

Histological studies on the effects of *Clinostomum marginatum* (Digenea, Clinostomidae) in its second intermediate host *Loricariichthys platymetopon* (Osteichthyes, Loricariidae) of the upper Paraná River, Brazil

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ABSTRACT. The histopathology of the infection of *Loricariichthys platymetopon* (Osteichthyes, Loricariidae) by *Clinostomum marginatum* (Digenea, Clinostomidae) is described in this study. The parasites were found encysted in the base of the fins, muscle, inner wall of the operculum, lips, gill arches, palate, bone plaques and eyes, forming small white-yellowish nodules. In all the locations the parasites were surrounded by a fibrous capsule consisting of a layer of dense connective tissue followed, in most of the cases, by a layer of loose connective tissue. The thickness of these layers varied with the location of the parasites. In some cases, infiltration by lymphocytes and macrophages within the connective layers was observed. When the parasites encysted under the integument a decrease of the epidermis height and disappearance of the dermis were also observed. The fat tissue near the cysts was invariably destroyed in a greater or lesser extent, and replaced by cell fragments in conjunction with granular material. In some cases this feature could be observed in places a little further away from the cysts and not only in their vicinity. Furthermore, in some cases digitations of destroyed fat tissue extending within the normal tissue were present. When the parasites encysted within the muscle tissue a slight degeneration of the muscle fibers was sometimes observed. In the eyes the parasites were encysted in the sclera, being enveloped by connective tissue, and causing deformity of the eyeball.

Key words: *Clinostomum marginatum*, Digenea, *Loricariichthys platymetopon*, fish, histopathology, Brazil.

RESUMO. Estudos histológicos sobre os efeitos de *Clinostomum marginatum* (Digenea, Clinostomidae) no segundo hospedeiro intermediário *Loricariichthys platymetopon* (Osteichthyes, Loricariidae) do alto rio Paraná, Brasil. Descreve-se a histopatologia da parasitose de *Loricariichthys platymetopon* (Osteichthyes, Loricariidae) por *Clinostomum marginatum* (Digenea, Loricariidae). Os parasitas foram observados encistados na base das nadadeiras, músculo, face interna do opérculo, lábios, arcos branquiais, palato, placas ósseas e olhos, formando pequenos nódulos branco-amarelados. Em todos os locais, os parasitas estavam circundados por uma cápsula de tecido conjuntivo denso seguida, na maior parte dos casos, por uma outra de tecido conjuntivo frouxo. A espessura desta cápsula variou com a localização dos parasitas e, em alguns casos, observava-se a presença de linfócitos e macrófagos. Quando os parasitas encistavam junto do tegumento, verificava-se uma redução da altura do epitélio, bem como o desaparecimento da derme. O tecido adiposo próximo dos cistos estava, invariavelmente, destruído em maior ou menor grau, por vezes em regiões um pouco afastadas dos cistos e não apenas na sua proximidade. Quando os parasitas encistavam no músculo, uma ligeira alteração das fibras musculares podia ocorrer. Nos olhos, os parasitas estavam encistados na esclerótica, estando envolvidos por tecido conjuntivo e causando deformação do globo ocular.

Palavras-chave: *Clinostomum marginatum*, Digenea, *Loricariichthys platymetopon*, peixes, histopatologia, Brasil.

There are a number of histopathological studies concerning the infection of fish by Digenea (Heckmann *et al.*, 1986; Hoffmann *et al.*, 1991; Lester, 1980; Lo *et al.*, 1980, 1981; Perera, 1992). For Brazilian fishes the pathology of the infections by *Nematobothrium scombrii*, *Ithyoclinostomum dimorphum* and *Microrchis oligovitelum* was described respectively by Eiras and Rego, 1987, and Pavanelli *et al.*, 1990, 1997.

Several species of the genus *Clinostomum* (Digenea, Clinostomidae) are frequent parasites of fishes which are the second intermediate hosts harbouring the encysted metacercariae in several organs (Lo *et al.*, 1980, 1981, 1982), while piscivorous birds are the final host. *Clinostomum marginatum* is a frequent parasite of the Loricariidae fish *Loricariichthys platymetopon* from the Paraná river at Brazil. According to Thatcher (1993), its definitive hosts in the neotropical region include bird species of the genus *Ardea*, *Jabiru*, *Cochlearis*, *Casmerodius*, *Nictanassa*, *Phalacrocorax*, *Anhinga*, *Nycticorax* and *Butorides*. In this paper a study on the histopathology of the infection in its second intermediate host, *L. platymetopon*, is reported.

Materials and methods

Four hundred and forty-five specimens of *Loricariichthys platymetopon*, *cascudo-chinelo*, were net fished from the floodplain of the Paraná River at Lagoas da Figueira, Pousada das Garças and Ressaco do Leopoldo, Brazil. The specimens were immediately transported to the laboratory, and a thorough examination of all the organs was made in order to detect the encysted parasites. The tissue samples containing the encysted metacercariae were fixed in buffered formalin, routinely processed for histology and stained by Haemalumen and Eosin (H&E).

Results

Three hundred and eleven out of the 445 (69,9%) specimens of *L. platymetopon* were parasitised by *C. marginatum*. The average intensity value of the infection was 30.3 cysts per host, varying between 1-218 cysts. The parasites were found encysted in the base of the fins, muscle, inner wall of the operculum, lips, gill arches, palate, upper jaw and eyes, forming small 2-3mm diameter white-yellowish nodules, easy to detect in macroscopical observation, each containing one parasite. The operculum and mouth were the most common locations of the parasites. In the fins, the parasites were frequent in dorsal, anal and pelvic fins, while only very rarely they were present in the caudal fin.

In all the organs examined the parasites were surrounded by a more or less developed concentric layer of dense connective tissue, followed or not by a layer of loose connective tissue.

In the gill arches the dense connective tissue capsule surrounding the parasites could be very thick (about 65-70 μm), the fibroblast being densely disposed, or thin (7-10 μm), these two different features being sometimes observed in close cysts (Figure 1a). In the first case, there was an intimate contact between the capsule and the parasite in most of its surface. Loose connective tissue was not observed. The fat tissue near the cysts was intensely destroyed. Its cells were completely broken and reduced to pieces, and the typical architecture of the tissue was changed into an irregular mass containing a great amount of cell remnants and debris. The fat cells beyond this zone had a normal appearance. This destruction was not limited to the vicinity of the cyst. Extensive and ramified regions of destruction of the fat tissue were observed irradiating from the cyst zone, extending the tissue destruction to places further away from the cyst (Figure 1b).

In the fins the parasites were located near their base. They were surrounded by a thin concentric layer of dense connective tissue of variable thickness (about 3-10 μm), followed by a layer of loose connective tissue whose thickness varied between 10-125 μm . Macrophages were present in this layer. The fat cells near the cyst were destroyed in a very small extent. However, the remaining fat cells presented an irregular and somewhat shrunken outline (Figure 1c). The inner wall of the dense connective tissue layer was in close contact with the parasite, apparently this contact being favoured by the small spines at the parasite surface (Figure 1d). The muscle fibers near the capsule were distorted, and a slight degeneration of the fibers was observed.

Within the muscle tissue the dense connective tissue capsule enveloping the parasite was thin (about 5-12 μm thick) and followed by a thin layer of loose connective tissue, which was separated from the muscle cells by fat tissue. In some cases, the fat cells were destroyed, as well as some muscle cells which were close to the parasite, and a considerable area of destroyed fat cells mixed with muscle fibers remnants and amorphous material was observed (Figure 2a,b). In other cases, no structural changes were observed in both fat and muscle tissues, which presented a normal structure.

In the inner wall of the operculum the parasites were surrounded by a concentric thin layer of dense connective tissue (about 10-15 μm thick). The fat

tissue was destroyed in a variable extent, showing places where the destruction was minimal to others where it was more intense and extensive. In this case, the cells were broken, the tissue desegregated and replaced by amorphous material and cell remnants (Figure 2c). The epidermis overlaying the cyst presented a reduction of its height (about 20-25 μm thick in contrast to the usual values of 45-85 μm), sometimes being reduced almost to the basal layer (Figure 2c). The dermal layer showed a progressive reduction, and in the places overlaying the cysts it had completely disappeared (Figure 2c). The inner surface of the connective capsule showed the presence of more or less long and very thin filiform projections, sometimes tuft-like, which were presumably caused by the abrasive effect of the surface spines of the parasite upon the connective cells (Figure 2d).

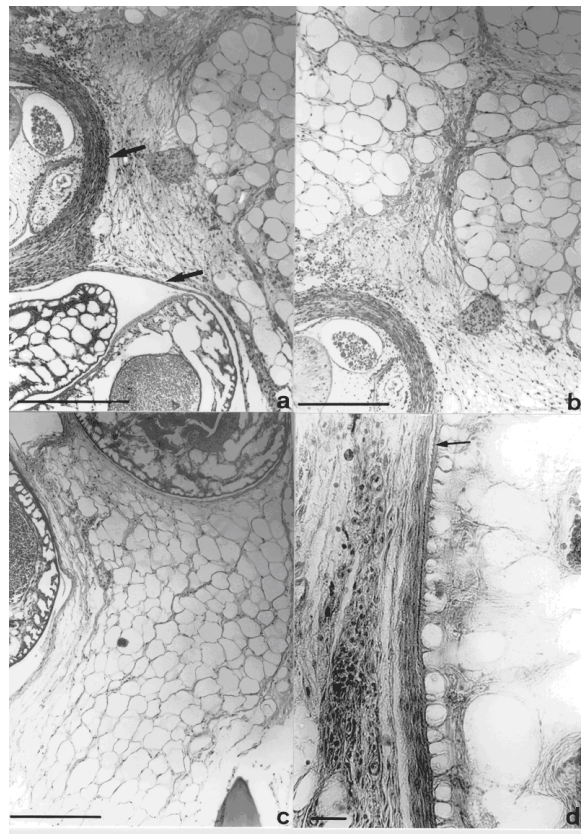


Figure 1. Photomicrographs of encysted *C. marginatum* in *L. platymetopon*. a: parasites in the gill arch surrounded by different sized layers of connective tissue (arrows), and destruction of the fat tissue; b: parasites in the gill arch showing ramified zones of necrosis extending into the fat tissue; c,d: parasites in the fins showing necrosis of the fat tissue and irregular outline of the fat cells, and the close contact between the parasite and the host connective tissue favoured by the parasite spines (arrow). H & E. Bars = 250 μm (a,b,c) and 12.5 μm (d).

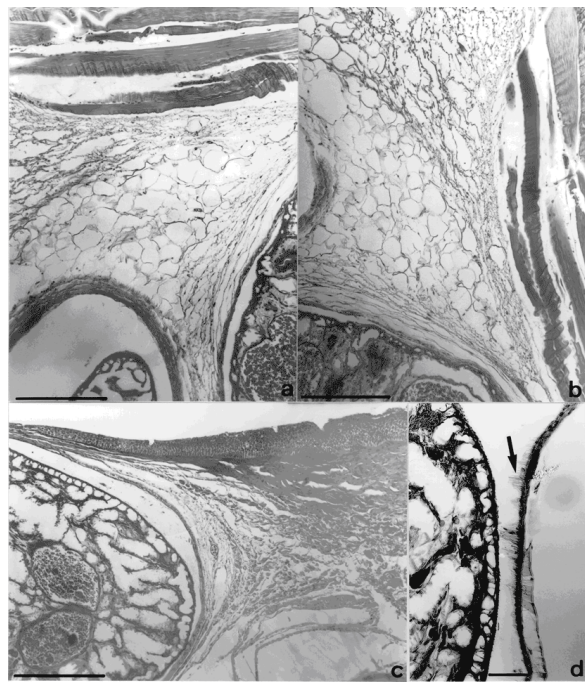


Figure 2. Photomicrographs of encysted *C. marginatum* in *L. platymetopon*. a, b: parasite in the muscle depicting extensive necrosis of fat tissue; c: parasite in the inner wall of the operculum – note the reduction of the height of the epidermis, progressive disappearance of the dermis, and fat cells destruction; d: filiform projections of the inner wall of the connective tissue capsule surrounding the parasite in the operculum (arrow). H&E. Bars = 250 μm (a,b,c) and 12.5 μm (d).

In the lips the parasites were immediately below the integument and surrounded by a thin (about 12 μm thick) concentric layer of connective tissue presenting intermediate characteristics between dense and loose connective tissue. Its internal limits were clearly defined, while externally they were sometimes difficult to distinguish. In several places of the parasite surface there was deposition of fine granular material. The integument above the parasitic cyst was thinner than normal, and reduced to a thin epidermis (about 20 μm thick in contrast to 60 μm in not parasitised places). Destruction of the fat cells as described above was present (Figure 3a).

In the parasites located in the upper jaw the surrounding layer of connective tissue presented intermediate characteristics between dense and loose connective tissue, having a thickness of about 15-35 μm , its exterior limits being imprecise. The fat tissue near the cyst was destroyed as in the organs mentioned above. However, that destruction was more intense and extended over a region equivalent to about 4 times the cyst area (Figure 3b).

In the palate, the parasites were also located immediately below the integument and surrounded

by a connective tissue layer (15–25 μm thick) which presented a variable density of fibers deposition, and overlaid by a thin epidermis. The close fat cells were destroyed as described above. In other cases, the connective layer was much thinner (about 3 μm thick) and, in that case, the fat cells were not necrotic but presented an irregular and shrunken outline (Figure 3c).

In the eye the parasites encysted in the sclera, in a lateral position near the cornea. They were surrounded by connective tissue, its fibers being more densely disposed in some places than in others, forming a capsule which was connected with the sclera. In this region the sclera presented a height reduction, and was partially destroyed, originating fibrillar amorphous material interspersed between the parasite and the sclera. The volume of the parasite caused eyeball distortion, which was flattened anteriorly, and presented an hernia-like formation towards the parasite (Figure 3d).

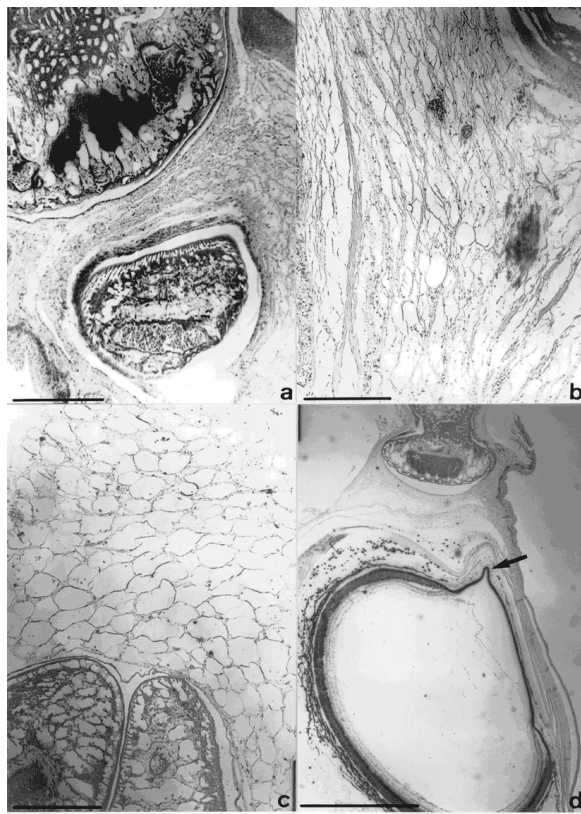


Figure 3. Photomicrographs of encysted *C. marginatum* in *L. platymetopon*. Parasite encysted in the lip (a) and upper jaw (b) showing destruction of the fat tissue; c: parasite in the palate, surrounded by a thin connective layer - note the irregular outline of the fat cells; d: parasite encysted in the eye causing flattening of the eyeball and hernia-like formation towards the parasite (arrow). H&E. Bars = 250 μm (a,b,c) and 1.2 mm (d)

Discussion

The prevalence values clearly show that the infection by *Clinostomum* spp. metacercariae in *L. platymetopon* is high. This is very likely due to the behaviour of the host. *Loricariichthys platymetopon* is a benthic species, therefore the infecting cercariae reach it much more easily.

The fact that the mouth and operculum were the most common locations of the parasites is probably due to the water current related to breeding activity, therefore facilitating the contact with the invasive cercariae. On the other hand, the low value found for the infection in the caudal fin, as compared with the other fins, is likely to be due to the more intense movements of the anal fin, therefore difficultating the penetration by the cercariae.

The host reaction to the parasite seems to be the same independently from the affected organ. All the parasites were found surrounded by well-defined fibrous layers. The variation of the thickness of this fibrous capsule may reflect the time of infection, being thicker in older parasites. Lo et al. (1982) found the encysted metacercariae of *Clinostomum complanatum* on *Plecoglossus altivelis* surrounded by a very thin cyst wall, about 2 μm thick, composed by a rather thin inner layer which was considered of parasite origin, and an outer layer similar to the host connective tissue. In our material, a layer of parasite origin was never observed.

The fatty changes observed in the different organs is not a common feature of Digenean metacercariae infections. Similar fatty changes were observed in the infection of *Haplochromis* spp. and *Bagrus docmac* by *Eustrongylides* sp (Paperna, 1974). To determine if this feature is peculiar of infections caused by *Clinostomum* metacercariae rather than due to a specific tissue reaction on a particular host, it would be necessary to verify if this feature is present in other host fish species. The variable extent and intensity of fat tissue destruction is also probably related to the duration of the infection.

For a long time it has been discussed that metacercariae may change the behaviour of the fish, as the second intermediate host, enhancing the chance that it will be eaten by the final host (Szidat, 1969). This adaptive strategy may be reached by causing the blindness of the fish which therefore are more susceptible to predation by piscivorous birds. In our specimens the location of the metacercaria on the eyes is not supposed to induce blindness. However, and due to the flatness of the cornea region and distortion of the eyeball, it is likely that visual acuity is more or less affected and may play an important role as a mechanism facilitating predation

by birds. Another factor that may change the behaviour of the fish, as the second intermediate host is the slight degeneration of the muscle fibers and the destruction of the fat cells in the fins. This fact makes the movements slow and also facilitates the predation.

The overall effects of the parasite upon the host condition seems to be not very detrimental. The parasites are encysted in limited places, and its repercussion on the host tissue is limited to the cyst vicinity. A serious deleterious effect is likely to occur, especially in small fish, only in the case of very high infections. However, Heckmann *et al.* (1986) found that wild fish with several hundreds of encysted metacercariae of *Posthodiplostomum minimum* in the liver, sinus venosus, heart and kidney, were often observed to suffer no obvious debilitating effects. On the other hand, Lo *et al.* (1982) postulated that when a large amount of cercariae of *C. complanatum* penetrate the fish body at the same time they may cause irritation and lesions to fish tissue, which may induce mortality of small fish. Furthermore, the same authors found that when *Plecoglossus altivelis* specimens were infected with *C. complanatum* metacercariae they became weak and grew more slowly than healthy fish, and always died during transportation (Lo *et al.*, 1982).

The histopathology of the infection caused by metacercariae of Clinostomidae parasitizing other species of fishes have been analyzed and will be present in further papers.

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Received on May 19, 1999.

Accepted on May 31, 1999.