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# Ovarian abnormality in a pathological case caused by *Myxidium* sp. (Myxozoa, Myxosporea) in onespot snapper fish *Lutjanus monostigma* (Teleostei, Lutjanidae) from the Red Sea

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## Abstract

To date, *Myxidium elmatboulii* Ali, Abdel-Baki et Sakran, 2006 (Myxozoa, Myxosporea) is the only species of the genus known from the Red Sea, and was originally described as a coelozoic parasite in the gall bladder of the belonid fish, *Tylosurus choram*. A *Myxidium* sp. closely similar to *M. elmatboulii* is described herein for the first time as a histozoic parasite in the ovary of the onespot snapper fish, *Lutjanus monostigma* (Teleostei, Lutjanidae) from the Red Sea coast of Saudi Arabia. The infected ovary was morphologically abnormal, with two protruding digitiform black cysts at its distal end, densely packed with mature plasmodia suspended in a mucoid liquid. Histological examination revealed that the cysts were extended deeply within the ovary, and each was surrounded by a capsule consisting of a relatively thick layer of fibrous connective tissues of host origin (host tissue reaction), and followed internally by a distinct black layer composed of melanomacrophages encircling the parasite mass; this layer clearly indicates the ability of this myxosporean parasite to induce a strong immune inflammatory response in the ovary of *L. monostigma*. Many small or developing cysts with the same characteristics were seen scattered in the connective tissue between the ovarian follicles. Plasmodia or spores of the parasite were not seen within the oocytes or within its developmental stages. The cysts occupied a considerable part of the ovary, and some areas of the ovarian tissues appeared to be vacuolated or degenerated. Thus, the typical ovarian structure of *L. monostigma* was greatly affected and lost its normal architecture. Therefore, the infection caused by this *Myxidium* sp. is presumed to negatively affect the reproductive capacity of the fish host.

## Keywords

Myxosporea, Myxozoa, *Myxidium*, pathological lesions, ovary fish, Red Sea

## Introduction

Myxosporeans are parasites of marine and freshwater fishes (Lom 1987, Lom and Dyková 1992), but few species of them are known to infect amphibians and reptiles (Lom and Dyková 1992, Canning *et al*. 1999, Garner *et al*. 2005).

Myxosporeans are usually coelozoic parasites (in body cavities or cavities of body organs), sometimes histozoic (wedged between tissue cells) or intracellular. Many species of them are highly pathogenic to their fish hosts (Lom 1985; Kent and Hedrick 1986; Dyková and Lom 1988; Hoffman 1990; Schäperclaus 1992; Alvarez-Pellitero and Sitjà-Bobadilla 1993, Kent *et al*. 1994, 2001; Lom and Dyková 1995; Noga 1995; Longshaw *et al*. 2005; Lom and Dyková 2006).

Myxosporeans of the Red Sea fishes are poorly known in the literature. Consequently, myxosporeans are one of the least known parts of the Red Sea fauna. Previous studies on myxosporeans in the region include those by Paperna and Overstreet (1981), Diamant and Paperna (1992), Diamant *et al*. (1994, 2004, 2005, 2007), Ali *et al*. (2001, 2003), Diamant and Palenzuela (2005), Ali *et al*. (2006, 2007a, b), Al-Quraishy *et al*. (2007) and Abdel-Ghaffar *et al*. (2008a, b). According to these, 18 myxosporean species belonging to 10 genera have been described from the Red Sea fishes.

*Myxidium* Bütschli, 1882 (Myxidiidae Thélohan, 1892) is one of the most common myxosporean genera, including over 205 species in marine and freshwater fishes, 8 species in amphibians and 4 in reptiles (Garner *et al*. 2005, Lom and Dyková 2006). Most of these species are typically coelozoic parasites (rarely histozoic), infecting the gall bladder, urinary bladder, or urinary tubules of the kidneys (Diamant *et al*. 1994, Lom and Dyková 2006, Jirků *et al*. 2006). To date, *Myxidium elmatboulii* Ali, Abdel-Baki et Sakran, 2006 is the only species of the genus known from the Red Sea, and described as a

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coelozoic parasite in the gall bladder of the belonid fish, *Tylosurus choram*. In the present work, we recorded and discussed a pathological case, in which a *Myxidium* species closely similar to *M. elmatboulii* was accidentally found in the ovary of the onespot snapper fish, *Lutjanus monostigma* (Teleostei, Lutjanidae), and caused some previously unreported pathological lesions and abnormalities. It is important to record and report new parasites and new pathological conditions when they are discovered because such information may be useful in the future as "baseline" data for assessing ecosystem health in the face of threats from global warming (Harvell *et al*. 2002).

## Materials and methods

During April and May of 2008, a total of 123 specimens of the onespot snapper fish, *Lutjanus monostigma* (Cuvier, 1828) (Teleostei, Lutjanidae), were examined in order to estimate female fecundity and dynamics of multiple spawning. These fish were caught by net in the Red Sea off the coast of Jeddah, Saudi Arabian, identified according to Randall (1983) and their names follow Froese and Pauly (2004/2008).

During laboratory examination and dissection, the ovary of a single fish seemed to be morphologically abnormal, and possessed two digitiform black cysts protruded from its wall; it was gently excised and placed on ice plate for morphological examination and picturing. A small piece of the cyst was gently removed and examined microscopically; for permanent preparation of spores, air-dried smears (ADS) of this piece were stained with Giemsa after fixation in acetone-free absolute methanol. Descriptions of spores followed the guidelines of Lom and Arthur (1989); measurements were based on 15 plasmodia and 20 fresh spores, and data were presented as mean ± SD. All measurements were given in micrometres, except where indicated. Drawings were made with the aid of a camera lucida.

Infected ovarian tissue taken for histological assessment were fixed in Bouin's fixative, embedded in paraffin wax, and 4 μm-thick sections were cut and stained with hematoxylin and eosin, then dehydrated, cleared and mounted on microscope slides using standard techniques.

Material examined: original specimens of *Myxidium elmatboulii* Ali, Abdel-Baki et Sakran, 2006 (syntypes on slide no. Myx.-15) deposited at the Museum of Zoology Department, Faculty of Science, Beni-Suef University, Beni-Suef, Egypt.

## Results

In a single specimen of examined fishes of *L. monostigma* (ca. 17 cm in total length and 115 g in weight), the normal ovary (Fig. 2A) appeared to be morphologically abnormal (Fig. 2B), and possessed two protruding digitiform black cysts at its distal end; a large one measuring 3.70 mm in diameter and 5.60 mm in height and a smaller one measuring 2.60 mm in diameter and 1.92 mm in height. On dissection, these cysts were found to be densely packed with mature plasmodia of a myxosporean parasite suspended in a mucoid liquid. The morphological characteristics of these plasmodia (Figs 2E, F) and the spores they contained (Fig. 1A, B and Fig. 2G) were typically consistent with the redefinition of genus *Myxidium* Bütschli, 1882 given by Lom and Noble (1984) and Lom and Dyková (2006). However, these characteristics and body measurements are typically conformable to those of *Myxidium elmatboulii*, which is so far, the only species of the genus known from the Red Sea (Ali *et al*. 2006).

***Myxidium* sp.** (Fig. 1A, B and Fig. 2E-G)

*Plasmodia* (Fig. 2E, F): Spherical, with thin hyaline ectoplasm and granular endoplasm observed in wet mounts, disporic (contained two tightly packed spores), 25.9 ± 1.8 (23.3–28.5) in diameter, and do not exhibit any motility.

*Spores* (Fig. 1 and Fig. 2G): Typical of the genus *Myxidium*. Mature spores smooth, fusiform or slightly sigmoid, composed of 2 identical valves. In sutural view, sutural ridge smooth, 9.0 ± 0.2 (0.8–1.0) thick, divided by a thin, slightly curved sutural line (bisects the spore) running down center of ridge, turning at angle of approximately 90° near each pole; oriented in opposite directions at opposite poles to form papillary projection around filament eversion pole of each shell valve. Polar capsules two, similar in size, distinctly pyriform, positioned at opposite poles of spore, tapering anteriorly to form prominent necks opening at the end of the spore tips; in sutural view, axis of each polar capsule diverts (in opposite directions) at an angle of 15–20° from longitudinal axis of spore. Polar filaments with 9 or 10 coils, perpendicular to the longi-

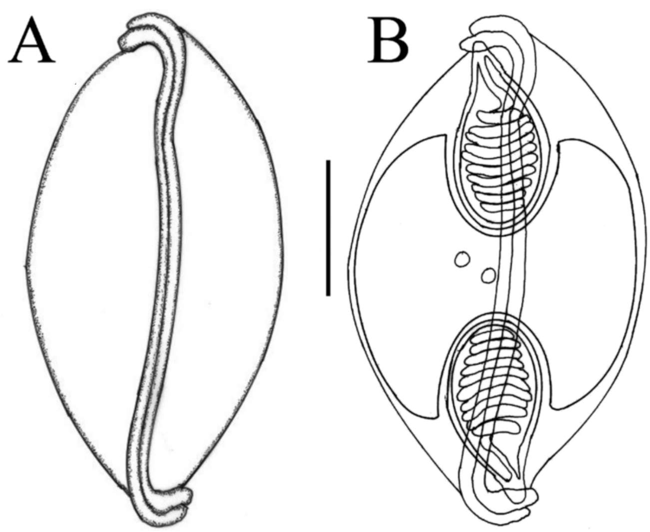


**Fig. 1.** Mature spore of *Myxidium elmatboulii* Ali, Abdel-Baki et Sakran, 2006. **A**. Sutural view of spore. **B.** Internal details of spore. Scale bar = 10 μm

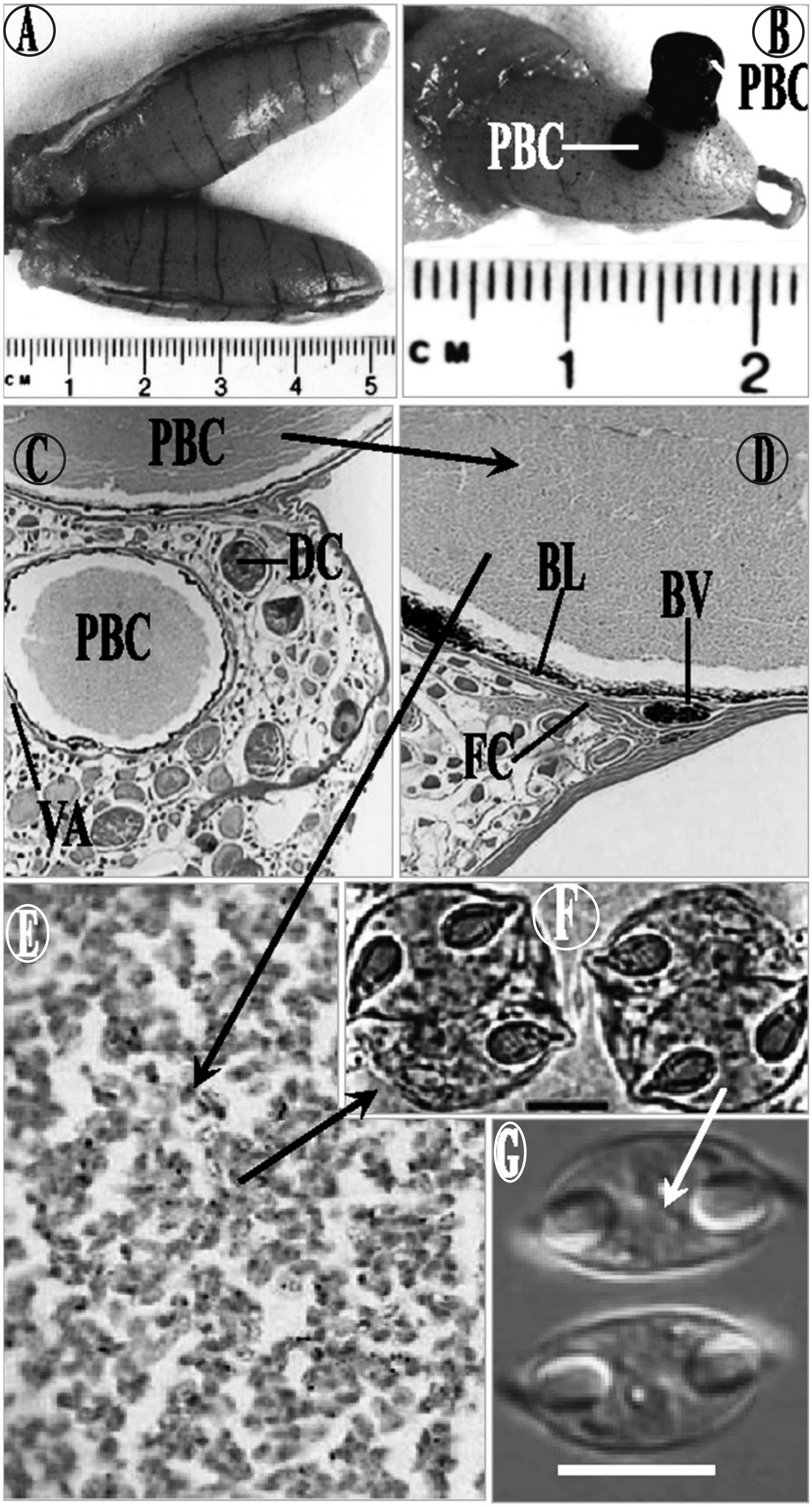


**Fig. 2. A.** Normal ovary of the fish *Lutjanus monostigma*. **B.** Morphologically abnormal ovary parasitized by a *Myxidium* sp., with two protruding digitiform black cysts (PBC) at its distal end. **C.** Histological section through the infected region (× 40). DC – developing cyst, PBC – protruding black cysts, VA – vacuolated area. **D.** Low power view (×100) of Fig. C. BL – black layer composed of aggregated melanomacrophages (melanin-laden macrophages, BV – blood vessel, FC – fibrous capsule surrounding the cyst. **E.** Higher power view (× 400) of cyst in Fig. D showing the high density of plasmodia. **F.** Disporic plasmodia, scale bar = 10 µm. **G.** Fresh spores (air-dried smear, scale bar = 10 µm)

tudinal axis of the polar capsule, visible in some formalin-fixed spores. Sporoplasm binucleate, filling spore cavity between the two polar capsules.

Spore measurements (n = 20): 21.5 ± 1.7 (18.0–25.0) in length × 10.6 ± 0.7 (8.0–13.2) in width × 10.2 ± 0.6 (7.4–13.0) in thickness. Polar capsules: 9.0 ± 0.8 (7.2–10.8) × 3.8 ± 0.6 (3.2–4.4).

*Histological examination*

Histological examination revealed that the two digitiform black cysts were extended deeply within the ovary of *L. monostigma*, and each was surrounded by a capsule consisting of a relatively thick layer of fibroblasts or fibrous connective tissues of host origin (host tissue reaction or fibrotic reaction against the aggregations of plasmodia), and followed internally by a distinct black layer composed of melanomacrophages (macrophages containing black and brown pigments called melanin, participating in immune reactions) encircling the parasite mass (immune inflammatory response against infection) (Fig. 2C, D). Many small or developing cysts with the same characteristics were also seen scattered in the connective tissue between the ovarian follicles (Fig. 2C); plasmodia or spores of the parasite were never seen within the oocytes or within their developmental stages. This suggests that plasmodia and spores are mainly associated with connective tissue within the ovary. As shown in Figure 2C, the black cysts and the developing ones occupied a considerable part of the ovary, and some areas within the ovarian tissues appeared to be vacuolated or degenerated, suggesting that cysts of the present *Myxidium* sp. can induce histological lesions in the neighboring ovarian tissue. Thus, the typical ovarian structure of *L. monostigma* was greatly affected and lost its normal architecture. Therefore, the infection caused by the present *Myxidium* sp. is presumed to negatively affect the reproductive capacity of the fish host.

## Discussion

Some *Myxidium* species are not entirely specific, i.e., infect one or more fish species, where they found in different organs and appear in both coelozoic and histozoic forms (Dyková *et al*. 1987, Lom and Dyková 1992); for example, in wild and cultured European eels, *Anguilla anguilla*, coelozoic plasmodia of *Myxidium giardi* Cépède, 1906 were found in the gall bladder, while histozoic plasmodia of the same species were found in the kidney, stomach and intestine (Copland 2006); *Myxidium rhodei* Leger, 1905 is known to occur in 32 fish species (Shulman 1984), in one of them, coelozoic plasmodia of *M. rhodei* were found in the gall bladder, while histozoic plasmodia were found in the intestinal mucosa, liver, spleen and kidney (Dyková *et al*. 1987). Based on this information and on the close morphological similarity and dimensions of the plasmodia and spores, and on the same geographical area, the present *Myxidium* sp. may represent a histozoic form of *M. elmatboulii*. However, recent studies using rDNA sequence have shown that myxozoans with similar appearing spores infecting different hosts and tissues usually represent different species. Thus, the present *Myxidium* sp. is considered as the first species of the genus *Myxidium* recorded from the sexual tissues of a teleost fish.

Presence of myxosporean species in sexual tissues of the teleost fishes is an unusual situation, and usually result in parasitic castration (partial or total destruction of host gonads, or partial or total inhibition of host gamete formation) as reported by several authors; *Myxobolus ovariae* Paperna, 1973 in ovaries of *Haplochromis gustrifons* invades and destructs mature eggs (Paperna 1973); *Sphaerospora testicularis* Sitjà-Bobadilla et Alvarez-Pellitero, 1990 in testes of the wild and cultured Mediterranean Sea bass, *Dicentrarchus labrax*, invades the testes and neighboring tissues, destructs the tubular tissue of testes and greatly reduces the production of spermatozoa, causing parasitic castration (Sitjà-Bobadilla and Alvarez-Pellitero 1990, 1993; Alvarez-Pellitero and Sitjà-Bobadilla 1993); *Kudoa paniformis* Kabata et Whitaker, 1981 in ovaries of the Pacific hake (*Merluccius productus*) negatively affect the reproductive effort, causing a decrease in fecundity proportional to the intensity of infection (Adlerstein and Dorn 1998); *Kudoa ovivora* Swearer et Robertson, 1999 in ovaries of the bluehead wrasse *Thalassoma bifasciatum* reduces the growth, fecundity and spawning activity of the infected females (Swearer and Robertson 1999). *Myxobolus dahomeyensis* Siau, 1971 in ovaries of *Tilapia zillii* induced destruction of the oocytes and host castration (Gbankoto *et al*. 2001).

In our case, plasmodia of a *Myxidium* sp. were found densely aggregated within fibrous cysts distributed in the connective tissue between the ovarian follicles of *L. monostigma*. However, plasmodia or spores were never seen within the mature oocytes or within their developmental stages. This suggests that plasmodia and spores of this species are mainly associated with connective tissue within the ovary. Parasite encapsulation is a host reaction aiming to isolate the parasite and to prevent its dispersal, and was described in several myxosporeans (Davies and Sienkowski 1988; Dyková and Lom 1988). Formation of cysts is probably controlled by the host's immune system. The fibrous capsule around the cyst is probably built as the first step of the host tissue reaction against the parasite. Thus, connective tissue may provide a sufficient source of facilitates for encapsulating the aggregated plasmodia and to isolate them from the ovarian tissue.

Migrating melanomacrophages (melanin-laden macrophages) could explain the dark layer encircling the densely aggregated plasmodia in the cysts within the ovary of *L. monostigma*. Melanomacrophages are normally located in melanomacrophage centers in the kidney and spleen, and are known to be involved in the defense mechanisms against various microbes (Roberts 1975, Agius 1985, Ferguson 1989). Roberts (1975) indicated that melanomacrophages migrate from hematopoietic tissues into blood vessels and then travel to infected tissues elsewhere in the fish. Thus, melanomacrophages encircling plasmodia in the cysts might play an important role in the defense mechanism against infection, and clearly indicate the ability of the present *Myxidium* sp. to induce a strong immune inflammatory response in the ovary of *L. monostigma*. The effects and origin of melanin in macrophages is controversial; some authors believe that macrophages do not possess the ability necessary for melanin synthesis and therefore have to engulf melanosomes or melanin from melanocytes (Zapata and Chiba 1996). Melanin is a complex polymer that can absorb and neutralize free radicals, cations and other potentially toxic agents (Edelstein 1971). Many articles refer to Edelstein's work, but new knowledge of the effects of melanin is scant.

Myxosporean fish parasites are capable of producing proteolytic enzymes responsible for tissue deterioration and possibly parasite encystation (Bilinski *et al*. 1984, Martone *et al*. 1999). In our opinion, the proteolytic action of these enzymes, the strong immune response, and the strong physical effect or mechanical compression accompanied the continuous growth of the densely aggregated plasmodia are the plausible reasons that orientate the developing cysts of the present *Myxidium* sp. to grow and to protrude from the ovary of *L. monostigma*, and which cause the ovary to become morphologically abnormal.

In conclusion, the establishment of *Myxidium* cysts in the connective tissue of the ovary of *L. monostigma* caused serious lesions, occupying a considerable area within the ovary and some areas within the ovarian tissues appeared to be vacuolated or degenerated; aggregations of plasmodia may induce tissue histolysis or degeneration around their cysts when they obtain their nutritional materials from the ovarian tissue. Thus, the typical ovarian structure of *L. monostigma* was greatly affected and lost its normal architecture. Therefore, the infection caused by the present *Myxidium* sp. seemed to negatively affect the reproductive capacity of the fish host.

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