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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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

examined, five (14.3%) had myxozoan plasmodia. Taxonomic analysis was performed integrating multiple characters, including morphometrical, biological traits, ssrDNA sequence data and host ecological characters. Myxospores of *M. iquitoensis* n. sp. were ovoid in shape from the frontal view and measured 17.6±1.2 µm (16.2–19.8 µm) in length and 10.5±0.7 µm (9.8–12 µm) in width. The two polar capsules were elongate in shape, equal in size and occupying almost half of the myxospore body. They measured 8.7±0.4 µm (6.9–9.3 µm) in length and 3.3±0.2 µm (3–3.6 µm) in width. The polar tubules presented six to seven turns. Molecular phylogenetic analysis revealed that the 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 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 *Otocinclus* from South America, as well the first report of these parasites infecting a 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 myxobolids, the low occurrence shown by the new myxozoan, and the fact that this is the only host known for this myxozoan, the conservation status of the new species of myxozoan is likely to be connected to the future survival of its host.

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

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

48 reductions of natural populations, and contributed to local extinctions (Ng and Tan, 1997;
49 Moreau and Coomes, 2006; Mohanty and Measey, 2019). In this context, over-
50 exploitation of wild ornamental fish for the aquarium trade has become an important
51 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,
55 with about 14,000 people depending directly on the aquarium trade (Moreau and Coomes,
56 2007). This activity is centralized in the city of Iquitos in the Department of Loreto, Peru
57 with approximately 28 established aquarium fish exporter firms, exporting ornamental
58 fish to 24 countries (Moreau and Coomes, 2007). Virtually all fishes in the trade are taken
59 from a variety of wild aquatic habitats such as main river channels, small tributaries,
60 floodplain oxbow lakes, swamps, flooded moriche palm forest, and remote upland
61 streams (Moreau and Coomes, 2007).

62 The plecos are members of the family Loricariidae. With more than 830 valid species,
63 they are the most species-rich family within the Order Siluriformes. These neotropical
64 freshwater catfishes are distributed throughout South America (Ribeiro et al., 2012).
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
67 fish exported from Peruvian Amazon, comprising over 29% of the total volume of the
68 international export (Moreau and Coomes, 2007). Species of *Otocinclus* are widely
69 distributed in South America, specifically east of the Andes, and often inhabit small
70 lowland tributaries of the main rivers associated with marginal vegetation (Schaefer,
71 1997). Among these, *Otocinclus cocama* Reis, 2004 known as “zebra otocinclus” is very
72 popular in the aquarium trade and it is one the most important aquarium fish exports from

73 Iquitos based on international sales (Moreau and Coomes, 2007). *O. cocama* is endemic
74 to Peruvian Amazon, specifically from the Yanayacu small stream, near district Jenaro
75 Herrera, Department of Loreto (Reis, 2004). As a direct consequence of the increasing
76 exploitation by the ornamental trade, with continuing decline of mature individuals, *O.*
77 *cocama* is currently considered an endangered species in Peru and it is included on the
78 Red List of Threatened Species-IUCN (Hidalgo and Chocano, 2016).
79 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, especially
82 parasites endemic to a small subset of host species. Following the fine scale of host-
83 parasite coextinction, parasites of *O. cocama* are likely to be similarly vulnerable.
84 However, little is known about parasites that infect this Amazonian ornamental fish,
85 especially the myxozoan parasites. Thus, it is important to clarify the parasite fauna of *O.*
86 *cocama*.
87 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 infect
90 virtually all vertebrate groups, including amphibians, reptiles, birds, and terrestrial
91 mammals (Okamura et al., 2015; Lisnerová et al., 2019). Among the myxozoans, the
92 genus *Myxobolus* Bütschli, 1882 is the most abundant, accounting for around 905 species
93 described within a wide geographical range (Eiras et al., 2014). Freshwater histozoic
94 *Myxobolus* species are widely recognized for their strict or high host-specificity (Molnár
95 and Eszterbauer, 2015).
96 In this study, we described a new host-specific freshwater histozoic myxozoan,
97 *Myxobolus iquitoensis* n. sp. infecting the gill filaments of the endangered fish *O. cocama*

98 from the Peruvian Amazon. Morphological and molecular features of the parasite are
99 provided herein.

100 **2. Materials and methods**

101 A total of 35 specimens of *Otocinclus cocama* were acquired from local fisherfolk of
102 aquarium ornamental fishes in September 2019, for parasitological survey. According to
103 the fisherfolk, the fishes were caught in the marginal vegetation in the small stream
104 Yanayacu, near Jenaro Herrera district, Loreto, Peru (4°58′58″S, 74°19′38″W). The fish
105 were transported live to the field laboratory at the Research Institute of the Peruvian
106 Amazon, where they were euthanized by pit transaction and examined using a light
107 microscope to verify the presence of lesions and parasites. The euthanasia method was
108 approved by Federal University of São Paulo—UNIFESP Ethics Committee (CEUA No.
109 9209080214; Federal Law No. 11794, dated 8 October 2008), in accordance to
110 international procedures.

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

120 For molecular characterization, plasmodia were removed from the infected tissue and
121 preserved in absolute ethanol. Extraction of genomic DNA (gDNA) was carried out from
122 a single plasmodium and was performed using a DNeasy® Blood & Tissue Kit (animal

123 tissue protocol) (Qiagen Inc., California, USA), in accordance with the manufacturer's
124 instructions. gDNA concentration was quantified in a NanoDrop 2000 spectrophotometer
125 (Thermo Scientific, Wilmington, USA) at 260 nm. Small-subunit ribosomal DNA (SSU
126 rDNA) was amplified using a two-round polymerase chain reaction (PCR) according to
127 Naldoni et al. (2019). The initial PCR was performed using universal eukaryotic primers
128 ERIB1, ACCTGGTTGATCCTGCCAG and ERIB10,
129 CTTCCGCAGGTTCACCTACGG (Barta et al., 1997), followed by a second round with
130 primer pairs ERIB1 with ACT1r, AATTTCACCTCTCGCTGCCA (Hallet and Diamant,
131 2001) and BOBF, TGTTACAGCATGGAACGAAC (Capodifoglio et al., 2019) with
132 ERIB10. PCR was carried out in a total volume of 25 µl consisting of 1 µl of DNA (10 to
133 50 ng), 0.5 µl of each specific primer (0.2 µM), 12.5 µl DreamTaq 2x PCR Master Mix
134 (Thermo Scientific, Massachusetts, USA) and 10.5 µl of ultrapure water. The
135 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 at 60 °C for 60 s, and extension at 72 °C for 120 s, following by a terminal extension at
139 72 °C for 5 min. A control reaction was processed in order to check for possible
140 contamination. The amplified products were subjected to electrophoresis in 1.0% agarose
141 gel (BioAmerica, California, USA) in a TAE buffer (Tris–Acetate EDTA: Tris 40 mM,
142 acetic acid 20 mM, EDTA 1 mM), stained with Sybr Safe DNA gel stain (Invitrogen by
143 Life Technologies, California, USA), and then analyzed with a Stratagene 2020E trans
144 illuminator. For sizing and approximate quantification of fragments, 1 Kb Plus DNA
145 Ladder (Invitrogen by Life Technologies, USA) was used. PCR products were purified
146 using USB® ExoSap-IT® (Thermo Fisher Scientific, Massachusetts, USA) in accordance
147 with the manufacturer's instructions. PCR products were sequenced using the same PCR

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

167 **3. Results**

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

Taxonomic summary

Phylum: Cnidaria Verrill, 1865

Class: Myxosporea Bütschli, 1881

Order: Bivalvulida Shulman, 1959

Family: Myxobolidae Thélohan, 1892

Genus: *Myxobolus* Bütschli, 1882

Species: *Myxobolus iquitoensis* n. sp.

Type host: *Otocinclus cocama* (Siluriformes: Loriicaridae)

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.

Prevalence: From 35 examined fish, five were infected (14.3%).

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, São Paulo, Brazil. The SSU rDNA sequence was deposited in GenBank under accession number MN995338.

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

Morphological description

Plasmodia of rounded shape and measuring up to 45 µm in diameter were found in the distal region of the gill filaments. Mature myxospores featured an ovoid shape from the

193 frontal view and measured 17.6±1.2 µm (16.2–19.8 µm) in length and 10.5±0.7 µm (9.8–
194 12 µm) in width (Fig. 1A-C). The two polar capsules were elongated in shape, equal in
195 size, occupying almost half of the myxospore body (Fig. 1A-B). They measured 8.7±0.4
196 µm (6.9–9.3 µm) in length and 3.3±0.2 µm (3–3.6 µm) in width. The polar tubules
197 presented six to seven turns (Fig. 1B and 2).

198 **Molecular characterization and phylogenetic analysis**

199 The sequencing of the SSU rDNA from the myxospores of *M. iquitoensis* n. sp., resulted
200 in sequence of 1926 bp with a GC content of 48.3%. BLASTn search revealed that the
201 sequence obtained did not match any known myxozoan species sequences available in
202 the GenBank database. The highest percentage of identity belonged to *Myxobolus*
203 *figueirae* Naldoni, Maia, Correa, Da Silva and Adriano, 2018 (query coverage 93%,
204 maximum identities 88%), a parasite of the Amazon pimelodid fish *Phractocephalus*
205 *hemioliopterus*. Phylogenetic analysis based on the most closely related myxozoan
206 sequences placed the sequence within a subclade containing myxobolid parasite species
207 of native South American fishes. In this same subclade, *M. iquitoensis* n. sp. appears as a
208 close species of *M. figueirae* (GenBank accession MG181226). Genetic distance analysis
209 considering only of SSU rDNA sequences of the myxobolids that cluster together in the
210 same clade (Fig. 3) with the new parasite herein described showed the largest genetic
211 divergence among these species, with a difference of 11% to *M. figueirae*, 14.9% to
212 *Myxobolus porofilus* Adriano, Arana, Ceccarelli and Cordeiro, 2002, 15.7% to *Myxobolus*
213 *curimatae* Zatti, Naldoni, Silva, Adriano, 2015 and 17.1% to *Myxobolus prochilodus*
214 Zatti, Arana, Maia and Adriano, 2016.

215 **4. Discussion**

Although, several myxosporean species have been described in South American fishes (Mathews et al., 2016; Velasco et al., 2019; Naldoni et al., 2020), there are few studies about the occurrence of this enigmatic group of parasites infecting ornamental fishes, particularly on native and endemic species from Amazon basin (Mathews et al., 2020). 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., 2017; Mathews et al., 2018). In the present study, based on integrating morphological, ecological (host endemism and geography locality), biological traits (host/organ-tissue specificity), and molecular phylogenetic analysis, we describe a new species of freshwater histozoic myxobolid, *Myxobolus iquitoensis* n. sp. infecting gill filaments of the endangered fish *O. cocama.*

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 parasites infecting a fish belonging to the Loricariidae family from Amazon basin. Thus, our results contribute to freshwater platysporines taxonomy and extend the knowledge about myxosporean parasites of ornamental fish from the Amazon basin. The morphological and morphometric comparison of mature myxospores of *M. 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 Table 1. Differences could also be observed in the genus/family of the parasitized host. 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.

241 Compared with other known *Myxobolus* species previously described from South
242 American freshwater fishes, the new species resembles *Myxobolus aureus* Carriero,
243 Adriano, Silva, Cecarelli and Maia, 2013, a parasite of *Salminus brasiliensis*. However,
244 the comparison showed that myxospores are slightly larger in length and width, being
245 17.6±1.2 ×10.5±0.7 µm for the new species, while 12.6±0.5×8.3±0.3 µm for *M. aureus*.
246 The two polar capsules of *M. iquitoensis* n. sp. are substantially larger at 8.7±0.4 µm ×
247 3.3±0.2 µm compared to those of *M. aureus* 5.7±0.3 µm × 2.9±0.2 µm and number of
248 coils in polar tubule are 6-7 turns to 7-8 for *M. aureus*. Furthermore, these species
249 demonstrated a large genetic divergence of 12.2% in their SSU rDNA. In addition, the
250 hosts of these two species are different, with *M. iquitoensis* n. sp. parasitizing a siluriform
251 fish belonging to the Loricariidae family, while *M. aureus* infects a characiform fish from
252 the Bryconidae family. Finally, the species infect different organs, with *M. iquitoensis* n.
253 sp. parasitizing gill filaments and *M. aureus* parasitizing the liver.

254 The phylogenetic analysis performed in our study showed SSU rDNA sequences of
255 *Henneguya* and *Myxobolus* species grouped together (Fig. 3). The absence of
256 phylogenetic separation between these two genera agrees with several other studies
257 conducted in many regions of the world (Kent et al., 2001; Carriero et al., 2013; Milanin
258 et al., 2018). This trend is noticeably observed in clade B, where *Henneguya*
259 *basifilamentalis* Molnar, Szekely, Mohamed and Shaharom-Harrison, 2006 and
260 *Henneguya mystusia* Sarkar, 1985 parasites of Siluriform bagrid catfishes, are positioned
261 in a clade composed mostly of species belonging to the genus *Myxobolus*. Our phylogeny
262 also evidenced a strong affinity of *Myxobolus* and *Henneguya* species to cluster, based
263 on the order and/or family of the host, as previously pointed out by other authors (Fiala,
264 2006; Naldoni et al., 2011; Carriero et al., 2013). In the phylogenetic tree, *M. iquitoensis*
265 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 et al., 2018). Although this data reveals geographic affinity, these two species showed a large genetic divergence of 11% as revealed by the pairwise analysis. Furthermore, the two species show noticeable differences in morphology, genus/family and the host organ they infect, as reported in Table 1. It is important to highlight that this is the first 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 available from myxosporeans infecting Amazonian siluriform hosts. Thus, future 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 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 described herein represents the only platysporine myxozoan reported to parasite this ornamental fish. According to previous studies, the relationship between parasite 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 al., 2008; Strona et al., 2013). Considering the endangered status of the host fish and 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 freshwater histozoic myxobolids, *M. iquitoensis* n. sp. may face the danger of coextinction with its host. In this context, the

291 establishment and implementation of a conservation programme in order to minimize
292 impacts on wild populations of *O. cocama* would also benefit the survival of their
293 endemic myxozoan.

294 **Conflicts of interest**

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

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305 **References**

306 Abrunhosa, J., Sindeaux-Neto, J.L., dos Santos, A.K., Hamoy, I., Matos, E., 2017.
307 *Myxobolus marajoensis* sp. n. (Myxosporea: Myxobolidae), parasite of the freshwater
308 catfish *Rhamdia quelen* from the Brazilian Amazon region. Braz. J. Vet. Parasitol. 26,
309 465–471. http://dx.doi.org/10.1590/S1984-29612017067.

310 Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J.H., Zhang, Z., Miller, W., Lipman,
311 D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search
312 programs. Nucleic Acids Res. 25, 3389–3402.

313 Atkinson, S.D., Bartholomew, J.L., Lotan, T., 2018. Myxozoans: Ancient metazoan
314 parasites find a home in phylum Cnidaria. Zoology 129, 66–68.
315 https://doi.org/10.1016/j.zool.2018.06.005.

316 Barta, J.R., Martin, D.S., Liberator, P.A., Dashkevicz, M., Anderson, J.W., Feighner,
317 S.D., Elbrecht, A., Perkins-Barrow, A., Jenkins, M.C., Danforth, D., Ruff, M.D., Profous-
318 Juchelka, H., 1997. Phylogenetic relationships among eight *Eimeria* species infecting
319 domestic fowl inferred using complete small subunit ribosomal DNA sequences. J.
320 Parasitol. 83, 262–271.

321 Capodifoglio, K.R.H., Adriano, E.A., Silva, M.R.M., Maia, A.A.M., 2019. The
322 resolution of the taxonomic dilemma of *Myxobolus colossomatis* and description of two
323 novel myxosporeans species of *Colossoma macropomum* from Amazon basin. Acta
324 Trop. 191, 17–23. https://doi.org/10.1016/j.actatropica.2018.12.026

325 Carriero, M.M., Adriano, E.A., Silva, M.R.M., Ceccarelli, P.S., Maia, A.A.M., 2013.
326 Molecular phylogeny of the *Myxobolus* and *Henneguya* genera with several new South
327 American species. PLoS One 8, e73713. https://doi.org/10.1371/journal.pone.0073713.

328 Dallas, T., Cornelius, E., 2015. Co-extinction in a host-parasite network: identifying key
329 hosts for network stability. Sci. Rep. 5, 13185. https://doi.org/10.1038/srep13185.

330 Dobson, A.P., Lafferty, K.D., Kuris, A.M., Hechinger, R.F., Jetz, W., 2008. Homage to
331 Linnaeus: how many parasites? How many hosts?. Proc. Natl Acad. Sci. 105, 11, 482–11
332 489. https://doi.org/10.1073/pnas.0803232105.

333 Eiras, J.C., Zhang, J., Molnár, K., 2014. Synopsis of the species of *Myxobolus*
334 Bütschli,1882 (Myxozoa: Myxosporea, Myxobolidae) described between 2005 and2013.
335 Syst. Parasitol. 88, 11–36. https://doi.org/10.1007/s11230-014-9484-5.

336 Fiala, I., 2006. The phylogeny of Myxosporea (Myxozoa) based on small subunit
337 ribosomal RNA gene analysis. Int. J. Parasitol. 36, 1521–1534.
338 https://doi.org/10.1016/j.ijpara.2006.06.016.

339 Hallett, S.L., Diamant, A., 2001. Ultrastructure and small-subunit ribosomal DNA
340 sequence of *Henneguya lesteri* n. sp. (Myxosporea), a parasite of sand whiting Sillago
341 analis (Sillaginidae) from the coast of Queensland, Australia. Dis. Aquat. Organ. 46, 197–
342 212.

343 Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and
344 analysis program for Windows 95/98/NT. Nucl. Acids Symp. Ser. 41, 95–98.

345 Hidalgo A.M., Chocano, L. 2016. *Otocinclus cocama*. The IUCN Red List of Threatened
346 Species. https://dx.doi.org/10.2305/IUCN.UK.2016-1.RLTS.T49829515A53817093.

347 Kent, M.L., Andree, K.B., Bartholomew, J.L., El-Matbouli, M., Desser, S.S., Devlin,
348 R.H., Feist, S.W., Hedrick, R.P., Hoffman, R.W., Khattra, J., Hallett, S.L., Lester, R.J.G.,
349 Longshaw, M., Palenzeula, O., Siddall, M.E., Xiao, C., 2001. Recent advances in our
350 knowledge of the myxozoa. J. Eukaryot. Microbiol. 48, 395–413.

351 Koh, L.P., Dunn, R.R., Sodhi, N.S., Colwell, R.K., Proctor, H.C., Smith, V.S., 2004.
352 Species coextinctions and the biodiversity crisis. Science 305, 1632–1634.

353 Lafferty, K., 2012. Biodiversity loss decreases parasite diversity: theory and patterns.
354 Philos. Trans. R. Soc. B. 367, 2814–2827. https://doi.org/10.1098/rstb.2012.0110.

355 Lisnerová, M., Fiala, I., Cantatore, D., Irigoitia, M., Timi, J., Pecková, H., Bartošová-
356 Sojková, P., Sandoval, C.M., Luer, C., Morris, J., Holzer, A.S. 2020. Mechanisms and
357 Drivers for the Establishment of Life Cycle Complexity in Myxozoan
358 Parasites. Biology 9, 10. https://doi.org/10.3390/biology9010010.

359 Lom, J., Arthur, J.R., 1989. A guideline for the preparation of species descriptions in
360 Myxosporea. J. Fish Dis. 12, 151–156.

361 Mathews, P.D., Madrid, R.R.M, Mertins, O., Rigoni, V.L.S., Morandini, A.C. 2020. New
362 *Myxobolus* (Cnidaria: Myxosporea) infecting the ornamental catfish *Corydoras schwartzi*
363 from the Purus River in Brazil. Eur. J. Taxon. 620, 1–14.
364 https://doi.org/10.5852/ejt.2020.620

365 Mathews, P.D., Mertins, O., Pereira, J.O.L., Maia, A.A.M., Adriano, E.A., 2018.
366 Morphology and 18S rDNA sequencing of *Henneguya peruviensis* n. sp. (Cnidaria:
367 Myxosporea), a parasite of the Amazonian ornamental fish *Hyphessobrycon loretoensis*
368 from Peru: A myxosporean dispersal approach. Acta Trop. 187, 207–213.
369 https://doi.org/10.1016/j.actatropica.2018.08.012.

370 Mathews, P.D., Naldoni, J., Adriano, E.A., 2017. Morphology and small subunit rDNA-
371 based phylogeny of a new *Henneguya* species, infecting the ornamental fish *Corydoras*
372 *leucomelas* from the Peruvian Amazon. Acta Trop. 176, 51–57.
373 https://doi.org/10.1016/j.actatropica.2017.07.017.

374 Mathews, P.D., Maia, A.A.M., Adriano, E.A., 2016. Morphological and ultrastructural
375 aspects of *Myxobolus niger* n. sp. (Myxozoa) gill parasite of *Corydoras melini*
376 (Siluriformes: Callichthyidae) from Brazilian Amazon. Acta Trop. 158, 214–219.
377 http://dx.doi.org/10.1016/j.actatropica.2016.03.016.

378 Matos, E., Videira, M., Velasco, M., Sanches, O., São Clemente, S.C., Matos, P., 2014.
379 Infection of the heart of *Pimelodus ornatus* (Teleostei, Pimelodidae), by *Myxobolus* sp.
380 (Myxozoa, Myxobolidae), Braz. J. Vet. Parasitol. 23, 543-546.

381 Milanin, T., Mathews, P.D., Mertins, O., Tavares, L.E.R., Silva, M.R.M., Maia, A.A.M.,
382 2018. Molecular phylogeny of the gill parasite *Henneguya* (Myxosporea: Myxobolidae)
383 infecting *Astyanax lacustris* (Teleostei: Characidae) from fish farm in Brazil. Microb.
384 Pathog. 123: 372–376. https://doi.org/10.1016/j.micpath.2018.07.037.

385 McKinney, M.L. 1999. High rates of extinction and threat in poorly studied taxa. Conserv.
386 Biol. 13, 1273–1281. https://doi.org/10.1073/pnas.0803232105

387 Mohanty, N.P., Measey, J., 2019. The global pet trade in amphibians: species traits,
388 taxonomic bias, and future directions. Biodivers. Conserv. 28, 3915–3923.
389 https://doi.org/10.1007/s10531-019-01857-x.

390 Molnár, K., Eszterbauer, E., 2015. Specificity of infection sites in vertebrate hosts. In:
391 Okamura, B., Gruhl, A., Bartholomew, J.L. (Eds.), Myxozoan Evolution, Ecology and
392 Development. Springer, Switzerland, pp. 295–313.

393 Molnár, K., Eszterbauer, E., Székely C., Dan, A., Harrach, B., 2002. Morphological and
394 molecular biological studies on intramuscular *Myxobolus* spp. of cyprinid fish. J. Fish
395 Dis. 25, 643–652.

396 Moreau, M.A., Coomes, O.T., 2007. Aquarium fish exploitation in western Amazonia:
397 conservation issues in Peru. Environ. Conserv. 34,12–22.
398 https://doi.org/10.1017/S0376892907003566.

399 Moreau, M.A., Coomes, O.T., 2006. Potential threat of the international aquarium fish
400 trade to silver arawana *Osteoglossum bicirrhosum* in the Peruvian Amazon. Oryx 40,
401 152–160. https://doi.org/10.1017/S0030605306000603.

402 Naldoni, J., Pereira, J.O.L., Milanin, T., Adriano, E.A., Da Silva, M.R.M., Maia, A.A.M.,
403 2020. Taxonomy, phylogeny and host-parasite interaction of two novel *Myxobolus*

404 species infecting *Brycon orthotaenia* from the São Francisco River, Brazil. 76:102061.
405 https://doi.org/10.1016/j.parint.2020.102061.

406 Naldoni, J, Zatti, S.A., Da Silva, M.R.M., Maia, A.A.M., Adriano, E.A., 2019.
407 Morphological, ultrastructural, and phylogenetic analysis of two novel *Myxobolus* species
408 (Cnidaria: Myxosporea) parasitizing bryconid fish from São Francisco River, Brazil.
409 Parasitol. Int. 71, 27–36. https://doi.org/10.1016/j.parint.2019.03.009.

410 Naldoni, J., Maia, A.A.M., Correa, L.L., Da Silva, M.R.M., Adriano, E.A., 2018. New
411 myxosporeans parasitizing *Phractocephalus hemioliopterus* from Brazil: morphology,
412 ultrastructure and SSU-rDNA sequencing. Dis. Aquat. Org. 128, 37–49.
413 https://doi.org/10.3354/dao03210.

414 Naldoni, J., Arana, S., Maia, A.A., Silva, M.R., Carriero, M.M., Ceccarelli, P.S., Tavares
415 L.E.R., Adriano, E.A., 2011. Host-parasite-environment relationship, morphology and
416 molecular analyses of *Henneguya eirasi* n. sp. parasite of two wild *Pseudoplatystoma*
417 spp. in Pantanal Wetland, Brazil. Vet. Parasitol. 177, 247–255.
418 https://doi.org/10.1016/j.vetpar.2010.12.008.

419 Ng, P.K.L., Tan, H.H., 1997. Freshwater fishes of Southeast Asia: potential for the
420 aquarium fish trade and conservation issues. Aquarium. Sci. Conserv. 1, 79–90.
421 https://doi.org/10.1023/A:1018335617835.

422 Okamura, B., Gruhl, A., Bartholomew, J.L., 2015. An introduction to Myxozoan
423 evolution, ecology and development. In: Okamura, B., Gruhl, A., Bartholomew, J.L.
424 (Eds.), Myxozoan Evolution, Ecology and Development. Springer, Switzerland, pp. 1–
425 20.

426 Prang, G., 2007. An industry analysis of the freshwater ornamental fishery with particular
427 reference to the supply of Brazilian freshwater ornamentals to the UK market. Uakari 3,
428 7–51. http://dx.doi.org/10.31420/uakari.v3i1.18.

429 Reis, R.E., 2004. *Otocinclus cocama*, a new uniquely colored loricariid catfish from Peru
430 (Teleostei: Siluriformes), with comments on the impact of taxonomic revisions to the
431 discovery of new taxa. Neotrop. Ichthyol. 2, 109-115. https://doi.org/10.1590/S1679-
432 62252004000300001.

433 Ribeiro, A.C., Lima, F.C.T., Pereira, E.H.L, 2012. A new genus and species of a minute
434 suckermouth armored catfish (Siluriformes: Loricariidae) from the Rio Tocantins
435 drainage, Central Brazil: The smallest known loricariid catfish. Copeia 4, 637–647.
436 https://doi.org/10.1643/CI-11-137.

437 Schaefer, S.A., 1997. The Neotropical cascudinhos: systematics and biogeography of the
438 *Otocinclus* catfishes (Siluriformes: Loricariidae). Proc. Acad. Nat. Sci. Philadelphia 148,
439 1–120.

440 Strona, G., Galli, P., Fattorini, S., 2013. Fish parasites resolve the paradox of missing
441 coextinctions. Nat. Commun. 4,1718. https://doi.org/10.1038/ncomms2723.

442 Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA 6: molecular
443 evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30, 2725–2729.
444 https://doi.org/10.1093/molbev/mst197.

445 Velasco, M., Sindeaux Neto, J.L., Videira, M., De Cássia Silva do Nascimento, L.,
446 Gonçalves, E.C., Matos, E., 2019. *Kudoa amazonica* n. sp. (Myxozoa; Multivalvulida), a
447 parasite of the esophageal musculature of the freshwater catfish, *Hypophthalmus*

448 *marginatus* (Siluriformes: Pimelodidae), from a river of the Amazon region. Microb.
449 Pathog. 130, 247-252. https://doi.org/10.1016/j.micpath.2019.03.017.

450 Zatti, S.A., Atkinson, S.D., Maia A.A.M., Corrêa, L.L., Bartholomew, J.L., Adriano
451 E.A., 2018. Novel *Myxobolus* and *Ellipsomyxa* species (Cnidaria: Myxozoa) parasiting
452 *Brachyplatystoma rousseauxii* (Siluriformes: Pimelodidae) in the Amazon basin, Brazil.
453 Parasitol. Int. 67, 612-621. https://doi.org/10.1016/j.parint.2018.06.005.

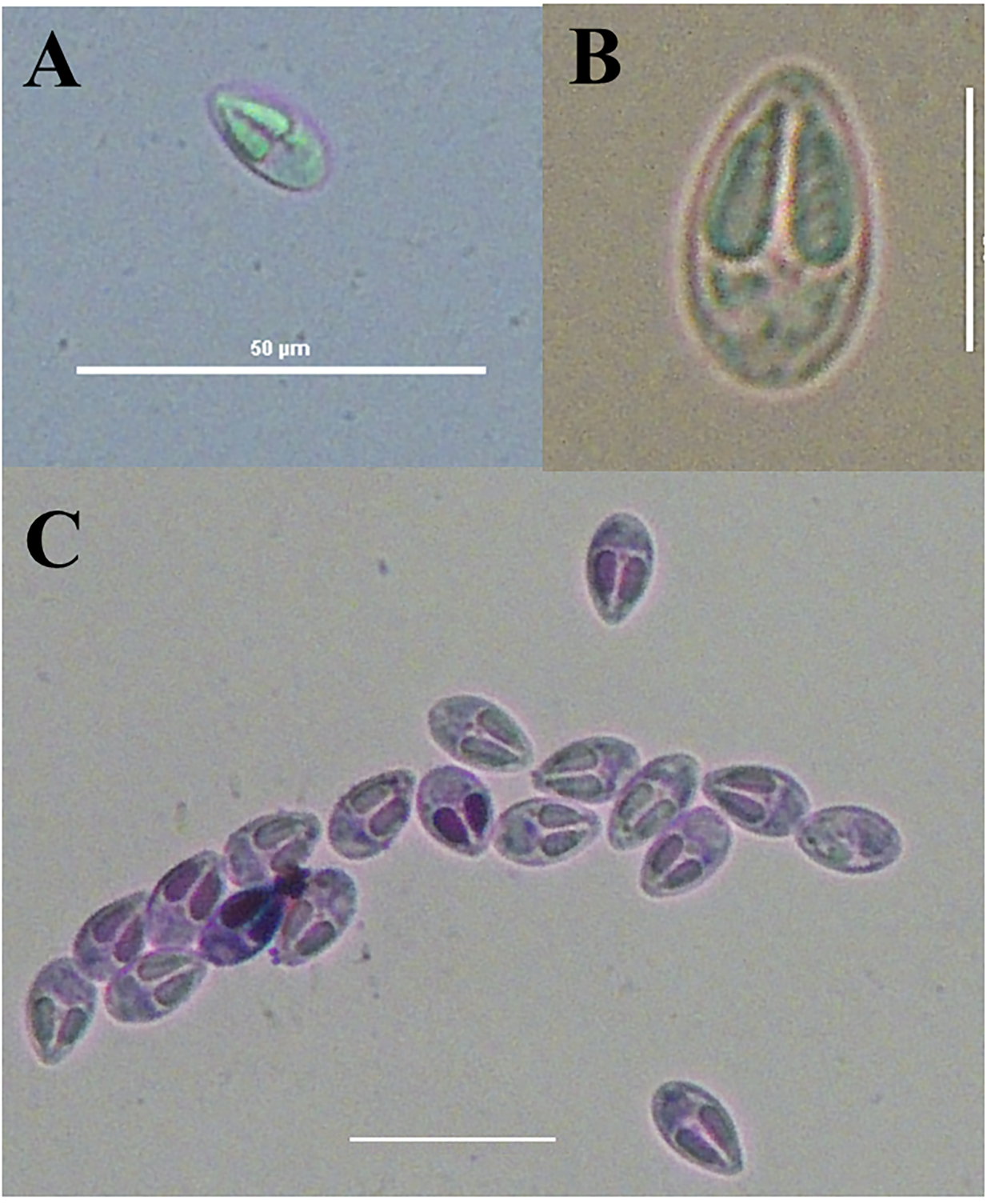
454 **Legends**

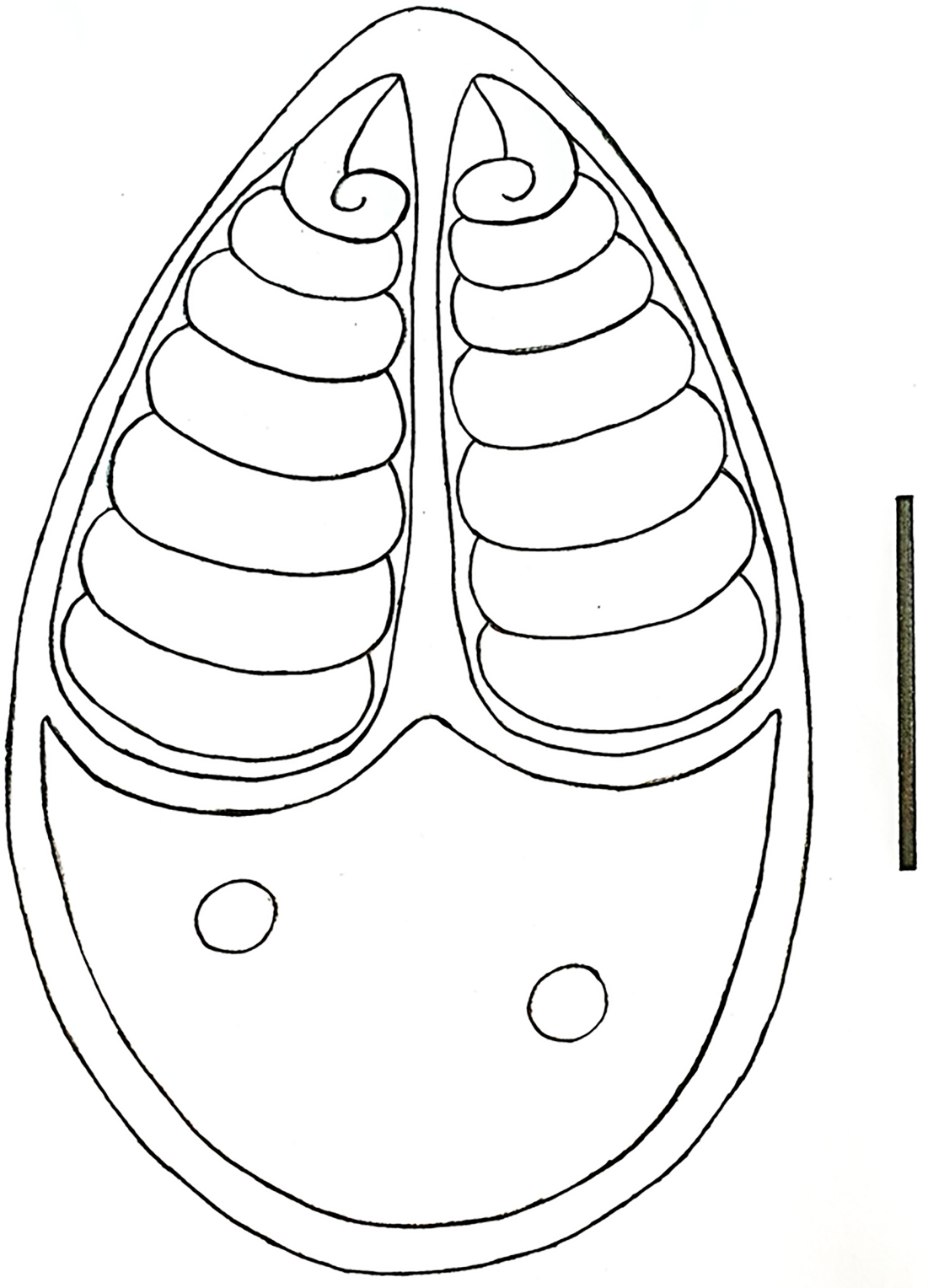
455 Fig.1. Light photomicrograph of mature myxospores of *Myxobolus iquitoensis* n. sp.,
456 parasite of gill filaments of *Otocinclus cocama*. A, Fresh mature myxospore. Scale bar:
457 50 µm. B, Formalin-fixed mature myxospore showing two equal elongated polar
458 capsules. Scale bar: 15 µm. C, Mature myxospores in frontal view stained in May-
459 Grünwald-Giemsa. Scale bar: 30 µm.

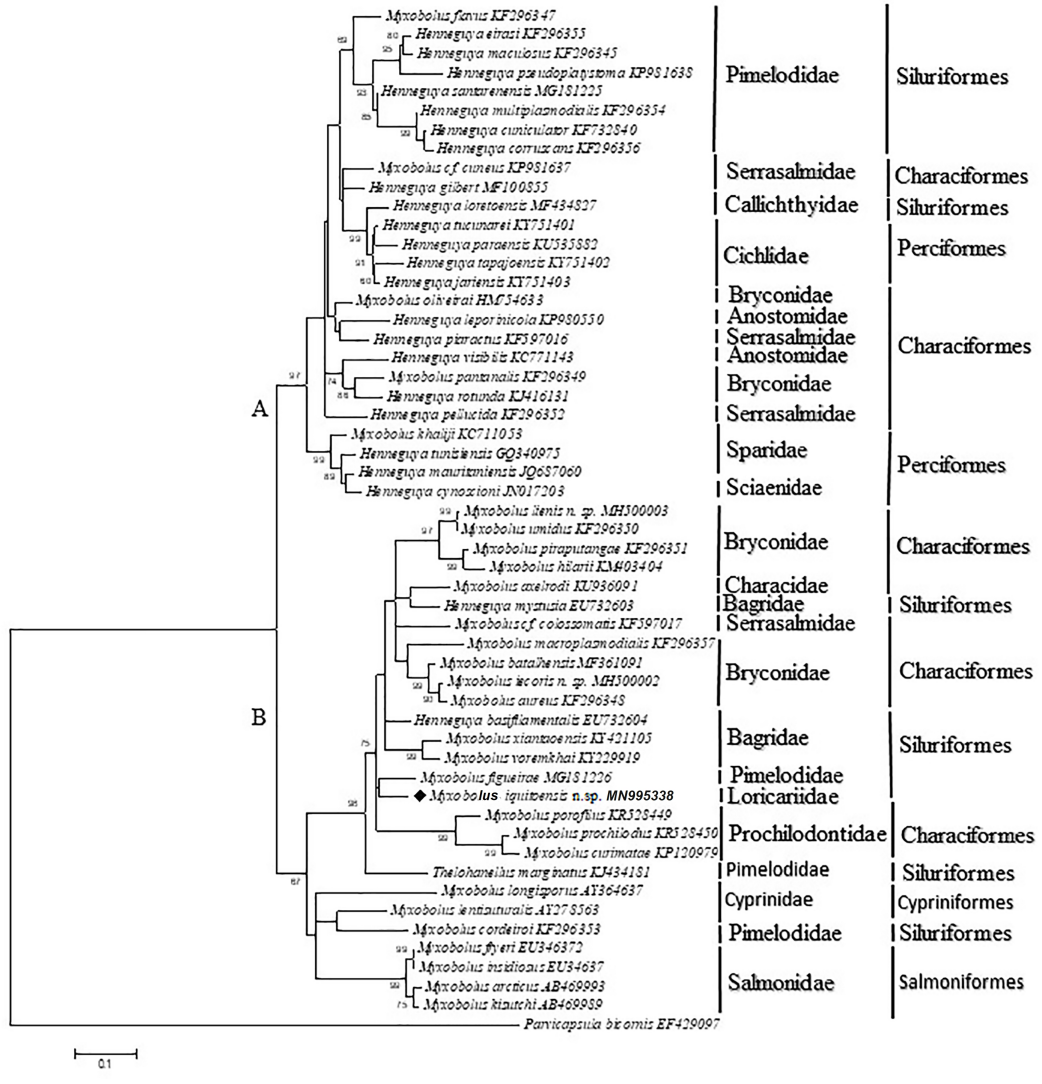
460 Fig. 2. Schematic representation of mature myxospore of *Myxobolus iquitoensis* n. sp.
461 parasite of gill filaments of *Otocinclus cocama*. Scale bar: 5 µm.

462 Fig. 3. Maximum Likelihood (ML) tree containing *Myxobolus iquitoensis* n. sp. and
463 closely related myxosporeans sequences deposited in GenBank based on partial small
464 subunit ribosomal DNA. The numbers above the nodes indicate bootstrap confidence
465 levels.

466 Table 1. Comparison of *Myxobolus iquitoensis* n. sp. with other *Myxobolus* species
467 described parasitizing siluriform fishes from Amazon basin. Spore dimensions, hosts,
468 infection sites, and collection sites are given. SL: spore length, SW: spore width, ST:
469 spore thickness, PCL: polar capsules length, PCW: polar capsules width, NCT: number
470 of coils in polar tubule, dashes: no data. All measurements are in µm.


A
50 µm
B
C




Myxobolus flavus KF296347
Henneguya eirasi KF296355
Henneguya maculosus KF296345
Henneguya pseudoplatystoma KP981638
Henneguya zantarenensis MG181225
Henneguya multiplasmodialis KF296354
Henneguya cuniculator KF732940
Henneguya cornuscans KF296356
Pimelodidae
Siluriformes
Myxobolus cf. cuneus KP981637
Serrasalmidae
Characiformes
Henneguya gilbert MF100855
Callichthyidae
Siluriformes
Henneguya loretoensis MF434827
Henneguya tucunarei KY751401
Henneguya paraensis KU535852
Henneguya tapajoensis KY751402
Henneguya jariensis KY751403
Cichlidae
Perciformes
Myxobolus oliveirai HM754633
Bryconidae
Henneguya leporinicola KP980550
Anostomidae
Henneguya piaractus KF597016
Serrasalmidae
Henneguya visibilis KC771143
Anostomidae
Myxobolus pantanalis KF296349
Henneguya rotunda KJ416131
Henneguya pellucida KF296352
Bryconidae
Serrasalmidae
Characiformes
A
Myxobolus khalili KC711058
Henneguya tunisiensis GQ340975
Henneguya mauritaniensis JQ687060
Henneguya cynoscioni JN017203
Sparidae
Sciaenidae
Perciformes
Myxobolus lienis n. sp. MH500003
Myxobolus umidus KF296350
Myxobolus piraputangae KF296351
Myxobolus hilarii KM403404
Bryconidae
Characiformes
Myxobolus axelrodi KU936091
Characidae
Henneguya mystusia EU732603
Bagridae
Siluriformes
Myxobolus cf. colossomatis KF597017
Serrasalmidae
Myxobolus macroplasmodialis KF296357
Myxobolus batalhensis MF361091
Myxobolus lacoris n. sp. MH500002
Myxobolus aureus KF296348
Bryconidae
Characiformes
Henneguya bazilfamentalis EU732604
Myxobolus xiantaoensis KY421105
Myxobolus voremkhai KY229919
Bagridae
Siluriformes
Myxobolus figueirae MG181226
Pimelodidae
◆ Myxobolus iquitosensis n.sp. MN995338
Loricariidae
Myxobolus porofilus KR528449
Myxobolus prochilodus KR528450
Myxobolus curimatae KP120979
Prochilodontidae
Characiformes
B
Thelohanellus marginatus KJ434131
Pimelodidae
Siluriformes
Myxobolus longisporus AY864637
Cyprinidae
Cypriniformes
Myxobolus lentiauralis AY278363
Myxobolus cordeiroi KF296353
Pimelodidae
Siluriformes
Myxobolus flyeri EU346372
Myxobolus insidiosus EU34637
Myxobolus arcticus AB469993
Myxobolus kisutchi AB469959
Salmonidae
Salmoniformes
Parvicapsula bicornis EF429097
0.1

Species	SL	SW	ST	PCL	PCW	NCT	Host	Family	Site of infection	Locality	References
***Myxobolus iquitoensis* 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. 2017
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