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ORIGINAL ARTICLE

# Ultrastructure of two microsporidians *Inodosporus* sp. and *Myospora* sp. co-infecting muscles of the Amazon River prawn *Macrobrachium amazonicum* (Heller, 1862)

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## Abstract

In the present study, we describe a co-infection of two microsporidians within the abdominal muscle of the Amazon River prawn *Macrobrachium amazonicum* from Brazil, detected through light and transmission electron microscopy and histopathological analysis. Two spore types and respective early developmental stages (meronts, sporonts, and sporoblasts) appeared grouped in numerous adjacent clusters among the muscle fibers of the host. Spores were initially divided into two morphotypes, Sp. 1 and Sp. 2, based on differences in shape, size, and internal organization. The Sp. 1 spores are pyriform to ovoid (4.3 ± 0.3 × 3.7 ± 0.4 μm in size) and resided in groups of eight within a sporophorous vesicle. Sp. 1 spores had bilayered walls and long tape-like external filaments with irregular morphology and size, forming a complex contiguous membranous system attached to the spore wall. The umbrella-like anchoring disc of the spores was in continuity with an anisofilar polar tube arranged in 7–8 (rarely 9) coils. The Sp. 2 spores were rod-like in shape (3.1 ± 0.5 × 0.8 ± 0.1 μm in diameter) and the polar tube had 7–8 coils. Their merogonic and sporogonic stages occurred within the sarcoplasm of the muscle cells in close contact with the myofibrils. Based on ultrastructural organization of the sporogonic stages, the Sp. 1 and Sp. 2 morphotypes probably belong to the genera *Inodosporus* and *Myospora*, respectively.

## Keywords

Crustacea, Microsporidia, muscle infection, spore dimorphism

## Introduction

Microsporidians are intracellular parasites that occur worldwide and infect a great variety of organisms (Lom and Dyková, 1992; Stentiford *et al.*, 2013a; 2018; Stentiford and Dunn, 2014; Newman, 2015). Most microsporidians are pathogenic, causing degradation of cellular organization that leads to the death of their hosts (Azevedo, 1987, 2001; Vivarès and Azevedo, 1988; Azevedo *et al.*, 2000; Stentiford *et al.*, 2007). Many of these species have been described based on the morphology of their life-cycle stages, mostly of the spores (Overstreet and Weidner, 1974; Lom *et al.*, 2000; Stentiford *et al.*, 2007). However, the description of new microsporidian species solely based on morphological criteria is falling out of favor (Stentiford *et al.*, 2013b), and the use of molecular markers is gradually solving many problems posed by previous techniques (Stentiford *et al.*, 2010; 2013b; 2018; Tang *et al.*, 2015; Ding *et al.*, 2016; Sokolova and Overstreet, 2018).

Some microsporidians, *e.g.*, *Nosema*-like, *Thelohania*-like, *Ameson, Nadelspora,* and *Vairomorpha* can simultaneously produce two morphologically distinct types of spores (Iwano and Ishihar, 1991; Sprague *et al.*, 1992; Sprague and Becnel, 1999; Stentiford *et al.*, 2013b; Stentiford and Dunn, 2014; Gruhevaya *et al.*, 2018; Itoh *et al.*, 2020). However, the development of dimorphic spores is not well clarified (Sprague *et al.*, 1992; Stentiford *et al.*, 2013b), and its occurrence in the same host specimen and organ or tissue is rare. Dimorphosporous microsporidia found in the same host (Iwano and Ishihara, 1991; Lom and Dyková, 1992; De Graaf *et al.*, 1994; Iwano and Kurtti, 1995; Stentiford *et al.*, 2013b; 2018; Stentiford and Dunn, 2014) were recently described in detail (Stentiford *et al.*, 2018; Itoh *et al.*, 2020), but the developmental occurrence of two spore types does not seem to be fully understood (Stentiford *et al.*, 2013b). Spore polymorphism in microsporidians is not uncommon in insect hosts (mainly Diptera - Becnel and Andrealis, 2014), fishes (Loubés *et al.*, 1979), and crustaceans (Stentiford and Dunn, 2014). Recently, a novel dimorphic microsporidian *Ameson iseebi* Itoh *et al.* (2020) infecting muscles of the Japanese spiny lobster from western Japan was described (Itoh *et al.*, 2020). Mortality in prawn and shrimp species (herein, we followed the classification proposed by Holthius (1980): the term "shrimp" is applied for Crangonidae and smaller species, and "prawn" is

used solely for Palaemonidae and larger forms) of commercial interest have been often associated with microsporidiosis (Bower *et al.*, 1994; Chávez-Sánchez *et al.*, 2002; Hudson *et al.*, 2001; Newman, 2015). To date, 13 microsporidian genera have been recorded infecting shrimps and prawns (Wang *et al.*, 2013; Ding *et al.*, 2016; Sokolova and Overstreet, 2018).

Prawns of the genus *Macrobrachium* Spence Bate, 1868 stand out in the family Palaemonidae due to their great biological and economic importance (New *et al.*, 2010). Only one commercially relevant species of this genus was reported to be infected by a microsporidian to date, the Oriental river prawn *Macrobrachium nipponense* De Haan, 1849 in Ding *et al.* (2016). In Brazil, 19 native species of *Macrobrachium* have been recorded in inland and coastal aquatic environments to date (Perroca *et al.*, 2021). Among these species, the Amazon River prawn *Macrobrachium amazonicum* (Heller, 1862) has the largest distribution (spread all over the river basins of Brazil) and is the most important in Brazilian aquaculture (Maciel and Valenti, 2009; Moraes-Valenti and Valenti, 2010). Some *M. amazonicum* populations have distinct life histories and phenotypes (*i.e.*, large-size amphidromous populations, and large-size and small-size hololimnetic populations), which gives ample plasticity in the use of the habitat by their representatives (Paschoal *et al.*, 2019; Paschoal and Zara, 2020).

In the present study, we describe the ultrastructure of life-cycle stages of two microsporidian species parasitizing specimens of a small-size hololimnetic population of *M. amazonicum*. Through light and transmission electron microscopy analysis, we point out that these microsporidians, tentatively named Sp. 1 and Sp. 2, probably belong to the genera *Inodosporus* and *Myospora*.

## Material and Methods

### *Sampling*

From October 2014 to January 2016, 5,575 specimens of *M. amazonicum* were collected in the Rio Grande (Paraná Basin), in the Furnas Hydroelectric Power Station reservoir (20°30'S 46°50'W) in the municipality of São José da Barra, in the state of Minas Gerais, southeastern Brazil. From these specimens, only 100 individuals showed abnormal color patterns (Fig. 1A–C), altered abdominal morphology and behavior, which can indicate the presence of microsporidians (Paschoal, 2017). Infected animals were anesthetized by chilling at -20 °C for 5 min. and had their carapace length (distance between the posterior margin of the ocular orbit and the midpoint of the posterior margin of the carapace) measured. Prawns and parasites were registered in the Brazilian National System of Management of Genetic Heritage (SisGen) under register number A533C83.

### *Light microscopy (LM)*

After anaesthesia, infected fragments of abdominal muscle were removed through dissection and fixed in a 4 % paraformaldehyde 0.2 M phosphate buffer solution (pH 7.2) for 24 hours. Fragments were subsequently washed twice in the same buffer, dehydrated in an ascending ethanol series (70-100 %), and embedded in glycol-methacrylate historesin Leica®. Resin blocks were cut (4–5 µm thick) and stained with hematoxylin-eosin (H&E) and periodic acid of Schiff (PAS) for general histological description and detection of microsporidian spores following Garcia (2002).

The spore count in each sporophorous vacuole (SpVc) was carried out using non-stained smears from the abdominal muscle of infected prawns. The SpVc were broken by applying light pressure on the cover slip using the blunt end of a needle for liberation of the spores, which were observed, counted, and measured under Zeiss Axiovision Z2 differential interference contrast (DIC) optics.

### *Transmission electron microscopy (TEM) observations*

Small fragments of infected abdominal muscle were fixed in 2.5 % glutaraldehyde in 0.08 M sodium cacodylate buffer solution (pH 7.2) for 4 hrs, washed, and post-fixed in 1 % $OsO_4$ solution in the same buffer for 2 hr. All these steps were carried out at 4 °C. Samples were "Enbloc" stained with 1 % aqueous uranyl acetate (overnight at 4 °C), dehydrated in an ascending acetone series (70–100 %) and embedded in Epon-Araldite® resin. Semi-thin sections were stained with toluidine blue Azur II, and suitable target areas were identified under LM. Ultrathin sections were obtained on an ultramicrotome Leica® UC7.

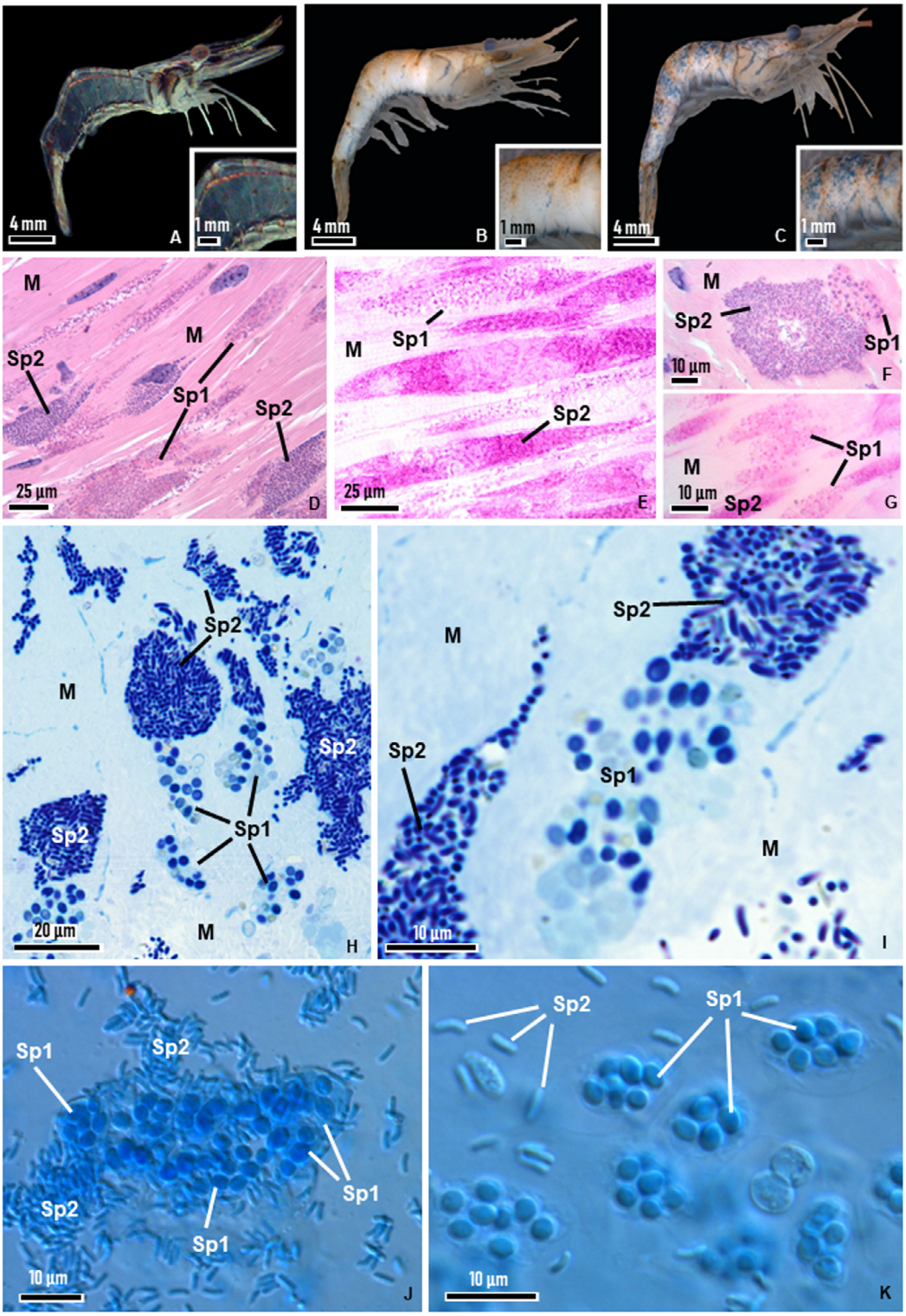


**Figure 1.** Macroscopic and light microscopy observations of microsporidians *Inodosporus* sp. and *Myospora* sp. infecting the abdominal muscles (M) of the Amazon River prawn *Macrobrachium amazonicum*: (**A**) The translucent appearance of an uninfected prawn. (**B**, **C**) Infected prawns showing opacity and whitening of the abdomen. Their chromatophores demonstrate abnormal color patterns, ranging from blue to orange (or both colors). Insets: Higher magnification of the abdominal area of the uninfected and infected prawns. (**D, E**) Spore clusters residing among muscle fibers: *Inodosporus* sp. (Sp. 1) and *Myospora*. sp. (Sp. 2). (**F, G**) Juxtaposed clusters of spores (Sp. 1 and Sp. 2). D and F: H&E; E and G: PAS. (**H, I**) Semi-thin sections showing distribution of Sp. 1 and Sp. 2 spore clusters. (**J**) A cluster composed of two spore morphotypes observed in DIC. Spores of the Sp. 1 are in the center of the cluster, while Sp. 2 spores are located at the periphery. (**K**) Isolated sporophorous vacuoles containing several spores of the Sp. 1 and free spores of the Sp. 2, observed in DIC.

The resulting sections were contrasted with aqueous uranyl acetate (2 %) and lead citrate (0.4 %), and observed in a JEOL 100CXII TEM, operated at 60 kV. The morphological analyses of the spores confirm that these parasites belong to phylum Microsporidia Balbiani, 1882.

## Results

*Light Microscopy*

Infected prawns were easily distinguished from normal translucent specimens due to the opaque, whitish discoloration of the abdomen, thorax, and limbs. Their chromatophores on all segments presented a high degree of pigmentation, exhibiting an aggregation of blue or orange pigment or, in some cases, both colors. The clinical signs of parasitosis in *M. amazonicum* are shown in Fig. 1A–C. The mean carapace length for infected males and females was 6.9 ± 1.1 and 7.6 ± 1.8 mm, respectively. The prevalence of the infection was low (1.79 %) and differed between sexes (female to male sex ratio = 1:3; $\chi^2$: 6.26 - $p < 0.05$).

The infection in the abdominal muscles was located in the intramuscular tissue. It was characterized by the presence of microsporidians showing simultaneous spore dimorphism in the same host. These two morphologically distinct spore types appeared isolated and clustered among the muscle fibers in close contact with the sarcolemma (Fig. 1D–I). In a preliminary identification, we designated the largest spores having pyriform to ovoid morphology as Sp. 1 (in order to facilitate the description) and the smaller rod-shaped to cylindrical spores as Sp. 2 (Fig. 1H, I). The Sp. 1 spores were enclosed in SpVc, each containing 8 spores (Fig. 1J, K).

*Transmission electron microscopy*

Our ultrastructural observations confirmed the LM observations: the presence of a co-infection by two microsporidian morphotypes (Fig. 2A, B). Some nuclei of the infected muscle fibers were picnotic, and the cytoplasm of the muscle fibers seemed ultrastructurally disorganized, with some visible degradation. The two morphotypes containing contiguous merogonic and sporogonic stages located side by side appeared randomly distributed within the sarcoplasm in close contact with the myofibrils (Fig. 2A, B).

Microsporidian Sp. 1.

The earliest life cycle stages, frequently organized in small clusters, were easily identified by the differentiation of the SpVc. Monokaryotic meronts, sporonts, and sporoblasts were observed in clusters containing a variable number of cells, sometimes located near mature spores (Figs. 2C–F). Early sporoblasts, with electron-dense walls, showed precursors of the tape-like filament appendages (Fig. 2D, E). These structures formed complex membranous systems attached to the spore wall, forming long extensions or tape-like filaments (Fig. 2C–F). Late sporoblasts have a denser cytoplasm with polar tube differentiation (Fig. 2F).

Maturing and mature spores exhibited typical microsporidian spore characters (Fig. 3A–G). Mature spores were 4.3 ± 0.3 (4.1-4.7) × 3.7 ± 0.4 (3.3-4.1) µm in diameter (n = 35). Their bilayered wall was 69 ± 0.2 (66–71) nm thick, consisting of an electron-dense exospore 30 ± 0.3 (26–34) nm thick, and a hyaline endospore layer 41 ± 0.5 (36.5–41.6) nm thick. The endospore touched the plasma membrane (Fig. 3A–G). The exospore was formed from three layers: a dense layer, 15 nm thick, followed by an electron-lucent layer ~8 nm thick, and another dense layer ~7 nm thick, which touched the endospore (Fig. 3D–G).

The anchoring disc was in continuity with the manubrial region of the polar tube (85 ± 0.2 nm in diameter), with 7–8 (rarely 9) coils in its basal region, surrounding the posterior vacuole (Fig. 3D–G). The apical region of the manubrium was surrounded by the polaroplast, which occupied about one-third of the length of the spore (Fig. 3B, C, E).

Three or 4 (rarely up to 6) long tape-like filaments with distinctive membranous appendages adhering to the exospore were projected into the SpVc giving rise to a complex membrane system entangled within the SpVc, surrounding the different developmental stages (meronts, sporonts, sporoblasts, and spores — Figs. 2D–F, 3A–C). The lumen of the polar tube contained a matrix formed by several concentric layers with different electron densities (Fig. 3G).

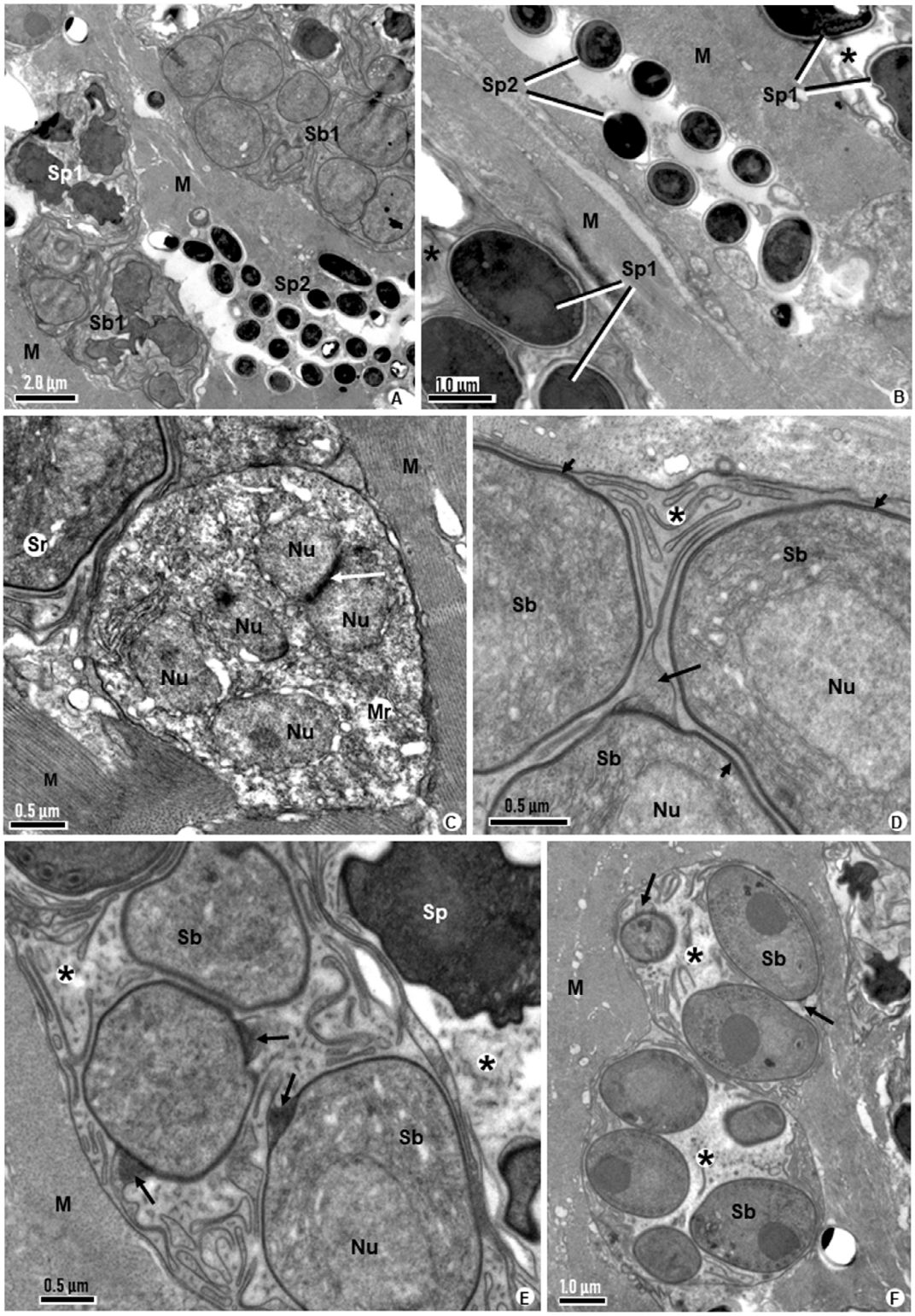


**Figure 2.** *Inodosporus* sp. (Sp. 1) and *Myospora* sp. (Sp. 2) observed in TEM. (**A**) Two morphologically different spores (Sp1 and Sp2) and intracellular stages (Sb1 - sporoblasts). (**B**) Two sporophorous vacuoles (*) containing Sp. 1 spores, and several Sp. 2 free spores located in the central position among muscle fibers. (**C**) Plasmodium with several nuclei and a meront (Mr) in the view. White arrow indicates the diplokaryon phase. Sr, sporont of the Sp. 1. (**D**) Sporophorous vacuole (*) showing early sporoblasts (Sb) of the Sp. 1 and their nuclei (Nu). A precursor of the tail-like appendage (long arrow) attached to the sporoblast wall (small arrows). (**E**) Two adjacent sporophorous vacuoles (*) in muscle (M): left vacuole contains early sporoblasts with the precursors of the tail-like appendages (arrows), right vacuole is filled with spores (Sp). (**F**) Two sporophorous vacuoles (*) containing late sporoblasts (Sb) some of which demonstrate precursors of the tail-like appendages on the sporoblast walls (arrows).

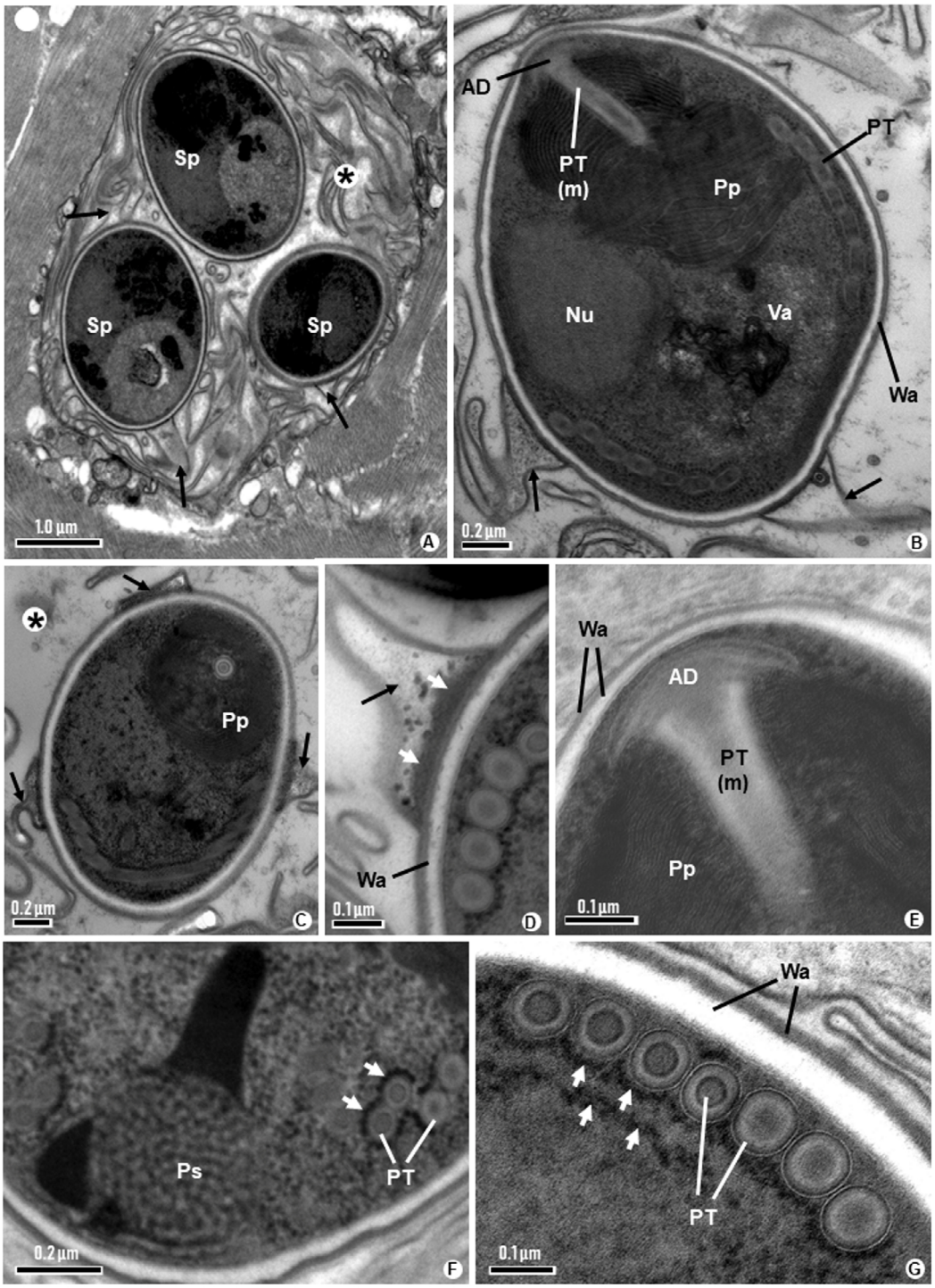


**Figure 3.** Ultrastructure of spores of *Inodosporus* sp. (**A**) Sporophorous vacuole (*) surrounded by muscle fibers, showing three spores (Sp), each surrounded by a complex membranous system, and tape-like filaments (arrows). (**B**) Longitudinal section through the spore showing the principal organelles and structures of the spore: spore wall (Wa), tape-like filaments (arrows), anchoring disc (AD) in continuity with the manubrial part of the polar tube (PT-m) and the polar tube coils (PT). The anterior portion of PT is enclosed by the polaroplast. Nucleus (Nu) and posterior vacuole (Va) are also in view. (**C**) Electron dense zones, to which appendages are attached, are located on the spore wall and indicated by arrows. (**D**) Zone of attachment (white arrows) of the tape-like filaments (black arrow) to the spore wall (Wa). (**E**) Apical region of the spore showing the spore wall (Wa), anchoring disc (AD), the manubrial portion of the polar tube (PT-m) and polaroplast (Pp). (**F**) Detail of the basal region of the spore showing the spore wall, the posterosome (Ps) and polar tube coils (PT) surrounded by several ribosome-like structures (white arrows). (**G**) Transverse sections of the polar tube (PT), located near the spore wall (Wa), showing adherent ribosome-like structures (white arrows). The transverse sections through the polar tube coils reveal variable ultrastructure.

The nucleus, with barely visible chromatin, was located in the central region of the spore (Fig. 3B). During the developmental phases, several membranous structures appeared in the SpVc around these cell stages (Figs. 2D–F, 3A–C). The morphological organization of the spores based on serial sectioning is presented in Fig. 6A.

Morphologically, it is reasonable to conclude that this parasite probably belongs to the genus *Inodosporus* Overstreet and Weidner, 1974. Compared to previously described species of this genus (Tab. 1), we observed some unique characters, which suggest that the present species is probably new.

Microsporidian Sp. 2.

The spores and other stages formed clusters in close contact to the myofibrils (Fig. 4A–C). All life cycle stages were diplokaryotic, and different stages were mixed in the same cluster (Fig. 4A, C). Some diplokaryotic meronts, sporonts, and sporoblasts were observed near the clusters containing mature diplokaryotic spores, and occasionally located near the nuclei of host cells (Fig. 4C). These stages were easily identified by the fact that they did not contain any appendages or ornaments on their walls, as is observed in Sp. 1. Some clusters containing diplokaryotic meronts, sporonts, and sporoblasts (Fig. 4A–C) were observed in close contact to myofibrils.

The spores were long, rod-shaped to cylindrical, with semi-spherical ends (Fig. 5A, C–E), 3.1 ± 0.5 (2.7–3.6) µm (n = 50) in length and 0.8 ± 0.1 (0.7–0.9) µm (n = 25) in diameter (Fig. 5C–E). The spore wall was 162 ± 9.2 nm (n = 25) thick, formed by an electron-dense exospore (9.8 ± 1.0 nm) and a hyaline endospore (30.0 ± 2.1 nm), which touched the cell membrane (Fig. 5C–E). The apical end of the spore contained the anchoring disc (umbrella-shaped) in continuity with the long manubrial region of the polar tube, with 7–8 (rarely 9) coils arranged in a single row around a rudimentary vacuole (Fig. 5E). The membranous polaroplast (Fig. 5D) and the polar tube surrounded the manubrium measuring ~0.2 µm in diameter. The first two coils presented ~78 (75–80) nm in diameter and gradually tapered to ~65 (60–69) nm (Fig. 5D). The manubrium was eccentric. The angle of tilt between the spore axis and the axis of the first coils of the polar tube was 34 ± 03° (n = 10). The organization of the spores seen through serial sectioning, is presented in Fig. 6B. Morphologically, compared to the only other described species having similar spore ultrastructure (Tab. 2), we conclude that this parasite probably belongs to the genus *Myospora* Stentiford *et al.* (2010).

## Discussion

Histological and ultrastructural data show that the studied organisms, with two morphologically distinct spores co-infecting Amazon River prawns, belong to the phylum Microsporidia (Azevedo, 1987; Vávra and Larsson, 1999; Azevedo *et al.*, 2000; Stentiford *et al.*, 2013b; 2018; Itoh *et al.*, 2020). Both microsporidians demonstrate morphology typical for the Phylum, but could be differentiated from each other by size, type of sporogony and numerous ultrastructural characters of spores and intracellular stages. These differences suggest that the discovered parasites belong to two different genera.

**Table 1.** Comparison of the spore characteristics of *Inodosporus* species.

| *Inodosporus* spp. | Host species | Country (region) | Habitat | N. of spores in SpVc | L × W (µm) | PTC | Spore app. | References |
|---|---|---|---|---|---|---|---|---|
| *I. spraguei* (former *Thelohania octospora*) | *Palaemon pugio* and *P. kadiakensis* | USA (Atlantic coast) | Estuarine | 8 | 2.9 x 2.0 and 2.9 x 2.1 | 4-5 | 3-4 | Overstreet and Weidner (1974) |
| *I. octospora* | *Palaemon serratus* | France and Portugal (Atlantic coast) | Marine | 8 | 2.5 × 1.3 | 5-6 | 3 | Azevedo *et al.* (2000) |
| *I. octospora* | *Palaemon serratus* | England (Atlantic coast) | Marine | 8 | 2.2 × 1.2 (*) | 5-6 | 3 | Stentiford *et al.* (2018) |
| *Inodosporus* sp. | *Macrobrachium amazonicum* | Brazil (Paraná Basin) | Freshwater | 8 | 4.3 × 3.7 | 7-8 (rarely 9) | 3-4 (rarely 6) | Present study |

(*) - calculated from their Fig. 5; app. - appendages; L - length; PTC - polar tube coils; SpVc - sporophorous vacuoles; W - width.

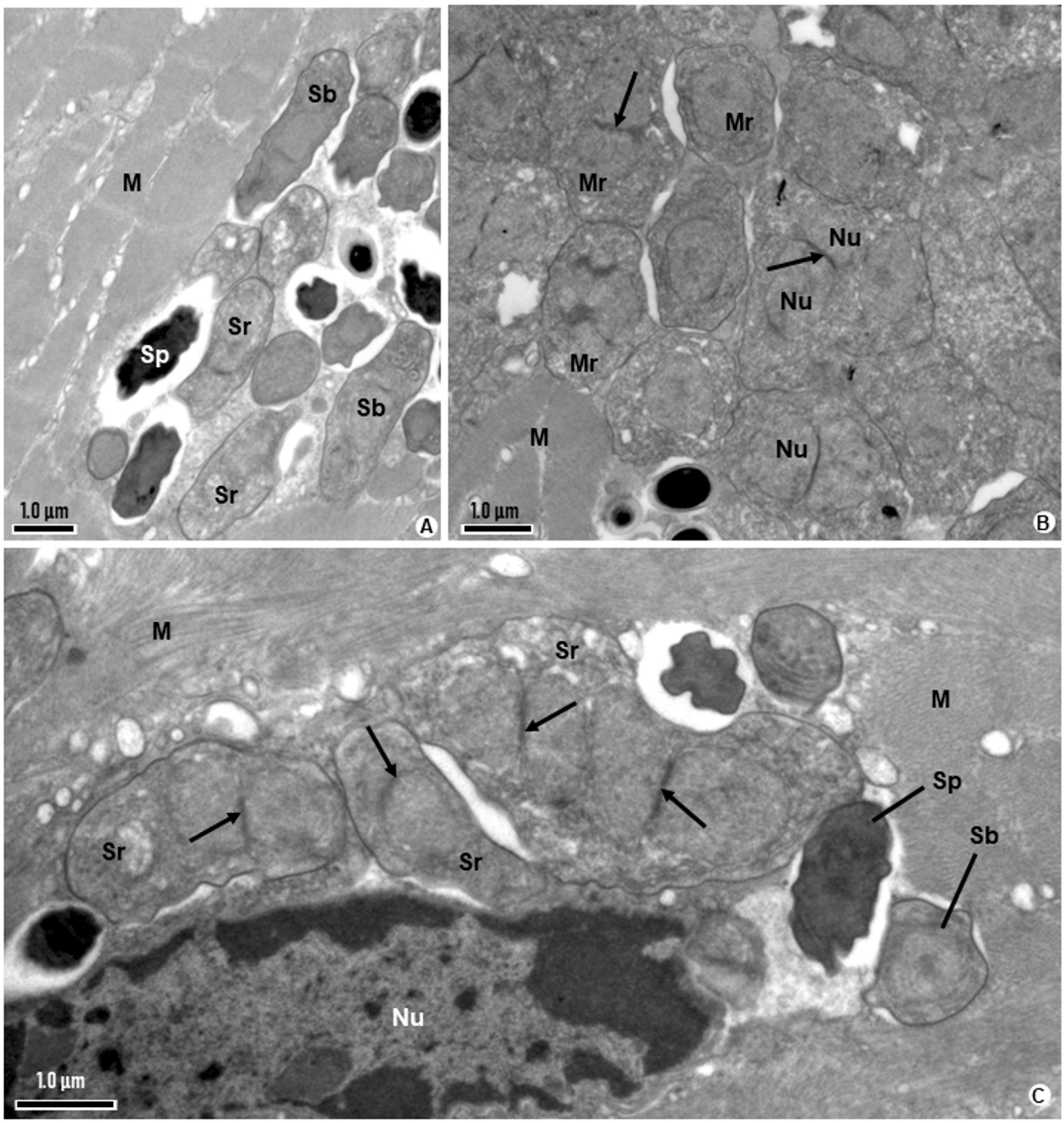


**Figure 4.** Ultrastructure of *Myospora* sp. (**A**) Spores (Sp), sporonts (Sr) and sporoblasts (Sb) in direct contact with muscle cells (M). (**B**) Meronts (Mr), some with diplokarya (arrows) are located in close contact with the cytoplasm of the muscle cell (M). (**C**) Detail of the muscle cell nucleus (Nu) surrounded by several sporonts (Sr), some of which are diplokaryons (arrows), a spore (Sp) and sporoblasts (Sb) in close contact with nuclear envelope. The muscle fibers show evident degradation.

The presence of dimorphic microsporidians infecting the same hosts, have been frequently described (Iwano and Ishihara, 1991; Iwano and Kurtti, 1995; Stentiford, 2008; Stentiford *et al.*, 2013b; Itoh *et al.*, 2020). However, dimorphic species infecting the same organ or tissue from the same host is a rare phenomenon and is here reported for the first time in Brazilian crustacean fauna.

The ultrastructural organization of the Sp. 1 spores demonstrates a complex membranous system around the spores, which fits the diagnosis of the genus *Inodosporus* (*cf.* Overstreet and Weidner, 1974;

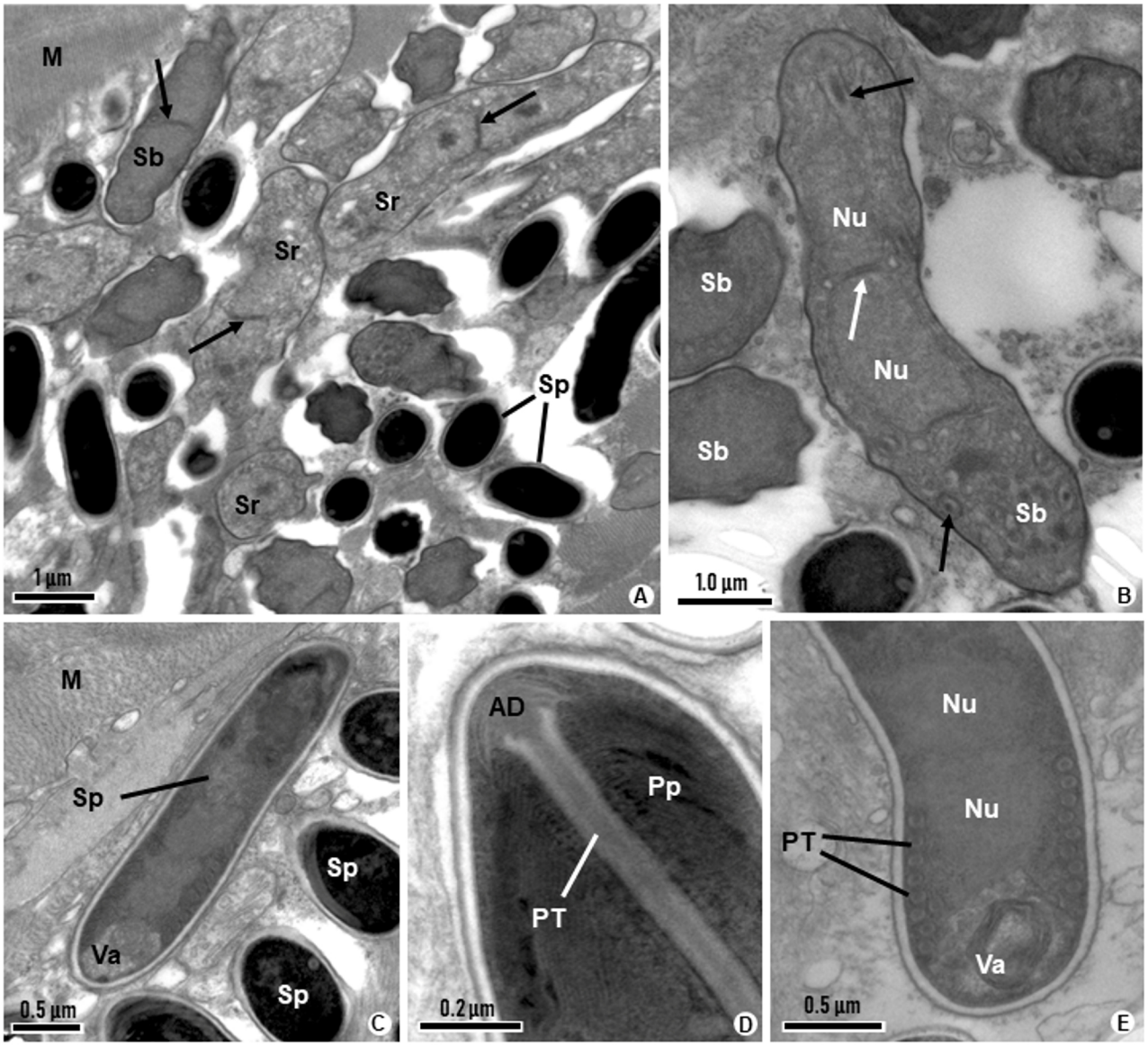


**Figure 5.** Ultrastructure of *Myospora* sp. (**A**) Different life cycle stages located among the muscles (M) showing the spores (Sp), surrounded by earlier intracellular stages, some displaying diplokaryons (arrows). Sr, sporonts. Sb, Sporoblasts. (**B**) Sporoblasts (Sb) sectioned at different levels. Diplokaryon is indicated by the white arrow, sections through the polar tube by the black arrow. (**C**) Sections through spores (Sp) showing posterior vacuole (Va) in contact with the muscle (M). (**D**) Longitudinal section of the apical region of the spore showing the anchoring disc (AD) and a manubrium part of the polar tube (PT), surrounded by the polaroplast (Pp). (**E**) Longitudinal section of the basal portion of the spore with two nuclei (Nu), the posterior vacuole (Va) and polar tube coils (PT).

Azevedo *et al.*, 2000; Stentiford *et al.*, 2018). Microsporidians with tailed spores were first reported in the grass shrimp *Palaemon serratus* (Pennant, 1777) from the European Atlantic coast and later in *Palaemon elegans* Rathke, 1836 from the Black Sea, and named *Thelohania octospora* Henneguy, 1892. Subsequent ultrastructural studies described spore tails in microsporidian parasites of the shrimp *Palaemon pugio* (Holthuis, 1949) from Mississippi (USA) and resulted in the introduction of a new genus, *Inodosporus*, and a new species, *Inodosporus spraguei* in Overstreet and Weidner (1974). Consequently, *Thelohania octospora* was recombined as *I. octospora* (Overstreet and Weidner, 1974; Azevedo *et al.*, 2000).

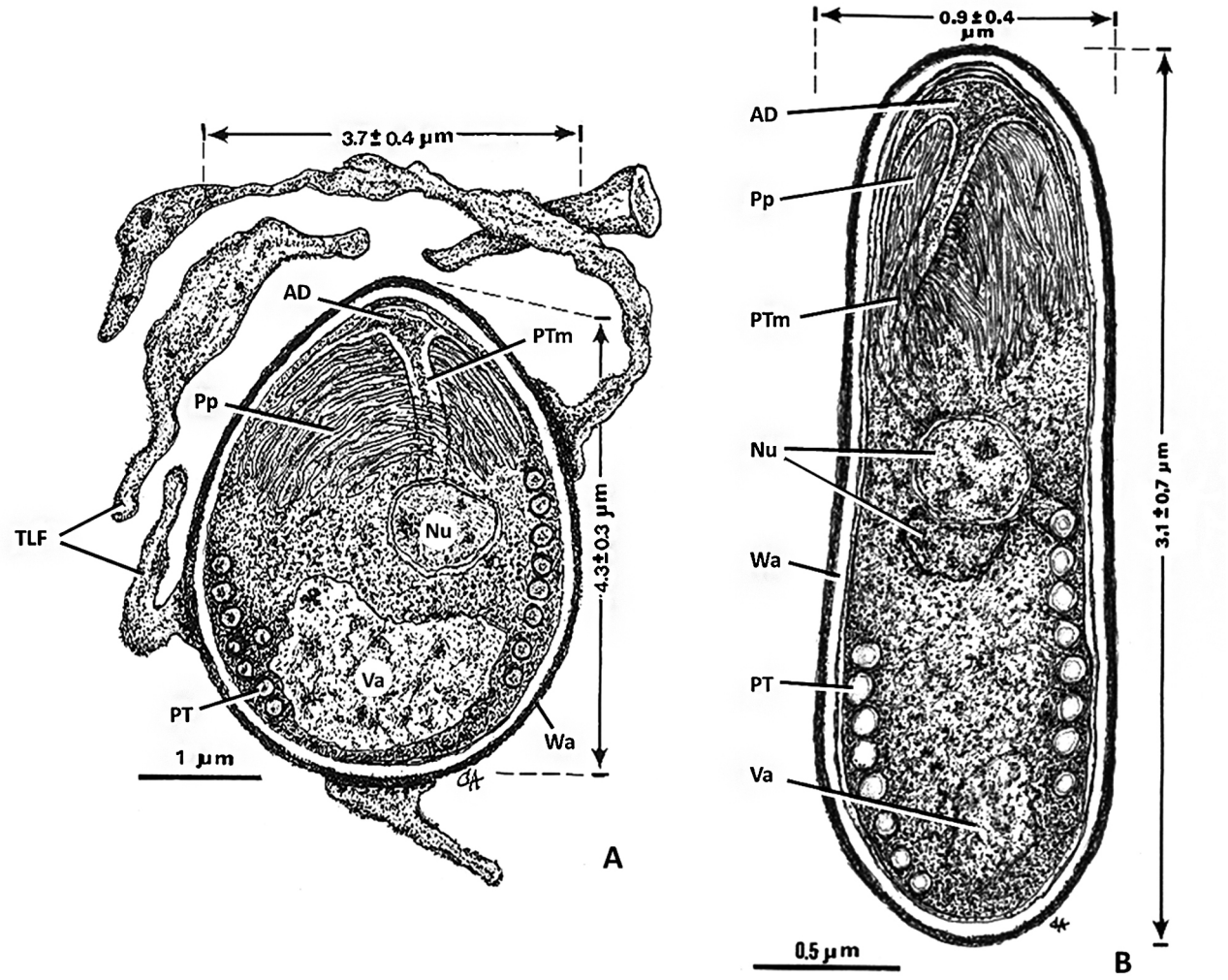


**Figure 6.** Schematic drawings of the studied microsporidian spores: (**A**) *Inodosporus* sp. and (**B**) *Myospora* sp. AD - anchoring disc; Nu - Nucleus; Pp - polaroplast; PT - polar tube; PTm - polar tube manubrium; TLF - tape-like filaments; Va - vacuole; Wa – wall.

**Table 2.** Comparison of the spore characteristics of *Myospora* species.

| *Myospora* spp. | Host species | Country (region) | Host habitat | L × W (µm) | PTC | References |
|---|---|---|---|---|---|---|
| *M. metanephrops* | *Metanephrops challengeri* | New Zealand (Pacific Ocean) | Marine | 3.4 x 0.9 | 9-11 | Stentiford *et al.* (2010) |
| *Myospora* sp. | *Macrobrachium amazonicum* | Brazil (Paraná Basin) | Freshwater | 3.1 x 0.5 | 7-8 | Present study |

L - length; PTC - polar tube coils, W - width.

Microsporidians with eigth sporoblasts further divided into eigth spores, each one possessing elongated external appendages (tail-like structures) and a pansporoblast with a persistent membrane, were placed within the family Nosematidae by Overstreet and Weidner (1974) and later in the family Thelohaniidae by Sprague *et al.* (1992). Recently, the synonymy of the genera *Kabatana* Lom, Dyková and Tonguthai, 2000 and *Inodosporus* was suggested (Stentiford *et al.*, 2018). Comparing morphology and ultrastructural organization of the microsporidian Sp. 1 in this study with spores of previously described *Inodosporus* spp., both spore dimensions and internal ultrastructural organization seem markedly different, suggesting that this may be a novel species (Tab. 1).

The microsporidian named Sp. 2 here, revealed morphological similarities to the genus *Myospora*: presence of diplokaryons at all stages (meronts, sporonts, sporoblasts and spores), development without SpVc formation, long rod-shaped to cylindrical spores with semi-spherical ends and similar measurements and internal organization of the spores (Stentiford *et al.*, 2010, 2013b).

We therefore report herein a co-infection of abdominal muscle in *M. amazonicum* with two morphologically distinct microsporidians, presumably belonging to two different genera. Moreover, we provide morphological and ultra-structural data on two microsporidia co-infecting an economically important species. Ultrastructural analysis suggests that the novel microsporidians belong to genera *Inodosporus* and *Myospora*, and likely represent two new species. The species descriptions are pending until genetic information is available and phylogenetic relations with other described microsporidians are clarified.

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## Conflicts of interest

The authors declare that they have no conflicts of interest.

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