Some Protozoan Parasites Infecting Catfish *Clarias gariepinus* Inhabiting Nile Delta Water of the River Nile, Dakahlia Province, Egypt

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Abstract: The present study was restricted to the freshwater catfish *Clarias gariepinus* inhabiting the Nile Delta water near Mansoura City in Egypt. The specimens of the catfish were collected monthly during this study some parasitic protozoa were identified as: *Trypanosoma alhussaini*, *Amphileptus sp*., *Chilodonella hexasticha*, *Vorticella sp*. and *Tetrahymena sp*. The aim of this work is for taxonomical, anatomical and morphological studies of the protozoan parasites infesting gill filaments, skin and blood of the catfish *Clarias gariepinus*. Morphometric data were also given for each species and interspecific variations were discussed. [Journal of American Science 2010; 6(9):676-696]. (ISSN: 1545-1003).

Keywords: *Trypanosoma – Amphileptus – Chilodonella vorticella – Clarias gariepinus* – River Nile – Egypt.

1. Introduction

One of the most important problems facing our world nowadays is food deficiency. The protein deficiency is one of the major global challenges facing the third world today. In Egypt, the continuous increase in human population requires more food production to meet the consequent increasing demands. With the increasing demands for animal protein, the poultry sources were expensive and insufficient. Fishes were considered to compensate the continuous lack of such element due to its comparatively low price. Protozoan parasites had been known for many years to infect several groups of fishes and cause great damage to their host fish.

In many instances, individuals of protozoan parasites provoke the secondary infection of other pathogens like viruses, fungi and bacteria and are the most dangerous parasitic group that probably cause more diseases in fish cultures than any other type of animal parasites (Hoffman, 1970). A moderate number of endoparasitic protozoa was found to infect fishes in Africa (Sarig, 1975; Paperna, 1980). The assessment of their pathogenicity is still lacking. Ectoparasites, including protozoa, are cited as the major problem in warm water pond fish culture where high temperature and organic content accelerate the life cycles of parasites and promote their spread (Sarig, 1975). Ectoparasitic protozoa represents one of the most hazardous threats to fish health. These parasites attack the fish and cause massive destruction of the skin and gill epithelium (Sterud et al., 2003; Enayat et al., 2008). Even moderate infection of these organisms may cause a fatal disease, since the infected fish lose their appetite and stop feeding (Meyer, 1966; Hoffman, 1970).

Parasites of the River Nile fishes attracted the attention of Egyptian parasitoligists long time ago (Fahmy et al., 1975; El-Naggar et al., 1993; El-Naggar et al., 1999). Recently, more work has been carried out in which the fish protozoa gain a lot of attention (Ali, 1992, 1996; Ahmed et al., 2000; El-Mansy and Bashtar, 2002; Enayat et al., 2008).

Haemoflagellates of the genus Trypanosoma are prevalent in freshwater fishes and are transmitted by leaches as vectors. Traditionally, fish trypanosome species were named by the host from which they were first isolated. More recently, it has been recognized (Letch, 1979; Woo and Black, 1984; Lom and Dykovà, 1992) that species definition for freshwater fish trypanosomes, based either on morphological criteria such as body length or on host range, is problematic because their morphology can vary and their host range appears to be relatively broad.

The host specificity of trypanosomes in freshwater fishes was tested by Lom (1973), who successfully transferred trypanosomes to fish hosts of several species and observed that non of the strains was found to be specific only for the host from which it was recorded. Mohamed (1978) reported two aspects to solve the problem of species determination in those parasites. The first aspect is the establishment of the species definition on morphological characters, since those are still the primary basis for any zoological systemic work. Measurements are among the most important and quantitative, morphological characteristics, but they should be interpreted in the light of the possible pleomorphism of the species concerned. The second

aspect is the host-restriction which is present in piscine trypanosomes.

Fanthman (1919) found trypanosome in the blood of *C. gariepinus*, in south Africa. Hoare (1932) described *T. mukasai* from *Haplochromis* spp., in Uganda. Baker (1960) found what he regarded as *T. mukasai* in five genera of fishes (Tilapia, Haplochromis, Bgmyrus, Mormyrus and Astatoreachromis), in Uganda. The author related all trypanosomes of African freshwater fish to *T. toddi* by Bouet (1909), *T. mukasai* by Hoare (1932) and *T. tobeyi* by Dias (1952). Smit et al. (2004) recorded harbour trypanosomes from 9 new hosts of fishes, captured in the Okavango Delta region of Botswana. Despite variations in their size and appearance, these trypanosomes were tentatively identified as *T. mukasai* (Hoare, 1932).

In most amphileptids, only the right body side, by which the ciliate moves across the surface, is ciliated. Literatures about genus *Amphileptus Ehrenberg*, 1830 are very scarce. Along the last 20 years, the genus Amphileptus was recorded only twice by Mitchell and Smith (1988) where they recorded *Amphileptus branchiarum* from *Notemigonous crysoleucas*, *Carrassius auratus* and *Ictalurus punctatus* reared in fish ponds in America and by Ghoneim (1998) who recorded *Amphileptus* sp1 from the skin of *Oreochromis aureus* and *Tilapia zillii* and *Amphileptus* sp2 from the gills of both host fishes. *Amphileptus branchiarum* seldom causes mortality in cultured fishes but may be responsible for epithelial hyperplasia and cell displacement in branchial tissue (Mitchell and Smith 1988).

Lin and Song (2004) reported that the genus *Apoamphileptus* has elongate, pyriform-shaped and slightly flattened body with one cross-striated band along the cytostome which is encircled by perioral kinety that does not extend to the posterior end of the cell.

Chilodonella spp. live on the skin and gills of fish. Two species of Chilodonella occur on freshwater fishes, *Chilodonella cyprini* (Moroff, 1902) occurring on the skin and gills of carp *Cyprinus carpio* (L) and *C. hexasticha* (Kiernik, 1909) on the skin and gills of tench (*Tinca tinca*). The identity of *C. cyprini* and *C. hexasticha* was supported by André (1912) who recorded these ciliates from *Carassius auratus* (L).

C. hexasticha has been found most often on warm water fish and also on fish in southern hemisphere, e.g. South Africa and Australia (Hoffman et al., 1979; Imai et al., 1984; Langdon et al., 1985; Paperna and Van As, 1983).

Van As and Basson (1988) reported that Childonella infestation occurs world-wide on warm and cold water fish species and has been responsible for large-scale mortalities at various places in the world. In heavy infestations on the skin and fins, the fish are emaciated and there is a darkening or dulling of the skin colour.

Vorticellidae are free-living or epizoic. The sessiline peritrichs found in fish are essentially ectocommensals or symphoriont that use their hosts as a living, moving substrate to settle where they may gain access to convenient source of food particles, organic debris and waterborne bacteria. They are specifically adapted, unlike free living sessilines, to the life on the surface of certain species of fishes (Lom and Dykovà, 1992).

There are over 100 species in the genus *Vorticella* (Linnaeus, 1767). Itabashi et al. (2002) reported that identification and differentiation between different species of the genus *Vorticella* is so difficult because of their various body shapes, variable sizes and high contractile nature which makes them among the most difficult of all ciliates to study and identify.

Lom and Dykovà (1992) reported that *T. pyriformis* is a natural parasite of amphibians and freshwater fish with a free living stage. There are many records of Tetrahymena infection in fish but their species determination is uncertain. Individuals of *T. pyriformis* was recorded by Corliss (1960) in the central nervous system of larva of *Cyprinus carpio*, *Abramis brama*, *Ameiurs sp*. and the spinal canal and musculature of rainbow trout. According to Schulman and Jankovski (1984), *T. pyriformis* may invade the fry of various fish hosts, through the injured tegument and may destroy not only surface tissues but also internal organs. Edgerton et al. (1996) recorded *T. pyriformis* in the haemal sinuses of the gills browsing on tissue fragments.

T. corlissi (Hoffman et al., 1975) is a free-living protozoan, apparently caused the death of large number of guppies (*Poecilia reticulates*) and occasionally other fishes, in aquaria and hatcheries at several locations.

Lom and Dykovà (1992) reported that *T. corlissi* is a histophagous parasite that can be found in the skin, muscle and body cavity. Ferguson et al. (1987) documented that cranial ulceration in yearling Atlantic salmon *Salmo salar* (L) was associated with great number of a holotrichous ciliate tentatively identified as *Tetrahymena sp*.

2. Material and Methods

Specimens of the catfish *Clarias gariepinus* were collected monthly from different localities in Nile Delta particularly Damietta branch near Mansoura city. Fishes were transported immediately alive to the laboratory, where they were kept alive in well aerated glass aquaria.

Smears were taken from skin, fins gills and suprabranchial organ to search for any ectoparasitic protozoan.

Smears containing protozoans were air dried, then impregnated with 2% aqueous solution of $AgNO_3$ for 10-15 minutes, followed by rinsing in distled water. The slides were then placed in white clean dish, covered with distilled water and exposed to UV (diffused day light) for about 2 hours (modification of Klein's dry silver impregnation method). To study the nuclear apparatus, the respective parasites were stained by Giemsa stain. The smears were fixed in methanol for 5-10 minutes, and stained with 5% Giemsa solution in phosphate buffer (pH 7.3) for 30-45 minutes. Smears were then examined, measured and photographed using Leitz research microscope.

Blood was collected either from the heart or from the tail peduncle. Thin blood films were made and air dried. These blood films were fixed in absolute methanol for 5-10 minutes, and stained with Giemsa solution in phosphate buffer (pH 7.3) for 30-45 minutes. Smears were then examined using bright 1field and Phase-contrast microscopy. All drawings of detected protozoans were made with the aid of a drawing eye-piece attached to Leitz microscope.

3. Results

Trypanosoma alhussaini Mohamed, 1978

This trypanosome was detected in the blood smears of *Clarias gariepinus*. It is a polymorphic trypanosome which shows three forms: small, intermediate and large form. All morphometric data of *T. alhussaini* are shown in Table 1. The large forms are the most abundant. The small and intermediate forms have thin elongated bodies (Figs. 1, 3, 4, 5). In the large forms (Figs. 2, 6, 7, 8), the body is also elongated and cylindrical in shape. All the forms have pointed extremes, the anterior end being more acute than the posterior one. The cytoplasm is finely granular and stained light blue with Giemsa stain. Vacuoles of varying sizes are present throughout the cytoplasm and their number is variable from one specimen to the other. The nucleus is situated mostly in the posterior half of the body or at least behind the middle of the body. It is oval-shaped and occupy the entire width of the body. The kinetoplast is oval or spherical in shape and subterminal in position. The free flagellum of the intermediate form is always short while in small and large forms is much longer.

Amphileptus sp.

This ciliate is recorded from the gills of the catfish *Clarias gariepinus*. The body of this ciliate is flat and remarkably long. All measurements of this parasite are shown in Table 2. The body length ranges from 40.7 to 70.4 µm and the width ranges from 15.4 to 25.3 µm. The body is lanceolate and laterally compressed (Figs. 9, 10, 11, 12). The cilia are found on one side of the body. The number of kineties ranges from 5 to 9. The cytostomial groove lies along the anterior edge of the body and does not exceed one third of the body length (Figs. 9, 10).

A comparatively large contractile vacuole is always found in the anterior region of the body (Figs. 11, 12). The nuclear apparatus consists of two macronuclei and a single micronucleus (Figs. 9, 10, 12). The two macronuclei have different sizes, one of them is comparatively larger than the other.

Chilodonella hexasticha Kiernik, 1909

This parasite is recorded from the gills of the catfish *Clarias gariepinus* and rarely found on the skin of the same host. The body of *C. hexasticha* is dorsoventrally flattened and oval-shaped (Figs. 13, 14, 15,16). The morphometrical data of this parasite are shown in Table 3. It measures 37.9 (29.7-50.6) µm in length and 27.7 (22.0-39.6) µm in width. The cytoplasm is coarsely granulated and the ventral body surface has less numerous and more loosely spaced kineties. There are no cilia on the dorsal surface. The ciliature of the ventral side is composed of two systems, the right and left ciliary kineties which are separated by a wide non-ciliary zone (Figs. 13, 14). The cytostome occurs at the anterior part of the non-ciliary zone (Figs. 13, 16). It leads into a conspicuous protrusible cytopharynx. The right ciliary kineties range from 7 to 8 while the left kineties range from 8 to 10 (Figs. 13, 14).

Two contractile vacuoles are present, one on the right and the other on the left side of the posterior region of the body (Figs. 13, 15, 16). The nuclear apparatus consists of comparatively large macronucleus and a single micronucleus (Figs. 13, 15, 16).

Vorticella sp.

This parasite is recorded from the gills of the catfish *Clarias gariepinus*. *Vorticella sp*. of the present study consists of two main parts, zooid and

scopula (Figs. 17, 18, 19, 20). All measurements of this parasite are shown in Table 4. The zooid is bell-shaped and measures 67.3 (59.4-86.9) µm in length and 66.6 (55.0-81.4) µm in width. There are no body cilia except at the epistomial disc (Figs. 17, 18, 19). The peristomial lip is more or less outwardly extended and measures 45.2 (30.8-50.8) µm in width. Large number of food vacuoles are clearly observed in Giemsa- stained specimens (Figs. 17, 18, 19). The nuclear apparatus consists of ribbon-shaped macronucleus that extends throughout the body length and measures 125 (99-176) µm in length and 7.1 (6.6-7.7) µm in width (Figs. 17, 18, 19). The micronucleus is oval-shaped and lies in close contact with the macronucleus (Fig. 17). It measures 16.1 (15.2-20) µm in length and 8.9 (8.5-10) µm in width. The scopula form large contractile stalk which measures 93.2 (40.6-105.5) µm in length. At the scopula region, several radiating ridges on the pellicle are found which appear to come into close contact with the base of the stalk (Figs. 17, 19). The stalk ends with a platelet that used for attachment to the substrate.

Tetrahymena sp.

This parasite is recorded from the gills of the catfish *Clarias gariepinus*. The infection with this parasite was usually mixed with *Chilodonella hexasticha* (Fig. 22). This ciliate moves during life like a spinning ball. The body is pyriform, radially symmetrical and relatively small in size (Figs. 21, 23). All measurements of this parasite are shown in Table 5. The whole body is covered with 14 (12-16) meridional kineties converging at the anterior body end around the apical loop (Fig. 23). The cytostome is small, pyriform and lies close to the anterior end (Fig. 21). The nuclear apparatus consists of macronucleus and micronucleus (Figs. 21, 24, 25). The micronucleus is closely adjacent to the macronucleus. There are several contractile vacuoles with varying sizes.

4. Discussion

Surveying the ectoparaitic protozoa and blood parasites from the catfish *Clarias gariepinus* revealed the parasitism of this host by 5 species representing 5 genera. The recorded parasites are:

***Trypanosoma alhussaini Mohamed*, 1978**

The present trypanosome is a polymorphic trypanosome with three forms (small, intermediate and large) and there is no doubt that these forms belong to one species. The three forms resemble each other in general characteristics, such as position of the nucleus, structure and staining reaction of the cytoplasm and presence of free flagellum. Tandon and Joshi (1973) mentioned polymorphism in *T. maguri* from the blood of *C. batrachus* from India but he did not describe the polymorphic forms separately. Mohamed (1978) described *T. mansouri* from the blood of *Chrysichthys auratus* and *Chrysichthys reuppelli* as a polymorphic trypanosome and *T. cyanophilum* from the same hosts as a dimorphic trypanosome. Qadri (1962) reported dimorphism of *T. batrachi* from the blood of *C. batrachus* but he did not provide names and mensural data. *T. choudhuryi* from *Tilapia mossambica* was described as monomorphic trypanosome (Mandal, 1977). Narasimhamurti and Saratchandra (1980) reported monomorphism in *T. qadri*. *T. magdalenae* was also described as monomorphic trypanosome (Grogl et al., 1980).

The present polymorphic trypanosome ranged from 31.9 to 62.7 µm in length and 1.7 to 2.2 µm in breadth. The cytoplasm is finely granular and stained light blue with Giemsa stain. The nucleus is situated mostly in the posterior half of the body or behind its middle region and ranged from 3.3 to 5.5 µm in length and 1.6 to 2.1 µm in breadth. The free flagellum of the intermediate form is always short while in small and large forms is much longer and measured 5.5 to 12.1 µm. Comparing the general features of the present trypanosome with those of other trypanosomes previously described from freshwater fishes, it appears that these features resemble those of *T. alhussaini* which was originally described by Mohamed (1978) from the Egyptian Nile catfish *C. gariepinus* (Syn: *C. lazera*), but Mohamed (1978) recorded it as a monomorphic trypanosome measuring 48.2 to 55.6 µm in length and 1.2 to 4.0 µm in breadth (Table 6). In Egypt, trypanosomes were described and identified as Trypanosoma tilapiae by Abu El-Wafa (1988) from *Oreochromis niloticus*, *Tilapia zillii*, *Schilbe mystus* and *Clarias sp*. Negm El-Din (1991) synonymized this species with *T. cyanophilum*, which was originally described by Mohamed (1978) from *Chrysichthys sp*. as a dimorphic trypanosome. Later, Ali (1992) recorded *T. cyanophilum* from *O. niloticus*, *O. aureus*, *Sarotherodon galili* and *Tilapia zillii* in Serow fish farm. Ahmed et al. (2000) recorded *T. cyanophilum* from *C. gariepinus* and *O. niloticus* but, the small form of *T. cyanophilum* was not found. Significant differences can be seen when comparing the morphology and cell body measurements of *T. cyanophilum* with the present trypanosome (Table 6). In *T. cyanophilum*, the cytoplasm stained very deep blue with Giemsa stain, a large vacuole appeared at the posterior end in front of the kinetoplast and the nucleus was situated in the anterior half of the body. In *T. alhussaini*, the

cytoplasm is finely granular and stained light blue with Giemsa stain. It contains vacuoles of various sizes and the nucleus is situated mostly in the posterior half or behind the middle of the body.

Amphileptus sp.

The present amphileptid has flat, lanceolate, laterally compressed and long body. The cytostomial groove lies along the anterior edge of the body and does not exceed one third of the body. The nuclear apparatus consists of two oval macronuclei and small micronucleus. According to Lom and Dykovà (1992), members of the genus *Amphileptus* have flat, leaf or lancet-like bodies bearing longitudinal or strongly arched ciliary rows on one side of the body, two rounded or oval macronuclei and a single micronucleus. Another closely related genus, *Pseudoamphileptus Foissner*, 1983 can be distinguished from the genus *Amphileptus Ehrenberg*, 1830 by the presence of a cytostomial slit which ends short before the posterior end of the body and the presence of contractile vacuoles which are arranged along the ventral and dorsal edges of the body. Lin and Song (2004) described a new pleurostomatid genus, *Apoamphileptus* which is diagnosed as belonging to the amphileptidea with spica on the dorsal and ventral surfaces of the right side of the cell and a single perioral kinety, which encircles the cytostome and does not extend to the posterior end of the cell. Apoamphileptus has elongate, pyriform and slightly flattened body with one cross-striated band along the cytostome and 2 to 6 (generally 4) large macronuclear nodules and one micronucleus. *A. branchiarum* was first described by Wenrich (1924) from the skin of various freshwater fishes and also amphibian larva. Later, Mitchell and Smith (1988) recorded *A. branchiarum* from the skin fish hosts namely *Notemigonous crysolencas*, *Carrassius auratus* and *Ictalurus punctatus* from fish ponds in America. The present *Amphileptus sp*. showed some resemblance to *A. branchiarum* in the shape of the body and nuclear apparatus. However, *A. branchiarum* has larger body dimensions (56-120 x 35-70) µm and a larger number of kineties (20-25).

The present *Amphileptus sp.* showed close resemblance to *Amphileptus sp*1 described by Ghoneim (1998) from the skin of *Oreochromis aureus* and *Tilapia zillii* inhabiting Damietta branch of the River Nile near Damietta and lake Manzalah (Table 7). However, Amphileptus sp1 Ghoneim (1998) has larger body dimensions (62.5-103 x 12.6-24.0) µm. Also, *Amphileptus* sp. of the present study differs from *Amphileptus sp* 2 described by Ghoneim (1998) in the shape of the body, body dimensions and number of kineties. *Amphileptus sp*. from the gills of *C. gariepinus* has long and lanceolate body and was found to possess (5-9) kineties whereas *Amphileptus sp2* of Ghoneim (1998) has oval body and possesses (5-7) kineties. *Amphileptus sp*. of the present study is differs from *A. piger* described by Sonntag and Foissner (2004) where the latter has small body dimensions (55 x 13) µm and a single contractile vacuole with a terminal excretory pore.

***Chilodonella hexasticha Kiernik*, 1909**

Three species of the genus *Chilodonella*, *C. cyprini* (Kazubski and Migala 1974), *C. hexasticha* (Hoffman et al., 1979; Kazubski and Migala, 1974) and *C. uncinata* (Migala and Kazubski, 1972) were known as freshwater fish parasites. Only two species namely *C. cyprini* (Syn: *C. piscicola*) and *C. hexasticha* were reported by Lom and Dykovà (1992) to be serious pathogens of freshwater fish. These two species, up to the seventies, were confused with each other. The problem of separation of these two species was dealt with for a long time. The arrangement of ciliary rows was indicated to be a very stable and useful character for the classification of ciliat protozoa (Corliss, 1979).

In the course of this study, specimens of *C. hexasticha* are characterized by its oval-shaped body and the number of kineties ranges from 7 to 8 in the right ciliary band and from 8 to 10 in the left ciliary band. The nuclear apparatus consists of round to oval macronucleus and oval micronucleus.

An extensive morphological study and a comprehensive review of the preceding literatures were made by Kazubski and Migala (1974) through which they recorded that *C. cyprini* Moroff, 1902 and *C. hexasticha Kiernike*, 1909 differ mainly in the number of kineties, being greater in *C. hexasticha*. Furthermore, in *C. cyprini* the arrangement of kineties are close one to the other and lying in nearly equal distances, while in *C. hexasticha*, the kineties are loosely arranged and distances between them are not equal. The inner kineties of the right system of *C. hexasticha* are outstanding, lying in much greater distances than the outer kineties in both systems. The pores of contractile vacuoles are situated at different kineties in both species. There are also some differences in the number of preoral rows, being 5 to 6 in *C. cyprini* and only 1 to 3 in *C. hexasticha*. Another difference concerns the body shape of this ciliates but this feature is not very prominent and can not be taken into account in species determination. Hoffman et al. (1979) reported that *C. hexasticha* from the gills of *Ictalurus punctatus* possesses 6 to 7 kineties in the right and left ciliary bands. Paperna and Van As (1983) found that the ciliary rows were 6 to 7 on both right and left sides of *C. hexasticha*.

Wiles et al. (1985) conducted an electron microscope study on *C. cyprini* and *C. hexasticha* and considered the number of ventral ciliary rows as the main criterion for species differentiation of chilodonellids, being 4 to 6 on each side of the ventral surface of *C. hexasticha* whereas in *C. cyprini*, the number was 10 to 11 on either side. However, Imai et al. (1984) recorded 5 to 6 distinct ciliary rows on the right side and 6 to 7 ciliary rows on the left side of *C. hexasticha* from the gills of a discus. There are 6 to 7 ciliary rows in the right and left side of *C. hexasticha* from the gills of *Neosilurus sp*., *Amniataba percoides*, *Leiopotherapon unicolor* and *Melanotaenia splendidi tatei* inhabiting Finke River in Australia (Langdon et al., 1985). Van As and Basson (1988) recorded that the rows of cilia being 5 to 8 in *C. hexasticha* and 8 to 14 in *C. cyprini*. According to Lom and Dykovà (1992), *C. hexasticha* differs from *C. cyprini* in the absence of a notch at the posterior body margin and in the less numerous kineties, being 5 to 7 in the right and 7 to 9 in the left ciliary band.

Rintamaki et al. (1994) observed 5 to 9 kineties in the right side and 6 to 11 kineties in the left side of *C. hexasticha* from the gills and skin of salmonid fish in Northen Finland. Ghoneim (1998) reported two populations of *C. hexasticha* (Table 8) and found that the number of ciliary rows in population A in right ciliary band is 7 to 8 and 8 to 9 in left ciliary band but the number of ciliary rows in population B is 4 to 5 in right and left ciliary bands. Ahmed et al. (2000) reported that there are 6 to 7 ciliary rows in the right side of *C. hexasticha* recorded from the skin of *Tilapia zillii* inhabiting River Nile at Cairo and Giza. The present study showed that *C. hexasticha* from the gills of *C. gariepinus* is slightly smaller than that recorded by Ghoneim (1998) and Ahmed et al. (2000). However, *C. hexasticha* of the present study was found to have greater number of kineties than population B of *C. hexasticha* (Ghoneim, 1998; Ahmed et al., 2000). The problem of differentiation between species of the genus *Chilodonella* needs more attention since the ranges of ciliary bands are still overlapping.

Vorticella sp.

Individuals belonging to the genus *Vorticella Linnaeus*, 1767 were detected in smears of the gill tissue of the catfish *Clarias gariepinus*. As far as our knowledge is concerned, this is the first record of *Vorticella sp*. from the gills of *C. gariepinus*. The problem faced the identification of this organism is that it is free living (ectocommensal) and not parasitic. Therefore, it is suggested that the organism has entered the gill chamber through the mouth with the gill ventilating water current. Subsequently, specimens of *Vorticella sp*. attach to the gill filaments using their stalks. For a long time, the taxonomy of the genus *Vorticella* Linnaeus, 1767 has been based on macro-morphological descriptions such as the shape and size of the cell, cilia and the form of macronuclei. It has been difficult to choose a morphological character as a phylogentic marker, the macro-morphological information being insufficient to identify the species, especially in the genus *Vorticella* (Itabashi et al., 2002). Warren (1986) provided drawings and descriptions of 82 species of *Vorticella* when he complied the taxonomic status of the many species. Itabashi et al. (2002) reported that 82 species did not include all *Vorticella* species.

The present specimens of *Vorticella sp*. consists of two main parts, zooid and scopula. The zooid is bell-shaped and ranges from 59.4 to 86.9 µm in length and 55 to 81.4 µm in width. The peristome lip is more or less outwardly extended and measures 30.8 to 60 µm in width. Moriyama et al. (1998) recorded that the vorticellid ciliates, such as *Vorticella*, *Carchesium* and *Zoothamnium* are composed of zooids and long stalks. Coiling of the stalk and the simultaneous change in shape of the zooids, namely, zooid contraction, generally occur in all or non fashion. The stalk coiling is produced by the contraction of spasmoneme, an intracellular fibrous organelle that resides in a helical form inside the elastic, cylindrical outer sheath of the stalk. Jones et al. (1970) studied the contraction of *V. difficilis*, *V. campanula* and *Carchesium sp*. by means of high speed cinematography. In their studies, no turning of the zooid was detected during the intermediate stalk contraction steps, although rotation was observed after the contraction has been completed. Maciejewski et al. (1999) reported that the stalked ciliated protozoan *Vorticella convalleria* possesses a highly contractile cytoskeleton consisting of spasmonemes and myonems. The major component of these contractile organelles is the calcium-binding protein (s) called spasmin. Cloning and characterization of spasmin would help in elucidating this contractile system. Moriyama et al. (1998) studied the contraction of zooid and stalk of living *Vorticella convallaria* by high-speed video cinematography. The contraction was monitored at a speed of 9000 frames per second to study the contractile process in detail. Complete stalk contraction required approximately 9 minutes.

Tetrahymena sp.

Jerome et al. (1996) reported that genus *Tetrahymena* comprises at least 33 species. Most species in this genus are free living. However, some

species of the genus *Tetrahymena* infect invertebrates and their invasion is frequently fatal to its host (Jerome et al., 1996). The literatures of tetrahymenids infesting fish are very scarce and according to Lom and Dyková (1992) the specific determination is mostly non-existent or uncertain. The present ciliate has a pyriform, radially symmetrical and small-sized body which measures 24.2 to 29.7 µm in length and 15.4 to 20.9 µm in width. The whole body is covered with 12 to 16 kineties. The nuclear apparatus consists of macronucleus and micronucleus. The previous features indicate that this parasite belongs to genus Tetrahymena (Furgason, 1940). The present *Tetrahymena sp*. is smaller than *T. pyriformis* where the body length of *T. pyriformis* ranges from 40 to 60 µm. However, *T. pyriformis* has greater number of kineties than *Tetrahymena sp*. of the present study. *T. rostrata* (Ferguson et al., 1987) has a greater body length ranging from 60 to 80 µm and large number of kineties ranging from 33 to 35 kineties. *T. corlissi* has a pyriform body, about 55 x 30 µm in size with 25 to 31 kineties and a caudal cilium which is stronger and longer than the other cilia (Lom and Dyková, 1992). Ghoneim (1998) recorded *Tetrahymena sp*. from the skin of *Oreochromis aureus* and *Tilapia zillii*. *Tetrahymena sp*. (Ghoneim, 1998) is slightly smaller than the present *Tetrahymena* specimens and the number of kineties ranges from 10 to 14 (Table 9).

Table (1): Measurements (in µm) of various parts for the three forms of *Trypanosoma alhussaini* collected from *Clarias gariepinus*.

Component parts of the parasite	Small form	Intermediate form	Large form
Total length of the parasite including free flagellum	35.9 (31.9-39.6)	43.2 (36.3-46.2)	48.6 (41.8-62.7)
Length of cell body	30.8 (28-31.9)	35.7 (32.0-39.6)	39.3 (33.0-51.7)
Breadth of cell body	1.8 (1.7-2.2)	1.8 (1.7-2.2)	2.2
Length of free flagellum	11.3 (11.0-12.1)	7.4 (5.5-8.8)	10.0 (8.8-11.0)
Length of nucleus	4.0 (3.3-4.4)	4.1 (3.3-4.4)	4.6 (3.3-5.5)
Breadth of nucleus	1.7 (1.6 - 2.1)	1.7 (1.6 - 2.1)	2.1
Distance from anterior margin of nucleus to anterior end of body	13.5 (13.2-14.3)	15.9 (15.4-17.6)	18.9 (16.5-23.1)
Distance from posterior margin of nucleus to kinetoplast	12.4 (12.1-13.2)	12.3 (12.1-13.2)	15.1 (13.2-18.7)
Length of kinetoplast	1.1	1.1	1.1
Breadth of kinetoplast	0.55	0.55	0.55
Distance from kinetoplast to posterior tip	1.1	1.6 (1.1-2.2)	1.2 (1.1-2.2)
Width of undulating membrane	1.1	1.1	1.4 (1.1-2.2)

Table (2): Morphometrical data (in µm) of *Amphileptus* sp. from the gills of *Clarias gariepinus*.

Parameter	*Amphileptus* sp.
Dimensions of body:	
Length	**40.7-70.4 (52.1 ± 10.2, 20)**
Width	**15.4-25.3 (22.2 ± 2.6, 20)**

Dimensions of first macronucleus:	
Length	**7.7-11.0 (9.0 ± 1.1, 20)**
Width	**5.5-7.7 (6.4 ± 0.7, 20)**
Dimensions of second macronucleus:	
Length	**5.5-9.9 (7.9 ± 1.4, 20)**
Width	**4.4-6.6 (6.0 ± 0.7, 20)**
Dimensions of micronucleus:	
Length	**2.2-3.3 (2.7 ± 0.5, 20)**
Width	**2.2-3.3 (2.3 ± 0.3, 20)**
Number of kineties	**5.0-9.0 (7, 20)**

Table (3): Morphometrical data (in µm) of *Chilodonella hexasticha* from the gills and skin of *Clarias gariepinus*.

Parameter	*Chilodonella hexasticha*
Dimensions of body:	
Length	29.7-50.6 (37.9 ± 6.5, 20)
Width	22.0-39.6 (27.7 ± 7.9, 20)
Dimensions of macronucleus:	
Length	11.0-17.1 (13.4 ± 2.4, 20)
Width	7.7-11.0 (10.1 ± 1.6, 20)
Dimensions of micronucleus:	
Length	3.3- 4.4 (3.9 ± 0.5, 20)
Width	2.2- 3.3 (2.7 ± 0.5, 20)
Number of kineties in:	
Right ciliary band	7-8 (8, 20)
Left ciliary band	8-10 (8, 20)

Table (4): Morphometrical data (in µm) of *Vorticella* sp. from the gills of *Clarias gariepinus*.

Parameter	*Vorticella* sp.
Dimensions of body:	
Length	**59.4-86.9 (67.3 ± 8.3, 20)**
Width	**55.0-81.4 (66.6 ± 11.0, 20)**
Dimensions of macronucleus:	
Length	**99.0-176.0 (125.0 ± 23.1,20)**
Width	**6.6-7.7 (7.1 ± 0.56,20)**
Dimensions of micronucleus:	
Length	**15.2-20 (16.1 ± 1.4, 20)**
Width	**8.5-10 (8.9 ± 0.5, 20)**
Length of peristomial disc	**30.8-50.8 (45.2 ± 8.2, 20)**
Length of stalk	**40.6-105.5 (93.2 ± 13.5, 20)**

Table (5): Morphometrical data (in µm) of *Tetrahymena* sp. from the gills of *Clarias gariepinus*.

Parameter	*Tetrahymena* sp.
Dimensions of body:	
Length	24.2-29.7 (27.2 ± 2.0, 20)
Width	15.4-20.9 (17.4 ± 1.7, 20)
Dimensions of macronucleus: Length	11.0-14.3 (12.3 ± 1.2, 20)
Width	6.6-9.9 (7.9 ± 1.1, 20)
Dimensions of micronucleus: Length	2.2-3.3 (2.4 ± 0.4, 20)
Width	1.1-2.2 (1.7 ± 0.5, 20)
Number of kineties	12-16 (14, 20)

Table (6): Comparison between cell body measurements (in µm) of the present study trypanosome, *T. alhussaini* (Mohamed, 1978) and *T. cyanophilum* (Mohamed, 1978).

Parameters	Present study			*Tryponosoma olhussaini* Mohamed , 1978	*T. cyomophilum* (Mohamed , 1978)	
	Small farm	Intermediate form	Large form		Small form	Large form
Total length including free flagellum	35.9 (31.9 - 39.6)	43.2 (36.3 - 46.2)	48.6 (44.2 - 62.7)	51.4 (48.2 - 55.6)	May reach 43	May reach 54
Length of cell body	30.8 (28 - 31.9)	35.7 (32 - 39.6)	39.3 (33.0 - 51.7)	41.2 (35.2 - 48.4)	25.9 (17.8 - 30.2)	
Breadth of cell body	1.8 (1.7 - 2.2)	1.8 (1.7 - 2.2)	2.2	1.9 (1.2 - 4.0)	1.4 (0.8 - 2.8)	
Length of free flagellum	11.3 (11.0 - 12.1)	7.4 (5.5 - 8.8)	10.0 (8.8 - 11)	8.1 (5.6 - 10.4)	Mostly absent (may reach 12.8 if present)	Mostly absent (may reach 11.6 if present)
Length of nucleus	4.0 (3.3 - 4.4)	4.1 (3.3 - 4.4)	4.1 (3.3 - 3.5)	3.7 (2.8 - 5.0)	2.8 (1.2 - 3.8)	3.6 (2.4 - 4.8)
Breadth of nucleus	1.7 (1.6 - 2.1)	1.7 (1.6 - 2.1)	2.1	1.7 (1.0 - 2.8)	1.3 (0.6 - 2.0)	3.2 (1.4 - 5.6)
Length of kinetoplast	1.1	1.1	1.1	1.1 (0.4 - 1.4)	0.94 (0.4 - 1.6)	0.66 (0.2 - 1.)
Breadth of kinetoplast	0.55	0.55	0.55	0.6 (0.4 - 0.8)	0.53 (0.2 - 1.5)	0.56 (0.2 - 0.8)
Distance from anterior margin of nucleus to anterior end of the body	13.5 (13.2 - 14.3)	15.9 (15.4 - 17.6)	18.9 (16.5 - 23.1)	20.2 (16.8 - 25.6)	9.9 (4.0 - 12.8)	14.0 (11.2 - 17.2)
Distance from posterior margin of nucleus to kinetoplast	12.4 (12.1 - 13.2)	12.3 (12.1 - 13.2)	15.1 (13.2 - 18.7)	16.3 (10.4 - 18.8)	12.8 (8.8 - 16.0)	16.7 (12.0 - 23.6)
Distance from kinetoplost to posterior tip	1.1	1.6 (1.1 - 2.2)	1.2 (1.1- 2.2)	1.1 (0.6 - 3.2)	0.5 (0.0 - 0.8)	0.5 (0.0 - 1.8)
width of undulating membrane	1.1	1.1	1.4 (1.1 - 2.2)	1.2 (0.6 - 1.6)	1.43 (0.8 - 2.8)	3.7 (2.2 - 6.2)

Table (7): Comparison between *Amphileptus* sp. of the present study and *Amphileptus* sp. of Ghoneim (1998).

Parameters	*Amphileptus* sp. of the present study	*Amphileptus* sp1 (Ghoneim, 1998)	*Amphileptus* sp2 (Ghoneim, 1998)
Dimensions of the body:			
Length	40.7-70.4	62.5-103	31.0-66.0
	(52.1 ± 10.2, 20)	(87.9 ± 10.0, 25)	(46.0 ± 11.0, 25)
Width	15.4-25.3	12.6-24.0	23.0-57.0
	(22.2 ± 2.6, 20)	(17.0 ± 3.0, 25)	(39.0 ± 10.0, 25)
Dimensions of ma1*:			
Length	7.7-11.0	7.0-10.7	6.9-13.0
	(9.0 ± 101, 20)	(8.5 ± 1.3, 25)	(9.6 ± 1.4, 25)
Width	5.5-7.7	6.7-8.8	5.1-9.5
	(6.4 ± 0.7, 20)	(7.3 ± 0.9, 25)	(7.5 ± 1.4, 25)
Dimensions of ma2*: Length			
	5.5-9.9	6.3-11.1	6.9-14.0
Width	(7.9 ± 1.4, 20)	(8.5 ± 1.2, 25)	(9.8 ± 1.5, 25)
	4.4-6.6	5.7-9.5	5.1-11.0
	(6.0 ± 0.7, 20)	(7.4 ± 1.1, 25)	(7.4 ± 1.4, 25)
Dimensions of mi*: Length			
	2.2-3.3	1.3-3.2	1.9-3.8
Width	(2.7 ± 0.5, 20)	(1.9 ± 0.5, 25)	(2.7 ± 0.5, 25)
	2.2-3.3	1.3-2.5	1.6-2.5
	(2.3 ± 0.3, 20)	(1.7 ± 0.4, 25)	(2.1 ± 0.3, 18)
Number of kineties	5-9 (7, 20)	6-10 (7, 13)	5-7 (6, 20)

*N.B. ma1, first macronucleus; ma2, second macronucleus; mi, micronucleus.

Table (8): Morphological comparison between *Chilodonella hexasticha* of the present study and that recorded by Ghoneim (1998) and Ahmed *et al.* (2000).

Parameter	Present study	Ghoneim, 1998 Population (A)	Ghoneim, 1998 Population (B)	Ahmed *et al.*, 2000
Dimensions of body:				
Length	29.7-50.6	32.0-55.0	32.8-56.8	50.2
	(37.9 ± 6.5, 20)	(44.0 ± 5.4, 25)	(40.0 ± 4.7, 26)	(49.2-50.6)
Width	22.0-39.6	32.0-45.0	17.7-37.9	33.2
	(27.7 ± 7.9, 20)	(38.0 ± 4.1, 25)	(22.8 ± 4.0, 26)	(31.8-34.6)

Dimensions of macronucleus:				
Length	11.0-17.1 (13.4 ± 2.4, 20)	----------	8.2-16.4 (13.4 ± 13.0, 26)	19.5 (18.5-20.6)
Width	7.7-11.0 (10.1 ± 1.6, 20)		6.3-15.8 (12.1 ± 12.0, 26)	16.9 (16.6-17.2)
Dimensions of micronucleus: Length				
	3.3-4.4 (3.9 ± 0.5, 20)	----------	2.5-4.4 (3.1 ± 3.1, 26)	2.3 (2.1-2.5)
Width	2.2-3.3 (2.7 ± 0.5, 20)		1.9-3.2 (2.6 ± 2.6, 26)	2.3 (2.1-2.5)
Number of kineties in:				
Right ciliary band	7-8 (8, 20)	7-8 (7, 25)	4-5 (4, 26)	6-7
Left ciliary band	8-10 (8, 20)	8-9 (9, 25)	4-5 (5, 26)	

Table (9): Comparison between the present *Tetrahymena* sp. and *Tetrahymena* sp. Described by Ghoneim (1998).

Parameter	*Tetrahymena* sp.	*Tetrahymena* sp. Ghoneim (1998)
Dimensions of body:		
Length	24.2-29.7 (27.2 ± 2.0, 20)	13.9-22.7(18.5±2.3, 25)
Width	15.4-20.9 (17.4 ± 1.7, 20)	6.3-15.8(10.5±2.6, 25)
Dimensions of macronucleus: Length		
Width	11.0-14.3 (12.3 ± 1.2, 20) 6.6-9.9 (7.9 ± 1.1, 20)	---------- ----------
Dimensions of micronucleus: Length		
Width	2.2-3.3 (2.4 ± 0.4, 20) 1.1-2.2 (1.7 ± 0.5, 20)	---------- ----------
Number of kineties	12-16 (14, 20)	10-14 (12, 25)

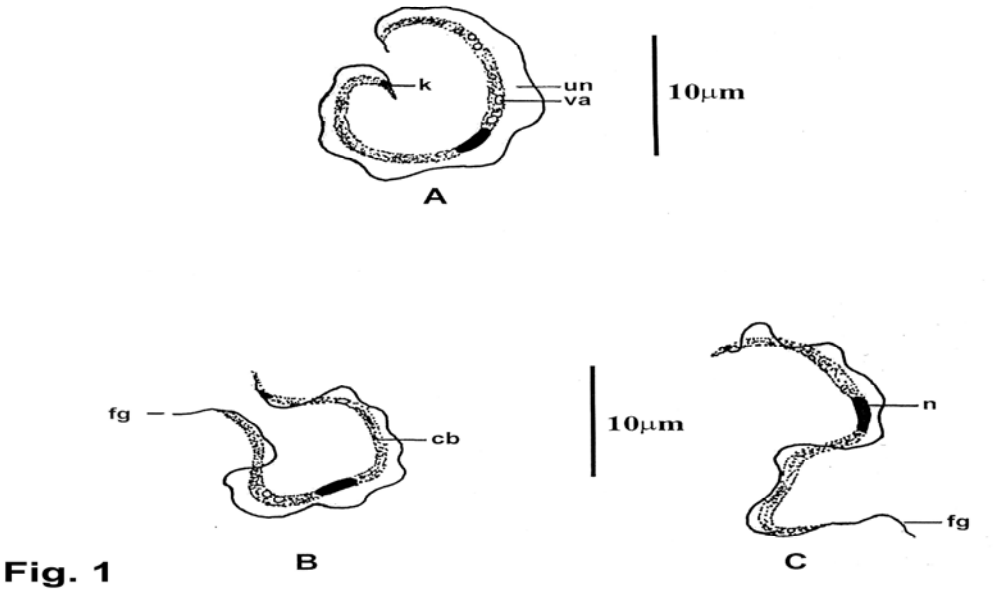


Fig. 1

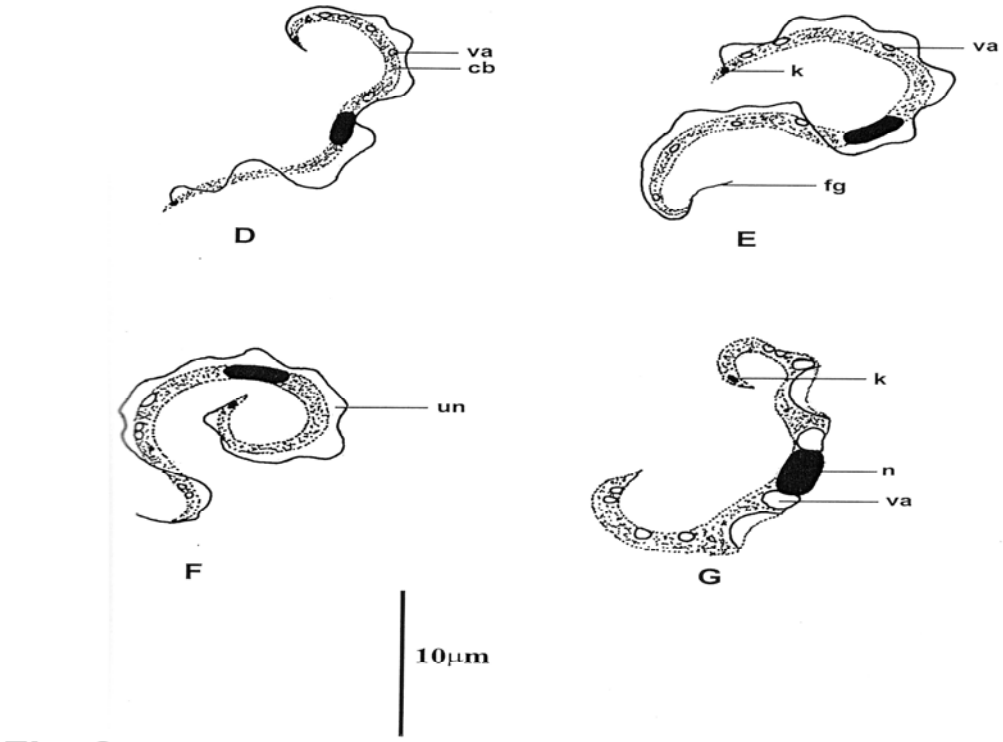
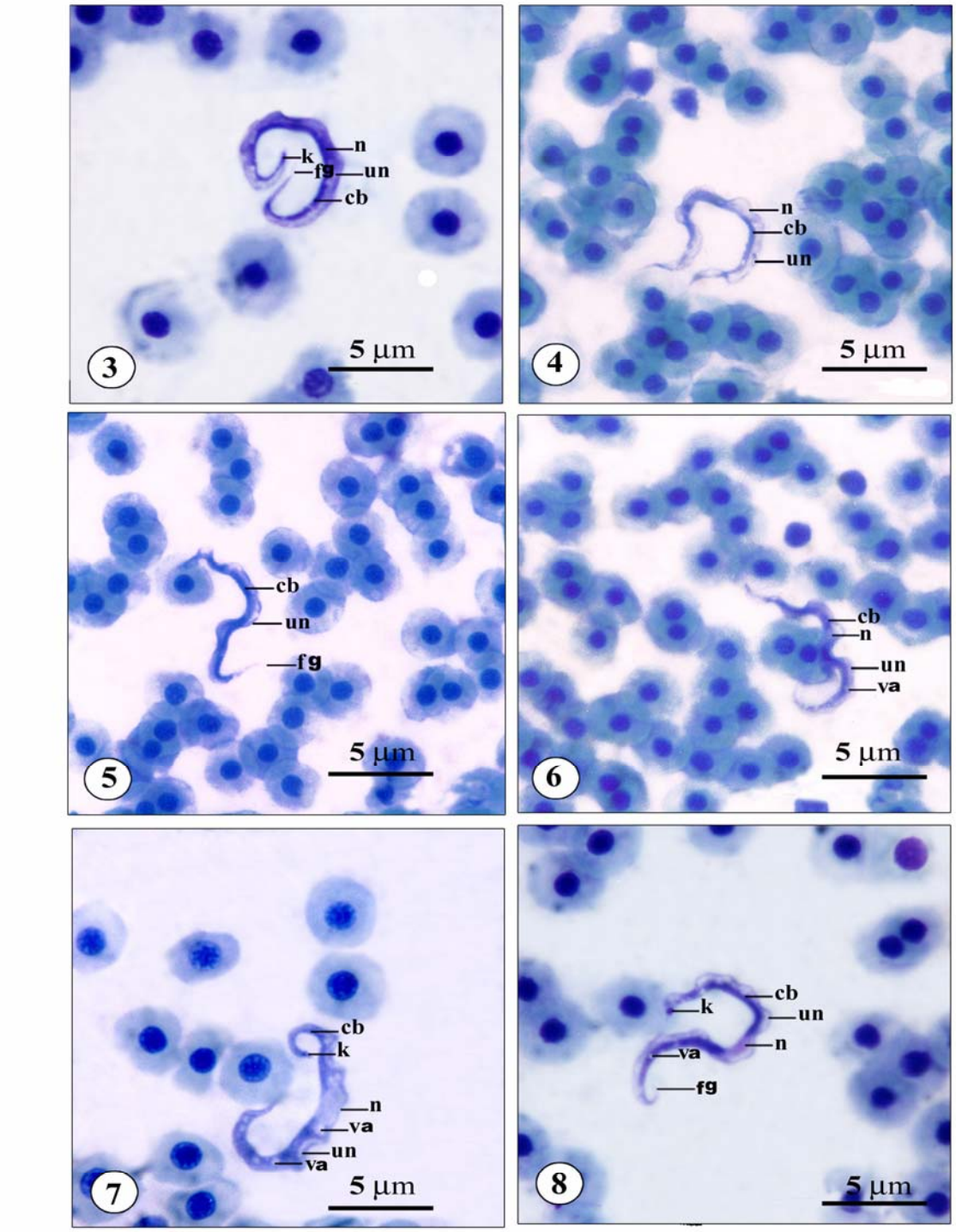


Fig. 2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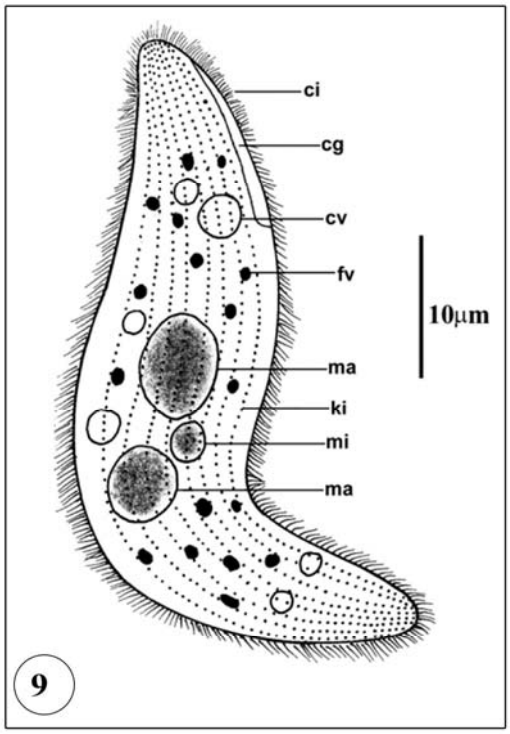
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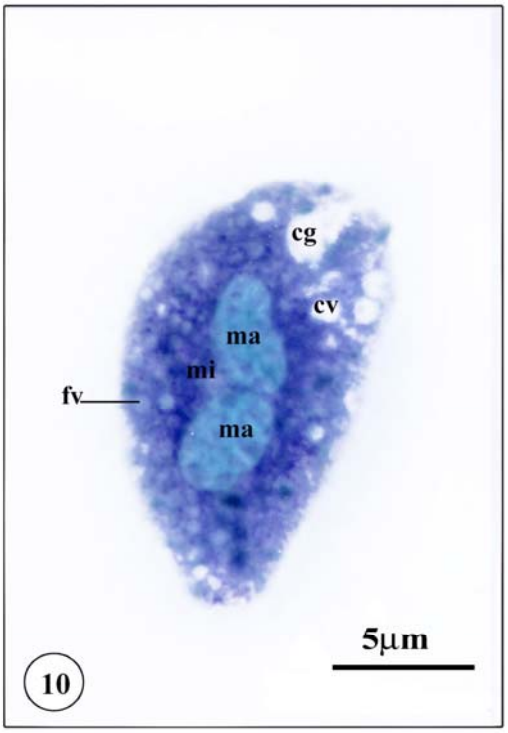
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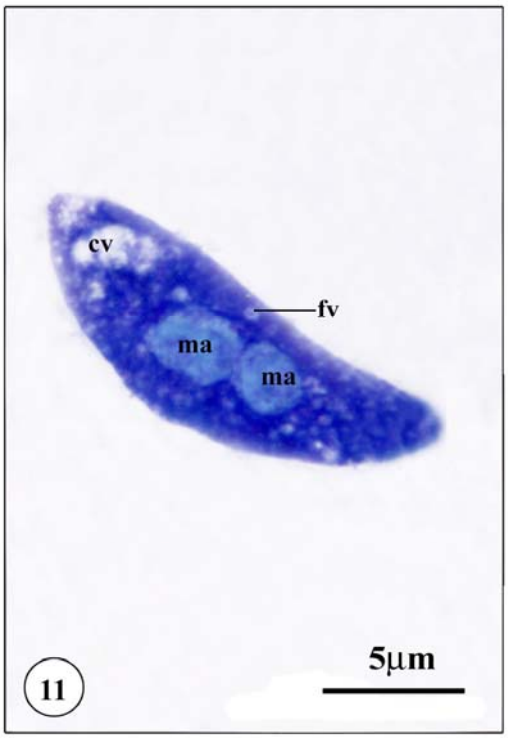
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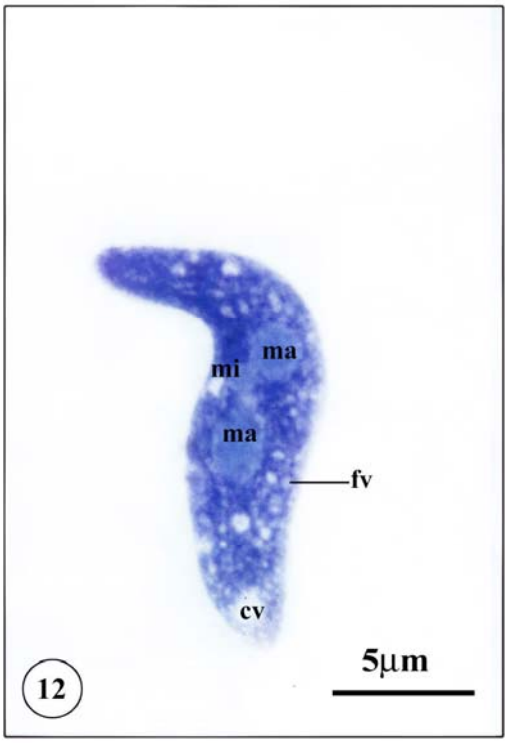
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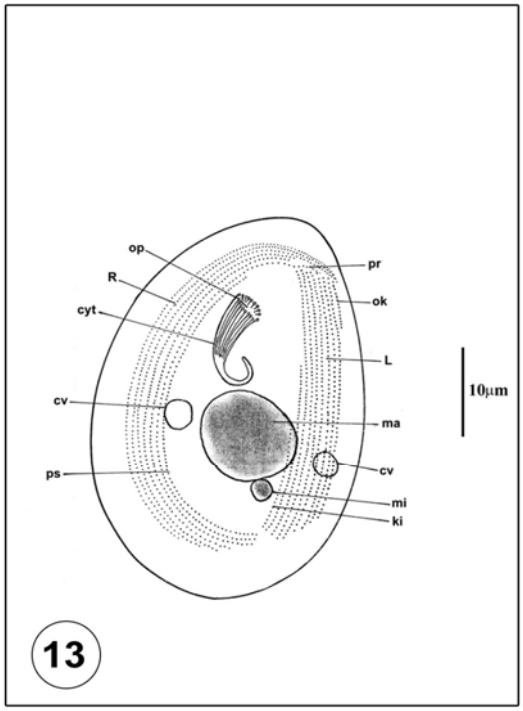
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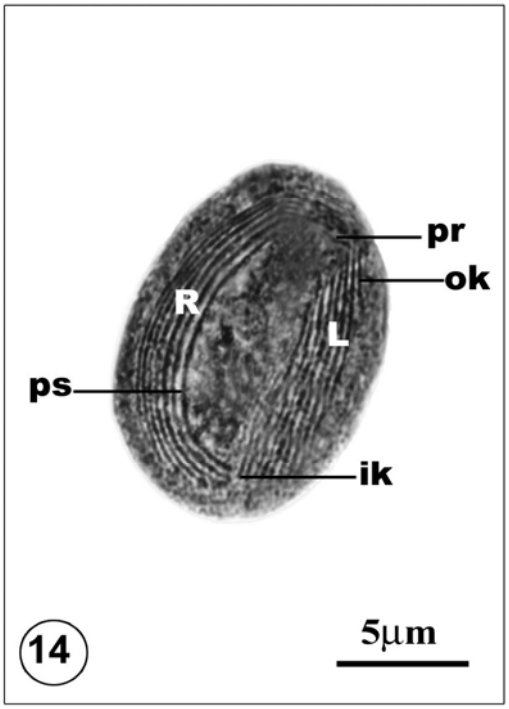
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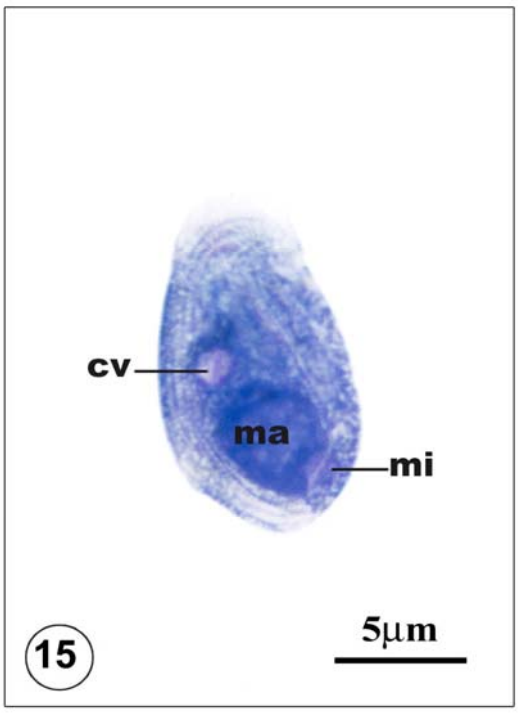
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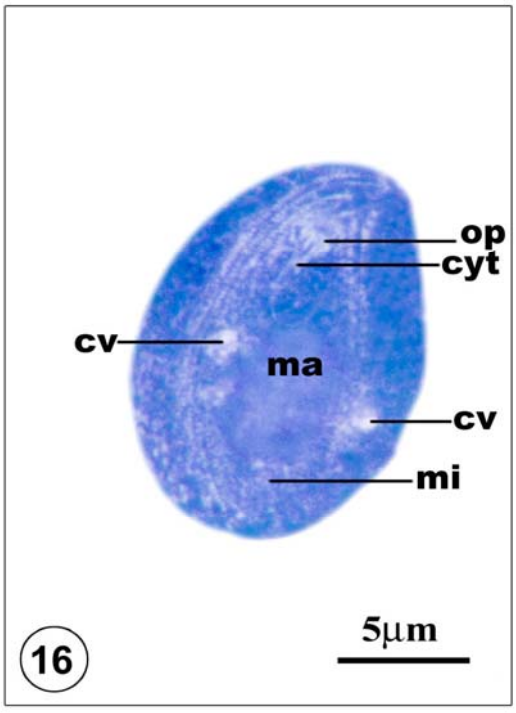
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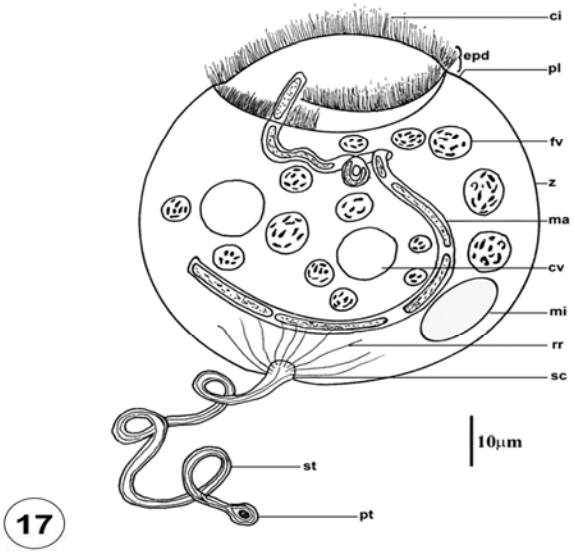
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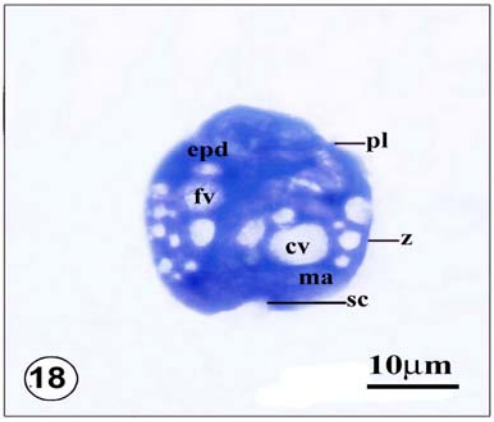
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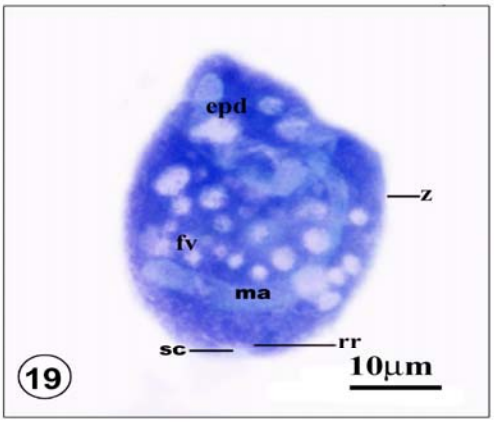
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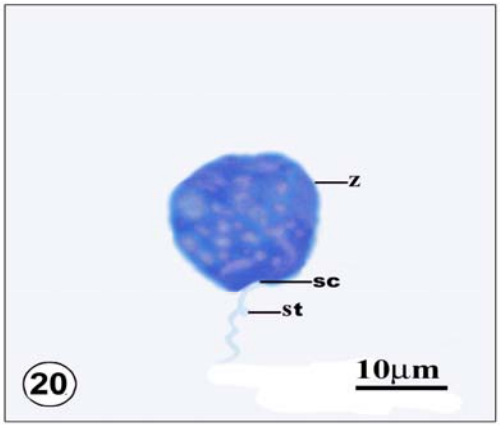
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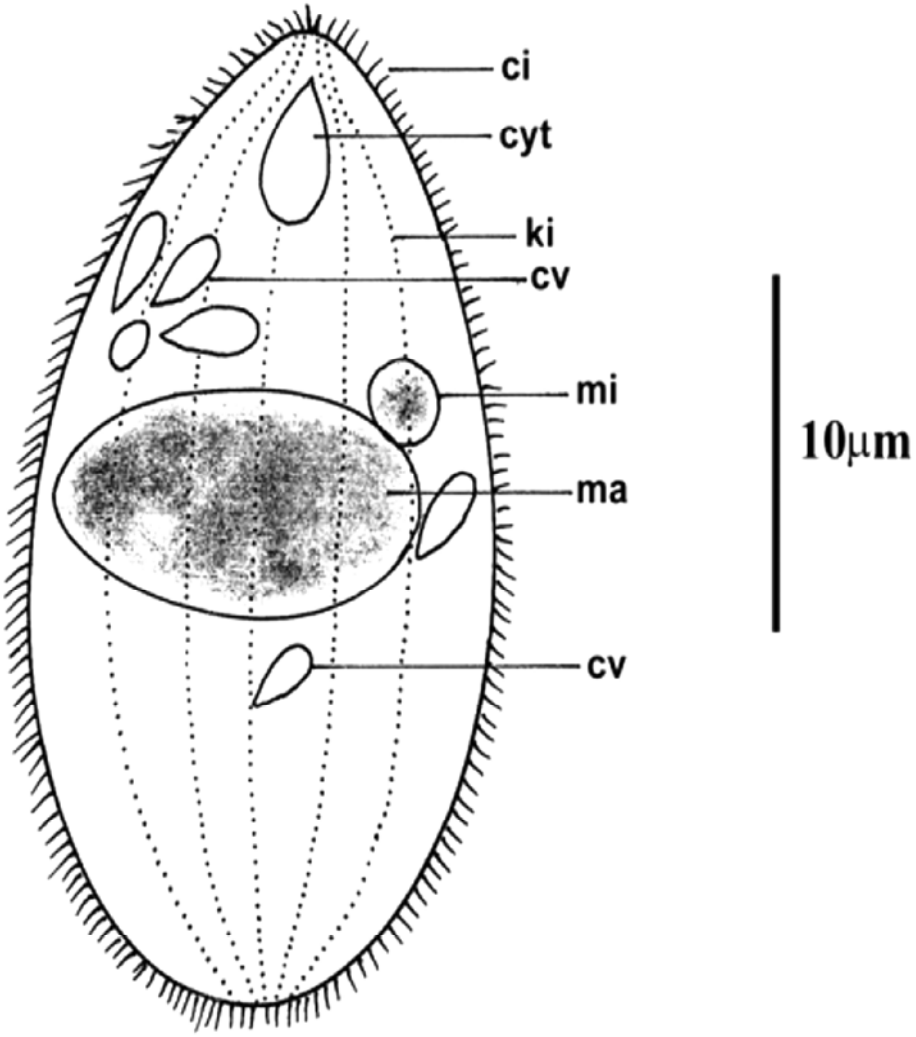
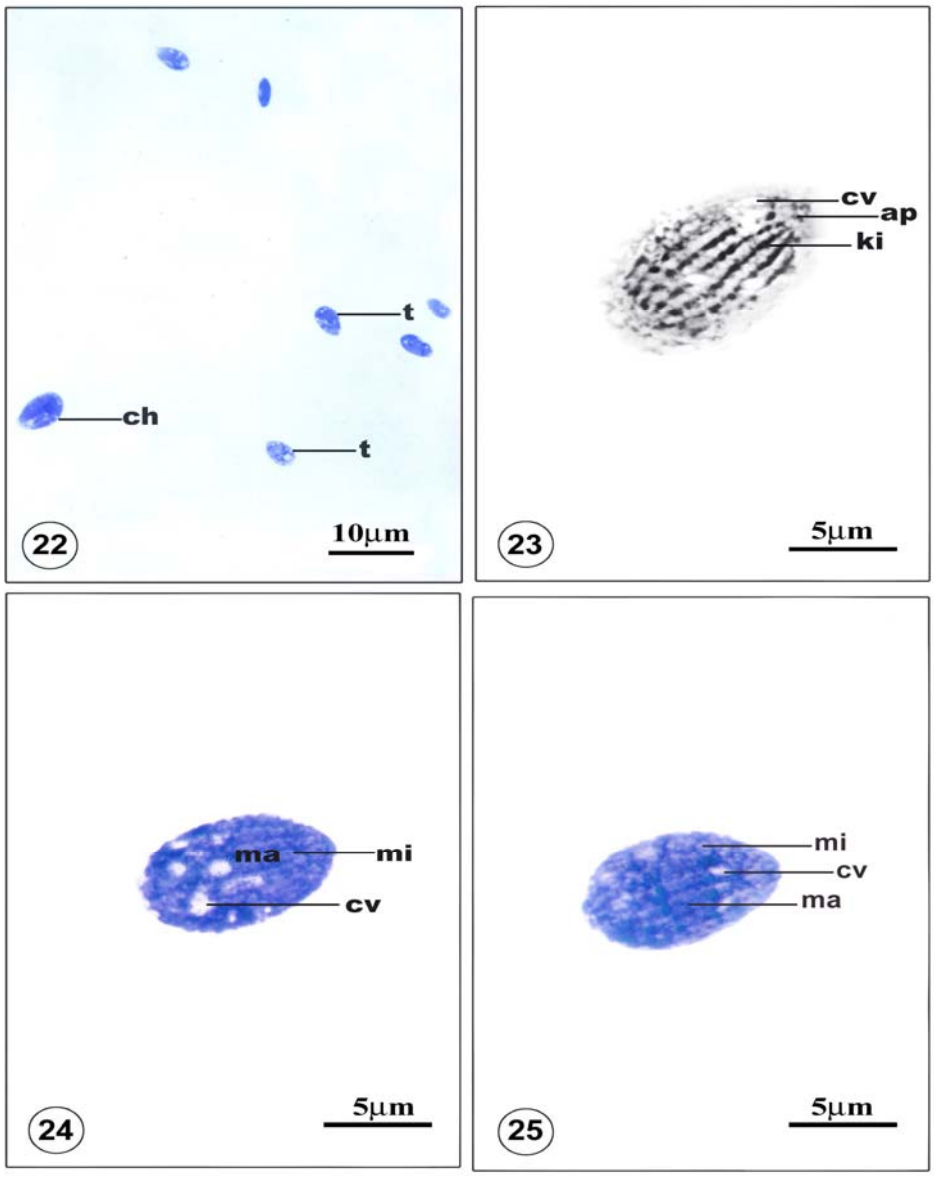


Fig. 21



Figures

Fig. (1): Schematic drawing of Trypanosoma alhussaini showing, small form (A) and intermediate form (B, C). cb; Cell body; fg, flagellum; k, kinetoplast; n, nucleus; un, undulating membrane; va, vacuole. Scale bar = 10µm

Fig. (2): Schematic drawing of Trypanosoma alhussaini showing large forms (D-G). cb, Cell body; fg, flagellum; k, kinetoplast; n, nucleus; un, undulating membrane; va, vacuole. Scale bare = 10µm

Fig. (3): Photomicrograph of Giemsa-stained specimen of Trypanosoma alhussaini showing the small form. cb, Cell body; fg, flagellum; k, kinetoplast; n, nucleus; un, undulating membrane. Scale bar = 5µm

Fig. (4): Photomicrograph of Giemsa-stained specimen of Trypanosoma alhussaini showing the intermediate form. cb, Cell body; n, nucleus; un, undulating membrane. Scale bar = 5µm

Fig. (5): Photomicrograph of Giemsa-stained specimen of Trypanosoma alhussaini showing the intermediate form. cb, Cell body; fg, flagellum; un, undulating membrane. Scale bar = 5µm

Fig. (6): Photomicrograph of Giemsa-stained specimen of Trypanosoma alhussaini showing the large form. cb, Cell body; n, nucleus; un, undulating membrane; va, vacuole. Scale bar = 5µm

Fig. (7): Photomicrograph of Giemsa-stained specimen of Trypanosoma alhussaini showing the large form. cb, Cell body; k, kinetoplast; n, nucleus; un, undulating membrane; va, vacuole. Scale bar =5µm

Fig. (8): Photomicrograph of Giemsa-stained specimen of Trypanosoma alhussaini showing the large form. cb, Cell body; fg, flagellum; k, kinetoplast; n, nucleus; un, undulating membrane; va, vacuole. Scale bar = 5µm
Fig. (9): Schematic drawing of Amphileptus sp. cg, Cytostomial groove; ci, cilia; cv, contractile vacuole; fv, food vacuole; ki, kinety; ma, macronucleus; mi, micronucleus. Scale bar = 10µm
Fig. (10): Photomicrograph of Giemsa-stained specimen of Amphileptus sp. cg, Cytostomial groove; cv, contractile vacuole; fv, food vacuole; ma, macronucleus; mi, micronucleus. Scale bar =5µm
Fig. (11): Photomicrograph of Giemsa-stained specimen of Amphileptus sp. Note the presence of two macronuclei (ma). cv, Contractile vacuole; fv, food vacuole. Scale bar = 5µm
Fig. (12): Photomicrograph of Giemsa-stained specimen of Amphileptus sp. Note the presence of numerous food vacuoles (fv). A large contractile vacuole (cv) is also found at the anterior end. ma, Macronucleus; mi, micronucleus. Scale bar = 5µm
Fig. (13): Schematic drawing of Chilodonella hexasticha. cv, Contractile vacuole; cyt, cytopharynx; ik, innermost kinety in the left ciliary band; L, left ciliary band; ma, macronucleus; mi, micronucleus; ok, outermost kinety in the left ciliary band; op, oral opening; pr, preoral kinety; ps, postoral kinety; R, right ciliary band. Scale bar = 10µm
Fig. (14): Photomicrograph of silver-impregnated specimen of Chilodonella hexasticha. ik, Innermost kinety in the left ciliary band; L, left ciliary band; ok, outermost kinety in the left ciliary band; pr, preoral kinety; ps, postoral kinety; R, right ciliary band. Scale bar = 5µm
Fig. (15): Photomicrograph of Giemsa-stained specimen of Chilodonella hexasticha. cv, Contractile vacuole; ma, macronucleus; mi, micronucleus. Scale bar = 5µm
Fig. (16): Photomicrograph of Giemsa-stained specimen of Chilodonella hexasticha. The cytopharynx (cyt) and its opening (op) are clearly visible. cv, Contractile vacuole; ma, macronucleus; mi, micronucleus. Scale bar = 5µm
Fig. (17): Schematic drawing of Vorticella sp. ci, Cilia; cv, contractile vacuole; epd, epistomial disc; fv, food vacuole; ma, macronucleus; mi, micronucleus; pt, platelet; pl, peristomial lip; rr, radiating ridges; st, stalk; sc, scopula; z, zooid. Scale bar = 10µm
Fig. (18): Photomicrograph of Giemsa-stained specimen of Vorticella sp. showing the zooid (z). Note the epistomial disc (epd) which consists of peristomial lip (pl) provided with cilia. cv, Contractile vacuole; fv, food vacuole; ma, macronucleus; sc, scopula. Scale bar = 10µm
Fig. (19): Photomicrograph of Giemsa-stained specimen of Vorticella sp. Note the prominent zooid (z) which possesses a relatively large ribbon-shaped macronucleus (ma). Note also the radial ridges (rr) which come into contact with the scopula (sc) from which the stalk is protruded. epd, Epistomial disc; fv, food vacuole. Scale bar = 10µm
Fig. (20): Phase-contrast micrograph of Giemsa-stained specimen of Vorticella sp. showing the zooid (z) and coiled contractile stalk (st) arising from scopula (sc). Scale bar = 10µm
Fig. (21): Schematic drawing of Tetrahymena sp. ci, cilia; cv, contractile vacuole; cyt, cytostome; ki, kineties; ma, macronucleus; mi, micronucleus. Scale bar = 10µm
Fig. (22): Photomicrograph of Giemsa-stained specimens showing a mixed infection of Tetrahymena sp. (t) with Chilodonella hexasticha (ch). Scale bar = 10µm
Fig. (23): Photomicrograph of silver-impregnated specimen of Tetrahymena sp. ap, apical loop; cv, contractile vacuole; ki, kineties. Scale bar = 5µm
Fig. (24): Photomicrograph of Giemsa-stained specimen of Tetrahymena sp. cv, contractile vacuole; ma, macronucleus; mi, micronucleus. Scale bar = 5µm
Fig. (25): Photomicrograph of Giemsa-stained specimen of Tetrahymena sp. cv, contractile vacuole; ma, macronucleus; mi, micronucleus. Scale bar = 5µm

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8/1/2010