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# BRAIN MYXOSPORIDIOSIS IN AFRICAN SHARPTOOTH CATFISH (*CLARIAS GARIEPINUS*).

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## ABSTRACT

This study was conducted to screening for *Myxobolus sp*. infections in the brain of African sharptooth catfish (*Clarias gariepinus*). A total of 120 fish were collected over one year from Al fath center in Assiut Governorate. Smears of all samples were examined parasitologically, and polymerase chain reaction (PCR) was used to confirm selected positive samples. Examination revealed the presence of *Myxobolus sp*. in 57 (47.5%) of examined fish. Prevalence of infection was highest in winter and spring (56.7%) and became low in autumn (30%). The effect of body weight revealed that, the highest prevalence rate (52.9 %) was occurred in middle group (301- 400g). Female fish seems to be more sensitive to infection (48.5%) than male (46.2%). Morphological characters and measurements of mature spores were recorded. The amplified 18S rDNA gene fragment by using a specific primer for Myxosporean was (869bp).

***Key words***: *Clarias gariepinus*, *Myxobolus sp*. Brain, Myxosporidiosis.

## INTRODUCTION

Myxozoa comprise an important group of fish pathogens. This phylum is composed of highly specialized metazoan parasites with an extremely reduced body size and structure (Fiala and Bartosová 2010). Myxosporidia are characterized by multicellular spores with polar capsules containing extrudable polar filaments spores (Woo, 1995) that mainly infect a wide range of aquatic hosts (Feist and Longshaw 2006).

Most myxozoans infecting fish are host-, tissue- and organ-specific parasites and in most cases they select a specific site for their development in the fish host body. (Lom and Arthur, 1989). Over 20 myxosporean species have been reported infecting the brain and spinal cord of teleosts (Hoffmannn *et al*., 1991; Frasca *et al*., 1999; Cho and Kim, 2003). This parasite can cause serious outbreaks of disease among fish species (Feist and Longshaw 2006) and negatively affect the health state of fish (Palenzuela *et al*., 1999; Munoz *et al*., 2000). Besides direct losses, parasites may have considerable impact on production, growth and behavior of fish, their resistance to other stress factors, susceptibility to predation, and reduction of marketability (Scholz, 1999, Pote *et al*., 2000).

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Sharptooth catfish, *Clarias gariepinus*, is a popular fish species dwelling the River Nile and the interconnecting lakes. They are abundant throughout the year (Hagras *et al.*, 2001), and have economic importance and good sensorial properties of meat (Maregoni, 2006).

The present study aimed to investigate the presence of Myxosporea parasites in brain of *Clarias gariepinus* in Assiut Governorate. In addition to study the effects of weight, season and sex on the susceptibility to infection.

## MATERIALS AND METHODS

**Fish:**

One hundred and twenty sharptooth catfish (*Clarias gariepinus*, Burchell, 1822) of different sexes and weights (Table 1) were randomly collected from the River Nile of Al fath center in Assiut Governorate through fish dealers. Fish were transported immediately to the laboratory of Assiut animal health research institute using special tanks supplied with aeration were they are measured, weighed, and subjected to external clinical examinations for detection of any abnormalities according to (Austin and Austin, 1987). Fish were dissected, and their brain organs examined for Myxosporean parasites.

**Table 1:** Groups of examined sharptooth catfish (*Clarias gariepinus*).

| | Weight | | | Sex | |
|---|---|---|---|---|---|
| **Category** | **100-300 gm** | **301-400 gm** | **401-600 gm** | **Male** | **Female** |
| **No. of examined fish** | 45 | 34 | 41 | 52 | 68 |
| **Total** | | 120 | | | 120 |

**Parasitological examination**:
Wet mounts of brain smear were examined microscopically for Myxosporean parasites. When parasites were found some of the fresh smears were air dried at room temperature, fixed in methyl alcohol, stained in 1:9 Giemsa solutions for 20 min. and examination under the oil immersion lens (Meyers *et al*., 1977 & Narasimhamurti and Kalavati, 1979). Identification of detected parasite was based on the morphological characters and their dimensions according to (Fomena and Bouix, 1997).

Photomicrographs of the detected spores were done and spores dimensions that include length, width; length and width of polar capsule were measured in micrometer (μm) using the Leica Application Suite (LAS EZ) program (measured ten spores and take the mean of them) (Lom and Arthur 1989).

**Molecular analysis:**
Genomic DNA was extracted using QIAamp DNA Mini kit (QIAGEN, Hilden, Germany), according to the manufacturer`s instructions. DNA was eluted in 100 μl elution buffer (AE) and kept at – 20 ºC until used. A fragment of 18S rDNA gene from the suspected Myxosporean parasite was amplified by nested polymerase chain reaction (nPCR) using the universal eukaryotic primer ERIB1:(5`-ACC TGG TTG ATC CTG CCA G -3`) & ERIB10: (5`-CTT CCG CAG GTT CAC CTA CGG -3`) and a Myxosporean specific 18S rDNA primers (Myxospec- F: (5`- TTC TGC CCT ATC AAC TWG TTG -3`) & Myxospec- R: (5`-GGT TTC NCD GRG GGM CCA AC -3`) according to Barta *et al*. (1997) and Fiala (2006). The initial amplifications were carried out with thermo PCR master mix following cycling profile: 95°C for 3 min (initial denaturation), then 35 PCR cycles of 95°C for 1 min (denaturation), 48°C for 1 min (annealing) and 72°C for 2 min (extension) with a final extension step of 72°C for 10 min. Two-microliters from the initial PCR products was used as a template for the nested PCR using 10 pmol each of Myxospec-F and Myxospec-R primers. PCR conditions were the same as in the first round but with an annealing temperature of 52°C. The amplification products were subjected to electrophoresis analysis with using negative extraction control, no-template control and DNA extracted from the malacosporean parasite Buddenbrockiaplumatellae to estimate the molecular size of the PCR amplicons (Altschul *et al*., 1997). This work was occurred in Molecular Biology Research Unit in Assiut University.

**Statistical analysis:**
Results were analyzed using (Prism 5, version 5.01, Graph pad software Inc.). The differences were considered significant if the (P-value <0.05) by using unpaired t-test or one way ANOVA test (tukey's compare all pairs of column).

## RESULTS

Parasitological examination of samples revealed the presence of *Myxobolus sp.* in brain of fifty-seven of examined *Clarias gariepinus* fish out of 120 (47.5%), (Fig.1 and Table 2).

**Epidemiological studies:**
**1- Host size:**
Prevalence were distributed as follows: 48.9 % in the group 100-300g, 52.9 % in the group 301-400 g and 39% in the group 401-600 g (Table 2 and Chart 1). Prevalence of *Myxobolus sp.* did not vary significantly with respect to fish size.

**2- Host sex:**
Females *Clarias gariepinus* had a highest infection rate of Myxosporidian parasites (48.5%) than male (46.2%), (Table 3 and Chart 1), although there was no significance difference in infection rate.

**3-Seasonal patterns of prevalence of infection:**
Percentage of seasonal variations of Myxosporidian infection in the examined brain fish was higher during winter and spring (56.7%) and started to decrease in summer (46.7%), while the lowest infection rate was observed during autumn 30% (Table 4).

**Morphological studies:**
The *Myxobolus* spores were ovoid in shape having a regular symmetrical shape, and anterior end slightly narrower than the posterior end. Their small triangular intercapuslar appendix was noted. Spore measurements: 9.21± 0.65 μm long, 7.73± 0.45 μm wide. The polar capsules were pyriform and slightly unequal in size in most cases larger polar capsule 3.62 ± 0.39 μm long, 2.5 ± 0.2 μm wide; smaller pollar capsule 3.2 ± 0.2 μm long, 1.5 ± 0.2 μm wide. They occupied the anterior half of the spore body cavity. They were sharply pointed at the anterior end and rounded posterior end. The polar filament usually made 3 coils and occasionally 4 coils inside the polar

capsule, they slightly obliquely to the axis of the polar capsule. Sporoplasm was granular, homogenous and cup-shaped filled the rest of the spore. An iodinophilous vacuole present, 2.1-3.41um in diameter (2.8 um in average) was observed in the sporoplasm in the majority of the spores examined (Fig.1A, B&C) and (Table 5).

**The Molecular studies**:

Molecular analysis based on 18 S SSU rDNA genes was performed to investigate the taxonomy and classification of examined sample (Fig 2). The amplified SSU rDNA gene region of the parasite was nearly 869bp amplicon in length. No amplification products were detected from the negative extraction control, no-template control or DNA extracted from the malacosporean parasite Buddenbrockiaplumatellae.

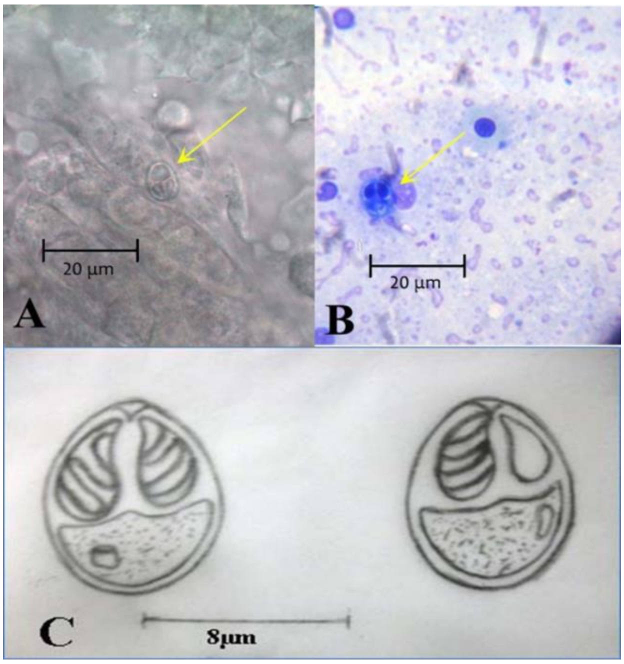



**Figure (1):** Showing *Myxobolus* sp.in brain of *Clarias gariepinus* by using oil immersion lens (x=100).
**A**: unstained *Myxobolus* sp. (Bar=20um).
**B**: stained *Myxobolus* sp. with Geimsa stain (Bar=20um**).**
**C**: Camera lucida drawings of mature spores of *Myxobolus sp* (Bar= 8 µm).

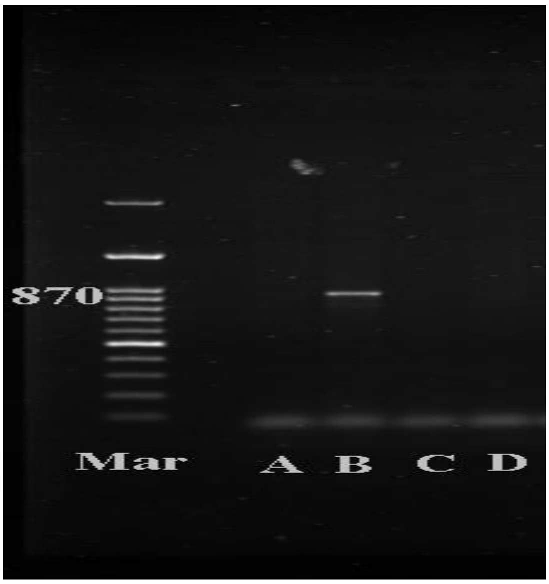



**Figure (2):** Agarose gel electrophoresis showing PCR amplified 18S rDNA product from using Myxosporean specific18S rDNA primers.
Mar= 100bp DNA marker, A=*Buddenbrockiaplumatellae*, B=sample, C= Negative extraction control, D= no-template control.

**Table 2:** Weight susceptibility to Myxosporidian infection in examined *Clarias gariepinus* in various seasons.

| Season / Weight | 100-300g (n=45) | 301-400g (n=34) | 401-600g(n=41) |
|---|---|---|---|
| **Autumn** | **2** | **5** | **2** |
| **Winter** | **6** | **4** | **7** |
| **Spring** | **9** | **3** | **5** |
| **Summer** | **5** | **6** | **3** |
| **Total** | **22** | **18** | **17** |
| **Percentage** | **48.9%** | **52.9%** | **39%** |

**Table 3:** Sex susceptibility to Myxosporidian infection in examined *Clarias gariepinus* in various seasons.

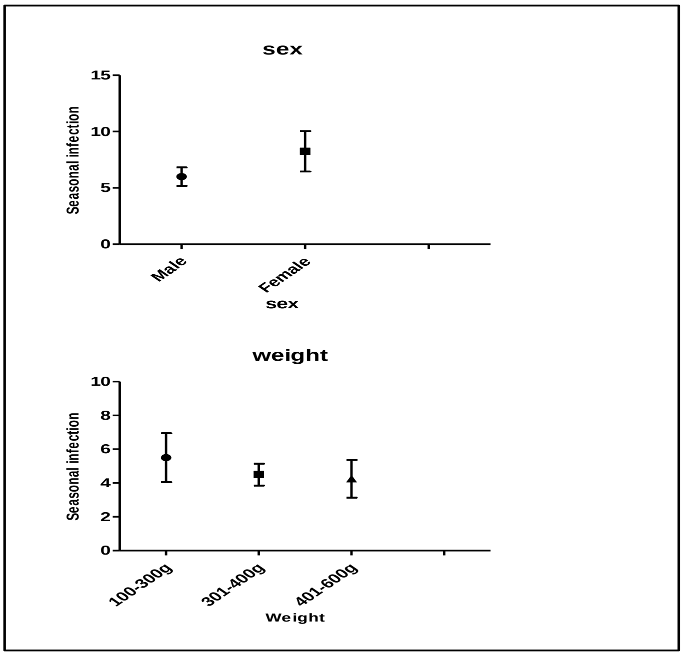
| Season / Sex | Male (n=52) | Female (n=68) |
|---|---|---|
| **Autumn** | **4** | **3** |
| **Winter** | **8** | **9** |
| **Spring** | **6** | **11** |
| **Summer** | **6** | **10** |
| **Total** | **24** | **33** |
| **Percentage** | **46.2%** | **48.5%** |

**Table 4:** Seasonal percentage of infection with Myxosporidian parasites in examined brain of *Clarias gariepinus.*

| Season | Autumn(n=30) | Winter(n=30) | Spring(n=30) | Summer(n=30) | Total(n=120) |
|---|---|---|---|---|---|
| **Infected fish** | **9** | **17** | **17** | **14** | **57** |
| **Percentage %)** | **30** | **56.7** | **56.7** | **46.7** | **47.5** |

**Table 5:** Comparison of measurements of spores of *Myxobolus sp.* previously described in some freshwater fishes in Africa (All measurements are provided in micrometers).

| *Myxobolus* spp | Host | Spore | | | Polar capsule | | N. of polar filament Coils | Organs |
|---|---|---|---|---|---|---|---|---|
| | | Shape | length | width | length | width | | |
| ***M. niloticus*** | *L. niloticus* | Elongate spore. polar capsules unequal | 10-12 | 6.5- 8 | Large: 5-7 small: 2.5-4.5 | Large: 2.5- 3.5 small: 1.5-2.0 | -------- | Fins |
| ***M. dossoui*** | *O. niloticus, T. zilli, T. mosambica* | Spherical spore Polar capsules are unequal in size | 8.5-11 | 8.0-10.5 | Large: 4.5-6.5 | Small: 3.5-5.0 | 7-9 5-6 | Gills |
| ***M. olio*** | *Barbus aspilus* | slightly pointed spore Polar capsules are unequal in size | 6.5 - 11.5 | 5-9.5 | Large: 4-7 x 2-5 | Small: 2-4 x 1.5-2.5 | -------- | Gills, kidneys and heart |
| **Present species** | *Clarias gariepinus* | Ovoid , Polar capsules are unequal in size | 8.5-9.8 (9.21± 0.65) | 7.3-8.2 (7.73± 0.45) | Large: 3.2- 4 (3.62± 0.39) | Small: 2.3-2.7 (2.5 ± 0.2) | 3-4 | Brain |




**(Chart 1)** Graph Shows body weight and sex susceptibility to Myxosporidian infection in examined *Clarias gariepinus*. Columns represent average seasonal infection in examined *Clarias gariepinus*. Data expressed by Mean ±SEM, where there is no significant different at p<0.05 using one-way ANOVA and using unpaired t test.

## DISCUSSION

The present study was carried out to investigate the brain infection with Myxosporidian parasites in *Clarias gariepinus* at Al fath center in Assiut Governorate.

Myxosporidian infecting nervous tissue do not elicit a significant host response (Langdon, 1990) who founded abnormal swimming behavior in juveniles but not adults from Western Australia fish which infested with *Myxobolus sp.* from brain.

The infection rate of *Myxobolus sp.*in brain of examined fish was 47.5 %. Myxosporean parasites are host, organ and tissue specific (Molnar, 1994), as a result of this specificity most Myxosporean develop in a given organ and in a tissue which occurs in different parts of the fish body (Masoumian *et al.*, 1996).The occurrence of Myxosporidian parasites depend on the presence of intermediate host, the degree of water pollution, the state of health or powers of resistance and availability of the hosts (El-Matbouli and Hoffmann, 1989). In addition, feeding habitats of catfish on fish, insect larvae, mollusks, planktonic organisms, benthic aquatic invertebrates and decomposing organic matter (Noga, 1996) may play a role. The percentage of infections with Myxosporidian parasites was 67 % in some Nile fish in Assiut Governorate Marwan (1980) &Abed (1987). In Pacific salmonids *M. arcticus* at prevalence approaching 100% (Kent *et al*., 1993; Awakura *et al*., 1995). In Qena Province, Egypt Mohammed *et al.* (2012) found that out of 246 fishes examined, 61 (24.8%) were infected with myxosporean parasites. In Giza province, Egypt Abdel-Gaber *et al.* (2017) conducted a survey of myxosporean parasites infecting Nile tilapia *Oreochromis niloticus*. Out of 100 fish specimens collected, 45 were found to be naturally infected with these parasites. Nevertheless, several investigators have detected high prevalence of infection with various Myxosporeans infecting different fish species (El-Matbouli *et al*., 1990; Lom and Dykova 1992; Abdel-Ghaffar *et al*., 1995; Saleh, 2015). This difference in percentage of infection may be due to difference in localities, from which the fish were collected, availability of invertebrate hosts, or other climatic conditions. Also these variations may be attributed to the environmental condition, degree of water pollution and water temperature.

The highest infection in fish in the present work was associated with middle and low weight. These results may be due to ill-developed immune system of small fish than older one. El-Mansy and Bashtar (2002) and Abdel-Ghaffar *et al.* (2008) stated that the highly infected fish attributed to weakness and disturbances in their immunity.

The current study found that female fish seems to be more susceptible to Myxosporidian infection than males. These results may be due to the physiology of females in the spawning period when they become readily vulnerable to parasites in the actinosporean stage, and immunological differences among host sexes as was suggested by Tombi and Bilong, 2004. A number of myxozoans exhibit sex specificity, infecting fish ovaries rather than testes may be caused by the specific need of the parasite, as oocytes and spermatozoa differ in composition and volume (Sitjà-

Bobadilla, 2009). These results were in line with those of Pampoulie *et al.*, 1999; Tombi and Bilong, 2004 who recorded the higher infection rate of females than males.

Concerning to the effect of seasonal variations of Myxosporidia infection in the examined fish, the highest percentages was recorded in winter and spring. Cold temperature proved to decrease the immune response and diseases resistance in fish (Abu El-Fadl, 2008). This fact may explain the highest infection rates in winter season. Similar results were recorded by many authors (Abed, 1987; Ali, 1999; Badran and Hashem, 2002; Mohamed, 2009 and Fawaz, 2013). The fluctuation of infection in various seasons may be due to the abundances of benthic organisms (Oligochaets) that vary with the seasonal variations of physical and chemical factors. These variations not only in species composition but also in densities and percentage were abundances (Tewabe, 2009).

The identification of the different species of Myxosporidian spores, especially those of *Myxobolus sp.* depend firstly on morphological and morphometric basis. The spores detected in the present work exhibited morphological characteristics of the genus *Myxobolus*, according to descriptions by Fomena and Bouix 1997. By comparing the shape and measurements of this species with different previously described species of *Myxobolus* we found that it is similar *M. niloticus*, *M. olio* and *M. dossoui*. *M. niloticus* spores were reported from the gills of *lobeo niloticus* by Fahmy *et al.* (1971) (LS: 11.2-13.5 WS: 5.6-7.5 LPC: 5.8-7.2‹WPC: 4.9-5.7µm) in Egypt. However, the present species has smaller size of both spore and polar capsule. As well, spores of *Myxobolus olio* were reported from gills, kidneys and heart of *Barbus aspilus* (slightly pointed spore LS: 6.5-11.5 WS: 5-9.5 LPC: 4-7 ‹WPC: 2-5µm) by Fomena and Bouix (1994) at South Cameroon, is almost similar with size of the present species. However the present species differ in having distinct intercapsular process and their anterior end is more or less rounded. While spores of *M. dossoui* were reported from gills of *O. niloticus, T. zilli,* and *T. mosambica* (Spherical in shape LS: 8.5-11.WS: 8-10.5 LPC: 4.5-6.5 ‹WPC: 3.5-5µm) by Sakiti *et al*. (1991) at Benin. The present species is ovoid in shape and its spore and polar capsule are smaller in size (Table 5).

Clearly there are significant differences between our species and the closely related species. Furthermore, our species infect different organ (brain) of examined fish.

Studying spores with molecular biological methods by the utilization of small subunit ribosomal DNA gene sequences which is the most sensitive approach for definitive species identification (Hallett *et al.*, 2006; Iwanowicz *et al.*, 2008).

## CONCLUSION

In conclusion, the *Myxobolus* species described in this study is different from other previously a known *Myxobolus* sp. found of brain of fish based on morphological and morphometric characteristics with target host. These require further confirm investigations including comparative ultra-structure study and molecular identification.

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## مرض الميكزوسبوريديا في مخ اسماك القراميط الافريقية (كلارياس جاربينيس)"

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الهدف من هذة الدراسة هو اجراء المسح الطفيلي والتحليل الجزيئى لعينات من مخ اسماك القراميط الأفريقية (كلارياس جاربينيس) فى محافظة أسيوط للتعرف علي الاصابة بطفيل الميكزوبولس. تم فحص عدد ١٢٠ سمكة مختلفة الجنس والوزن علي مدار العام بمعدل ١٠ سمكات شهريا ووجد ان عدد ٥٧ سمكة تحمل الاصابة بطفيل الميكزوبولس في المخ. وكانت معدلات الاصابة في اخر فصل الخريف قليلة ثم ازدادت في فصل الصيف حتي وصلت الي اعلي معدلاتها في فصل الشتاء والربيع ، وسجلت اعلي نسبة أصابة في الأوزان المتوسطة والصغيرة. كما كانت الأناث أكثر عرضة للأصابة بطفيل الميكزوسبورديا عن الذكور. وقد تم الوصف المورفولوجى الخاص بالبوغيات الناضجة. وقد أسفرت مقارنة مواصفات النوع الذى وجد فى مخ الاسماك المصابة مع الانواع الاخرى من سلالة ميكزوبولس عن وجود اختلافات مورفولوجية. وقد خضعت العينة المصابة بطفيل الميكزوبولس لإجراء تفاعل البلمرة المتسلسل واعطت نتيجة ايجابية لجين هذا الطفيل .