

Molecular Phylogeny and taxonomy of a new Myxobolus species from the endangered ornamental fish, Otocinclus cocama endemic to Peru: A host-parasite coextinction approach

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4	Patrick D. Mathews ^{a,b*} , Omar Mertins ^c , Tiago Milanin ^d , Luis L. Espinoza ^e , Anai P.												
5	Gonzales-Florese ^f , Fabienne Audebert ^b , André C. Morandini ^a												
6	^a Department of Zoology, Institute of Biosciences, University of São Paulo, 05508-090,												
7	São Paulo, Brazil												
8	^b Unité Biologie des Organismes et Écosystèmes Aquatiques, Sorbonne Université,												
9	MNHN, CNRS, IRD, UCA, CP 26, 75231 Paris, France												
10	^c Department of Biophysics, Paulista Medical Scholl, Federal University of São Paulo,												
11	04023-062 São Paulo, Brazil												
12	^d Department of Basic Sciences, Faculty of Animal Science and Food Technology,												
13	University of São Paulo, 13635-900, Brazil												
14	^e Laboratory of Biology and Molecular Genetics, Faculty of Veterinary Medicine,												
15	National University of San Marcos, 2800 San Borja, Lima, Peru												
16	^f Post-Graduate Program in Tropical Biodiversity, Federal University of Amapá, 68903-												
17	419 Macapá, Brazil												
18	*Corresponding author at: Department of Zoology, Institute of Biosciences, University												
19	of São Paulo, 05508-090 São Paulo, Brazil. E-mail address:												
20	patrickmathews83@gmail.com (P.D. Mathews).												
21	ABSTRACT												

A new *Myxobolus* species is described infecting gill filaments of the endangered
ornamental fish *Otocinclus cocama* from Peruvian Amazon. In a total of 35 fish

24 examined, five (14.3%) had myxozoan plasmodia. Taxonomic analysis was performed 25 integrating multiple characters, including morphometrical, biological traits, ssrDNA 26 sequence data and host ecological characters. Myxospores of *M. iquitoensis* n. sp. were 27 ovoid in shape from the frontal view and measured $17.6\pm1.2 \ \mu m \ (16.2-19.8 \ \mu m)$ in 28 length and $10.5\pm0.7 \,\mu\text{m}$ (9.8–12 μm) in width. The two polar capsules were elongate in 29 shape, equal in size and occupying almost half of the myxospore body. They measured 30 $8.7\pm0.4 \ \mu m \ (6.9-9.3 \ \mu m)$ in length and $3.3\pm0.2 \ \mu m \ (3-3.6 \ \mu m)$ in width. The polar tubules presented six to seven turns. Molecular phylogenetic analysis revealed that the 31 32 obtained ssrDNA sequence did not match any existing sequences in GenBank but showed *M. iquitoensis* n. sp. to be a close species of *M. figueirae*. Nonetheless, the 33 34 ssrDNA sequences of those species show large genetic divergence. This is the first description and phylogenetic study of a myxozoan parasitizing fish of the genus 35 36 Otocinclus from South America, as well the first report of these parasites infecting a 37 fish belonging to the Loricariidae family from Amazon basin. Considering the endangered status of the host, the high degree of host-specificity of freshwater histozoic 38 myxobolids, the low occurrence shown by the new myxozoan, and the fact that this is 39 the only host known for this myxozoan, the conservation status of the new species of 40 myxozoan is likely to be connected to the future survival of its host. 41

42 Keywords: Cnidaria; Myxosporea; Myxobolus iquitoensis n. sp.; Peruvian Amazon

43 **1. Introduction**

The worldwide aquarium industry moves millions of fishes each year around the world and is an important source of income in many local markets (Prang, 2007). Nonetheless, as a function of growing global demand of the pet trade, exports of species that are biologically unsuited to heavy exploitation has increased. This has resulted in dramatic reductions of natural populations, and contributed to local extinctions (Ng and Tan,
1997; Moreau and Coomes, 2006; Mohanty and Measey, 2019). In this context, overexploitation of wild ornamental fish for the aquarium trade has become an important
conservation menace (Moreau and Coomes, 2007).

52 The Amazon basin is the most important source of ornamental freshwater fishes for the 53 international aquarium industry (Prang, 2007; Moreau and Coomes, 2007). Among 54 countries of the Amazon region, Peru is the second largest exporter of ornamental fish, with about 14,000 people depending directly on the aquarium trade (Moreau and 55 Coomes, 2007). This activity is centralized in the city of Iquitos in the Department of 56 Loreto, Peru with approximately 28 established aquarium fish exporter firms, exporting 57 58 ornamental fish to 24 countries (Moreau and Coomes, 2007). Virtually all fishes in the trade are taken from a variety of wild aquatic habitats such as main river channels, small 59 60 tributaries, floodplain oxbow lakes, swamps, flooded moriche palm forest, and remote 61 upland streams (Moreau and Coomes, 2007).

The plecos are members of the family Loricariidae. With more than 830 valid species, 62 they are the most species-rich family within the Order Siluriformes. These neotropical 63 freshwater catfishes are distributed throughout South America (Ribeiro et al., 2012). 64 65 Within Loricariidae, species belonging to the genus Otocinclus Cope, 1871 are small 66 loricariids not exceeding 5 cm in length and represent one of the most important aquarium fish exported from Peruvian Amazon, comprising over 29% of the total 67 volume of the international export (Moreau and Coomes, 2007). Species of Otocinclus 68 69 are widely distributed in South America, specifically east of the Andes, and often inhabit small lowland tributaries of the main rivers associated with marginal vegetation 70 71 (Schaefer, 1997). Among these, Otocinclus cocama Reis, 2004 known as "zebra otocinclus" is very popular in the aquarium trade and it is one the most important 72

aquarium fish exports from Iquitos based on international sales (Moreau and Coomes, 2007). *O. cocama* is endemic to Peruvian Amazon, specifically from the Yanayacu small stream, near district Jenaro Herrera, Department of Loreto (Reis, 2004). As a direct consequence of the increasing exploitation by the ornamental trade, with continuing decline of mature individuals, *O. cocama* is currently considered an endangered species in Peru and it is included on the Red List of Threatened Species-IUCN (Hidalgo and Chocano, 2016).

Models of coextinction have suggested parasites to be one the most menaced ecological 80 groups (Strona et al., 2013). According to Dallas and Cornelius (2015) extinction and 81 declining of wild hosts could result in secondary extinctions of their parasites, 82 83 especially parasites endemic to a small subset of host species. Following the fine scale of host-parasite coextinction, parasites of O. cocama are likely to be similarly 84 vulnerable. However, little is known about parasites that infect this Amazonian 85 86 ornamental fish, especially the myxozoan parasites. Thus, it is important to clarify the 87 parasite fauna of O. cocama.

Myxozoans are microscopic parasitic cnidarians of worldwide distribution and they 88 represent about 20% of the cnidarian biodiversity known to date (Atkinson et al., 2018). 89 Although predominantly known to infect wild and cultured fishes, myxozoans can 90 91 infect virtually all vertebrate groups, including amphibians, reptiles, birds, and 92 terrestrial mammals (Okamura et al., 2015; Lisnerová et al., 2019). Among the myxozoans, the genus Myxobolus Bütschli, 1882 is the most abundant, accounting for 93 around 905 species described within a wide geographical range (Eiras et al., 2014). 94 95 Freshwater histozoic Myxobolus species are widely recognized for their strict or high host-specificity (Molnár and Eszterbauer, 2015). 96

97 In this study, we described a new host-specific freshwater histozoic myxozoan,
98 *Myxobolus iquitoensis* n. sp. infecting the gill filaments of the endangered fish *O*.
99 *cocama* from the Peruvian Amazon. Morphological and molecular features of the
100 parasite are provided herein.

101 **2. Materials and methods**

102 A total of 35 specimens of Otocinclus cocama were acquired from local fisherfolk of aquarium ornamental fishes in September 2019, for parasitological survey. According to 103 104 the fisherfolk, the fishes were caught in the marginal vegetation in the small stream Yanayacu, near Jenaro Herrera district, Loreto, Peru (4°58′58″S, 74°19′38″W). The fish 105 106 were transported live to the field laboratory at the Research Institute of the Peruvian 107 Amazon, where they were euthanized by pit transaction and examined using a light 108 microscope to verify the presence of lesions and parasites. The euthanasia method was approved by Federal University of São Paulo-UNIFESP Ethics Committee (CEUA 109

110 No. 9209080214; Federal Law No. 11794, dated 8 October 2008), in accordance to111 international procedures.

For morphological and morphometric characterization, the mature myxospores were 112 fixed in 10% formalin and transported to Department of Zoology, University of São 113 114 Paulo, Brazil. The analyses were performed based on the criteria outlined by Lom and Arthur (1989). Measurements and photographs were taken from 30 myxospores using a 115 compound microscope Leica DM1000 LED equipped with Leica Application Suite 116 117 version 1.6.0 image capture software. Smears containing free myxospores were air dried, fixed with methanol and stained with Giemsa solution to mount on permanent 118 slides that were deposited in the collections of the Museum of Zoology of the 119 University of São Paulo - USP, São Paulo, Brazil. 120

121 For molecular characterization, plasmodia were removed from the infected tissue and 122 preserved in absolute ethanol. Extraction of genomic DNA (gDNA) was carried out 123 from a single plasmodium and was performed using a DNeasy® Blood & Tissue Kit (animal tissue protocol) (Qiagen Inc., California, USA), in accordance with the 124 manufacturer's instructions. gDNA concentration was quantified in a NanoDrop 2000 125 126 spectrophotometer (Thermo Scientific, Wilmington, USA) at 260 nm. Small-subunit ribosomal DNA (SSU rDNA) was amplified using a two-round polymerase chain 127 reaction (PCR) according to Naldoni et al. (2019). The initial PCR was performed using 128 universal eukaryotic primers ERIB1, ACCTGGTTGATCCTGCCAG and ERIB10, 129 CTTCCGCAGGTTCACCTACGG (Barta et al., 1997), followed by a second round 130 with primer pairs ERIB1 with ACT1r, AATTTCACCTCTCGCTGCCA (Hallet and 131 132 Diamant, 2001) and BOBF, TGTTACAGCATGGAACGAAC (Capodifoglio et al., 2019) with ERIB10. PCR was carried out in a total volume of 25 µl consisting of 1 µl of 133 134 DNA (10 to 50 ng), 0.5 µl of each specific primer (0.2 µM), 12.5 µl DreamTaq 2x PCR Master Mix (Thermo Scientific, Massachusetts, USA) and 10.5 µl of ultrapure water. 135 The amplification of the partial SSU rDNA was performed in the Mastercycler® nexus 136 (Eppendorf, Hamburg, Germany) and the PCR cycle consisted of an initial denaturation 137 step at 95 °C for 5 min, followed by 35 denaturation cycles at 95 °C for 60 s, annealing 138 139 at 60 °C for 60 s, and extension at 72 °C for 120 s, following by a terminal extension at 140 72 °C for 5 min. A control reaction was processed in order to check for possible contamination. The amplified products were subjected to electrophoresis in 1.0% 141 agarose gel (BioAmerica, California, USA) in a TAE buffer (Tris-Acetate EDTA: Tris 142 143 40 mM, acetic acid 20 mM, EDTA 1 mM), stained with Sybr Safe DNA gel stain (Invitrogen by Life Technologies, California, USA), and then analyzed with a 144 Stratagene 2020E trans illuminator. For sizing and approximate quantification of 145

146 fragments, 1 Kb Plus DNA Ladder (Invitrogen by Life Technologies, USA) was used. 147 PCR products were purified using USB® ExoSap-IT® (Thermo Fisher Scientific, 148 Massachusetts, USA) in accordance with the manufacturer's instructions. PCR products were sequenced using the same PCR primers. Additional primers MC5, 149 CCTGAGAAACGGCTACCACATCCA and MC3, GATTAGCCTGACAGATC 150 151 ACTCCACGA (Molnár et al., 2002) were used to connect overlapping fragments. Sequencing was performed with a BigDye® Terminator v3.1 cycle sequencing kit 152 153 (Applied Biosystems Inc., California, USA) in an ABI 3730 DNA sequencing analyzer. The obtained sequences were visualized, assembled and edited using BioEdit 7.1.3.0 154 software (Hall, 1999). A basic local alignment search was conducted (BLASTn) to 155 156 verify the similarity of our sequence with other sequences of myxozoans available in the 157 National Center for Biotechnology non-redundant nucleotide database (Altschul et al., 1997). Phylogenetic analysis was conducted using the 53 most closely related 158 159 myxobolid myxosporeans sequences with BLAST similarity > 80%, included 29 sequences of Myxobolus species, 23 sequences of Henneguya species and 1 of 160 Thelohanellus species. Parvicapsula bicornis (EF429097) sequence was used as 161 outgroup. The sequences were edited and aligned with ClustalW within BioEdit version 162 7.1.3.0 (Hall, 1999) and phylogenetic relationships were performed using maximum 163 164 likelihood (ML) methods with a Kimura 2-parameter (K2P) evolution sequence model 165 in the MEGA 6.0 program (Tamura et al., 2013). Bootstrap analysis (1000 replicates) was employed to assess the relative robustness of the tree branches. Other alignments, 166 including species that clustered together with the O. cocama isolate in the phylogenetic 167 168 tree, were used to produce a pairwise similarity using MEGA 6.0.

169 **3. Results**

Among the 35 specimens of *O. cocama* examined in the present study, the gill filaments
of five (14.3%) harbored plasmodia of an unknown parasite belonging to the genus *Myxobolus*. Plasmodia were not found in any other organ and no clinical signs were
observed in the parasitized organ. The description of the novel species is provided
below.

- 175 Taxonomic summary
- 176 Phylum: Cnidaria Verrill, 1865
- 177 Class: Myxosporea Bütschli, 1881
- 178 Order: Bivalvulida Shulman, 1959
- 179 Family: Myxobolidae Thélohan, 1892
- 180 Genus: *Myxobolus* Bütschli, 1882
- 181 Species: *Myxobolus iquitoensis* n. sp.
- 182 Type host: *Otocinclus cocama* (Siluriformes: Loriicaridae)
- 183 Site of infection: Gill filaments
- Type locality: Yanayacu brook, Municipality of Jenaro Herrera (4°58′58″S,
 74°19′38″W), Department of Loreto, Peru.
- 186 Prevalence: From 35 examined fish, five were infected (14.3%).
- 187 Type of material: Syntypes-Air-dried slide stained with Giemsa (MZUSP 8507)
- deposited in the collection of Museum of Zoology of the University of São Paulo USP,
- 189 São Paulo, Brazil. The SSU rDNA sequence was deposited in GenBank under accession
- 190 number MN995338.

Etymology: The specific name (*M. iquitoensis*) is based on the geographic area (Iquitoscity), which is the center of aquarium fish trade of the Peruvian Amazon.

193 Morphological description

Plasmodia of rounded shape and measuring up to 45 μ m in diameter were found in the distal region of the gill filaments. Mature myxospores featured an ovoid shape from the frontal view and measured 17.6±1.2 μ m (16.2–19.8 μ m) in length and 10.5±0.7 μ m (9.8–12 μ m) in width (Fig. 1A-C). The two polar capsules were elongated in shape, equal in size, occupying almost half of the myxospore body (Fig. 1A-B). They measured 8.7±0.4 μ m (6.9–9.3 μ m) in length and 3.3±0.2 μ m (3–3.6 μ m) in width. The polar tubules presented six to seven turns (Fig. 1B and 2).

201 Molecular characterization and phylogenetic analysis

The sequencing of the SSU rDNA from the myxospores of *M. iquitoensis* n. sp., 202 203 resulted in sequence of 1926 bp with a GC content of 48.3%. BLASTn search revealed 204 that the sequence obtained did not match any known myxozoan species sequences 205 available in the GenBank database. The highest percentage of identity belonged to 206 Myxobolus figueirae Naldoni, Maia, Correa, Da Silva and Adriano, 2018 (query coverage 93%, maximum identities 88%), a parasite of the Amazon pimelodid fish 207 208 Phractocephalus hemioliopterus. Phylogenetic analysis based on the most closely 209 related myxozoan sequences placed the sequence within a subclade containing myxobolid parasite species of native South American fishes. In this same subclade, M. 210 211 iquitoensis n. sp. appears as a close species of M. figueirae (GenBank accession MG181226). Genetic distance analysis considering only of SSU rDNA sequences of the 212 213 myxobolids that cluster together in the same clade (Fig. 3) with the new parasite herein described showed the largest genetic divergence among these species, with a difference 214

- 216 Cordeiro, 2002, 15.7% to Myxobolus curimatae Zatti, Naldoni, Silva, Adriano, 2015
- and 17.1% to *Myxobolus prochilodus* Zatti, Arana, Maia and Adriano, 2016.

218 **4. Discussion**

219 Although, several myxosporean species have been described in South American fishes 220 (Mathews et al., 2016; Velasco et al., 2019; Naldoni et al., 2020), there are few studies 221 about the occurrence of this enigmatic group of parasites infecting ornamental fishes, 222 particularly on native and endemic species from Amazon basin (Mathews et al., 2020). 223 Indeed, from the Peruvian Amazon, a key source of wild freshwater fishes to the global aquarium market, only two myxozoan species have been described (Mathews et al., 224 225 2017; Mathews et al., 2018). In the present study, based on integrating morphological, ecological (host endemism and geography locality), biological traits (host/organ-tissue 226 specificity), and molecular phylogenetic analysis, we describe a new species of 227 freshwater histozoic myxobolid, Myxobolus iquitoensis n. sp. infecting gill filaments of 228 229 the endangered fish O. cocama.

230 To our knowledge, this study reports for the first time a myxosporean infecting a fish of the genus Otocinclus from South America, being notably as well the first report of these 231 parasites infecting a fish belonging to the Loricariidae family from Amazon basin. 232 233 Thus, our results contribute to freshwater platysporines taxonomy and extend the knowledge about myxosporean parasites of ornamental fish from the Amazon basin. 234 The morphological and morphometric comparison of mature myxospores of M. 235 236 *iquitoensis* n. sp. with those from the other *Myxobolus* species described that infect siluriform fish from the Amazon basin, showed remarkable differences as reported in 237 Table 1. Differences could also be observed in the genus/family of the parasitized host. 238

Indeed, it has been reported that the host fish represents an important taxonomic clue and that *Myxobolus* species display a strong tendency to cluster, based on host phylogenies (Fiala, 2006; Carriero et al., 2013). Thus, a single species is typically limited to closely related hosts within Genus/Family/Order, which is another important feature of *M. iquitoensis* n. sp as a new species, since *O. cocama* represents its unique host.

Compared with other known Myxobolus species previously described from South 245 246 American freshwater fishes, the new species resembles Myxobolus aureus Carriero, Adriano, Silva, Cecarelli and Maia, 2013, a parasite of Salminus brasiliensis. However, 247 the comparison showed that myxospores are slightly larger in length and width, being 248 249 $17.6\pm1.2 \times 10.5\pm0.7 \mu m$ for the new species, while $12.6\pm0.5\times8.3\pm0.3 \mu m$ for *M. aureus*. 250 The two polar capsules of *M. iquitoensis* n. sp. are substantially larger at $8.7\pm0.4 \,\mu\text{m} \times$ $3.3\pm0.2 \ \mu\text{m}$ compared to those of *M. aureus* $5.7\pm0.3 \ \mu\text{m} \times 2.9\pm0.2 \ \mu\text{m}$ and number of 251 252 coils in polar tubule are 6-7 turns to 7-8 for *M. aureus*. Furthermore, these species 253 demonstrated a large genetic divergence of 12.2% in their SSU rDNA. In addition, the hosts of these two species are different, with M. iquitoensis n. sp. parasitizing a 254 255 siluriform fish belonging to the Loricariidae family, while M. aureus infects a 256 characiform fish from the Bryconidae family. Finally, the species infect different 257 organs, with *M. iquitoensis* n. sp. parasitizing gill filaments and *M. aureus* parasitizing the liver. 258

The phylogenetic analysis performed in our study showed SSU rDNA sequences of *Henneguya* and *Myxobolus* species grouped together (Fig. 3). The absence of phylogenetic separation between these two genera agrees with several other studies conducted in many regions of the world (Kent et al., 2001; Carriero et al., 2013; Milanin 263 et al., 2018). This trend is noticeably observed in clade B, where Henneguya basifilamentalis Molnar, Szekely, Mohamed and Shaharom-Harrison, 2006 and 264 265 Henneguya mystusia Sarkar, 1985 parasites of Siluriform bagrid catfishes, are positioned in a clade composed mostly of species belonging to the genus Myxobolus. 266 267 Our phylogeny also evidenced a strong affinity of Myxobolus and Henneguya species to 268 cluster, based on the order and/or family of the host, as previously pointed out by other authors (Fiala, 2006; Naldoni et al., 2011; Carriero et al., 2013). In the phylogenetic 269 270 tree, *M. iquitoensis* n. sp. appears weakly supported in a subclade as a close species of M. figueirae, a parasite of the Amazonian catfish from the Pimelodidae family (Naldoni 271 et al., 2018). Although this data reveals geographic affinity, these two species showed a 272 273 large genetic divergence of 11% as revealed by the pairwise analysis. Furthermore, the 274 two species show noticeable differences in morphology, genus/family and the host 275 organ they infect, as reported in Table 1. It is important to highlight that this is the first 276 phylogenetic study of a myxobolid parasite of Otocinclus genera and Loricariidae family from Amazon basin and, as a matter of fact, there are few SSU rDNA data 277 available from myxosporeans infecting Amazonian siluriform hosts. Thus, future 278 279 molecular data and phylogenetic studies of the many yet-to-be-discovered Myxobolus species from these underrepresented hosts and other groups of native and endemic 280 281 Amazonian fishes should help resolve the evolutionary context of *M. iquitoensis* n. sp.

Moreover, it is now recognized that parasitic fauna can decline with biodiversity losses (Koh et al., 2004; Dobson et al., 2008; Lafferty et al., 2012). Indeed, models of coextinction have identified parasites as one of the most menaced ecological groups, representing an unseen majority of species extinctions (Dobson et al., 2008; Strona et al., 2013). Although, *O. cocama* is currently considered an endangered species in the IUCN, little or nothing is known about its parasitic fauna, and the *Myxobolus* species 288 described herein represents the only platysporine myxozoan reported to parasite this ornamental fish. According to previous studies, the relationship between parasite 289 290 specialization and host vulnerability are important factors that predict the risk of a parasite species becoming extinct together with its host (McKinney, 1999; Dobson et 291 292 al., 2008; Strona et al., 2013). Considering the endangered status of the host fish and 293 that the same is the only known habitat for *M. iquitoensis* n. sp., together with the low occurrence shown by this new myxozoan and the high degree of host-specificity of 294 freshwater histozoic myxobolids, M. iquitoensis n. sp. may face the danger of 295 coextinction with its host. In this context, the establishment and implementation of a 296 conservation programme in order to minimize impacts on wild populations of O. 297 298 cocama would also benefit the survival of their endemic myxozoan.

299 Conflicts of interest

300 The authors declare that they have no conflict of interest.

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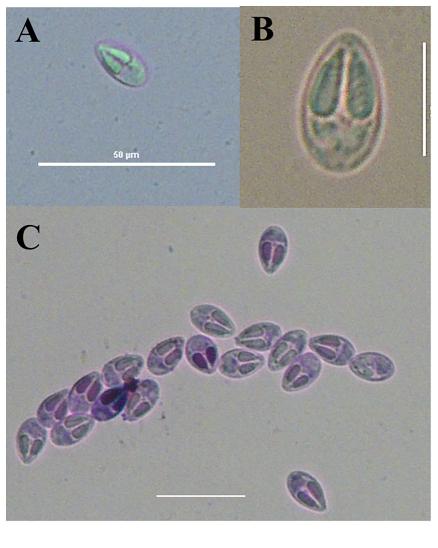
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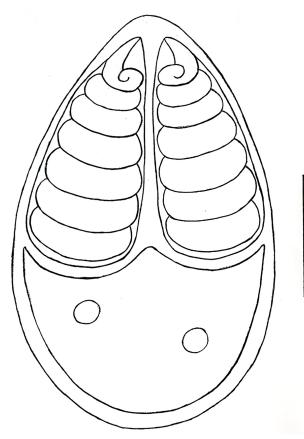
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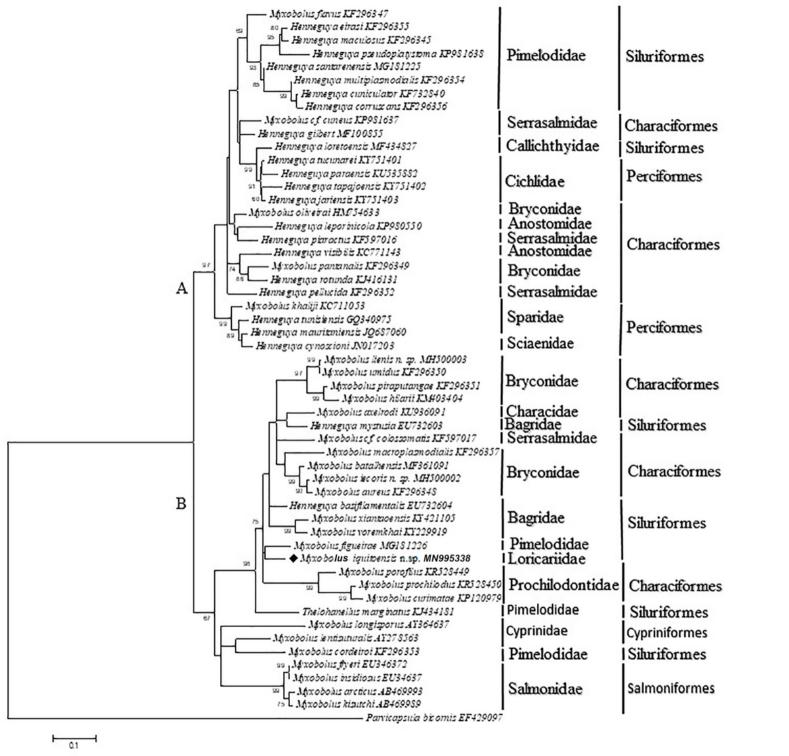
Fig.1. Light photomicrograph of mature myxospores of *Myxobolus iquitoensis* n. sp.,
parasite of gill filaments of *Otocinclus cocama*. A, Fresh mature myxospore. Scale bar:
50 μm. B, Formalin-fixed mature myxospore showing two equal elongated polar
capsules. Scale bar: 15 μm. C, Mature myxospores in frontal view stained in MayGrünwald-Giemsa. Scale bar: 30 μm.

- 471 Fig. 2. Schematic representation of mature myxospore of *Myxobolus iquitoensis* n. sp.
 472 parasite of gill filaments of *Otocinclus cocama*. Scale bar: 5 μm.
- Fig. 3. Maximum Likelihood (ML) tree containing *Myxobolus iquitoensis* n. sp. and
 closely related myxosporeans sequences deposited in GenBank based on partial small
 subunit ribosomal DNA. The numbers above the nodes indicate bootstrap confidence
 levels.
- Table 1. Comparison of *Myxobolus iquitoensis* n. sp. with other *Myxobolus* species
 described parasitizing siluriform fishes from Amazon basin. Spore dimensions, hosts,
 infection sites, and collection sites are given. SL: spore length, SW: spore width, ST:
 spore thickness, PCL: polar capsules length, PCW: polar capsules width, NCT: number
 of coils in polar tubule, dashes: no data. All measurements are in μm.

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Species	SL	SW	ST	PCL	PCW	NCT	Host	Family	Site of infection	Locality	References
<i>Myxobolus iquitoensis</i> n. sp.	17.6±1.2 (16.2-19.8)	10.5±0.7 (9.8-12)	-	8.7±0.43 (6.9-9.3)	3±0.2 (3-3.6)	6-7	Otocinclus cocama	Loricariidae	Gill filaments	Yanayacu brook, Peru	This study
Myxobolus adrianoi	22.4±0.3	16.3 ± 0.1	-	14.3 ±0.2	6.5 ± 0.1	5	Corydoras schwartzi	Callichthyidae	Intestine	Purus River, Brazil	Mathews et al. 2020
Myxobolus figueirae	9.5 (9.1-10)	6.4 (5.8-6.9)	4.5 (4.4-4.5)	4.1 (3.5-4.6)	2.1 (1.7–2.6)	7–8	Phractocephalus hemioliopterus	Pimelodidae	Skin	Tapajós River, Brazil	Naldoni et al. 2018
Myxobolus tapajosi	15 (13.5-17)	10.7 (9.6-11.4)	-	5.8 (4.6-7.1)	3 (2.3-3.8)	6-7	Brachyplatystoma rousseauxii	Pimelodidae	Gill filaments	Tapajos River, Brazil	Zatti et al. 2018
Myxobolus marajoensis	10.9 (10.0- 11.6)	5.1 (4.2-5.4)	-	5.3 ± 0.6	1.6 ± 0.36	-	Rhamdia quelen	Heptapteridae	Intestine	Marajó Island, Brazil	Abrunhosa et al. 20
Myxobolus niger	11.3±0.4	6.8 ±0.2	4.1 ± 0.2	5.0 ± 0.3	2.0 ± 0.1	6–7	Corydoras melini	Callichthyidae	Gill arch	Negro River, Brazil	Mathews et al. 2016
Myxobolus sp.	8 ± 0.2	5.8 ± 0.4	3.4 ±0.2	3.6 ± 0.3	1.2 ± 0.2	-	Pimelodus ornatus	Pimelodidae	Heart	Arari River, Brazil	Matos et al. 2014

