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Redescription and pathological effects of myxozoan parasite *Thelohanellus ophthalmicus* Halder et al., 1983 infecting *Glossogobius giuris.*

Redescripción y efectos patológicos del parásito mixozoario *Thelohanellus ophthalmicus* Halder et al., 1983 que infecta a *Glossogobius giuris*.

N.J. NANDINI [1*] , SINY G BENJAMIN [2] , P. NATARAJAN [3]

*1Department of Zoology, University College, University of Kerala, Thiruvananthapuram, Kerala

[2]Department of Zoology, All Saints'College, University of Kerala, Thiruvananthapuram, Kerala

[3]Aquatic Sciences and Aquaculture, College of Natural and Computational science, Hawassa University, Ethiopia

Corresponding author's e mail: nandininnj@gmail.com

ABSTRACT

Myxozoan parasites cause serious diseases and massive destruction of fishes in aquaculture and natural populations. During the present study, *Thelohanellus ophthalmicus* infecting the skeletal muscle of *Glossogobius giuris* has been described. The histopathological alterations indicated that the muscle parasite *T. ophthalmicus* is potentially pathogenic to the host fish *G. giuris* and high parasite load could compromise body functions. The presence of two types of plasmodia was established during the study. The presence of plasmodia without encapsulation was very rare in myxozoan infection. The multiple plasmodia with single continuous encapsulation were reported for the first time from India. The pathological importance of changes caused by myxozoan parasites greatly depend on the intensity of parasite colonization, the size of plasmodia and the number of spores.

Key words: Myxozoan parasite, *T. ophthalmicus,* Skeletal muscle, Histopathology

RESUMEN

Los parásitos mixozoos causan enfermedades graves y destrucción masiva de peces en la acuicultura y en las poblaciones naturales. Durante el presente estudio, se ha descrito que *Thelohanellus ophthalmicus* infecta el músculo esquelético de *Glossogobius giuris*. Las alteraciones histopatológicas indicaron que el parásito muscular *T. ophthalmicus* es potencialmente patógeno para el pez huésped *G. giuris* y una alta carga parasitaria podría comprometer las funciones corporales. Durante el estudio se estableció la presencia de dos tipos de plasmodios. La presencia de plasmodios sin encapsulación fue muy rara en la infección por mixozoos. Los plasmodios múltiples con encapsulación única continua se informaron por primera vez en la India. La importancia patológica de los cambios causados por parásitos mixozoos depende en gran medida de la intensidad de la colonización del parásito, el tamaño de los plasmodios y el número de esporas.

Palabras clave: Parásito mixozoario, *Thelohanellus ophthalmicus*, Músculo esquelético, Histopatología

INTRODUCTION

The pathogenicity of myxozoan parasites depends largely on the outcome of the dynamic interaction of the parasite and its host. Myxozoan parasites harm fishes by parasiting organs and tissues resulting in strange external appearances and deformalities. They form cream colored plasmodia and its size may vary depending upon the tissue they infect and also on the myxozoan species (Ahmad & Kaur H, 2018, Székely *et al*., 2021). *Thelohanellus ophthalmicus* are histozoic parasites and their cysts are usually arranged with the longer axis of the muscle bundles of the host. The cysts of different sizes are` visible to the naked eye. This strange external appearances and deformations cases the host fish to lose their high commercial value. This species is recorded for the second time from Kerala. The histopathology of *T. ophthalmicus* provided very important and useful data concerning changes in cellular and sub cellular structure of skeletal muscle much earlier than external notification. Studies on pathology associated with infection by myxosporidian parasites were carried out (Ogawa *et al*., 1992, Yokoyama *et al*., 1996, Viozzi *et al* Viozzi and Flores, 2003, Lonshow *et al*., 2005, Gupta and Kaur 2017, Kaur and Katoch, 2016, Kaur *et al*., 2017, Saha and Bandopadya, 2018.

MATERIALS AND METHODS

During the present study a total of 355 host fishes *G. giuris* were examined for one year from Veli lake. It is one of the prominent lakes in Kerala, which is located in the southern part of Kerala. Out of 355 fishes 214 fishes were found to be infected with myxozoan parasites. Prevalence was calculated according to Bush *et al*. (1997). The fishes were autopsied and vital organs were examined microscopically for the presence of myxosporidian parasites and cysts. For histological studies, the skeletal muscle infected with *T. ophthalmicus* was fixed in 10% buffered formalin. They were made in to blocks. Sections of 5-7 µm thickness were routinely stained with haematoxylin/eosin. Stained sections were studied under a compound research microscope. Microphotography was made employing Leica DMLS microscope using Leica DFC 295 camera.

RESULTS AND DISCUSSION

The current study examined a total of 355 fishes from Veli lake, located in the southern part of Kerala, India. Out of which 214 fishes were found to be infected with myxozoan parasite. Prevalence of infection was recorded to be 60.28%. The cysts of *T. ophthalmicus* were found distributed throughout the body of the host. The distribution of cysts in the host body was presented in Fig. 1-2. On the basis of the details of spores (Fig. 3-8) and measurements listed in Table 1, the myxozoan parasite found in the skeletal muscle of *G. giuris* was identified as *T. ophthalmics* (Halder *et al*., 1983).

Thelohanellus ophthalmicus (Halder *et al*., 1983) Phylum: Myxozoa

Class	: Myxosporea
Order	: Bivalvulida
Sub order	: Platysporina
Family	: Myxobolidae
Genus	*: Thelohanellus* (Kudo 1983) Species *: ophthalmicus* (Halder *et al*., 1983)

Table 1 Measurements of *T. ophthalmics* spores (µm)

Character	Range	Mean
Spore body		
Length	11.34 - 12.96	12.15
Width	4.86 - 7.29	6.50
Thickness	4.86 - 6.48	5.80
Polar capsule		
Length	4.86 - 7.29	6.31
Width	3.07 - 3.84	3.22
Polar filament		
Length	48.60 - 61.56	52.32

Description

Plasmodia: Elongated, cylindrical, milky weight, upto 10mm in length, occurred on the muscle throughout the body. They were arranged with the longer axis of cyst coinciding with the long axis of muscle bundles. These polysporic cyst like plasmodia contained only fully formed spore. Spore development stages were not found.

Spore: Spores pyriform with tapering anterior end and border rounded and posterior end in valvular view (Fig. 3-8) lenticular in sutural view (Fig. 3-6). The spore valves smooth symmetrical, uniformly thick m*et al*ong a prominent sutural ridge. Polar capsule single, pyriform, occurred on the anterior end of the sspore. A small dense body was often found associated with the posterior region of the polar capsule and seemed to be capsulogenous nucleus. A polar capsule contained 6 to 8 turns of polar filaments. The polar filaments extruded through the anterior end was thin, tube like and uniformly thick (Fig. 7-8). Sporoplasm was finely granular, cup shaped, occupied the posterior region of the spore and contained two round sporoplasmic nulei in the centre and an iodinophilous vacuole below it. No mucus envelope was detected around the spore.

Thelohanellus ophthalmicus was first reported by Haldar, Das and Sharma in 1983 from the internal musculature as well as from the sclera of eye of Catla catla in Nadia, Krishna Nagar, West Bengal, India. The myxosporidian observed from the muscle G. giuris during the course of the present investigation is similar to *T. ophthalmicus* in its spore characters except for slight morphometric differences.

This by itself is not significant enough to differentiate the two forms. This could be due to the host variation of the species. This myxosporidian is therefore identified and reported here as *Thelohanellus ophthalmicus* Halder, Das and Sharma, 1983. This species is recorded for the second time from Kerala.

Histopathology

Histological observation of the skeletal muscle infected with *T. ophthalmics* shared the presence of two types of plasmodia viz. interfibrilar and intrafibrilar. The parasites formed ovoid or elongated plasmodia between the muscle fibres (interfibrilar). The interfibrilar plasmodia were located within the host connective tissue lining (Fig. 9). Plasmodia within the host muscle fibres (intrafibrilar type) were rare and invisible to the naked eye (Fig.10). The sarcoplasm was eventually replaced by the intrafibrilar plasmodia. The interfibrilar plasmodia were found to occupy the entire muscle layer. The developed plasmodia fractured the muscle layer and replaced it in the infected area. Sporogony was asynchronous and vegetative developmental stages were found towards the periphery of plasmodium with mature spores in the centre of plasmodim (Fig.11). Growth of plasmodia resulted in marked enlargement of infected muscle fibres and pressure atrophy of adjacent myofibrils (Fig.12). The histopathological study showed relatively young plasmodia with few hundred spores to large and matured plasmodia with thousands of developed spores (Fig. 13). No developmental stages were observed in fully developed plasmodium; only the mature spores were thickly or loosely aggregated inside the plasmodia (Fig 14). No host response was noted towards developing plasmodial stages. After completion of spore formation, host reaction was induced. The infected myofibrils were destroyed and in some cases remnant of myofibrils were observed within the inflammatory tissue (Fig. 15). Inflammatory responses included vacuolation of muscle fibres (Fig. 16) and degeneration of muscle fibres (Fig. 17). Pathological findings included atrophy of muscle bundles (Fig. 18), edema between muscle bundles (Fig. 19) and splitting of muscle fibres (Fig. 20). Infected muscle fibres had consistently undergone necrosis (Fig. 21). Granulomatous tissue was noticed in the vicinity of fractured plasmodium (Fig. 22).

Histopathological sections revealed the presence of some unusual interfibrilar plasmodia. Both the usual and unusual types were often found in the same individual. In unusual plasmodia, the encapsulation of host tissue was not uniform and not complete. The encapsulation was found to be irregular and discontinuous in some regions (Fig. 23). At these regions, the parasite is in direct contact with the host muscle tissue. In some cases, the encapsulation was absent and ectoplasm grew irregularly (Fig. 24). An unusual occurrence of more than one plasmodia with a continuous single encapsulation was found (Fig. 25).The histological changes associated with the infection of T ophthalmics indicated marked pathogenicity in the host fish G. giuris. Heavy infection of the parasite in the musculature of host disrupted the anatomical and functional unity of muscle fibres. The present histopathological studies showed infection with small and large plasmodia in the skeletal muscle fibres. The extremely large number of myxozoan cysts in the muscle may be due to the depressed immune system of the host fish (Cone *et al*., 1997). It is evident from the present study that, host responses to the parasite are usually not initiated until sporogenesis of the myxospores is complete.

On completion of sporogenesis spores are subjected to a vigorous granulomatous response. These results are consistent with the earlier reports on muscle infecting Myxobolus spp. of juvenile cyprinids (Longshaw *et al*., 2005).

The infection in the musculature clearly showed muscle destruction not only of the infected muscle fibre but also in the surrounding tissues. In the present study, some plasmodia showed hypertrophy of the enveloping epithelia, which might be an attempt of this layer to accommodate the enlarged plasmodial mass. Similar mechanism of infection by Myxobolus fahmii in the gills of *Barbs bynni* was also reported (Ali *et al*., 2002). From the present study, the major factor contributing to tissue changes is intensity of infection. Mild infection provokes minor tissue and cellular reaction and high parasitic load results in extensive histopathological changes (Kuperman *et al*., 2001). The present study revealed the presence of two types of plasmodia in the skeletal muscles of the host fish. The presence of plasmodia with incomplete encapsulation appears to be rare among myxozoan infections in fishes and it seems likely that the parasite is quite new to the host and that the host-parasite relationship is still unstable (Viozzi and Flores, 2003). The present investigation first reported the presence of plasmodia without or incomplete encapsulation and multiple plasmodia with continuous encapsulation from India.

Fig. 1

Fig.2

Distribution of histozoic cysts of *T. ophthalmicus*

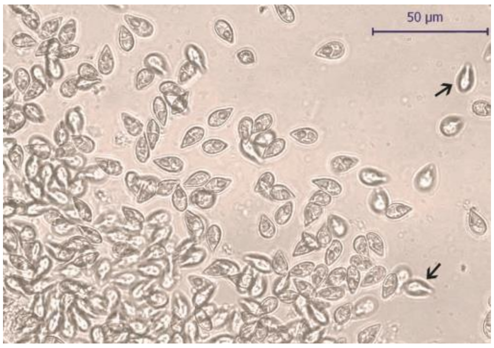

Fig. 3

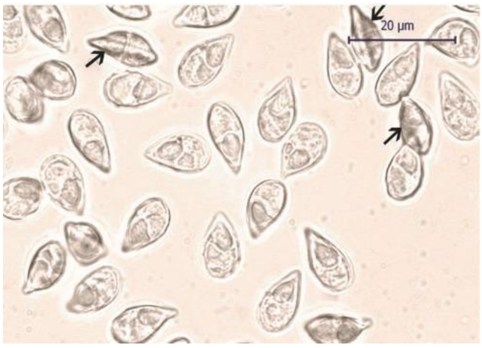

Fig. 4

T. ophthalmicus, Spores- valvular and sutural view (arrows)

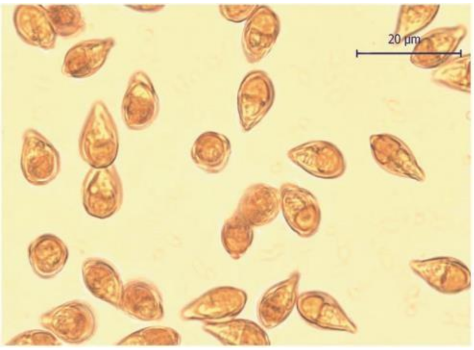

Fig. 5

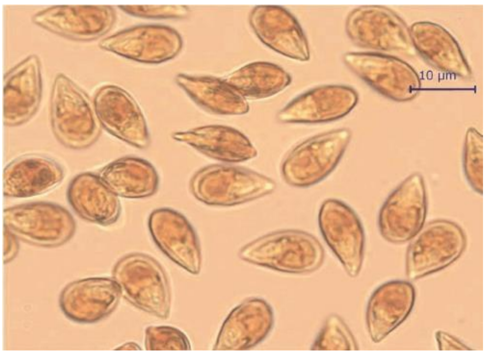

Fig. 6

T. ophthalmicus, Spores- valvular and sutural view (giemsa stained)

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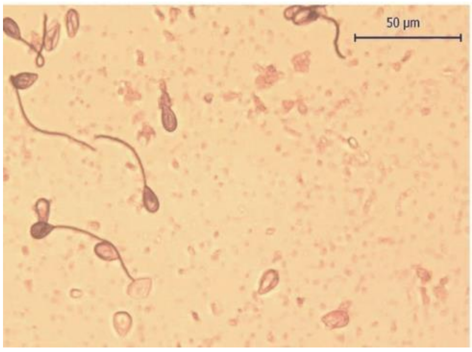

Fig. 7

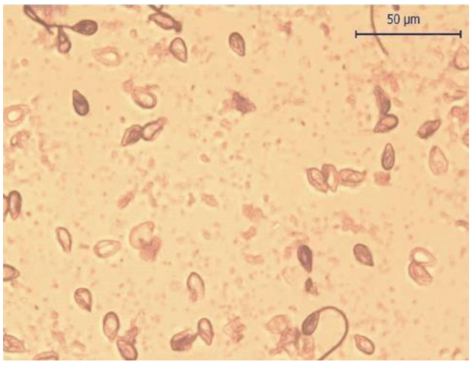

Fig. 8

T. ophthalmicus, Spores with extruded polar filaments (giemsa stained)

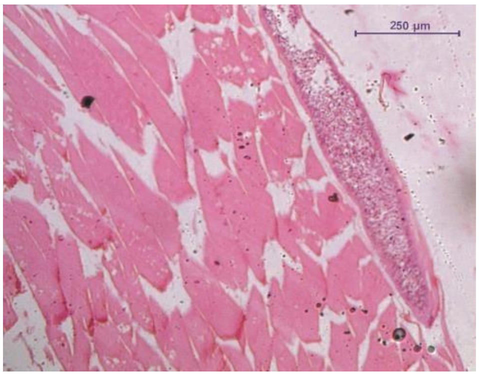

Fig. 9 Interfibrilar plasmodium

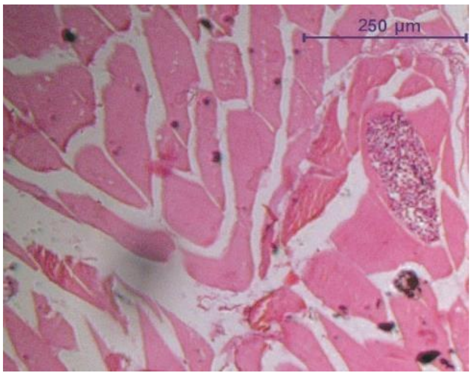

Fig. 10 Intrafibrilar plasmodium

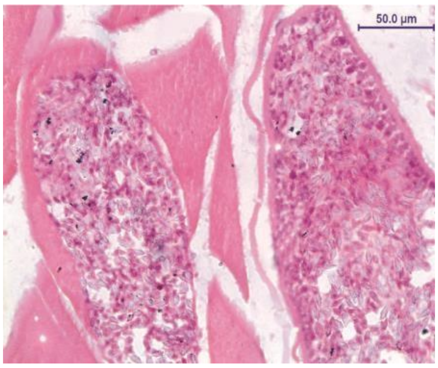

Fig. 11 Plasmodia with vegetative stages towards periphery and mature spores in the centre.

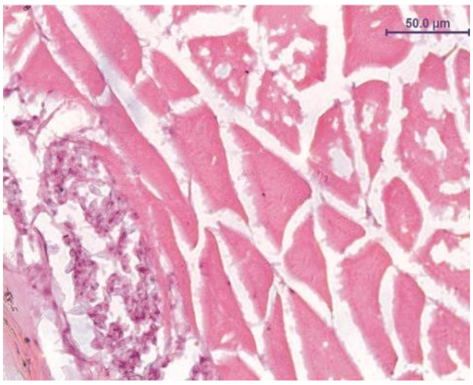

Fig. 12 Enlargement of infected muscle fibres and pressure atrophy of myofibrils

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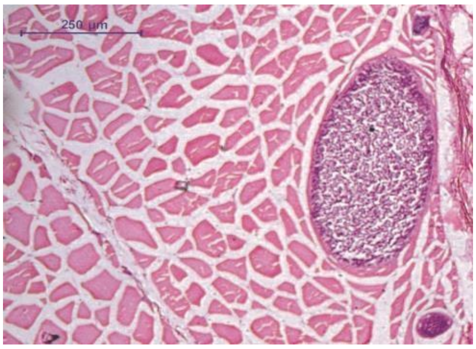
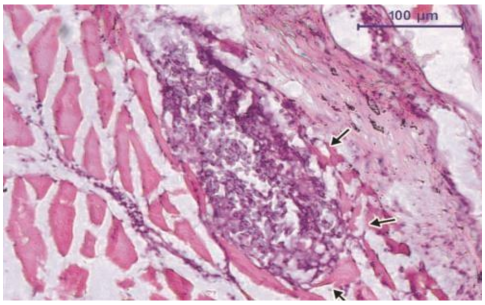
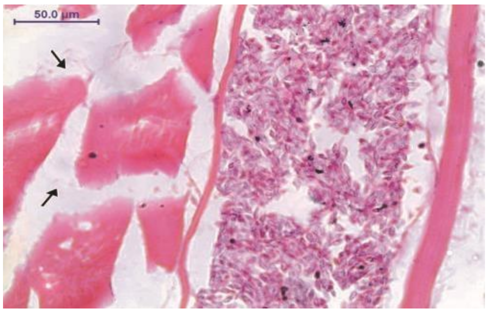


Fig. 13 Young and mature plasmodia

Fig 14 Mature plasmodia with thickly packed spores





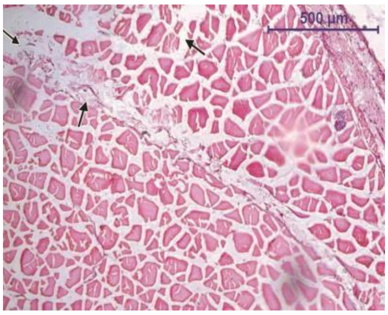


Fig. 17 Degeneration of muscle fibres

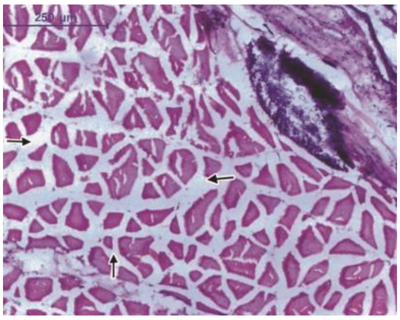


Fig. 18 Atrophy of muscle bundles

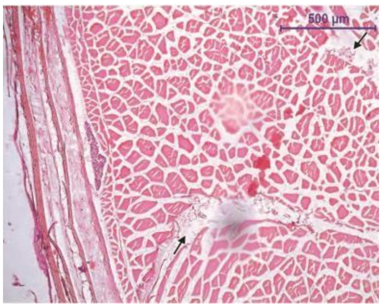


Fig. 19 Edema between muscle bundles

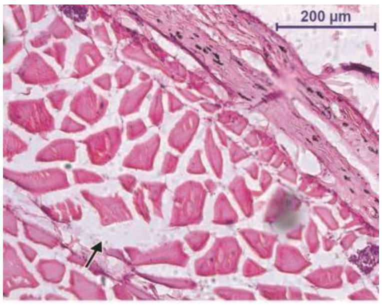


Fig. 20 Splitting of muscle fibres

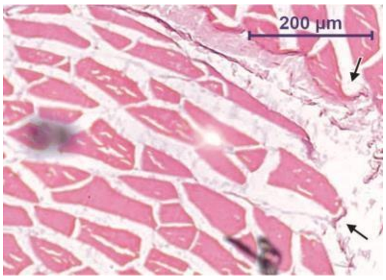


Fig. 21 Necrosis of muscle bundle

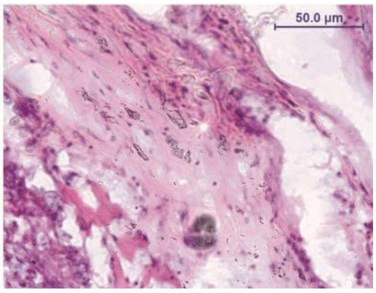


Fig. 22 Granulomatous response near ruptured Plasmodium

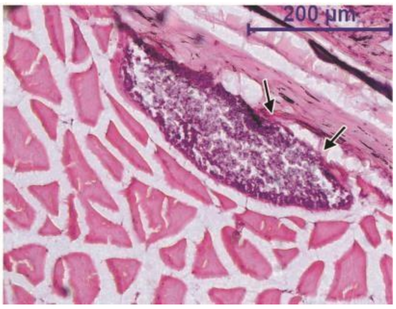


Fig. 23 Abnormal plasmodium with discontinuous Encapsulation

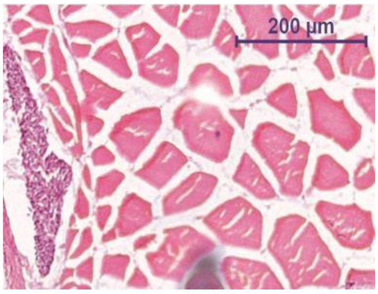


Fig. 24 Abnormal plasmodia without Encapsulation

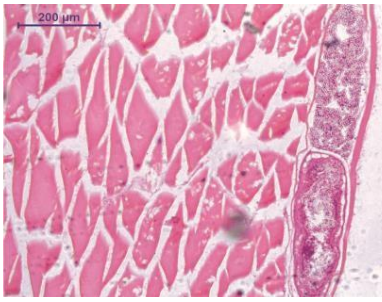


Fig. 25 Abnormal multiple cysts with single encapsulation

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Conflict of interest

The authors declare no conflict of interest.

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