

The infection of freshwater fishes with three species of *Henneguya* in Qena, Upper Egypt

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ABSTRACT

It is so for the first time to do general survey of protozoan parasites that infect freshwater fishes in Qena Province. The total number of fishes examined were 581 belonging to 10 families represented by 14 Species during the period from January 2006 to May 2008 . Three species of *Henneguya* were recorded from two species of fishes. The first species is suggested to be a new one called *Henneguya mandouri* sp.nov. The second species is *Henneguya assiuti*, Mandour *et al.*, 1988 and the third one is *Henneguya nilotica*, Marwan, 1998.

Key words: Freshwater fishes, *Henneguya mandouri* sp.nov., *Henneguya assiuti*, *Henneguya nilotica*, Qena , Upper Egypt.

INTRODUCTION

The genus *Henneguya* Thelohan, 1892 is one of the largest nine genera in the family Myxobolidae. The present parasites are placed in the genus *Henneguya* according to the morphological characteristics as described by Kudo (1966). In Africa, about 100 species of Myxosporean parasites were currently known (Fomena and Bouix, 1997). Examination of some freshwater fishes from Chari and Logone Rivers of Chad (Central Africa) revealed the presence of Myxosporean species of the genus *Henneguya* Thelohan, 1892. *Henneguya ghaffari* Ali, 1999 was recovered from gills and intestine of the Nile perch *Lates nilotica* from Chad in Senegal. Obiekezie and Schmahl, (1993) reported that *Henneguya latero* capsulate in the skin of cat fish (*Clarias gariepinus*) and *Heterobranchus bidorsalis* in Nigeria. In Egypt, the River Nile fish were first examined for Myxosporean parasites by Fahmy *et al.* (1975). then by Abed (1987), Iman *et al.*, (1987), Abdel-Ghaffar *et al.* (1995a, b, 1998), Ali (1998, 1999, 2000). Current and

Janovy (1976) studied the ultra-structure of inter lamellar *Henneguya exilis* in the channel cat fish. Abdel Ghaffar *et al.*, (1995) reported some *Henneguya* spp, from cat fishes. Mandour *et al.* (1988) described *Henneguya assiuti* collected from gills and respiratory trees of cat fish (*Clarias lazera*) .Ashmawy *et al.*, (1989) reported the occurrence of *Henneguya branchialis* in the gills, intestine and secondary respiratory organ of *Clarias lazera* collected from Behera water bodies. Abdel Ghaffar and his colleges in 1995 described the same parasite in Giza Province. Abed, (1997) studied the ultra-structure of the spores of *Henneguya assiuti* in the gill filaments and respiratory trees of the Nile fish (*Clarias lazera*) by light, scanning and transmission electron microscopy at Assiut. Marawan, (1998) described *Henneguya nilotica* from *Clarias lazera* in Assiut Province. Bianca *et al.*, (2003) described *Henneguya curvata* n.sp. from gills of *Serrasalmus spilopheura* in Brazil. Carlos and Edilson (2003) described a new species of *Henneguya pilosa* located in the gill filaments of

white piranha *Serrasalmus altuvei* Ramirey, 1965 in Brazil.

MATERIALS AND METHODS

Several species of freshwater fishes in Qena City were captured during the period from January 2006 to May 2008. They were collected from different freshwater bodies in Qena, including Qus, Qeft, El-Tramssa, El-Maana, Dandara, Dishna and Nagh-Hamadi.

Fishes were brought alive to the laboratory and examined for *Henneguya* species by a light microscope. Impression smears were made from gills, fins, liver, kidney, gonads and intestine then were fixed and stained with Giemsa's stain.

Scanning electron microscopic studies

Cysts were fixed in phosphate buffer 3.5% gluteraldehyde at pH 7.4 for 3-4 hours, post fixed in 1% Osmium tetroxide for 2 hours, washed in Na-phosphate buffer (pH 7.4). Fixed cysts were gently crushed in phosphate buffer to dislodge the spores that were transferred to glass cover slips which were mounted on copper stubs, gold coated and examined.

RESULTS

During the present study, 3 species of *Henneguya* were identified as following:

1- *Henneguya mandouri* sp nov.

The cysts of this parasite were collected from the middle and the base of the gill filaments of 32 out of 40 *Lates niloticus* fish (80%). With light microscopic level only large forms of spores were detected which have an ovoid body with a rounded anterior end Fig (1,2) which measured (11-13) x (6.0-7.5) μm , the tails were composed of 2 bifurcated filaments, measuring about (40-53) μm in length (Table 1). The polar capsules were

equal in size and pyriform, situated at the anterior end, measuring about (3.1-4.3 x 1.5-2.2) μm . The ratio of polar capsule / spore length were (0.23-0.36) μm , the total length of spores (51-66) μm , length / posterior process ratio was (0.2-0.3) μm . Polar filament coils are (3-5) as shown in fresh spores (Fig. 3) the length of polar filament is (12.5-23) μm (Fig. 4). The sporoplasm granulated, occupied 75% of the spore cavity with one iodophilous vacuole and 2 clearly visible sporoplasmic nuclei (Fig.5). This species of *Henneguya* was studied by SEM which revealed that the plasmodia were whitish, ellipsoidal, and oval in shape, measuring (0.2-1 x 0.1-0.5) mm (Fig. 6), the spores were resolved more clearly by SEM, and revealed that the surface of spores were smooth (Figs.7-9) the two caudal appendages are equal, some spores are joined and appear as one appendage (Fig. 8), but in others were composed of 2 bifurcated filaments (Figs.9,10). Enlarged spore showed the site of the polar capsules and the polar filaments, the cyst wall (Cw), suture (S) the anterior groove (AG), the posterior groove (PG), and the edges of the spore, (E) as in (Fig. 11).

2- *Henneguya assiuti* Mandour *et al.* (1988):

This parasite was collected from gills of *Clarias gariepinus*, where 7 out of 28 fish examined (25%) were infected with the parasite. Cysts are oval to round in shape, yellowish in colour, filled with fluid containing a suspension of mature spores which appear milky white by naked eye. Mature spores were pyriform in shape, measuring about (9.8-13.5 x 4.2-5.5) μm . The posterior end was prolonged into more or less extended process to form 2 often equal caudal appendages, measuring about (30.4-37.2) μm ., the polar capsules appear equal in length (7.2- 7.9 x 1.5-2) μm as in Fig (12) or

unequal especially in length (Fig.13). Polar filaments are coiled (8-9 coils) inside the polar capsules and the iodophilic inclusions are seen in fresh spores stained with iodine as in Fig. (14).

3- *Henneguya nilotica* Marwan (1998):

This parasite was observed in 2 out of 28 *Clarias lazera* (7.14 %). The spherical cysts were observed on the tips of the secondary respiratory organ, measuring (1.0-3.0) mm., mature spores are ellipsoidal with a blunt anterior end and a tapering posterior end, measuring about (13.5-15 x 4.5-5.6) μm , the spore is surrounded by a homogenous dense sheath when the spores stained with Giemsa's stain. Sheathed spores have dissimilar staining reaction that may take deep violet, purple or blue colour Figs. (15,16). When the outer sheath was ruptured, the parasite was stained dark blue, while the sheath took a light

violet color as in Figs. (17,18). Sheathed spores had the same body length as uncoated ones, but the later appeared broader reaching (4.5-5.6) μm , while their posterior tails were shorter reaching 19 in length, the two processes were seen adhering to each other as in Fig.(18). Each spore had two equal polar capsules which were elongated, thin and occupy approximately half the body length, they measured (6.5-7.9 x 1.5-2.5) μm . Polar capsules were seen adhering to each other at the anterior end of the spore and separated a part in the lateral sides of the spore. Spores treated with Lugol's Iodine solution showed one or two iodophilous vacuoles and 8-9 coils of polar filaments as in Fig. (14). These vacuoles were irregular in shape, situated in-between the two polar capsules, directly behind them or at the posterior third and adhering to the lateral side of the spore.

DISCUSSION

1-*Henneguya mandouri* sp. nov., present species from *Bagrus bayad*:

According to the available literature, 4 species of *Henneguya* have been reported namely *Henneguya assiuti* Mandour *et al.*, 1988; *Henneguya nilotica* Marwan, 1998; *Henneguya ghaffari* Ali, 1999 and the fourth unnamed species *Henneguya* sp. (indetermined) Abed, 1987. *Henneguya ghaffari* Ali, 1999 and *Henneguya* sp. Abed, 1987 are more or less similar to the present parasite. According to Table (2) it is quite clear that the present species of *Henneguya* appears to be a new one to which the name, *Henneguya mandouri* sp. nov. is suggested while, Qena Province is a new locality.

Specific diagnosis

Host: *Lates niloticus*

Site of infection: Gill.

Locality: Qena (a new locality)

Type material: Deposited in the department of Zoology, Faculty of Science, South Vally University, Qena.
2-*Henneguya assiuti* Mandour *et al.*, 1988 from *Clarias gariepinus*:

Mandour *et al.*, (1988) described *Henneguya assiuti* from *Clarias lazera* in Assiut Province by a prevalence of 21.02 % while in present study the same parasite was collected from the same host in Qena Province where the incidence of infection was is 25%. The spores described above are identified as *Henneguya assiuti* in account of the marked similarities between the spores of those described by Mandour *et al.*, (1988) and Abed (1997). Mandour *et al.* (1988) reported that cysts occurred in gills and secondary respiratory organ; while Abed (1997) and Marwan (1998) and in the present work cysts were reported from the gills only *Henneguya assiuti* may be related to *Henneguya* sp. Abed, (1987) which was described from the

gills of *Lates niloticus* due to the length of the spore and the long posterior processes, but differs in having bigger polar capsules reaching (7-9) μm in length, in comparison with the parasite of *Lates niloticus* which measures (3-4) μm . Also, the polar capsule of *Henneguya assiuti* has 8-9 polar filament coils while in *Henneguya* sp. there are 3-5 polar filaments coils. The total length of *Henneguya* sp. is larger than the present one.

3- *Henneguya nilotica* Marwan, 1998 from *Clarias gariepinus*:

Marwan (1998) described *Henneguya nilotica* as a new species from *Clarias lazera* in Assiut Province where the parasite was obtained from the secondary respiratory organ of 18.18 % of specimens collected from Assiut Province. During the present study the incidence of infection was 7.14 % in the same host. The spores that described above are identified as

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Henneguya nilotica on account of the marked similarities between the spores of the parasite with those described by Marwan (1998).

Table (2): shows a comparison between some previously described *Henneguya* species in the two fresh water fish species: *Lates niloticus* and *Clarias lazera*.

It is clear that the spores in the two descriptions are closely comparable both in their structure and measurements. *Henneguya nilotica* is similar to *Henneguya testicularis* that is a sheathed myxozoan (Azevedo *et al.*, 1997) which infects the testis of the Amazonian fish *Moenkhausia oligolepis*, but this parasite is unique in its site of infection, locality and host. Mature spores of this parasite have short caudal processes compared with the present sheathed species, being 13-14.5 μm .

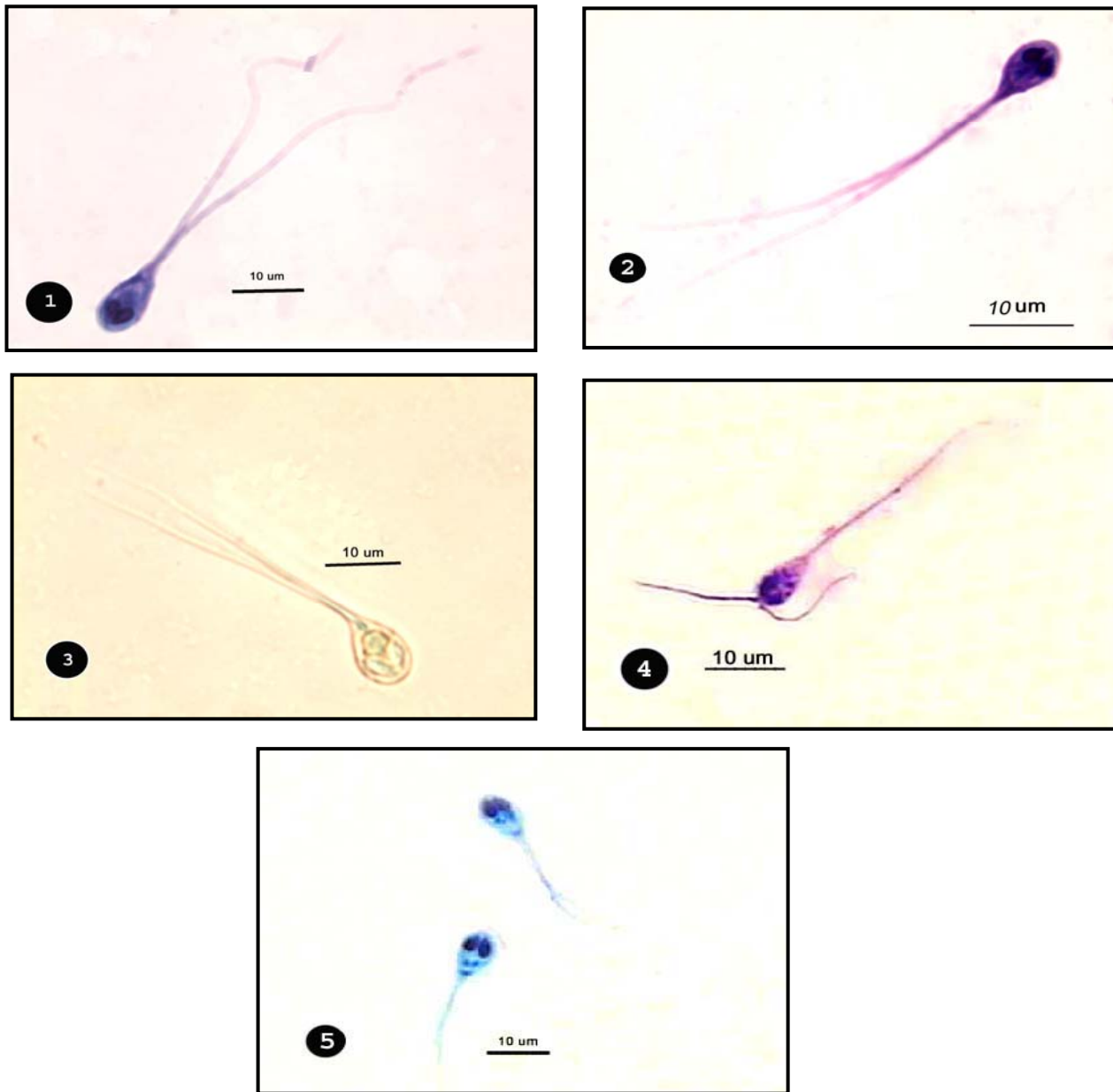
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Table (1): Showing the measurements by μm . of the three described species of *Henneguya* in the present work.

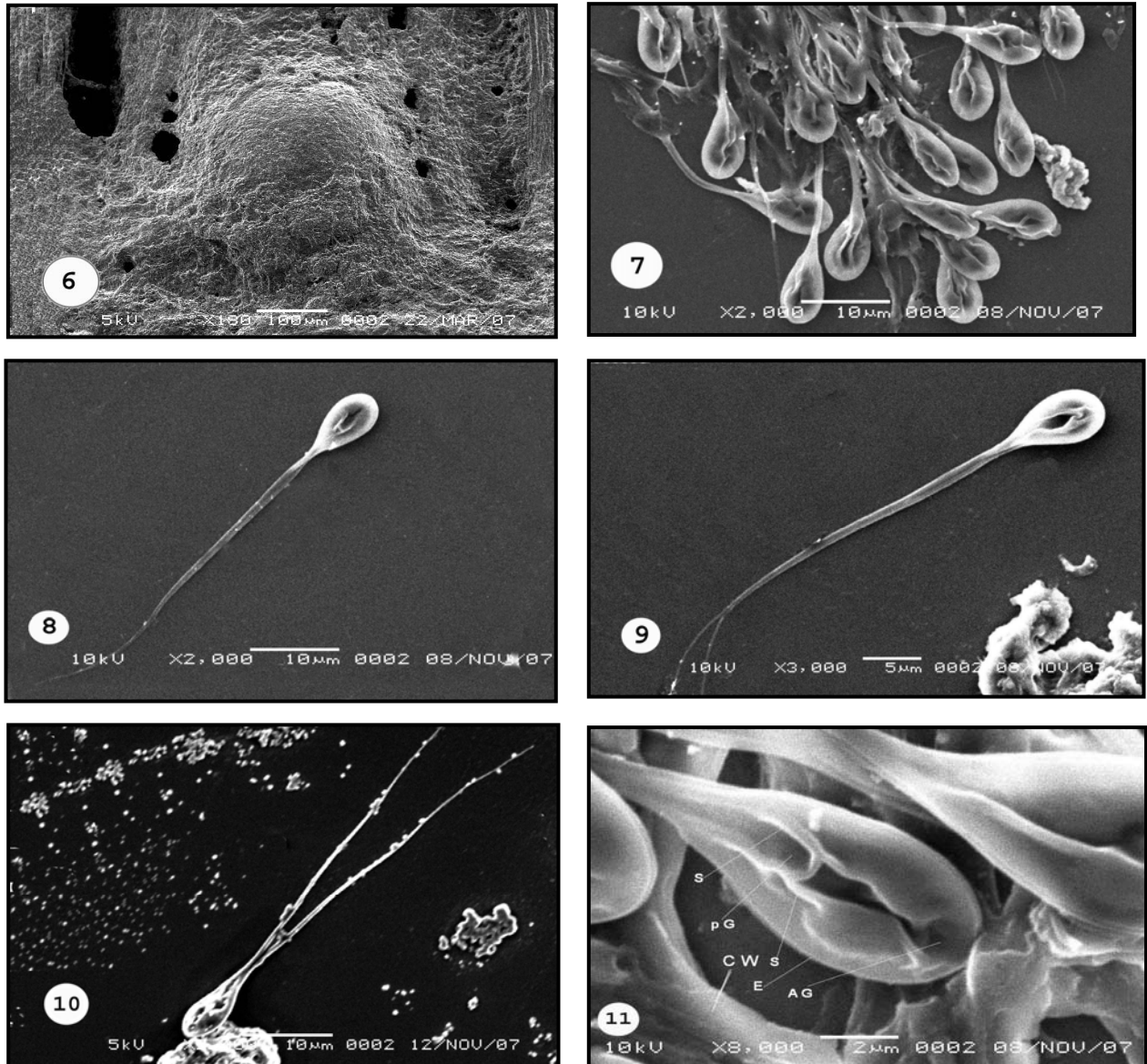
<i>H. species</i> Criteria	<i>Henneguya mandouri</i> sp. nov. (present parasite)	<i>Henneguya assiuti</i> Mandour <i>et al.</i> , 1988	<i>Henneguya nilotica</i> Marwan, 1998
Host	<i>Lates niloticus</i>	<i>Clarias gariepinus (lazera)</i>	<i>Clarias gariepinus (lazera)</i>
Site of infection	Gills	Gills	Secondary respiratory organ
Length	11-13	9.8-13.5	13.5-15
Width	6.0-7.5	4.2-5.5	4.5-5.6
Polar capsules length	3.1-4.3	-equals: 7.2-7.9 -unequal: 6.8-7.6, 4.8-5.5	6.5- 7.9
Polar capsules width	1.5-2.2	1.5-2.0 in both cases	1.5-2.5
Posterior process	40-53	30.4-37.2	18-22
Total length	51-66	40.2-50.7	31-37
Length/posterior process ratio	0.2-0.3	0.3-0.34	0.7-0.8
Iodine vacuole	have one iodophilous vacuole	have one iodophilous vacuole	have one or two iodine vacuole (1.7-3.2) μm in length
Polar filament coils	3.0-5.0	8-9	7-9
Polar filament length	12.5-23	-----	-----
Ratio of polar capsule/spore length	0.23-0.36	0.7- 0.8	0.45-0.52

Table (2): A comparison between some previously described *Henneguya* species in the two freshwater fish species: *Lates niloticus* and *Clarias lazera*, where: H-*Henneguya*, SBL-spore body length, PCL- polar capsule length, PFC- polar filament coils, PP- posterior process, TBL- total body length. And all measurements by μm .

Parasite	Host	Site	Locality	Mean measurements					Remarks
				SBL	PCL	PFC	PP	TBL	
<i>H. clariae</i> Abolarin, 1971	<i>Clarias lazera</i>	Gills	West Africa	22 X 6.5	11 X 3	---	66	---	-----
<i>H. sp.</i> Abed, 1987	<i>Lates niloticus</i>	Gills, brain, intestine and liver	Assiut ARE	11.8 X 6.3	3.14 X 1.34	3-5	-----	52.29	Collected from the River Nile
<i>H. assiuti</i> Mandour <i>et al.</i> , 1988	<i>Clarias lazera</i>	Gills & SRO	Assiut ARE	12.3 X 5.1	6.0 X 1.54	9	---	45.5	Collected from the River Nile
<i>H. branchialis</i> Ashmawy <i>et al.</i> , 1989	<i>Clarias lazera</i>	Gills & SRO	Behera & Giza ARE	14.5 X 5.6	6.2 X 2.2	4-10	17.3	-----	-----
<i>H. saoudi</i> Marwan, 1998	<i>Clarias lazera</i>	Intestine	Assiut ARE	17.3 X 5.7	- 5.78 X 2.5 - 5.0 X 2.5	6-7	13.98	31038	Spores with unequal polar capsules
<i>Henneguya massi</i> Kostoingue <i>et al.</i> , 2001	<i>Lates niloticus</i>	Gills	Chad, central Africa	8-9 X 5-6	2-3 X 1-2	----	12-14	20-23	Caudal appendages short, well separated, spore was oval with rounded anterior and attenuated posterior end
<i>Henneguya nilotica</i> Marwan, 1998	<i>Clarias lazera</i>	SRO	Assiute ARE	12.6-17.4	7.2-8.4 X 1.5-2.7	-----	22-30	-3502 45.6	Sporoplasm with 1-3 small vacuoles
<i>Henneguya Ghaffari</i> Ali, 1999	<i>Lates niloticusa</i>	Gills	Egypt	11-13 X 7.5	5.2X 3.2	---	36-53	48- 66.5	Polar capsules pyriform
<i>Henneguya assiuti</i> , present parasite	<i>Clarias lazera</i>	Gills	Qena ARE	9.8-13.5 X 4.2-5.5	- 7.2-7.9 X 1.5-2.0 - 4.8-5.5 X 1.5-2.0	8-9	30.4- 37.2	40.2- 50.7	The two polar capsules are equal in some spores and unequal in others
<i>Henneguya nilotica</i> , present parasite	<i>Clarias lazera</i>	SRO	Qena ARE	13.2-15 X 4.5-5.6	6.5-7.9 X 1.5-2.5	7-9	18-22	31-37	Spores sheathed, two pyriform process found in the posterior end of spore body
<i>Henneguya mandouri</i> sp. nov., present parasite	<i>Lates niloticus</i>	Gills	Qena ARE	11-13 X 6-7.5	3.1-4.3 X 1.5-2.2	3-5	40-53	51-66	Spores with unequal polar capsules



Figs. (1-5): Light photographs of mature spores of *Henneguya mandouri* sp. nov. in *Lates niloticus*; Figs. 1,2: Spores stained with Giemsa's stain, showed the ovoid body of the spores with rounded anterior end, polar capsules, and also showed the caudal process length, Fig. 3: Fresh spore by using Lugol's iodine solution showed the sporoplasm, Fig. 4: Spore stained with Giemsa's stain, showed the polar filament when it is extruded, Fig. 5: Spores stained with Giemsa's stain showed the presence of two sporoplasmic nuclei.



Figs. (6-11): SEM micrograph of mature spores of *Henneguya mandouri* sp. nov.; Fig. (6): SEM micrograph showing the site, size, and shape of *Henneguya mandouri* sp. nov. (Present parasite) cyst on gills of infected *Lates niloticus* fish, Fig. 7: SEM micrograph of *Henneguya mandouri* sp. nov. showing irregular masses of spores embedded in plasmodia, Fig. 8 : Showed that the two caudal appendages are joined with each other and appear as one appendage, Fig. 9: Showed that the caudal appendage composed of two bifurcated filaments, where the separation is near to its end, fig. 10: The separation of caudal appendage is in the anterior part of it. Fig. 11: Is an enlarged spore showing the cyst wall (CW), suture (S), the anterior groove (AG), the posterior groove (PG), and the edges of spore (E).

اصابة اسماك المياه العذبة بثلاثة انواع من طفيل هينوجويا فى قنا، صعيد مصر

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للمرة الاولى تم فى هذا البحث اجراء مسح للطفيليات الاولية التى تصيب اسماك المياه العذبة فى قنا. وكان العدد الكلى للاسماك التى تم جمعها هو 581 وتنتمى الى 10 عائلات وشكلت 14 نوع، وذلك فى الفترة من يناير 2006 وحتى مايو 2008. كما تم تسجيل اصابة نوعين من الاسماك بثلاثة انواع من طفيل هينوجويا. النوع الاول يقترح انه نوع جديد ويسمى هينوجويا ماندورى. والنوع الثانى هو هينوجويا اسيوطى، (مندور واخرون 1988) واما النوع الثالث هو هينوجويا نيلوتيكيا، مروان 1998