Isopods Cymothoidae ectoparasites of fish from the Amazon

Lucena Rocha Virgilio1,2*; Marcos Sidney Brito Oliveira3; Lorrana Santana Almeida2; Ricardo Massato Takemoto2; Luis Marcelo Aranha Camargo1,6; Dionatas Ulises de Oliveira Meneguetti1,6

1 Programa de Pós-graduação em Biodiversidade e Biotecnologia - Bionorte, Universidade Federal do Acre – UFAC, Rio Branco, AC, Brasil
2 Laboratório de Ecologia Aquática, Universidade Federal do Acre – UFAC, Campus Floresta, Cruzeiro do Sul, AC, Brasil
3 Programa de Pós-graduação em Biodiversidade Tropical – PPGBio, Universidade Federal do Amapá – UNIFAP, Macapá, AP, Brasil
4 Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura, Laboratório de Ictioparasitologia, Universidade Estadual de Maringá, Maringá, PR, Brasil
5 Instituto de Ciências Biomédicas, Universidade de São Paulo – ICB5 USP, Monte Negro, RO, Brasil
6 Laboratório de Medicina Tropical, Universidade Federal do Acre – UFAC, Rio Branco, AC, Brasil

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Abstract
Most freshwater species of Cymothoidae are distributed in South America. They have mainly been recorded in the eastern and western regions of the Amazon River basin. However, in this ecosystem, the biodiversity of this group may be greater if the entire Amazon basin is considered. In this regard, the aim of the present study was to provide an updated list of isopod species of the family Cymothoidae that are found in fish in the Brazilian Amazon region and to report on new fish host occurrences and expanded geographical distributions for cymothoid isopods that parasitize fish in the southwestern Brazilian Amazon region. The parasites found in fish specimens were collected, fixed and identified later. We found eight species of Cymothoidae parasitizing different host fish species in the southwestern Amazon region. However, we found 14 species of Cymothoidae throughout the Brazilian Amazon region. Three additional species are thus reported here, which increases the number of species of Cymothoidae in this region to 17. These additional species are also new records for Brazil. Therefore, this study has contributed to expand the knowledge about the distribution and diversity of Cymothoidae in the Amazon basin.

Keywords: Amazon River basin, geographical distribution, Cymothoidae, freshwater fish, diversity.

Resumo
A maioria das espécies de água doce de Cymothoidae está distribuída na América do Sul e foi registrada principalmente nas regiões Leste e Oeste da bacia do Rio Amazonas. Entretanto, nesse ecossistema, a biodiversidade desse grupo pode ser maior se toda a bacia amazônica for considerada. Nesse sentido, o objetivo do presente estudo foi fornecer uma lista atualizada de espécies de isópodes da família Cymothoidae que são encontrados em peixes na Região Amazônica brasileira e relatar novas ocorrências de peixes hospedeiros, ampliando as distribuições geográficas para isópodes cimotoides que parasitam peixes na região Sudoeste da Amazônia brasileira. Os parasitos encontrados em espécimes de peixes coletados foram analisados e posteriormente identificados. Foram encontradas oito espécies de Cymothoidae parasitando diferentes espécies de peixes hospedeiros na região Sudoeste da Amazônia. No entanto, foram encontradas 14 espécies de Cymothoidae em toda a Região Amazônica brasileira. Três novos relatos de espécies foram observados, o que aumenta o número de espécies de Cymothoidae nessa região para 17. Essas espécies relatadas, também são novos registros para o Brasil. Portanto, este estudo ajudou a expandir o conhecimento sobre a distribuição e diversidade de Cymothoidae na bacia amazônica.

Palavras-chave: Bacia do Rio Amazonas, distribuição geográfica, Cymothoidae, peixes de água doce, diversidade.
Isopods parasitizing fish

Introduction

The family Cymothoidae comprises around 40 genera and 380 species that have been described. Some species occur in both marine and freshwater environments (Ahyong et al., 2011; Smit et al., 2014). They are obligate parasites, and mostly use fish as their hosts, although some parasitize shrimps (Smit et al., 2014). Unlike other groups of parasites, isopods have a large body (~10 to 50 mm). Moreover, these parasites have morphological characteristics specific to parasitic life, such as buccal appendages with setae similar to the robust curved terminal and subterminal spines that serve to attach the parasite to the host tissue as well, and nails modified into powerful claws to attach to the host (Brusca et al., 2001).

Most species of Cymothoidae attach to the integument, fins, tongue, gills and buccal cavity (e.g. those in the genera *Anphira*, *Asotana*, *Braga*, *Cymothoa*, *Livoneca*, *Paracymothoa* and *Telotha*) (Thatcher, 1988; Araujo et al., 2009; Gomiero et al., 2012; Martin et al., 2016; Murrieta-Morey et al., 2016; Oliveira et al., 2017). Other species drill holes in the host integument (e.g. those in the genera *Riggia* and *Artystone*) (Magalhães et al., 2018; Oliveira et al., 2019). The host specificity in those parasites varies according to the genus and may be high (e.g. genera *Cymothoa* and *Mothocya*) or low (e.g. genera *Braga* and *Nerocila*) (Brusca et al., 2001; Tavares-Dias et al., 2014).

The highest diversity of cymothoid isopods is concentrated in the tropics, with well-known distribution in marine environments. However, they have only been poorly studied in freshwater ecosystems (Smit et al., 2014). Most freshwater species (around 13) are distributed in South America, mainly in the Amazon River basin (Thatcher et al., 2003). These species were collected in its eastern region (around 9) and central region (around 6), which concentrate the highest cymothoid diversity (Thatcher, 1993; Magalhães et al., 2018; Oliveira et al., 2017; Esteves-Silva et al., 2020). Furthermore, the northwestern Amazon region is the only area in which the occurrence of *Asotana magnifica* Thatcher, 1988 has been reported, parasitizing the mouth cavity of a specimen of *Serrasalmus* sp. (Thatcher, 1988) (Table 1).

Table 1. Cymothoidae species in fish of the Brazilian Amazon.

| Parasite species | Host species | IS | Locations | References |
|------------------|--------------|----|-----------|------------|
| *Anphira* | *Serrasalmus silpleura* | Gill chamber | Solimões River, AM; Ilha de Maracá - RR | Thatcher (1993), Murrieta-Morey et al. 2016 |
| *Pygocentrus nattereri* | Gill chamber | Solimões River, AM; Ilha de Maracá - RR | Thatcher (1993), Murrieta-Morey et al. 2016, Vital et al. (2011) |
| *Serrasalmus altispinis* | Gill chamber | Solimões River, AM | Murrieta-Morey et al. (2016) |
| *Vanamea symmetrica* | *Serrasalmus silpleura* | Gill chamber | Solimões River, AM | Murrieta-Morey et al. 2016 |
| *Serrasalmus elongatus* | Mouth cavity | Amazon River, AM | Thatcher (1993) |
| *Triportheus albus* | Gill chamber | Solimões River, AM | Araújo & Thatcher (2003) |
| *Triportheus flavus* | Gill chamber | Solimões River, AM | |
| *Anphira xinguensis* | *Ossubtus xinguense* | Gill chamber | Xingu River, PA | Thatcher (1995) |
| *Metynnis lippincottianus* | Gill | Xingu River, PA | Magalhães et al. (2018) |
| *Metynnis hypsauchen* | Gill | Xingu River, PA |
| *Metynnis altidorsalis* | Gill | Xingu River, PA |
| *Artystone minima* | *Nannostomus beckfordi* | Tegumentar orifice | Upper Rio Negro, AM | Thatcher & Carvalho (1988) |
| *Artystone trysibia* | *Caquetaia spectabilis* | Tegumentar orifice | Jari River, AP | Oliveira et al. (2019) |

AM: Amazonas state; AP: Amapá state; RR: Roraima state; PA: Pará state; TO: Tocantins state; MT: Mato Grosso state; NI: Not informed.
| Parasite species | Host species | IS | Locations | References |
|------------------|--------------|----|-----------|------------|
| **Geophagus brasiliensis** | Tegumentar orifice | Roraima River, RR | Van Name (1936) |
| **Serrasalmus sp.** | Mouth cavity | Uaricoera River, RR | Thatcher (1988) |
| **Asotona magnifica** | Mouth cavity | Upper Araguaí River, AP | Thatcher (1996) |
| **Acestrorhynchus microlepis** | Mouth cavity | Upper Araguaí River, AP | Thatcher (1996) |
| **Acestrorhynchus guyanensis** | Mouth cavity | Upper Araguaí River, AP | Thatcher (1996) |
| **Braga amapaensis** | Mouth cavity | Negro River, AM | Araujo et al. (2009) |
| **Braga cichlae** | Mouth cavity | Jari River, AP | Oliveira et al. (2017) |
| **Braga fluviatilis** | Mouth cavity | Jari River, AP | Oliveira et al. (2017) |
| **Salminus maxillosus** | NI | Xingu River, MT | Lemos de Castro (1959) |
| **Loricaria anus** | NI | Xingu River, MT | Lemos de Castro (1959) |
| **Braga nasuta** | Tegument | Fish-farming in Parauapebas, PA | Jesus et al. (2017) |
| **Braga patagonica** | Gill | Igarapé Fortaleza River, AP | Alcântara & Tavares-Dias (2015) |
| **Plagioscion squamosissimus** | Mouth cavity; gills | Middle Solimões, AM | Tavares-Dias et al. (2014) |
| **Pygocentrus nattereri** | Mouth cavity; gills | Middle Solimões, AM | Tavares-Dias et al. (2014) |
| **Colossoma macropomum** | Mouth cavity; gills | Middle Solimões, AM | Tavares-Dias et al. (2014) |
| **Serrasalmus sp.** | Mouth cavity; gills | Middle Solimões, AM | Tavares-Dias et al. (2014) |
| **Serrasalmus rhombeus** | Mouth cavity; gills | Middle Solimões, AM | Tavares-Dias et al. (2014) |
| **Mylossoma duriventre** | Mouth cavity; gills | Middle Solimões, AM | Tavares-Dias et al. (2014) |
| **Brycon amazonicus** | Mouth cavity; gills | Middle Solimões, AM | Tavares-Dias et al. (2014) |
| **Chaetobranchopsis orbicularis** | Mouth cavity; gills | Middle Solimões, AM | Tavares-Dias et al. (2014) |
| **Hydrolycus scombreoides** | Mouth cavity; gills | Middle Solimões, AM | Tavares-Dias et al. (2014) |
| **Colossoma macropomum** | Gill | Igarapé Fortaleza River, AP | Tavares-Dias et al. (2014) |
| **Acestrorhynchus falcatus** | Gill | Igarapé Fortaleza River, AP | Tavares-Dias et al. (2014) |
| **Colossoma macropomum** | Gill | Fish-farming in Macapá, AP | Dias et al. (2015) |
| **Curimata incompta** | Gills and fins | Igarapé Fortaleza River, AP | Neves et al. (2016) |
| **Chaetobranchus flavescens** | Gill | Igarapé Fortaleza River, AP | Bittencourt et al. (2014) |
| **Colossoma macropomum** | Fins | Jari River, AP | Gonçalves et al. (2018) |
| **Serrasalmus altispinis** | Mouth cavity | Jari River, AP | Oliveira et al. (2017) |
| **Leporinus fasciatus** | Gill | Matapi River, AP | Neves & Tavares-Dias (2019) |
| **Livoneca guianensis** | Mouth cavity | Jari River, AP | Oliveira et al. (2017) |
| **Riggia puyensis** | Ancistrus sp. | Tegumentar orifice | Xingu River, PA | Magalhães et al. (2018) |

AM: Amazonas state; AP: Amapá state; RR: Roraima state; PA: Pará state; TO: Tocantins state; MT: Mato Grosso state; NI: Not informed.
Isopods parasitizing fish

The species of Cymothoidae that parasitize fish in the southwestern Brazilian Amazon region (e.g. the state of Acre) were hitherto unknown. This region shows high heterogeneity of aquatic environments and high fish diversity (Begossi et al., 1999), which are ideal for accommodating great richness of Cymothoidae. Based on this principle, it is important to develop studies that expand the knowledge about the distribution of these ectoparasite crustaceans in this region of the Amazon basin. This will not only contribute information on the biodiversity of the Neotropical region, but also can help prevent fish losses in farming systems, where these organisms are known pests (Tavares-Dias et al., 2015).

Therefore, the aim of this study was to provide an updated list of isopod species of the family Cymothoidae that are found in fish in the Brazilian Amazon region and to report on new fish host occurrences and expanded geographical distributions for cymothoid isopods that parasitize fish in the southwestern Brazilian Amazon region.

Material and Methods

Collection sites

The fish were caught in the Juruá (7°40’34.1” S; 72°39’39.5” W), Crôa (7°71’48.30” S; 72°53’34.98” W), and Môa rivers (7°37’18” S; 72°47’47” W), all located in the municipality of Cruzeiro do Sul, state of Acre, and Paranã river (7°17’13”S 72°36’49”W) in the municipality of Guajara, in state of Amazonas, Brazil. This was done under a permit from the Brazilian Institute for the Environment and Renewable Natural Resources (no. 59642-2/2019) (Figure 1).

Sampling

The fish specimens were caught using gillnets of 80 m in length and 3.0 m in height, with meshes of 1.5 cm, 2.5 cm, 3.5 cm and 5.5 cm between opposite nodes. The nets were installed in the early afternoon and were kept there for 12 hours with fish collections every two hours. The two nets used each had a mesh size of 12 mm, height of 2 m and opening size of 12 m. The nets were launched ten times per collector at each collection point. A beach trawl was also used, measuring 9 m in length and 2.4 m in height, with 13 mm mesh on the wings.

Figure 1. Location of occurrence places of Cymothoidae species occurrence sites throughout the Brazilian Amazon.
Isopods parasitizing fish

Each fish was initially identified in the field and its mouth, nostrils, operculum, gills, and integument were examined macroscopically to check for the presence of isopod parasites. The fish were then stored in water-filled boxes with an oxygenator and were transported to the laboratory, where they examined for parasites using a stereomicroscope. After this, the fish were fixed in 10% formalin and were identified in accordance with the specialized literature (Silvano, 2001; Buckup et al., 2007; Queiroz, 2010). The parasites found were fixed in 70% alcohol, sent to the laboratory at Embrapa Amapá and identified in accordance with the specialized literature, as listed in Table 1.

Database review

A review was conducted in the SciELO, ISI, Scopus, Science Direct, Zoological Records, CAB Abstracts Archive, and Google Scholar databases, in order to describe the distribution of Cymothoidae species in the Brazilian Amazon region (Table 1). The geographical coordinates of each study were ascertained using Google Earth and were used to generate a geographical distribution map of the family Cymothoidae in the Brazilian Amazon region, using the QGIS software, version 3.12.2.

Results

We collected a total of 2551 fish specimens, belonging to five orders, 23 families and 83 species. Out of the total number of fish collected, only 15 individuals (0.4%) in 10 species (12%) were parasitized by Cymothoidae (Tables 2 and 3).

Table 2. Fish species collected in rivers of the southwestern Amazon, Brazil.

| Order/Family/Species | Môa River | Paranã River | Juruá River | Crôa River |
|----------------------|-----------|--------------|-------------|------------|
| Characiformes        | 00        | 0            | 0           | 0          |
| Acestrorhynchidae    | 0         | 0            | 0           | 0          |
| Acestrorhynchus cf. falcatus (Bloch, 1794) | 0 | 8 | 6 | 2 |
| Acestrorhynchus falcirostris (Cuvier, 1819) | 4 | 0 | 2 | 0 |
| Acestrorhynchus heterolepis (Cope, 1878) | 7 | 0 | 0 | 0 |
| Acestrorhynchus cf. microlepis (Schomburgk, 1841) | 3 | 0 | 0 | 0 |
| Acestrorhynchus sp. | 8 | 23 | 0 | 2 |
| Anostomidae          | 0         | 0            | 0           | 0          |
| Leporinus jamesi (Garman, 1929) | 2 | 2 | 2 | 3 |
| Pseudanos cf. gracilis (Kner, 1858) | 0 | 0 | 0 | 0 |
| Rhytiodus cf. argenteofuscus (Kner,1858) | 2 | 0 | 0 | 0 |
| Rhytiodus microlepis (Kner, 1859) | 0 | 0 | 0 | 1 |
| Characidae           | 0         | 0            | 0           | 0          |
| Brachychalcinus paranaibae (Reis, 1989) | 0 | 0 | 0 | 0 |
| Brachychalcinus retrospina Boulenger, 1892 | 4 | 0 | 0 | 8 |
| Charax cf. pauciradiatus (Günter, 1864) | 7 | 2 | 2 | 2 |
| Moenkhausia cf. jamesi (Eigenmann, 1908) | 0 | 0 | 1 | 0 |
| Roeboides myersi (Gill, 1870) | 8 | 0 | 3 | 2 |
| Tetrogonopterus argenteus Cuvier, 1816 | 7 | 0 | 0 | 0 |
| Curimatidae          | 0         | 0            | 0           | 0          |
| Curimatella meyeri (Steindachner, 1882) | 6 | 27 | 5 | 2 |
| Order/Family/Species | Môa River | Paranã River | Juruá River | Crôa River |
|----------------------|-----------|-------------|-------------|------------|
| Cyphocharax cf. festivus Vari, 1992 | 57 | 111 | 3 | 0 |
| Cyphocharax cf. plat anus (Günther, 1880) | 0 | 0 | 0 | 0 |
| Potamorhina cf. Iatior (Spix & Agassiz, 1829) | 5 | 0 | 34 | 0 |
| Psectrogaster amazonica Eigenmann & Eigenmann, 1889 | 0 | 0 | 0 | 3 |
| Cynodontidae | 0 | 0 | 0 | 0 |
| Cynodon gibbus (Agassiz, 1829) | 0 | 2 | 0 | 0 |
| Erythrinidae | 0 | 0 | 0 | 0 |
| Erythrinus erythrinus (Bloch & Schneider, 1801) | 0 | 1 | 0 | 0 |
| Hoplias malabaricus (Bloch, 1794) | 19 | 38 | 26 | 7 |
| Hemiodontidae | 0 | 0 | 0 | 0 |
| Anodus elongatus Agassiz, 1829 | 5 | 0 | 0 | 3 |
| Prochilodontidae | 0 | 0 | 0 | 0 |
| Prochilodus nigricans Spix & Agassiz, 1829 | 50 | 14 | 22 | 31 |
| Serrasalmidae | 0 | 0 | 0 | 0 |
| Metynnis luna Cope, 1878 | 0 | 0 | 57 | 0 |
| Myloplus cf. arnoldi Ahl, 1936 | 0 | 14 | 0 | 0 |
| Myloplus cf. rubripinnis (Müller & Troschel, 1844) | 16 | 0 | 0 | 0 |
| Pygocentrus nattereri Kner, 1858 | 0 | 2 | 2 | 13 |
| Serrasalmus rhombeus (Linnaeus, 1766) | 0 | 0 | 0 | 0 |
| Triportheidae | 0 | 0 | 0 | 0 |
| Triportheus albus Cope, 1872 | 14 | 0 | 0 | 0 |
| Triportheus angulatus (Spix & Agassiz, 1829) | 4 | 2 | 12 | 10 |
| Clupeiformes | 0 | 0 | 0 | 0 |
| Pristigasteridae | 0 | 0 | 0 | 0 |
| Pellona flavipinnis (Valenciennes, 1837) | 1 | 0 | 0 | 0 |
| Gymnotiformes | 0 | 0 | 0 | 0 |
| Gymnotidae | 0 | 0 | 0 | 0 |
| Electrophorus electricus (Linnaeus, 1766) | 0 | 0 | 0 | 2 |
| Rhamphichthyidae | 0 | 0 | 0 | 0 |
| Rhamphichthys marmoratus Castelnau, 1855 | 1 | 1 | 0 | 1 |
| Cichiliformes | 0 | 0 | 0 | 0 |
| Cichlidae | 0 | 0 | 0 | 0 |
| Biotodoma cupido (Heckel, 1840) | 0 | 7 | 0 | 6 |
| Biotodoma cf. wavrini (Gosse, 1963) | 0 | 0 | 0 | 0 |
| Chaetobranchus flavescens Heckel, 1840 | 16 | 0 | 49 | 81 |
| Cichla nigromaculata Jardine & Schomburgk, 1843 | 9 | 0 | 2 | 6 |
| Crenicichla semicincta Steindachner, 1892 | 11 | 0 | 0 | 0 |
### Table 2. Continued...

| Order/Family/Species | Môa River | Paranã River | Juruá River | Crôa River |
|----------------------|-----------|--------------|-------------|------------|
| **Isopods parasitizing fish** |           |              |             |            |
| Heros cf. severus Heckel, 1840 | 0 | 2 | 0 | 0 |
| Laetacara sp. | 2 | 4 | 0 | 0 |
| Satanoperca cf. jurupari Heckel, 1840 | 0 | 0 | 0 | 0 |
| **Scinidae** |           |              |             |            |
| Plagioscion squamosissimus Heckel, 1840 | 1 | 0 | 0 | 0 |
| **Siluriformes** |           |              |             |            |
| Asprendinidae | 0 | 0 | 0 | 0 |
| Bunocephalus coracoideus Cope, 1874 | 0 | 3 | 0 | 0 |
| **Auchenipteridae** |           |              |             |            |
| Ageneiosus inermis (Linnaeus, 1766) | 3 | 4 | 0 | 1 |
| Auchenipterichthys sp. | 15 | 3 | 0 | 0 |
| Auchenipterichthys thoracatus (Kner, 1858) | 59 | 1 | 0 | 0 |
| Centromochlus sp. | 0 | 0 | 0 | 0 |
| Trachelyopterus cf. galeatus (Linnaeus, 1766) | 0 | 2 | 0 | 0 |
| Trachelyopterus sp. | 0 | 185 | 1 | 14 |
| **Callichthyidae** |           |              |             |            |
| Brochis multiradiatus (Orcés V., 1960) | 5 | 0 | 13 | 2 |
| Megalechis cf. thoracata (Valenciennes, 1840) | 0 | 1 | 29 | 0 |
| **Doradidae** |           |              |             |            |
| Agamyxis pectinifrons (Cope, 1870) | 0 | 53 | 0 | 0 |
| Amblydoras affinis (Kner, 1855) | 0 | 12 | 0 | 0 |
| Anadoras cf. weddellii (Castelnau, 1855) | 0 | 0 | 0 | 3 |
| Nemadoras cf. cristinae Sabaj Pérez, Arce H., Sousa & Birindelli, 2014 | 16 | 0 | 0 | 0 |
| Nemadoras cf. humeralis (Kner, 1855) | 30 | 1 | 0 | 4 |
| Opsodoras boulengeri (Steindachner, 1915) | 18 | 1 | 0 | 0 |
| Ossancora asterophyso Birindelli & Sabaj Pérez, 2011 | 13 | 733 | 45 | 8 |
| Oxydoras niger (Valenciennes, 1821) | 0 | 0 | 0 | 0 |
| Platydoras cf. armatulus (Valenciennes, 1840) | 2 | 8 | 0 | 0 |
| Platydoras costatus (Linnaeus, 1758) | 0 | 0 | 0 | 0 |
| Pterodoras granulosus (Valenciennes, 1821) | 0 | 0 | 0 | 0 |
| Trachydoras sp. | 2 | 3 | 0 | 5 |
| Tenellus ternetz (Eigenmann, 1925) | 21 | 0 | 0 | 0 |
| Tenellus trimaculatus (Boulenger, 1898) | 7 | 0 | 6 | 0 |
| **Loricariidae** |           |              |             |            |
| Aphanotorulus cf. unicolor (Steindachner, 1908) | 0 | 0 | 0 | 0 |
| Calophysus macropterus (Lichtenstein, 1819) | 3 | 0 | 0 | 0 |
| Hypoptopoma cf. steindachneri Boulenger, 1895 | 2 | 0 | 0 | 0 |
Isopods parasitizing fish

We collected a total of 15 specimens of Cymothoidae, belonging to eight species: *Artystone bolivianensis* Thatcher & Schindler, 1999; *Artystone trysibia* Schioedte, 1866; *Braga fluviatilis* Richardson, 1911; *Paracymothoa parva* Taberner, 1976; *Braga* sp. Schioedte & Meinert, 1881; *Braga patagonica* Schiödte & Meinert, 1884; *Braga brachmanni* Stadler, 1972; and *Livoneca guianensis* Van Name, 1925. All the parasites were recorded for the first time in these host fish species. *Artystone bolivianensis*, *B. brachmanni* and *P. parva* were recorded for the first time parasitizing fish in Brazil (Table 3).

Among the eight species of Cymothoidae collected, four were found in the Paranã River, two in the Moa River, one in the Juruá River and one in the Crôa River. *Braga fluviatilis* and *L. guianensis* were observed to parasitize the highest number of hosts, such that *L. guianensis* occurred in all four rivers. The mean intensity of the species of Cymothoidae were similar to each other, i.e. one parasite per fish (Table 3).

Out of the 15 individuals of Cymothoidae collected, ten were females (73.3%). The highest prevalence of specimens were recorded for *B. fluviatilis* (three specimens) and *L. guianensis* (three specimens). Most of these isopods were found in the fish integument and mouth cavity. However, *P. parva* and *Braga* sp. occurred in the anal cavity of the hosts and *B. patagonica* in the gills (Table 3).

We found a total of 34 host fish species for these species of Cymothoidae in the Brazilian Amazon region. Eight species of these isopods were found in fish in the state of Amazonas, five in the state of Amapá, four in the state of Pará, two in the state of Roraima and one in the state of Mato Grosso. Across the Brazilian Amazon region, the isopod *B. patagonica* showed the widest geographical distribution and largest number of host species (Table 1).

| Order/Family/Species | Môa River | Paranã River | Juruá River | Crôa River |
|----------------------|-----------|--------------|-------------|------------|
| Loricaria cataphracta Linnaeus, 1758 | 6 | 0 | 0 | 0 |
| Loricaria sp. | 40 | 0 | 0 | 0 |
| Loricariichthys sp. | 95 | 5 | 21 | 0 |
| Peckoltia cf. vittata (Steindachner, 1881) | 0 | 0 | 0 | 0 |
| Spatuloricaria sp. | 0 | 0 | 0 | 0 |
| Squilloforma cf. squalina (Jardine, 1841) | 1 | 0 | 0 | 0 |
| Sturisoma cf. lyra (Regan, 1904) | 10 | 0 | 0 | 0 |
| Sturisoma cf. tenuirostris (Steindachner, 1910) | 2 | 0 | 0 | 0 |
| Sturisoma sp. | 2 | 0 | 0 | 0 |
| Pimelodidae | 0 | 0 | 0 | 0 |
| Hemisorubim sp. | 0 | 3 | 0 | 0 |
| Hypophthalmus dentatus Spix & Agassiz, 1829 | 0 | 0 | 0 | 0 |
| Pimelodelia cf. humeralis Slobodian, Akama & Dutra, 2017 | 0 | 0 | 2 | 1 |
| Pimelodina flavipinnis Steindachner, 1876 | 0 | 0 | 1 | 0 |
| Pimelodus blochii Valenciennes, 1840 | 8 | 0 | 5 | 3 |
| Pinirampus pirinampu (Spix & Agassiz, 1829) | 2 | 0 | 1 | 0 |
| Sorubim lima (Bloch & Schneider, 1801) | 1 | 0 | 0 | 5 |
| Tetraodontiformes | 0 | 0 | 0 | 0 |
| Tetraodontidae | 0 | 0 | 0 | 0 |
| Colomesus asellus (Müller & Troschel, 1849) | 37 | 0 | 1 | 0 |

Table 2. Continued...
Isopods parasitizing fish

Discussion

Parasites of the family Cymothoidae are distributed throughout the Amazon River basin, in which the largest numbers of species have been reported in the central and eastern regions of this basin, where these cymothoids parasitize a great diversity of hosts belonging to the families Acestrorhynchidae, Anostomidae, Arapaimidae, Bryconidae, Curimatidae, Cynodontidae, Triportheidae, Serrasalmidae, Loricariidae, Pimelodidae, Lebiasinidae, Cichlidae, Erythrinidae and Sciaenidae (Table 1). Our results from the southwestern Amazon region, together with data from the central and eastern Amazon regions, depict an area with great richness of species of Cymothoidae, totaling 17 species recorded in fish in the Brazilian Amazon region.

However, it is essential to note that the biogeographical status of freshwater cymothoids may still be underestimated (Smit et al., 2014) because of the extensive area of the Amazon region. Many areas of this region have never been studied to assess the fauna of this group of parasites.

In the present study, not all the fish species acted as hosts for these cymothoids. The species parasitized generally have low abundance, since out of the 84 fish species analyzed, only 10 were parasitized by cymothoids, with low values for mean abundance and intensity. Immunological, behavioral, environmental and genetic characteristics may explain this complex parasite-host interaction (Poulin, 2013; Aneesh et al., 2013; Tavares-Dias et al., 2015).

| Hosts | Parasites | Locality | Sex of parasites | IS | EF/PF | MA | MI | TNP |
|-------|-----------|----------|-----------------|----|-------|----|----|-----|
| Auchenipterichthys thoracatus | Braga fluviatilis | Môa River, AC | M | Tegument | 22/1 | 0.045 | 1 | 1 |
| Biotodoma wavrini | Artystone bolivianensis | Paranã River, AM | F | Tegumentar orifice | 2/1 | 0.500 | 1 | 1 |
| Brachychalcinus retrospina | Braga fluviatilis | Môa River, AC | M | Mouth cavity | 10/1 | 0.100 | 1 | 1 |
| Brachychalcinus retrospina | Paracymothoa parva | Môa River, AC | F | Anus | 10/1 | 0.100 | 1 | 1 |
| Curimatella meyeri | Brago sp. | Môa River, AC | F | Anus | 67/1 | 0.015 | 1 | 1 |
| Laetacara sp. | Artystone trysibia | Paranã River, AM | F, F | Anus and tegumentar orifice | 2/2 | 1.000 | 1 | 2 |
| Leporinus jamesi | Livoneca guianensis | Paranã River, AM | F | Mouth cavity | 2/1 | 0.500 | 1 | 1 |
| Leporinus jamesi | Braga fluviatilis | Môa River, AC | M | Mouth cavity | 2/1 | 0.500 | 1 | 1 |
| Pimelodella cf. humeralis | Livoneca guianensis | Juruã River, AC | F | Mouth cavity | 22/1 | 0.045 | 1 | 1 |
| Pimelodella cf. humeralis | Braga patagonica | Paranã River, AM | F | Gill | 2/1 | 0.500 | 1 | 1 |
| Rhytiodus microlepis | Livoneca guianensis | Crôa River, AC | F | Tegument | 1/1 | 1.000 | 1 | 1 |
| Sturisoma lyra | Braga brachmanni | Môa River, AC | F, M | Mouth and tegument | 1/2 | 1.000 | 1 | 2 |
| Tonellus ternetzi | Livoneca guianensis | Môa River, AC | F | Mouth cavity | 8/1 | 0.125 | 1 | 1 |

AM: Amazonas state, AC: Acre state. IS: infestation site, MA: mean abundance, MI: mean infestation, EF: examined fish, PF: parasitized fish, P: prevalence, MI: mean intensity, MA: mean abundance, TNP: total number of parasites.
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and act as important factors in the selection of a host fish. Recently, it was observed that the defenses of some fish species are important for preventing colonization by cymothoids. These defenses include the ability of the host to remove the parasite using its mouth or through rapid movements with its body. In some cases, fish can eat these isopods, thereby eliminating the risk of parasitism (Alberto et al., 2009). Another factor that can influence the degree of infestation is the short period for which this parasite can survive freely in the environment. Free living requires a large energy investment, to search for a host fish, and the time that this takes can lead these parasites to death (Leonardos & Trilles, 2003).

**Artystone bolivianensis, B. brachmanni** and **P. parva** are new species records for the Amazon and Brazil. **Artystone bolivianensis** was previously described parasitizing *Otocinclus vestitus* Cope, 1872, in Santa Cruz de la Sierra, Bolivia (Thatcher & Schindler, 1999). **Braga brachmanni** was previously described parasitizing *Ancistrus cirrhosus* Valenciennes, 1836, in Argentina (Stadler, 1972). **Paracymothoa parva** was previously described parasitizing *Hyphessobrycon callistus* (synonym of *Hyphessobrycon eques* Steindachner, 1882) (Taberner, 1977) and subsequently parasitizing *Metynnis maculatus* Kner, 1858 (Taberner, 1977), also in Argentina.

New reports on Cymothoidae in the Amazon region can be expected, given the high diversity of environments and host fish in this region (Hata et al., 2017; Tavares-Dias et al., 2015). In this context, when research is conducted in areas where few studies had previously been conducted, new reports of known species are expected for that region, in addition to species not yet described.

The data from the present study expanded the geographical distribution of *B. patagonica* to the southwestern part of the Brazilian Amazon region. *Braga patagonica* has wide geographical distribution, occurring in different tributaries of the Amazon River basin (e.g. the middle Solimões River, Amazon River, Jari River and Matapi River). This species parasitizes a variety of hosts belonging to the families Acestrorhynchidae, Anostomidae, Bryconidae, Curimatidae, Cynodontidae, Serrasalmidae, Cichlidae, Erythrinidae and Sciaenidae (Table 1). The generalist nature of this species of Cymothoidae demonstrates its low parasitic specificity (Tavares-Dias et al., 2014, 2015).

In general, in the Amazon region, genera of Cymothoidae such as *Anphira* and *Vanamea* have been found infesting the gill cavities of hosts, while *Artystone* and *Riggia* have been found inside holes formed by the parasite in the tegument of the fish, mostly in the pelvic region of these hosts (Table 1). However, *Braga* species can be found in several places in the host, such as the mouth and gill cavities, and integument surface. The ways in which the parasite becomes attached to the host fish can influence the specificity of species of Cymothoidae regarding their host choice (Hata et al., 2017). Cymothoids parasitizing the external surfaces of their hosts, such as the gill and mouth cavities, are more generalist, and can become attached to many fish species (Bruce, 1986; Hadfield et al., 2015). This was observed in the present study, in which species of the genera *Braga* and *Livoneca* were seen to infest the integument, gills and mouth cavities in many fish species.

Encapsulation in the host integument as the means of attachment is characteristic of *Artystone* and *Riggia* (Thatcher et al., 2003; Junoy, 2016; Oliveira et al., 2019) and can be considered to be a host-parasite specific characteristic (Tsai & Dai 1999; Yamano et al., 2011; Hata et al., 2017). Data in the literature show that species are generalists, parasitizing fish species of the families Anostomidae, Ariidae, Characidae, Cichlidae, Heptapteridae, Loricariidae and Pimelodidae (Junoy, 2016). However, in the Brazilian Amazon region, the *Artystone* species that was observed can be considered to be a specialist because it was found parasitizing only Cichlidae fish (Oliveira et al., 2019). Studies on integrative, comparative and molecular biology may be the approach that should be followed in order to understand these complex interactions.

**Artystone trysibia**, *Braga* sp. and *P. parva* were found in the anal cavity of their hosts (Table 2). Among the cymothoids reported in Brazil, only *Riggia acuticaudata* Thatcher, Lopes & Froehlich, 2002 was found parasitizing the anal cavity of its host, a specimen of *Ancistrus* sp. (Oda et al., 2015). Therefore, the present study suggests that because the anal cavity is an opening and these parasites have the habit of living in cavities, this site becomes an important place of refuge and the parasites may use excreted food waste in their diet.

In conclusion, through this study, the occurrence and distribution of Cymothoidae in the Brazilian Amazon are expanded, with new records of host fish and infestation sites. Therefore, this study contributes towards future studies on the diversity, distribution and specificity of cymothoids in the Amazon region, which is the most biodiverse region on the planet.
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