An Acad Bras Cienc (2025) 97(2): e20240590 DOI 10.1590/0001-3765202520240590
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

ANIMAL SCIENCE

Isonebula clavuslongus n. sp. (Isopoda: Cymothoidae) parasitizing *Myloplus ternetzi* (Norman, 1929), a fish of the family Serrasalmidae, in the Brazilian Amazon region

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Abstract: Crustaceans of the family Cymothoidae are important components of the invertebrate fauna of marine and freshwater environments. The genus *Isonebula* Taberner, 1977, has only two described species (*Isonebula maculatus* (Taberner, 1977) and *Isonebula acanthopleon* Taberner 1998), which are both parasites of freshwater fish in South America. During a field survey of fish parasites in the Araguari River, state of Amapá, Brazilian Amazon region, we found a new species of the genus *Isonebula* infesting the mouth (on the host's tongue) of *Myloplus ternetzi* (Norman, 1929), a characiform of the family Serrasalmidae, for which we proposed the name *Isonebula clavuslongus* n. sp. *Isonebula clavuslongus* n. sp. differs from its congeneric species through the larger size of the dactyl of its 6th pereopod, with an inner measurement of 4.50 mm. Additionally, it possesses 7 joints in the second antenna, whereas 9 joints were reported for *I. acanthopleon* and *I. maculatus*. The present study thus expands the knowledge of the diversity of Cymothoidae in the Amazon region to 26 species, and of the genus *Isonebula*, with 3 species, which stands out as one of the genera with the lowest number of known species among Neotropical Cymothoidae that parasitize freshwater fish.

Key words: Araguari River, ectoparasites, crustaceans, freshwater fish, new species.

INTRODUCTION

The family Cymothoidae Leach, 1814, comprises 70 genera with 395 valid species (WORMS 2024). *Cymothoa* Fabricius, 1793, is its most diversified genus, with 81 known species, while *Isonebula* Taberner, 1977, is one of the least diverse genera, with only two described species. These are *I. maculatus* (Taberner, 1977) and *I. acanthopleon* Taberner 1998, which were both described parasitizing freshwater fish in Argentina (Taberner 1998, 1977).

The Amazon region has been little studied with regard to its species richness within Cymothoidae Leach, 1814, given that some localities remain unexplored regarding knowledge of parasites of this family. The most recent study on cymothoids of the Brazilian Amazon region, which used samples collected in the field and also data compiled from the literature, showed that this region is home to 17 species, among which five are in the genus *Braga* Schioedte & Meinert, 1881; three are in the genera *Anphira* Thatcher, 1993, and *Artystone* Schioedte, 1866; and one is in each of the genera *Asotona* Schioedte & Meinert, 1881, *Livoneca* Leach, 1818, *Paracymothoa* Lemos de Castro, 1955, *Riggia* Szidat, 1948, and *Vanamea*

Thatcher, 1993. All of these species are parasites of freshwater fish (Virgilio et al. 2020).

With the aim of increase the knowledge of the diversity of fish parasites in the Amazon region, we conducted sample collection in the Araguari River, state of Amapá, Brazil. During this sampling, we found a new species of Cymothoidae belonging to the genus *Isonebula,* which was fixed to the tongue of a specimen of *Myloplus ternetzi* (Norman, 1929), a characiform fish of the family Serrasalmidae. *Myloplus ternetzi* is a herbivorous fish that consumes fruits, seeds and flowers, presents sexual dimorphism and reproduces during the rainy season of the Amazon region. Its geographical distribution encompasses Suriname, French Guiana and the Brazilian Amazon basin as far upstream as the Curuá-Una River in the state of Pará (Planquette et al. 1996).

The ZooBank Life Science Identifier (LSID) of this publication is: urn:lsid:zoobank.org:pub:DD64E2DE-56C5-40CD-9DE2-7C3E3A575F16.

MATERIALS AND METHODS

Fish and collection site

The fish were collected in the upper stretch of the Araguari River, between the municipalities of Serra do Navio and Ferreira Gomes, in the state of Amapá, in the Brazilian Amazon region (Figure 1). The collections took place in July and November 2014. For fish collection, gill nets of different mesh sizes and hook lines were used.

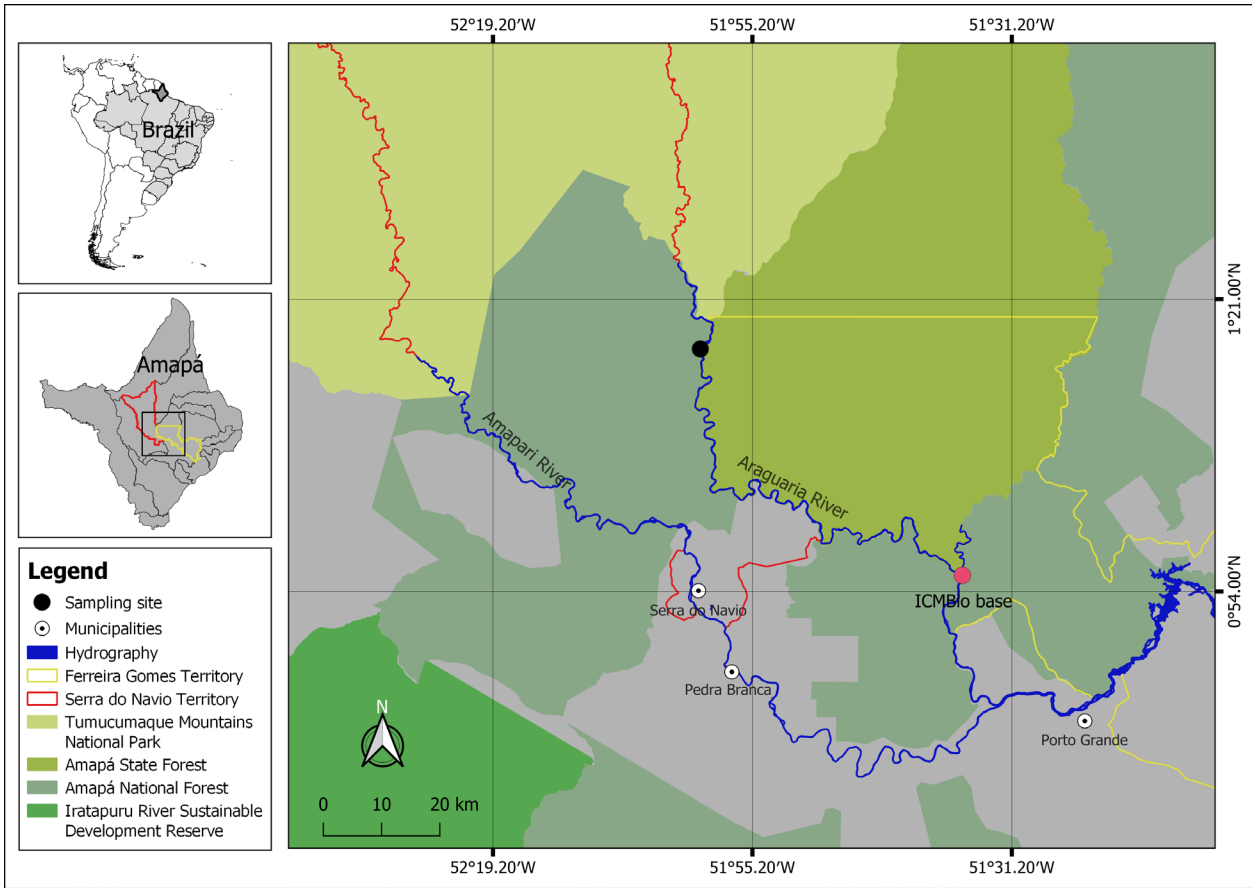

Figure 1. Geographic location of the collection site of *Myloplus ternetzi* and *Isonebula clavuslongus* n. sp. in the Araguari River, municipality of Serra do Navio, State of Amapá, Amazonia, Brazil.

Afterwards, the weight (g) and total length (cm) of each fish were measured. A total of 558 specimens of fish distributed in 10 families and 26 species were collected and analyzed. Among these fish, 29 specimens were of the species *M. ternetzi* (18.2 ± 2.2 cm and 259.0 ± 48.3 g), and a single individual was parasitized by the new species of Cymothoidae that were are proposing here.

Ethical approval

Fish collection was authorized by ICMBio (Protocol number 42203-1), and the Ethics Committee on Animal Use of the Laboratório de Biologia de Peixes da Universidade Federal do Amapá (UNIFAP) approved the procedures involving animals (Protocol number 47757715.5.0000.0003).

Collection procedures and analysis of parasites

Immediately after capture, each fish was transferred to a tray and the possible sites of infection, such as the mouth, gills, opercula, tegument, fins and anus were assessed to ascertain any presence of ectoparasite crustaceans. The crustaceans thus collected were then fixed in 70% ethanol. Parts of the parasite (mouthparts, antennae, pereopods, pleopods and uropods) were clarified for analysis of morphological structures using 5% potassium hydroxide (Oliveira et al. 2021).

The pleopods underwent an initial battery of 5% potassium hydroxide (20 to 30 minutes at 60°C - this time varies according to the size of the structure) (Oliveira et al. 2021), subsequently, they were transferred to pure Lactic Acid until complete muscle degradation (5 to 10 minutes - varies according to the size of the structure), aiming only to maintain the pleopods exoskeleton, then the pleopods were subjected to dehydration in 70% ethyl alcohol (5 minutes), 80% (5 minutes), 90% (5 minutes) and two batteries of absolute alcohol (5 minutes each), and finally they were clarified in pure Eugenol (2-methoxy-4-prop-2-enylphenol; phenol). Permanent slides were mounted using Damar gum medium (Chick & Cassella 2011) diluted in xylene (ratio: 4g of Damar gum/1 mL xylene) and dried at room temperature for 72 hours.

The terminology used for the body and appendages of the isopods was in accordance with what had been used in previous studies (Taberner 1998, 1977). The average measurements, expressed in millimeters (mm), were reported followed by the range and the number of samples (n) measured. Illustrations and measurements were made with the aid of a drawing tube coupled to a microscope (Motic BA210). Subsequently, the illustrations were prepared in the CorelDraw 2021 software.

To compare the size of the dactyls of the proposed new species with those of the other known species of *Isonebula*, it was necessary to make estimates of the inner length of the largest dactyls, given that the authors who previously described species of *Isonebula* did not mention any type of measurement of these morphological structures. To make these estimates, we used prints of the drawings of the dactyls and exported them to the CorelDraw 2021 software. Then, based on the scale size of the drawings (1 mm), we used the distance measurement tool of the software to calibrate the measurements. Lastly, using the rule of three, we calculated the inner measurement of the dactyls that were used for comparison.

RESULTS

Taxonomic summary
Class Malacostraca Latreille, 1802
Order Isopoda Latreille, 1816
Family Cymothoidae Leach, 1814
Genus *Isonebula* Taberner, 1977

Isonebula clavuslongus n. sp. (Figure 2-4).

Description of the female

Diagnosis of the female: One female specimen was analyzed, measuring 25.5 mm in total length and 9.8 mm in total width (largest size, measured at the 5th pereonite). Triangular head, measuring 1 mm in length (measured from the limit of the 1st pereonite to the point of the head) and 1 mm in width (measured at the widest portion, i.e. level with the eyes), not immersed in the 1st pereonite, with a broad rounded forehead, strongly curved downwards, with a rounded point. Eyes median and subrounded, located in the posterior part of the lateral borders of the head, totally visible when viewed from above (Figure 2a-c). First antenna with 8 joints, among which the last joint is smaller and thinner than the others, but all are thicker than those of the second antenna (Figure 3a). Second antenna with 7 joints, among which the last joint is thinner than the others (Figure 3b). Mandible (Figure 2c) with mandibular palpus containing three segments and absence of hairs on the surface; the third segment has a rounded shape. Maxilla formed by two laminae, both armed with three small distal teeth (Figure 3d). Maxilla with three terminal teeth (Figure 3e-f). Maxilliped with borders ornamented with diminutive setae. Trilobate internal lamella; the distal lobule is the largest, with approximately 50 feathery setae, one on top of another (i.e. approximately 25 in the first plane and 25 in the second plane), while the intermediate lobule has approximately 15 feathery setae (Figure 3g).

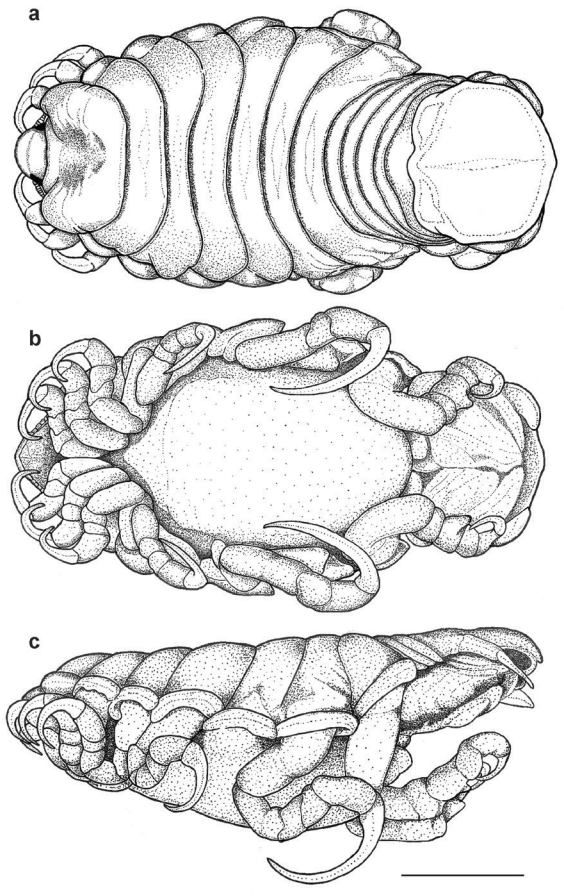

Figure 2. ***Isonebula clavuslongus*** **n. sp. a) dorsal view; b) ventral view; c) lateral view. Scale bar 4 mm.**

Pereon measuring 12.5 mm in total length. 1st to 5th pereonites convex, as projecting forwards; 6th pereonite not projecting forward; and 7th pereonite very convex, forming an arch of approximately 90°, projecting backwards. 1st pereonite longer than the others, with slight depression in the central region; posterior margin very convex, with two rounded points projecting forwards to form to form two entrances.

Pleotelson with all its pleonites presenting sharp points. 1st pleonite very immersed in the 7th pereonite; sharp points of the 1st pleonite directed downwards but not going beyond the line of the 2nd pleonite. 2nd to 5th pleonites of differing sizes, gradually diminishing from the 2nd to the 5th pleonite, with points ending one on the next like inverted steps. 2nd to 4th pleonites with points slightly curved, directed backwards, while the 5th pleonite has strongly curved points directed upwards that overlie the pleotelson.

Telson measuring 4.0 mm in length and 4.0 mm in width, very keeled (height 1.0 mm), with a small, slightly rounded point.

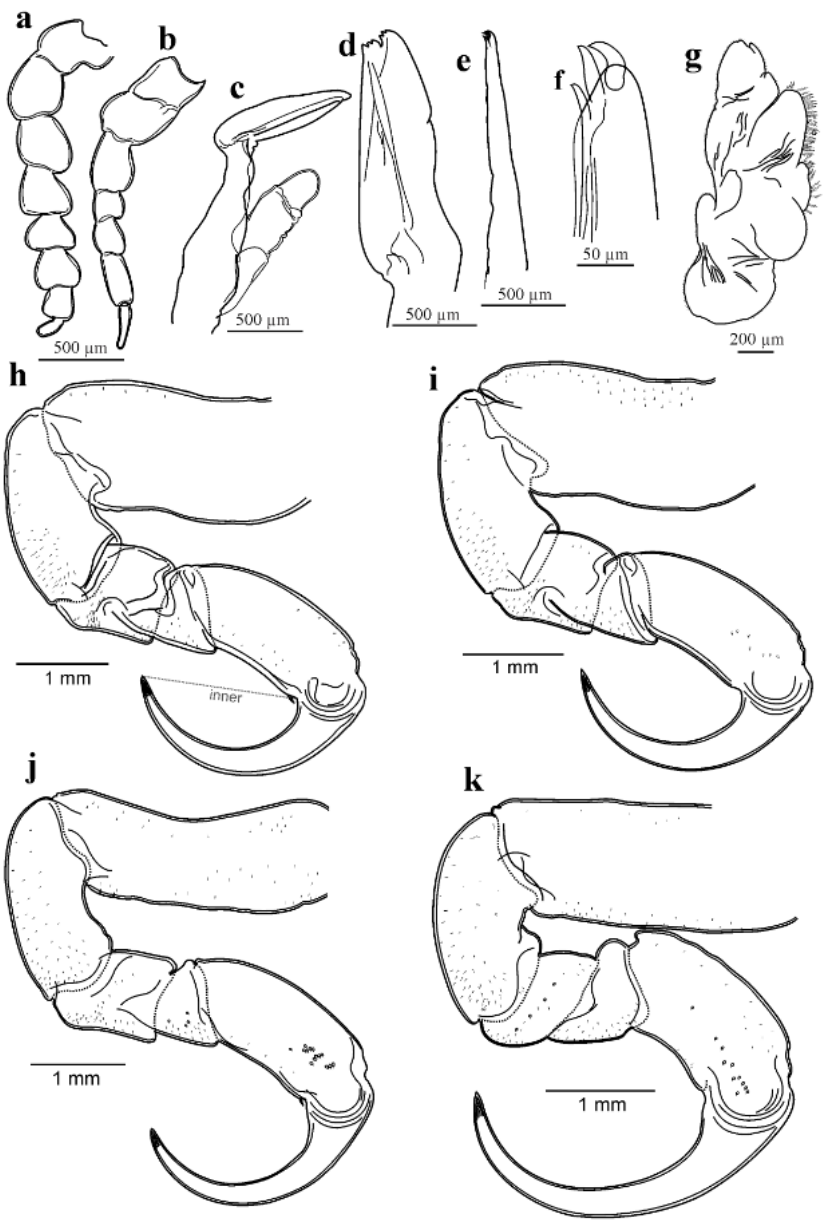

Figure 3. a) 1st antenna; b) 2nd antenna; c) mandible; d) maxilla; e-f) maxillule; g) maxilliped; h) 1st pereopod; i) 2nd pereopod; j) 3rd pereopod; k) 4th pereopod.

1st pereopod with several small spines similar to the hairs on the propod (close to the edges), carpus (dispersed on one of the edges), merus (concentrated at the anterior and posterior points), ischium (some distributed in the lateral region, but mainly concentrated in the region close to the merus) and base of the coxa (a small row on one of the edges). Propod armed with one well-developed spine, located in the distal region close to the confluence with the dactyl. Dactyl of the 1st pereopod with inner measurement (measured from the point of the nail to the internal base of the dactyl) of 1.80 mm (Figure 3h).

2nd pereopod with several small spies similar to hairs on the carpus (concentrated at the lower margin), merus (distributed at the lower and upper edges, but mainly concentrated at the lower points), ischium (some distributed in the lateral region, but mostly concentrated

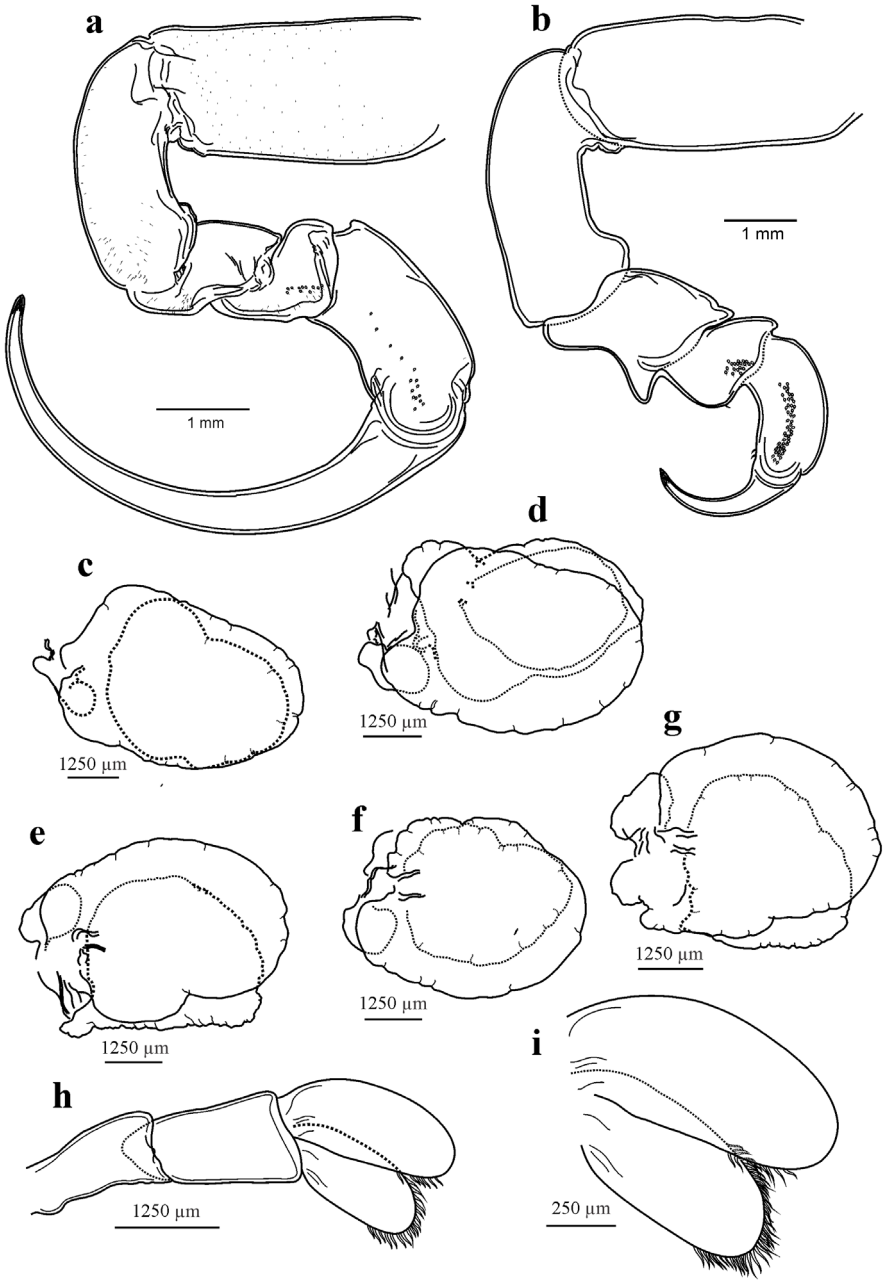

Figure 4. a) 6th pereopod; b) 7th pereopod; c) 1st pleopod; d) 2nd pleopod; e) 3rd pleopod; f) 4th pleopod; g) 5th pleopod; h-i) uropod.

towards the merus) and base of the coxa (mainly concentrated at the upper edge). Propod with seven spines distributed in a row in the medial-lateral region and one well-developed spine in the distal region close to the confluence with the dactyl. Dactyl of the 2nd pereopod with inner measurement of 1.83 mm (Figure 3i).

3rd pereopod with small spines similar to hairs on the propod (a small row on the lower edge), carpus (concentrated at the lower edge close to the point), merus (mainly distributed at the lower edge, but with a small transverse row), ischium (distributed in the medial-lateral region, but mainly concentrated in the distal region close to the merus) and base of the coxa (dispersed, but with slight concentration at the edges). Propod containing 14 spines in the lateral region, forming three small groups

(three spines, five spines and five spines) and one spine slightly further away, and armed with one well-developed spine in the distal region close to the confluence with the dactyl. Dactyl of the 3rd pereopod with inner measurement of 1.78 mm (Figure 3j).

4th pereopod with small spines similar to hairs on the propod (some distributed on the edges), carpus (agglomerated on one of the edges), merus (concentrated at one of the edges and distributed in a small transverse row), ischium (distributed in the lateral region but mainly concentrated in the region close to the merus) and base of the coxa (some dispersed but mostly concentrated at the edges). Propod containing a row with 11 spines in the lateral region. Merus with a transverse row containing four spines at one side. Dactyl of the 4th pereopod with inner measurement of 2.33 mm (Figure 3k).

5th pereopod was lost during collection in the field, and possibly remained on the host at the time of removing the parasite.

6th pereopod with small spines similar to hairs on the carpus (concentrated on one of the edges), merus (concentrated on one of the edges), ischium (distributed in the lateral region but mainly concentrated in the region close to the merus) and base of the coxa (dispersed, but with slight concentration mostly at the edges). Propod containing a row with 15 spines distributed in the lateral region. Carpus containing a row with 11 spines at the side. Dactyl of the 6th pereopod with inner measurement of 4.50 mm (Figure 4a).

7th pereopod does not have any small spines similar to hairs, as observed on the 1st, 2nd, 3rd, 4th and 6th pereopods. Propod with 52 spines distributed longitudinally in the lateral region, armed with two well-developed spines in the distal region, close to the confluence with the dactyl. Carpus with 19 spines at the side, concentrated in the basal region close to the propod, and armed with one well-developed spine at the lower point. Merus with terminations containing a very prominent rounded point. Dactyl of the 7th pereopod with inner measurement of 1.28 mm (Figure 4b).

The 1st, 3rd, 4th and 5th pleopods are bilaminar and the 2nd pleopod is trilaminar. 1st to 5th pleopods have a small lobule at the external margin; both branches are lamellar, the internal one is smaller. 1st pleopod wit slightly elongated laminae, while the others have rounded laminae (Figure 4c-g).

Uropod with rounded endopodite and exopodite. Endopodite ornamented with bristles in the final portion. Exopodite ornamented with bristles on the internal side, only in the lateral portion, without reaching the final portion. Exopodite longer than the endopodite.

Type host: *Myloplus ternetzi* (Serrasalmidae).

Infection site: Mouth (on the host's tongue).

Type locality: Araguari River, municipality of Serra do Navio, state of Amapá, Brazil (1°16'24.25" N 52°0'1.08" W).

Specimen deposited: Museu de Diversidade Biológica (MDBio), ZUEC-Cru 6620.

ZooBank Life Science Identifier (LSID): urna:lsid:zoobank.org:act:A9E5D1D5-51C6-470D-A663-B7081638EDA6.

Etymology: The species name refers to the 6th pair of dactyls, which are very long (Latin, *clavus* = nail + *longus* = long).

Remarks

Isonebula clavuslongus n. sp. differs from the existing two congeneric species mainly through the greater size of the 6th pereopod (inner measurement = 4.50 mm). In *I. maculatus*, the largest dactyls are the 5th (inner measurement = 0.94 mm) and 6th (inner measurement = 0.86 mm), while in *I. acanthopleon* the largest dactyl is the 1st (inner measurement = 1.75 mm). The new species also differs from the others through

the merus of the 7th pereopod, which terminates with a very prominent rounded point; and through the propod of the 1st pereopod, which is armed with one well-developed spine located in the distal region, close to the confluence with the dactyl, a feature that is absent from the other species. Moreover, *I. clavuslongus* n. sp. has 7 joints in the second antenna, whereas *I. acanthopleon* and *I. maculatus* each have 9 joints. *Isonebula clavuslongus* n. sp. also differs from *I. acanthopleon* in that it does not have pointed extension separated from the telson.

DISCUSSION

Cymothoids have wide distribution, including records in South America (Wilson 2008), such that 25 species have been catalogued as parasites of Amazonian freshwater fish (Thatcher 2006, Virgilio et al. 2020). However, *Isonebula clavuslongus* n. sp. is the first species of its genus to be reported in this biome.

Until now, the genus *Isonebula* contained just two species: *I. maculatus*, a parasite of the serrasalmid *Metynnis maculatus* (Kner, 1860) in the Paraná River, in the province of Corrientes, Argentina; and *I. acanthopleon*, a parasite of the characiforms of the family Curimatidae, *Cyphocharax platanus* (Gunther, 1880), *Cyphocharax spilotus* (Vari, 1987) and *Cyphocharax voga* (Hensel, 1869) in La Plata River, Buenos Aires, Argentina (Taberner 1998, 1977). Given that we found that *I. clavuslongus* n. sp. occurred exclusively on the tongue of the serrasalmid *M. ternetzi*, despite extending our examinations to 26 species belonging to 10 fish families of the Amazon region, we can speculate that this parasite exhibits some level of host specificity.

Initially, the genus *Isonebula* was proposed as *Nebula* (Taberner 1977). However, *Nebula* is a name that was first attributed to a genus of Lepidoptera. Thus, around 21 years later, in describing *I. acanthopleon*, the same author renamed the genus, which became called *Isonebula* (Taberner 1998). Now, around 25 years after the second species was described, we are proposing a third species for the genus *Isonebula*.

We described *Isonebula clavuslongus* n. sp. using morphological characteristics alone, because it was not possible to isolate DNA from the sample for molecular analysis. The sample was conserved in 70% alcohol for around 10 years and its DNA had degraded, possibly because of the high temperatures of the geographical region where this parasite sample had been stored (Amazon region, Brazil); moreover, the specimen had not been kept cooled. Nonetheless, the morphological characteristics of the specimen were amply robust for a new species to be proposed, considering that this new species has a dactyl of the 6th pereopod that is extremely long, with an inner measurement of 4.50 mm, thus differing totally from the congeneric species. Furthermore, this characteristic has not been documented in any species of Cymothoidae so far.

Robust morphological data sustain this proposal of a new species of *Isonebula*, which diverges from its congeneric species mainly through the large size of the 6th dactyl. The present study increases the number of known species of *Isonebula* to three and thus contributes towards the knowledge of the diversity of these parasites in this important Neotropical region, which presents great diversity of parasites. Thus, we have increased the number of species of Cymothoidae known to parasitize fish in the Amazon region to 26 species. The genus *Isonebula*, now with three species, continues to stand out as one of the genera with the smallest number of known species among Neotropical Cymothoidae that parasitize freshwater fish.

Acknowledgments

We thank the Fundação de Amparo à Pesquisa do Amapá (FAPEAP) for its financial support under the project Monitoramento da Atividade Pesqueira no Médio Rio Araguari (Process number 004/2013 and 005/2014); Conselho Nacional de Pesquisa e Desenvolvimento Tecnológico (CNPq), for the productivity grant to Tavares-Dias, M. (Process number 301911/2022-3), Adriano E. A. (307485/2023-4) and the Financiadora de Estudos e Projetos (FINEP) (Process number 0117005600); We would also like to thank CNPq for the Post-Doctoral scholarship granted to Marcos S. B. Oliveira (Process number 150039/2023-9) and FAPEAP for funding the project "Estudo da taxonomia, diversidade e filogenia de parasitos isópodes Cymothoida (Cymothoidae, Corallanidae e Bopyridae) de água doce da Amazônia brasileira" granted to Marcos S. B. Oliveira through the financing notice "Programa de Apoio à Fixação de Jovens Doutores no Brasil" (no 06/2022 - FAPEAP/CNPq), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Finance Code 001.

REFERENCES

CHICK AIR & CASSELLA JP. 2011. Gum Damar as a substitute for Canada Balsam in mounting media for microscopical specimens. Entomol Mon Mag 147: 111-114.

OLIVEIRA MSB, CORRÊA LL, ADRIANO EA & TAVARES-DIAS M. 2021. Integrative taxonomy of a new species of *Therodamas* (Ergasilidae) infecting the Amazonian freshwater fish *Leporinus fasciatus* (Anostomidae). Parasitol Res 120: 3137-3147.

PLANQUETTE P, KEITH P & LE BAIL P-Y. 1996. Atlas des Poissons d'eau Douce de Guyane (Tome 1), Paris, 429 p.

TABERNER R. 1977. *Nebula maculatus* gen. nov. sp. (Crustacea, Isopoda, Cymothoidae) ectoparasito de *Metynnis maculatus* (Kner 1860) Berg 1897 (Pisces, Characidae). Phys 36: 141-146.

TABERNER R. 1998. *Isonebula acanthopleon* sp. n. (Isopoda, Cymothoidae) nuevo ectoparásito de peces curimátidos. Rev Bras Zool 15: 307-313.

THATCHER VE. 2006. Amazon Fish Parasites, Moscow: Pensoft, 508 p.

VIRGILIO LR, OLIVEIRA MSB, ALMEIDA LS, TAKEMOTO RM, CAMARGO LMA & MENEGUETTI DUO. 2020. Isopods Cymothoidae ectoparasites of fish from the Amazon. Rev Bras Parasitol Veterinária 29: 1-13.

WILSON GDF. 2008. Global diversity of Isopod crustaceans (Crustacea; Isopoda) in freshwater. Hydrobiologia 595: 231-240.

WORMS. 2024. Isopoda. In: World Register of Marine Species.

How to cite

OLIVEIRA MSB, ALVES AM, LIMA ES, ADRIANO EA & TAVARES-DIAS M. 2025. *Isonebula clavuslongus* n. sp. (Isopoda: Cymothoidae) parasitizing *Myloplus ternetzi* (Norman, 1929), a fish of the family Serrasalmidae, in the Brazilian Amazon region. An Acad Bras Cienc 97: e20240590. DOI 10.1590/0001-3765202520240590.

Manuscript received on June 1, 2024;
accepted for publication on January 7, 2025

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