Morphological and Histopathological Characterization of *Myxobolus cyprinicola* in the Asian Catfish, *Silurus triostegus* (Heckel, 1843), from Basrah, Iraq

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Received 20/12/2025 Accepted 24/01/2026 Published 27/01/2026

Abstract

Background: Myxozoan parasites are among the most widespread and pathogenic fish parasites. In Iraq, several *Myxobolus* species have been reported, yet histopathological impacts remain poorly understood. Aim: The purpose of this work is to offer the first record, morphological characterization, and histological assessment of *Myxobolus cyprinicola* infecting the Asian catfish *Silurus triostegus* from the Hammar Marshes in southern Iraq. Methods: A total of Asian catfish were examined, of which were found to be naturally infected. The spores were examined morphologically, measured, and identified using standard taxonomic keys. Infected intestinal tissues were processed for histology using paraffin embedding and H&E staining.

Results: *M. cyprinicola* plasmodial cysts (0.4–1.7 mm) were found in the lamina propria and circular muscle layer. Spores were ellipsoidal (10–12.7 × 9–9.7 µm) with distinct sutural lines and pyriform polar capsules. Histopathological lesions included epithelial atrophy, villous fusion, hemorrhage, muscular degeneration, and vascular congestion.

Conclusion: This study documents *M. cyprinicola* for the first time in Iraq and identifies *Silurus triostegus* as a new host species. Its presence in intestinal wall indicates significant tissue damage and highlights the parasite's pathogenic potential.

Keywords: *Myxobolus cyprinicola*, *Silurus triostegus*, histopathology, Hammar Marshes, Iraq, myxozoa.

P-ISSN: 1812-237X, E-ISSN: 2788-5720, https://ijaqua.uobasrah.edu.iq/index.php/jaqua/index

Introduction

Since their discovery in the early 19th century, myxozoans have attracted great attention. To date, more than 2,600 myxozoan species, representing approximately 20% of cnidarians, have been described worldwide (Okamura, Hartigan and Naldoni, 2018; Eiras *et al.*, 2021) Although the taxonomic methods for myxozoans are improved continuously, it is still challenging to identify many species with similar morphology (Kaur and Singh, 2012). Myxozoans are the members of phylum Cnidaria, parasitic in nature infecting fish as definitive host both in natural and farmed environments (Okamura *et al.*, 2015). Spores morphology is the major criterion used for the identification and description of new species of Myxosporidia with additional criteria being vegetative stage characteristics, host specificity, organ specificity and geographic location (Lom and Arthur, 1989; Lom and Dyková, 1992; Molnár, 1994; Molnár, 2002; Molnár and Eszterbauer, 2015)

In freshwater fish, species of the genus *Myxobolus* are generally histozoic. Some are host and/or organ specific, but others are found in several host species and/or organs (Lom and Dyková, 2006). Although it is widely known that many *Myxobolus* species cause asymptomatic infections in fish, there is increasing evidence that some species are highly pathogenic and cause various damages to their hosts (Dyková and Lom, 2007). A substantial body of research worldwide has examined the histopathological impacts of parasitic infections in fish. Mosoumian *et al.* (1996) documented *Myxobolus nodulointestinalis* in the intestines of Bunne fish and reported distinct histological modifications within the intestinal wall associated with infection. In a subsequent investigation, Molnar (2000) studied *Myxobolus introchondralis* in the gills of common carp, identifying spore-filled cysts embedded in the supporting cartilage of the gill arch. Notably, no significant histopathological reaction was observed in relation to the presence of the parasite. Later, Molnar (2002) described *M. cypinicola* in common carp, noting that its spore cysts were situated within the lamina propria of the intestinal wall, leading to cell compression and localized congestion.Furthermore, Dyková *et al.* (2003) recorded *M. longisporus* in the gills of common carp and characterized the presence of spore cysts within the gill lamellae. The authors highlighted pronounced pathological effects, including cellular necrosis and fusion of adjacent lamellae, with the severity of these alterations increasing alongside the size and number of spore cysts.

Although several Iraqi studies have addressed the identification and description of fish parasites, relatively few have thoroughly investigated their histopathological consequences. Al-Nuaimi (1997) provided detailed accounts of the histopathological effects of several parasites infecting European catfish from the Tigris River in Mosul. Spore cysts of *Myxobolus iranicus* were observed in the spleen, encapsulated by fibrous tissue accompanied by marked macrophage infiltration. Additionally, cysts of *M. koi* were identified in the skin, gills, intestines, and liver.

Rahemo (1997) reported the pathogenicity of two protozoan species. *Ichthyophthirius multifiliis* caused pronounced hyperplasia in gill tissues of *Cyprinion macrosomus*, while

Myxobolus pfeifferi in *A. marmid* was encapsulated by dense fibrous tissue, leading to hypertrophy and deformation of gill filaments.In another study, Al-Dossary (1999) examined histopathological alterations in the ovaries and intestines of Khushni fish infected with (*Myxobolus* sp.). The ovaries exhibited extensive infiltration by spore cysts, accompanied by numerous necrotic oocytes. In the intestines, spore cysts occupied the majority of the intestinal wall layers, resulting in villous erosion and hemorrhage attributed to parasite-induced tissue disruption.

The Asian catfish *Silurus triostegus* and the European catfish *Silurus glanis* are among the most ecologically and economically significant freshwater catfish species in Iraq. *S. triostegus* is widely distributed in the Shatt al-Arab, Shatt al-Basra, and the southern marshes of Iraq, extending through the Euphrates and Tigris river basins up to Haditha on the Euphrates and Samarra on the Tigris (Al-Daham, 1977; Al-Dubaikal, 1986). According to the comprehensive review conducted by Mhaisen & Khamees (1995) on the pathogenic agents and parasites of catfish in Iraq, analyses of 18 studies on Asian catfish revealed infections with 15 protozoan species and 29 trematode species, underscoring the diversity and prevalence of parasitic fauna associated with this host. Mhaisen and Al-Jawda (2020) Surveying 123 references concerning the occurrence of species of the genus *Myxobolus* infecting fishes of Iraq, till the end of 2019, showed the occurrence of 97 *Myxobolus* species as well as some unidentified *Myxobolus* species from 43 valid fish species. These *Myxobolus* species were reported from three marine fish species and 40 freshwater fish species of Iraq.

The present study aims to provide a detailed morphological description and histopathological assessment of *Myxobolus cyprinicola* infecting *Silurus triostegus* from southern Iraq.

Materials And Methods

Collection and Examination of Asian Catfish (*Silurus triostegus*)

Samples of Asian catfish were collected(323 fishes) from the study area located in the southern part of the Hammar Marsh, specifically in the region locally known as Hor Alawi (Al-Masshab). Live fish were transported to the laboratory and placed in 50-liter plastic tanks filled with dechlorinated tap water.Recently dead fish were subjected to immediate external examination to prevent the loss of ectoparasites following host death. Subsequently, external examination of live fish was performed, followed by internal inspection according to the procedures recommended by Amlacher (1970) and Lucky (1977).

Isolation of Parasitic Protozoa

Different tissues were examined externally and internally. Upon confirmation of the presence of protozoan parasites, smears were prepared and fixed using 96% methanol. The smears were stained with Giemsa stain following the procedures used for the preparation of fish blood films as described by Coulombe (1970) and Lucky (1977).

The classification of protozoan parasites followed the criteria outlined by Kudo (1971) and Kreier (1977).A Leitz Biomed compound microscope and a Wild M3B dissecting microscope were used for microscopic examinations. Identified parasite specimens were illustrated using a camera lucida.

Isolation and Preparation of Infected Tissues

Infected tissue samples were carefully removed and immediately transferred into a fixative solution. The method of Al-Hajj (1998) was followed for preparing samples intended for paraffin embedding and light microscopy, with some modifications.Some tissue samples were placed in 10% formalin, after which the fixed specimens were washed with tap water. The samples were then passed through a graded series of ethyl alcohol for dehydration, cleared in toluene, and infiltrated with molten paraffin. Tissues were subsequently embedded in paraffin blocks. Sections of 6–7 µm thickness were prepared and stained using Harris hematoxylin and water-soluble eosin, according to the technique described by Bancroft & Steven (1974), with minor modifications. After staining, tissue sections were mounted on clean glass slides using an adequate amount of Canada balsam, then covered with a clean coverslip. The slides were examined using an Altay light microscope, and photomicrographs were taken using either a Nikon photomicroscope or an LG digital camera.

Results

First Record of *Myxobolus cyprinicola* in Iraq

The present study records *Myxobolus cyprinicola* for the first time in Iraq.The classification of this parasite was confirmed based on morphological characteristics described in (Kudo, 1971; Kreier, 1977; Molnar ,2002).

Kingdom: Protista
Phylum: Protozoa
Class: Cnidosporida
Order: Myxosporida
Family: Myxobolidae
Genus: *Myxobolus*
M. cyprinicola Reuss , 1906

Description of the Parasite *Myxobolus cyprinicola* Reuss, 1906

Plasmodia of this parasite were observed within the intestinal wall of six infected Asian catfish specimens, whose total lengths ranged between 23–51 cm. These plasmodia appeared spherical in shape, with diameters ranging from 0.4–1.7 mm.

The following description and morphometric measurements are based on observations from 10 spores:

The spores was ellipsoidal in frontal view, while the lateral view reveals a lemon-shaped outline. The spore valves are relatively thick, smooth, and symmetrical. The sutural line is

distinct and clearly visible, with the posterior sutural edge being more prominent than the anterior one.

Spore dimensions ranged between 10–12.7 µm in length and 9–9.7 µm in width, while the thickness measured 5.3–6.2 µm.

The polar capsules are pyriform and equal in size, measuring 5–5.7 µm in length and 3–3.5 µm in width. The polar filaments are clearly visible, coiled inside each capsule in eight turns perpendicular to the longitudinal axis. When discharged, the polar filament measured 33–45 µm in length.

A triangular-shaped appendix is present in the space between the polar capsules at the anterior region of the spore.

The spore cytoplasm occupies approximately two-thirds of the posterior region and contains two nuclei along with a large iodinophilous vacuole (Fig. 1).

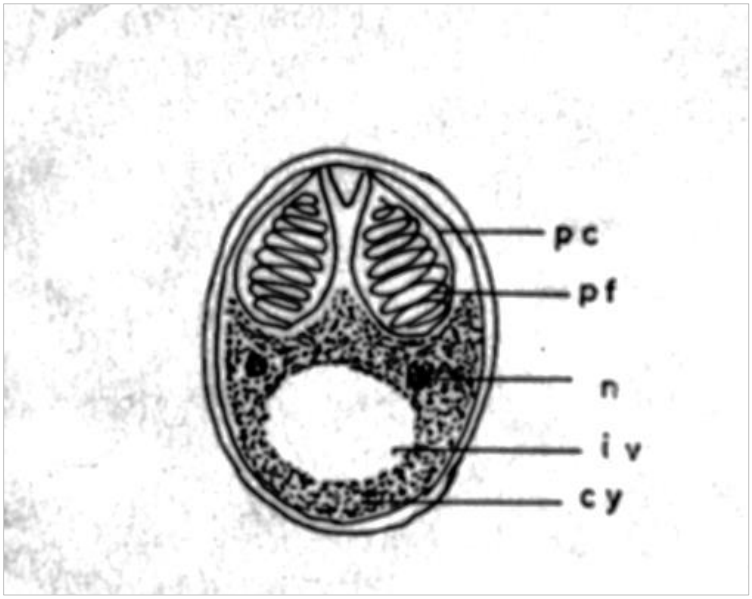

Figure 1. A schematic illustration showing the anterior view of the spore of the parasite *M. cyprinicola* (cy:cytoplasm, iv: iodinophilous vacuole, n: nucleus pc: polar capsule, pf: polar filament) Scale bars represent 10 µm.

Histopathological Effects

Macroscopically, the plasmodial cysts (plasmodia) of *M. cyprinicola* were observed as white nodules on the intestinal wall of the Asian catfish. Histological examination revealed the presence of these plasmodial cysts in two distinct locations within the intestinal wall. The first location was within the circular muscle layer, where the cysts caused separation of this layer into two portions: an upper layer located above the cysts and a lower layer beneath them, accompanied by atrophy of the muscle fibers adjacent to the plasmodial cysts (Fig. 2). The second location was in the lamina propria of the

intestinal villi, where the presence of the cysts led to atrophy of the overlying columnar epithelial cells, congestion of the underlying blood vessels, slight hemorrhage and fusion of intestinal villi (Fig. 3)

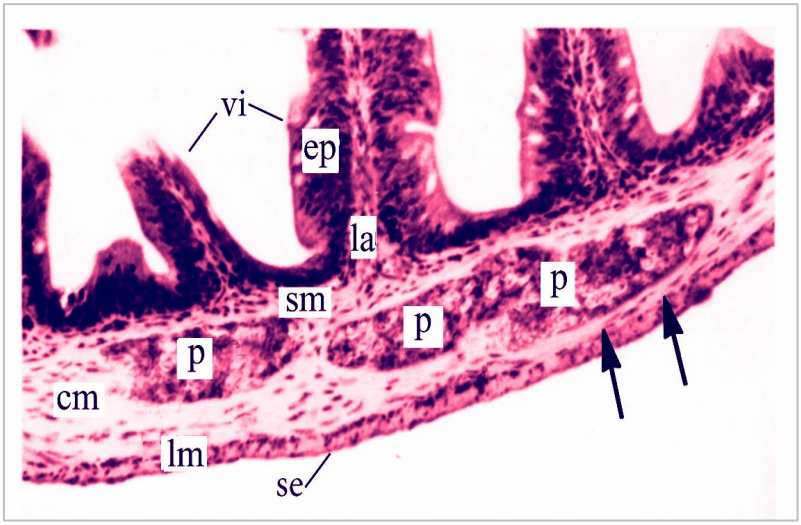

Figure 2. A cross-section showing the plasmodial cyst (p) and atrophy of the muscle fibers (arrows). The section also demonstrates the layers of the intestinal wall: circular muscle layer (cm), epithelial layer (ep), lamina propria (la), longitudinal muscle layer (lm), serosa (se), submucosa (sm), villi (vi). Magnification: 200×.

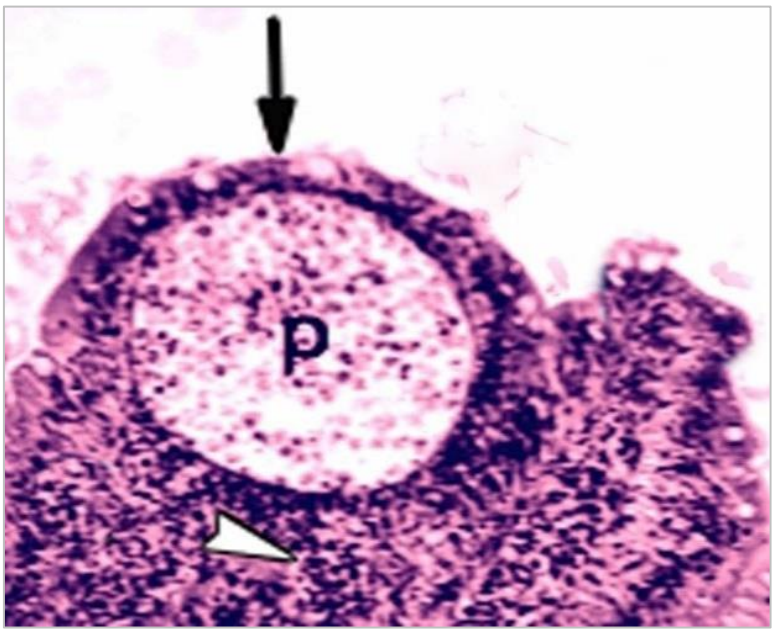

Figure 3. A cross-section showing the plasmodial cyst (p) within a villus, with atrophy of the epithelial cells (arrow) and vascular congestion (arrowheads). Magnification: 300×.

Discussion

Molnar (2002) reported that the number of species of the genus *Myxobolus* recorded worldwide has now approached 500 species. In 1984, Dones and Shulman documented 25 species of *Myxobolus* in common carp (*Cyprinus carpio*) in the former Soviet Union. Later, Chen and Ma (1998) reported 50 species of the genus *Myxobolus* in fish from China. Regarding global records of *M. cyprinicola*, Lom and Dyková (1988) reported this species in common carp in the former Czechoslovakia, while Molnar (2002) recorded it in Hungarian fishes. Additionally, Dones and Shulman (1984) reported ten cyprinid fish species as hosts for *M. cyprinicola* in the former Soviet Union, with common carp being considered the primary natural host of this parasite. The first record of the genus *Myxobolus* in Iraq was reported by Herzog (1969), who identified *M. oviformis* in *Alburnus chalcoides*, *Luciobarbus esocinus*, *B. grypus*, and *C. macrostomum*, and *M. mulleri* in *Barbus heckelii*. He also reported an undescribed *Myxobolus* sp. in *B. esocinus, B. grypus* and *B. heckelii*. Later, Fattohy (1975) recorded *M. pfeifferi* in the gills of *Acanthobrama marmid* in the Tigris River near Mosul. Subsequent studies, particularly in southern Iraq, confirmed this species, including those by Khamees (1983), Mhaisen *et al.* (1986), Al-Daraji (1986), Al-Daraji and Al-Salim (1986, 1989), and Al-Daraji and Al-Salim (1990). The scientific of fish names have been updated according to Fricke *et al.* (2025) and Freyhof *et al.* (2025). The most recent record of *Myxobolus* in Iraq was provided by Jori (2006), who reported *M. mesopotamae* and *Myxobolus* sp. in Asian catfish. The *M. cyprinicola* recorded in the present study shows morphometric characteristics consistent with those described by Molnar (2002), who redescribed this parasite in common carp in Hungary. Therefore, the first record of *M. cyprinicola* in Iraq, and Asian catfish (*Silurus triostegus*) represents a new host species added to the parasite's known host range.The occurrence of this parasite in Asian catfish (a member of the Siluridae family) is notable because previous records indicated its specificity for cyprinid hosts (family Cyprinidae). This suggests the potential transmission of the parasite into Iraqi freshwater systems via the importation of live European carp (from Romania, Yugoslavia, and Poland) as broodstock for artificial breeding in fish hatcheries, which likely facilitated the spread of this parasite into Iraqi aquatic environments. Histopathological examination revealed the presence of *M. cyprinicola* plasmodial cysts in two locations within the intestinal wall: the mucosal and muscular layers. These findings are consistent with previous reports. Molnar (2002) observed *M. cyprinicola* plasmodia in the lamina propria of common carp intestines, while Masoumian *et al.* (1996) and Lom *et al.* (1992) reported cysts in the muscular layer of the intestinal wall. Other studies have reported additional sites; for example, Al-Nuaimi (1997) and Al-Dossary (1999) reported that plasmodial cysts of *M. colossomatis* and *Myxobolus* sp. occupied most layers of the intestinal wall in the fish examined, whereas Molnar and Bekesi (1993) observed cysts of *M. colossomatis* in the serosal layer of *Colossoma macropomum* intestines.

Al-Nuaimi (1997) suggested that the location of plasmodial cysts within different intestinal layers depends on the parasite species, its ability to penetrate tissue layers, the host's defense mechanisms, and tissue responses. In some connective and muscular tissues, fibrous white fibers or muscle fibers may form around the cysts as a host reaction, limiting parasite spread. The histopathological effects caused by this parasite, including congestion and hemorrhage, are consistent with those reported by Molnar and Kovacs-Gayer (1985) in their study of *M. cyprini*, indicating localized inflammation near the site of infection.

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الخصائص المظهرية والامراضية النسجية لـ *Myxobolus cyprinicola* في اسماك الجري الآسيوي *Silurus triostegus* (Heckel, 1843) في البصرة-العراق

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تاريخ الإستلام: 2025/12/20 تاريخ القبول: 2026/01/24 تاريخ النشر : 2026/01/27

المستخلص

الخلفية: تُعد الطفيليات البوغية (Myxozoa) من أكثر طفيليات الأسماك انتشارًا وإمراضًا. وفي العراق، تم تسجيل عدة أنواع من جنس *Myxobolus*، إلا أن التأثيرات النسجية المرضية لها ما تزال غير مفهومة بشكل كافٍ.

الهدف: تقديم أول تسجيل، ووصف مظهري، وتقييم نسجي مرضي مفصل لطفيلي *Myxobolus cyprinicola* الذي يُصيب سمكة الجري الآسيوي *Silurus triostegus* في أهوار الحمار بجنوب العراق.

المواد وطرائق العمل: جرى فحص عدد من أسماك الجري الآسيوي المصابة طبيعيًا. تم فحص الأبواغ مظهريا وقياسها والتعرّف عليها باستخدام المفاتيح التصنيفية الاساسية. كما جرى تحضير الأنسجة المعوية المصابة للفحص النسجي باستخدام طريقة الطمر بالبارافين والتصبيغ بالهيماتوكسيلين والإيوسين.(H&E)

النتائج: وُجدت الأكياس البلازمودية لـ *M. cyprinicola* (0.4–1.7 مم) في الصفيحة الأصيلة (lamina propria) وطبقة العضلات الدائرية. كانت الأبواغ بيضوية الشكل (10–12.7 × 9–9.7 ميكرومتر) مع خطوط درزية واضحة ومحافظ قطبية كمثرية الشكل. وشملت الآفات النسجية المرضية ضمور النسيج الظهاري، اندماج الزغابات، النزف، تنكس العضلات، واحتقان الأوعية الدموية.

الاستنتاج: وثقت هذه الدراسة تسجيل *M. cyprinicola* لأول مرة في العراق، وشخصت *Silurus triostegus* كمضيف جديد لهذا الطفيلي. كما يشير وجوده في جدار الامعاء إلى إحداثه أذى نسيجياً ملحوظاً، مما يبرز قدرته الإمراضية.

الكلمات المفتاحية: *Myxobolus cyprinicola*, *Silurus triostegus*، علم الأنسجة المرضية، أهوار حمار، العراق، myxozoa.