

**First Record of Microsporidium *Neonosemoides* Sp. and some Ciliates Infecting *Chrysichthys 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 *Chrysichthys 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 multifiliis*. 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.

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

### 1. 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 (2005) microsporidia infect most invertebrate phyla and all classes of vertebrate. As mentioned by Klaus (2005) 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).

Genus *Neonosemoides* is one of microsporidian genera parasitizing freshwater fishes and at the same time produce xenoma which is 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* in *Tilapia zillii* from Benin and Faye and Toguedaye, (2005) recorded 4 unidentified species in carangid fishes from Senegal.

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). It 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. multifiliis* 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.

### 2. 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 alive 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 ( $\mu\text{m}$ ) mean  $\pm$  SD (range). Figures were drawn with aid of camera lucida.

### 3. Results

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

Phylum: Microsporidia

Genus: *Neonosemoides*

*Neonosemoides* sp.

Xenomias are white spherical, inhabiting gills range in size from 50-70  $\mu\text{m}$  (mean 60  $\mu\text{m}$ ) in diameter. Xenomias consists of a simple lamellar wall measures 2.2  $\mu\text{m}$ , 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 xenomias appears as "a bag of spores". (Figs. 1A & 3A)

#### Spore description

Spores are egg-shaped with bluntly rounded poles (Fig. 1B). It measures  $3.2 \pm 0.2$  (3.0-3.4)  $\mu\text{m}$  in length X  $1.6 \pm 0.3$  (1.3-1.8)  $\mu\text{m}$  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  $\mu\text{m}$  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)  $\mu\text{m}$  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

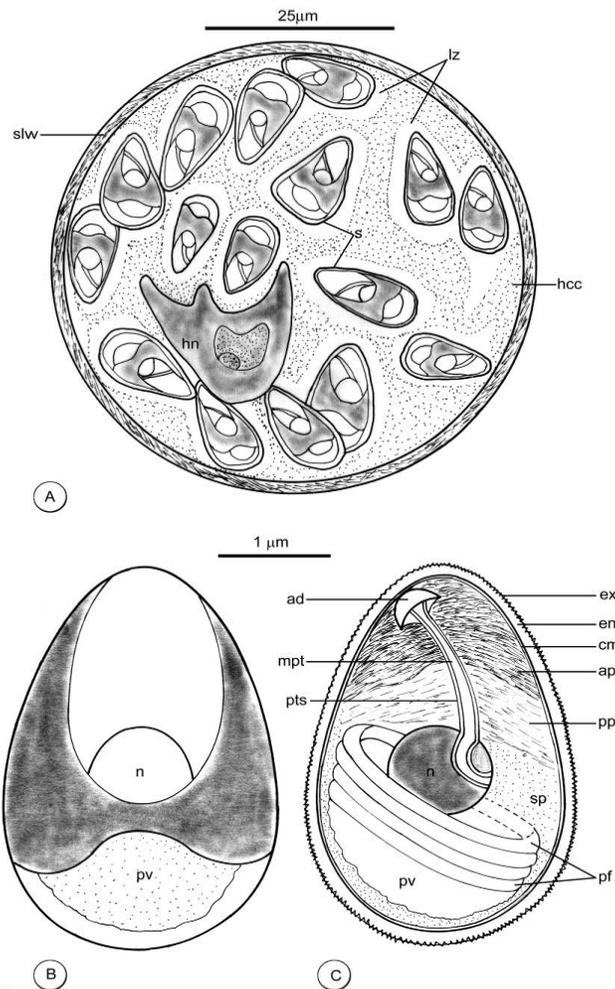
This ciliate is solitary large parasite, inhabiting gills with cup-shaped body measures  $57.6 \pm 3.6$  (54-61.2)  $\mu\text{m}$  in length X  $49.9 \pm 3.1$  (46.8-53)  $\mu\text{m}$  in width. Epistomial disc is vaulted and slightly elevated above the peristomial disc. The peristomial disc is narrow and encircles the epistomial disc. The macronucleus is ribbon shaped, sinuous and measures  $48.4 \pm 4.4$  (44-52.8)  $\mu\text{m}$  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  $35.2 \pm 2$  (33.1-73.2)  $\mu\text{m}$  in length X  $36.3 \pm 2$  (34.4-38.1)  $\mu\text{m}$  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  $33.6 \pm 5$  (28.6-38.5)  $\mu\text{m}$  in length X  $5.5 \pm 0.8$  (4.6-6.2). The giant micronucleus situated in close contact with the macronucleus and measures  $11.3 \pm 1.4$  (9.9-12.6)  $\mu\text{m}$  in length X  $2.2 \pm 0.4$  (1.9-2.7)  $\mu\text{m}$  in width. Scopula attached to the host skin directly by a secretory layer of sticky material. Infundibulum is small and extends between the two nuclei by cytopharynx. There is a non ciliated groove situated anteriorly (Figs. 2C & 3D).

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

Parameter	<i>N. tilapiae</i> Sakiti and Bouix, 1987	Present Species
Xenoma size	120-800	50-70
Xenoma spores number	Many micro and macrospores	16 macrospores
Nucleus	Multinuclei	One with three lobes
Spore length	2.5-3	3-3.4
Spore width	1.5-2	1.3-1.8
Polar filament coils	4-5	4
Host	Cichlid <i>Tilapia zillii</i>	Bagrid <i>Chrysichthys auratus</i>
Site	Gills	Gills
Locality	Benin (West Africa)	Egypt



**Figure 1**

**Fig. (1). Diagram of xenoma of *Neonosemoides* 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.**

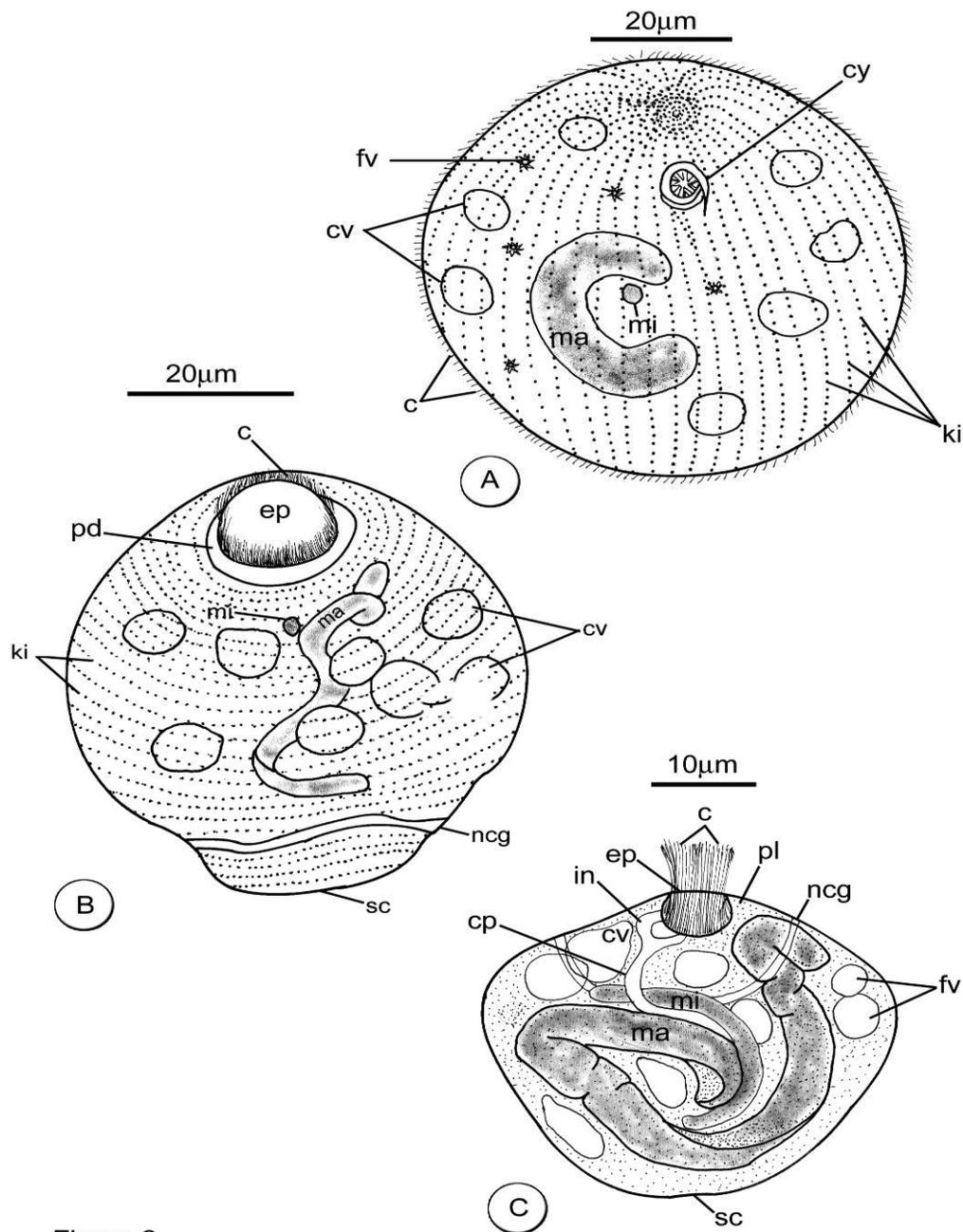


Figure 2

Fig. (2). Diagram of *Ichthyophthirius multifiliis* (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.

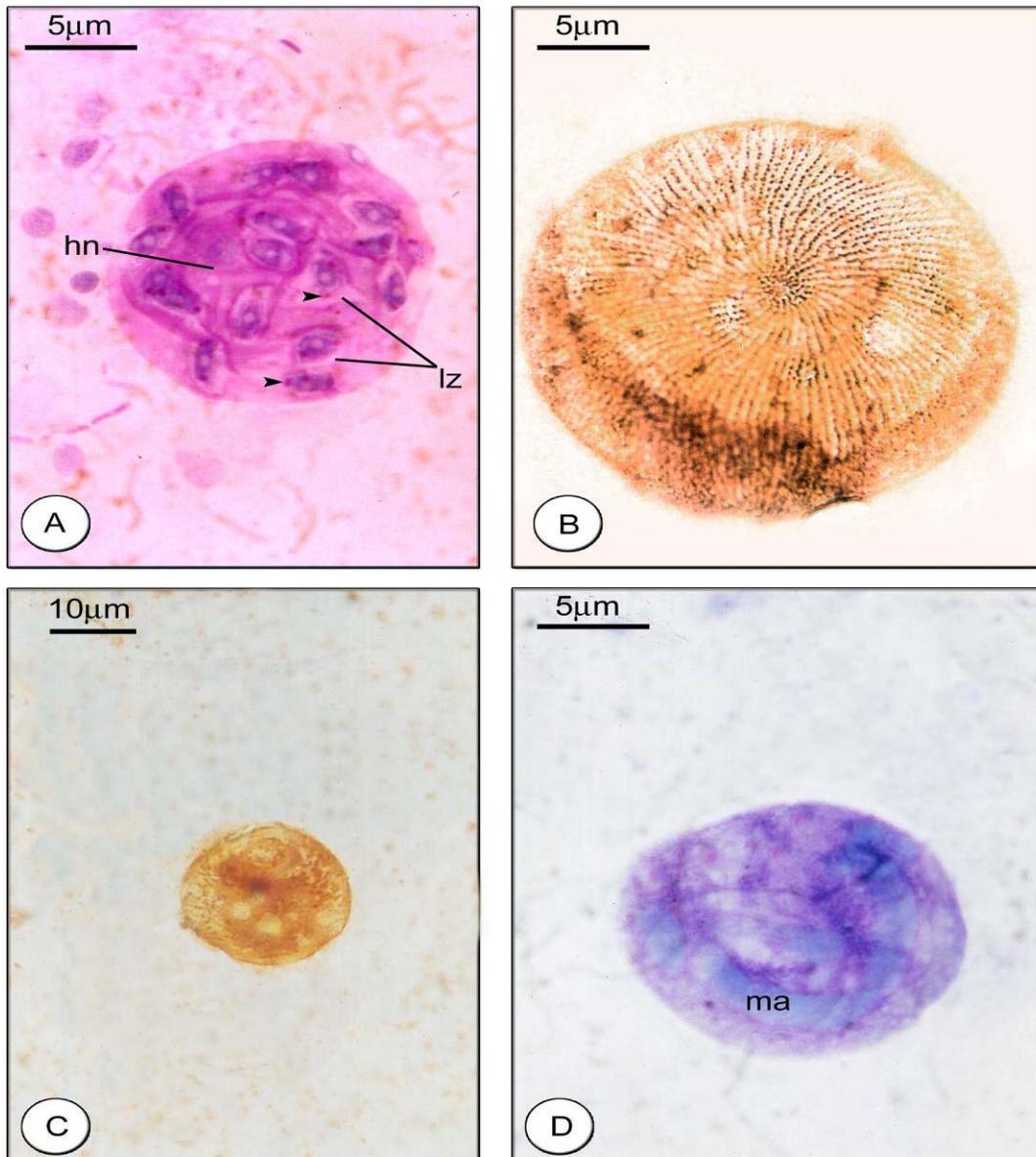


Figure 3

Fig. (3). Giemsa stain zenoma (A). Note the presence of anchoring disc as a red granule (arrowhead), silver impregnation *Ichthyophthirius multifiliis* (B) and *Schyphidia* sp. 1(C) and Giemsa stain *Schyphidia* sp. 2(D). Note the sinuous ribbon-shaped macronucleus.

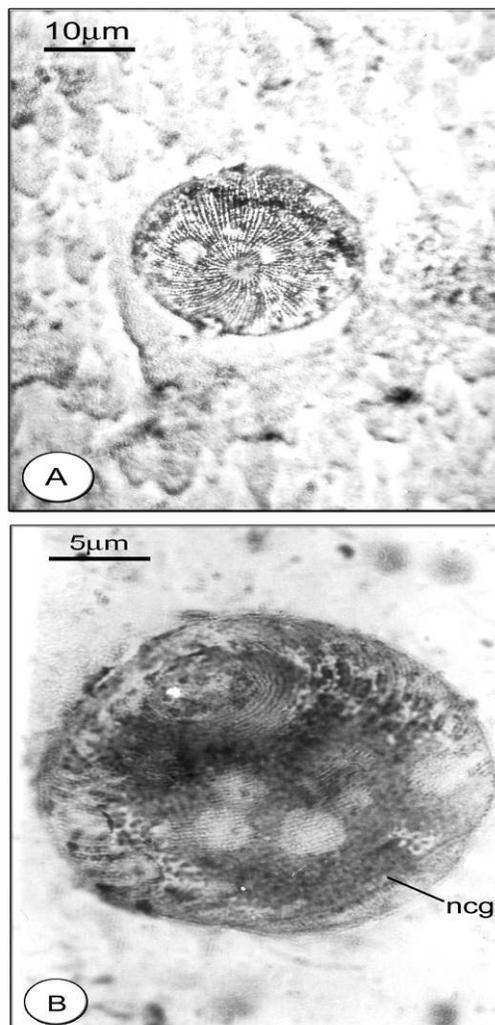


Figure 4

**Fig. (4).** Phase contrast microscope photograph of living specimens of *I. multifillis* (A) and *Schyphidia* sp. 1(B). Note the non ciliated groove.

Abbreviations for all figures

ad:	Anchoring disc	fv:	Food vacuole	ncg:	Non ciliated groove
ap:	Anterior part of polaroplast	hcc:	Host cell cytoplasm	pd:	Peristomial disc
c:	Cilia	hn:	Hypertrophic nucleus	pf:	Polar filament
cm:	Cell membrane	in:	Infundibulum	pl:	Peristomial lip
cp:	Cytopharynx	ki:	Kineties	pp:	Posterior part of polaroplast
cv:	Contractile vacuole	lz:	Light zones	pts:	Polar tube sleeve
cy:	Cytostome	ma:	Macronucleus	pv:	Posterior vacuole
en:	Endospore	mi:	Micronucleus	s:	Spores
ep:	Epistomial disc	mpt:	Manubrium part of polar tube	sc:	Scopula
ex:	Exospore	n:	Nucleus	slw:	Simple lamellar wall
				sp:	Sporoplasm

#### 4. 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 group (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 belongs to genus *Nosemoides* (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, 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. multifiliis*

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. multifiliis* 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 is the presence of 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 measurements 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).

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