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Histopathology effects of *Myxobolus pethericii* Fomena, Lekeufack Folefack and Tang II, 2007 on *Ctenopoma petherici* Günther, 1864, in the Sangé River, Littoral Region, Cameroon

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Abstract

Histological method, becoming common in diagnostic methodology, provides information on effect of parasite and help predicting therapeutic measures for aquatic animals. *Ctenopoma petherici* caught in Sangé River were examined for myxozoan infections. Cyst measurements were taken and prevalence was estimated. Formalin-fixed organs were dehydrated through a graded ethanol series and embedded in paraffin. Obtained sections were stained with hematoxylin and eosin. From examined fish, 93.33% harbored elongated, ovoid or spherical cysts measuring 70 - 660 µm long × 40 - 390 µm wide in one or more of the following organs: gills, heart, stomach, liver, gonads, eyes, skin, bones, muscles, intestine, mesentery, fins, opercula, esophagus, labyrinth organs, kidney, swimbladder, and gallbladder. Cysts were arranged anarchically on affected organs and their development was asynchronous. Histological observations revealed cysts implantation mostly in loose connective tissue on which they induce one or more of the following: hyperplasia, swelling, alteration and lack of adhesion between tissue layers. In some cases, cysts were responsible of mechanical compression and distortion of blood vessels surrounding affected tissue. In many cases, significant portion of affected organs was replaced by numerous and voluminous cysts leading to tissue destruction and reduction of organ's functional area. Generally, few sign of fish immune response to the presence of cysts was observed. Histological examination of *Ctenopoma petherici* appears as an important tool for rapid diagnosis to ensure farming production. Therefore, monitoring *M. pethericii* in farmed fish using histological method would help in early and proper drug therapy and avoid it dissemination.

1. Introduction

The Anabantid fish *Ctenopoma petherici* Günther, 1864 is a freshwater fish found mainly in Asia and Africa. In Africa, it is found in Sudanian basins, West African basins, and Central African basins, where it stands as an important food and economic resource [1]. It great importance in human nutrition is due to it abundance on the markets, its sale at derisory prices, and its high protein content [2]. Furthermore, *C. petherici*'s high reproductive capacity and rapid growth make it a good candidate for fish farming [1]. To optimize the growth of *C. petherici* in fish farm, it is important to know the potential treats of this fish species in order to develop effective prevention and control methods [2]. Among these threats, myxosporidia infections figure prominent [3]. To the best of our knowledge, there are three myxosporidia species known around the world as parasites of *C. petherici*, they are *Myxobolus pethericii* [4], *Henneguya pethericii* [5] and *Myxidium petrocephali* [6]. In *C. petherici*, *M. pethericii* was found affecting sixteen organs, *H. pethericii* was found in four organs while *M. petrocephali* was found parasitizing only one organ [2]. Studies have demonstrated that *M. pethericii* can become a serious threat to the culture of this nutritional and commercially important fish species [2, 7].

According to Feist et al. [8], myxozoan infections can result in large-scale histopathological features depending on parasite species, infectious stage and host susceptibility. Based on the site selection by myxozoan species within a host organ, histozoic and coelozoic species of myxozoans are distinguished [3]. Histozoic species form cyst and spores in tissue of specific organs, whereas for coelozoic species a part of the process of sporogony takes place in the lumen of certain organs. Histozoic species elicit greater pathological response compared to coelozoic species [8]. Available data suggests strict host specificity (oioxeny) for *M. pethericii* developing cysts on different organs of *C. petherici* [2, 4, 7] but no histopathology details have been reported on the affected organs. Histopathology studies indicates that the plasmodia of myxozoan species can be found in diverse tissues of their host with inflammatory [9, 10] or no inflammatory responses [11]. Therefore, many myxozoans species have a preference for particular tissues for spore development within fish organs [12, 13, 14, 15, 16]. Given knowledge on the reaction between the fish and myxozoan plasmodium, a better understanding of the features leading to the pathogenicity of *M. pethericii* on *C. petherici* may be obtained by

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investigating host-tissue reaction resulting from *M. pethericii* infection in different host organs.

In the present study, the histopathological changes in the structure of the tissues in some organs of *C. petherici* infected by *M. pethericii* have been examined under the light microscope.

2. Materials and Methods

2.1. Data on study site

Sixty specimens of *C. petherici* were collected from January to September 2022, in the Sangé River (a tributary of Nkam River) at Tondé (4° 12' - 4° 17' N and 10° - 10° 8' E). Tondé is a village located in the Yabassi sub-Division, Nkam Division, Littoral Region, Cameroon, and central Africa. The climate in Tondé is equatorial, characterized by high humidity and two seasons: a short dry season (December to February) and a long rainy season (March to November).

2.2. Fishing, fish conservation, fish dissection and parasites collection

Examined fish were captured using gill nets. Fish caught were immediately immersed in a 10% formalin solution.

In the laboratory, the search for myxosporidia started with careful examination of the external organs of the fish (scales, fins, skin, opercules and eyes) with the naked eye, then with an Olympus BO61 binocular stereoscopic microscope to detect and count cysts on each organ. After opening the fish's gill and abdominal cavities, all internal organs (gills, labyrinth organs, heart, kidneys, spleen, liver, gonads, swimbladder, gallbladder, urethra, urinary bladder, digestive tract, mesenteries, muscles, etc.) were individually set apart and examined under a stereoscopic microscope for cysts detection and enumeration. Some of the cysts found were set apart, then dilacerated in a drop of water between the slide and coverslip, and their contents were examined under the 40X objective of a light microscope.

2.3. Tissue processing

The method used to obtain histological sections to be examined under a light microscope was proposed by Wolfe [17]. Parasitized tissues containing myxosporidia cysts were dehydrated using six alcohol baths of increasing degrees: 1 bath of 70% ethanol for 1 hour; 2 baths of 95% ethanol for 1 hour and 1 hour 30 minutes respectively; 3 baths of 100% ethanol for 1 hour, 1 hour 30 minutes and 2 hours respectively. Tissue clearing was done in two xylene baths for 1 hour and 1 hour 30 minutes respectively after which impregnation with 60°C melted paraffin was done for 4 hours and 30 minutes. Tissue samples were embedded into the paraffin and allowed to solidify so as to create a tissue "block" suitable for sectioning. Thin sections (5 to 8 µm thickness) of tissue samples were obtained using manual rotary reichert-jung 2030 microtome. The sections obtained were immersed in a hot 0.5% gelatinized water bath at 42 °C, then spread on a glass slide and dried in an oven for at least 12 hours at 45 °C. Tissue sections were stained with hematoxylin and eosin. Hematoxylin was the nuclear stain while eosin was the cytoplasmic stain. The stained sections were successively dehydrated in 3 baths of 100% ethanol for 10 minutes each, and then cleared successively in 3 baths of xylene for 5 minutes each. A few drops of Eukitt synthetic resin were then applied to each section, and the whole was covered with coverslips. The stained tissues sections were studied under an optical microscope. Photographs of parasitized organs were taken using a Nikon COOLPIX S2800 digital camera, and histological sections were photographed using an Olympus BH-2 optical microscope equipped with a microphotography device.

2.4. Cyst measurements and prevalence estimation

Cyst measurements were taken from the largest and smallest cysts. According to Bush et al. [18], the prevalence was estimated as the number of specimens of *C. petherici* infected with one or more cyst of *M. pethericii* divided by the total number of *C. petherici* examined.

3. Results

From the population of *Ctenopoma petherici* examined, 93.33% of specimens harbored cysts in one or more of the following organs: gills, heart, stomach, liver, gonads, eyes, skin, bones, muscles, intestine, mesentery, fins, opercula, esophagus, labyrinth organs, kidney, swimbladder, gallbladder. These cysts are whitish and polysporous. They are elongated or ovoid or spherical, vary in size and can reach 2.8 mm in diameter.

- Fins infection

From the population of *C. petherici* examined, 16.7% were found with cyst of *M. pethericii* on their fins. This parasite species forms ovoid cysts in the soft rays of the fins (Figure 1a). All fins are potential sites for cyst implantation. These cysts, measure 100 - 200 µm long × 50 - 100 µm wide, and are distributed anarchically on the fins (Figure 1a). Up to 22 cysts have been enumerated on a parasitized host individual. Histological observations confirm that the cysts of this parasite species are implanted in the layer of loose connective tissue between the bony rays of the fin (Figure 1b).

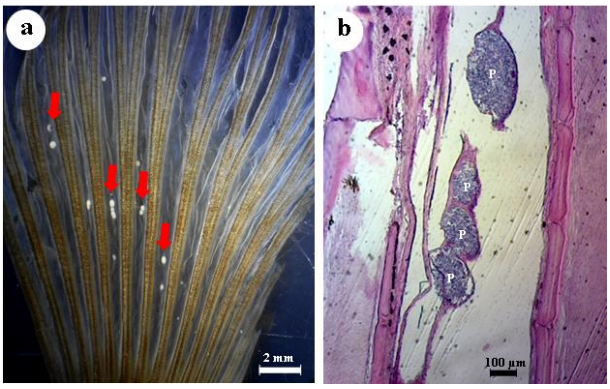

Figure 1: Microphotographs of fin fragment of *Ctenopoma petherici* showing plasmodia of *Myxobolus pethericii*. **a**: fin portion showing anarchically arranged plasmodia (arrow); **b**: histological section of a fin fragment showing plasmodia (P) situated in the layer of loose connective tissue between bony rays.

-Skin infection

Cysts of *M. pethericii* were found on the skin (Figure 2a) of 40% of *C. petherici* examined. These cysts are ovoid or subspherical, measuring 540 - 560 µm long × 260 - 300 µm wide. They are distributed on the skin in no particular order. Up to 30 cysts have been numbered on a parasitized host. Histological observations revealed that the cysts are implanted in the connective tissue of the dermis adjacent to the epidermis (Figure 2b). The plasmodium is surrounded by a thin membrane and its cavity is uniformly filled with spores (Figure 2b). No sign of fish immune response to the presence of the cyst was observed.

- Eye infection

Examination of 60 specimens of *C. petherici* revealed the presence of one or two cysts of *M. pethericii* in the eye of three individuals, representing a 5% infestation rate. Infection of the fish may be unilateral or bilateral. The cysts are ovoid, 270 - 570 µm long × 200 - 290 µm wide. They are implanted in the sclera of the eye (Figure 3a).

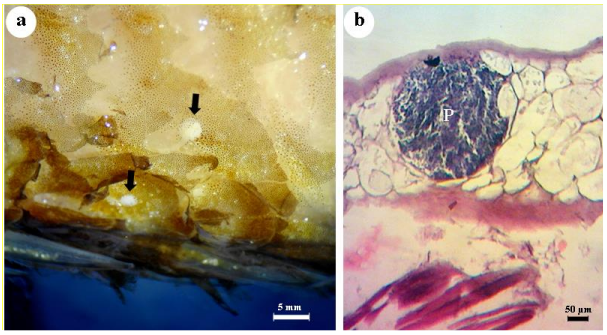

Figure 2: Microphotographs of skin fragment of *Ctenopoma petherici* showing plasmodia of *Myxobolus pethericii*. **a**: skin fragment showing cysts (arrow); **b**: histological section of skin fragment showing cyst (P) located in the dermis

Examination of histological sections of the eyeball revealed that, unlike healthy fish specimens (Figure 3b), in parasitized specimens, the cysts of this parasite species are implanted in the multilayered connective tissue of the cornea, occupying the entire width of the sclera (Figure 3c). The presence of these cysts induces hyperplasia of the connective tissue resulting in swelling (arrows) on both sides of this tissue (Figure 3c). Cyst development is asynchronous. There is also an alteration, and lack of adhesion between the different layers of tissue surrounding the cyst (Figure 3d). High magnification of the parasitized tissue section reveals the presence of immune cells at the cyst periphery, reflecting host immune reaction (Figure 3e). Within the cysts, mature spores (MS) are located in the median zone. In contrast, immature spores (IS) embedded in a gelatinous substance are found in the peripheral zone (Figure 3f).

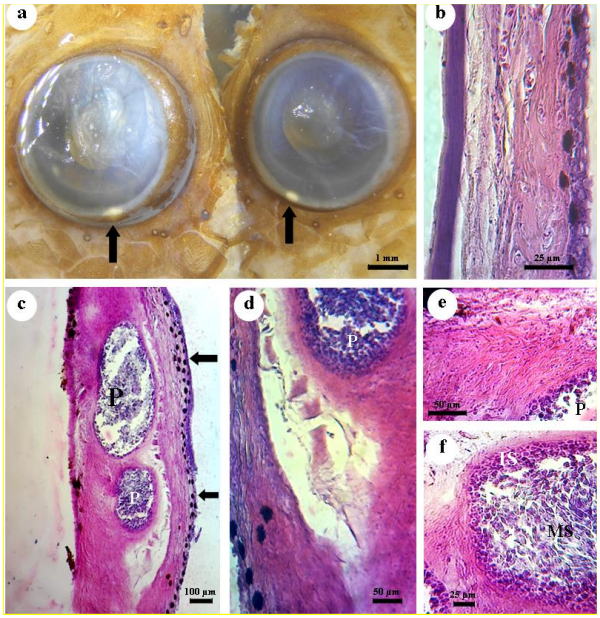

Figure 3: Microphotographs of plasmodia of *Myxobolus pethericii* developing on eye of *Ctenopoma petherici*. **a**: plasmodia implanted in eyes (black arrows); **b**: histological section of eye portion of *C. petherici* showing unparasitized sclera; **c**: histological section of a sclera portion bearing two plasmodia (P) of *M. pethericii* and showing the swelling of the sclera (black arrows); **d**: portion of the sclera showing the lack of adhesion between the different layers of tissue surrounding the plasmodium; **e**: observe monocytes influx at the periphery of the plasmodium (P); **f**: plasmodium of *M. pethericii* showing the location of mature spores (MS) in the medial part and immature spores (IS) at the periphery.

- Gills infection

Parasitism by *M. pethericii* in the gills of *C. petherici* was revealed at lamellae and gill arch level for a total infestation rate of 91.6%.

➢ **Gill filament infection**

Thirty-one specimens of *C. petherici* (51.7%) showed parasitized gill lamellae (Figure 4a). The arrangement of cysts on the gill is random. In these host individuals, the parasite forms ovoid or subspherical cysts. They measure 80 - 150 µm long × 70 - 100 µm wide. There are 1 to 6 cysts per gill lamellae and around 50 per holobranchia. Histological observations reveal that, unlike gills devoid of cysts (Figure 4b), in parasitized fish, the cysts of *M. pethericii* are implanted in gill lamellae (Fig. 4c). These cysts cause hyperplasia of the gill lamellae and deformation of adjacent gill lamellae (Figure 4d). Engulfed gill lamellae are also destroyed (Figure 4d).

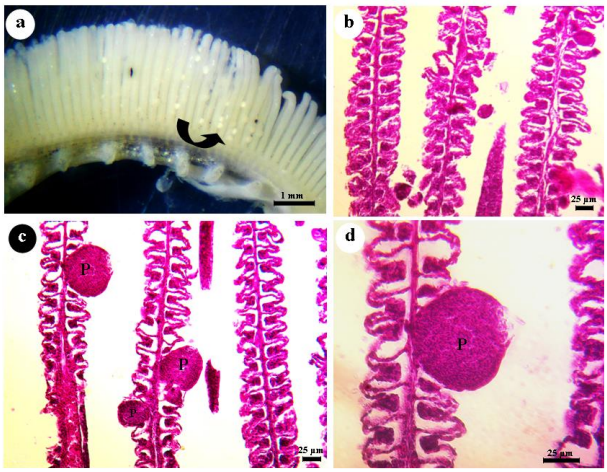

Figure 4: Microphotographs of gill portions of *Ctenopoma petherici* showing plasmodia of *Myxobolus pethericii* on gill filament. **a**: gill portion showing plasmodia location in the gill filament (black arrows); **b**: histological section of healthy gill lamellae; **c**: histological section revealing plasmodia (P) located in gill lamellae; **d**: histological section showing a plasmodium (P) that has engulfed gill lamellae and is responsible of the deformation of adjacent gill lamellae.

➢ **Gill arch infection**

Ovoid cysts of *M. pethericii* were found on the gill arch (Figures 5a-5b) of 25 specimens of *C. petherici* (41.7%). The cysts were arranged anarchically in the gill arch and measured 300 - 600 µm long × 270 - 390 µm wide. Up to 7 cysts can be numbered per gill arch. Histological examination shows that, the cysts of *M. pethericii* are implanted in the cartilaginous tissue of the gill arch (Figures 5c-5d). Cysts development is asynchronous (Figure 5c) and leads to mechanical compression of adjacent vessels (Figure 5c; arrowhead). The development of these cysts also leads to progressive destruction (Figure 5d; arrowheads) of the surrounding tissue.

- Stomach infection

The cysts of *M. pethericii* were observed on the stomach wall (Figures 6a-6c) of 63.3% of *C. petherici* examined. These ovoid cysts measured 180 - 235 µm long × 110 - 200 µm wide. Up to 22 anarchically arranged cysts were counted in a parasitized stomach. Histological examination reveals encrustation of the cyst in the glandular tissue (Figure 6d) and progressive destruction of the latter.

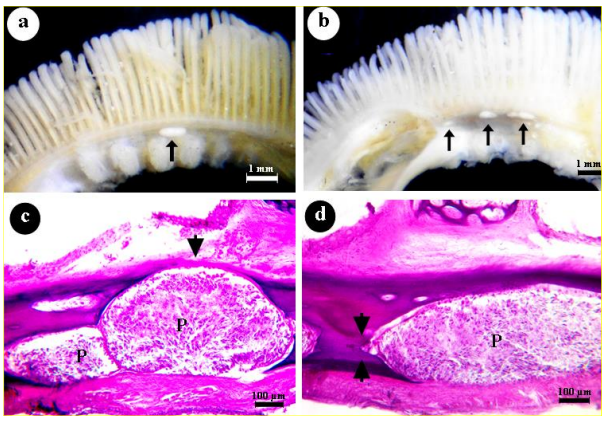

Figure 5: Microphotographs of gill portions of *Ctenopoma petherici* showing plasmodia of *Myxobolus pethericii* on the gill arch. **a-b**: gill portions showing plasmodia located in the gill arch (arrows); c-d: histological section revealing plasmodia (P) location in the cartilaginous tissue of the gill arch.

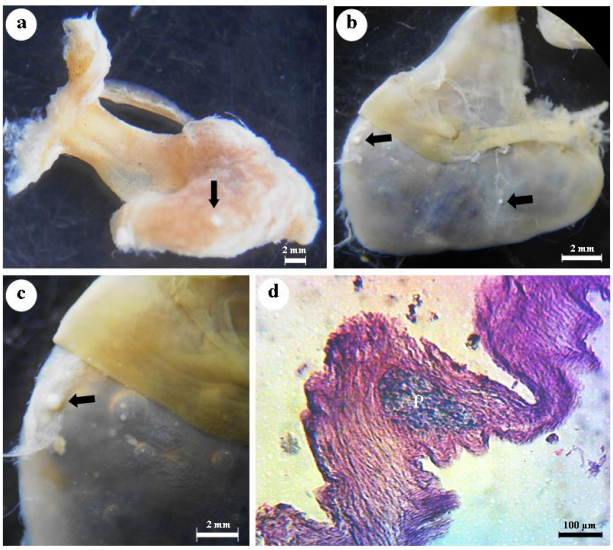

Figure 6: Microphotographs of the stomach of *C. petherici* parasitized by *M. pethericii*. **a-c**: plasmodia arranged anarchically on the stomach wall (arrows); d: histological section showing plasmodium (P) location in stomach glandular tissue.

- Intestine and mesentery infections

Numerous cysts of *M. pethericii* were macroscopically visible on the intestinal wall and mesentery (Figures 7a-b) of 40% of the *C. petherici* examined. These ovoid cysts measured 150 - 300 µm long × 135 - 150 µm wide on the intestinal wall, and 300 - 350 µm long × 145 - 180 µm wide on the mesentery. In a 3 cm section of parasitized intestine and mesentery, up to 13 and 43 cysts were counted respectively. The cysts were randomly distributed over these organs (Figures 7a-b). Histological analysis of unaffected (Figure 7c) and affected (Figure 7d) portions of the intestine revealed that the plasmodia of *M. pethericii* were developed in the circular muscle layer (Figure 7d). A plasmodium is surrounded by a layer of connective tissue and its development induces mechanical compression on the adjacent muscle tissue, even protruding (arrows) into the intestinal villi (Figure 7d). Histological examination of the parasitized portion of the mesentery (Figures 7e-f) shows cysts implanted in the walls of the mesentery vessels that irrigate the intestine (Figures 7e-f).

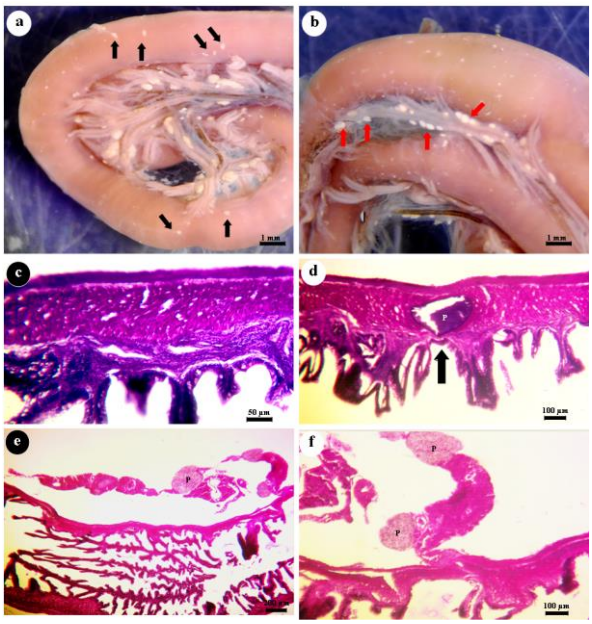

Figure 7: Microphotographs of portions of the intestine and mesentery of Ctenopoma petherici parasitized by *M. pethericii*. **a-b**: Plasmodia implanted on intestine (black arrows) and mesentery (red arrows); **c-f**: histological sections showing a healthy portion of the wall of intestine (**c**), plasmodium (P) located in the layer of circular muscle of the intestinal wall (d), plasmodium (P) located in the mesentery wall (**e-f**).

- Liver infection

Examination of *C. petherici* revealed that 66.7% of them had liver infected with *M. pethericii*. In parasitized individuals, this parasites species forms voluminous ovoid cysts (Figures 8a-b). Isolated or grouped, these cysts are anarchically distributed in the liver (Figures 8a-b) and measured 300 - 525 µm long × 275 - 325 µm wide. The cyst load may exceed 200 cysts per parasitized liver (Figures 8a-b). Observations made on the whole organ (Figures 8a-b) or on the histological section (Figure 8c) reveal that, in an infected liver, cysts are either attached to the periphery of the organ or completely implanted within it. Cyst development in the liver is asynchronous (Figure 8c). Unlike the non-parasitized liver (Figure 8d), in the parasitized liver, cysts are implanted at the vicinity (Figures 8e-f) and along a significant segment of the blood vessels (Figure 8f). These cysts cause mechanical compression, and distortion of adjacent blood vessels and hepatocytes (Figures 8e-f). In many *C. petherici*, a significant portion of the liver has been replaced by numerous and voluminous cysts of *M. pethericii* (Figures 8a-f), reducing the functional area of the infected organ.

- Gallbladder infection

From *C. petherici* examined, 5% were found carrying cyst of *M. pethericii* on the gallbladder (Figures 9a, b). These ovoid and elongated cysts measured 80 - 150 µm long × 50 - 90 µm wide. Up to 4 cysts, distributed anarchically over this organ, were counted on a parasitized host.

- Swimbladder infection

Ten specimens (16.6%) of *C. petherici* revealed the presence of cysts of *M. pethericii* on the swimbladder. These elongated or ovoid cysts, measured 212 - 290 µm long × 106 - 150 µm wide. In a parasitized fish specimen, 8 to 47 cysts can be counted and arranged anarchically on this organ (Figures 10a-b). Examination of histological sections revealed that, unlike the healthy portion (Figure 10c) of the gasbladder wall, the parasitized portion has *M. pethericii* cysts in the loose connective

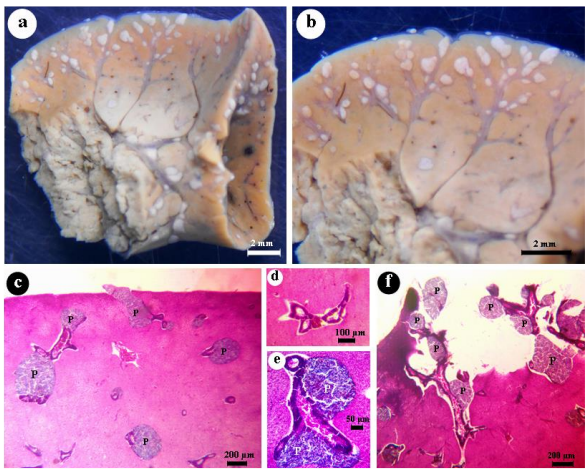

Figure 8: Microphotographs of liver of *C. petherici* parasitized by *M. pethericii*. **a-b**: voluminous and numerous white spot plasmodia implanted in the liver; **c-f**: histological sections of the liver illustrating asynchronous development of plasmodia (**c**), a healthy portion of the liver (**d**), location of plasmodia in the vicinity (**e**) and along a significant length (**f**) of blood vessels. P: plasmodia.

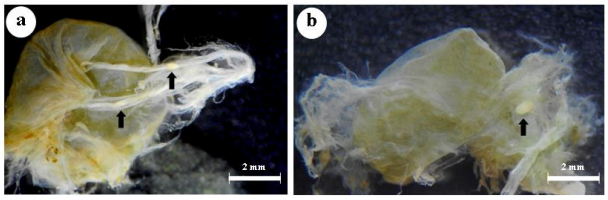

Figure 9: Microphotographs of the gallbladder of *C. petherici* parasitized by *M. pethericii*. **a-b**: large plasmodia (arrows) located in the gallbladder wall.

tissue between the outer tunica and the submucosa of the gasbladder (Figure 10d). The presence of the cyst leads to stretching and deformation of the outer tunic (Figure 10e).

- Ovaries infection

Examination of *C. petherici* revealed 41.6% female specimens with ovaries parasitized by *M. pethericii*. Ovarian infestation was bilateral and characterized by the presence of cysts or diffuses spores. Cysts were ovoid and measure 390 - 570 µm long × 200 - 270 µm wide. These cysts, isolated (Figure 11a; red arrows) or grouped (Figure 11a; black arrow), are anarchically implanted in the organ (Figure 11a).

Although it was impossible to determine the exact number of cysts in some cases when they were grouped together, in other cases 30 to 150 cysts per parasitized ovary were numbered. Histopathological analysis of the ovaries revealed implantation of the cysts in the oocytes (Figure 11b). Some plasmodia found in the ovaries completely filled the oocytes. When healthy (Figure 11b; red arrowheads) and infected (Figure 11b; black arrowheads) oocytes were compared, a structural difference was observed. In healthy oocytes, the yolk is present and occupies the entire oocyte content, in contrast to the infected oocyte in which the parasite occupies the entire internal cavity (Figure 11b). The oocyte may contain one (Figure 11c; red arrowhead) to four (Figure 11c; black arrowhead) cysts. The cysts develop asynchronously (Figure 11c).

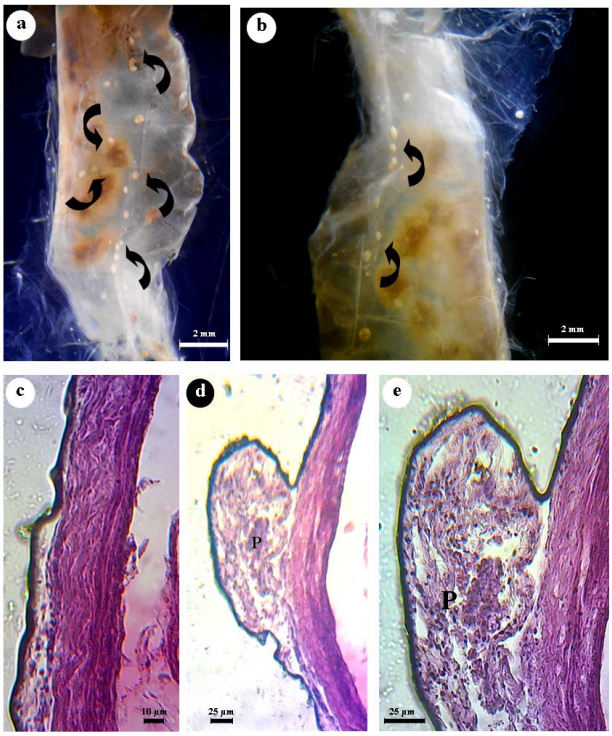

Figure 10: Microphotographs of plasmodia of *M. pethericii* on the swimbladder of *Ctenopoma petherici*. **a-b**: plasmodia (arrows) implanted on the wall of the swimbladder; **c-e**: histological sections illustrating a healthy portion of the wall of the swimbladder (**c**), location of plasmodia (P) in loose connective tissue (d) responsible of stretching and deformation of the outer tunica (**e**).

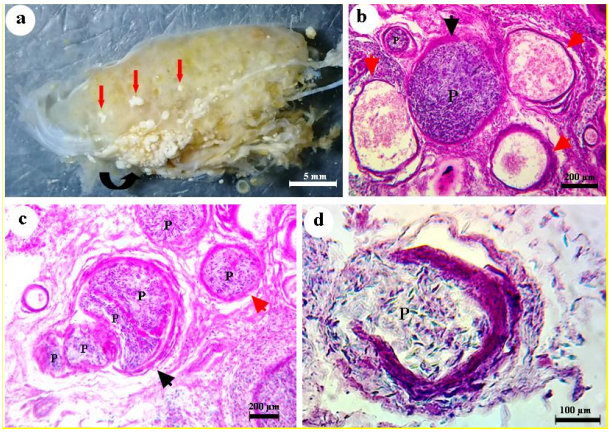

Figure 11: Microphotographs of plasmodia of *M. pethericii* in the ovary of *C. petherici*. a: isolated (red arrows) or grouped (black arrow) plasmodia implanted anarchically in the ovary; b: histological section of the ovary illustrating plasmodium (P) location within an oocyte (black arrowhead) surrounded by healthy oocytes (red arrowheads) ; c: histological section of the ovary showing asynchronous development of plasmodia (P) and oocytes containing one plasmodium (red arrowhead) and four plasmodia (black arrowhead); d: rupture of the wall of a mature plasmodium(P) releasing spores into the oocyte cavity.

Cyst development leads to oocyte hypertrophy (Fig. 11c) and rupture of the mature cyst wall results in the release of spores into the oocyte cavity (Figure 11d). It has also been observed that after rupture of the follicular

epithelium, spores are released into the ovarian interstitium (Figures 11b-d)

- Testes infection

After opening the abdominal cavity of *C. petherici*, 18.3% of male revealed the presence of cysts of *M. pethericii* in the testes (Figure 12a). Male fish infested with *M. pethericii* may harbor up to 7 cysts in the testes. Infection is bilateral. The spherical cysts are anarchically arranged in the organ and measure 344 - 660 µm long × 244 - 390 µm wide. Histological observations revealed that in the non-parasitized portion of the testis (Figure 12b), it was possible to observe the normal architecture of the seminiferous tubules where spermatogenesis take place. In parasitized fish, the plasmodia were located in the seminiferous tubule (Figure 12c). A plasmodium of *M. pethericii* found in the testis is surrounded by a thin membrane and does not induce any inflammatory reaction in *C. petherici* (Figure 12d).

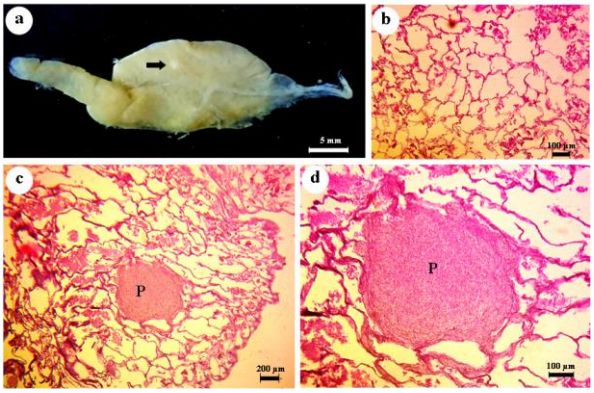

Figure 12: Microphotographs of the testis of *C. petherici* parasitized by *M. pethericii*. a: Plasmodia (arrow) implanted in the testis; b-d: histological sections illustrating a healthy portion of the testis (b) and the location of the plasmodia (P) in the seminiferous tubule (c-d).

- Operculum infection

In the population of *C. petherici* examined, 21.7% of individuals revealed the presence of cysts of *M. pethericii* in the inner surface of the operculum (Figure 13a). These small and ovoid cysts are 190 - 290 µm long × 90 - 200 µm wide. Infestation of the fish is bilateral. Up to 18 anarchically arranged cysts can be numbered per parasitized host individual.

- Internal cavity and bones infection

In a specimen of *C. petherici*, ovoid cysts of *M. pethericii* were found in the epithelial tissue lining the fish's internal cavity (Figure 13b). Cysts were also found in the bones (Figure 13c). These cysts are anarchically arranged in the bone and measured 150 - 300 µm long × 100 - 250 µm wide.

- Labyrinth organ infection

The labyrinth organ of five *C. petherici* specimens (8.3%) revealed the presence of *M. pethericii* cysts (Figure 13d). These cysts are ovoid, isolated or grouped, anarchically implanted in the organ, and measured 210 - 290 µm long × 100 - 150 µm wide. Although it was impossible to determine the exact number of cysts in some cases (Figure 13d) when they were grouped together, in other cases it was possible to count 1 to 8 cysts per parasitized organ.

- Esophagus infection

Two *C. petherici* specimens were found with cysts of *M. pethericii* on the esophagus wall (Fig. 13e). These cysts measured 80 - 250 µm long × 50 - 150 µm wide and were distributed anarchically. Up to 5 cysts can be numbered per parasitized organ.

- Heart infection

The cysts of *M. pethericii* were found on the heart (Figure 13f) of 20% of *C. petherici* examined. These ovoid cysts are 240 - 360 µm long × 150 - 250 µm wide. One to five cysts can be counted per parasitized organ.

- Kidney infection

The kidneys of nine specimens (15%) of *C. petherici* revealed the presence of cysts of *M. pethericii* (Fig. 13g). These ovoid cysts are anarchically arranged in the organ and measured 70 - 260 µm long × 40 - 120 µm wide. There are 1 to 3 cysts per parasitized kidney.

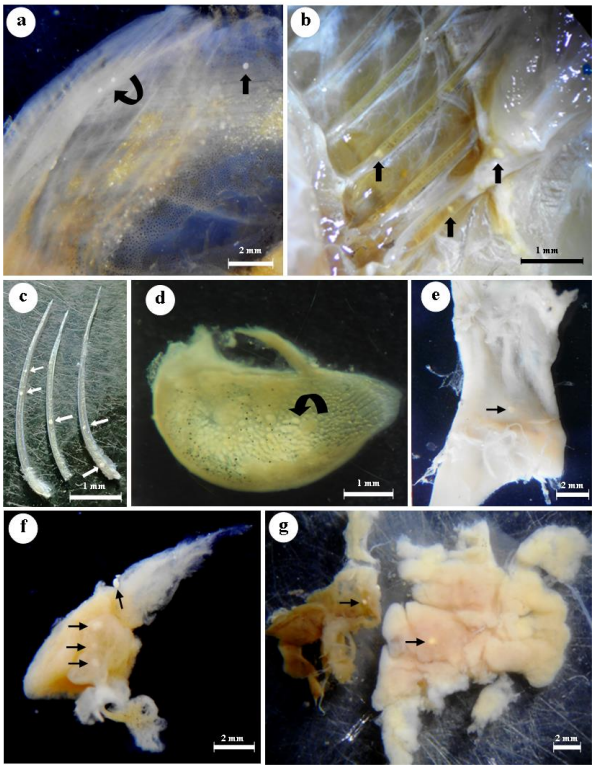

Figure 13: : Microphotographs of some organs or organ fragments of *C. petherici* parasitized by *M. pethericii*. Plasmodia (arrows) implanted in the inner face of the operculum (a); in the epithelial tissue lining the internal cavity of the fish (b), in the bones (c), in the labyrinth organs (d), in the wall of the esophagus (e), in the heart (f) and in the kidney (g) of the fish.

4. Discussion

Among myxosporidia, the genus *Myxobolus* is numerically the largest group. Up to 1027 of the approximately 2600 species of myxosporidia described worldwide [19] are known from the genus *Myxobolus* [20, 21, 22]. Morphometric observations revealed consistency in the characteristics of spores obtained from cysts found on infected organs. This suggests that the spores belong to a single species responsible for simultaneous infestations in several organs. The characteristics of this parasite's species correspond to the data provided by Fomena et al. [4] on *Myxobolus pethericii*.

Molnár [23] believes that species of the genus *Myxobolus* have relatively strict host and organ specificity. In the present study, the specificity of *M. pethericii* to *Ctenopoma petherici* corresponds to the above idea. However, cysts were found in numerous organs, suggesting that organ specificity is an insignificant factor compared with host specificity for the identification of *M. pethericii*. Lekeufack Folefack and Fomena [2] demonstrated that there is a variation in the prevalence and mean cyst

load of *Myxobolus pethericii* between organs in *C. petherici*. This finding suggests that the pathological significance of *M. pethericii*-induced changes would depend on the intensity of parasite colonization, cyst size, and type of organ affected.

The cysts of *M. pethericii* were found between the bony rays of the fin, and *C. petherici* showed no inflammatory reaction to their presence, as also reported by Molnár [12]. As the fish's fin is an organ of less vital importance, the presence of cysts of *M. pethericii* between the fin rays of *C. petherici* can only result in general weakness and slow swimming movements, which may ultimately render the host fish vulnerable to predation.

Infection of the skin by myxosporidia is rare but easily observable. *Myxobolus pethericii* forms conspicuous plasmodia on the skin of *C. petherici*. By forming large cysts on the skin of *C. petherici*, *M. pethericii* can reduce the market value of its host. Several species of *Myxobolus* found on skin and subcutaneous tissue have been reported as pathogenic. *Myxobolus drjagini* Akhmerov, 1954, responsible for whirling disease in *Hypophthalmichthys molitrix* in China, forms small plasmodia on the skin covering the operculum, head, and oral cavity of its host [24]. *Thelohanellus wuhanensis* Xiao and Chen, 1993, a serious pathogen in *Carassius auratus gibelio*, not only forms large cysts on the skin of affected fish but also causes the death of infected fry [25].

As revealed in the present work with the development of *M. pethericii's* cysts in the cornea of the eye, the presence of cysts of myxosporidia is common in the cornea and sclera in fish [26]. Although Muzzal [27] estimates that young specimens of *Perca flavescens* can die from ocular infection with *Myxobolus scleropercae*, we believe that even high-intensity ocular infection can lead neither to the death of *C. petherci* specimens nor to the complete destruction of the eye. However, corneal deformation in *C. petherci* can lead to myopia, hypermetropia, or other types of ocular pathology [28]. In addition, the presence of cysts in the cornea and cyst-induced swelling of the sclera can impair the eyesight of parasitized fish and increase their chances of being captured by predators. The influx of monocytes to the site of implantation of *M. pethericii* plasmodia in *C. petherici* would reflect the host phagocytosis reaction due to the presence of this parasite.

The gill of *C. petherici* has proved to be the organ most affected by *M. pethericii*, probably because this organ is a suitable site for spore release [29]. The development of *M. pethericii* in the gills corresponds to the intralamellar and cartilaginous types according to the classification of Molnár [12]. Intralamellar development is known to be the most common type of infection in gills, and several *Myxobolus* species show this type of development [20, 21, 22]. It has long been known that certain species of *Myxobolus* prefer the cartilage of the gill arch as developmental site for cyst [30]. However, there are few histopathological data on the species that parasitize this gill structure. Data from the present work indicate that *M. pethericii* can have a deleterious effect on its host by reducing the functional respiratory surface of the gills [12].

Although few species are exclusive digestive tract pathogens, members of several myxosporidia genera have been found in the digestive tract of fish. Almost 60 species of *Myxobolus* affect the digestive tract of their hosts [20, 21, 22]. Most of these species affect the lamina propria underlying the mucosal epithelium of the gut. The present study revealed the development of *M. pethericii* cysts within the circular muscle layer of the intestine, the glandular tissue of the stomach and the mesentery. This type of parasitic development leads to the replacement of a large surface of the digestive tract by cysts. Heavy colonization of the *C. petherici* digestive tract by cysts of *M. pethericii* can lead its muscles to tighten and narrow the tube, thus disrupting digestion [31]. Moreover, the presence of cysts on the mesentery impedes the transport of nutrients from the intestine to the bloodstream, with the immediate risk of fish morbidity.

Several species of the genus *Myxobolus* have been described in fish liver [32, 33, 34], but specific infection of the liver by species forming larger and numerous cysts is relatively rare. In many cases, infection of the liver is simultaneous with that of other organs and materializes through the presence of diffuse spores. To the best of our knowledge, no histological observations have been made in a liver affected by myxosporidia. The presence of vegetative stages in the liver of *C. pethericii* confirms that this organ is a normal site of sporogenesis for *M. pethericii*. This work has shown that *M. pethericii*, by developing numerous and voluminous cysts in the liver, induces important anatomical and physiological changes in *C. petherici*. Direct development of *M. pethericii* cysts directly on the outer layer of blood vessels can lead to partial or complete obstruction of the lumen of hepatic arterioles, resulting in severe functional disorders of the liver. Within the population of *C. petherici* examined, the cysts of *M. pethericii* were frequently located in the bile-collecting duct of the liver, and spores were diffused in the bile. According to Dyková and Lom [35], the developmental stages of myxosporidia found in bile originate in the hepatic vascular system, passing through the bile ducts to reach the gallbladder. The presence of *M. pethericii* spores in the bile can therefore be seen as a consequence of its development in the liver and the wall of the bile-collecting duct. Coelozoic myxosporidia infects the gallbladder, and many of them have been described without histological examination of the bladder or bile ducts. The presence of *M. pethericii* in the gallbladder would have a negative impact on the quality of the bile produced by *C. petherici*.

Myxobolus pethericii forms large plasmodia in the connective tissue of the swimbladder wall, corroborating the observations of Holzer and Schachner [36] that several species of myxosporidia form cysts in the swimbladder wall. No distinct inflammatory response was observed at the site of *M. pethericii* infection. The absence of a significant inflammatory response to *M. pethericii* reveals that its plasmodia are not exposed to the host immune system. However, massive infestation followed by cyst rupture can lead to a vigorous inflammatory response and bladder dysfunction, with the direct consequence of impaired swimming performance and eventually fish death.

Infections by myxosporidia of the genus *Myxobolus* have not been described very often in gonads as in other organs [20, 21, 22]. It appears from this study that, by infecting the ovaries and testes of *C. petherici*, *M. pethericii* does not express host sex specificity. Although this is not the case with *M. pethericii*, sex specificity of a parasite species is thought to be a consequence of the parasite's specific needs, as oocytes and spermatozoa differ in composition and volume [37]. In the ovary of *C. petherici*, the increase in cyst size and number due to the maturation and multiplication of *M. pethericii* spores induces a lytic action of the oocyte. This occurs because the parasite is conveniently located inside oocytes that are full of nutrients and where the host immune response is absent [37]. The presence of *M. pethericii* in the seminiferous tubules of *C. petherici* can lead to testicular hypertrophy, occlusion of the tubular lumen, and damage to Sertoli cells [38]. Infection of the oocyte or seminiferous tubule by *M. pethericii* appears more dangerous than infestation of the ovarian or testicular stroma [37, 39, 40]. The data from the present study suggest that cyst production by *M. pethericii* in the reproductive organs of *C. petherici* makes this myxosporidia a potential dangerous species capable of negative impact on the survival of this Anabantid fish in the Sangé River. Sitjà-Bobadilla [37] indicate the importance of regular checking of myxosporidia in wild and farmed fish,

particularly those species that cause damage to the host gonads, as they can affect the host's reproductive capacity through castration [41].

The kidney, which receives the vast majority of post-branchial blood, should receive all pathogens present in the blood. At least 200 (≈19.5%) species of myxosporidia belonging to the genus *Myxobolus* have been described in fish kidneys. This organ is therefore a frequent infection site for *Myxobolus*; but it is still difficult to provide accurate diagnosis to known whether the spores were produces in the kidney or whether they have been transported there by the bloodstream and captured by macrophages [3]. Many authors believe that kidneys only act as a deposition site for Myxosporidia species, where spores from the bloodstream are collected, retained, and destroyed in melano-macrophage centers [16, 42, 43]. The present study revealed the presence of well-developed cysts of *M. pethericii* in the kidneys of *C. petherici*, demonstrating that sporogenesis effectively takes place in this organ. Molnár [16] named five specific sites for the development of myxosporidia in kidneys. Without any histopathological description, it is impossible in this work to speculate on the potential type of damage caused by *M. pethericii* in kidneys and other organs of *C. petherici* in which histological sections could not be done.

5. Conclusion

The present study reveals the adverse effects of *Myxobolus pethericii* on *Ctenopoma petherici* in a natural environment. Histopathological changes show that a high parasite load could compromise the function of affected host organs. In *C. petherici*, tissue surrounding the cysts may undergo hyperplasia, swelling, alteration and lack of adhesion between it layers. Likewise, cysts of *M. pethericii* are also responsible for mechanical compression and distortion of blood vessels surrounding affected tissue. The presence and dispersion of *M. pethericii* needs to be systematically monitored in fish farms.

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