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1 **Taxonomy and 18S rDNA-based phylogeny of *Henneguya multiradiatus* n. sp.**
2 **(Cnidaria: Myxobolidae) a parasite of *Brochis multiradiatus* from Peruvian**
3 **Amazon**

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24

25 ABSTRACT

26 A new myxozoan species belonging to the genus *Henneguya* was isolated from the
27 serous membrane of the visceral cavity of the hog-nosed catfish *Brochis multiradiatus*
28 from Peruvian Amazon. Whitish plasmodia, macroscopically visible, were found in four
29 of the thirty examined fishes. Mature myxospores were ellipsoidal in shape in frontal
30 view and had a total length of $44.5 \pm 0.6 \mu\text{m}$ (43.9–45.1), spore body measured $18.7 \pm$
31 $0.9 \mu\text{m}$ (16.8–19.6) in length, $7.1 \pm 0.2 \mu\text{m}$ (6.6–7.4) in width and $5.5 \pm 0.3 \mu\text{m}$ (4.9–
32 5.6) in thickness. The two polar capsules were elongated and equal in size, measuring
33 $9.1 \pm 0.1 \mu\text{m}$ (8.8–9.4) in length and $1.7 \pm 0.1 \mu\text{m}$ (1.6–1.8) in width, occupying half of
34 the myxospore body. Polar tubules coiled in 10 to 11 turns perpendicular to the long
35 axis of the polar capsule. The caudal appendage was not bifurcated and measured $25.8 \pm$
36 $0.6 \mu\text{m}$ (24.7–26.5) in length. The sequencing of the 18S rDNA gene resulted in 1400
37 bp and this sequence did not match any of the myxozoans available in GenBank.
38 Phylogenetic analysis placed the new species in a well-supported subclade of
39 *Henneguya* spp. infecting callichthyid fishes, with *Henneguya loretoensis* being the
40 closest species. This study is the first description of a myxozoan species, *Henneguya*
41 *multiradiatus* n. sp. from a fish of the genus *Brochis*.

42 **Keywords:** Cnidaria, Endocnidozoa, Hog-nosed catfish, Myxosporean, 18S rDNA
43 sequencing, Peru

44 1. Introduction

45 Myxozoans are microscopic endoparasites with complex life cycles involving
46 invertebrate and vertebrate hosts [1]. Recently, due to synapomorphies and cnidarian-
47 specific genes, myxozoans were placed within the phylum Cnidaria and currently
48 represent about 20% of the known cnidarian species diversity around the world [1].

49 While predominantly known to infect freshwater and marine fishes, myxozoans can also
50 infect other groups of vertebrates such as amphibians, reptiles, birds, and terrestrial
51 small mammals [2]. Within fish-infecting myxozoans, *Henneguya* Thélohan, 1892, is
52 one of the largest genera, with more than 195 described species within a wide
53 geographical range [3]. *Henneguya* spp. are histozoic parasites and they show high host-
54 specificity and organ- or tissue tropism, with some species inducing severe
55 henneguyosis in wild and cultured fish [4–7].

56 It is well known that the growing global pet trade, including aquarium market, plays a
57 role in the spread of pathogens [8]. According to Hallett et al. [9], the aquatic pet trade
58 would very likely stand as a primary mode for international transport of myxozoan
59 parasites. In fact, there are some reports about the introduction of myxosporeans
60 through commercial transfers of pets with some cases of ecological and economic
61 impacts [10–12]. As such, there is a growing need for myxozoans screening during
62 parasitological surveys in these aquatic pet animals.

63 The armored catfish corydoradines are members of the family Callichthyidae. With over
64 190 described species, it is one of the most diverse siluriform assemblages in the
65 Neotropics [13]. These neotropical freshwater catfishes are endemic to South America
66 [13]. Among these corydoradines, *Brochis multiradiatus* Orcés V., 1960 is found in the
67 Napo River in Ecuador and Peru [14]. It can reach up to 6.7 cm in length and is largely
68 marketed in the aquarium trade, being one of the most popular in the Chinese
69 ornamental fish market [15]. Although corydoradines are highly appreciated in the
70 international aquarium trade, there are few parasitological surveys about this fish,
71 especially those concerning myxozoan parasites.

72 To our knowledge, nothing is known about myxozoan parasites infecting any member
73 of the genus *Brochis*. Herein, based on morphological, biological/ecological traits and

74 molecular data, we described a new *Henneguya* species infecting the ornamental fish *B.*
75 *multiradiatus* from the Amazon region of Peru, a key supply region of wild ornamental
76 fishes for the international aquarium trade.

77 **2. Material and methods**

78 In September 2019, thirty wild specimens of *B. multiradiatus* were collected by a local
79 fisherman. The fishes were caught in the Napo River, in an adjacent area of the village
80 Cabo Pantoja (0°57'25"S, 75°27'11"W), Department of Loreto, Peru. The fish were
81 transported alive to the field laboratory where they were euthanized by neural pithing
82 and examined. The procedures were approved by the Federal University of São Paulo-
83 UNIFESP Ethics Committee (CEUA No. 9209080214), in accordance with Brazilian
84 law for scientific use of animals (Federal Law No. 11794, dated 8 October 2008). The
85 family and current status of the host fish (valid species name or synonym) were
86 reviewed using FishBase [14].

87 Morphometric analyses were performed on mature myxospores following the criteria
88 outlined by Lom and Arthur [16]. Measurements and photographs were taken from 30
89 myxospores using a computer equipped with Axiovision 4.1 image capture software
90 coupled to an Axioplan 2 Zeiss microscope (Carl Zeiss AG, Oberkochen, Germany).
91 The dimensions of the myxospores were given in micrometers (μm) and expressed as
92 the mean \pm standard deviation, followed by the range in parentheses. Prevalence of
93 infection [(number of infected fish/total number of fishes examined) $\times 100$] was
94 calculated according to Bush et al. [17]. Smears containing free myxospores were air-
95 dried, fixed with methanol and stained with Ziehl-Neelsen to mount on permanent slides
96 that were deposited in the cnidarian collection of the zoology Museum of the University
97 of São Paulo - USP, São Paulo, Brazil (MZUSP).

98 For molecular studies, a plasmodium was dissected from the host tissue and fixed in
99 absolute ethanol. The genomic DNA was extracted from a single plasmodium using
100 DNeasy® Blood & Tissue Kit (Qiagen, Valencia, USA), in accordance with the
101 manufacturer's instructions. The DNA concentration was measured using a NanoDrop
102 2000 spectrophotometer (Thermo Scientific, Wilmington, USA). Polymerase chain
103 reactions (PCRs) were performed in accordance with Milanin et al. [18] and conducted
104 in a final volume reaction of 25 µL, which comprised 10–50 ng of extracted DNA, 0.2
105 pmol for each primer, 12.5 µL of Dream Taq Green PCR Master Mix (Thermo
106 Scientific) and nuclease-free water. Partial 18S rDNA sequence was amplified using the
107 primer pairs ERIB1 [19] - ACT1r [20] and Myxgen4F [21] - ERIB10 [19] (Table 1),
108 which amplified two fragments containing approximately 1.000 and 1.200 bp
109 respectively. PCR amplification was done by initial denaturation at 95 °C for 5 min,
110 followed by 35 cycles at 95 °C for 1 min, 64 °C (ERIB1-ACT1r) or 58 °C (Myxgen4F-
111 ERIB10) for 1 min, 72 °C for 2 min, and then final elongation at 72 °C for 5 min. PCRs
112 were performed in an AG22331 Hamburg Thermocycler (Eppendorf, Hamburg,
113 Germany). PCR products were subjected to electrophoresis in 2.0% agarose gel
114 (BioAmerica, Irvine, USA) in a TAE buffer (Tris-Acetate EDTA: Tris 40 mM, acetic
115 acid 20 mM, EDTA 1 mM), stained with Sybr Safe DNA gel stain (Invitrogen by Life
116 Technologies, Carlsbad, USA), and then analyzed with a Stratagene 2020E trans
117 illuminator (Stratagene California, San Diego, USA). The size of the amplicons was
118 estimated by comparison with the 1 Kb Plus DNA Ladder (Invitrogen by Life
119 Technologies). PCR products were purified using USB® ExoSap-IT® (Thermo Fisher
120 Scientific) in accordance to the manufacturer's instructions and sequenced using the
121 same PCR primers plus two additionally MC5 and MC3 primers used to overlap the
122 obtained amplification fragments [22]. Sequencing was performed at Paulista Medical

123 School, Federal University of São Paulo, using a BigDye® Terminator v3.1 Cycle
124 Sequencing kit (Applied Biosystems Inc., California, USA) in an ABI 3130 automatic
125 DNA analyzer (Applied Biosystems Inc.™). The obtained sequence was visualized,
126 assembled and edited using BioEdit 7.1.3.0 software [23]. A standard nucleotide
127 BLAST (blastn) search was conducted to verify the similarity of the sequence obtained
128 in this study with other sequences available in GenBank [24]. Phylogenetic analysis was
129 conducted using the most closely related myxozoans sequences with similarity > 80%.
130 The sequences were aligned with ClustalW within BioEdit version 7.1.3.0 [23].
131 Phylogenetic analysis was performed using Maximum Likelihood (ML) method with a
132 Kimura 2-parameter (K2P) evolution sequence model in MEGA 6.0 [25]. Bootstrap
133 analysis (1000 replicates) was employed to assess the relative robustness of the
134 branches in ML tree. *Ceratonova shasta* sequence was used as outgroup. The pairwise
135 method with the p-distance model in MEGA 6.0 [25] was performed to evaluate the
136 genetic distance between the myxozoan species clustering together with the new
137 sequence obtained.

138 **3. Results**

139 Whitish plasmodia macroscopically visible measuring 2.1 to 3 mm in diameter and
140 containing large quantities of myxospores belonging to the genus *Henneguya* were
141 found in the serous membrane of the visceral cavity of wild specimens of *B.*
142 *multiradiatus* (Fig. 1A). The prevalence of the myxozoan parasites in these fish hosts
143 was 13.3% (4/30) and no signs of diseases were observed in the infected individuals.

144 ***Henneguya multiradiatus* n. sp.**

145 **Taxonomic summary**

146 Phylum: Cnidaria Verrill, 1865

147 Class: Myxosporea Bütschli, 1881

148 Order: Bivalvulida Shulman, 1959

149 Family: Myxobolidae Thélohan, 1892

150 Genus: *Henneguya* Thélohan, 1892

151 Type host: *Brochis multiradiatus* (Orcés V., 1960) (Siluriformes: Callichthyidae)

152 Site of infection: Serous membrane of the visceral cavity

153 Type locality: Napo River, adjacent area of the village Cabo Pantoja (0°57'25"S,

154 75°27'11"W), Department of Loreto, Peru.

155 Prevalence: 13.3 % (4/30).

156 Type of material: Syntypes-air-dried slide stained with Ziehl-Neelsen deposited in the

157 cnidarian collection of Zoology Museum of the University of São Paulo - USP, São

158 Paulo, Brazil (MZUSP 8508). Partial 18S rDNA sequence (1400bp) was deposited in

159 GenBank under accession number MT415832.

160 Etymology: The specific name (*H. multiradiatus*) is based on host species name.

161 **Morphological characterization**

162 Mature myxospores were ellipsoidal in shape from the frontal view, measuring $44.5 \pm$

163 $0.6 \mu\text{m}$ (43.9–45.1) in total length, $18.7 \pm 0.9 \mu\text{m}$ (16.8–19.6) in spore body length, 7.1

164 $\pm 0.2 \mu\text{m}$ (6.6–7.4) in width and $5.5 \pm 0.3 \mu\text{m}$ (4.9–5.6, n=5) in thickness (Figs. 1B and

165 2). Two elongated polar capsules, equal in size, measuring $9.1 \pm 0.1 \mu\text{m}$ (8.8–9.4) in

166 length and $1.7 \pm 0.1 \mu\text{m}$ (1.6–1.8) in width, occupying half of myxospore body (Figs.

167 1B-C and 2). Polar tubules had 10 to 11 coils, perpendicular to long axis of polar

168 capsule (Figs. 1C and 2). Non-bifurcate caudal appendage, measuring $25.8 \pm 0.6 \mu\text{m}$
169 (24.7–26.5) in length (Figs. 1B and 2).

170 **Molecular characterization and phylogenetic analysis**

171 Partial 18S rDNA sequence obtained from mature myxospores of *H. multiradiatus* n. sp.
172 resulted in 1400 bp with a CG content of 49.4% and did not match any other myxozoans
173 available in GenBank. The comparison of the new sequence obtained by BLAST
174 revealed the highest sequence similarity to *Henneguya loretoensis* Mathews, Naldoni
175 and Adriano 2017 (GenBank accession number MF434827.1, query coverage 99%,
176 maximum identities 98.2%). The phylogenetic analysis placed *H. multiradiatus* n. sp. in
177 a well-supported subclade of *Henneguya* parasites of callichthyids fish, including
178 *Henneguya loretoensis*, and *Henneguya guanduensis* Abdallah, Azevedo, Luque and
179 Bomfim 2007 (Fig. 3). This subclade, was close to a lineage formed by *Henneguya*
180 species described from Amazonian cichlids. 18S rDNA pairwise comparisons between
181 *H. multiradiatus* n. sp. described and the closest relatives resulted in 1.8% to *H.*
182 *loretoensis*, 5.3% to *H. guanduensis*, 6.1% to *Henneguya peruviansis* Mathews,
183 Mertins, Pereira, Maia and Adriano 2018, 7.6% to *Henneguya jariensis*, 9.5% to
184 *Henneguya tapajoensis*, 7.2% to *Henneguya tucunarei* Zatti, Atkinson, Maia,
185 Bartholomew and Adriano 2018 and 8.6% to *Henneguya paraensis* Velasco, Videira,
186 Nascimento, Matos, Gonçalves and Matos 2016.

187 **4. Discussion**

188 The Peruvian Amazon is the second largest portion of the Amazon rainforest
189 concentrating a high aquatic biodiversity, with several commercially exploited fish
190 species for human consumption and global aquarium trade [26,27]. However, little is
191 known about the diversity of myxozoans in this region, with only three species

192 described to date: *Henneguya loretoensis*, *Henneguya peruviansis* and *Myxidium*
193 *peruviansis* [28–30]. In contrast, over 40 myxozoan species have been described in the
194 Brazilian Amazon region [31–33], indicating that there are likely many more myxozoan
195 parasites to be identified in Peruvian Amazon, considering the recognized high
196 diversity. This study reports, for the first time, a myxozoan species infecting a fish from
197 the genus *Brochis* in the Amazon basin.

198 For robust identification and description of new myxosporeans taxa, it has been
199 recommended, that the studies integrate multiple characters, including morphology,
200 biological traits, host ecology factors and molecular data [34]. Following these
201 delineated criteria for classifying myxozoans, we described a novel *Henneguya* species,
202 *Henneguya multiradiatus* n. sp. from an important Amazonian ornamental fish. In the
203 morphological comparison, considering the twenty-two *Henneguya* species previously
204 described to infect fishes from Amazon basin, the most morphologically similar species
205 to the new species were *Henneguya torpedo* Azevedo, Casal, Matos, Alves and Matos,
206 2011 and *Henneguya testicularis* Azevedo, Corral and Matos, 1997. Nevertheless, these
207 differ from *H. multiradiatus* n. sp. in myxospore body length ($28.5 \pm 0.3 \mu\text{m}$ in length
208 for *H. torpedo*, $14.0 \mu\text{m}$ for *H. testicularis* and $18.7 \pm 0.9 \mu\text{m}$ to the new species),
209 number of coils of the polar tubule (12 to 13 in *H. torpedo*, six to seven in *H.*
210 *testicularis* and ten in the new species) and in the length of the caudal appendage, which
211 is substantially larger in the new species ($19.6 \pm 0.4 \mu\text{m}$ in *H. torpedo*, $13.5 \mu\text{m}$ in *H.*
212 *testicularis*, and $25.8 \pm 0.6 \mu\text{m}$ in *H. multiradiatus* n. sp.). Plasmodia of *H.*
213 *multiradiatus* n. sp. were located in the serous membrane of the visceral cavity, while
214 *H. torpedo* and *H. testicularis* were described infecting brain, spinal cord, and testis
215 respectively [35,36]. On the same premise, host species and locality of collection are
216 indispensable traits for accurately distinguishing new histozoic platysporines species,

217 since host-specificity and geography may play an important role in speciation [37].
218 Accordingly, differences were observed in the infected host, *e.g.*, *H. multiradiatus* n. sp.
219 infecting *B. multiradiatus*, a Siluriform fish; *H. torpedo* infecting *Brachyhypopomus*
220 *pinnicaudatus* (Hopkins 1991), a Gymnotiform fish; and *H. testicularis* infecting
221 *Moenkhausia oligolepis* (Günther 1864), a Characiform fish. Besides, differences in the
222 locality of these species were noted, with *H. multiradiatus* n. sp. found in the Napo
223 River, a tributary of the upper Amazon River, near the village of Cabo Pantoja in
224 Department of Loreto, Peru, and *H. torpedo* and *H. testicularis* found in the lower
225 Amazon River, both near Belém city in Pará state, Brazil. In addition to the large
226 geographic distance between these species (2, 965 km in straight line), the nonmigratory
227 behavior of their hosts is an important ecological character for the separation of these
228 species, taking in account that host behavior may drive both parasite endemism and the
229 radiation within the Amazon basin [32]. Unfortunately, there is no molecular data
230 available for *H. torpedo* and *H. testicularis* in GenBank, so it is not possible to compare
231 them with *H. multiradiatus* n. sp. However, differences observed in the integrative
232 comparison, including morphology, geography, biological traits in conjunction with the
233 ecological characters of their hosts, are sufficient arguments to conclude that *H.*
234 *multiradiatus* n. sp. is a separate species.

235 In our phylogenetic analysis, *Henneguya* spp. showed tendency to cluster, largely based
236 on vertebrate host fish family, and this corroborates the results pointed out by other
237 authors who evidenced that host group is a strong evolutionary signal within the
238 Myxobolidae [32,38,39]. *Henneguya multiradiatus* n. sp. was placed in a well-
239 supported subclade of parasites of callichthyids fishes, with *H. loretoensis* as the closet
240 related species (Fig. 3). This close phylogenetic relationship was corroborated by the
241 pairwise analysis, which showed a slight genetic divergence of 1.8% on their 18S rDNA

242 sequences. Nevertheless, there is no exact value for determining the level of genetic
243 variation in 18S rDNA that equates to species differentiation within this enigmatic
244 group of parasites [38,40]. In this context, species differentiation should be assessed for
245 each individual case and always with the aid of biology and/or ecology traits of the
246 organisms, such as their morphology, tissue and/or organ tropism, host species,
247 geography, and host ecological aspects such as endemic character, migratory behavior
248 and distribution [16,40]. In our study, remarkable morphometrical differences can be
249 observed between these two corydoradines *Henneguya* parasites, with myxospores
250 substantially larger in total length and width in the newly identified species (44.5 ± 0.6
251 $\mu\text{m} \times 7.1 \pm 0.2 \mu\text{m}$ vs $36.2 \mu\text{m} \times 5.1 \mu\text{m}$ for *H. loretoensis*), larger body length ($18.7 \pm$
252 $0.9 \mu\text{m}$ vs $14.3 \pm 0.1 \mu\text{m}$ for *H. loretoensis*), longer polar capsules ($9.1 \pm 0.1 \mu\text{m}$ vs 5.1
253 $\pm 0.1 \mu\text{m}$ in *H. loretoensis*), higher number of coils in the polar tubule (ten to eleven
254 coils vs five coils in *H. loretoensis*) and the caudal appendage which is substantially
255 longer ($25.8 \pm 0.6 \mu\text{m}$ vs 21.9 ± 0.1 in *H. loretoensis*). Furthermore, *H. multiradiatus* n.
256 sp. the caudal appendage is not bifurcated, as occurs in *H. loretoensis*. Differences can
257 also be outlined concerning the host-, tissue- and organ infected, with the new species
258 proposed found in the serosa layer of the visceral cavity of *B. multiradiatus* n. sp.
259 whereas *H. loretoensis* is found in gill filaments of *Corydoras leucomelas* Eigenmann
260 and Allen, 1942, a host of a different genus. While these two corydoradines parasites
261 inhabit the Amazon biome of Peru (*H. multiradiatus* n. sp. in Napo River and *H.*
262 *loretoensis* Nanay River), they are separated by 381 km from each other. Another
263 important point to consider in establishing *H. multiradiatus* n. sp. as a new species, is in
264 regards to the endemic character and the absence of migratory reproduction,
265 characteristics which are highly recognized to corydoradines fish [33].

266 In the present study, an integrative taxonomy approach was employed in the description
267 of an unknown myxosporean. Given that these criteria are compelling evidence for the
268 characterization of new species [16,40], we confidently considered that this isolate is a
269 new species, *H. multiradiatus* n. sp. Hence, this new data contributes to increase the
270 knowledge of the myxosporean diversity from the Amazon biome, as well to clarify the
271 relationships of myxozoan parasites of corydoradines, an economic important
272 assemblage of catfishes in the lucrative global aquarium industry.

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463 Legends

464 **Table 1.** Primers used in the amplification and sequencing of the 18S rDNA gene of
465 *Henneguya multiradiatus* n. sp.

466 **Table 2.** Comparative data of *Henneguya multiradiatus* n. sp. with other *Henneguya*
467 species parasites of Amazon fish. Spore dimensions, infection sites, and fish host are
468 given. TL: total length; BL: body length; APCL: caudal appendage length; SW: spore
469 width; ST: spore thickness; PCL: polar capsule length; PCW: polar capsule width;
470 NCT: number of coils of polar tubules; –: no data. All measurements are range in μm
471 and/or means \pm SD.

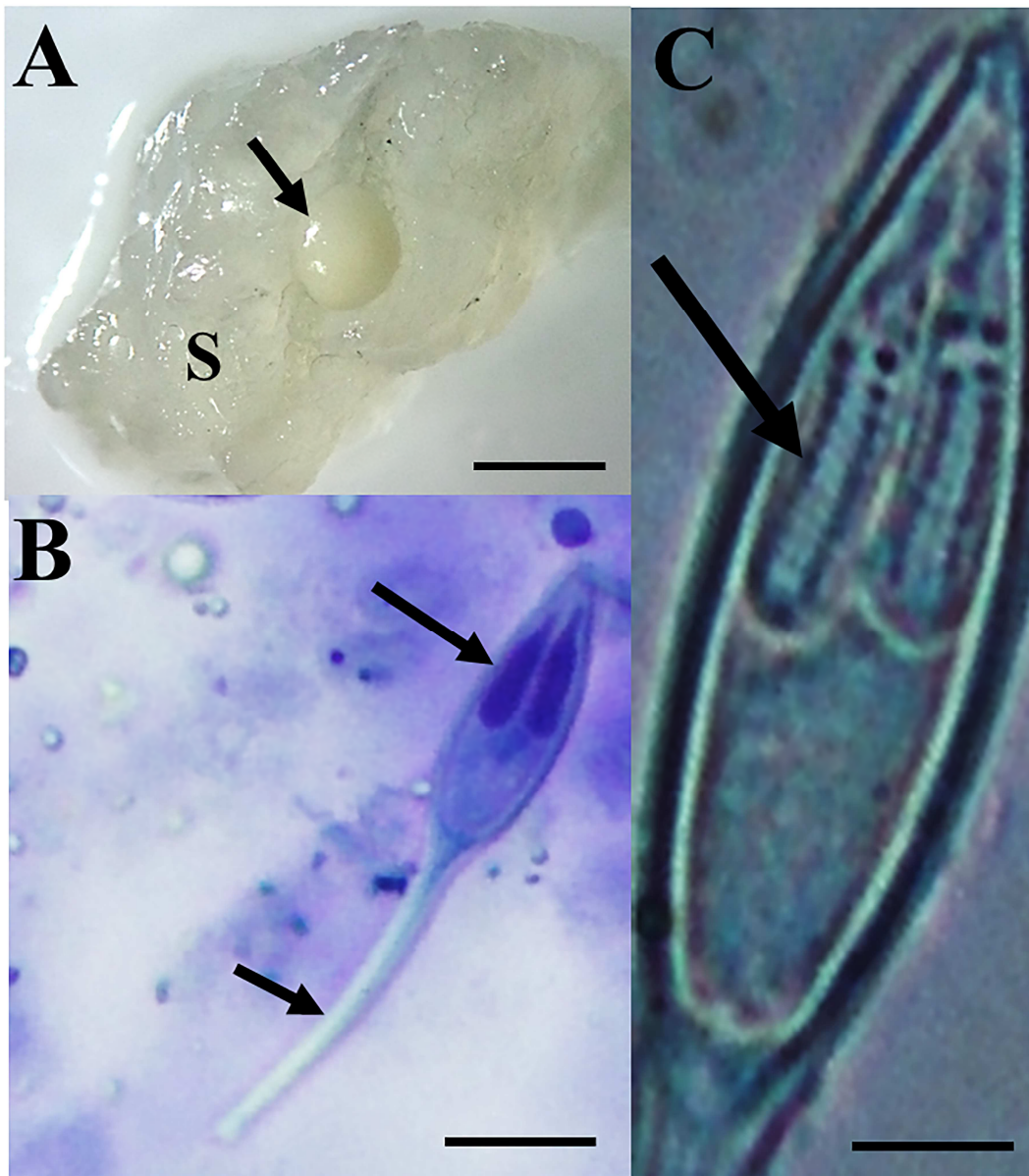
472 **Fig. 1.** Plasmodium and myxospores of *Henneguya multiradiatus* n. sp. from *Brochis*
473 *multiradiatus*. A- Whitish plasmodium macroscopically visible (black arrow) in the
474 serosa of the visceral cavity (S). Scale bar= 3 mm. B- Mature myxospore stained with

475 Ziehl-Neelsen showing two elongated equal polar capsules (black large arrow) and
476 caudal appendage not bifurcated (black small arrow). Scale bar= 10 μm . C: Myxospore
477 showing polar coiled tubules (black arrow) perpendicular to the long axis of the polar
478 capsule. Scale bar= 3 μm .

479 **Fig. 2.** Schematic representation of myxospore of *Henneguya multiradiatus* n. sp. from
480 *Brochis multiradiatus*. Scale bar= 7 μm .

481 **Fig. 3.** Maximum likelihood phylogenetic tree based on partial 18S rDNA sequences
482 containing *Henneguya multiradiatus* n. sp. and closely related myxozoans based on
483 BLAST. GenBank accession numbers and host family are given in front of species.
484 Bootstrap values above 50 are indicated at the nodes.

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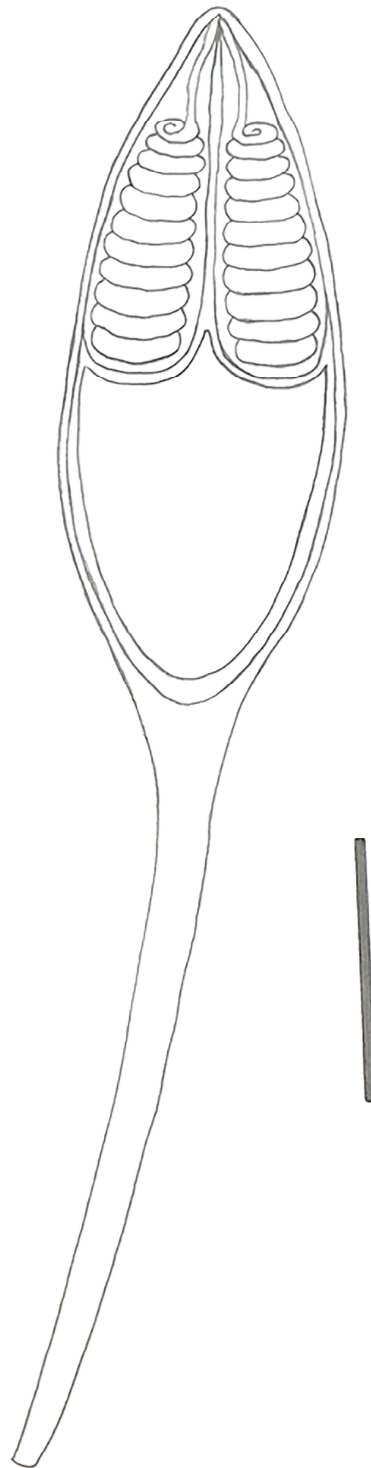


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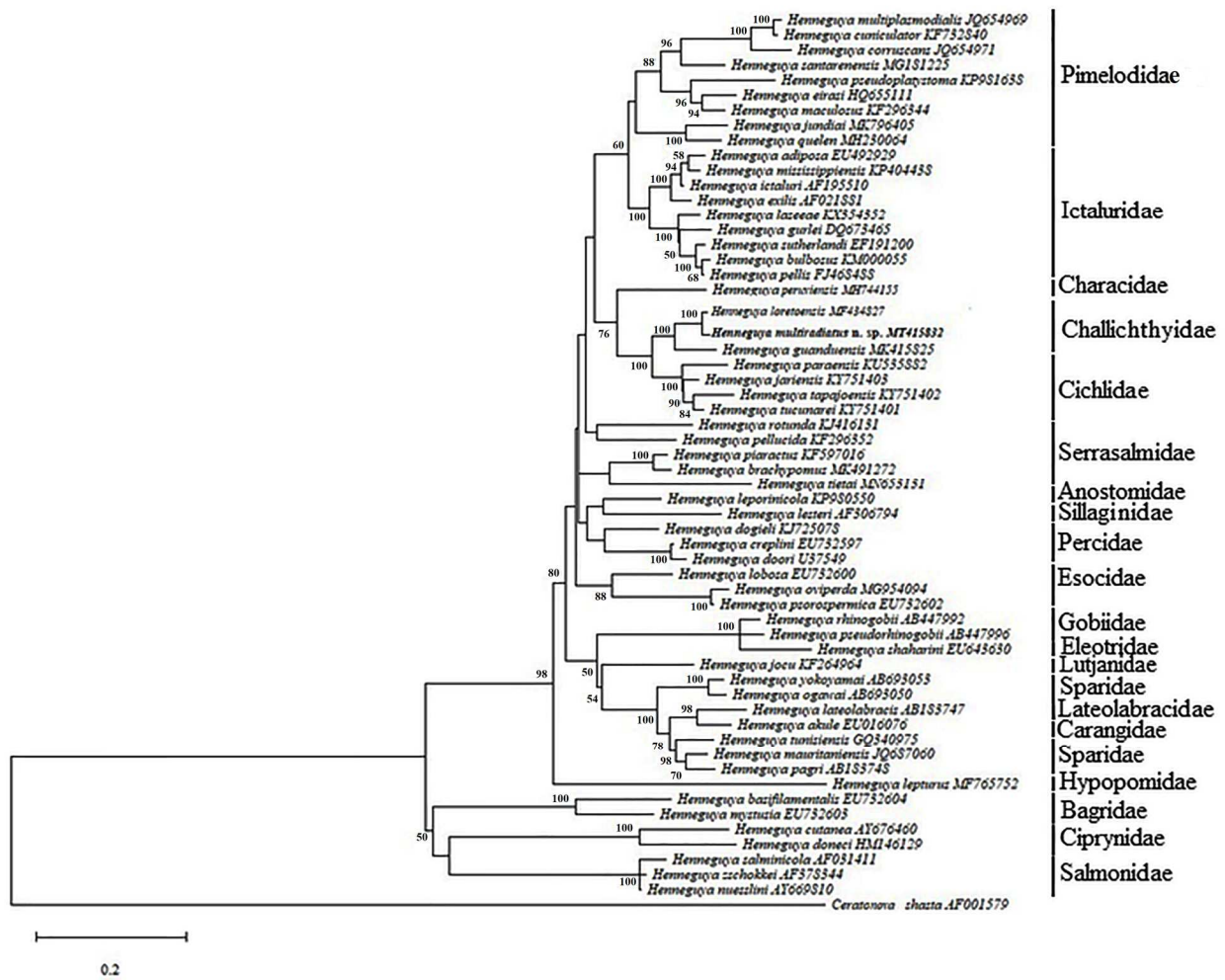
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Primers	Sequences (5'-3')	References
ACT1r	AATTTACCTCTCGCTGCCA	Hallett and Diamant [20]
ERIB1	ACCTGGTTGATCCTGCCAG	Barta et al. [19]
ERIB10	CTCCGCAGGTTACCTACGG	Barta et al. [19]
Myxgen4F	GTGCCTTGAATAAATCAGAG	Diamant et al. [21]
MC5	CCTGAGAAACGGCTACCACATCCA	Molnár et al. [22]
MC3	GATTAGCCTGACAGATCACTCCACGA	Molnár et al. [22]

Species	TL	BL	APCL	SW	ST	PCL	PCW	NCT	Site of infection	Fish species
<i>Henneguya multiradiatus</i> n. sp. [This study]	44.5 ± 0.6 (43.9–45.1)	18.7 ± 0.9 (16.8–19.6)	25.8 ± 0.6 (24.7–26.5)	7.1 ± 0.2 (6.6–7.4)	5.5 ± 0.3 (4.9–5.6)	9.1 ± 0.1 (8.8–9.4)	1.7 ± 0.1 (1.6–1.8)	10-11	Abdominal cavity serosa	<i>Brochis multiradiatus</i>
<i>Henneguya peruviansis</i> [30]	24.2 ± 1.3 (22.9–25.5)	13.4 ± 0.9 (12.5–14.3)	10.7 ± 1.2 (9.5–11.9)	3.9 ± 0.1 (3.8–4.0)	–	3.3 ± 0.2 (3.1–3.5)	1.6 ± 0.2 (1.4–1.8)	4-5	Gill filaments	<i>Hyphessobrycon loretoensis</i>
<i>Henneguya loretoensis</i> [29]	36.2 ± 0.2 (36.1–36.3)	14.3 ± 0.1 (14.2–14.4)	21.9 ± 0.1 (21.8–22)	5.1 ± 0.2 (4.9–5.3)	–	5.1 ± 0.2 (4.9–5.3)	2.4 ± 0.3 (2.1–2.7)	5	Gill filaments	<i>Corydoras leucomelas</i>
<i>Henneguya tucunareii</i> [32]	43.8 ± 4.1 (36.1–49.6)	14 ± 0.8 (12.1–15.7)	28.1 ± 4.3 (19.6–35.6)	6.1 ± 0.7 (4.9–7.8)	–	3.4 ± 0.5 (2.5–4.6)	1.98 ± 0.3 (1.3–2.6)	3–4	Gill filaments	<i>Cichla monoculus</i>
<i>Henneguya tapajoensis</i> [32]	54.6 ± 3.9 (47.2–62.2)	16.4 ± 1.2 (14.5–19.1)	39 ± 3.9 (31.7–46.5)	7 ± 0.4 (5.7–9.3)	5 ± 0.1 (4.8–5.1)	4.2 ± 0.5 (2.9–5.0)	2.1 ± 0.4 (1.5–2.8)	4–5	Gill filaments	<i>Cichla pinima</i>
<i>Henneguya jariensis</i> [32]	46.7 ± 1.5 (43.9–49.2)	13.4 ± 0.7 (11.9–14.6)	33.1 ± 1.7 (30.2–37.0)	6.5 ± 0.5 (4.9–7.3)	–	4 ± 0.3 (3.4–4.3)	2 ± 0.1 (1.7–2.4)	4	Fins	<i>Cichla monoculus</i>
<i>Henneguya paraensis</i> [41]	42.3 ± 0.3 (41.6–42.9)	12.8 ± 0.42 (12.3–13.2)	29.5 ± 0.73 (28.7–30.2)	8.6 ± 0.32 (8.18–8.92)	–	7.4 ± 0.16 (6.67–7.5)	2.6 ± 0.1 (2.52–2.68)	5–7	Gill filaments	<i>Cichla temensis</i>
<i>Henneguya melini</i> [42]	40.8 ± 0.3 (40.3–41.1)	15.5 ± 0.2 (15.3–15.7)	25.3 ± 0.1 (25.2–25.4)	4.7 ± 0.1 (4.6–4.8)	–	4.8 ± 0.5 (4.3–5.3)	1.7 ± 0.3 (1.4–2)	5–6	Gill filaments	<i>Corydoras melini</i>
<i>Henneguya aequidens</i> [43]	41 ± 1.5	15 ± 0.9	27 ± 0.6	6 ± 0.8	–	3 ± 0.3	3 ± 0.3	4–6	Gill filaments	<i>Aequidens plagiozonatus</i>
<i>Henneguya torpedo</i> [36]	48.62 ± 0.51 (48.3–48.9)	28.53 ± 0.36 (28.3–30.1)	19.64 ± 0.44 (19.2–19.9)	7.25 ± 0.31 (7–7.5)	3.06 ± 0.2 (2.9–3.1)	6.41 ± 0.26 (6.3–6.6)	1.84 ± 0.19 (1.7–1.9)	5–6	Brain and spinal cord	<i>Brachyhypopomus pinnicaudatus</i>
<i>Henneguya arapaima</i> [44]	51.6 ± 3.4 (48.4–53.1)	14.2 ± 0.8 (13.5–15.2)	38.3 ± 2.9 (38–41.2)	5.7 ± 0.5 (5.1–6.1)	4.9 ± 0.2 (4.7–5.3)	6.5 ± 0.2 (6.3–6.8)	6.3 ± 0.1 (6.3–6.8)	5	Gill arch	<i>Arapaima gigas</i>
<i>Henneguya rondoni</i> [45]	17.7 (16.9–18.1)	7 (6.8–7.3)	10.7 (10.3–11)	3.6 (3–3.9)	2.5 (2.2–2.8)	2.5 (2.2–2.8)	0.85 (0.79–0.88)	6–7	Lateral nerves	<i>Gymnorhamphichthys rondoni</i>
<i>Henneguya rhamdia</i> [46]	50 ± 1.8	13.1 ± 1.1	36.9 ± 1.6	5.2 ± 0.5	–	4.7 ± 0.4	1.1 ± 0.2	10–11	Gill filaments	<i>Rhamdia quelen</i>
<i>Henneguya schtzodon</i> [47]	28.9 (27–30)	13.1 (12–14)	16.3 (15–17)	3.3 (3–4)	–	5.4 (5–6)	1.3 (1–1.5)	8–10	Kidney	<i>Schtzodon fasciatum</i>
<i>Henneguya friderici</i> [48]	33.8 (28.7–39.3)	10.4 (9.6–11.8)	23.3 (19.1–28.7)	5.7 (4.8–6.6)	4.9	4.9 (4.25–5.9)	2.1 (1.59–2.62)	7–8	Gut, gill, kidney and liver	<i>Leporinus friderici</i>
<i>Henneguya astyanax</i> [49]	47.8 ± 0.71	15.2 ± 0.77	32.6 ± 1.11	5.7 ± 0.71	4.2 ± 0.31	5.0 ± 0.13	1.5 ± 0.07	8–9	Gill filaments	<i>Astyanax bimaculatus</i>
<i>Henneguya curimata</i> [50]	35.4 (4.2–36)	16.6 (16–17.4)	19.1 (18.3–19.9)	6.2 (5.8–6.6)	–	3.3 ± 0.02 (2.7–3.6)	1.5 ± 0.04 (1.1–1.9)	10–11	Kidney	<i>Curimata inornata</i>
<i>Henneguya testicularis</i> [35]	27.5 (27–28.5)	14 (14–14.5)	13.5 (13–14.5)	6.5 (6–6.5)	–	9 (8.5–9.5)	2 (2–2.5)	12–13	Testicle	<i>Moenkhausia oligolepis</i>
<i>Henneguya malabarica</i> [51]	28.3 (26.6–29.8)	12.6 (11.8–13.1)	17.1 (16.2–18.9)	4.8	–	3.7 (3.0–4.3)	1.8 (1.6–2.2)	6–7	Gill filaments	<i>Hoplias malabaricus</i>

<i>Henneguya adherens</i> [52]	32.3 (30.7–35.1)	12.4 (10.5–13.8)	20.5 (18–21.7)	5.8 (5.1–6.5)	–	3.1 (2.8–3.5)	1.2 (1–1.6)	3–4	Gill filaments	<i>Acestrorhynchus falcatus</i>
<i>Henneguya amazonica</i> [53]	59.3 ± 0.56 (55–65.9)	13.9 ± 0.16 (11.5–14.9)	45.4 ± 0.61 (41.7–52.1)	5.7 ± 0.06 (5.2–6.3)	–	3.3 ± 0.02 (2.7–3.6)	1.5 ± 0.04 (1.1–1.9)	6	Gill lamellae	<i>Crenicichla leplota</i>

