First Record Of Microsporidium Neonosemoides Sp. And Some Ciliates Infecting Chrysichthus Auratus (Bagridae) From The Damietta Branch Of River Nile, Egypt

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Abstract: The present study was carried out as a general survey for the possible ectoparasites that can infect the Nile fish Chrysichthus auratus. A total of 52 fish specimens were collected from Damietta branch of River Nile. Examination of the investigated fish revealed that, fish were infected with four ectoparasitic species belonging to three genera. These species were: Neonosemoides sp., Scyphidia sp. 1, Scyphidia sp. 2 and Ichthyophthirius multifilis. The first three species were recorded for the first time in Egypt. The recovered parasites have pathological effects on the host fish with subsequent economic losses were discussed.


Key words: Neonosemoides sp., Ciliates, Chrysichthus auratus, River Nile. Egypt.

Introduction
Microsporidia are obligate intracellular parasites. Infected cells usually enlarge to accommodate the proliferating parasite. In 1968 Wiessenberg coined the term "xenoma" on the host cell with completely changed structure and the parasite proliferating inside it. According to Klaus Rohde (2005) microsporidia infect most invertebrate phyla and all classes of vertebrate. There are 17 genera are known to infect fishes; 13 genera infect marine fishes and 4 genera infect freshwater fishes: Heterosporis (Schubert, 1969), Nosemoides (Vinckier, 1975), Neonosemoides (Faye, Toguebaye and Bouix, 1996) and Pseudoloma Matthew, Brown, Larison, Bishop-Stewart, Rogers and Kent, 2001 (Klaus Rohde 2005).

Genus Neonosemoides is one of microsporidian genera parasitizing freshwater fishes and at the same time produce xenoma which play an important agent of diseases in commercial fishes.

Although there is considerable information on the species of microsporidia (Lom and Dykova, 1992; Sprague et al., 1992; Lom, 2002; Lom and Nilsen, 2003), little is known about those from Africa. Sakiti and Bouix, 1987 recorded Neonosemoides tilapia from Tilapia zillii from Benin and Faye and Toguedaye, 2005 recorded 4 unidentified species from carangid fishes from Senegalse.

External protozoa are cited as major problem in freshwater fishes; sessilines ciliates like genus Scyphidia utilize gills and skin as a substrate for attachment.

On the other hand mobilina ciliates like genus Ichthyophthirius which is an obligate parasite of gills, skin and fins has a worldwide distribution (Paperna, 1980) also has been found to cause the white spot disease which is accompanied by severe morbidity and eventually end with fish mortality (Hoffman, 1970). Abu-El Wafa, (1988) and Koura et al., (1997), described I. multifilis from some freshwater fishes. This study aims to contribute to the ciliates fauna infecting Chrysichthys auratus with special emphasis on genus Neonosemoides as a first record in Africa and to establish a background for further studies.

Materials and Methods
A total of 52 fish of Chrysichthys auratus were collected from Damietta branch of River Nile near El-Mansoura. The collected fishes were transported to the laboratory in tank with good aeration. Fishes were kept a live until required in aerated glass aquaria. Fishes were identified according to Bashai and Khalil (1997).

Fish skin, fins and gills were firstly examined by the naked eye for detection of any macroscopically visible lesions. Samples of mucus were scraped gently from the skin, fins and gills, then spread on a clean slide and freshly examined under phase-contrast microscope for the presence of ectoparasitic protozoans. Some of the positive slides were air-dried and stained according to Klein;s dry silver impregnation method. Other positive slides were also air-dried, fixed with absolute methanol and stained with 10% Giemsa stain.

Detected protozoa were examined freshly, stained and identified according to Shulman (1984) and Lom and Dykova (1992 & 2005). All measurements were taken in micrometers (μm) mean
± SD (range). Figures were drawn with aid of camera lucida.

Results
The detected protozoan parasites were classified into two main phyla; Microsporidia and Ciliophora as following:

Phylum: Microsporidia
Genus: Neonosemoides
Neonosemoides sp.
Xenomas are white spherical, inhabiting gills range in size from 50-70 μm (mean 60 μm) in diameter. Xenoma consists of a simple lamellar wall measures 2.2 μm, contains only 16 mature macrospores in direct contact with the cytoplasm of the host cells and the three lobes hypertrophic nucleus of host cell. All spores in generally are surrounded by a light zone. Fully formed xenoma appears as “a bag of spores”. (Figs. 1A & 3A).

Spore description
Spores are egg-shaped with bluntly rounded poles (Fig. 1B). It measures 3.2±0.2 (3.0-3.4) μm in length X 1.6±0.3 (1.3-1.8) μm in width. The spore has a thin outer finely corrugated layer (exospore), thin inner layer (endospore) and an inner most simple cell membrane. The spore consists of three parts which determine the anterior-posterior polarity of the spore (Fig. 1C).

The anchoring disc (polar cap) is mushroom cap like-shaped and stained as a red granule by Giemsa stain (Lom & Dykova, 1992), which is highly characteristic of the group (Fig. 3A). It is eccentric (subapically) located.

The polar tube; is the first part and is inserted into the base of the polar cap. The manubrium part of the polar tube extends from the cap obliquely backwards. There is an outer sheath around the polar tube, acting as a sleeve, through which the tube slides while extruding.

The isofilar polar filament forms 4 regular and helically arranged coils around the surface of the posterior vacuole in the posterior half of the spore. The second part is the polaroplast; lamellar organelle consisting of an anterior region of closely packed membranes and posterior region of more loosely packed membranes that surrounding the basal part of the polar tube. The third part, is the posterior vacuole, that lies inside the coils of the polar tube and occupies more than one-third of the spore cavity.

The remaining space within the spore and between the polaroplast and the posterior vacuole is occupied by the infective germ itself, the sporoplasm. The nucleus is single, spherical and centrally located between the polaroplast and the posterior vacuole.

Phylum: Ciliophora
I-Genus: Ichthyophthirius
I. multifiliis
This parasite appears as a rounded-shaped ciliated organism (Figs. 2A, 3B & 4A). In heavily infested fish, this parasite could be easily detected by the naked eye inhabiting the gills, skin and fins. It is white in colour, tiny dots, exhibits a sluggish movement and measures 44.2-90.6 μm in diameter (mean 67.4). The body is uniformly covered by dense rows of cilia. The number of meridional kineties are ranged from 77-98 (mean 88), converging anteriorly and apically raised into a pointed elevation. The cytoplasm appears to be grossly granulated containing many small food vacuoles, the horse-shoe macronucleus measures (32.3-44.6) μm in length (mean 38.5) and lies in middle of the body. A rounded micronucleus is almost adhering to the macronucleus. There are many contractile vacuoles.

II-Genus: Scyphidia
Scyphidia sp. 1
This ciliate is solitary large parasite, inhabiting gills with cup-shaped body measures 57.6±3.6 (54-61.2) μm in length X 49.9±3.1 (46.8-53) μm in width. Epistomial disc is vaulted and slightly elevated above the peristomial disc. The peristomial disc is narrow and encircles the epistomial disc. The macronucleus is ribbon shaped, sinuous and measures 48.4±4.4 (44-52.8) μm in length. Micronucleus is very small. There are some scattered contractile vacuoles. Transverse striations of pellicle conspicuous and ranged from 80-110 (mean 95). There is non ciliated groove near the narrow scopula (Figs. 2B, 3C & 4B).

Scyphidia sp. 2
This peritrich is solitary parasite, inhabiting gills with cup-shaped body and measures 35.2±2(33.1-73.2) μm in length X 36.3 ±2(34.4-38.1) μm in width. Peristomial disc is narrow. Both epistomial disc and peristomial lips are at the same level. The macronucleus is ribbon-shaped, sinuous, occupies almost all the body cavity and measures 33.6±5(28.6-38.5)μm in length X 5.5±0.8(4.6-6.2). The giant micronucleus situated in close contact with the macronucleus and measures 11.3±1.4(9.9-12.6) μm in length X 2.2±0.4(1.9-2.7) μm in width. Scopula attached to the host skin directly by a secretory layer of sticky material. Infundibulum small and extends between the two nuclei by cytopyhrinx. There is a non ciliated groove situated anteriorly (Figs. 2C & 3D).

Discussion
1- Genus Neonosemoides

Neonosemoides sp.

The more conspicuous characteristics of the spore, the shape, wall, polaroplast, polar filament and posterior vacuole are used to distinguish microsporidia from other taxonomic groups (Sprague et al., 1992). According to the site of infection the present xenomas were found on the gills of freshwater fish Chrysichthys auratus, so it belong to genus Neonosemoides (Lom and Dykova, 1992). Recently (Lom and Dykova, 2005) reported that genera of microsporidia that comprise xenoma-forming species can be grouped in several categories according to xenoma wall, hypertrophic nucleus and type of spores inside xenoma. Accordingly the present investigated xenomas belong to genus Neonosemoides. Type and only species recorded in this genus is Neonosemoides tilapiae from Tilapia zillii (Sakiti and Bouix, 1987 and Faye et al., 1996) from Benin (West Africa). Comparing the present species with N. tilapiae, it was found many differences as listed in Table (1). So the present species assigned to the same genus but further ultrastructure and molecular study need to reveal the exact taxonomic assignment of this species.

The pathogenic effects induced by Microsporidia in host include physical disruption of cells due to occupation of intracellular space, host cell hypertrophy, change to host cell metabolism and reorganization of host cell components. The direct effects include increased mortality (Klaus Rohde, 2005). In the present work parasites are generally surrounded by a light zone the existence of which, is to be explained by the action of their proteolytic enzymes, which dissolve the host protoplasm around parasites and render it suitable for assimilation.

2- Genus: Ichthyophthirius

I. multifilisi

The parasite is identified by its characteristic horse-shoe shaped macronucleus in addition to the coarsely granular and vacuolated cytoplasm. Abu El-Wafa (1988) described I. multifilisi from different species of fishes but with smaller measurements (28 μm in diameter). He also found the same species in the grass carp Ctenopharyngodon idella with the measurements much larger (about 710 μm in diameter). The present study (67.4 μm) is similar to Koura et al. (1997) described the parasite from Oreochromis niloticus (57.5 μm).

The first symptom of heavy infection, white spots appear over the entire body “white spots disease”. Fins begin to fray, skin starts being eroded, gills are pale (anemia). Scales may detach, eyes sunken, fish hardly move followed by death (Lom and Dykova, 1992).

3- Genus: Scyphidia

Scyphidia sp. 1

The present investigated parasite is resemble in shape and measures to Scyphidia doliaris Chernova, 1977 (cited in Schulman 1984), but the latter has one contractile vacuole, epistomial disc is below the peristomial disc level and there is no non ciliated groove. This species is first record in Egypt.

Scyphidia sp. 2

Scyphidia sp. investigated during this study was characterized by the cup-shaped body, ribbon-shaped irregularly twisted macronucleus, occupies almost all the cell cavity. The most characterized feature was the detection of the giant micronucleus. The present Scyphidia sp. 2 is similar in shape and macronucleus to Scyphidia sp. described by Ahmed et al. (2000), but the present parasite have-smaller size and has giant micronucleus. The present parasite is closely resemble S. globularis described by Solomatova, 1977 (cited in Shulman, 1984), but the latter has a smaller macronucleus besides the micronucleus not detected. This species is first record in Egypt.

The pathogenicity of genus Scyphidia is attributed to disturbance in the respiratory process of the infected fishes, leading to asphyxia. (Paperna, 1980).

Explanation of figures

Fig. (1). Diagram of zenoma of Neonosemoide sp. (A) showing 16 macrospores, three lobes of hypertrophic nucleus and light zones. Mature spore (B) with characteristic egg-shaped and posterior vacuole. Mature spore (C) in details.

Fig. (2). Diagram of Ichthyophthirius multifilisi (A). with characteristic round-shaped, horse-shoe macronucleus and meridional kineties. Scyphidia sp. 1(B) with transverse striation of pellicle. Scyphidia sp. 2(C) with cup-shaped body. Note the ribbon-shaped and sinuous macronucleus and giant micronucleus.

Fig. (3). Giemsa stain zenoma (A). Note the presence of anchoring disc as a red granule (arrowhead), silver impregnation Ichthyophthirius multifilisi (B) and Scyphidia sp. 1(C) and Giemsa stain Scyphidia sp. 2(D). Not the sinuous ribbon-shaped macronucleus.

Fig. (4). Phase contrast microscope photograph of living specimens of I. multifilisi (A) and Scyphidia sp. 1(B). Note the non ciliated groove.
Abbreviations for all figures
ad: Anchoring disc
ap: Anterior part of polaroplast
c: Cilia
cm: Cell membrane
cp: Cytopharynx
cv: Contractile vacuole
cy: Cytostome
en: Endospore
cp: Epistomial disc
cv: Exospore
fv: Food vacuole
hcc: Host cell cytoplasm
hn: Hypertrophic nucleus
in: Infundibulum
ki: Kineties
lz: Light zones
ma: Macronucleus
mi: Micronucleus
mpt: Manubrium part of polar tube
n: Nucleus
ncg: Non ciliated groove
pd: Peristomial disc
pf: Polar filament
pl: Peristomial lip
pp: Posterior part of polaroplast
pts: Polar tube sleeve
pv: Posterior vacuole
s: Spores
sc: Scopula
slw: Simple lamellar wall
sp: Sporoplasm

Table (1): Comparative description of Neonosemoides tilapiae with the present species. (Measurements are in micrometers).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>N. tilapiae Sakiti and Bouix, 1987</th>
<th>Present Species</th>
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<tbody>
<tr>
<td>Xenoma size</td>
<td>120-800</td>
<td>50-70</td>
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<tr>
<td>Xenoma spores number</td>
<td>Many micro &amp; macrospores</td>
<td>16 macrospores</td>
</tr>
<tr>
<td>Nucleus</td>
<td>Multinuclei</td>
<td>One with three lobes</td>
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<td>Spore length</td>
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<td>Spore width</td>
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<td>Polar filament coils</td>
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<td>Bagrid Chrysichthys auratus</td>
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<tr>
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<td>Locality</td>
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References

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