornamented with regularly arranged spines of similar size up to the height of the first pair of legs. Paired respiratory areas on ventral surface of lobes; anterior respiratory area small and circular to ovoid; posterior respiratory area with 4 indentations on internal margin. Thorax indistinctly 4-segmented, with distinctive light

brown pigment spots, distended with eggs, ventral surface ornamented with scales and dorsal surface smooth (Fig. 2E).

First antenna (Fig. 1B and 4A) comprising 2 sections; stout 2-segmented proximal section possessing large spines; slender distal section with setae. First segment bearing a stout, large,

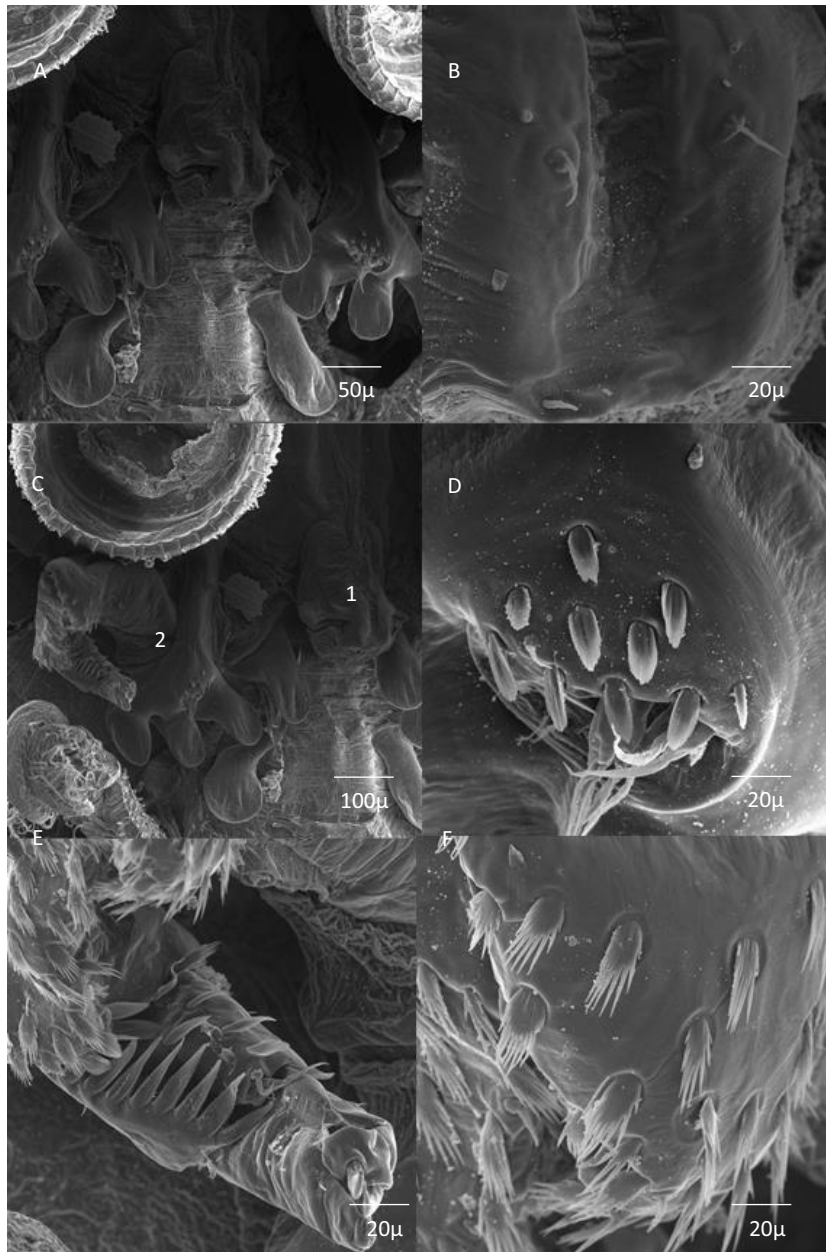


Figure 3. A - Female of *Argulus yawewitus* sp. n., mouth tube and second maxillae; B – Pre-oral papilla; C – 1 mouth tube and 2 second maxillae; D – second maxilla basis; E – Detail of the ornamentation of the second maxillae; and F – Scales of second maxilla.

posterior, slightly incurved spine; 2nd segment massive, without medial spine, large hook-like terminal spine and anterior spine, lacking hook. Second antenna (Fig. 1B and 4A) 5-segmented, fleshy. First 2 segments larger; remaining 3 thin, cylindrical; basal segment bears posterior spine. First segment bearing large, pointed posterior spine, a tuft of setae on rounded protrusion and terminally a large protrusion with three apical setae. Second segment short, approximately half of the first with a tuft of setae. Third segment the longest with nine thin setae. Fourth segment without ornamentation. Fifth segment with three terminal spines.

First maxilla forming large suction cup. Support rods composed of two sclerites of a rectangular shape (Fig. 1E and F). Anterolateral portion of suction cup with a thin membrane. Second maxilla 5-segmented. Basal segment subtriangular with three rectangular teeth broad and spatulate, basal nodule with three simple setae. Second segment with pectinate scales in the mid-posterior portion. Third segment smaller than the second with two

conical spines distally. Fourth flat segment, two thirds of the length of the third segment. Fifth segment with two claw-shaped terminal spines and a projection.

Retractile pre-oral spine elongated located midway between first maxilla; tip extending to midway between post-antennal spine when retracted; ventral surface of spine without scales. Mouth tube less than twice as long as broad, with few scales scattered around base; denticulate mandibles visible within mouth tube. Pair of accessory spines located between the second maxilla (Fig. 3 - A and C).

Pair of thoracic spines located between the first pair of legs. First to 4th pairs of legs biramous and of near equal size; sympods indistinctly 2-segmented. Flagellum present on 1st and 2nd legs; extending medially from origin on dorsal surface at base of exopod; both rami and flagella armed with 2 rows of plumose setae. Natatory lobe on 4th leg produced laterally. Simple scales present on natatory legs (Fig. 2 - F).

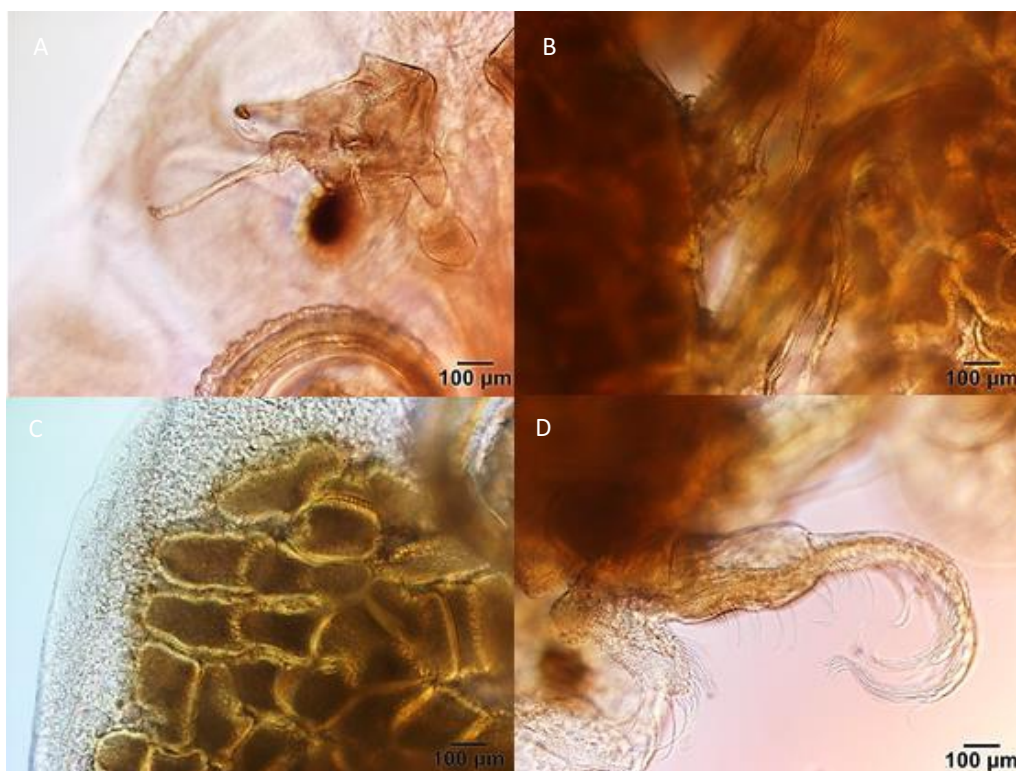


Figure 4. Light microscopy photo female of *Argulus yawewitus* sp. n. A – Antenna and antennule; B – natatory flagellum of the first and second pair of legs; C - eggs in the carapace; and D – fourth legs.

Adult male (Fig. 5)

Based on ten males (INPA - CR 2550). Body shape rounded (Fig 5AB) with paired incisions of anterolateral carapace to form defined frontal region and comprising cephalon, four thoracic somites, and abdomen, similar to that of female. Total length (tl) 3,6 - 3,99 ($3,7 \pm 0,16$) mm, about 21% smaller than female (Figura 5). Dorsal surface of yellow color with light brown designs, softer than in females. Elliptical carapace, slightly longer than wide. Carapace length (cl) 2,61 - 3,21 ($2,9 \pm 0,22$). Total length / carapace length ratio (tl / lc) 1,2 - 1,4 ($1,3 \pm 0,08$).

Thorax indistinctly 4-segmented, smaller, narrower and less prominent than the female's, with few light brown pigment spots. Abdomen sub-oval, lobes tapering to bluntly rounded tips, separated by sinus 1/6 length of abdomen; furcal rami small, located adjacent to midline at base of anal sinus; paired testes oval. Dorsally few light brown pigment spots.

Cephalic appendages and first pairs of legs like those of female. Second to fourth pairs of legs modified by accessory copulatory structures. Second leg bearing two cup-shaped covered by simple scales on posterior face of protopod. Third leg bearing two cup-shaped covered by simple scales on anterior face of protopod and two cup-shaped sockets on posterior face located directly above peg on anterior surface of basis of 4th leg. Anterior surface of basis of fourth leg bearing peg-like structure and posterior natatory lobes fringed with plumose setae.

Attachment area: body surface, fin base, gills and oral cavities.

Etymology. In the Amazon, the Branchiura are known by the fishing community as small ray, the specific name, *yawewitus* derived from the “yawewit” is the Tupi, Brazilian indigenous language word for ray.

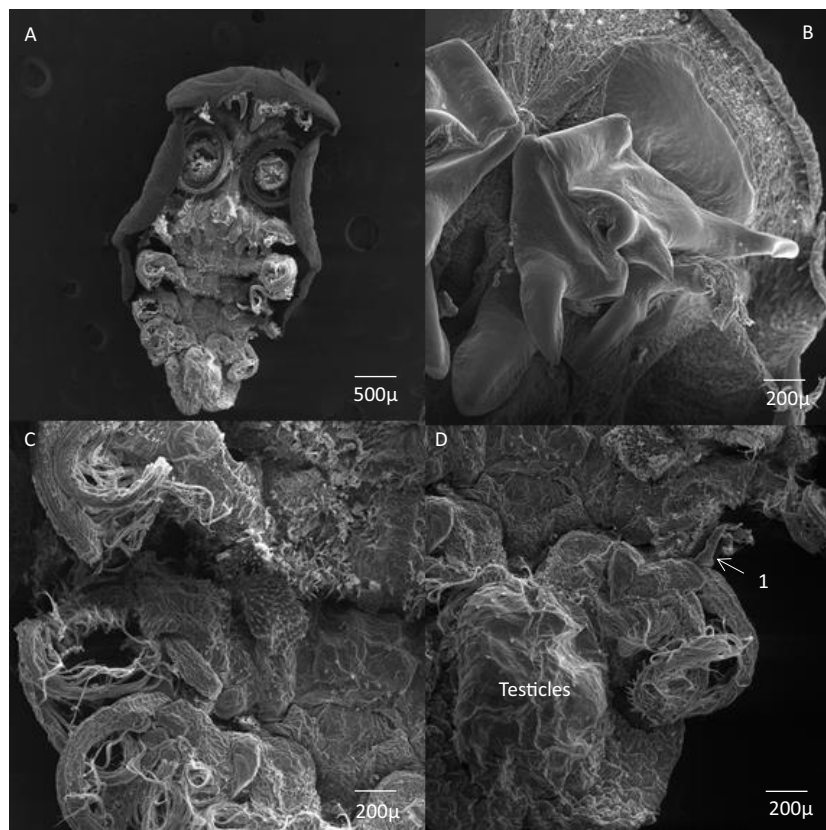


Figure 5. A - Male of *Argulus yawewitus* sp. n.; B - Antenna and antennule; C - Legs; D - Testicles, 1(Copulatory organ accessory).

DISCUSSION

For Brazil, 13 species of *Argulus* are cited: *A. nattereri* Heller, 1857; *A. elongatus* Heller, 1857; *A. salminei* Krøyer, 1863; *A. paulensis* Wilson, 1924; *A. carteri* Cunningham, 1931; *A. multicolor* Steckoven, 1937; *A. pestifer* Ringuélet, 1948; *A. juparanaensis* Lemos de Castro, 1950; *A. spinulosus* Silva, 1980; *A. amazonicus* Malta & Santos-Silva, 1989; *A. chicomendesi* Malta & Varella, 2000; *A. ybatecobe* Souza, Porto & Malta, 2017; *A. celioi* Souza, Porto & Malta, 2018. For Amazônia six species: *A. multicolor*, *A. pestifer*, *A. juparanaensis*, *A. amazonicus*, *A. chicomendesi*, *A. ybatecobe*, and *A. celioi*. *Argulus yawewitus* sp. n. is the second species of *Argulus* cited for *A. gigas*, the first was *A. chicomendesi*.

The morphology of *A. yawewitus* sp. n. resembles *A. flavescens* Wilson, 1916 in its pigmentation pattern, size and shape of the carapace, eggs distribution in the carapace lobes, and ornamentation of the second maxilla basal nodule. However, whereas in *A. flavescens* the accessory and post maxillary spines are conical, in *A. yawewitus* n. sp. they are rectangular teeth with rounded edges similar to *A. striatus* Cunningham, 1913.

Ventral surface of the cephalic region and anterior part of the carapace ornamented with regularly arranged spines, to the height of the first pair of legs, similar to *A. vittatus* Rafinesque-Schmaltz 1814, *A. angusticeps* Cunningham, 1913 and *A. exiguus* Cunningham 1913. The first antenna is like *A. fryeri*.

According to World Register of Marine Species (WORMS) there are 159 *Argulus* species with 17 synonymised species (Walter & Boxshall, 2021). Except for *A. japonicus* Thiele, 1900 originally from Asia, but spread to all continents of the world except Antarctica. There are 152 Branchiura species in the world: four in Australia and Oceania, eleven in Europe, 28 in South America, 35 in Africa, 37 in Asia and 47 in North America (Neethling & Avenant-Oldewage, 2016).

The suction cups, or so-called sucking disks, develop from the first maxillae on each side of the preoral sting. They have a flat rim covered by a

chitinous exoskeleton with thickenings like ribs radiating outward. These ribs are variously broken up into segments, which are characteristic for a given species. They may be a series of long or short rods, segments simulating imbricate plates, or an elongate segment followed by a series of overlapping plates. The number of support rods of first maxilla varies only slightly for a given species. They are used for identification purposes (Meehan, 1940).

The number of support rods of first maxilla in the *Argulus* species ranges from two to 35 sclerites. *Argulus melanostictus* Wilson, 1935 from the Pacific Ocean collected in the United States and Thailand is the species that has the highest number of sclerites ranges from 33-35 (Meehan 1940; Wilson, 1944). In most species the number of sclerites ranges from 3 to 21. The minimum number of sclerites is two and was found in two North American species: *A. appendiculosus* Wilson, 1907 and *A. americanus* Wilson, 1902 (Wilson, 1904). Four species of South America: *A. violaceus* Thomsen, 1925; *A. pestifer* Ringuélet, 1948; *A. moratoi* Souza, Porto & Malta 2019; *A. ventanensis* Tanzola & Villegas-Ojeda, 2017 (Thomsen, 1925; Ringuélet, 1948; Souza & Malta, 2018ab; Souza *et al.*, 2019; Tanzola & Villegas-Ojeda, 2017). And no species from Africa has only two sclerites on the support rods. *Argulus yawewitus* sp. n. is the fifth species of *Argulus* that has the minimum number (2) of support rods of the first maxilla. They are composed of two sclerites of a rectangular shape.

Second to fourth pairs of legs modified by accessory copulatory structures. Second leg bearing two cup-shaped covered by simples scales on posterior face of protopod. Third leg bearing two cup-shaped covered by simples scales on anterior face of protopod and two cup-shaped sockets on posterior face located directly above peg on anterior surface of basis of 4th leg. Anterior surface of basis of fourth leg bearing peg-like structure and posterior natatory lobes fringed with plumose setae.

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In the male, chitinous prominences with thorns on the legs resemble *A. giordanii* Brian, 1959. The accessory sexual structures, flag-like protrusions, present on legs 2 and 3 also occurred in two other species of *Argulus*, were found in *A. ernsti* Weibezahn & Cobo, 1964 from Venezuela, two on the second and two on the third pair of legs, still with different shapes (Weibezahn & Cobo, 1964).

Argulus yawewitus sp. n. is a unique species, among its peculiarities we can mention: ornamentation of antennas 1 and 2, rods supporting the first maxilla, second maxilla, ornamentation of the buccal cone, accessory and post-maxillary spines and ornamentation of the male's legs.

Argulus yawewitus sp. n. may easily be distinguished from other species of *Argulus* by a combination of the following characters: the format and ornamentation of the first and second antennas position of the smaller respiratory area is anterior to the larger one and (2) the ribs of the suction cups are composed of 9 to 11 imbricated plates only. In Wilson's (1904) account, there are 22 species of *Argulus* including *A. indicus* with their respiratory areas arranged as in *A. quadristriatus*. Among the seven species of *Argulus* known to occur in India, *A. giganteus* and *A. indicus* have this type of respiratory areas. In *A. kurafugu* and *A. scutiformis* from Japanese fishes, Yamaguti & Yamasu (1959) reported such an arrangement of the respiratory areas. Of the above cited species, only *A. melanostictus*, *A. pugettensis*, *A. niger*, *A. melanostictus* and *A. giganteus* have the arrangement of the respiratory areas combined with the fact that the ribs of the suction cups are exclusively formed of imbricated plates, as in the present species. However, they differ from *A. quadristriatus* in the much greater number of these plates in each rib, viz., 30 in *A. melanostictus*.

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