

Light and Scanning Electron Microscopic Studies of Trypanosomes Parasites Infecting Freshwater Fishes in Qena Governorate, Egypt

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ABSTRACT

Freshwater fishes were sampled from different localities in the River Nile at Qena Governorate (Qus, Qift, Dandara, El-Trammsa, El-Maana, Dishna and Nag-Hammady). Investigation revealed that *Trypanosoma* were found parasitized blood of many fish species. Out of 581 blood smears examined, 143 revealed trypanosomes infection with a prevalence of 24.6%. The infected fishes are belonging to seven species and four families. Trypanosomes parasites were found belonged to three species with different infection rates: *T. mukasai* Van As *et al.* 2004 (28.3%); *T. cyanophilum* Mohamed, 1978 (52.9%) and *T. cataractae* Putz, 1972 (1.5%). A new *Trypanosoma* species was found infecting *Ch. ruppelli* fish with an infection rate of 1.96%. Histological sections showed the presence of the adult stage of *T. mukasai* in between liver cells. Also *T. mukasai* had been examined using scanning electron microscope.

Keywords: Fishes, Freshwater, *Trypanosma*, Infection rate, Qena, Egypt

INTRODUCTION

Genus *Trypanosoma* belongs to haemoflagellates is prevalent in both marine and fresh water fishes; they are living principally extracellularly in the blood and tissue fluids (Hoffman, G.L., 1970; Duijn, C.V., 1973; Al-Salim, N.K., 1980 and Overath, *et al.*, 1999).

Most species of trypanosomes in the natural hosts produce no serious damage, however; several species cause pathogenic diseases of considerable medical and economic importance. This is particularly true in the tsetse fly areas of Africa, where sleeping sickness of man and trypanosomiasis in cattle, sheep, horses and goats are amongst the major obstacles to economic and social progress.

In Europe, Laveran, A. and Mesnil, F. (1901) described two forms of *Trypanosoma* remake from the pike *Esox lucius* in France, Keysselitz, G. (1906) found the same parasite in the same host

fish in Germany and England respectively, Hawking, F. (1957) found Trypanosomes in the English fresh water perch. Qadri, S.S. (1962) described *T. winchesiense* in the blood of the mirror carps. Prevalence of *T. granulorum* consistently high in eels from the lake sites in western Ireland (Zintl, *et al.*, 1997). Akmirza, A. and Tepecik, R.E. (2007) had been studied the seasonal variation in some haematological parameters in naturally infected and uninfected roach (*Rutilus rutilus*) with *Cryptobia tincae* in Marmara region, Turkey.

Minchin, E.A. (1909) described the polymorphic trypanosome (*T. percae*) from the English *percea* var. *Canadensis* from Canada. The hemoflagellate *T. catostomi* was described by Daly, J.J. and Degiusti, D.L. (1971) from the blood of white sucker (*Catostomus commersoni*) from Flemings Creek in Washtenaw country, Michigan, *T. catostomi* was

described in 33 of 285 white suckers (prevalence 11.6 %) (Lom, G., 1979 and Woo, P.T.K., 1987 and 1994). *T. carassii* is a wide spread parasite of carp and other cyprinid as well as some non-cyprinid fresh water fish in West Virginia, USA (Lom, J. and Dykova, I., 1992). *T. immanis* has been found parasitizing *Loricariichthys anus* from Rio Grande do Sul, Brazil (Froes, *et al.*, 1978).

In Asia, China, *T. clariae* had been recorded -for the first time- from *C. macrocephalus* by Mantel, R. (1905). The research concerning trypanosomes in fishes had many steps. Qadri, S.S. (1955) described *T. striate* from *Ophicephalus striatus* in Hyderabad, India. Hassan, R. and Qasim, S.Z. (1962) reported *T. punctati* from *Ophiocephalus punctatus* in India. Qadri, S.S. (1962) described *T. batrachi* from the Indian fish *C. batrachi*. Trypanosomes occur in *C. lazera*, in the Near East, but not in Cichlids or Cyprinids. Trypanosomes have also been reported from introduced *Or. mossambicus* in India (Mandal, A.K., 1977).

Regarding to Arabic countries, A few new species of trypanosome has been recorded and described in Iraq (Warsi, A.A. and Fattohy, A.L., 1976; Fattohy, Z.L., 1978; Al-Jafry, A.R. and Rahema, Z.I.F., 1982; Al Salim, N.K., 1985; Al-Jafry, *et al.*, 1988 and Al-Salim, *et al.*, 1990 and 1992).

Prior to 1960 Knowledge of Trypanosomes infecting fresh water fishes in Africa was limited to probably 13 publications describing almost as many species These records were form fishes in French, West Africa, Congo, Sudan, Uganda Mosambique and Natal (Baker, J.R., 1960, 1961). Careful scrutiny of these reports led to conclude that only three of these species could be considered valid: *T. toddi* Bouet, 1909, *T. mukasai* Hoare, 1932 and *T. tobeyi* Dias, 1952. Even so, Baker, J.R. (1960) noted that a "small difference in nuclear

position" was the only character separating *T. toddi* from *T. tobeyi*, he also reported that *T. toddi* was not remarkably different from *T. mukasai* and *T. tobeyi* might prove asynonym of the same trypanosome.

Since 1961, new species of trypanosomes from fresh-water fishes have been described from South Africa and Egypt (Pienaar, U.D.V., 1962 and Negm- El-Din, M.M., 1997, 1998), and *T. froesi* Lima, 1976, from the Rivers and lagoons of southern Africa (Paperna, I. 1996 and Smit, *et al.*, 2000). However, elucidation of the life cycle of *T. mukasai* involving its transmission to a variety of wild-caught host fishes has led to the conclusion (Negm- El-Din, M.M., 1998) that most records of fresh water fish trypanosomes across Africa may be of a single species, (*T. mukasai*).

Sudan is one of the earliest African countries in which fish trypanosomes had been reported, where trypanosomes recorded by Neave, S. (1906) in the blood of *Mugil* sp., *B. bayad* and *Synodontis schall*; and in blood of *Ophicephalus obsccurus*, in the *mugil* sp., *B. bayad*, *S. schall*, *Ch. auratus*, *C. anguillaris* and *Ti. zilli* by Wenyon, C.M. (1909). In 1976, Saoud came with some new hosts (*Mormyrus kannume*, *B. bayad*, *B. docmac*, *Ch. auratus*, *C. anguillaris*, *C. lazera*, *S. schall*, *S. batensoda*, *S. membanceus*, *S. serratus*, *S. khartomensis*, *S. filamentosus* and *Ti. zilli*) harboring trypanosomes caught from the Nile River in the Sudan.

In other African countries fish trypanosomes had been found parasitizing *C. angolensis* in the Congo by Dutton, *et al.* (1907). Bouet, (1909) described *T. toddi* from *C. anguillaris* in French West Africa. He believed that *T. toddi* was the same as that described by Dutton, *et al.* (1907). Fantham, H.B. (1919) recorded trypanosomes in the blood of *C. gariepinus* in South Africa. Baker, J.R. (1960) described *T. mukasai* in five genera of fish in Uganda. He

concluded that all Trypanosomes of African fresh-water fish could be related to *T. toddi* Bouet, 1909, *T. mukasai* Hoare, 1932 and *T. tobeyi* Dias, 1952. In Lake Victoria, *Or. variabilis* and *Or. esculenta* were infected with trypanosomes, *Or. niloticus* in Lake George (Baker, J.R., 1960 and 1961). From the silver catfish *Schilbe intermedius* Ruppell, 1832, tentatively identified *T. mukasai*, in the Okavango Delta region of Botswana (Smit, *et al.*, 2000). *T. mukasai* from different fishes in the Okavango Delta, *Or. andersonii*, *Serranochromis angusticeps*, *Se. macrocephalus*, *Se. robustus*, *C. gariepinus*, *C. theodora*, *Parauchenoglanis ngamensis*, *S. nigromaculatus*, *S. vanderwaali*, *Sc. intermedius* (Smit, *et al.*, 2004). *Trypanosoma* infection was observed in *C. gariepinus*, Lagos Nigeria, where the overall prevalence was 7.23 %; while the overall prevalence of trypanosome infection in *S. Clarias* was 35.24 % (Hassan, *et al.*, 2007).

In Egypt, Fresh-water fish's trypanosomes had a little focused. Mohammed, M.A., 1978 had described *T. alhaussainii* from *C. lazera* caught from the Nile near Cairo. Marwan, A.M. (1980) described this species in *C. lazera* and other three species (*T. synodonti* n. sp. from *S. schall*, *T. bagri* n. sp. from *B. bayad*, and *T. mansouri* Mohammed, 1978 in *Ch. auratus*) from the River Nile at Assiut, Upper Egypt.

The present study aimed to identify and describe the blood parasites of the genus *Trypanosoma* parasitizing freshwater fishes in Qena Governorate, Egypt.

MATERIALS AND METHODS

Freshwater fishes were captured from different localities of the River Nile in Qena Governorate. The collected fishes were identified according to the Publication of National Biodiversity Unit No. 9, 1997. Blood smears from the

caudal veins of each fish have been made. Air -dried blood smears were fixed in methanol and stained using Giemsa's stain and then examined for Trypanosomes.

From the infected fish with *T. mukasai* in *B. bayad* small pieces of liver, spleen and kidney were washed in saline solution 0.7%, fixed in formol-alcohol glacial acetic acid, dehydrated in ascending series of ethyl alcohol. The specimens have been cleared in xylene, impregnated in paraplast wax, embedded and sectioned. After removing of wax the sections have hydrated in descending series of ethyl alcohol. The sections have been stained in Ehrlich's hematoxylin stain and differentiated then stained in Eosin stain. After completion dehydration, clarified, mounted in Canada balsam, the sections have examined using light microscope.

The infected blood with *T. mukasai* in *B. bayad* has spread on a cover slip, fixed in phosphate buffered 5% gluteraldehyde (PH 7.4), post-fixed in 1% osmium tetroxide. The smears washed in Na-phosphate buffer (PH 7.4) then processed in drying apparatus, subsequently mounted on copper studs and gold coated. The smears had been examined using JEOL JSM-T5 kv scanning electron microscope (SEM).

RESULTS

Trypanosomes were found parasitized many fish species. Out of 581 examination blood smears, 143 revealed trypanosomes infection with a prevalence of 24.6%. The infected fishes are belonging to four Families: Bagridae (*B. bayad*, *Ch. rueppelli*, *Ch. auratus*); Cichlidae (*Ti. zilli*, *Or. niloticus*); Clariidae (*C. lazera*); Cyprinidae (*L. niloticus*).

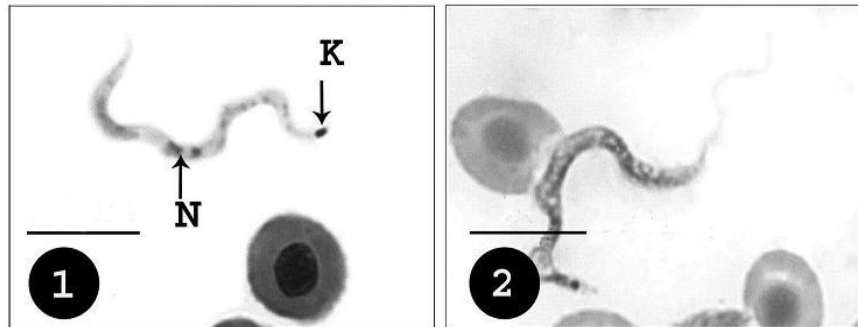
1- *T. mukasai* Van As *et al.*, 2004 (Figs. 1-8)

T. mukasai had been found parasitizing blood in different species of fishes (*B. bayad*, *Ch. auratus*, *C.*

garipepinus, *Ti. zilli*, *Or. niloticus* and *L. niloticus*). Generally, the infection rate was 29.3%. Description of these trypanosomes species in each infected host species are:

A- *T. mukasai* in *B. bayad* (Figs. 1&2).

T. mukasai was observed in 92 (86.7%) out of 106 examined *B. bayad* fishes. Neither polymorphic nor division stages were seen in the blood smears. One form only has been observed and described.



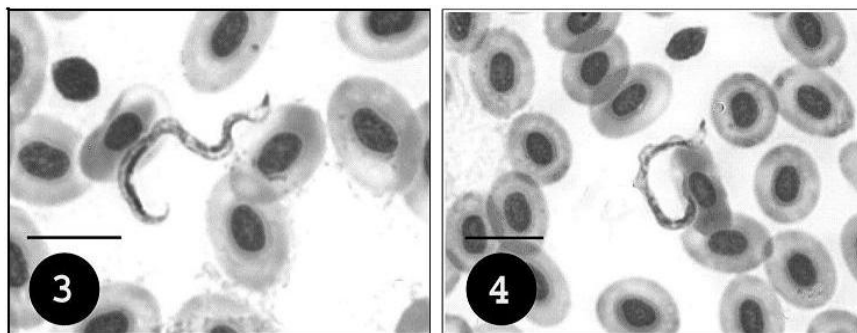
Figs. 1&2: Photomicrograph of *T. mukasai* Van As *et al.* 2004 in *B. bayad* fish; Fig. 1. Showed an oval kinetoplast, pigments, and an elongate nucleus (S. bar 10 µm); Fig. 2. Showed a rounded kinetoplast, undulating membrane, free flagellum, and pigments (S. bar 10 µm).

The body is generally thin, elongated and cylindrical with pointed anterior end while the posterior end is fairly blunt. It measures 32.1- 40.7 µm in length and the free flagellum length ranged from 16.5 to 17.2 µm when present while it couldn't recognized in some samples. It has a distinct undulating membrane. The cytoplasm stained faint blue and includes blue violet granulated bands near the edge opposite to the undulating membrane. The nucleus has an elongated form, oval structure situated in the anterior half of the body. The nucleus lies at the end of the first half or slightly anterior to the middle of the body, it measured 3.0-4.4 and 1.0-2.1 µm in length and width, respectively. The nuclear index (distance between middle of

nucleus and posterior end divided by distance between middle of nucleus and anterior end) was 1.0-1.6 µm. Kinetoplast has a round or oval form lies almost entirely at the posterior end of the body. In a very few cases the kinetoplast is slightly sub-terminal and lying a very short distance from the posterior end of the body. It measured 0.56 to 0.71 µm in length and 0.5-0.6 µm in width. The flagellum originates from the kinetoplast and extends along the undulating membrane which is thrown into a number of folds. The undulating membrane is conspicuous and forms 5 -9 folds, and extended anteriorly to form the free flagellum.

B- *T. mukasai* in *Ch. auratus* (Figs 3&4).

The prevalence was 3 (37.5%) out of 8 *Ch. auratus* fishes examined.



Figs. 3&4: Photomicrograph of *T. mukasai* in blood of *Ch. auratus* (S. bar 10 µm).

The body is relatively long and cylindrical with pointed anterior and posterior end it is (34.5-35.7) μm in length and the flagellum length ranged from 9.5 to 13.8 μm . The cytoplasm stained faint violet and showing many staining granules those aggregated to form a marginally located band which is basophilic in reaction and stained deeper than the rest of cytoplasm. The nucleus rounded or oval shaped. It is small in size (1.4-1.8 μm in length and 0.7-1.2 μm in width). It lies in the anterior half of the body. The nuclear index was (1 – 1.25) μm . It has a large kinetoplast measured 2.0-3.5 and 1.5-2.3 μm in length and width, respectively. The deeply stained extends to the pointed posterior end of

the body. The flagellum arises from the kinetoplast and extends forming undulating membrane which is thrown into (3 – 7) folds; it is broad in certain parts and narrows in others; free flagellum is generally rather short and not easily stained.

C- *T. mukasai* in *C. lazera* (Fig. 5)

T. mukasai has been observed in 6 (21.4 %) out of 28 *C. lazera* fishes investigated.

It has a long body, body length ranged from 38.5 to 41 μm in length and the free flagellum ranged from 9 to 11.5 μm length. The body has a rounded or pointed posterior end and tapering anterior end.

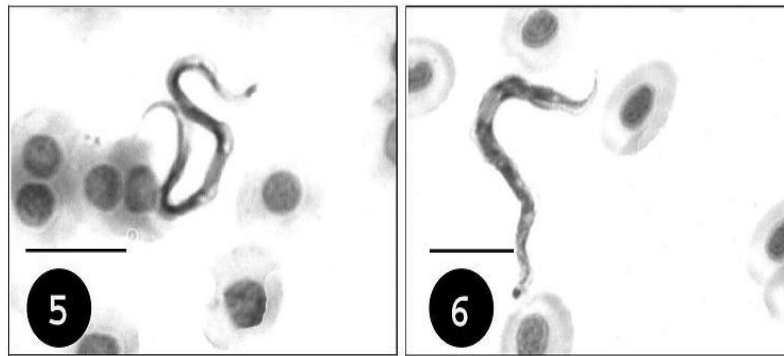


Fig. 5: Photomicrograph of *T. mukasai* in blood of *C. gariepinus* (*Lazera*) showing the elongate nucleus and rounded kinetoplast; Fig. 6. Photomicrograph of *T. mukasai* in blood of *Or. niloticus* fish (S. bar 10 μm). E- *T. mukasai* in *Ti. zilli* (Fig. 7)

The cytoplasm stained faint violet and has numerous basophilic deeply stained granules aggregated to form a marginally located band. Also the cytoplasm contains many vacuoles at the posterior part of the body. The nucleus has an oval or slightly elongated shape and lies nearest to the anterior end. It measured 3 – 4 μm in length and 2.1 – 2.7 μm . The nuclear index was 1.3 – 1.5 μm . Kinetoplast has a rounded or oval shape lying at the posterior end of the body. It measured 1.0 – 1.8 μm in length and 0.5 – 1.0 μm in width. The undulating membrane showed 3 – 6 folds.

D – *T. mukasai* in *Or. niloticus* (Fig. 6)

Out of 180 *Or. niloticus* fishes examined, 8 (4.4%) fishes were found harboring *T. mukasai*.

The body is relatively long and cylindrical with pointed anterior end. The body length ranged from 41-43.5 μm and 1.8-2.5 μm in width. There was no ability to detect and measure the free part of the flagellum clearly. The violet stained cytoplasm contains numerous staining granules in anterior and posterior parts of the body. It contains an elongated oval nucleus, 2.7-3.2 in length and 1.5-1.8 μm in width. It lies in the anterior half of the body and the nuclear index is 1-1.4 μm .

There is a small and spherical kinetoplast, it is 0.7-0.9 μm in length and 0.7-0.9 μm in width. It lies at the posterior end of the body and deep violet stained. The undulating membrane thrown into 5-9 folds. The free part flagellum is generally short.

E- *T. mukasai* in *Ti. zilli* (Fig. 7).

This species of trypanosome was observed in 4 (6.1%) out of 66 *T. zilli* fishes. The elongated body contains an elongate nucleus lies near to the anterior end of the body. Also the body contains many vacuoles and stained granules.

The body is cylindrical, 40.2-42.5 μm in length with pointed anterior end and blunt posterior end. Cytoplasm contains vacuoles and stained granules. The elongated nucleus measures 3.5-4 μm in length and 1.1-1.8 μm . It stained

violet and lies slightly anterior to the middle of the body. The nuclear index was 1.1-1.3 μm . The kinetoplast has terminal position near to the posterior end of the body. It stained blue, oval or round in shape and measured 0.7-0.9 μm in width and 0.7-1.3 μm in length. The free part flagellum is generally short.

F- *T. mukasai* in *L. niloticus* (Fig. 8)

Only one (6.6%) out of 15 *L. niloticus* examined was found to be infected with *T. mukasai*.

The body is relatively long and cylindrical with pointed anterior and posterior ends; (50-53) μm in length, free flagellum length is (11-14) μm . The deep violet stained cytoplasm contains many vacuoles in both anterior and posterior parts of the body. The nucleus has spherical to oval in shape.

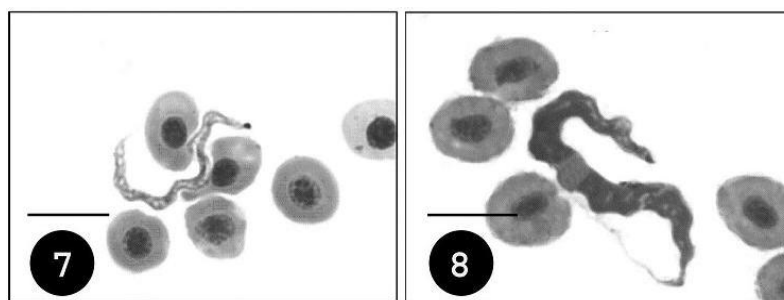


Fig. 7: Photomicrograph of *T. mukasai* in blood of *Ti. zilli* (bar 10 μm); Fig. 8: Photomicrograph of *T. mukasai* in blood of *L. niloticus* (S. bar 10 μm).

It lies in the posterior half of the body and measured 4-4.3 μm in length 3-4 μm in width. The nuclear index was 1-1.2 μm . The elongated kinetoplast measured 1-1.2 μm in length and 0.5-0.7 μm in width. It has sub-terminal in position closed to the posterior end of the body.

(2)- *T. cyanophilum* Mohamed, 1978 (Figs. 9-11)

T. cyanophilum were observed in 27 (52.9%) of 51 specimens of *Ch. ruepelli*. It is a polymorphic trypanosome species had been found in three forms; small, medium, and the large forms. Small form (Fig. 9).

This form has been detected in the blood of all infected specimens. The thin and elongate body measured 24 - 26.7 μm in length and 3.2 - 3.5 μm in width. The body has pointed anterior end and slightly rounded posterior end and contains many vacuoles. The free part flagellum length is 7 - 9 μm . The cytoplasm contains fine granules and many vacuoles. The oval shaped nucleus measured 2.8-3.2 μm in length and 3.1-3.5 μm in width. The nuclear index was 1.3-1.63 μm . The rod-like kinetoplast measured 1.3-1.8 μm in length and 0.5-0.7 μm in width. It has terminal or slightly sub-terminal position.

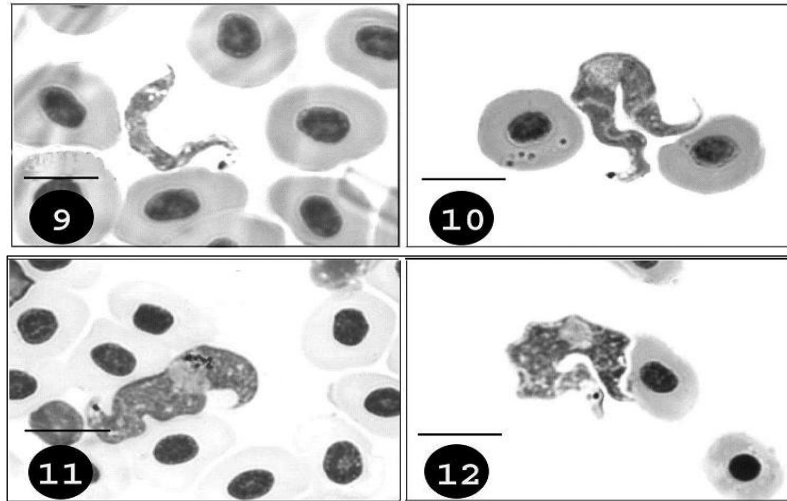
Medium form (Fig. 10)

This stage has been found in the blood of all infected fish species. The body is longer (37 μm in length) more wide (4.7 μm) comparing with the small form. The body has a pointed anterior end. Cytoplasm contains fewer vacuoles. It contains a large nucleus measured 4.8 μm in length and 4.5 μm in width. The

nuclear index was 1.2 μm . It contains a terminal or slightly sub-terminal oval shaped kinetoplast. The free part of flagellum has been not easily recognized.

The large form (Fig. 11)

The large form of *T. cyanophilum* was rare and observed only in one infected *Ch. ruepelli*.



Figs. 9-11: Photomicrograph of *T. cyanophilum* Mohamed, 1978 in the blood of *Chrysichthys ruepelli* (S. bar 10 μm); Fig. 9. Small form; Fig. 10. Medium form; Fig. 11. Large form.

Fig. 12: Photomicrograph of *T. cataractae* Putz, 1972 in the blood of *Ti. zilli* (S. bar 10 μm).

The body is large and wide. The body measured 34 μm in length and 0.8 μm in width. The free part of the flagellum measured 11.5 μm . The body has a pointed anterior end and a rounded posterior end. It contains a large size nucleus that measured 6.5 μm in length and 5.6 μm in width.

There are seven large stained granules in the posterior part of the nucleus and many other small stained granules spread inside the body in addition to many vacuoles. The nuclear index is 1.7 μm . The kinetoplast was strongly shifted to the posterior tip with a measurement of 1.1 μm in length and 0.8 μm in width.

(3)- *T. cataractae* Putz, 1972 (Figs. 12&13).

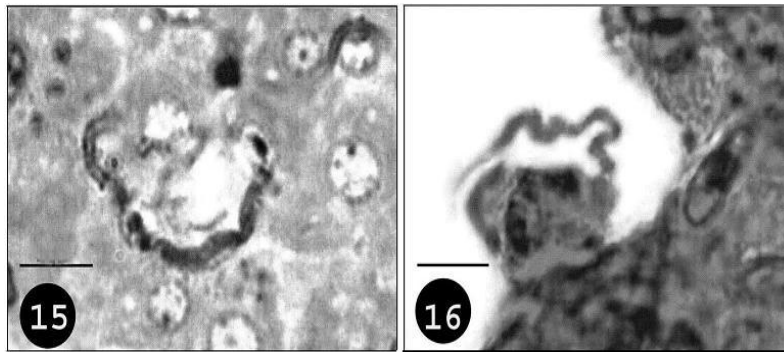
One fish only (1.5%) out of 66 *Ti. zilli* examined was *T. cataractae*. The

body is roughly ovoid or rounded similar to "C" shaped without a flagellum. It measured 17-20 μm in length and 6.5-8.0 μm in width and mostly found adhering to the red blood cells.

(4)- *Trypanosoma* species (Fig. 14)

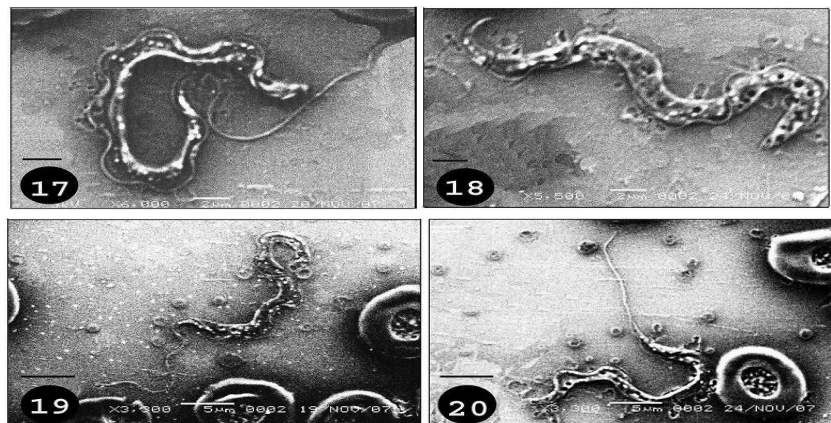
This trypanosome species was observed in one fish only (1.96%) out of 51 *Ch. ruppelli* fish. No polymorphism or division stages were seen in the blood of this specimen. The body is wide and both ends are pointed. It measured 48.5 μm in length and 5.5 μm in width and the free part of the flagellum couldn't be recognized. There are many stained granules near the body margin and others spread inside the body. The nucleus is oval in shape and measured 4.5 and 3.8 μm . The nuclear index is 1.6 μm . The kinetoplast measured 1.0 and 0.7 μm .

Histological sections of *T. mukasai* stage of *T. mukasai* in between liver cells (Figs. 15&16).
 Van As *et al.* 2004 parasitizing liver of *B. bayad* showed the presence of the adult



Figs. 15&16: Photomicrograph of sections from liver of *B. bayad* fish infected with *T. mukasai* showed the presence of trypanosomes between liver cells (S. bar 10 µm).

Using scanning electron microscope to investigate the topography of the blood stage of *T. mukasai*, showed the general shape and the undulating membrane, and also showed the positions of many vacuoles inside the body (Figs. 17-20).



Figs. 17-20: Scanning electron micrographs of *T. mukasai* in *B. bayad* fish; Fig. 17. Showed the undulating membrane, length of free flagellum, and the site from which the flagellum arises (S. bar 2 µm); Fig. 18. Showed vacuoles inside the body, and revealed that the flagellum coils around the body within the undulating membrane (S. bar 2 µm); Fig. 19. Showed free flagellum and the undulating membrane (S. bar 5 µm); Fig. 20. Showed the pointed anterior end of the body in addition to vacuoles and the free flagellum (S. bar 5 µm).

DISCUSSION

T. mukasai Van As *et al.* 2004

Baker, J.R. (1960) recorded *T. mukasai* in *T. esculenta*, *Or. variabilis*, and *B. docmac* from Lake Victoria. The same author recorded *T. tobeyi* from *C. angolensis* but suggested that *T. tobeyi* is synonymous of *T. mukasai*. Negm-El-Din, M.M. (1998) considered these both trypanosomes species are belong to the same species. He mentioned a certain

characters those agreed with the data in the present study as *T. mukasai* (Table 3).

Pienaar, U.D.V. (1962) reported *T. clariense* from *C. gariepinus* fish caught from South Africa. Usually, Pienaar, U.D.V. (1962) reported the same species in his study of blood parasites of South Africa reptiles, total body length (35-48) µm and width (1.3-2.7) µm, this parasite falls within the ranges of those from *C. gariepinus* in the Okavango

Delta, as well as those of *T. mukasai*. He also mentioned the difficulty in staining the free flagellum, a characteristic found in the majority of the Okavango specimens. Based on Pienaar, U.D.V. (1962) original description (no type material was deposited, the name *T. clariense* may therefore also prove to be a junior synonym of *T. mukasai*).

Saoud, M.F.A. (1976) recorded without description, trypanosomes from *C. anguilaris* and *C. lazera* in the White Nile in Sudan. Mohammed (1978) described *T. alhaussainii* from *C. lazera* caught from the Nile near Cairo, A. R. Egypt. By comparing the measurements of this species with those of *T. mukasai* and the present species in *C. lazera* it was found that the three species are similar. Smit, *et al.* (2004) reported *T. mukasai* in 9 host fishes: *Or. andersonii*, *Se. angusticeps*, *Se. macrocephalus*, *Se. robustus*, *C. gariepinus*, *C. theodora*, *P. ngamensis*, *S. nigromaculatus*, and *S. vanderwaali*.

In the present study *T. mukasai* is recorded from 6 fish species: *Or. niloticus*, *Ti. zilli*, *L. niloticus*, *Ch. auratus*, *C. lazera* and *B. bayad*.

Dutton, *et al.* (1907) found Trypanosomes in *C. angolensis* caught at Leopoldville in the Congo. They described three forms of Trypanosomes, small, medium, and large, and all of them were characterized by the possession of a large four-lobed granular kinetoplast, a long oval granular nucleus and spherical longitudinal striations or myonemes. Mohammed, M.A. (1978) described *T. alhaussainii* from *C. lazera* caught from the Nile near Cairo, A. R. Egypt. In Mohammed's opinion *T. alhaussainii* is certainly distinct from *T. toddi* but shows some similarity to *T. andradesilvae* from which it can be differentiated by the wider body, longer free flagellum and the position of the nucleus. Marwan, A.M. (1980) described trypanosomes from *C. lazera* at Assiut, A. R. Egypt where only two forms; the large form is almost

similar to Mohammed's account of *T. alhaussainii* which was described as a monomorphic trypanosome. Smit, *et al.* (2004) described *T. mukasai* from *C. gariepinus* (syn *C. lazera*) from Botswana.

In the present work, only one form of trypanosomes is represented with long cylindrical body measured 44-52.5 µm in length. Nucleus is spherical to oval, situated in the posterior half of the body. And this form is similar to *T. alhaussainii*, 1978 and the large form of trypanosomes of Marwan, A.M. (1980) from the same host and each of them are similar to *T. mukasai* Smit, *et al.*, 2004 so that the present species is identified as *T. mukasai*.

***T. mukasai* in *B. bayad*:**

The earliest record of trypanosomes from *B. bayad* is that of Neave, S. (1906) who reported and figured these parasites in that host caught from the Nile at Sudan. Wenyon, C.M. (1908) also reported trypanosomes in *B. bayad* caught from the Sudanese Nile. Saoud, M.F.A. (1976) reported trypanosomes in *B. bayad* and *B. docmac* caught from the White Nile in the Sudan. All these previous authors only reported trypanosomes in *Bagrus* fishes but no description or specific identification were given. Marwan, A.M. (1980) described trypanosomes from *B. bayad* and *B. docmac* was the first detailed description of trypanosomes from *Bagrus* fishes, and named it *T. bagrii*. Trypanosomes from *B. bayad* fish in the present work may be related to trypanosomes described by Marwan, A.M. (1980) from *Ch. auratus* which belongs to the same family of fish. But *T. mansouri* described before from *Chrysichthys* fishes are shorter and broader from those described from *B. bayad* (Table 1). Certain differences can also be seen in the shape and size of kinetoplast where in *T. mansouri* the kinetoplast is large, elongated and extends to the pointed posterior end of the body, but in the present trypanosomes

from *B. bayad*, the kinetoplast is rounded to oval in structure, and in some cases there is a distance between it and the posterior end of the body. Trypanosomes described here differ from *T. alhaussaini* described from *C. lazera* (Table 1), where *T. alhaussaini* is broader and longer than *Bagrus* trypanosomes, and also they differ in the position and shape of nucleus and in the position of kinetoplast. When comparing the present trypanosome with *T. bagrii*, Marwan, 1980 (Table 1) it was found that the present trypanosome is similar to the second form of *T. bagrii* (Marwan, A.M., 1980) in both features and measurements and both of them are similar to *T. mukasai* Smit, *et al.* (2004) and they only differ in the host, so that *T. bagrii* Marwan, 1980 and the present *Trypanosoma* in *B. bayad* might be a synonymous with *T. mukasai*.

***T. mukasai* in *Ch. auratus*:**

Mohammed, M.A. (1978) described *T. mansouri* from *Ch. auratus* which were caught from River Nile near Cairo. The incidence of trypanosomes in *Ch. auratus* in Mohammed's work was 60%. In Marwan study (1980) on *Ch. auratus* caught from the Nile in Assiute, Upper Egypt, the incidence of trypanosomes was 35.76%.

In the present study on *Ch. auratus*, the specimens were caught from the Nile in Upper Egypt at Qena, the incidence of trypanosomes is 37.5%. Smit, *et al.* (2004) described *T. mukasai* from different fishes (Table 2). Trypanosomes in the present study can be identified as *T. mukasai*, where it differs from *T. mansouri* in being monomorphic and the length and width of the nucleus in the present trypanosome is lesser than *T. mansouri*. Also the kinetoplast is larger and wider than in *T. mansouri*; while it is similar to *T. mukasai* in being monomorphic and other measurements (Table 1).

T. cyanophilum* Mohamed, 1978 in *Ch. rueppelli

It appeared from the above description that the species under consideration can be identified as *T. cyanophilum* Mohammed, 1978. This species was previously described from *Ch. rueppelli* caught from the River Nile near Cairo, and the incidence of infection was 44.7%. Marwan, A.M. (1980) reported the same species of *T.* in *Ch. rueppelli* also but at Assiut, A. R. Egypt and the incidence of infection was 35.76%.

In the present work, *Ch. rueppelli* caught from the Nile in Upper Egypt at Qena, where the incidence of infection is 52.9% (higher than Mohammed's and Marwan incidence), the above description fits fairly well with that of Mohammed, M.A. (1978) and Marwan, A.M. (1980) except in minor morphological details which are summarized in table (1). Previously, trypanosomes were recorded from *Chrysichthys* fishes from the Nile by Wenyon, C.M. (1909) and Saoud, M.F.A. (1976). Both records are from Sudan but the parasites were neither described nor named by those authors. Mohammed, M.A. (1978) offered a detailed description for *T. cyanophilum*, and described it as a deep blue staining trypanosome with Giemsa's stain. The only other species with this staining characteristic is *T. percae* Brumpt, 1906 which was re described few years later by Minchin, E.A. (1909). Apart from differences in the last species which belongs to different sub orders of fishes, *T. percae* is considerably longer and thinner than *T. cyanophilum*. Mohammed, M.A. (1978) and Marwan, A.M. (1980) reported two forms (small and large) of *T. cyanophilum* from *Ch. rueppelli* fishes, but in the present work three forms are described, (small, medium and large) and their measurements are summarized in Table (3).

T. cataractae* Putz, 1972 from *Ti. zilli

According to Lom, J. and Dykova, I. (1992) *T. cataractae* Putz, 1972 infects four cyprinids- *Rhinichthys cataractae*, *R. antatulus*, *Exoglossum maxillingua* and *Campostoma anomalum* in West Virginia (USA). The body length is 17 µm and the body width is 2 µm and was described as showing no distinguishing features.

In the present work *Trypanosoma* species which is observed in *Ti. zilli*, family Cichlidae, and measures 17 – 20 µm in length; and 6.5 – 8.0 µm in width agrees with *T. cataractae* which was described by Putz, R.E. (1972).

***Trypanosoma* new species parasitising *Ch. rueppelli* in the present study**

Comparing this species with other trypanosomes in freshwater fishes, it was found that it resembles *T. striati* Qadri, 1955 in shape and size which was 32-54 × 2-8 µm in the first and 48.5 × 5.5 µm in the present species, the two are similar also in having a distinct nucleus in the anterior half of the body, but the free flagellum in *T. striati* Qadri, 1955 was 8 – 11 µm; while in the present species it is not distincted. In addition that *T. sriati* Qadri, 1955 was observed in *Oph. striatus* from India, but the present species is observed in *Ch. rueppelli* in

Qena, Egypt (a new locality). It resembles *T. immanis* Froes, *et al.*, 1978 which was found in *L. anus* from Rio Grande do Sul, Brazil in size and in the site of nucleus, and in the distance between the kinetoplast and the posterior end of the body, but they differ in the body length, where the present species is 48.5 µm in length but the previous one was 105-132 µm. Also the free flagellum in the previous species was 6-7 µm while in the present species it is not distinct. Also this species resembles the adult stage of *T. gargantua* Laird, 1951 which was recorded from *Raja nosuta* from New Zealand in body width which was 4.6-13.7 µm in the previous trypanosomes and 5.5 µm in the present species. Also the two are without a free flagellum and posses a distinct nucleus which lies in the anterior half of the body. But they differ in body length where it was 66.7-131 µm in *T. gargantua* and 48.5 µm in the present species.

From the previous comparison, the present species differs from the previous trypanosomes in some respects, so that the present trypanosome could be identified as a new trypanosome species called *T. rueppelli* according to its host (*Ch. rueppelli*).

Table 1: Dimensions by µm of some previously described *Trypanosoma* spp. In fresh water fishes in Egypt (KL Kinetoplast length; KW Kinetoplast width; KN Distance between the posterior end of nucleus and the anterior end of kinetoplast; NL Nucleus length; NW Nucleus width; NA Distance between the anterior end of nucleus and the anterior nucleus and the anterior tip of the body; FF Free flagellum; BL Body length without flagellum; TL Total body length with flagellum; BW Body width).

	<i>Trypanosoma mansouri</i> Mohammed, 1978			<i>Trypanosoma alhaussainii</i> Mohammed, 1978		<i>Trypanosoma bagrii</i> Marwan, 1980	
	<i>Chrysiichthys auratus</i>			<i>Clarias lazera</i>		<i>Bagrus bayad</i>	
	Form I	Form II	Form III	Small form	Large form	Form I	Form II
KL	0.5-1.0	0.7-1.1	0.85-1.3	1.0-1.6	0.7-1.2	0.5-0.8	0.4-0.65
KW	0.5-0.7	0.7-0.9	0.77-0.5	0.3-0.6	0.7-1.5	0.5-0.7	0.4-0.7
KN	15.5-19	15.9-18.4	14.9-10.5	14-18	12.2-15.6	12.4-19.2	10.5-19
NL	2.5-4.0	3.5-5.0	2.2-3.9	3.5-5.0	2.5-3.0	3.0-4.5	1.0-3.0
NW	1.5-3.0	2.5-3.0	1.04-2.5	1.6-3.3	1-2	1.0-1.5	0.5-1.25
NA	15.7-20	16.2-19	7.9-11.6	14-18	6.6-7.1	9.25-13.2	12.5-15.5
FF	4.5-11	10-11.5	13.5-7.0	6.5-12	8.5-12.5	7.9-15.5	0.0
BL	31.5-42.5	33.5-37	19-26.5	35-37.5	24-25.5	31.5-34	28-36
TL	34.4-47	43.5-48.5	29-35.7	41-55	32.5-37	39-49.5	28-36
BW	2.5-3.2	3.5-4.0	1.06-2.8	2.5-3.5	2.0-3.0	1.5-3.0	1.5-2.5

Table 2: *Trypanosoma mukasai* Smit et al., 2004 from Botswana (TL Total length with flagellum; BL Body length without flagellum; BW Body width; NA Distance between middle of nucleus and anterior body end; NL Nucleus length; PN Distance between middle of nucleus and posterior body end; PK Distance between middle of kinetoplast and posterior body end; KN Distance between middle of kinetoplast and the middle of nucleus; NI Nuclear index = PN / NA).

	TL	BL	BW	NA	NL	PN	PK	KN	NI
<i>Oreochromis andersonii</i>	45.2-63.3	37.7-53.6	3.9-5.6	17.3-27.3	2.9-3.7	19.3-28.2	1.1-1.8	17.9-26.4	1.1
<i>Serranochromis angusticeps</i>	51.6-57.1	43.9-48.2	3.2-6.2	17.3-24.8	3.6-4.6	23.6-29.1	1.6-2.9	19.4-26.8	1.2
<i>Serranochromis macrocephalu</i>	51.5-61.6	44.6-50.8	3.9-6.1	19.1-23.6	3.9-5.0	25.5-27.2	2.5-3.2	22.3-24.7	1.2
<i>Serranochromis robustus</i>	29.5-70.1	23.6-60.9	1.4-5.7	8.2-27.3	5.8-2.6	15.5-33.6	0.9-2.2	14.5-31.5	1.2
<i>Clarias gariepinus</i>	30.1-52.4	21.7-41.5	1.2-3.6	8.6-22.1	1.9-4.1	13.1-22.2	0.8-1.7	12.2-20.5	1.2
<i>Clarias theodora</i>	32.7-62.5	22.7-54.9	1.8-3.5	10.9-26.9	3.2-5.5	30.4-11.8	1.4-2.1	28.4-10.5	1.1
<i>Parauchenoglanis ngamensis</i>	33.1-80.8	30.8-75.4	2.3-11.5	11-40	3.2-5.0	40-15	1.5-6.2	13.4-33.8	1.2
<i>Synodontis nigromaculatus</i>	36.6-54.0	28-43.6	2.0-4.0	10.3-20.2	2.7-4.6	27.3-17.7	1.1-2.2	16.2-25.8	1.4
<i>Synodontis vanderwaali</i>	32-48	26.7-40.9	1.1-3.0	10-18.6	2.2-3.7	23.3-14.6	0.9-1.4	13.6-22.1	1.3
<i>Schilbe intermedius</i>	35.3-51.6	31.6-42.5	2.5-4.7	19.5-13.1	2.6-4.4	18-26.7	1.3-2.1	16-25.2	1.4

Table 3: Measurements (in μm) of trypanosomes in the present study (TL Total body length with flagellum; BL Body length without flagellum; BW Body width; FF Free flagellum; NL nucleus length; NW Nucleus width; KN Distance between middle of nucleus and kinetoplast; PK- Distance between middle of kinetoplast and posterior body end; PN Distance between middle of nucleus and posterior body end; NA Distance between middle of nucleus and anterior body end).

	<i>Trypanosoma mukasai</i> Van As et al., 2004						<i>Trypanosoma cyanophilum</i> Mohammed, 1978			<i>Trypanosoma ruppelli</i> present study
	<i>Clarias lasera</i>	<i>Oreochromis raitoticus</i>	<i>Tilapia nilotica</i>	<i>Labeo raitoticus</i>	<i>Chrysichthys auratus</i>	<i>Bagrus docud</i>	Form I	Form II	Form III	<i>Chrysichthys ruppelli</i>
TL	47.5-52.5	----	----	56-61	45-49.5	45.3-58.9	31-35.7	----	45.5	----
BL	38.5-41	41-43.5	40.2-42.5	50-53	34.5-35.7	32.1-40.7	24-26.7	37.0	34	48.5
BW	2.0-2.5	1.8-2.5	2.0-3.2	4.0-4.2	1.2-2.5	1.4-2.2	3.2-3.5	4.5	0.8	5.5
FF	9.0-11.5	----	----	6.0-7.5	11-14	16.5-17.2	7.0-9.0	----	11.5	-----
NL	3.0-4.0	2.7-3.2	3.5-4.0	3.0-4.0	1.4-1.8	3.0-4.4	2.8-3.2	4.8	6.5	3.8
NW	2.1-2.7	1.5-1.8	1.1-1.8	4.0-4.3	0.7-1.2	1.0-2.1	3.1-3.5	4.5	5.6	4.5
KN	20-22	20.5-23	22-23	23-25	14-15.5	15.5-17.2	11.2-12.7	19.5	20.2	26.4
PK	1.7-2.2	1.0-1.4	0.7-1.3	0.8-1.0	1.5-2.7	0.9-1.5	0.7-0.9	0.8	1.5	3.2
PN	23-24	21.5-24	22.5-24	27.5-29	18-19.5	15.5-19.7	14.7-15.5	21	21.7	30
NA	15.5-17	17-21	17.5-19.5	24-25.7	15.5-16.5	12-14.6	9.5-11.2	17	12.3	18.5
KL	1.0-1.8	0.7-0.9	0.7-1.3	1.0-1.2	2.0-3.5	0.56-0.71	1.3-1.8	1.2	1.1	1.0
KW	0.5-1.0	0.7-0.9	0.7-0.9	0.5-0.7	1.5-2.3	0.5-0.6	0.5-0.7	0.9	0.8	0.7
NI	1.3-1.5	1.0-1.4	1.1-1.3	1.0-1.2	1.0-1.25	1.0-1.6	1.3-1.63	1.2	1.7	1.6

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Abbreviation used in this article

Name	Abbreviation
01- <i>Trypanosoma</i> -----	<i>T.</i> -----
02- <i>Bagrus bayad</i>	<i>B. bayad</i>
03- <i>Chrysichthys rueppelli</i>	<i>Ch. Rueppelli</i>
04- <i>Chrysichthys Auratus</i>	<i>Ch. Auratus</i>
05- <i>Tilapia zilli</i>	<i>Ti. zilli</i>
06- <i>Oreochromis niloticus</i>	<i>Or. niloticus</i>
07- <i>Clarias lazera</i>	<i>C. lazera</i>
08- <i>Labeo niloticus</i>	<i>L. niloticus</i>
09- <i>Synodontis schall</i>	<i>S schall</i>
10- <i>Synodontis clarias</i>	<i>S clarias</i>
11- <i>Schilbe intermedius</i>	<i>Sc. Intermedius</i>
12- <i>Ophicephalus obscurus</i>	<i>Oph. Obscurus</i>
13- <i>Serranochromis robustus</i>	<i>Se. robustus</i>

ARABIC SUMMARY

دراسات باستخدام المجهر الضوئي والمجهر الإلكتروني الماسح للتريبانوسوما (*Trypanosoma*) التي تصيب اسماك المياه العذبة في محافظة قنا، مصر

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تم أخذ عينات اسماك المياه العذبة من مناطق مختلفة لنهر النيل في محافظة قنا (قوص، فقط، دندرا، الترامسه، دشنا ونجع حمادى). بالفحص وجد ان (*Trypanosoma*) متطفلة في دم عديد من الاسماك. من خلال فحص ٥٨١ سحبة دم وجد ان ١٤٣ عينة مصابة بطفيل (*Trypanosoma*) بنسبة اصابة ٢٤.٦%. الاسماك المصابة وجدت تنتمي الى سبعة انواع و اربعة عائلات. طفيليات (*Trypanosoma*) وجدت تنتمي الى ثلاثة انواع بنسب اصابة مختلفة: *T. mukasai* Van As et al., 2004 (30%) ، *T. cyanophilum* ، *T. cataractae* Putz, 1972 (52.9%) و *T. cataractae* Putz, 1972 (1.5%). بالاضافة الى نوع جديد من (*Trypanosoma*) يصيب سمك (*Ch. ruppelli*) بنسبة اصابة 1.9%. القطاعات النسيجية اظهرت وجود اطوار بالغة من *T. mukasai* بين خلايا الكبد. ايضا تم فحص *T. mukasai* باستخدام المجهر الإلكتروني الماسح.