

# *Henneguya melini* n. sp. (Myxosporea: Myxobolidae), a parasite of *Corydoras melini* (Teleostei: Siluriformes) in the Amazon region: morphological and ultrastructural aspects

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Received: 27 March 2016 / Accepted: 10 May 2016 / Published online: 20 May 2016  
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**Abstract** A new species of myxozoan, *Henneguya melini* sp. n. (Myxosporea: Myxobolidae), was described based on morphologic and ultrastructural features. This is a parasite of the ornamental freshwater fish *C. melini* from the Rio Negro, and it was found in five of 30 (16.7 %) *C. melini* examined. The parasite was found in the gill filaments, and the plasmodia had form of round to ellipsoid, with mature and immature spores inside them. The average spore body was  $15.5 \pm 0.2$   $\mu\text{m}$  in length,  $4.7 \pm 0.1$   $\mu\text{m}$  in width, and the tail measured  $25.3 \pm 0.1$   $\mu\text{m}$  in length. The spores showed typical features of the genus *Henneguya*, with two valves of equal size and two symmetrical polar capsules of  $4.8 \pm 0.7$   $\mu\text{m}$  in length and  $1.7 \pm 0.3$   $\mu\text{m}$  in width. Each polar capsule had a polar filament with five to six turns. Based on morphology (morphologic and ultrastructural data) of the plasmodia and spores and the fact that this is the first report of a *Henneguya* species in a fish species of the genus *Corydoras*, it was considered a new myxozoan species.

**Keywords** Myxozoa · *Henneguya* · Taxonomy · Fish · Amazon

## Introduction

Interest in the Myxozoa as significant fish parasites is still increasing with the growing importance of fish farm and wild fisheries around the world. *Henneguya* Thélodan, 1982 (Myxozoa: Myxosporea) is one of the genera from family Myxobolidae with the highest diversity (Eiras 2002). Currently, more than 200 species are known (Eiras 2002; Eiras and Adriano 2012), and in recent years, South America has been the continent where more than 50 % of the new species reported (Eiras and Adriano 2012). Species of the genus *Henneguya* are predominantly histozoic, invading different organs and causing considerable pathological changes (Dyková and Lom 1978; Molnár 1998; Pote et al. 2000; Adriano et al. 2005; Naldoni et al. 2009; Barassa et al. 2012; Morsy et al. 2012).

*Corydoras melini* Lönnberg and Rendahl, 1930 (English name = bandit corydoras), is a Siluriformes freshwater fish belonging to the Callichthyidae family that harbor freshwater fishes with occurrence restricted to the Neotropical Region (Panama and South America) (Reis 1998). *C. melini* is endemic to the upper Rio Negro and Rio Meta Basins in Brazil and Colombia (Reis 1998; Froese and Pauly 2011), and it is popular in the aquarium trade industry (Froese and Pauly 2011). Despite the commercial importance of species of the genus *Corydoras*, only two myxozoans have been reported infecting them (Mathews et al. 2015, 2016).

Herein, as part of a continuous study of freshwater myxozoans biodiversity, was described a new species of Myxozoa, *Henneguya melini* n. sp., which was found in the gill filaments of *C. melini* from the Rio Negro, Amazonas

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State, Brazil. The parasite was characterized based on critical morphological features using light and transmission electron microscopy.

## Materials and methods

Thirty wild *C. melini* specimens (ranging from 4.5 to 5 cm in length) caught in the Rio Negro, municipality of Santa Isabel do Rio Negro (0° 24' 50" S, 65° 01' 08" O), Amazonas state, Brazil, were examined in June 2014. Fish were transported live to the field laboratory, where they were euthanized in a benzocaine solution overdose (400 mg L<sup>-1</sup>) (methodology approved by the ethics research committee of the Federal University of São Paulo – UNIFESP, CEUA N 9209080214), in accordance with Brazilian law (Federal Law No. 11.794, dated 8 October 2008 and Federal Decree No. 6899, dated 15 July 2009).

All organs and body fluids were examined for myxosporeans infection. Mature myxospores were examined on fresh wet mounts by light microscopy. Morphological and morphometric analyses were performed on mature myxospores obtained from two different plasmodia based on the criteria outlined by Lom and Arthur (1989). Measurements were taken of 30 myxospores using a computer equipped with Axivision 4.1 image capture software coupled to an Axioplan 2 Zeiss microscope. Smears containing free spores were air-dried and stained with Giemsa solution and mounted in a low-viscosity mounting medium (Cytoseal™) on permanent slides.

For ultrastructural examination, plasmodia were fixed for at least 12 h in 2.5 % glutaraldehyde with 0.1-M buffered cacodylate (pH 7.4). After this, the plasmodia were washed in the same buffer and post-fixed with osmium tetroxide (OsO<sub>4</sub>). All procedures were performed at 4 °C. After dehydration in an ascending ethanol series, the samples were embedded in EMbed 812 resin (Electron Microscopy Sciences, Hatfield, PA, USA). Ultrathin sections, double stained with uranyl acetate and lead citrate, were examined in an LEO 906 electron microscope operating at 60 kV.

## Results

Five out of 30 (16.7 %) specimens of *C. melini* caught from the Rio Negro had plasmodia of an unknown *Henneguya* species in the gill filaments. The intensity ranged of one to three cyst per fish and they were not found in any other organs.

*Henneguya melini* n. sp. (Figs. 1, 2, 3, and 4).

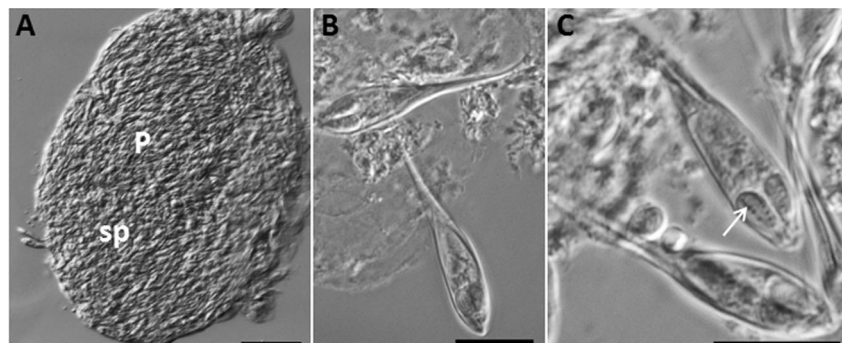
## Description

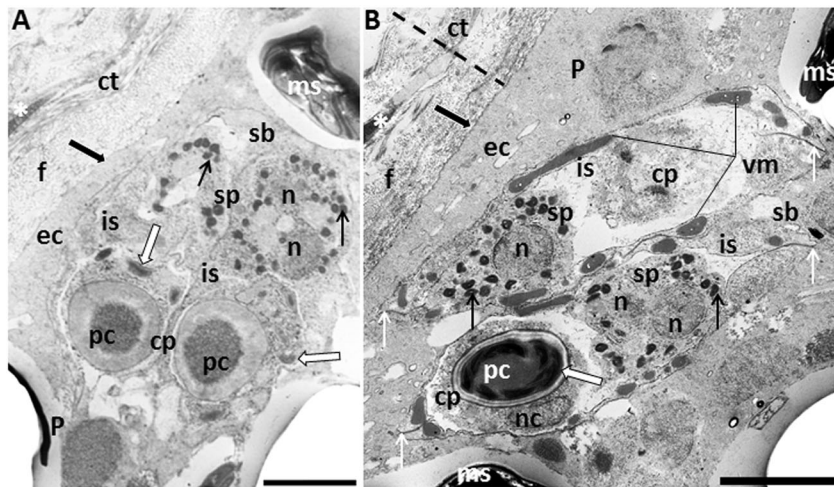
Round to ellipsoidal cysts measured up to 260 μm in diameter and with numerous myxospores were found in the gill filaments of *C. melini* (Fig. 1a). Mature myxospores were ellipsoidal in shape from the frontal view and had a total length of 40.8±0.3 μm (40.3–41.1), a body length of 15.5±0.2 μm (15.3–15.7), width of 4.7±0.1 μm (4.6–4.8), and the tail measured 25.3±0.1 μm (25.2–25.4) in length. The two polar capsules were elongated and equal in size, and have 4.8±0.5 μm (4.3–5.3) in length and 1.7±0.3 μm (1.4–2.0) in width. They occupied only the anterior third of the myxospore body, and helical filament with five to six coils was arranged obliquely to the longitudinal axis (Figs. 1b, c and 4).

Ultrastructural analysis showed a connective capsule surrounding the plasmodia, which was composed by collagen fibrils and some fibroblasts (Figs. 2 and 3a, b). The wall of the plasmodia consisted of a single membrane and the periphery of the ectoplasm had several pinocytic channels (Figs. 2 and 3b). The development of the plasmodia was asynchronous, with numerous mature spores in the central area and young developmental in the periphery (Figs. 1a, 2, and 3). Sections of immature myxospores indicated a polar capsule with five to six coils positioned obliquely to the longitudinal axis of the capsule and binucleated sporoplasms that contained several sporoplasmosomes (Figs. 2 and 3a). Capsulogenic and valvogenic cells were readily recognized in the developing sporoblast by the presence of bulbous capsular primordia and associated external tube in the first and by valve-forming materials in the second (Fig. 2).

Type host: *Corydoras melini* Lönnberg and Rendahl, 1930 (Callichthyidae: Corydoradinae).

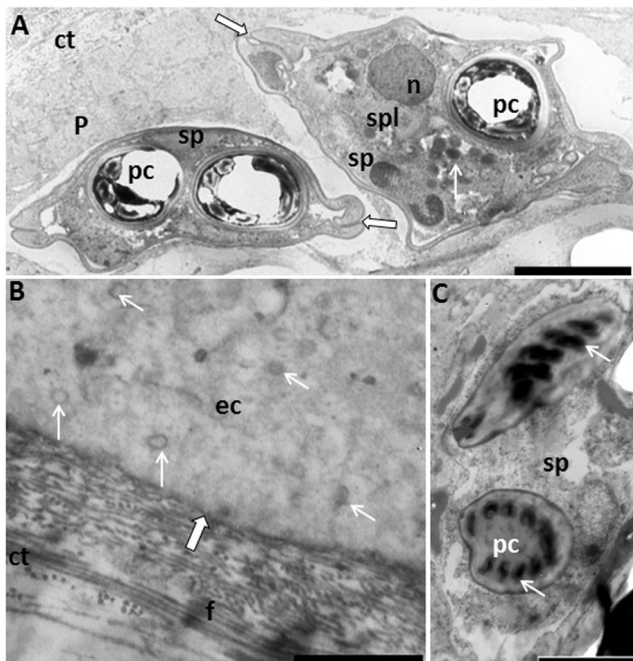
**Fig. 1** Light photomicrographs of *Henneguya melini* n. sp. parasite of the gill filaments of *Corydoras melini*. **a** Plasmodium (*P*) with numerous spores inside (*sp*). Scale bar = 40 μm. **b, c** Mature spores. Note in **c** the polar filaments inside of the polar capsules (white arrow). Scale bars = 10 μm





**Fig. 2** Electron micrography of plasmodia (*P*) of *Henneguya melini* n. sp. parasite of the gill filaments of *Corydoras melini* showing the host–parasite interface. Externally can be seen connective tissue layer (*ct*) with collagenic fibers (*f*) and fibroblasts cells (*asterisk*), surrounding the plasmodial wall (*large black arrows*). Internally are seen a thin ectoplasm (*ec*), fragments of mature spores (*ms*), and disporic sporoblasts (*sb*) with immature spores (*is*). In **a**, note young sporoblast

developmental stage with polar filaments (*large white arrows*) still out of the polar capsules (*pc*). In **b**, the sporoblast is in more advanced developmental stage, with the polar filament inside of the polar capsule, abundant valve-forming material (*vm*), and conspicuous sutural line (*white thin arrows*). The binucleate sporoplasms (*sp*) have numerous sporoplasmosomes (*thin black arrows*). *cp* capsulogenic cells, *n* nucleus Scale bars = 2  $\mu$ m



**Fig. 3** Electron micrography of plasmodia (*P*) of *Henneguya melini* n. sp. parasite of the gill filaments of *Corydoras melini*. **a** Almost mature spores (*sp*). Note the sutural lines (*large arrows*), polar capsules (*pc*), and sporoplasm (*spl*) with its nucleus (*n*) and sporoplasmosomes (*thin arrow*). Scale bar = 2  $\mu$ m. **b** Amplified area of **a** showing longitudinal and transversal distributions of the collagenic fibers (*f*) in the connective tissue layer (*ct*), single membrane of the plasmodial wall (*large arrow*), and pinocytotic channels (*thin arrows*) in the ectoplasm (*ec*). Scale bar = 1  $\mu$ m. **c** Spores (*sp*) with details of the polar filaments (*arrows*) in the polar capsules (*pc*). Scale bar = 2  $\mu$ m

#### Site of infection: Gill filaments

Type locality: Rio Negro, Municipality of Santa Isabel do Rio Negro, state of Amazonas, Brazil.

Prevalence: Five out of 30 (16.7 %).

Type material: glass slides with stained spores (syntype) deposited in the collection of the Museum of Zoology of State University of Campinas, “Adão José Cardoso,” State University of Campinas (UNICAMP), state of Sao Paulo, Brazil (accession number Zuec Myx 51).

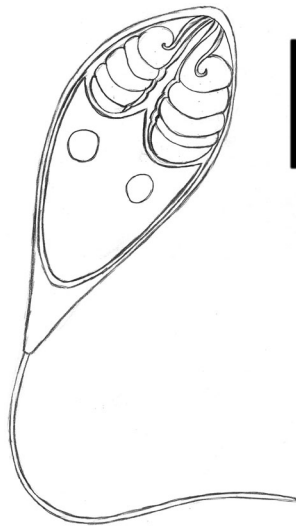
Etymology: The specific name is derived from the name of the host species (“melini”).

#### Discussion

Several studies have reported infections of species of the genus *Henneguya* Thélodan, 1982, in Amazonian fishes (Rocha et al. 1992; Azevedo and Matos 1995; Casal et al. 1997; Vita et al. 2003; Feijó et al. 2008; Videira et al. 2015). However, to our knowledge, this is the first report of a *Henneguya* species parasitizing fish of the family Callichthyidae in the Amazon basin and the first report of this parasite infection in *Corydoras* fish in South America. In the present study, based on morphological features obtained by light and electron microscopy, *Henneguya melini* n. sp. is described parasitizing gill filaments of *C. melini*, a callichthyid fish endemic to the upper Rio Negro and Rio Meta basins in Brazil and Colombia (Reis 1998; Froese and Pauly 2011).

The morphological and morphometric data of *H. melini* n. sp. were compared firstly with all *Henneguya* spp. described





**Fig. 4** Schematic representation of mature spores of *Henneguya melini* n. sp. from the gill filaments of *Corydoras melini*. Scale bar = 5  $\mu$ m

previously infecting South America Siluriformes freshwater fish (Matos et al. 2005; Abdallah et al. 2007; Eiras et al. 2009; Naldoni et al. 2009; Naldoni et al. 2011; Adriano et al. 2012; Carriero et al. 2013; Naldoni et al. 2014). The most similar species to the new species were *Henneguya maculosus* Carriero et al., 2013, which infects *Pseudoplatystoma corruscans* and *Henneguya eirasi* Naldoni et al., 2011, which infect *Pseudoplatystoma fasciatum* and *Pseudoplatystoma corruscans*. However, these differ from *H. melini* n. sp. in the size of the spore (13.3  $\pm$  0.7  $\mu$ m in length to *H. maculosus*, 37.1  $\pm$  1.8  $\mu$ m to *H. eirasi* and 40.8  $\pm$  0.3  $\mu$ m to the new species), number of coils in the polar filament (six to seven to *H. maculosus*, 12 to 13 *H. eirasi* and five to six to the new species), and because the tail is substantially greater in length (25.3  $\pm$  0.1  $\mu$ m in the new species and 17.5  $\pm$  1.0  $\mu$ m in *H. maculosus* and 23.6  $\pm$  2.2  $\mu$ m in *H. eirasi*). Considering *Henneguya guanduensis* Abdallah et al., 2007, which was described from *Hoplosternum littorale*, the closest host species to *C. melini*, the comparison showed shorter and wider spores for *H. guanduensis*, with 33.6  $\times$  6.5  $\mu$ m, while 40.8  $\times$  4.7  $\mu$ m for *H. melini* n. sp. The tail was smaller for *H. guanduensis* with 19.0 versus 25.3  $\mu$ m for *H. melini* n. sp., and still the two polar capsules of *H. guanduensis* are unequal in size, while they are equal in *H. melini* n. sp.

Compared with *Henneguya* species which infect Siluriformes fish from other regions of the world (Sarkar 1985; Landsberg 1987; Kostoingue et al. 2001; Reed et al. 2003; Molnár et al. 2006; Iwanowicz et al. 2008; Griffin et al. 2008; Rabie et al. 2009; Morsy et al. 2012), *H. melini* n. sp. resembles those of *Henneguya suprabranchiae* Landsberg, 1987, and *Henneguya gurleyi*, Kudo, 1919, which infect the freshwater catfish *Clarias lazera* and *Ameiurus nebulosus*, respectively (Landsberg 1987; Iwanowicz et al. 2008).

However, a small number of noticeable morphologic differences were observed, such as spore tail length (25.3  $\mu$ m for *H. melini* n. sp.; 41.1  $\mu$ m for *H. gurleyi* and 29.0  $\mu$ m for *H. suprabranchiae*) and the number of turns of the polar filaments (five to six turns in *H. melini* n. sp., 10 to 11 turns in *H. suprabranchiae* and nine turns in *H. gurleyi*). Other differences may also be observed in the width of the spores (4.7  $\mu$ m for *H. melini* n. sp., 3.0  $\mu$ m for *H. suprabranchiae*, and 5.4  $\mu$ m for *H. gurleyi*).

The morphology of *H. melini* n. sp. also was compared with *Henneguya* species that have been reported infecting gills of not Siluriformes fishes from Amazon Basin. The species described in this paper has a total size similar to that of *Henneguya aequidens* Videira et al., 2015, and *Henneguya striolata* Casal et al., 1997. However, *H. aequidens* has longer tail (27  $\pm$  0.6  $\mu$ m), is greater in width (6  $\pm$  0.8  $\mu$ m), and the polar capsules are smaller in length (3  $\pm$  0.3  $\mu$ m) (Videira et al., 2015). In the same way, *H. striolata* has more number of filament turns (13 to 14) and the polar capsules are smaller in width (1.1–1.3  $\mu$ m) (Casal et al., 1997). Regarding the body width of spore, polar capsule width, or length and shape, *H. melini* n. sp. resembles *Henneguya astyanax* Vita et al., 2003, *Henneguya friderici* Casal et al., 2003, *Henneguya amazonica* Rocha et al., 1992, *Henneguya adherens* Azevedo and Matos, 1995, *Henneguya malabarica* Azevedo and Matos, 1996, and *Henneguya schizodon* Eiras et al., 2004. Nevertheless, it has been noticed that the parasite was readily distinguishable by lower number of polar filament turns (five to 6 for *H. melini* n. sp.; 8–9 for *H. astyanax*, 7–8 for *H. friderici*, 6–7 for *H. malabarica*, and 8–10 for *H. schizodon*). Differences may also be observed in the length of the tail of the spores (25.3  $\mu$ m for *H. melini* n. sp.; 18.5  $\mu$ m for *H. adherens*, 41.7  $\mu$ m for *H. amazonica*, 32.6  $\mu$ m for *H. astyanax*, 19.1  $\mu$ m for *H. friderici*, and 16.3  $\mu$ m for *H. schizodon*). In the same way, considering the shape and size of myxospores and polar capsules, number of polar filament turns, host, and tissue affinity, *H. melini* n. sp. differs from all other *Henneguya* spp. parasites of freshwater fish found out of the Amazon basin, at least, in one of those characteristics (Eiras 2002; Eiras and Adriano 2012).

Ultrastructural studies on the plasmodial wall of species of the *Henneguya* genus are of major important for understanding host–parasite interactions and to show the characteristic features of this important group of parasites (Current and Janovy 1976; Hallett and Diamant 2001; El-Mansy and Bashtar 2002; Abdel-Ghaffar et al. 2008). The plasmodial wall of *H. melini* n. sp. is bordered by a single membrane and presents pinocytotic channels in the ectoplasma zone of the plasmodium, which are clearly involved in nutrient acquisition, to supporting the developmental forms inside the plasmodia (Hallett and Diamant 2001; El-Mansy and Bashtar 2002; Naldoni et al. 2014). Sporogenesis of *H. melini* n. sp. has many similarities with other previously described *Henneguya* species (Ali 1999; Casal et al. 2003;

Abdel-Ghaffar et al. 2008; Naldoni et al. 2011); however, there was only a thin layer of the plasmodia with young developmental stages and mature spores viewed near the peripheric area, characterizing asynchronic development, but with slow development. The plasmodia were surrounded by connective tissue capsule; with fibroblast and collagen fibers, slow layers disposed transversal and longitudinally, as observed in other studies (Adriano et al. 2005; Matos et al. 2005; Sitjà-Bobadilla 2008; Iwanowicz et al. 2008).

The new species has been characterized only on morphological/morphometric, host, and geographic area data; we recognize the importance of molecular data in the descriptions of new myxosporeans taxa. However, some difficulties were encountered to standardizing PCR reactions to this species, which, added to the few samples obtained and limitations to accessing new samples of the same regions, making it impossible to provide sequencing data of *H. melini* n. sp. So, future molecular and phylogenetic studies are highly recommended, since they will permit stronger taxonomic comparison, as well as show the phylogenetic position of this species in the evolutionary context of myxosporeans.

In the present study, the prevalence and intensity of *H. melini* n. sp. were relatively low, with only 16.7 % of the specimens of *C. melini* being infected and with an intensity of infection ranging from one up to three plasmodia. This finding is in agreement with other *Henneguya* spp. from South American Siluriformes from natural environment. Martins et al. (2004) reported a prevalence of 13.4 % for *Henneguya* sp. parasite of *Pimelodus maculatus*, Naldoni et al. (2011), who reported for *Henneguya eirasi* a prevalence of 17.1 % in specimens of *P. corrucans* and *P. fasciatum*, and Carriero et al. (2013), who reported a prevalence of 16 and 19 % for *Henneguya maculosus* in *P. reticulatum* and *P. corrucans*. However, the results in the present study are in contrast with those described by Naldoni et al. (2009), who reported a high prevalence, which ranged of 75 up to 100 % for *Henneguya Pseudoplatystoma* infecting the farmed pintado hybrid (*P. corrucans* × *P. fasciatum*).

In our study, no disease symptoms were observed in the infected fish specimens. However, several species of the family Myxobolidae have showed to induce pathogeny in their hosts (Molnár 1998; El-Mansy and Bashtar 2002; Mohammed et al. 2002; Adriano et al. 2005; Feist and Longshaw 2006; Ali et al. 2007; Naldoni et al. 2009). Thus, as *C. melini* is commonly used in aquariums, there is a need of constant monitoring of the fish for diagnosis and timely control of infections by myxosporean to better prevent future disease outbreaks.

**Acknowledgments** This study was supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo - FAPESP (Proc. No. 2013/21374-6-Adriano E. A.). Mathews P.D. was supported by a doctoral scholarship from FAPESP (Proc. No. 2013/14656-5). Adriano E.A. received a research productivity grant from the Brazilian Fostering Agency CNPq (Proc. No. 305630/2013-0). CAPES (Coordenação de

Aperfeiçoamento de Pessoal de Nível Superior) provides support to the Doctoral Program in Animal Biology of UNICAMP. The authors thank Dr. Geraldo Bernardino, secretary of Fishing of the Amazonas State, for help in the logistic of the field works and Dr. Christopher Berger for reviewing the language of the text.

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