

Description of *Triangulamyxa psittaca* sp. nov. (Myxozoa: Myxosporea), a New Parasite in the Urinary Bladder of *Colomesus psittacus* (Teleostei) from the Amazon River, with Emphasis on the Ultrastructure of Plasmodial Stages

Sónia ROCHA¹, Graça CASAL^{1,2}, Patrícia MATOS³, Edilson MATOS⁴, Mohamed DKHIL^{5,6} and Carlos AZEVEDO^{1,5}

¹Department of Cell Biology, Institute of Biomedical Sciences (ICBAS/UP), and Laboratory of Pathology, Centre for Marine and Environmental Research (CIIMAR/UP), University of Porto, Portugal; ²Departamento de Ciências, Instituto Superior de Ciências da Saúde, Gandra, Portugal; ³Edilson Matos Research Laboratory, Federal University of Pará, Belém, Brazil; ⁴Carlos Azevedo Research Laboratory, Federal Rural University of Amazonia, Belém, Brazil; ⁵Department of Zoology, College of Science, King Saud University, Riyadh, Saudi Arabia; ⁶Department of Zoology and Entomology, Faculty of Sciences, Hewan University, Egypt

Summary. A fish-infecting myxosporean was found in the urinary bladder of the teleostean *Colomesus psittacus*, collected from the Amazon River, Brazil. Specimens were sampled in three different periods: May and June, with water temperature ranging from 18–23°C; August, with water temperature ranging from 24–28°C; and November and December, with water temperature ranging from 29–32°C. Upon observation, several fish displayed abnormal behaviour, consisting of erratic movements, and mortality was recorded among them. Necropsy of all sampled fishes revealed hypertrophy of the urinary bladder only among specimens previously displaying the irregular behaviour. Microscopic analysis of this organ confirmed the parasitic infection, resulting in the observation of spores floating free in the urine, and numerous plasmodia attached to the epithelium of the urinary bladder. Light and ultrastructural studies allowed recognition of the spores and plasmodia morphological characteristics. Coelozoic plasmodia were polysporic with varying organizational structure, according to the sampling period. Spores were equilaterally triangular with rounded ends in valvar view, measuring $8.8 \pm 0.4 \mu\text{m}$ ($n = 30$) in length and $8.4 \pm 0.5 \mu\text{m}$ ($n = 30$) in width, and displaying a ridge surface pattern. Two polar capsules were observed in the anterior end of the spores, measuring 3.1–3.2 μm in diameter. The spores were morphologically identified as belonging to the recently described genus *Triangulamyxa*. Further observation and comparison to the morphological features described for *Triangulamyxa amazonica*, the only other species within this genus, allowed us to conclude our parasite as a new species, herein named *Triangulamyxa psittaca* sp. nov. from the Amazon River, Brazil. Also, three different stages were distinguished in the plasmodium evolution, based on the observed morphological features at the three sampling periods. Fish sampled during May and June displayed small plasmodia (up to ~15–20 μm long), containing early stages of sporogonic development. Fish sampled during November and December presented larger plasmodia (up to ~850 μm long), which appeared flattened against and lining the urinary bladder epithelial cells and contained the later stages of sporogonic development, including some mature spores. Fish sampled during August presented plasmodia displaying intermediate morphological features between those observed in infected fish from the other sampling periods. Several immature and mature spores were among the different developmental stages. The

Address for correspondence: Carlos Azevedo, Department of Cell Biology, Institute of Biomedical Sciences, University of Porto, Lg. Prof. Abel Salazar no. 2, 4099-003 Porto, Portugal; E-mail: azevedoc@icbas.up.pt

parasite-host interface evolution is described throughout the different observed stages, with emphasis on the formation of septate junctions. Considering several previous reports, as well as the different environmental conditions during the sampling periods, the plasmodium development here described appears to be influenced by environmental factors, namely water temperature.

Key words: Ultrastructure, plasmodia, myxosporean, *Triangulamyxa psittaca* sp. nov., parasite, urinary bladder, freshwater fish, *Colomesus psittacus*.

INTRODUCTION

The Class Myxosporidia Bütschli, 1881 of the Phylum Myxozoa Grassé, 1970 is an assemblage of more than 2180 parasite species (Lom and Dyková 2006), with new species being frequently added. Widely distributed, this class contains the causative agents of some of the most severe and expanding parasitic diseases for marine and freshwater fish (Kent *et al.* 2001). Notwithstanding the effort made in improving the description of myxosporean species, little knowledge is available from these parasites infecting South American freshwater fishes, including those from Brazil. The few existing reports are based only on light microscopy and diagrammatic drawings of spores (Lutz 1889, Cunha and Fonseca 1917, Nemeček 1926, Penido 1927, Pinto 1928, Guimarães 1931, Walliker 1969, Kent and Hoffman 1984, Gioia and Cordeiro 1996, Molnár *et al.* 1998, Cellere *et al.* 2002), and lack the useful description of the parasites development, namely the extrasporogonic stages (Molnár and Békési 1993; Molnár *et al.* 1998; Azevedo *et al.* 2002, 2005; Casal *et al.* 2002, 2003; Cellere *et al.* 2002; Vita *et al.* 2003; Adriano *et al.* 2009a, b). Recently, the employment of more effective and reliable microscopic procedures, lead to the establishment of a new myxosporean taxon from this geographical area. Based on morphological and ultrastructural comparative features, the genus *Triangulamyxa* was created within the family Ortholineidae Lom and Noble, 1984, upon the report of the then new species *Triangulamyxa amazonica*, from the intestine of the freshwater teleostean *Sphoeroides testudineus* (Azevedo *et al.* 2005). Spores of *Triangulamyxa* are equilaterally triangular in valvar view with rounded ends and ridged surface. Two subspherical polar capsules are present in the anterior portion of the spores, and the sporoplasm is binucleate. Coelozoic in freshwater fishes, plasmodia are polysporic and display a variable number of spores and other developmental stages that are found floating free in the lumen or attached to the epithelium of the hosts

organ, through numerous peripheral extensions (Lom and Dyková 1992, Azevedo *et al.* 2005).

Reports of myxosporean species inhabiting tropical regions have concluded some environmental conditions as important factors influencing the parasites development (Booker and Current 1981, Haaparanta *et al.* 1994, Molnár 1998, Canning *et al.* 1999, Molnár and Székely 1999). However, few of those references relate to species infecting the Brazilian fish fauna (Azevedo *et al.* 2005, 2009a, b, 2011a, b; Adriano *et al.* 2009a, b; Casal *et al.* 2009).

Amongst the documented species, there is no report of a myxosporean parasitizing fish from Brazil or other South American countries with similar plasmodium development and consequent hypertrophy of the host organ, as described in the present work.

MATERIALS AND METHODS

Thirty five specimens (23 females and 12 males) of the freshwater fish *Colomesus psittacus* Schneider, 1801 (Teleostei, Tetraodontidae) (common Brazilian name: Baiacú), were collected in the low Amazon River, near the city of Cameté (02°14'S, 49°30'W) in the State of Pará, Brazil. Sampling occurred in three different periods. The first sampling period occurred during the months of May and June; the second during August; and the third during the months of November and December. Upon arrival to the laboratory, fish were kept alive for 5 days for observation, in aquaria using water collected from the capture site at the same temperature range as in the original site. All the specimens were necropsied, and only samples of the urinary bladder (UB) and urine were taken for parasitological evaluation, because of its abnormal appearance. No other organs were examined and no bacteriological analyses were performed.

Smears of small fragments from the UB and urine were examined by light microscopy (LM), including differential interference contrast (DIC) optics. For transmission electron microscopy (TEM), small fragments of epithelial tissue from the parasitized UB were fixed in 5% glutaraldehyde buffered in 0.2 M sodium cacodylate (pH 7.2) at 4°C for 20 h, washed overnight in the same buffer at the same temperature, and post-fixed in 2% OsO₄ buffered with the same solution for 3 h, again at the same temperature. The biological material was then dehydrated in an ascending ethanol series followed by propylene oxide, and embedded in Epon. Semithin sections were stained with methylene blue–Azur II for LM, and ultra-

thin sections were double contrasted with uranyl acetate and lead citrate and observed and photographed in a JEOL 100 CXII TEM operated at 60 kV.

RESULTS

During the period of observation, several fish displayed abnormal behaviour consisting of erratic movements, and mortality was recorded among them. All the animals displaying these movements, upon necropsy, exhibited an outstanding macroscopic hypertrophy of the UB, and resulted to be infected by a myxosporean parasite. Microscopic observation revealed the presence of myxosporean spores (Fig. 1) floating free in the UB fluid in the samples taken in August and November–December. No bacteria were observed in the fluid. The histological study revealed no hyperplasia of the UB and the presence of plasmodia attached to the epithelium in the three sampling periods (Figs 2–16). Table 1 shows the prevalence of infection and the type of plasmodium found in each period, as described below. Spores observed at DIC clearly displayed an equilaterally triangular shape with rounded ends in valvar view, two polar capsules and ornamental ridges in the valves (Figs 1, 15). According to these morphological characteristics of the spores, the myxosporean was ascribed to the recently described genus *Triangulamyxa*. Morphological and ultrastructural comparison with the only species thus far belonging to the genus, *Triangulamyxa amazonica*, allow us to determine the current species as a new one, *Triangulamyxa psittaca* sp. nov.

Three plasmodial developmental stages are described, based on the observation of several morphological and ultrastructural differences between plasmodia collected from the three sampling periods. Due to a scarcity of observations relating to sporogenesis, we lack a better description of the different sequential

stages occurring during this process. Nevertheless, we could observe the existence of polysporic development; since pansporoblasts encased more than two sporoblasts within the pericyte envelop (Fig. 14).

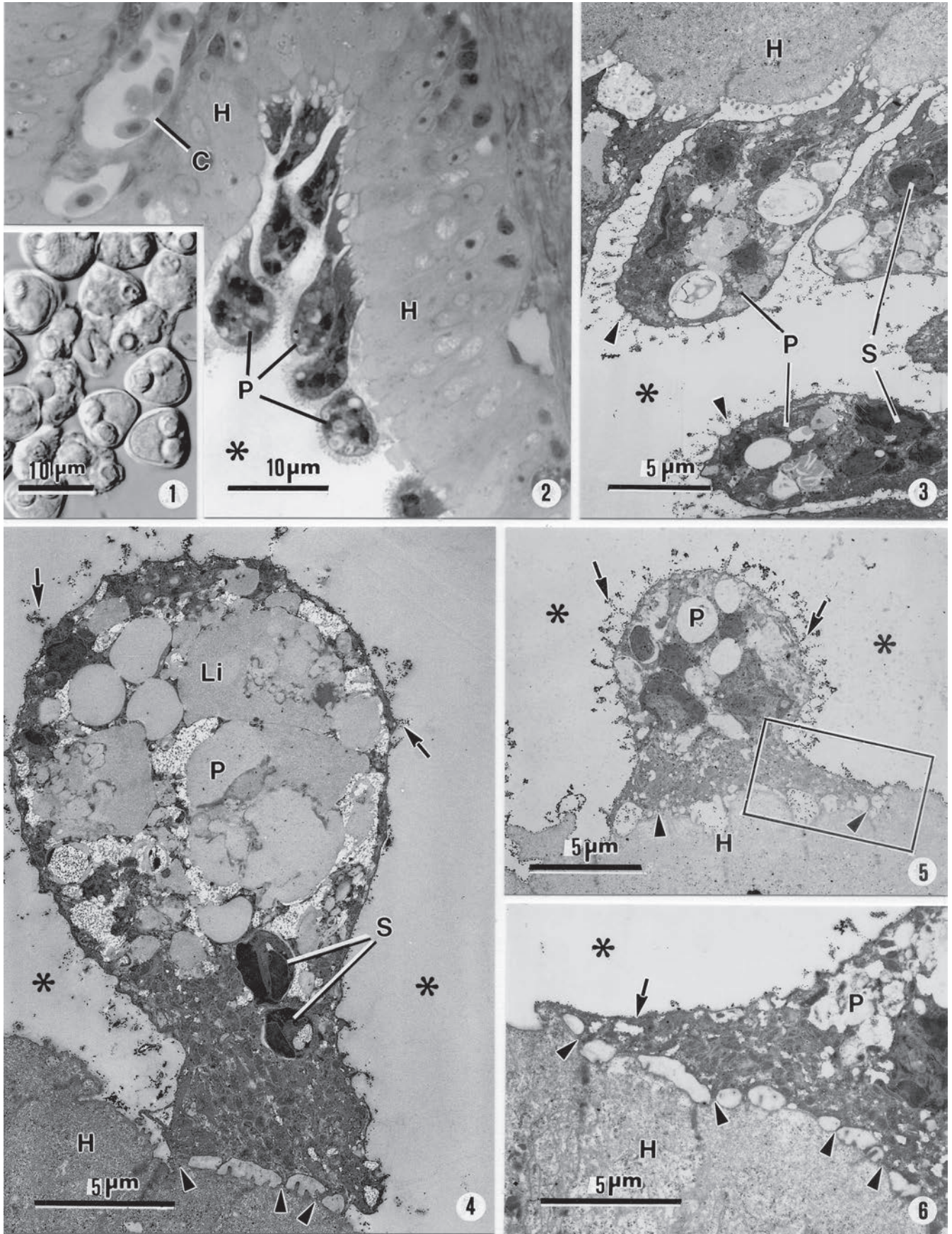
Stage 1 of the plasmodium

The stage 1 of the plasmodium corresponds to the observations from infected hosts sampled during May and June, when water temperature was lower. In this stage, plasmodia measured up to 15–20 μm in length and up to 8–12 μm in width (Figs 2–5), being smaller than the plasmodia found in hosts captured during the other sampling periods. Plasmodia contained early stages of sporogenic development and were pyriform-like shaped, tapering basally to contact the host tissue. The basal outline was irregular due to the presence of 7 to 12 pseudopodia, some of which in close contact with the UB epithelium (Figs 2–5). The apical surface of the plasmodia also presented numerous fine hair-like pseudopodia projecting towards the UB lumen (Figs 2–6). Dense structures later identified as immature spores, some unidentified developmental stages and several large lipid droplets were observed in the cytoplasm (Figs 3–5). The nucleus was hardly seen among the different cytoplasmic structures observed. Some capillaries located among the UB epithelium appeared compressed (Fig. 2).

Although the described characteristics were consistent for plasmodia in this stage, TEM allowed the observation of slightly different developmental aspects (Figs 3–6). In some sections, the plasmodia appeared more rounded in shape, with only a small basal portion contacting the UB epithelium (Figs 3, 4), whereas in others it was possible to observe a marked lateral growth of the basal pseudopodia, augmenting the contact surface with the UB epithelium (Figs 5, 6). Septate junctions were frequently observed between the pseudopodia and the host epithelium (Fig. 6).

Table 1. Sampling details and prevalence of infection of *Triangulamyxa psittaca* sp. nov. in *Colomesus psittacus*. See the text for the description of the three stages of the plasmodium (P). S – spores.

Sampling period	Water temperature range (°C)	Sample size (n)	Prevalence of infection (%)	Parasite stages
May–June	18–23	14	35.7	P-1
August	24–28	4	50	P-2, S
November–December	29–32	17	64.7	P-3, S



Stage 2 of the plasmodium

The stage 2 of the plasmodium corresponds to the observations from infected hosts sampled during August. In this stage, plasmodia presented transitional morphologic aspects between those described from hosts sampled during the other two periods. Plasmodia appeared to be flattening, displaying a larger contact surface with the UB epithelium than the plasmodia observed in stage 1 (Figs 7, 8). Pseudopodia were also longer and thinner, attaining up to ~ 10 µm in length, and were ramified and anastomosed (Figs 7, 8). In some sections, septate junctions were observed in the contact zone with the epithelial cells of the host (Figs 7, 8).

Stage 3 of the plasmodium

The stage 3 of the plasmodium corresponds to the observations from infected hosts sampled during November and December, when water temperature is higher. The observed plasmodia were much larger in size, if compared to the measurements obtained in stage 1 and stage 2, measuring up to ~ 850 µm in length and 10–20 µm in thickness (Figs 9, 10). Plasmodia appeared elongated as if flattened against the UB epithelial cells, forming a thin irregular layer over the simple columnar UB epithelium (Figs 9, 10). The irregularity of the layer was due to the presence of space ridges in the host tissue (Fig. 10). The parasite-host interface was maintained through numerous cytoplasmic bridges and pseudopodia between the plasmodia and the epithelial cells (Figs 9–11), and presented a plasmalemma reinforced by a homogenous dense layer (Fig. 15). In stage 3, several pseudopodia, including anastomosed pseudopodia, projected towards the UB lumen (Figs 10–12), instead of the fine hair-like pseudopodia observed in the other stages. Some sections displayed yet another type of liaison between the pseudopodia membranes and

the UB epithelium membrane, as they formed septate junctions constituted by parallel rows and corresponding to a regular periodicity of junctional proteins (Figs 12, 13). The plasmodia in this stage also contained a variable number of spores (up to ~ 54 were observed in sequential semithin sections) randomly distributed in the cytoplasm and displaying apparent lysed aspects (Figs 11, 14); thus suggesting a polysporic pansporoblast origin.

The schematic drawing (Fig. 16) represents the morphological evolution of the plasmodia according to our observations during the three sampling periods.

Triangulamyxa psittaca sp. nov. (Figs 1–16)

Type host: *Colomesus psittacus* Schneider, 1801 (Teleostei, Tetraodontidae).

Host size: Average of 30 cm in total length.

Type locality: Amazon estuarine region, near the city of Cametá (02°14'S, 49°30'W) in the State of Pará, Brazil.

Site of infection: Urinary bladder and urine.

Prevalence: 18 of 35 fishes sampled during the three sampling periods were parasitized (51.4%) with no observed difference in prevalence between sexes.

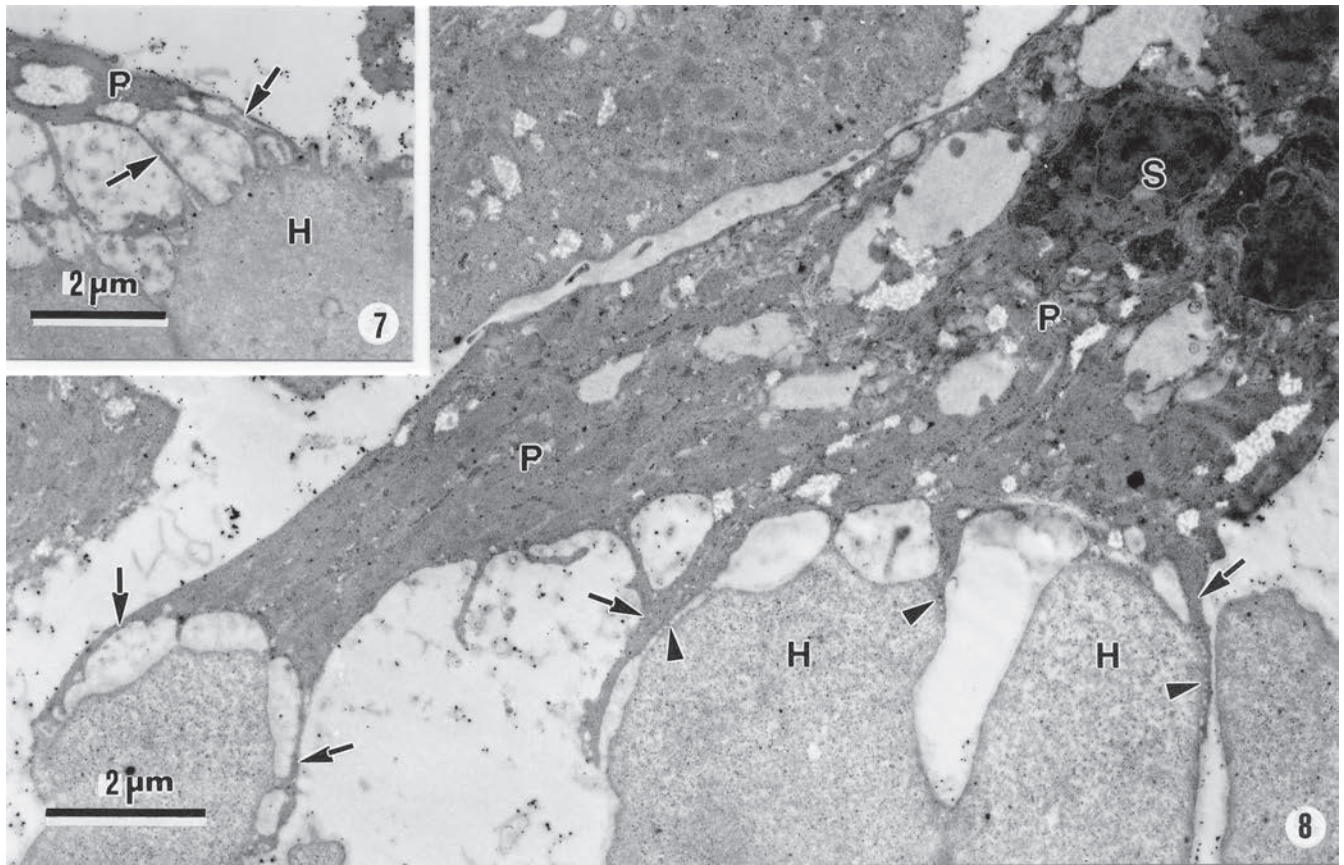
Type specimens: One slide of semithin sections containing mature spores and plasmodia displaying other developmental stages of the hapantotype was deposited in the Myxozoa Type Slide Collection at the "Instituto Nacional de Pesquisa da Amazônia – INPA", Manaus, Brazil, under "INPA" no. 010/11.

Etymology: The specific name is derived from the scientific name of the host species.

Description of the spores: For the description of spores, light microscopy (including DIC) (Figs 1, 2, 9, 10), TEM (Figs 3–8, 11–15) and a schematic drawing (Fig. 16) were used. Spores appeared equilaterally triangular with rounded ends in valvar view (Fig. 1), and measured $8.8 \pm 0.4 \mu\text{m}$ ($n = 30$) in length and 8.4 ± 0.5



Figs 1–6. Micrographs of fresh smears and histological sections of *Triangulamyxa psittaca* sp. nov., from the urinary bladder of *Colomesus psittacus* sampled during May and June. **1** – free fresh mature spores observed at DIC optics; **2** – semithin section showing several plasmodia in stage 1, located in the lumen and attached to the host epithelium. A capillary (C) is located near the base of the epithelium; **3** – TEM micrograph of plasmodia from stage 1, attached to the epithelium and containing some spores and numerous fine hair-like pseudopodia (arrowheads) projected into the lumen; **4** – ultrastructural detail of the stage 1 of the plasmodium showing sectioned spores and some large lipid droplets, as well as numerous peripheral fine hair-like pseudopodia (arrows) projected into the lumen. The tapering basal portion of the plasmodium is attached to the host epithelium by some pseudopodia (arrowheads); **5** – stage 1 plasmodium showing extending basal region in closed attachment (arrowheads) with the epithelium. The periphery of the plasmodium in contact with the lumen has numerous fine hair-like pseudopodia (arrows). The box highlights an area similar to the one detailed in figure 6; **6** – ultrastructural detail of the stage 1 plasmodium, showing the basal extending pseudopodia (arrow), closely contacting the epithelium. Some of the contact zones correspond to septate junctions (arrowheads). H – host epithelium, P – plasmodium, S – spore, Li – lipids, * – lumen of the urinary bladder.



Figs 7, 8. TEM micrographs of the stage 2 of the plasmodium of *Triangulamyxa psittaca* sp. nov., from the urinary bladder of *Colomesus psittacus*, sampled during August. **7** – part of the basal region of a plasmodium showing several types of pseudopodia (arrows) contacting the epithelium; some of which form septate junctions in the contact zone; **8** – detailed ultrastructural aspect of a plasmodium contact zone between the pseudopodia (arrows) and the host epithelium. Some zones of contact form septate junctions (arrowheads). H – host epithelium, P – plasmodium, S – spore.

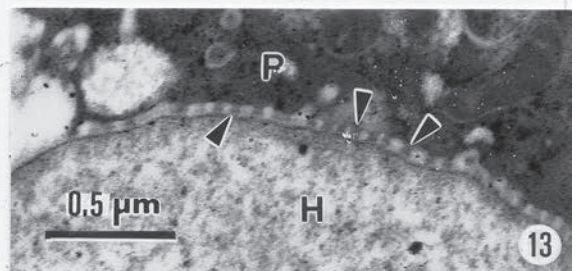
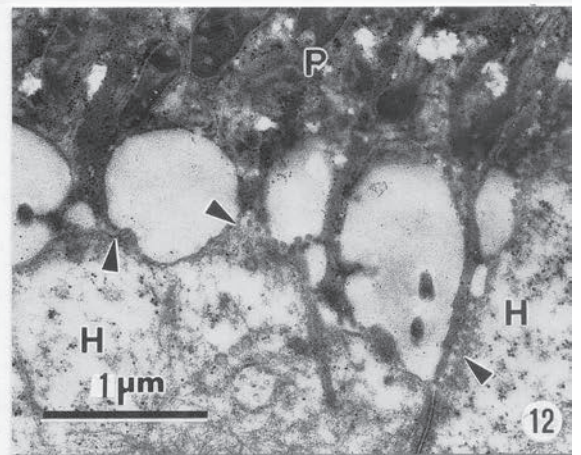
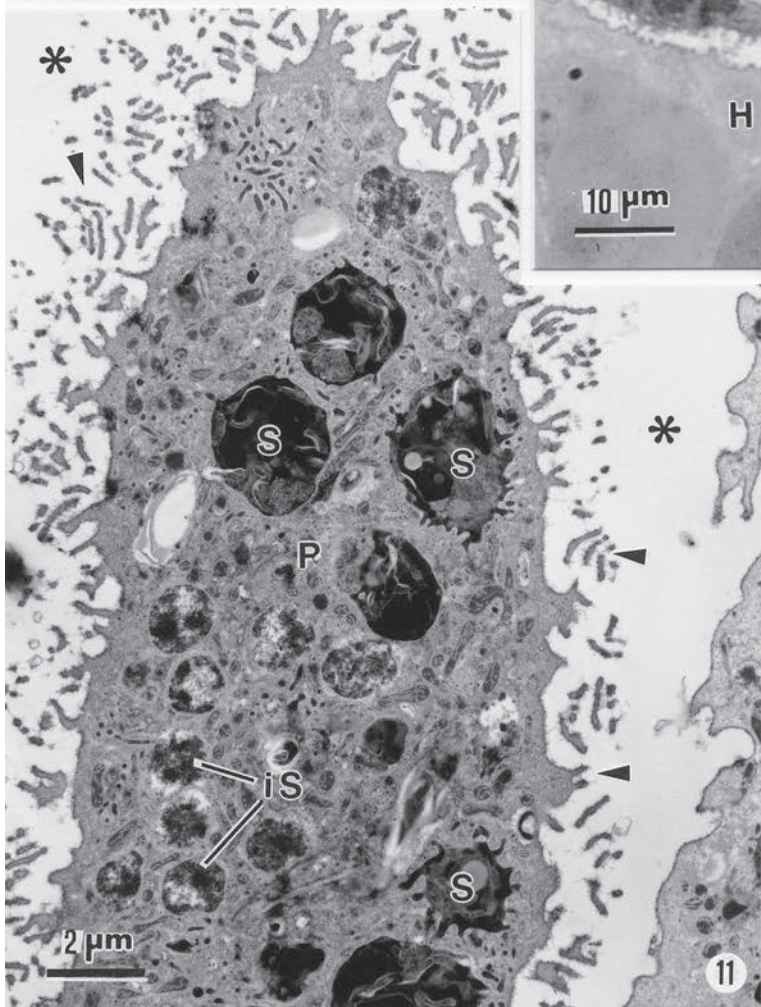
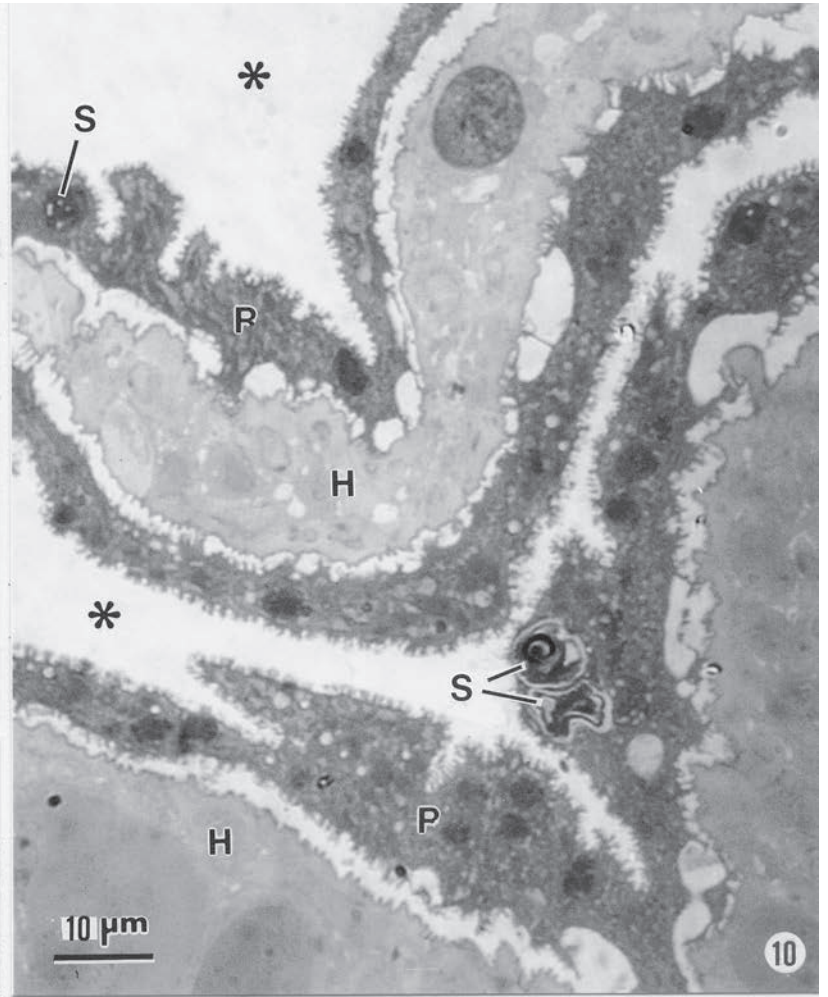
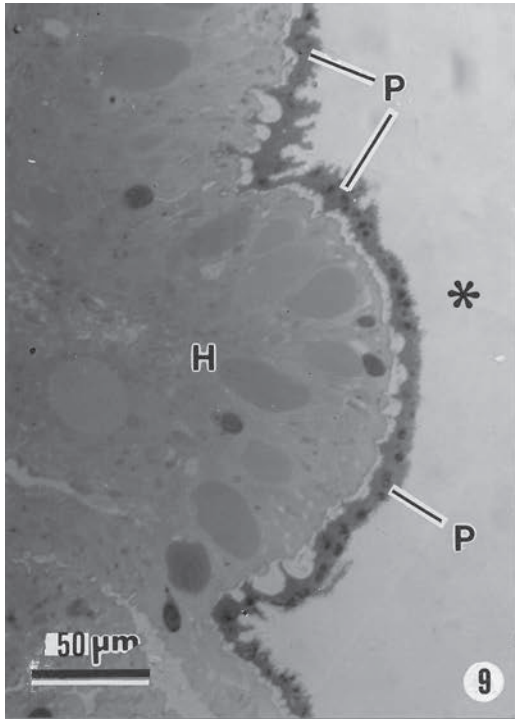


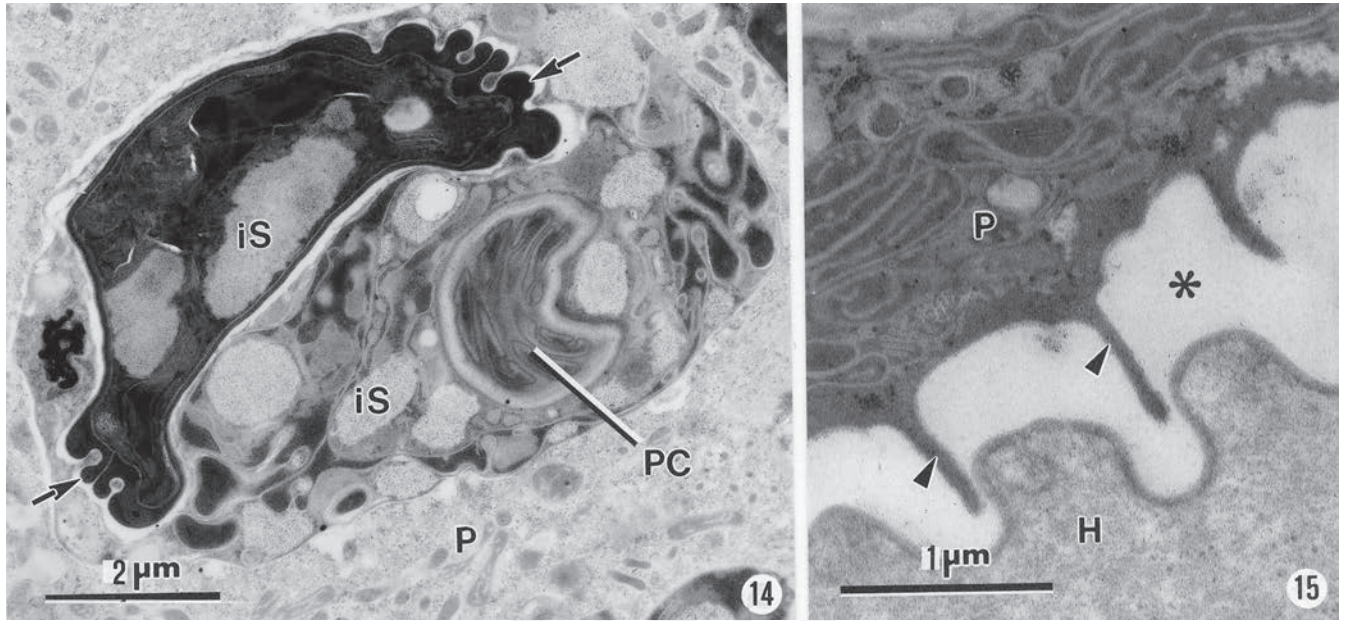
Figs 9-13. Light and TEM micrographs of the stage 3 of the plasmodium of *Triangulamyxa psittaca* sp. nov., from the urinary bladder of *Colomesus psittacus* sampled during the months of November and December. **9** – semithin section of the simple columnar epithelium showing a large and flattened plasmodium covering its entire surface and projecting into the lumen; **10** – semithin section showing a plasmodium covering the entire surface of the epithelium; **11** – TEM micrograph showing a plasmodium with several immature and mature spores. The periphery of the plasmodium shows numerous sections of ramified and anastomosed pseudopodia (arrowheads) projected into the lumen; **12** – details of the different forms of contact between the pseudopodia of the plasmodium and the cytoplasmic membrane of the urinary bladder epithelium; some of which form septate junctions (arrowheads); **13** – detail of the contact zone between the membrane of the plasmodium and the membrane of the epithelial cell, forming a large septate junction (arrowheads). H – host epithelium, P – plasmodium, S – spore, iS – immature spore, * – lumen of the urinary bladder.

µm (n = 30) in width. The spores' wall was comprised of two valves uniting together along a straight sutural line, and displayed a surface ridged pattern. Within the spores, two polar capsules measuring 3.1–3.2 µm in diameter were observed located in the anterior end, and lacking intercapsular space between them.

DISCUSSION

The main criterion used for the description and determination of new myxosporean species is morphology, including spores and polar capsules measurements and features (Lom and Hoffman 2003, Lom and





Figs 14, 15. TEM micrographs of the stage 3 of the plasmodium of *Triangulamyxa psittaca* sp. nov., from the urinary bladder of *Colomesus psittacus* sampled during the months of November and December. **14** – two juxtaposed immature spores within the plasmodium. A sectioned polar capsule and the primordial wall ridges (arrows) can be seen; **15** – detail of the plasmodium periphery showing the pseudopodia (arrowheads) interdigitated with the epithelial cells. H – host epithelium, P – plasmodium, iS – immature spore, PC – polar capsule, * – lumen of the urinary bladder.

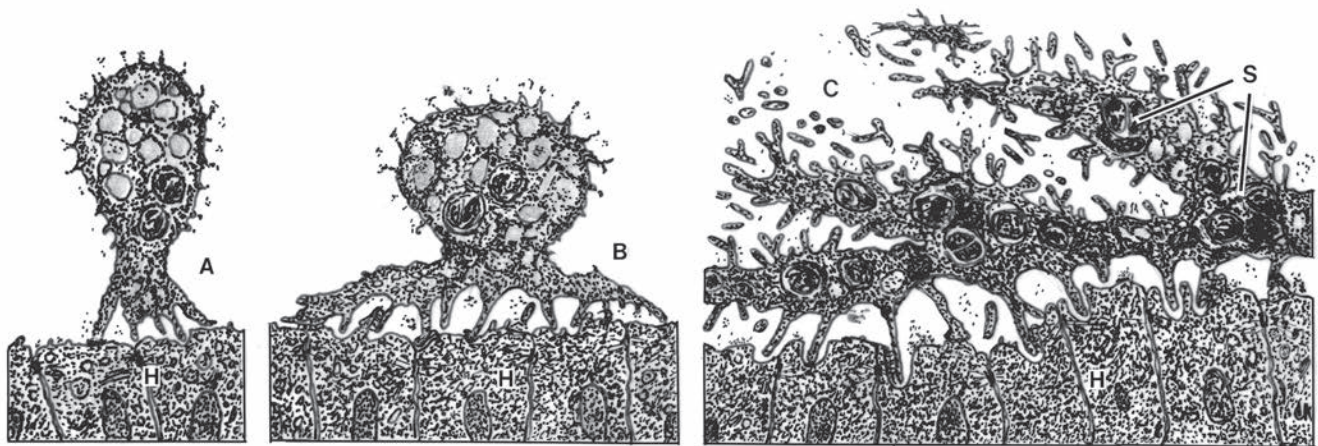


Fig 16. Schematic drawing of the sequential developmental evolution of the plasmodium of *Triangulamyxa psittaca* sp. nov. adhered to the urinary bladder of *Colomesus psittacus*, according to the three sampling periods. **A** – stage 1 plasmodium displaying rounded shape and tapering basely to contact the host epithelium through the establishment of some pseudopodia; **B** – stage 2 plasmodium displaying a larger contact surface with the host epithelium and several more pseudopodia; **C** – stage 3 plasmodium flattened against the host epithelium and displaying a much developed contact zone. The spores contained within pansporoblasts present polysporic development. H – host epithelium, S – spore.

Dyková 2006). Nevertheless, the distinct ultrastructural aspects displayed by the vegetative stages are most useful upon classification, since these structures provide

valuable information concerning the parasites development (Lom and Noble 1984; Lom and Dyková 1992, 1993, 2006; Canning *et al.* 1999; Lom and Hoffman

2003). Specificity for host species and site of infection is also considered when describing a new species (Lom and Noble 1984; Lom and Dyková 1992, 2006).

Analysis of morphological features, such as spore shape, wall structure and ridges organization, determined our parasite as belonging to the recently described genus *Triangulamyxa*, within the family Ortholineidae (Lom and Noble 1984). Further ultrastructural comparison to *Triangulamyxa amazonica* (Azevedo *et al.* 2005), thus far the only species established in this genus, revealed several specific differences in both spores and plasmodia. The spores, equilaterally triangular with rounded ends in valvar view, differed in dimension, as well as in several other aspects. *Triangulamyxa amazonica* spores were smaller (~ 8.5 µm long and ~ 7.6 µm wide), with ridged surface walls and a slightly curved sutural line. The spores observed in our study measured ~ 8.8 µm long and ~ 8.4 µm wide and the sutural line was straight. *Triangulamyxa amazonica* polar capsules were also smaller (2.5–2.8 µm in diameter) and separated by an intercapsular space (~ 1.3 µm), when compared to the polar capsules observed in our parasite, measuring 3.1–3.2 µm in diameter and lacking the intercapsular space between them. The apparently lysed aspect of the spores in TEM observations did not allow recognition of the number of polar filament coils in our species. Congruent with the spores morphology, the development of the plasmodium also displays several morphological and ultrastructural differences between this two species, namely in shape, dimensions and internal organization. The plasmodium described for *Triangulamyxa amazonica* was smaller, contained variable number of spores (up to ~ 18 were described) and often appeared free in the intestinal lumen or contacting the epithelial cells of the intestinal tract through the insertion of fine adhesion processes. The contact surface corresponded to a small portion covered by numerous microvilli and displaying hemidesmosome-like structures. Also, the plasmodium structure was similar in all the observed developmental stages (Azevedo *et al.* 2005). The plasmodium observed in our studies varied greatly in structure throughout the developmental stages, always contacting the epithelium of the UB and containing a higher number of spores (up to ~ 54 in semithin sections). During the earliest stages of development, correspondent to the stages 1 and 2, the plasmodium was smaller (up to ~ 15–20 µm long) and pyriform-like, tapering basally to contact the epithelial cells through the establishment of pseudopodia. In the later stage of development, correspondent to stage

3, the plasmodium appeared flattened, thus forming a layer that nearly covered the entire epithelium, and consequently attaining larger dimensions (up to ~ 850 µm long). Again, pseudopodia were observed in the parasite-host interface of the plasmodium in stage 3. In our species, septate junctions were present in all the observed stages, but were more evident in stage 2 and stage 3 sections. Host species and site of infection were also different for our species and *Triangulamyxa amazonica*. Although both species are coelozoic in freshwater fish species, *Triangulamyxa amazonica* infects the intestinal tract of *Sphoeroides testudineus* (Azevedo *et al.* 2005), while our parasite infects the UB of *Colomesus psittacus*. Based in all these morphological differences, as well as in the specificity for site of infection and host species, the myxosporean here described represents a new species of the genus *Triangulamyxa*, thus named *Triangulamyxa psittaca* sp. nov.

Despite the existence of some reports relating to the presence and description of coelozoic myxosporeans infecting Brazilian fishes, none refers to the ultrastructural evolution of the plasmodium (Molnár *et al.* 1998; Adriano *et al.* 2002, 2009a, b; Azevedo *et al.* 2005, 2009a, b, 2011a). In fact, this report is the first ultrastructural study of a plasmodial development occurring in a coelozoic myxosporean species collected from South America. During sporogenesis, the major occurring events in the plasmodium development are both an increase of size and the occurrence of pronounced surface alterations (Lom and Dyková 1995, 1996; Canning *et al.* 1999). Surface alterations are characterized by the development of pinocytic channels into the histozoic plasmodium ectoplasm (Current 1979, Current *et al.* 1979, Cho *et al.* 2004, Azevedo *et al.* 2011a), and the differentiation of peripheral extensions in the coelozoic plasmodium (Sitjà-Bobadilla and Alvarez-Pellitero 1993, 2001; Canning *et al.* 1999). Considering previous reports, the myxosporean parasite here described possesses a most interesting plasmodial development. The marked morphological differences between the three developmental stages described in this study, in which an increase of size and the formation of a large stratified layer nearly covering the entire UB epithelium occur, represent the most relevant ultrastructural comparative feature relating to other species. The majority of previously reported studies describe species with plasmodium ultrastructural aspects completely unlike the ones observed in our study (Sitjà-Bobadilla and Alvarez-Pellitero 1993, 2001; Lom and Dyková 1995; Canning *et al.* 1999; Cho *et al.* 2004; Azevedo *et al.* 2005; Adriano *et al.* 2006). Lom *et al.*

(1986) described similar ultrastructural aspects to those found in our stage 3 of the plasmodium, for the trophozoites of *Hoferellus gilsoni*, observed in the urinary tract of the European eel *Anguilla anguilla*. *Hoferellus gilsoni* also displayed elongated plasmodia firmly attached to the UB epithelium, but the adherence zones lacked pseudopodia in the parasite-host interface. Instead, evaginations of the host cell were drawn into corresponding invaginations of the parasite surface, sometimes forming desmosome-like junctions; and thus compensating the absence of cellular peripheral extensions and pinocytic activity for nutritional intake. On the contrary, similarly to several other coelozoic myxosporeans, *Triangulamyxa psittaca* sp. nov. plasmodium displays several peripheral extensions in the interface zone with the host epithelial cells, which are usually associated with the intensification of trophic functions (Lom 1969; Current *et al.* 1979; Sitjà-Bobadilla and Alvarez-Pellitero 1993, 2001; Lom and Dyková 1996; Canning *et al.* 1999).

It comes as no surprise that environmental factors influence the myxosporean life cycle, and that specific physical, chemical and biological conditions are necessary for the parasite successful development. We suggest water temperature as the influencing factor in our study, since this physical parameter has been correlated with the development of vegetative stages in several myxosporean species. Temperature appears to influence the shape of the plasmodia, the type and extension of attachment to the host cells and the development of the parasite-host interface (Booker and Current 1981, Haaparanta *et al.* 1994, Molnár and Székely 1999). In our observations, plasmodia evolved markedly increasing its dimensions between the samples collected in May and June and those collected in November and December. In the natural environment, one of the most preponderant changing variables between these time periods is water temperature range. As the fish were kept in water brought from the original site and maintained at the same range of water temperature verified during sampling, plasmodium evolution appears influenced by this physical parameter, attaining its more advanced evolutionary forms when water temperature increases, as described for other species (Molnár and Székely 1999, Viozzi and Flores 2003). However, some myxosporean species are reported to develop mature plasmodia when the water temperature range is lower (Molnár 1998).

Several studies consider that the basic structure and sequential stages of plasmodial development is similar between different myxosporean species (Lom and Dyková 1992, Canning *et al.* 1999). When analysing

our results and considering the abundance of myxosporean species that parasitize fishes inhabiting the tropical regions, we speculate that the plasmodial development here described may not be unique. Further studies on Brazilian myxosporean species may still be surprising when it comes to increasing the available knowledge referring to the development of vegetative stages. Also, studies should consider the possible influence of other environmental factors of this region in the parasitic development. The abnormal behaviour and mortality displayed by the infected sampled fishes leads us to suspect that *Triangulamyxa psittaca* sp. nov. is a pathogen. Nevertheless, our observations are not statistically significant to infer such a conclusion, as we aimed only at the ultrastructural description of this new parasite. Further studies should be performed in order to elucidate this observation.

Acknowledgements. This study was partially supported by Eng^o António de Almeida Foundation (Porto-Portugal), “CNPq” and “CAPES” (Brazil). We would like to thank Newton de Souza, technician of UFF (Oriximiná, Brazil) for the help in collecting the fish samples, and João and Joana Carvalheiro (ICBAS/UP) for the iconographic work. We would also like to thank the anonymous reviewers for the precious help provided in improving our manuscript. The performed methodology complies with the current laws of both countries.

REFERENCES

- Adriano E. A., Arana S., Alves A. L., Silva M. R. M., Ceccarelli P. S., Henrique-Silva F., Maia A. A. M. (2009a) *Myxobolus cordeiroi* n. sp., a parasite of *Zungaro jahu* (Siluriformes: Pimelodiade) from Brazilian Pantanal: Morphology, phylogeny and histopathology. *Vet. Parasitol.* **162**: 221–229
- Adriano E. A., Arana S., Carriero M. M., Naldoni J., Ceccarelli P. S., Maia A. A. M. (2009b) Light, electron microscopy and histopathology of *Myxobolus salminus* n. sp., a parasite of *Salminus brasiliensis* from the Brazilian Pantanal. *Vet. Parasitol.* **165**: 25–29
- Adriano E. A., Arana S., Ceccarelli P. S., Cordeiro N. S. (2002) Light and scanning electron microscopy of *Myxobolus porofilus* sp. n. (Myxosporea: Myxobolidae) infecting the visceral cavity of *Prochilodus lineatus* (Pisces: Characiformes: Prochilodontidae) cultivated in Brazil. *Folia Parasitol.* **49**: 259–262
- Adriano E. A., Arana S., Cordeiro N. S. (2006) *Myxobolus cuneus* n. sp. (Myxosporea) infecting the connective tissue of *Piaractus mesopotamius* (Pisces: Characidae) in Brazil: histopathology and ultrastructure. *J. Soc. Franc Parasitol.* **13**: 137–142
- Azevedo C., Casal G., Garcia P., Matos P., Teles-Grilo L., Matos E. (2009a) Ultrastructural and phylogenetic data of *Chloromyxum riorajum* sp. nov. (Myxozoa), a parasite of the stingray *Rioraja agassizii* in Southern Brazil. *Dis. Aquat. Org.* **85**: 41–51
- Azevedo C., Casal G., Marques D., Silva E., Matos E. (2011a) Ultrastructure of *Myxobolus brycon* n. sp. (Phylum Myxozoa), parasite of the piraputanga fish *Brycon hilarii* (Teleostei) from Pantanal (Brazil). *J. Eukaryot. Microbiol.* **58**: 88–93

- Azevedo C., Casal G., Mendonça I., Matos E. (2009b) Fine structure of *Henneguya hemiodopsis* sp. n. (Myxozoa), a parasite of the gills of the Brazilian teleostean fish *Hemiodopsis microlepes* (Hemiodontidae). *Mem. Inst. Oswaldo Cruz, Rio de Janeiro* **104**: 975–979
- Azevedo C., Corral L., Matos E. (2002) *Myxobolus desaequalis* n. sp. (Myxozoa, Myxosporea), parasite of the Amazonian freshwater fish, *Apteronotus albifrons* (Teleostei, Apterontidae). *J. Eukaryot. Microbiol.* **49**: 485–488
- Azevedo C., Corral L., Matos E. (2005) Ultrastructure of *Triangulamyxa amazonica* n. gen. and n. sp. (Myxozoa, Myxosporea), a parasite of the Amazonian freshwater fish, *Sphoeroides testudineus* (Teleostei, Tetodontidae). *Eur. J. Protistol.* **41**: 57–63
- Azevedo C., Ribeiro M., Clemente S. S. C., Casal G., Lopes L., Matos P., Al-Quraishy S. A., Matos E. (2011b) Light and ultrastructural description of *Meglitschia mylei* n. sp. (Myxozoa) from *Myleus rubripinnis* (Teleostei: Serrasalminidae) in the Amazon river system. *J. Eukaryot. Microbiol.* (In press)
- Booker O. J., Current W. L. (1981) *Myxobilatus mictospora* (Kudo, 1920) (Myxozoa: Myxosporea) in the largemouth bass (*Micropeterus salmoides* Lacépède): plasmodium morphology and fine structure. *J. Parasitol.* **67**: 859–865
- Canning E. U., Curry A., Anderson C. L., Okamura B. (1999) Ultrastructure of *Myxidium trachinorum* sp. nov. from the gallbladder of the lesser weever fish *Echiichthys vipera*. *Parasitol. Res.* **85**: 910–919
- Casal G., Garcia P., Matos P., Monteiro E., Matos E., Azevedo C. (2009) Fine structure of *Chloromyxum menticirrhoi* n. sp. (Myxozoa) infecting the urinary bladder of the marine teleost *Menticirrhus americanus* (Sciaenidae) in Southern Brazil. *Eur. J. Protistol.* **45**: 139–146
- Casal G., Matos E., Azevedo C. (2002) Ultrastructural data on the spore of *Myxobolus maculatus* n. sp. (Phylum Myxozoa), parasite from the Amazonian fish *Metynnis maculatus* (Teleostei). *Dis. Aquat. Org.* **51**: 107–112
- Casal G., Matos E., Azevedo C. (2003) Light and electron microscopic study of the myxosporean, *Henneguya friderici* n. sp. from the Amazonian teleostean fish, *Leporinus friderici*. *Parasitology* **126**: 313–319
- Cellere E. F., Cordeiro N. S., Adriano E. A. (2002) *Myxobolus absonus* sp. n. (Myxozoa: Myxosporea) parasiting *Pimelodus maculatus* (Siluriformes: Pimelodidae), a South American freshwater fish. *Mem. Inst. Oswaldo Cruz, Rio de Janeiro* **97**: 79–80
- Cho J. B., Kwon S. R., Kim S. K., Nam Y. K., Kim K. H. (2004) Ultrastructure and development of *Ceratomyxa protopsettae* Fujita, 1923 (Myxosporea) in the gallbladder of cultured olive flounder, *Paralichthys olivaceus*. *Acta Protozool.* **43**: 241–250
- Cunha A. M., Fonseca O. (1917) Sobre os myxosporidios dos peixes do Brazil. *Brazil-Médico.* **31**: 321 (in Portuguese)
- Current W. L. (1979) *Henneguya adiposa* Minchew (Myxosporida) in the channel catfish: Ultrastructure of the plasmodium wall and sporogenesis. *J. Protozool.* **26**: 209–217
- Current W. L., Janovy Jr. J., Knight S. A. (1979) *Myxosoma funduli* Kudo (Myxosporida) in *Fundulus kansae*: Ultrastructure of plasmodium wall and of sporogenesis. *J. Protozool.* **26**: 574–583
- Gioia I., Cordeiro N. S. (1996) Brazilian myxosporidians' check-list (Myxozoa). *Acta Protozool.* **35**: 137–149
- Guimarães J. R. A. (1931) Myxosporideos da ictiofauna brasileira. Thesis, Faculdade de Medicina, São Paulo, Brasil, 1–50 (in Portuguese)
- Haaparanta A., Valtonen E. T., Hoffmann R. W. (1994) Pathogenicity and seasonal occurrence of *Henneguya creplini* (Protozoa, Myxosporea) on the gills of perch *Perca fluviatilis* in central Finland. *Dis. Aquat. Org.* **20**: 15–22
- Kent M. L., Andree K. B., Bartholomew J. L., El-Matbouli M., Desser S. S., Devlin R. H., Feist S. W., Hedrick R. P., Hoffmann R. W., Khattra J., Hallett S. L., Lester R. J. G., Longshaw M., Palenzuela O., Siddall M. E., Xiao C. (2001) Recent advances in our knowledge of the Myxozoa. *J. Eukaryot. Microbiol.* **48**: 395–413
- Kent M. L., Hoffman G. L. (1984) Two new species of Myxozoa, *Myxobolus inaequus* sp. n. and *Henneguya theca* sp. n. from the brain of a South American knife fish, *Eigemannia virescens* (V.). *J. Protozool.* **31**: 91–94
- Lom J. (1969) Notes on the ultrastructure and sporoblast development in fish parasitizing myxosporidian of the genus *Sphaeromyxa*. *Z. Zellforsch.* **97**: 416–437
- Lom J., Dyková I. (1992) Protozoan Parasites of Fishes. Developments in Aquaculture and Fisheries Science 26. Elsevier, Amsterdam, Netherlands, 159–235
- Lom J., Dyková I. (1993) Scanning electron microscopic revision of common species of the genus *Chloromyxum* (Myxozoa: Myxosporea) infecting European freshwater fishes. *Folia Parasitol.* **40**: 161–174
- Lom J., Dyková I. (1995) New species of the genera *Zschokkella* and *Ortholinea* (Myxozoa) from the Southeast Asian teleost fish, *Tetraodon fluviatilis*. *Folia Parasitol.* **42**: 161–168
- Lom J., Dyková I. (1996) Notes on the ultrastructure of two myxosporean (Myxozoa) species, *Zschokkella pleomorpha* and *Ortholinea fluviatilis*. *Folia Parasitol.* **43**: 189–202
- Lom J., Dyková I. (2006) Myxozoa genera: definition and notes on taxonomy, life-cycle terminology and pathogenic species. *Folia Parasitol.* **53**: 1–36
- Lom J., Hoffman G. L. (2003) Morphology of the spores of *Myxosoma cerebralis* (Hofer, 1903) and *M. cartilaginis* (Hoffman, Putz, and Dunbar, 1965). *J. Parasitol.* **89**: 653–657
- Lom J., Molnár K., Dyková I. (1986) *Hoferellus gilsoni* (Debaissieux, 1925) comb. n. (Myxozoa, Myxosporea): redescription and mode of attachment to the epithelium of the urinary bladder of its host, the European eel. *Protistology* **4**: 405–413
- Lom J., Noble E. R. (1984) Revised classification of the Class Myxosporea Bütschli, 1881. *Folia Parasitol.* **31**: 193–205
- Lutz A. (1889) Über ein *Myxosporidium* aus der Gallenblase brasilianischer. *Centralbl. Bakt. u Parasit.* **5**: 84–88
- Molnár K. (1998) Taxonomic problems, seasonality and histopathology of *Henneguya creplini* (Myxosporea) infection of the pike-perch *Stizostedion lucioperca* in Lake Balaton. *Folia Parasitol.* **45**: 261–269
- Molnár K., Békési L. (1993) Description of a new *Myxobolus* species, *M. colossomatis* n. sp. from the teleost *Colossoma macropomum* of the Amazon River basin. *J. Appl. Ichthyol.* **9**: 57–63
- Molnár K., Ranzani-Paiva M. J., Eiras J. C., Rodrigues E. L. (1998) *Myxobolus macroplasmodialis* sp. n. (Myxozoa: Myxosporea), a parasite of the abdominal cavity of the characid teleost, *Salminus maxillosus*, in Brazil. *Acta Protozool.* **37**: 241–245
- Molnár K., Székely Cs. (1999) *Myxobolus* infection of the gills of common bream (*Abramis brama* L.) in Lake Balaton and in the Kis-Balaton reservoir, Hungary. *Acta Vet. Hung.* **47**: 419–432
- Nemeczek A. (1926) Beiträge zur Kenntnis der Myxosporidien fauna Brasiliens. *Arch. Protist.* **54**: 137–149

- Penido J. C. N. (1927) Quelques nouvelles Myxosporidies parasites de poissons d'eau douce du Brésil. *C. R. S. Brésil Biol.* **97**: 850–852
- Pinto C. (1928) Mixosporideos e outros protozoários intestinais de peixes observados na América do Sul. *Arch. Inst. Biol. S. Paulo.* **1**: 101–126 (in Portuguese)
- Stijà-Bobadilla A., Alvarez-Pellitero P. (1993) *Zschokkella mugilis* n. sp. (Myxoporea: Bivalvulida) from mullets (Teleostei: Mugilidae) of Mediterranean waters: light and electron microscopic description. *J. Eukaryot. Microbiol.* **40**: 755–764
- Stijà-Bobadilla A., Alvarez-Pellitero P. (2001) *Leptothea spari-darum* n. sp. (Myxosporea: Bivalvulida), a parasite from cultured common dentex (*Dentex dentex* L.) and gilthead sea bream (*Sparus aurata* L.) (Teleostei: Sparidae). *J. Eukaryot. Microbiol.* **48**: 627–639
- Viozzi G. P., Flores V. R. (2003) *Myxidium biliare* sp. n. (Myxozoa) from gall bladder of *Galaxias maculatus* (Osmeriformes, Galaxiidae) in Patagonia (Argentina). *Folia Parasitol.* **50**: 190–194
- Vita P., Corral L., Matos E., Azevedo C. (2003) Ultrastructural aspects of the myxosporean *Henneguya astyanax* n. sp. (Myxozoa: Myxobolidae), a parasite of the Amazonian teleost *Astyanax keithi* (Characidae). *Dis. Aquat. Org.* **53**: 55–60
- Walliker D. (1969) Myxosporidea of some Brazilian freshwater fishes. *J. Parasitol.* **55**: 942–948

Received on 2nd August, 2011; revised on 13th October, 2011; accepted on 15th October, 2011

