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Henneguya garavelli n. sp. and Myxobolus peculiaris n. sp. (Myxozoa: Myxobolidae) in the gills of Cyphocharax nagelli (Osteichthyes: Curimatidae) from Rio do Peixe Reservoir, São José do Rio Pardo, São Paulo, Brazil

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Abstract

The present work describes myxozoans found in *Cyphocharax nagelli* (Characiformes: Curimatinae) commonly called "sagüiru" collected from Rio do Peixe Reservoir, São José do Rio Pardo, São Paulo, Brazil. From a total of 38 examined fish, 24 were infected with *Henneguya garavelli* n. sp. (63% prevalence) and two with *Myxobolus peculiaris* n. sp. (5% prevalence) in the gills. Spores were studied by staining and fresh spores were observed by differential interference contrast optics. *Henneguya garavelli* n. sp. differs from *Henneguya iheringi*, *Henneguya occulta*, *Henneguya cesarpintoi*, *Henneguya santae*, *Henneguya pisciforme*, *Henneguya amazonica*, *Henneguya striolata*, *Henneguya malabarica*, *Henneguya piaractus* and also *Henneguya chydadea* in spore length and from *Henneguya travassosi*, *Henneguya adherens*, *Henneguya malabarica*, *Henneguya piaractus* and also *Henneguya chydadea* in polar capsule length and tail length. *Myxobolus peculiaris* n. sp. was very different when compared to other species of *Myxobolus* in its morphology and the biggest size of spore body. The authors present tables with comparative measurements of Brazilian myxozoan parasites.

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Keywords: Henneguya garavelli n. sp.; Myxobolus peculiaris n. sp.; Brazilian fish; Cyphocharax nagelli; Species comparison

1. Introduction

In Brazil, Pinto (1928a,b) described *H. linearis* in the gills of *Rhamdia sebae* and *Pseudoplatystoma*

* Corresponding author. E-mail address: mlaterca@cca.ufsc.br (M.L. Martins). fasciatum, Henneguya occulta in Callichthyidae fish, H. wenyoni in Astyanax fasciatus and Henneguya iheringi in Serrasalmus spillopleura. Guimarães and Bergamin (1934) observed Henneguya santae in Tetragonopterus santae. Azevedo and Matos (1989) have studied Henneguya infection in the gills of Hoplosternum litorale while Rocha et al. (1992)

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observed Henneguya amazonica in Crenicichla lepidota. Henneguya adherens was present in Acestrorhynchus falcatus (see Azevedo and Matos, 1995) and Henneguya malabarica in Hoplias malabaricus (see Azevedo and Matos, 1996). Gioia and Cordeiro (1996) assembled Brazilian myxosporean in a checklist. Martins and Souza (1997) reported Henneguya piaractus in the gills of Piaractus mesopotamicus and reported severe mortality caused by Henneguya infections in the gills of P. mesopotamicus (Martins et al., 1997). The genus Myxobolus harbour a great number of described species, most of them in fishes from Eurasia and North America (Molnár and Békési, 1993). Until now, 20 species occurs in wild and cultured Brazilian fish. Thus, 18 species were reported in wild fish while M. colossomatis was observed in cultivated P. mesopotamicus, hybrid tambacu (P. mesopotamicus male × Colossoma macropomum female) and Astyanax bimaculatus (Martins et al., 1998, 1999b).

The present work describes new species of *Henneguya* Thélohan, 1892 and *Myxobolus* Bütschli, 1882 in native freshwater fish *Cyphocharax nagelli* from Rio do Peixe Reservoir in Brazil.

2. Material and methods

Thirty eight specimens were collected with net from the Rio do Peixe Reservoir in the City of São José do Rio Pardo, SP, Brazil in September 29, 2000 and transported to laboratory in ice. Four hours after capture the fish were examined. The gills were placed on Petri plates with 0.65% saline solution for microscopic observation. Gill fragments were compressed between a slide and a cover slip for smear preparation. The smear was air-dried at room temperature, fixed by immersion in undiluted methylic alcohol and stained by 1:9 Giemsa solution for 10 min. Myxozoan identification was performed according to Lom and Noble (1984), Lom and Arthur (1989) and Martins et al. (1999a). For the visualization of the iodophilic vacuole, the spores were fixed in a 10% buffered formalin solution and stained by Lugol's solution. A total of 20 cysts, 70 spores of Henneguya and 30 spores of Myxobolus were measured (µm) and drawn with a camera lucida and a light microscope. Forty spores of Henneguya and 20 spores of *Myxobolus* were measured in fresh conditions. For measurement and description, fresh spores were mounted in glycerine gelatine for observation by differential interference contrast optics with an Olympus BX 60 microscope. Prevalence was calculated according to Bush et al. (1997).

3. Results

3.1. Henneguya garavelli n. sp.

Cyst: the cysts presented dark colour with circular to ellipsoidal shape of 60.9 ± 13.7 (42.8–63.2) length and 34.7 ± 7.3 (24.5–42.8) width (Figs. 1–3).

Spore characteristics: the parasitological examination showed, from a frontal view, elongated spindle-shape spores, provided with long bifurcated tail when observed in fresh conditions. Two polar capsules situated on the anterior extremity with insignificant difference in size, each of them contained an anterior polar filament. The sporoplasm and an iodophilic vacuole in the interior of the spore stained by Lugol were observed. The spore



Fig. 1. Line drawing in frontal view of *Henneguya garavelli* n. sp. (A) and *Myxobolus peculiaris* n. sp. (B) from the gills of *Cyphocharax nagelli* collected in Rio do Peixe Reservoir, São Paulo, Brazil.



Fig. 2. A mature spore of *Henneguya garavelli* n. sp. observed by differential interference contrast optics. Note bifurcated tail. Fresh mount (bar = 10 µm).



Fig. 3. Spore of *Henneguya garavelli* n. sp. showing extruded polar filament. Stained by Giemsa (bar = 10μ m).

characteristics were: total length with tail 28.9 ± 2.5 (24.8–31.6); body spore length 11.5 \pm 1.4 (8.8–13.6); body spore width 5.0 \pm 1.2 (4.0–7.2); polar capsule length 4.3 \pm 0.3 (3.6–4.8); polar capsule width 1.5 \pm 0.2 (1.2–1.7); number of coils of polar filament 8–9; distance of anterior extremity of the spore to the polar capsule 0.8 \pm 0.2 (0.5–1.6); tail length 17.4 \pm 2.4 (12.8–20.8). Polar filament length 29.5 \pm 3.7 (24.0–32.8).

Fresh spore characteristics: total length with tail 46.6 \pm 2.4 (41.2–51.5); body spore length 13.6 \pm 1.0 (12.0–14.4); body spore width 4.0 \pm 0.1 (3.9–4.1); polar capsule length 5.4 \pm 0.4 (4.8–6.0); polar capsule

width 1.2 ± 0.1 (1.0–1.5); tail length 33.0 ± 2.6 (29.2–37.5); in a sutural view the spores are flattened with 3.2 ± 0.5 (2.4–4.0) thickness.

The name *H. garavelli* n. sp. is proposed to homage Dr. Julio Cesar Garavello (Federal University of São Carlos, SP, Brazil). The comparison of fresh and fixed spores with those Brazilian species described on fish is shown in Table 1.

Type host: *Cyphocharax nagelli* Steindachner, 1881 Site of infection: gill filaments (prevalence 63%) Locality: Rio do Peixe Reservoir, São Paulo, Brazil Specimens deposited: FIOCRUZ-Av. Brasil 4365, 21045-900, Rio de Janeiro, Brazil. CHIOC no. 34986, fixed gills 34818

Table 1					
Comparative measurements of	species of	Henneguya	described in	í fish,	in Brazil

Species	Total L	Spore L	Spore W	P.C.L.	P.C.W.	I.V.	Tail L	Host	Locality	Author
H. iheringi	-	22.0	6.0	3.4	2.0	Yes	-	Serrasalmo spilopleura	São Paulo	Pinto (1928a)
H. occulta	38.0	18.0	9.0	8.0	-	-	20.0	Loricaria sp.	Rio de Janeiro	Pinto (1928b)
H. wenyoni	21.0	10.2	5.2	3.7	1.5	Yes	10.8	Astyanax fasciatus	São Paulo	Pinto (1928b)
H. cesarpintoi	-	13.5	4.2	2.6	0.8	No	-	Astyanax fasciatus	São Paulo	Guimarães (1931)
H. travassosi	27.3 ± 0.7	10.6 ± 0.2	4.3 ± 0.3	3.6 ± 0.3	_	No	16.7 ± 0.8	Astyanax fasciatus and Leporinus copelandi	São Paulo	Guimarães and Bergamin (1933)
H. santae	21.0 ± 1.1	9.6 ± 0.5	5.3 ± 0.4	3.0 ± 0.3	-	Yes	11.8 ± 1.1	Tetragonopterus santae	São Paulo	Guimarães and Bergamin (1934)
H. psorospermica	_	_	-	-	-	_	_	Carp, lambari and tilapia	Paraná	Schönhofen et al. (1983)
H. pisciforme	31.0 ± 1.4	20.4 ± 1.5	6.1 ± 0.5	4.3 ± 0.6	1.7 ± 0.4	Yes	10.6 ± 1.3	Hypressobrycon anisitsi	São Paulo	Cordeiro et al. (1983/1984)
H. theca	40.6–52.6	20.3-28.4	3.0-4.1	9.8-12.5	1.0 - 1.5	Yes	20.3-24.2	Eigemannia virescens	Brazil	Kent and Hoffman (1984)
H. intracornea	66.7 ± 2.2	42.4 ± 2.3	6.6 ± 0.7	8.6 ± 0.6	2.4 ± 0.3	Yes	24.3 ± 2.1	Astyanaz scabripinnis Mugil liza and M. curema	São Paulo	Gioia et al. (1986) Godinho et al. (1988)
Henneguya sp.	- 58 7 ^a	13.5	53	_	_	_	45.2	Hoplosternum littorale	Amazonas	Azevedo and Matos (1980)
Henneguya sp.	/0.0	14.2	5.0	58	1.8		35.7	Pimelodus maculatus	São Paulo	Cordeiro et al. (1989)
H. amazonica	59.3 ± 3.0^{a}	$14.2 \\ 13.9 \pm 1.0$	5.7 ± 0.4	3.3 ± 0.1	1.5 ± 0.2	No	45.4 ± 3.3	Crenicichla lepidota	Amazonas	Rocha et al. (1992)
H. adherens	30.7–35.1 ^a	10.5-13.8	5.1-6.5	2.8-3.5	1.0-1.6	_	18.0-21.7	Acestrorhynchus falcatus	Pará	Azevedo and Matos (1995)
H. malabarica	26.6-29.8	11.8–13.1	4.8x3.6	3.0-4.3	1.6-2.2	Yes	16.2–18.9	Hoplias malabaricus	Amazonas	Azevedo and Matos (1996)
H. testicularis	27.0-28.5	14.0-14.5	6.0-6.5	8.5-9.5	2.0-2.5	_	13.0-14.5	Moenkhausia oligolepis	Pará	Azevedo et al. (1997)
H. piaractus	47.6–56.3	11.8–13.6	3.2–3.9	6.3–7.1	0.9–1.6	Yes	39.7-43.6	Piaractus mesopotamicus	São Paulo	Martins and Souza (1997)
H. striolata	39.3–45.6 ^a	14.4-17.0	4.9–5.9	5.1-7.0	1.1–1.3	No	23.6-29.8	Serrasalmus striolatus	Amazonas	Casal et al. (1997)
H. leporinicola	18.4-40.9	5.5-8.7	3.6-4.9	2.0-3.5	1.2 - 2.0	Yes	12.9-32.2	Leporinus macrocephalus	São Paulo	Martins et al. (1999a)
H. curimata	34.2-36.1	16.0-17.4	5.8-6.6	6.3 ± 0.3	1.2 ± 0.2	_	18.3-19.9	Curimata inormata	Pará	Azavedo and Matos (2002)
H. astyanax	47.8 ± 0.7	15.2 ± 0.8	5.7 ± 0.7	5.0 ± 0.1	1.5 ± 0.1	_	32.6 ± 1.1	Astyanax keithi	Pará	Vita et al. (2003)
H. chydadea	17.6-20.0	8.8-11.2	3.2-5.6	3.2-4.4	1.2-1.6	No	8.0–9.6	Astyanax altiparanae	São Paulo	Barassa et al. (2003)
H. curvata	41.7 ± 2.7	16.4 ± 0.8	4.7 ± 0.2	7.8 ± 0.3	1.4 ± 0.2	Yes	25.3 ± 2.3	Serrasalmus spilopleura	São Paulo	Barassa et al. (2003)
H. friderici	28.7–39.3	9.6–11.8	4.8-6.6	4.2–5.9	1.6–2.6	-	19.1–28.7	Leporinus friderici	Pará	Casal et al. (2003)
H. garavelli	$46.6\pm2.4^{\rm a}$	$13.6\pm1.0^{\rm a}$	$4.0\pm0.1^{\rm a}$	$5.4\pm0.4^{\rm a}$	$1.2\pm0.1^{\rm a}$	Yes	$33.0\pm2.6^{\rm a}$	Cyphocharax nagelli	São Paulo	Present work

 \overline{L} , length; W, width; P.C., polar capsule; I.V., presence or not of iodophilic vacuole. ^a Measurements of fresh spores.



Fig. 4. Spore of *Myxobolus peculiaris* n. sp. from the gills of *Cyphocharax nagelli* collected in Rio do Peixe Reservoir, São Paulo, Brazil. Stained by Giemsa (bar = 10 μm).

3.2. Myxobolus peculiaris n. sp.

The gill smears revealed a few number of myxozoan parasites of the genus *Myxobolus* Bütschli, 1882. The sporoplasm and an iodophilic vacuole were found in the interior of the spore stained by Lugol. The spore characteristics were: spore length 23.1 \pm 0.1 (23.0–23.2); spore width 14.8 \pm 0.4 (14.4–15.2); the two polar capsules are of equal size with 10.7 \pm 0.1 (10.5–10.9) length and 4.4 \pm 0.4 (4.0–4.8) width; the distance of anterior extremity of the spore to the polar capsule is 1.6 \pm 0.0 (1.6). The polar filament was easily visible with the microscopic examination showing four to five coils in the polar capsule (Figs. 1 and 4).

Fresh spore characteristics: spore length 25.2 \pm 0.15 (25.0–25.3) and spore width 15.4 \pm 0.36 (15.0–15.5).

The name *M. peculiaris* n. sp. is proposed from the size of body spores compared to other descriptions. The comparison of fresh and fixed spores with those species already described on fish gills, in Brazil, is shown in Table 2.

Type host: *Cyphocharax nagelli* Steindachner, 1881 Site of infection: gill filaments (prevalence 5%)

Locality: Rio do Peixe Reservoir, São Paulo, Brazil Specimens deposited: FIOCRUZ, CHIOC no. 34987, fixed gills 34834

4. Discussion

Little information is found about myxozoan prevalence in fish collected from the nature or aquaculture in Brazil. Some data revealed prevalences of 20% Henneguva pisciforme in Hyphessobrycon anisitsi (Cordeiro et al., 1983/1984); 11.5% Henneguya intracornea in Astyanax scabripinis (Gioia et al., 1986); 55.5% H. adherens in A. falcatus (Azevedo and Matos, 1995); 6.7% H. malabarica in Hoplias malabaricus (Azevedo and Matos, 1996); 5.6-97.3% H. piaractus in cultivated P. mesopotamicus, Colossoma macropomum and the hybrid tambacu (P. mesopotamicus male \times C. macropomum female (Martins et al., 1999b); 28% H. testicularis in Moenkhausia oligolepis (Azevedo et al., 1997); 2.5-8.3% M. absonus and M. porofilus in Pimelodus maculatus and Prochilodus lineatus (Cellere et al., 2002; Adriano et al., 2002) and 88.3% H. chydadea in Astyanax altiparanae (Barassa et al., 2003). Studies with parasite fauna of bream (Abramis brama) a common fish of Central Europe (Lake Balaton and Kis-Balaton Reservoir) showed 38.0% of prevalence of *M. bramae* in the gills (Molnár and Székely, 1999). Later, Molnár (2000) reported prevalences of 11.5% of M. margitae, 14.0% of M. alburni and 15.5% of M. obesus in the gills and fin of bleak (Alburnus alburnus) fish collected from the River Danube and Lake

Species	Spore L	Spore W	P.C.L.	P.C.W.	Host	Locality	Author
M. noguchii	13.6	8.5	6.8	2.2	Serrasalmo spilopleura	São Paulo	Pinto (1928a)
M. stokesi	8.5	5.3	3.4	1.7	Pimelodella sp.	São Paulo	Pinto (1928a)
M. inaequalis	5.2	3.3	-	-	Piramutaba blochi and Synodontis schall	South America	Pinto (1928b)
M. lutzi	10.0	7.0	-	-	Poecilia vivipara	Rio de Janeiro	Pinto (1928b)
M. chondrophilus	6.0	4.5	3.0	-	Sardinella anchovina	Rio de Janeiro	Pinto (1928b)
M. associates	15.0	10.0	7.0	_	Leporinus mormyrops	Minas Gerais	Pinto (1928b)
M. cunhai	9.0–11.0	4.0-6.0	-	-	<i>Pygocentris piraya</i> and <i>Pimelodus clarias</i>	Mato Grosso	Pinto (1928b)
M. pygocentris	15.0-16.0	9.0-11.0	9.0-11.0	3.0-4.0	Pygocentris piraya	Mato Grosso	Pinto (1928b)
M. kudoi	8.5-8.9	6.5-7.3	3.5-4.2	1.3–2.0	Nematognatha sp.	São Paulo	Guimaraes and Bergamin (1938) cited by Walliker (1969)
M. serrasalmi ^a	12.5-18.0	7.0-10.0	6.0–9.0	2.5-4	Serrasalmus rhombeus	Amazonas	Walliker (1969)
M. serrasalmi ^b	7.0–9.5	3.5-5.0	5.0-7.5	1.0-2.0	Serrasalmus rhombeus	Amazonas	Walliker (1969)
<i>Myxobolus</i> sp. <i>Myxobolus</i> sp.	9.0–11.0 8.0–10.0	5.0–6.5 4.0–7.0	5.0–6.0 3.5–5.0	1.5–2.0 1.0–2.5	Serrasalmus sp. Colossoma bidens	Amazonas Amazonas	Walliker (1969) Walliker (1969)
M. inaequus	15.6-22.0	7.8–9.3	9.4-13.0 3.9-5.5	3.1-3.9	Eigemannia virescens	Brazil	Kent and Hoffman (1984)
Myxobolus sp.	8.2	5.6	3.8	1.9	Pimelodus maculatus	São Paulo	Cordeiro et al. (1989)
M. colossomatis	11.4-12.2	6.6–7.2	5.8-6.6	1.8-2.5	Colossoma macropomum	Ceará	Molnár and Békési (1993)
M. braziliensis	10.2 ± 0.5	5.3 ± 0.3	5.3 ± 0.2	1.4 ± 0	Bunocephalus coracoideus	Pará	Casal et al. (1996)
M. macroplasmodialis M. colossomatis M. colossomatis	10.5–12.0 12.7–13.6 12.7–13.5	8.0–9.0 5.5–6.2 5.8–6.2	4.0–5.0 6.6–7.3 6.4–7.2	2.0–3.0 1.4–1.7 1.2–1.7	Salminus maxillosus Hybrid tambacu Astyanax bimaculatus	São Paulo São Paulo São Paulo	Molnár et al. (1998) Martins et al. (1998) Martins et al. (1998)
M. absonus M. porofilus M. maculatus	$\begin{array}{c} 15.7 \pm 1.5 \\ 5.7 \pm 0.3 \\ 19.7 23.0 \end{array}$	$\begin{array}{c} 10.2 \pm 0.7 \\ 4.8 \pm 0.2 \\ 7.9 – 9.5 \end{array}$	$\begin{array}{c} 6.4 \pm 0.7 4.2 \pm 0.6 \\ 1.6 \pm 0.1 \\ 11.813.8 \end{array}$	$\begin{array}{c} 3.6 \pm 0.5 2.5 \pm 0.5 \\ 1.1 \pm 0.1 \\ 3.03.6 \end{array}$	Pimelodus maculatus Prochilodus lineatus Metynnis maculatus	São Paulo São Paulo Pará	Cellere et al. (2002) Adriano et al. (2002) Casal et al. (2002)
M. peculiaris	$25.2\pm0.1^{\rm c}$	$15.4\pm0.4^{\rm c}$	10.7 ± 0.1	4.4 ± 0.4	Cyphocharax nagelli	São Paulo	Present work

Table 2 Comparative measurements of species of Myxobolus described in fish, in Brazil

L, length; W, width; P.C., polar capsule. ^a Macrospores. ^b Microspores. ^c Measurements of fresh spores.

Balaton, Hungary. The prevalence observed in this work (63%) was higher than the majority observed in natural environment as stated above.

Comparing to the present species, considerable difference was observed in the spore length of H. iheringi (Pinto, 1928a), H. occulta (Pinto, 1928b), H. cesarpintoi (Guimarães, 1931), H. santae (Guimarães and Bergamin, 1934), H. pisciforme (Cordeiro et al., 1983/1984), Henneguva sp. (Azevedo and Matos, 1989; Cordeiro et al., 1989), H. amazonica (Rocha et al., 1992), H. striolata (Casal et al., 1997) and H. leporinicola (Martins et al., 1999a) (Table 1) and total length, polar capsule length and tail of Henneguya chydadea (Barassa et al., 2003). Similar measurements were found when compared to H. travassosi (Guimarães and Bergamin, 1933), H. adherens, H. malabarica and H. piaractus (Azevedo and Matos, 1995, 1996; Martins and Souza, 1997) but differ in the polar capsule length and tail length. Moreover, the cyst appearance was also different when compared to the others described species. Finally, the present description differs from others in the World such as H. postexilis (Minchew, 1977), H. exilis (Minchew, 1977), H. shaharini (Shariff, 1982), H. mystusia (Sarkar, 1985), H. bopeleti (Fomena and Bouix, 1987), H. waltairensis (Narasinhamurti and Kalavati, 1975) and H. sebasta (Moser and Love, 1975) in total length, spore length, polar capsule length and tail length. Once more, difference in measurements among length of the body spores, total length of the spore and length of the polar capsule were related by Pavanelli et al. (1998) fish from the Paraná River.

It can also be observed that *M. peculiaris* n. sp. differ in all measurements of species described from the other countries and localities (Table 2). Myxobolus basilamellaris (Lom and Molnár, 1983), Myxobolus inaequus (Kent and Hoffman, 1984) and Myxobolus nuevoleonensis (Segovia-Salinas et al., 1995) were different in size and morphology of the polar capsule when compared to M. peculiaris. In spite of similar shape of *M. cultus* described by Yokoyama et al. (1995); Myxobolus macroplasmodialis by Molnár et al. (1998), Myxobolus magellanicus by Flores and Viozzi (2001), Myxobolus porofilus (Adriano et al., 2002) spore length, spore width, polar capsule length and polar capsule width were smaller than the present description. Our specimens also differ from Myxobolus maculatus described by Casal et al. (2002) and *Myxobolus absonus* described by Cellere et al. (2002) in spore shape, higher length and width and by having equal polar capsules. Comments on the size of the body spores of *M. peculiaris* n. sp. are needed. Until then we have not observed spores of *Myxobolus* with this size, characteristics confirmed by differential interference contrast optics. This fact is important because of the considerable difference in size of *M. peculiaris* n. sp. when compared to the other described species.

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