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Isolation and Identification of some fresh Protozoa from surface water sources and taps in Dakahlyia Governorate, Egypt

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ABSTRACT

The River Nile represents the main source of water in Egypt, used for irrigation, drinking, and fisheries. Therefore, it is so necessary to undergo frequent monitoring of the protozoan biodiversity in fresh water to avoid epidemic diseases. Accordingly, this work aims to isolate and identify the expected protozoan species in water samples collected from the River Nile and taps at four sites in Dakahlia Governorate. Water samples were collected using the concentration technique, followed by modified Ziel-Neelsen stain and Giemsa staining methods for detecting protozoan species using bright light and phase-contrast microscopy. 12 protozoan species belonging to the subphylum Sarcodina, Masigophora, and Cilliophora were recorded. Moreover, examination of water samples revealed that drinking water was contaminated by six species of protozoa. A description of the locomotive and cyst stages is carried out for most of the detected protozoa. **Conclusion:** This study proved that drinking water must be carefully analyzed to avoid the presence or growth of protozoa

Keywords: Protozoa, Srcodina, Mastigophora, Ciliophora, River Nile, Egypt

INTRODUCTION

Water is life. It is used for different purposes, including drinking, irrigation, fisheries, and industrial uses, as well as for preserving the integrity of the world's ecosystem. Access to safe drinking water is a basic right for all human beings **(1).** There has always been water pollution as a natural occurrence. A water body might get contaminated by forest fires, hurricanes, volcanoes, or a significant fall of leaves. However, these organic compounds undergo spontaneous biodegradation **(2).** Many agents that are chemical, microbiological, or parasitic can contaminate water sources **(3).** There are two types of water found in nature: surface water and groundwater. In most river systems, the surface water is found in the headwaters. Although the numbers of bacteria and pathogens are typically low, some bacteria, protozoa, or algae may be present **(4)**.

Because there are inadequate sanitation facilities in impoverished countries, human feces are the main source of water contamination **(5).** Biological pollutants, usually referred to as pathogens, might be bacteria, viruses, or parasites in water sources. In poor countries, it causes several fatal childhood ailments **(6).** About 18% of the world's population does not have access to clean drinking water, and nearly 40% does not have proper sanitation **(7)**. Moreover, poor water management practices, habitat modification (such as clearing forests and converting land for agriculture and highways) and shifting ecological circumstances all contribute to

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the degradation of water quality and the ensuing rise in the spread of vector-borne diseases. More stagnant surface water is produced by the building of dams and reservoirs, insufficient drainage in irrigation plans, or subpar urban water management. These still pools of water serve as the perfect habitat for mosquitoes, which spread several fatal or crippling illnesses, including malaria **(8).**

The quantity and quality of the water are so important for health care. Water tables are dropping on every continent, and one-third of the world's population already resides in nations that experience moderate to severe water stress. If current trends continue, it is predicted that two out of every three people on Earth will reside in places where water quality is unaffected, and the availability of clean drinking water is a significant problem for rural and low-income populations. 1.1 billion people worldwide lack access to a clean water source, such as rainfall collected from a protected spring, groundwater extracted from a borehole, or a protected drilled well. Too little water makes it virtually impossible to maintain the necessary sanitary conditions in the home, which can lead to the outbreak of debilitating or fatal diseases **(9).** The majority of developing countries have poor sanitary conditions. Human excrement especially that of children is contaminated with a wide variety of germs, including viruses and parasite eggs **(10)**. According to **Kudo, most** of the freshwater protozoan parasites were recorded **(11).** Most previous studies on the physicochemical and heavy metal characteristics of the water quality of the river Nile concentrated on how pollution affected the numbers and distribution of zooplankton (**12; 13; 14).** Several researches have been reported and identified some protozoa in tap water from Dakahlyia Governorate Egypt. For instance, four species of *Emeria* **(15)** and one species *Vannella* (**16)** were recorded and recognized in drinking water for the first time in Egypt**.** It was found that regular protozoan fauna monitoring of the Damietta branch and other freshwater canals in the Dakahlyia Governorate was essential for the sustainable use of the River Nile and its branches in the Nile Delta region.

One common genus of protozoa that are amoebo-flagellate is *Naegleria*. It is a live, independent entity. There aren't many pathogenic *Naegleria* species for animals or people. One main example that affects both people and different animal species is *N. folweri*. This species is a facultative pathogen known as an amoeba, which may multiply into several living generations without harming its host. Warm water swimming and other forms of intimate contact with warm water allow both pathogenic and non-pathogenic *Naegleria* to enter the body through the nose. After entering the body, the amoeba uses motility to travel to the brain, where it causes neuronal damage and ultimately results in Primary Amoebic Meningoencephalitis (PAM) in humans **(17)**.

Protozoans belonging to the genus *Acanthamoeba* Volkonsky 1931 are found in various natural environments, including soil, dust, swimming pools, seawater, natural thermal water, tap water, and even human nasal mucosa **(18;19; 20; 21; 22; 23; 24)**. Furthermore, due to their capacity to cause dangerous and lethal infections in humans and animals, the majority of *Acanthamoeba* species are free-living potential pathogens that have drawn a lot of interest in recent years **(25, 26)**. According to **Visvesvaraya**, the life cycle of Acanthamoeba consists of two stages: an active trophozoite stage that shows vegetative growth and a dormant cyst stage with little metabolic activity **(27).** According to **Khan,** the genus *Acanthamoeba* is the cause of skin lesions, sinus infections, keratitis that can impair eyesight, and granulomatous amoebic encephalitis, an uncommon but deadly form of encephalitis **(28).** *Acanthamoeba* cysts were reported to be greatly resistant to most antimicrobial agents and subsequently difficult to treat **(29)**. The presence of *Acanthamoeba* in drinking water might represent a double risk since some *Acanthamoeba* species are pathogenic and could harbor pathogenic strains of *Pseudomonas*, *Helicobacter*, and *Legionella* **(30).** Furthermore, *Acanthamoeba* cysts are resistant to

desiccation, high temperatures, and disinfectants **(31)**. *Acanthamoeba* species have been found in freshwater sources, drinking water taps, soil, lakes, swimming pools, water cooling towers, and the atmosphere in Egypt **(32).**

Members of the family Arcellidae are enveloped by a simple shell; form lobopodia or filopodia, within which the body can be completely withdrawn. Shell has usually a single aperture through which pseudopodia protrude, and vary in shape and structure. However, a chitinous or pseudo-chitinous membrane forms the basis of all. It may be thickened. Species of the genus *Arcella* have a transparent, chitinous test, with hexagonal markings, colorless to brown. It looks circular, angular, or stellate in front view. While in profile looks plano-convex or semicircular; variously ornamented. The aperture is circular, central, and inverted like a funnel. The protoplasmic body does not fill the test and is connected with the latter by many ectoplasmic strands. Slenderlobopodia, few, digitate, simple or branched. This genus has two or more nuclei and several contractile vacuoles. However, few species of *Arcella* are living in freshwater **(33)**.

Species of the genus: *Actinophrys* **(34)** are characterized by spheroidal body. The cytoplasm is highly vacuolated, especially ectoplasm, and often symbiotic zoochlorellae. This genus has a central nucleus and one to many contractile vacuoles. In a straight axopodia, numerous axial filaments terminate at the surface of the nucleus. Members of this genus live in fresh water. *Actinophrys sol* was described by **Chantangsi** from fresh water in Thailand **(35),** while *A.sol* was described by **Mikrjukov and Patterson** in a Brackish water pond swan pool from Falmouth, England **(36).**

The genus *Sphaerastrum* includes protozoan species characterized by a thick gelatinous mantle covering the greater part of axopodia **(11).** Additionally, Members of the genus *Euglena* are unicellular flagellate protists that have a central granule and an eccentric nucleus. *Euglena* lacks a cell wall. Instead, it has a pellicle **(34).** Species of *Euglena* are found in fresh and marine waters.

Members of the genus Coleps are characterized by bodies that are cylindrical or barrel-shaped and are covered in characteristic calcified cuirasses (armored plates with small lateral plate processes organized in longitudinal rows). Additionally, their caudal cilia are generally longer than other somatic cilia, and they occasionally have anterior and/or posterior spines **(37; 38).** Therefore, this work attempts to isolate and identify the existing protozoan species from water samples collected from the River Nile and taps of 4 sites in Dakahlia Governorate.

MATERIALS AND METHODS

Forty water samples were collected seasonally randomly from different localities in the Nile Delta region, Dakahlyia Governorate, Egypt. These sites included the River Nile at Met Khamis and Gamaleia and tap water from Awish El-Hagar and Belkas. The collected water samples were directly transferred to the Invertebrate Laboratory, Zoology Department, Faculty of Science, Mansoura University. Samples were processed within 24-48hr of the collection as described by **(39).** Briefly, each water sample was filtered through a cellulose acetate filter 0.45 µm mesh diameter using a magnetic funnel and suction pump. The filtrate was diluted in 10 ml filtered freshwater and centrifuged at 3000 rpm for 10 minutes. A drop of the precipitate was transferred to a clean slide, covered with a coverslip, and examined using a Leitz Laborlux 20 EB phase-contrast research microscope. Also, an examination was carried out by using various staining techniques, in which sediments were fixed in 10 %formalin and then stained by Modified Ziel-Neelsen stain and Giemsa stain. All recorded species were identified according to the previous method described by **Patterson (40).** All specimens were investigated, photographed, and then drawn using a Leitz Laborlux 20 EB phase-contrast microscope fitted with a camera. Additionally, the dimensions of each species were evaluated with the aid of a micrometer-calibrated ocular lens at different magnifications.

RESULTS

In the present study, 12 Protozoan species were detected from the River Nile and tap water of four different sites in Dakahlia governorate. (Table 1).

I- Sub-phylum: Sarcodina

1- Genus: *Naegleria*

N. fowleri

The trophozoite form of. *N. fowleri* is usually elongated with one main hyaline cap (H.C) that appears during locomotion (Fig.1a, 1a1). Additionally, contractile vacuoles (C.V) and food vacuoles (F.V) were recorded. The elongated body has a large spherical nucleus (N) containing a large, centrally placed, dense nucleolus (NU), and lobular pseudopodia (L.P). A body was held to be the parabasal body (P) that is always present at the base of the protrusion or flagella (F). Flagella were formed by filamentous protrusion of endoplasm; one pair of flagella is produced from a single protrusion. The amoeboid stage measures 16,8 µm in length. Flagellated form was recorded in the present study from the tap water.

2- Genus: *Acanthamoeba*

***Acanthamoeba* sp.**

The *Acanthamoeba* sp. of the present study is recorded in two forms, the locomotive (trophozoite) form and the cyst form (Figs.1b, 1b1, 1c, 1c1 1d, 1d1). The body of the locomotive form is typically circular (Figs.1b,1b1) but during locomotion, it appears sometimes wider than long or longer than wide. The cytoplasm is differentiated into an outer thin region called hyaloplasm (HY) and an inner region filling most space of the cell called granuloplasm (GR) (Figs.1b, 1b1). The body of the locomotive form is characterized by the presence of a small amoeboid elongated to broad hyaline filamentous projections and numerous food vacuoles (F.Vs). The body has a hyaline anterior zone of ectoplasm (hyaline cap) (H.C) with anterior filose sub-pseudopodia (F.Su.P). It measures about 14µm in length and 11.2µm in width (Figs. 1b, 1b1).

The cyst stage of *Acanthamoeba sp*. differs from one species to another. The cyst stage of *A. castellanii* is a doubled wall (D.W); the outer smooth wall and inner wrinkled wall with highly resistant to environmental conditions (Figs.1c, 1c1). The cyst stage of *A. hyaline* is spherical with two walls; an outer wrinkled wall (O.W.W) and an inner smooth wall (I.S.W). The cyst measures 14µm in diameter (Figs. 1d, 1d1). Both trophozoite and cyst were recorded from the tap water.

3- Genus: *Arcella*

A. discoides

The body of this species is characterized by its brown chitinous test (or shell); and hexagonal marking (H.M) and it appears circular in front view. The circular aperture is inverted centrally like a funnel. The body protoplasm does not fill the test and is connected with the latter by many ectoplasmic strands; slender lobopodia (L). The body has two or more nuclei (N) and several contractile vacuoles (C.V). It measures 14µm in diameter and was recorded from the tap water (Figs 2a,2a1).

4- Genus: *Sphaerastrum*

S. fockei

The body is characterized by a thick gelatinous mantle (G.M), and eccentric nucleus (N). The body measures 25µm in diameter and was recorded from the tap water (Figs. 2b&2b1).

5-Genus: Actinophrys

A. sol

The body is a spherically symmetrical heliozoan. The cytoplasm is vacuolated, and the endoplasm is granulated with numerous small vacuoles. A large central nucleus (C.N.) has a peripheral nucleolus. The arms (Ar) protrude from the ectoplasm while the axons (A) are protruded from the nucleus. The body measures 40 µm in diameter and was recorded from River Nile (Figs.2c,2c1).

The cyst of this species has two layers: an outer layer (O.L) with siliceous plates (S.P) and an organic inner layer (Or.I.L). The cytoplasm is granular (G.C). It measures 20µm and was recorded from the tap water (Figs. 2d,2d1).

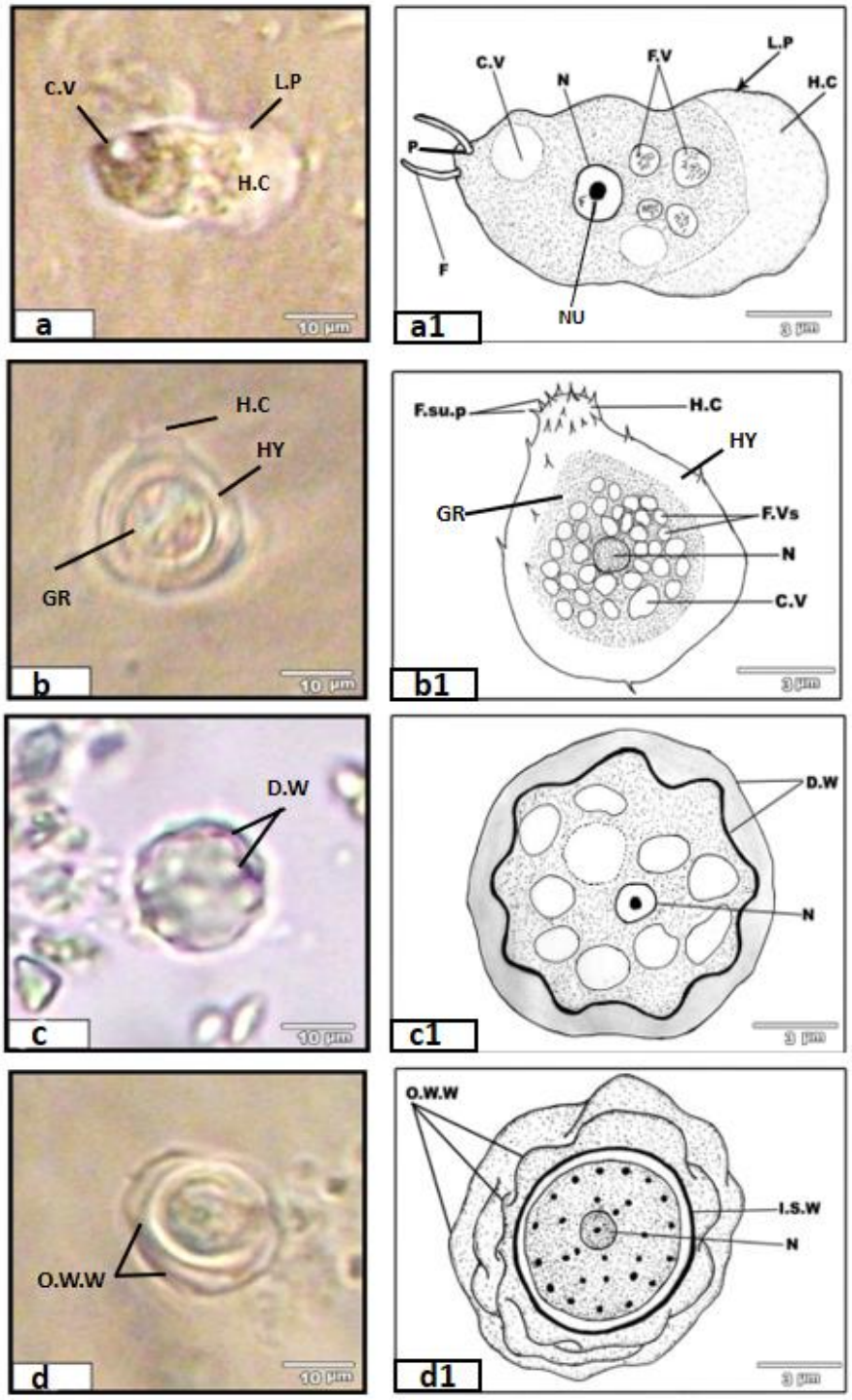

Fig.1 Phase-contrast microscope photographs of the locomotive stages of *Naegleria fowleri* (a), *Acanthamoeba* sp.(b), cyst stages of *Acanthamoeba* sp. (c and d). Schematic drawing of the trophozoite stage of *Naegleria fowleri* (a1), trophozoite stage of *Acanthamoeba* sp. (b1), *Acanthamoeba* cyst (c1 and d1).

Abbreviations: CV, Contractile vacuoles; D.W, double wall; F, flagellum; F.su.p filose subseudopodia projection; F.Vs, food vacuoles; GR, granuloplasm; H.C, hyaline cap; HY, hyaloplasm I.S.W, inner smooth wall; L.P, lobular pseudopodia; N, nucleus; NU, nucleolus; O.W.W outer wrinkled wall; P, parabasal body.

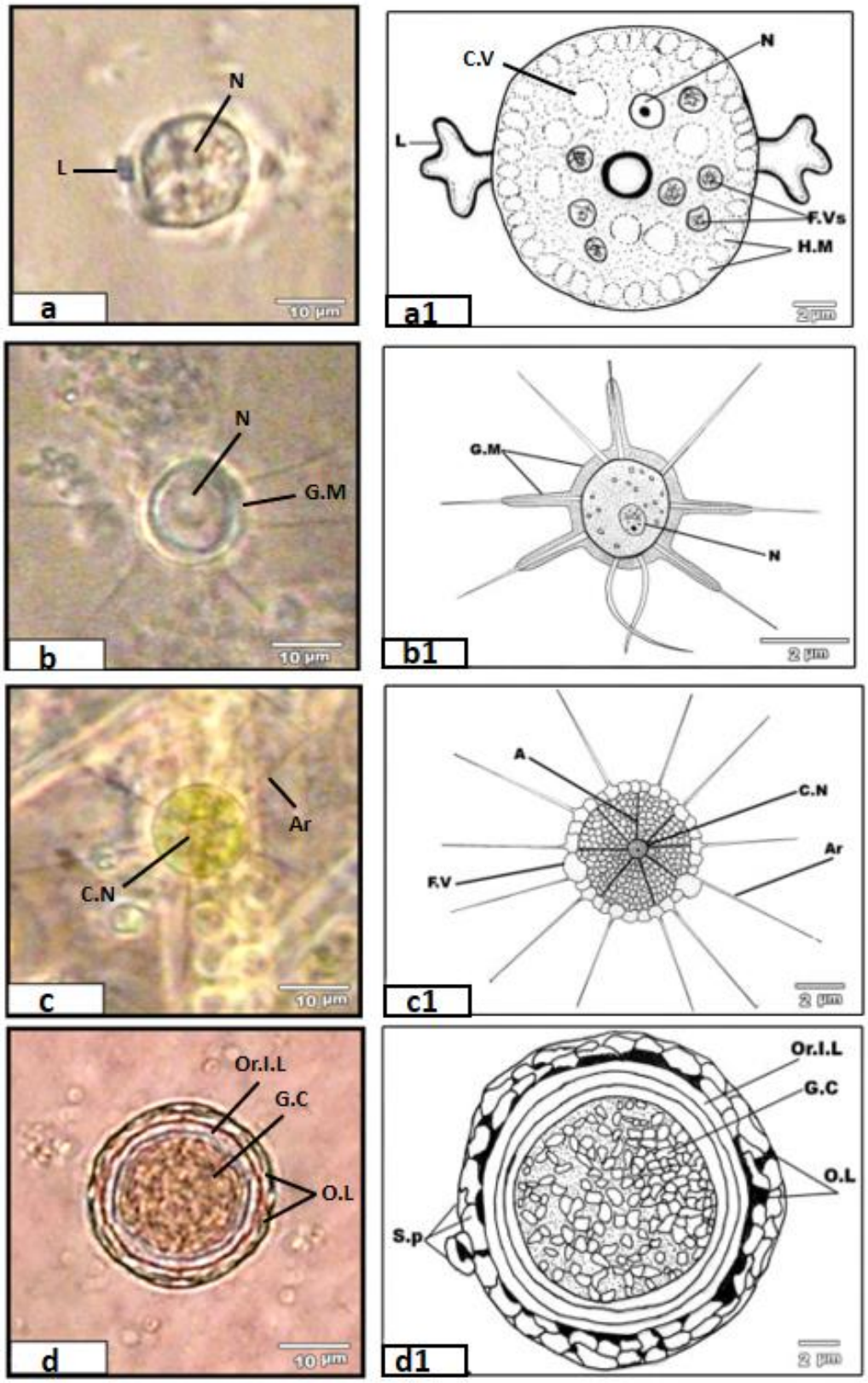

Fig.2 Phase-contrast microscope photographs of *Arcella discoides*.(a), *Sphaerastrum fockei*. (b), *Actinosphaerium sol.*(c), *Actinosphaerium sol* cyst (d). Schematic drawing of *Arcella discoides* (a1), *Spearastrum fockei* (b1), *Actinophrys sol* (c1), *A. sol* cyst (d1).

Abbreviations: A, axoneme; Ar, arm; F.Vs, food vacuoles; G.C, granular cytoplasm; G.M, gelatinous mantle; H.M, hexagonal markings; L, lobopodia; N, nucleus O.L, outer Layer; Or.I.L, organic inner Layer; S. P, siliceous plates

II Sub- Phylum: Mastigophora.

1-Genus: *Euglena*

E. pisciformis

The body is elongated, characterized by chloroplast (Ch) which gives it green color, flagellum (F), and lateral eye spot(S). It measures 20µm in diameter and was recorded from the River Nile water (Figs. 2a, 2a1).

2-Genus: *Phacus*

P. pleuronectes

The body is asymmetrical flattened leaf-like and green in color with a short posterior slightly curved caudal (C). The pellicle is quite rigid and is composed of longitudinal periplasts (L.P). The chloroplasts are small with two spherical paramylum grains (P.G). The body has contractile vacuoles and red-pigmented stigma(S) and flagellum (F) appear as long as the body. The body measures 40µm in length and 30µm in width. It was recorded from the River Nile water (Fig. 3b, b1).

P. onyx

The body is oval, with a strong curved caudal (C) at the posterior end. Longitudinally periplast striated, dorsal ridge is present and two paramylum grains are situated at the center. The eye spot is star in shape (S.S). The body measures 66.7 µm in length and 46 µm in width. It was recorded from the River Nile water (Fig.3 c&c1).

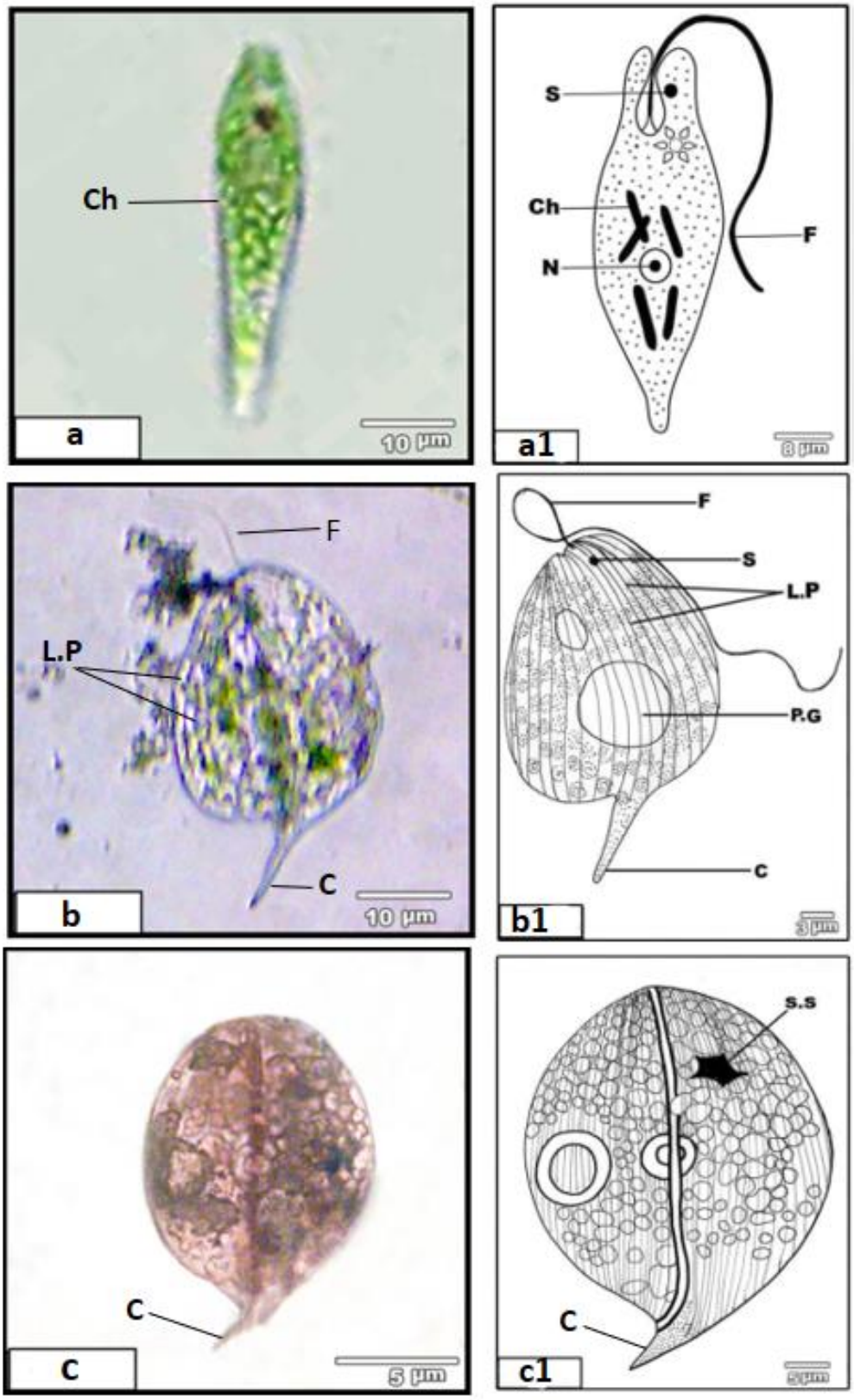

Fig.3 Phase-contrast microscope photographs of *Euglena* sp.(a), *Phacus pleuronectes* (b). Light microscope photograph of *P. onyx* stained with Trichrome. Schematic drawing of *Euglena* sp.(a1), *Phacus pleuronectes* (b1), *P. onyx* (c1).

Abbreviations: C, caudal; Ch, chloroplast; F, flagellum; L.P, longitudinal proteinaceous; N, nucleus; P.G, paramylum grain; S, eye spot; S.S, star eye spot

III Sub-Phylum: Ciliophora.

1-Genus: *Tokophrya*

Members of this genus are characterized by developing suctorial tentacles in attaching species and the absence of cilia.

T. infusionum

The body is characterized by the distribution of suctorial tentacles (Te) with a mouth, over the entire body. Moreover, a short stalk (S) and attaching disk were recorded. The nucleus (N) is large and oval. Additionally, a lot of small contractile vacuoles were recorded. It measures 9.2 µm in length and 6.9 µm in width. *T. infusionum* and was recorded from the tap water (Figs.4a, a1).

2-Genus: *Eodinium*

E. lobatum

The body is barrel in shape. Dorsal zone membranelles (D.Z.M) are on the same level as adoral zone membranelles (A.Z.M); and skeleton plate (S.P). The macronucleus (Ma) is straight. In contrast, the micronucleus is small and spherical (Mi). Two contractile vacuoles (C.V) were recorded. The body measures 40µm in length and 25µm in width. It was recorded from the tap water (Figs. 4b, b1).

3-Genus: *Colpoda*

C.cucullus

The body is oval with anterior keels (K) with eight indentations (I). The body has a lateral cytostome (Cy), and macronucleus (Ma) with a stellate endosome and micronucleus (Mi). Trichocycts (T) are large usually with food vacuoles. Contractile vacuoles (C.V) are terminal. It measures 40µm in diameter and was recorded from the River Nile water (Figs.4c, c1).

4- Genus: *Coleps*

C. bicuspis

The present *Coleps bicuspis* is barrel in shape which is divided into anterior main tiers (AMt) and posterior main tiers (PMt). The cytostome lies at the anterior end which is surrounded by slightly longer cilia with oral aperture (O.A). Regularly body has arranged ectoplasm plates; 16 rows of platelet windows (P.W), two posterior cuspis (Cs), and huge cilia (Ci). It measures 55µm in length and was recorded from River Nile water (Figs. 5a, 5a1).

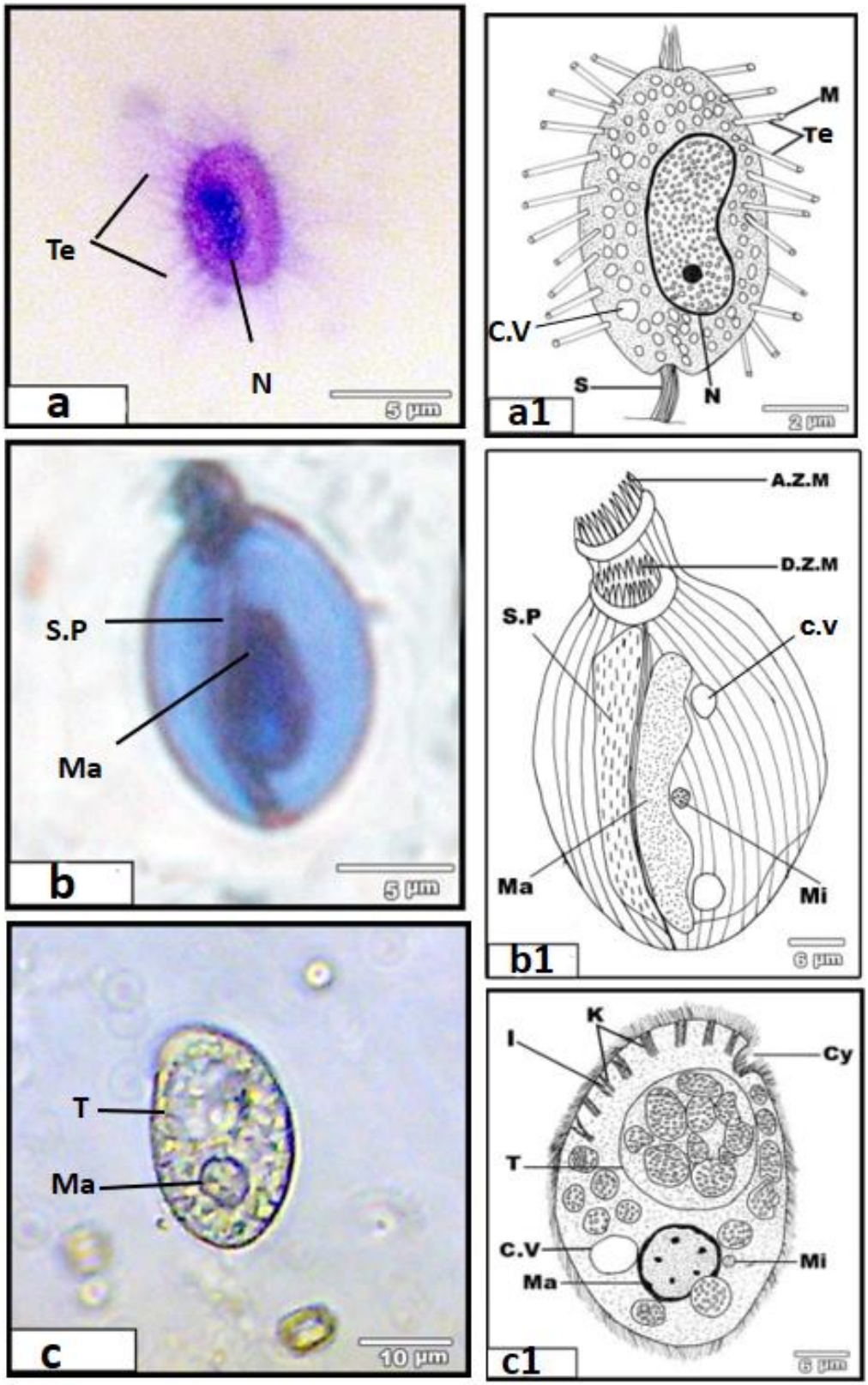

Fig.4 Light microscope photograph of *T. infusionum* stained by Trichrome and *Eodiniumlobatum* stained by Modified Zeihl-Neelsen (b). Phase-contrast micrograph of *Colpoda cucullus*.(c). Schematic drawing of *T. infusionnum* (a1), lateral view of Eodinium *lobatum* (b1), *Colpoda cucullus* (c1).

Abbreviations: A.Z.M, adoral zone membrenella.. C.V, contractile vacuole; Cy, cytostome; D.Z.M, dorsal zone membrenella; I, indentation; K, keels; M, mouth; Ma., macronucleus; Mi., micronucleus N, nucleus; S, stalk; T, trichocyst; Te, tentacles; S.P, skeletal plate.

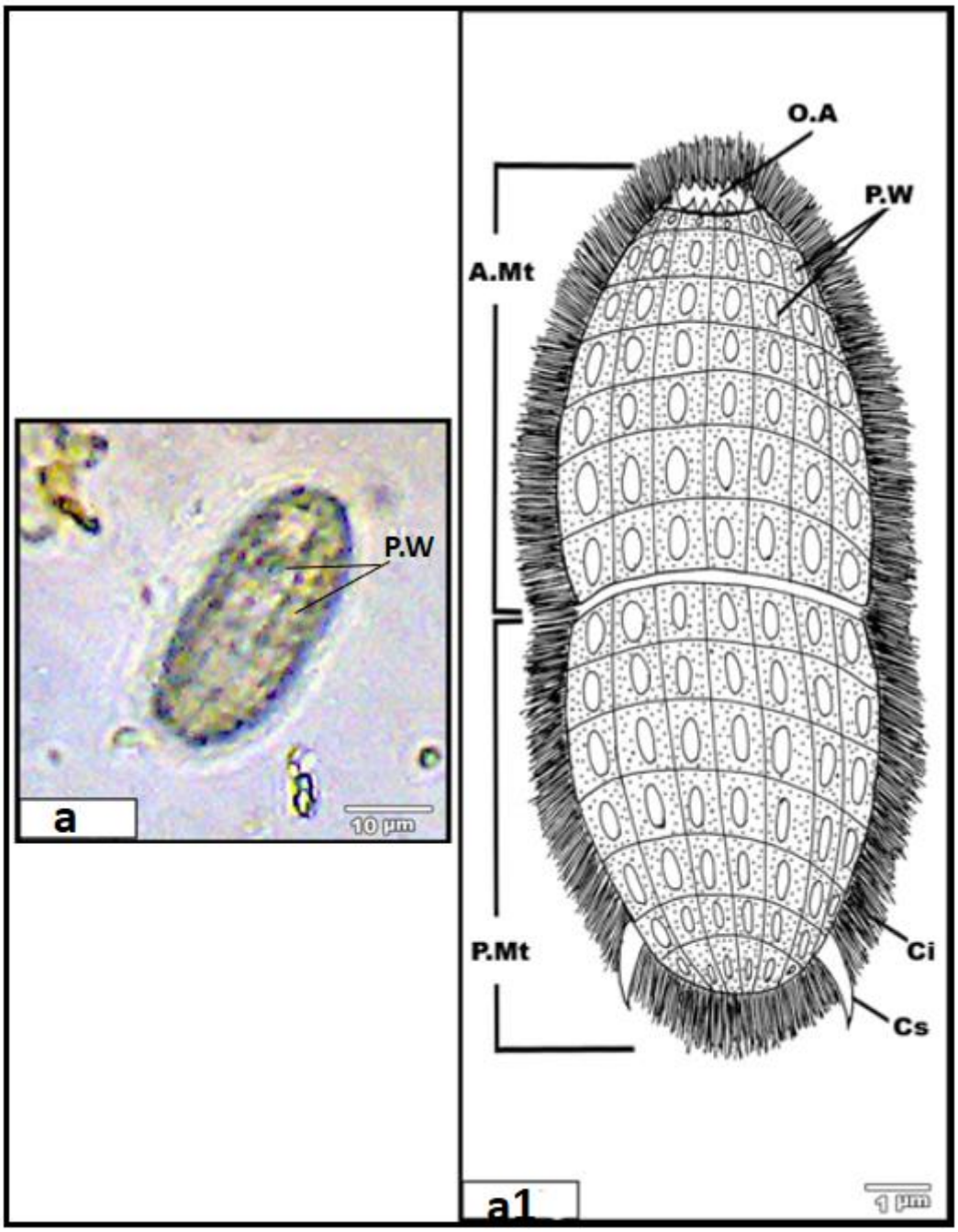

Fig.5 Phase-contrast microscope photographs of *Coleps bicuspis* (a). Schematic drawing of *Coleps bicuspis* (a1).

Abbreviations: AMT, anterior main tier; Ci, cilia; Cs, cuspis; OA, oral apparatus; PMT, posterior main tier; P.W, plate window.

Table 1: Occurrence of pathogenic and non-pathogenic protozoa different water sources:

Organism	Water source	Site
Naegleria fowleri	**Tap water**	**Awish- Elhagar**
Acanthamoeba sp.	**Tap water**	**Belkas & Elgamaleia**
Arcella discoides	**Tap water**	**Belkas**
Sphaerastrum fockei	**Tap water**	**Belkas**
Actinophrys sol	**River Nile**	**Mit- khamis**
Euglena pisciformis	**River Nile**	**Mit– khamis**
p.pleuronectes	**River Nile**	**Belkas**
p.onyx	**River Nile**	**Gamaleia**
Tokophrya infusionum	**Tap water**	**Belkas**
Eodinium lobatum	**Tap water**	**Elgamaleia**
Colpoda cucullus	**River Nile**	**Belkas**
Coleps bicuspis	**River Nile**	**Belkas**

DISCUSSION

In the present study, 12 freshwater protozoans have been isolated from River Nile and tap water samples including four sites of Dakahlyia Governorate, Egypt. These species included *Naegleria fowleri, Acanthamoeba sp., Arcella discoides, Sphaerastrum fockei, Tokophrya infusionum,* and *Eodinium lobatum* From the tap water, and *Actinophrys sol, Euglena pisciformis, p.onyx, p.pleuronectes, Colpoda cucullus* and *Coleps bicuspis* from the River Nile water.

The trophozoite of *Naegleria fowleri* appeared elongated with characteristic contractile and food vacuoles, lobular pseudopodia, and one pair of flagella. The obtained description goes parallel with the findings of **Martinez** who reported similar observations except absence of flagellum extended from pseudopodia **(41).** The presence of one pair of flagella in the trophozoite of this species may be involved in movement against the water current direction. **Ithoi et al.** described *Naegleria* trophozoite from water pools in Malaysia **(42)**. Also, **Leiva et al** studied a survey to evaluate the prevalence of *N. fowleri* in water sources of León, Nicaraguan **(43).**

Acanthamoeba trophozoit is a small filose amoeba elongated to broad, hyaline and filamentous projections. The body has a hyaline anterior zone of protoplasm (hyaline cap) and filose anterior sub-pseudopodia, a thick outer ectoplasm

layer, and numerous food vacuoles. *Acanthamoeba* sp. has a small refractive cyst that has double thick walls nearly circular, an outer ectocyst layer, and an oval or some irregular inner endocyst. Trophozoites and cysts in the present study were recorded from the tap water. *Acanthamoeba sp*. in the present study is similar to *A. sohi* described by **Kyung-il Im, and Ho-Joon Shin** from freshwater fishes in Korea **(44).** *Acanthamoeba rhysodes, A. glebae, A. culbertsoni, A. astronyxis,* and *A. palestinensis* were identified by **Sadaka et al.** from freshwater sources in Alexandria **(45).** Additionally, **Lorenzo-Morales et al.** reported finding *Acanthamoeba* spp. in water sources in the Nile Delta region **(46).** Furthermore, *Acanthamoeba* spp. was identified from the tap water sources in Cairo by **Al-Herrawy et al. (32).** *Acanthamoeba* spp. were found in the tap water and tanks of the Fayoum Governorate by **Sakran et al. (47).** The Egyptian population in Dakahlyia Governorate would likely be seriously threatened by the presence of these pathogenic organisms, which could result in morbidities and fatalities **(48).** Water contamination issues were especially noticeable in rural Egyptian villages, where sewage, industrial discharges, and human activity all had an impact on the water supplies. Higher densities and varieties of pathogens in surface water sources are anticipated in regions with minimal or nonexistent sewage and industrial discharge treatment **(12).** *A. castallanii* cyst which was recorded in the present study is similar to that described by **Stratford and Griffiths** from culture under different conditions **(49).**

The Cyst of *A.hyalina* is spherical with double walls; the outer is wrinkled and the inner smooth. **Ithoi et al.** also described *Acanthamoeba* cysts from water pools in Malaysia that have double thick walls consisting of outer wrinkled (ectocyst) and inner stellated (endocyst) layers **(42).**

Arcella in the present study has a chitinous test with hexagonal markings and a circular aperture inverted centrally like a funnel. The protoplasmic body does not fill the test and is connected with the latter by many ectoplasm strands. The body has two or more nuclei with several contractile vacuoles, brown in color, very small in size measures 14µm in length, and recorded from tap water. The obtained description goes parallel with the findings of **Swindles** who reported similar observations except for the measurements and the appearance where he was recorded as *A.discoides* 78-105µm in length and transparent in color **(50).**

Sphaerastrum fockei cell body of the present study has an eccentric nucleus and a thick, gelatinous mantle covering it and the body is only 25µm in size. On the other hand, *S.focki*, which was recorded by **Kudo** in freshwater as colonies, measures 30µm **(11).**

The body of heliozoan *Actinophrys sol* is spherically symmetrical. The cytoplasm is vacuolated, and endoplasm is granulated with small numerous vacuoles and a large central nucleus with peripheral nucleoli material. The arms protruded from the ectoplasm while the axonemes protruded from the nucleus. The cell body is small and was recorded from River Nile. *A. sol* in the present study was similar to *A.sol* described by **Mikrjukov and Patterson** in a Brackish water pond swan pool from Falmouth, England **(36)** while **Chantangsi** was described *as Actinophrys sol* from fresh water in Thailand **(35).** The cell body of *A.sol* which was recorded in the present study measures 40µm while the recorded *A. sol* measures 70 µm. The cyst of this species has two layers; an outer layer with siliceous plates, an inner organic layer, and granular cytoplasm (**40).**

Actinosphaerium eichhorni of the present study has a scattered nucleus in the periphery of the endoplasm and two large contractile vacuoles. Axial filaments arise from a narrow zone of dense cytoplasm and ectoplasm. The cell body has a very small size of 14µm. The present *A. eichhorni* is similar to that recorded by **Swindles** in fresh water and measures more than 200µm **(50).**

The present study revealed that the body of *Phacus pleuronectes*is flattened and asymmetrical

with a short posterior caudal slightly curved. The pellicle is quite rigid and is composed of longitudinal periplasts that prevent the elastic metabolic movements and dorsal rigid is absent. The chloroplasts are small and two spherical paramylon bodies are present. The body has contractile vacuoles, a red-pigmented stigma, and a long flagellum. The present findings showed quite a similarity to those previously recorded in freshwater by **Kudo and Kosmala et al. (11; 51)**, while **Delgado et al.** described *P. Pleuronectes* from Caura River in Venezuela **(52)** and **Alves-da-Silva et al.** recorded *P. Pleuronectes* in fresh water from Brazil **(53).** Moreover, the body of *P. onyx* is oval with a strong caudal curve at the posterior end. Longitudinal periplast striated, with two paramylon grains situated at the center. *P. onyx* is characterized by a star eye spot in shape. The present findings are quite similar to those previously recorded by **Delgado et al.** in the Caura River from Venezuela **(52)** and **Alves-da-Silva et al.** described in fresh water from Brazil **(53).**

Burt made a comparative study of five species of the genus *Colpoda*. The body of all members of this genus was often reniform with a right border semi-circular and flattened ventral side. The present species *C. cucullus* can be identified from the other four species by the anterior keel with 8-10 indentations and macronucleus with a stellate endosome **(54).**

Coleps bicuspis is barrel-shaped and is divided into anterior main tiers and posterior main tiers. The cytostome lies at the anterior end which is surrounded by slightly longer cilia with oral aperture. Regularly, the body has arranged ectoplasm plates; 16 rows of platelets, two posterior cuspis, and huge cilia. *C.bicuspis* which was recorded in the present study is similar to that reported by **Kudo** in fresh water **(11).** Additionally, **Lynn** described *C.bicuspis*and from fresh water from France **(55).**

CONCLUSION

In the present study, several protozoa were detected in the River Nile and tap water collected from four sites in Dakahlia Governorate, Egypt. Twelve protozoa species were recorded and described in this study using a phase-contrast microscope. These species included *Naegleria fowleri, Acanthamoeba sp., Arcella discoides, Sphaerastrum fockei, Tokophrya infusionum*, and *Eodinium lobatum* From the tap water, and *Actinophrys sol, Euglena pisciformis, p.onyx, p.pleuronectes, Colpoda cucullus* and *Coleps bicuspis* from the River Nile water. Further studies are needed to evaluate the main reasons for the existence of protozoa in these areas of study.

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REFERENCES

1. **WHO (1993) World Health Organization:** The control of schistosomiasis. Second Report of WHO Expert Committee, Technical Report Series No. 830, World Health Organization, Geneva, pp. 1-86.
2. **Smyth, J.D. (1994):** Introduction to Animal Parasitology. 3rd edition. Cambridge University Press, Cambridge, England.
3. **Millichap, J.G. (1995):** Is our water safe to drink? A guide to drinking water hazards and health risks, PNB publishers, Chicago.
4. **Mac Donnell, L.J. (1996):** Water Quality Land Water Law Review, 31(2): 329-348.
5. **WHO (1996) World Health Organization:** Guidelines for drinking- water quality 2nd ed. Geneva.
6. **Ford, T. (1999):** Microbial safety of drinking water: United states and global perspectives. Environ Health Persepect, 107 (11): 191-206.

7. WHO (1998) World Health Organization: The World Health Report. Life. In the 21st century. A vision for all Geneva.

8. Cooper, R.C. (1991): Disease risk among sewage plant operators: a Review Sanitary Engineering and Environmental Health Research Laboratory, Report 91-1. Berkeley: University of California.

9. WHO (2000) World Health Organization: Global water supply and sanitation assessment 2000 report. Geneva.

10. Clinton, W.J. (1997): Executive Order 13045: Protection of children from environmental health and safety risks. Washington, DC.

11.Kudo, R.R. (1977): Parasitology. 3rd edition. Thomas c.c. Publisher. U.S.A. pp. 1-1174.

12. Khallaf EA, Galal M, Elsbbagh S, Nabet NM (2014): A study of the physicochemical and biological characteristic of raw water, fltrated and treated water at a water treatment plant in Shebin Elkom, Menoufya, Egypt. EJABF 18(1):115–125.

13. Khalifa NS, Bendary RE (2016): Composition and biodiversity of zooplankton and macrobenthic populations in El-Rayah El-Menoufya, Egypt. Int J App Env Sci 11(2):683–700

14. Fishar MR, Mahmoud NH, El-Feqy FA, Gaber KMG (2019): Community composition of zooplankton in El-Rayah El-Behery, Egypt. EJABF 23(1):135–150. https://doi.org/10.21608/EJABF.2019. 26815

15. Reda E.S, El-Abbassy S A. and Marzouk A. M. (2015): First record of *Eimeria spp*. (coccidian: Eimeridae) Drinking water from Dakahlia Province, Egypt. Journal of Environment Sciences. 44 (2): 333- 343.

16. Marzouk A. M, Mashaly M. I, Reda E. S. and El-Naggar M. M. (2024): The record of Vannella species (Vannellidae, Discosea, Amoebozoa) from freshwater sources in Dakahlyia Governorate, Egypt. Archives of Microbiology. 206:113

17. Francine and Carbal (2007): The immune response to Naegleria fowleri amebae and pathogenesis of infection. FEMS Immunol Med Microbiol. 51(2):243-259.

18. Rivera F, Lares F, Gallegos E et al (1989): Pathogenic amoebae in natural thermal waters of three resorts of Hidalgo, Mexico. Environ Res 50:289–295.

19. Rivera F, Lares F, Ramirez E et al (1991): Pathogenic *Acanthamoeba* isolated during an atmospheric survey in Mexico City. Rev Infect Dis 13(5): S388–S389. https://doi.org/10.1093/clind/13.supplement_5. s388

20. Michel R, Hauroder-Philippczyk B, Muller K, Weishaar I (1994): *Acanthamoeba* from human nasal mucosa infected with an obligate intracellular parasite. Eura J Protistol 30:104-110.

21. Tawfeek GM, Bishara SAH, Sarhan RM, Taher EE, Khayyal AE (2016): Genotypic, physiological, and biochemical characterization of potentially pathogenic *Acanthamoeba* isolated from the environment in Cairo, Egypt Parasitol Res 115(5):1871-1881.

22. Lass A, Guerrero M, Li X, Karanis G, Ma L, Karanis P (2017): Detection of Acanthamoeba spp. in water samples collected from natural water reservoirs, sewages, and pharmaceutical factory drains using LAMP and PCR in China. Sci Total Environ 15:489-494. https://doi.org/10.1016/j.scitotenv.2017.01.046

23. Carnt NA, Subedi D, Lim AW, Lee R, Mistry P, Badenoch PR (2020): Prevalence and

seasonal variation of *Acanthamoeba* in domestic tap water in greater Sydney, Australia. Clin Exp Optom 103:782–786.

24. Wopereis DB, Bazzo ML, Macedo JP et al (2020): Free-living amoebae and their relationship to air quality in hospital environments: Characterization of *Acanthamoeba* spp. obtained from air-conditioning systems. Parasitol 147:782-790. https://doi.org/10.1017/S0031182020000487

25. Rocha-Azevedo B, Silva-Filho FC (2007): Biological characterization of a clinical and an environmental isolate of *Acanthamoeba polyphaga*: analysis of relevant parameters to decode pathogenicity. Arch Microbiol 188:441–449.

26. Visvesvara GS, Moura H, Schuster FL (2007): Pathogenic and opportunistic free-living amoebae: *Acanthamoeba* spp., *Balamuthia mandrillaris*, *Naegleria fowleri*, and *Sappinia diploidea*. FEMS Microbiol Immunol 50(1):1-26.

27. Visvesvara GS (1980): Free-living pathogenic amoebae. In: Lennette EH, Balows A, Hausler WJ, Truant JP. editors. Manual of clinical microbiology. 3. Washington, D. C: American Society for Microbiology, pp 704–708

28. Khan NA (2006) *Acanthamoeba*: biology and increasing importance in human health. FEMS Microbiol Rev 30:564-595.

29. Radford CF, Minassian DC, Dart JKG (2002): *Acanthamoeba keratitis* in England and Wales: incidence, outcome, and risk factors, Br J Ophthalmol 86(5):536-542. https://doi.org/10.1136%2Fbjo.86.5.536

30. Balczun C, Scheid P (2017): Free-living amoebae as Hosts for and Vectors of Intracellular Microorganisms with Public Health Significance. Viruses, 9 (65): 1-18. https://doi.org/10.3390/v9040065

31. Marciano-Cabral F, Cabral GA (2003): *Acanthamoeba* spp. as agents of disease in humans. Clin Microbiol Rev 16:273-307**.**

32. Al-Herrawy AZ, Khalil MI, El-Sherif SS, Omar FA, Lotfy WM (2017): Surveillance and molecular identification of *Acanthamoeba* and *Naegleria* species in two swimming pools in Alexandria University, Egypt. Iran J Parasitol 12(2):196-205.

33.Jollos, V. (1924): Untersuchungen ueber Variabilität and Verebung bei *Arcellen*. Archiv für Protistenkunde , 49:307.

34. Ehrenberg, Christian Gottfried (1830). "Beiträge zur Kenntniss der Organisation der Infusorien und ihrer geographischen Verbreitung, besonders in Sibirien" [Contributions to the knowledge of the organization of the infusoria and their geographical distribution, especially in Siberia]. Abhandlungen der Königlichen Akademie der Wissenschaften zu Berlin (in German): 1–88.

35. Chantangsi, C. (2002): *Actinophrys* "A well-known sun animalcule". Micscape Magazine.

36. Mikrjukov K.A. and Patterson D. J. (2011): Taxonomy and Phylogeny of Heliozoa. III. *Actinophryids*. Acta Protozoology, 40: 3 – 25.

37. Dragesco J, Dragesco-Kernéis A. (1991). Free-living ciliates from the coastal area of Lake Tanganyika (Africa). *European Journal of Protistology*, **26**(3-4): 216-235 https://doi.org/10.1016/S0932-4739(11)80144-6

38. Obolkina LA. (1995). New species of the family Colepidae (Prostomatida, Ciliophora) from Lake Baikal. *Zoologlchesky Zhurnal*, 74(9): 3-1

39. Aldom, J.E. and Chagla, A.H. (1995): Recovery of *Cryptosporidium* oocysts from water by a membrane filter dissolution

method. Letters in Applied Microbiology, 20:186-187.

40. Patterson, D.J. (1996): Free- Living Freshwater Protozoa. Manson Publishing Ltd London. pp.1-224.

41. Martinez, A.J. (1996): Free-Living Amebas: *Naegleria*, *Acanthamoeba* and *Balamuthia*. In: Baron, S. ed. Medical Microbiology. Section 4. Chapter 81.

42. Ithoi1, I.; Ahmad, A.F.; Mak, J.W., Veeranoot Nissapatorn, V.; Lau, Y. and Mahmud, R. (2011): Morphological characteristics of developmental stages of *Acanthamoeba* and *Neagleria* species before and after staining by various techniques. Southeast Asian Journal of Tropical Medicine and Public Health. 42 (6) 1327-1338.

43. Leiva, B.; Clasdotter, E.; Linder, E. and Winiecka-Krusnell, J.(2008): Free-living *Acanthamoeba* and *Naegleria spp*. *amebae* in water sources of León, Nicaraguan Review of Tropical Biology, 56(2):439-46

44. Kyung-il Im, Ho-Joon Shin (2003): *Acanthamoeba sohi*, n. sp., a pathogenic Korean isolate YM-4 from a freshwater fish. Korean Journal of Parasitology.41 (4):8-88.

45. Sadaka HA, El-Nassery SF, Awadalla HN (1994) Isolation and identification of free-living amoebae from some water sources in Alexandria. J Egypt Soc Parasitol 24(2):247-257.

46. Lorenzo-Morales J, Ortega-Rivas A, Martinez E (2006): *Acanthamoeba* isolates belonging to T1, T2, T3, T4 and T7 genotypes from environmental freshwater samples in the Nile Delta region, Egypt. Acta Trop 100(1-2):63-69.

47. Sakran TF, El-Shahawy GA, Shalaby MA, Sabry HY, Matooq PM, Elmallah AM (2017): Detection rates of waterborne protozoa in water sources from Fayoum Governorate. Parasitol Int 10:30–33.

48. WHO (2011) World Health Organization, guidelines for drinking-water quality. Microbial Aspects. 4th edition, WHO Library Cataloguing-in-Publication Data, Geneva.

49. Stratford M.P. and Griffiths A.J. (1978): Variations in the Properties and Morphology of Cysts of *Acanthamoeba castellanii* . Journal of General Microbiology, 108: 33-37.

50. Swindles, G.T. (2003): Testate amoebae, peat bogs and pastclimates.Queen's University of Belfast Geography homepage. Micscape Magazine.

51. Kosmala, S.; Bereza, M.; Milanowski, R.; Kwiatowski, J. and Zakryś, B. (2007): Morphological and molecular examination of relationships and epitype establishment of *Phacus pleuronectes*, *Phacus orbicularis*, and *Phacus hamelii*. Journal of Phycology, 43: 1071–1082.

52. Delgado, J. G. and Sánchez, L. (2007): Euglenophyta from lower basin of the Caura River, Venezuela. Acta Botánica Venezuelica, 30 (2).

53. Alves-da-Silva, S.M. and Bicudo, C.E.M. (2009): *Cryptoglena*, *Monomorphina* and *Phacus* (Euglenophyceae) of a reservoir in the State of Rio Grande do Sul, southern Brazil. Brazilian Journal of Botany, 32(2).

54. Burt, R. L. (1940): Specific diagnosis of the genus *Colpoda* with special reference to the standardization of experimental material. Transactions American Society for Microbiology, 59: 414.

55. Lynn, D.H. (1985): Cortical ultrastructure of *Coleps bicuspis* Noland, 1925 and the phylogeny of the class Prostomatea (Ciliophora). Biosystems, 18(3-4):387-397.