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Research Article

A Preliminary Faunistics observation on the fresh water ciliates from outside protected area of Simlipal Biosphere Reserve, Devkund, Mayurbhanj, Odisha.

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Abstract:
Ciliates important members in eukaryotes microbial loop and are accepted to be a first-rate tool for assessing the occurrence of the pollution. Most of the ciliate species in river, lakes, and ponds be either primarily or solely bacteriovores feeding on a good form of microorganism. In India 106 species of ciliates belonging to 58 Genera and 36 families are reported. A Numerous scientific studies are created on the impact of various microorganism diets on the speed of procreation. Much abundant has been written on the ecological role that ciliates fulfill within the earth. But Most of the limnological studies in Odisha have focused on a few taxa of large, permanent water bodies, and pond ecosystems, and are neglected. We present here a faunal inventory, with representative photographs, for DeoRiver and small ponds near Devkund area outside Simlipal Biosphere Reserve, reporting over 23 species of strictly aquatic fresh water ciliate belonging to 18 Genus, 14 family, 10 Order and 6 class. This study focus on the critical information to manage and conserve the functional property of the fresh water ecosystem for the long term, particular in the area that are vulnerable to human activities.

Keywords: Ciliates, habitat conservation, River ecosystem.

Introduction

The ciliates occur in almost every kind of aquatic environments as these are important to ecosystem and it plays a critical role in function of the ecosystem. It is florish in abundant nutrients, suitable moisture, and appropriate microhabitats are available[9, 25].About 4,500 free-living ciliate morphospecies have been described, and 83–89% of the ciliate diversity is still undescribed[15, 26]. The useful forms of the ciliate constitute important links in the food web and play a critical role in metabolism of aquatic ecosystem.These occupy some significant that they are dominant, within a community the position of protozoa among the consumer. Their important is to use of bacteria as a source of food. Ciliates are very dynamic in structure, their number of cells change rapidly by cell division, excystment and encystment. The ciliateare employed very well in biological and medical research, act as indicators of pollution and

petroleum deposits and they are above all good natural enemies of harmful bacteria, aiding in soil fertility. Ciliates with their large size, short life cycle, high reproduction rate and lack of cell wall help in detecting the water, soil quality, and the environmental impacts in a short timescale [1, 18, 27, 28].

The Indian Subcontinent is rich in biodiversity as it has diverse ecosystems. Despite this richness, data concerning freshwater ciliate diversity from India is rather scarce; few reports based on morphological identifications are available [2, 6, 5, 7, 19, 10, 27]. And a few novel genera and species of ciliates have been reported from Odisha region [10]. Knowledge of biodiversity of ciliates is becoming increasingly important for managing ecosystems and develop better model as their significant role has become better known [3, 5, 12, 24].

As freeliving ciliates play an important role in the aquatic ecosystem and form an important component of the environment monitoring surveillance. These ciliophorans show their significance as biological indicators and occupy an important position in the aquatic food chain. In Odisha,in all 31 species of ciliates, belonging to 3 classes, 12 orders, 36 families and 38 genera have been recorded by several investigators since 1840s. [11, 10]and most of the samples were collected from the south part of Odisha (Ganjam, Puri). But no serious survey was conducted for the ciliates of Similipal Biosphere Reserve area or outside protected area and hence this survey was conducted near Devkund tourism area outside protected area of Simlipal Biosphere Reserve, Mayurbhanj Odisha. The taxonomic compositions of fresh water ciliates diversity through the systematic biodiversity survey will emphasis these microbial eukaryotic communities very much which influence the health of the fresh water ecosystem.

Methods:

For the study of free living ciliates the water samples were collected from different stations ofDeoRiver near Devkund area.Water samples were collected in wide mouth, sterilized glass bottles. Samples were brought to the laboratory, filtered immediately using Nytex nets to remove copepods, crustaceans, debris, and other unwanted materials. Water samples were maintained at room temperature for 3–4 days. Mostly thesamples were collected during morning and evening.

Culture methods –

For cultivation various media are used such as

	Culture Method	Species
1	Hay infusion	*Paramecia, Coleps, Chilodonella, Amphileptus, Stylonechia and Vorticella.*
2	Wheat infusion	*Vorticella, Stylonechia and Coleps*
3	Rice infusion	*Vorticella.*

In-vitro Culture:

Water samples were collected from the period June 2022 to April 2023. Water sample is obtained from different water bodies contains a mixer of organism, by adding any type of culture media (Wheat or rice infusion) can usually rise the number of certain ciliate species due to increaseof the bacterial population in water sample. However the rough sample containing several ciliates and unknown bacterial and algal floras can be obtained inseveral weeks. Selection of a particular ciliate species and culturing it in the isolation of organisms other than its prey may obtain a greater degree of control over a culture. The choice of culture medium depends largely upon what the ciliate feeds. Many feed upon bacteria and that is why many of the media commonly used are designed to encourage the growth of bacterial populations. Different culture media and methods published by [22, 23, 16].

Identification of the freshwater ciliates isolated from the sample was done in-vivo under the Stereoscopic Microscope. Collected water sample were placed in Petri dishes and observed under a stereo zoom microscope in order to detect organisms belonging to the genus and

divide them according to their main morphotype. The resulting populations were then maintained at 18 - 20° C in their original medium, periodically enriched with rice grains, modified Cerophyl medium [14]inoculated with Roultella planticola (Gamma proteo bacteria). The monoclonal cultures were acquired by isolating single cells from the original populations. These cells were briefly washed for several times in sterile distilled water. Clonal cultures of Blepharisma, species was maintained in the laboratory at 22-24°C in a medium made of hay infusion, Cerophyl, Na_2HPO_4, and Stigma sterol and distilled water inoculated with *Roultellaplanticola* was added to the medium to promote the growth of bacteria which served as the primary food source for the ciliates. The green algae *Dunaliellatertiolecta* was employed as food for ciliates. Morphological study was done for ciliate cells which were picked from monoclonal culture were harvested from the culture medium and observed Culture were examined under low power and then in to high power microscope taking care to focus at all levels in the culture watching for movements of any kind. Dry silver impregnation was used to study infra-ciliature of the ciliates. Methyl cellulose has been found too many advantages, as it arrest the movement, ciliates can be identified by their appearance.

Morphological studies

Cells were observed under high-power oil immersion objective with bright field, phase contrast [3]and Feulgenstaining [13, 17]. Measurements were done using eyepiece micrometer. Classification, identification, nomenclature, and terminology of ciliate species were done according to [4, 8, 15, 20, 21].

Results and discussion

Ciliate diversity

A total of 23 species belonging to 6 classes, 10 orders, 14 families and 18 genera were identified from freshwater habitats in the Devkund region (Figs 1–2, Table 1).

1. *Aponotohymenaaustralis*(Foissner) [14]

Body Size: in vivo 110–140 x 30–45µm, length to width ratio 4:1;

Diagnosis: body flexible, colorless, prolate ellipsoidal, dorsoventrally flattened, subpelliculargranules located mainly along the rows of cirri and dorsal kineties, arranged in small groups imparting yellowish orange colouration to the cell;

Nuclei: two macronuclei, six to eight micronuclei;

Cirri :18frontal-ventral-transverse cirri which include 3 frontal, 1buccal, 4frontoventral, 3postoral ventral, 2pretransverse, 5 transverse cirri (arranged in a linear row). Posteriorly with an average of 40 and 38 cirri, respectively; 6 rows of dorsal kineties.

2. *Gastrostyla sp*.

Body Size: in vivo about 95–100 x 30–32 µm, length towidth ratio 3:1; flexible,

Diagnosis:dorsoventrally flattened;

Nuclei: 2 ovoid macronuclei, 2 to 6 spherical micronuclei

Cirri: 24–30 frontoventral transverse cirri, number 7 frontoventral and postoral ventral cirri arranged in lightly oblique frontoventral row, 2pretransverse, 5 transverse cirri, 6 rows of dorsal kineties,3 to 4 caudal cirri at the posterior ends

3. ***Oxytrichagranulifera*** **(Foissner) [15]**

Body Size: in vivo 68–109 x 22–25 µm,

Diagnosis: Body broadly oval, both left and right sides straight and almost parallel, both anterior and posterior ends rounded, dimensions 50-54 µm X 25-30 µm;

Nuclei: two macronuclei, two micronuclei;

Cirri: frontal cirri 9, ventrals 5 and anals 5, peristome extending to almost middle of the body, macrounclei long, rod-shaped and two in number, contractile vacuole single and located at one side of the peristome.

4.***Paraurostylacoronata***

Body Size: in vivo 180–200 x 60–70 µm, length to width ratio 3:1;

Diagnosis: body flexible, diffused green coloured appearance, pink coloured anterior and posterior extremities, dorsoventrally flattened

Nuclei: two macronuclei, four micronuclei;

Cirri: 7 frontal cirri, one buccal cirrus, six to eight (usually seven) longitudinal rows

5. ***Tetmemenapustulata***

Body Size: Size in vivo 100–120 x 50–60 µm,

Diagnosis: Body rigid with both the end rounded

Nuclei: two macronuclei, two micronuclei;

Cirri: 18 frontal-ventral-transverse cirri which include 3 frontal, 1buccal, 4 frontoventral (arranged in oblique hook-shaped row), 3 postoralventral placed equidistant to each other, 2pretransverse, 5 transverse; 3 caudal cirri not equidistant.

6. *Tetmemenasaprai* [17]

Body Size: in vivo 130–150 x 50–60 µm,

Diagnosis: Body rigid with no cortical granules, body lanceolate anteriorly and rounded posteriorly.

Nuclei: two macronuclei, two to four micronuclei;

Cirri: 18 frontal-ventral-transverse cirri which include 3 frontal, 1buccal, 4frontoventral, 3postoral ventral, 2pretransverse, 5 transverse; one left and one right row of marginal cirri with 22 and 28 cirri, respectively; 3 caudal cirri not equidistant

7.*Tetmemena sp.*

Body Size: in vivo 90–100 x 40–50 µm,

Diagnosis: Body rigid, dorso-ventrally flattened, broad anterior end and a rounded tapering posterior end.

Nuclei: two macronuclei, two micronuclei;

Cirri: 18 frontal-ventral-transverse cirri which include 3 frontal, 1buccal, 4frontoventral, posterior frontoventral cirri forms a small arc, 3 postoral ventral, 2 pretransverse, 5 transverse cirri,3 caudal cirri equidistant to each other.

***8. Urosomoida sp.* [15]**

Body Size: in vivo 50–65 x 17–27 µm

Diagnosis: Body flexible, dorsoventrally flattened, cell broadest at the mid body, tapering posteriorly.

Nuclei: two macronuclei, two micronuclei;

Cirri: 16 frontal-ventral-transverse cirri with 3 frontal, 1 buccal, 4frontoventral, 3 post oral ventral, 2pretransverse, 3 transverse cirri, 3 caudal cirri, one each at the posterior ends.

9.*Gonostomum sp.*

Body Size: in vivo 60–65 x 20–30 µm.

Diagnosis: Body flexible, ellipsoid, both ends more or less narrowly rounded.

Nuclei: two macronuclei, four micronuclei;

Cirri: 3large frontal cirri, 1buccal cirrus, 1 short fronto-terminal row with 3-8 cirri, 3 rows of frontoventral cirri with first row having 2-4 cirri, second row having 2-5 cirri and third row having 6–10 cirri, 2-6 transverse cirri, 3 caudal cirri, one each at the posterior ends.

10. *Anteholostichamonilata*

Body Size: in vivo about 140–170 x 35–39 µm.

Diagnosis: Body flexible, slender to ellipsoidal in shape, anterior end more or less narrowed, posterior broadly rounded.

Nuclei: 13–18 macronuclei, 3-7 micronuclei;

Cirri: 3-6 frontal cirri, 1buccal, 2-4frontoterminal cirri, 18–27 cirral pairs in mid ventral row of cirri, 6-8 transverse cirri; caudal cirri absent;

11. *Pseudourostylacristata*

Body Size: Size in vivo about 200–230 x 70–100 µm.

Diagnosis: Body flexible, slender, ellipsoidal with both ends broadly rounded, extrusomes present.

Nuclei: 15–83 macronuclear nodes and 2-9 micronuclei.

Cirri: Singebuccal cirrus, 18–22 frontal cirri, 2-3frontoterminal cirri, midventralcomplex having 20–27 pairs of cirri, 7-9 transverse cirri, 5 rows of left and 4 rows of right marginal cirri; 8–10 rows of dorsal, caudal cirri absent.

12. *Urostylagrandis*

Body Size: in vivo about 240–260 x 120–140 µm.

Diagnosis: Body flexible, elongate body with both ends rounded.
Nuclei: numerous macronuclei with 2-4 micronuclei.
Cirri: 8 frontal cirri, 7 buccal cirri, 3 rows of parabuccal cirri, midventral complex having 17–18 pairs of cirri, 7 transverse cirri, 5 rows of right marginal cirri with an average of 28 cirri in the Innermost row and 38 cirri on the outermost row, caudal cirri absent.

13. *Aspidisca sp*.
Body Size: in vivo about 40–60 x 20–30 µm.
Diagnosis: small sized, ovoid, inflexible body with four dorsal ridges.
Nuclei: one macronucleus horseshoe-shaped and one or two micronuclei;
Cirri: 7 frontal cirri, 5 transverse cirri.

14. *Euplotesaediculatus*
Body Size: in vivo about 107–119 x 72–82 µm.
Diagnosis: body rectangular, rigid, dorso-ventrally flattened with inconspicuous dorsal grooves, pellicle colourless with refractive granule.
Nuclei: macronucleus C-shaped with an arched back, 1 spherical micronucleus.
Cirri: 9frontoventral cirri, 5 transverse cirri, 2 left marginal cirri, 2 caudal cirri, 8 dorsolateral.

15. ***Euplotes sp.***
Body Size:in vivo about 49–52 x 40–46 µm.
Diagnosis: body oval, pellicle colourless.
Nuclei: macronucleus C-shaped, one spherical micronucleus.
Cirri: 10 frontoventral, 5 transverse, 2 left marginal, 2 caudal cirri.

16. *Blepharismasinosum*
Body Size: in vivo 100–120 x 30–40 µm.
Diagnosis: body spindle-shaped with tapered anterior end, pellicle flexible with numerous cortical granules arranged in five to seven longitudinal rows.
Nuclei:moniliform macronucleus with 4-7 nodules connected by nuclear bridges, macronuclear nodule shape varies from spherical to ellipsoidal with 10–20 spherical micronuclei dispersed throughout the cytoplasm.
Cirri: 20–30 cirri.

17. *Chilodonella sp.*
Body Size: in vivo 45–55 x 25–35 µm.
Diagnosis: body dorsoventrally flattened, oval in shape, distinct pre-oral beak at the anterior end cytoplasm transparent; the cell has a flat ventral surface and an arched dorsal surface.
Nuclei: single spherical to ellipsoidal macronucleus located near the posterior end with two or three micronuclei.
Cirri: ventralsurface is ciliated with many numbers of somatic kineties placed longitudinally, the dorsal surfaceis un-ciliated.

18. *Paramecium multimicronucleatum*
Body Size: in vivo 200–220 x 55–65 µm.
Diagnosis: body slipper-shaped with cilia covering the entire body, trichocyst present.

19. *Cyclidium sp.*
Body Size: in vivo 20–30 x 13–15 µm.
Diagnosis: body elongated-ovoid, ventral side almost straight with dorsal evenly convex, apical end free from cilia, anterior end large and flat, posterior end broadly rounded; cytoplasm colorless.
Nuclei:2 macronuclear nodules, spherical in shapeand adjacent to each other, 1 micronucleus;
Cirri: 11–12 somatic kineties arranged longitudinally; contains single caudal cilium.

20. *Vorticella sp.*
Body Size: in vivo 55–80 x 40–50 µm.
Diagnosis: inverted bell shaped body with a long coiled stalk of length 100 µm and diameter of 3–4 µm; central part of cell is filled with refractile reserve granules.
Nuclei:single long and worm-like macronucleus with a single spherical micronucleus.
Cirri: ring of cilia on the oral end.

21. *Coleps sp.*
Body Size: in vivo 110–120 x 60–70 µm.
Diagnosis: barrel shaped body, typically covered with spikes, anterior end broad and posterior end moderately rounded.
Nuclei:one macronucleus, one micronucleus.
Cirri: longitudinally placed 20–25 cirri.

22. *Colpoda magna*

Body Size: in vivo 110–120 x 125 µm.

Diagnosis: body having shape of bean or kidney, the anterior body broad and blunt and the posterior half is tapering.

Nuclei:one macronucleus, one micronuclei.

23. *Colpoda sp.*

Body Size: in vivo 40–50 x 30–40 µm.

Diagnosis: body ellipsoidal to broadly ellipsoidal with distinct concavity at the oral opening.

Nuclei: one single ellipsoidal macronucleus with a single micronucleus attached.

Cirri: 15 ciliary rows arranged longitudinally.

Discussion

This study provides the first report on a community of free-living ciliated protists from the aquatic ecosystem of Devkund outside Simlipal Biosphere Reserve area. This subterranean groundwater ecosystem represents a hotspot of biodiversity that still needs to be fully explored with particular reference to microbial eukaryotes such as protist ciliates. A total of 23 Species of ciliates were identified during the Study period. A majority of the species reported form this study belong to Oxytichidae(Table 1). It is concluded that extensive research should be made to assess the whole diversity of less studied microbes.

Table 1. List of 23 free living ciliate species isolated from freshwater habitats in Devkund region (Classification according to Adl*et al.,* 2019 [4]).

Species	Family	Order	Class
Aponotohymenaaustralis	Oxytrichidae	Hypotrichia	Spirotrichea
Gastrotyla sp.			
Oxytrichagranulifera			
Paraurostylacoronate			
Tetmemenapustulata			
Tetmemenasaprai			
Tetmenena sp.			
Urosomoida sp.			
Gonostomum sp.	Gonostomatidae		
Anteholostichamonilata	Holostichidae		
Pseudourostylacristata	Pseudourostylidae	Urostylida	
Urostylagrandis	Urostylidae		
Aspidisca sp.	Aspidiscidae	Euplotida	
Euplotesaediculatus	Euplotidae		
Euplotes sp.			
Blepharismasinosum	Blepharismidae	Heterotrichida	Heterotrichea
Chilodonella sp.	Chilodonellidae	Chlamydodontida	Phyllopharyngea
Parameciu multimicronucleatum	Parameciidae	Peniculida	Oligohymenophorea
Cyclidium sp.	Cyclidiidae	Pleuronematida	
Vorticella sp.	Vorticellidae	Sessilidae	
Coleps sp.	Colepidae	Prorodontida	Prostomatea
Colpoda magna	Colpodidae	Colpodida	Colpodea
Colpoda sp.			
18 genera 23species	14 families	10 order	6 classes

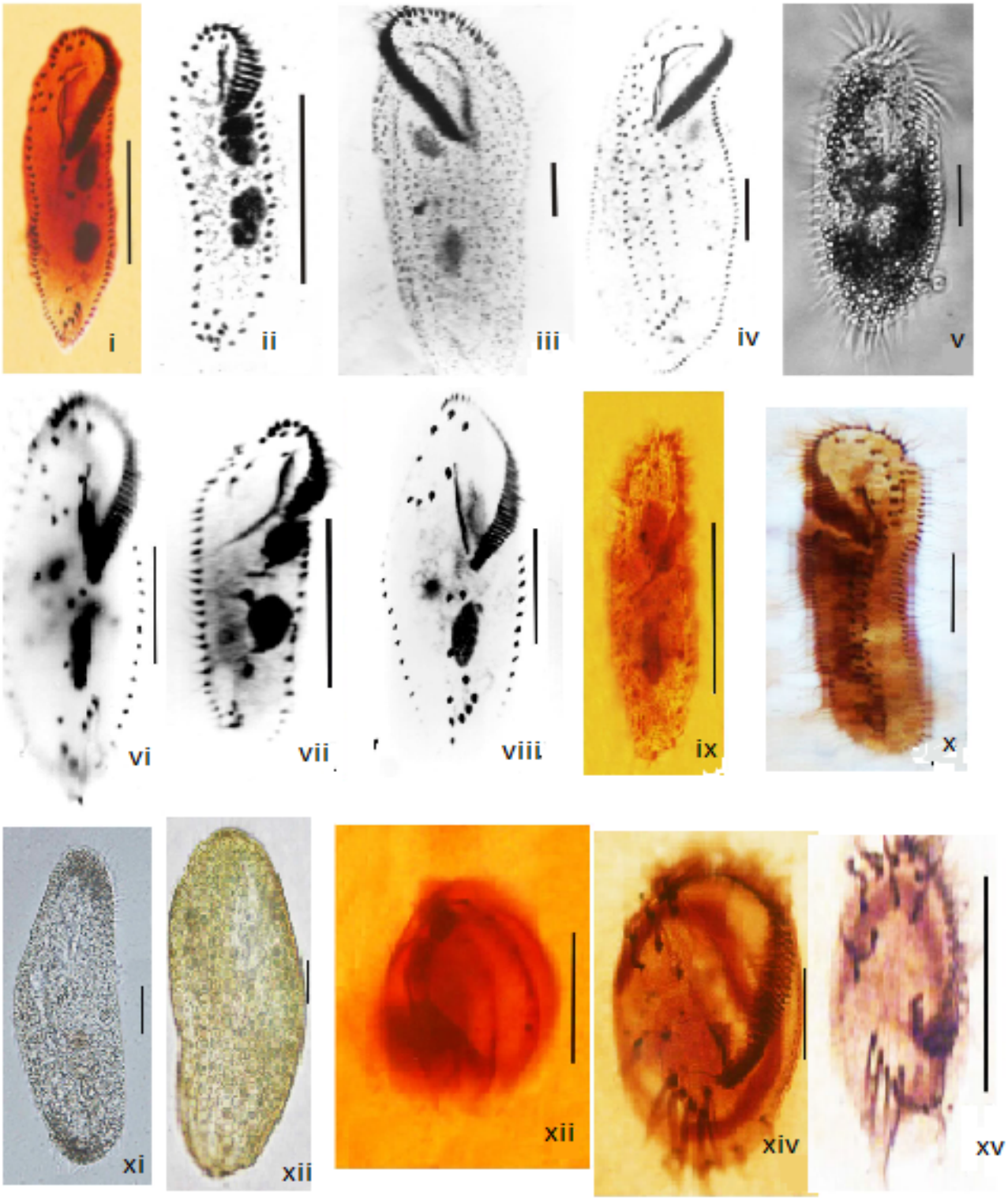

Figure1 i)Aponotohymena austral ii) Gastrotylasp iii) Oxytrichagranulifera iv)Paraurostyla coronate v)Tetmemenapustulata vi)Tetmemenasaprai vii)Tetmenena sp. viii)Urosomoida sp. ix)Gonostomum sp. x)Anteholostichamonilata xi)Pseudourostylacristata xii)Urostylagrandis xiii)Aspidisca sp. xiv)Euplotesaediculatusxv) Euplotes sp.

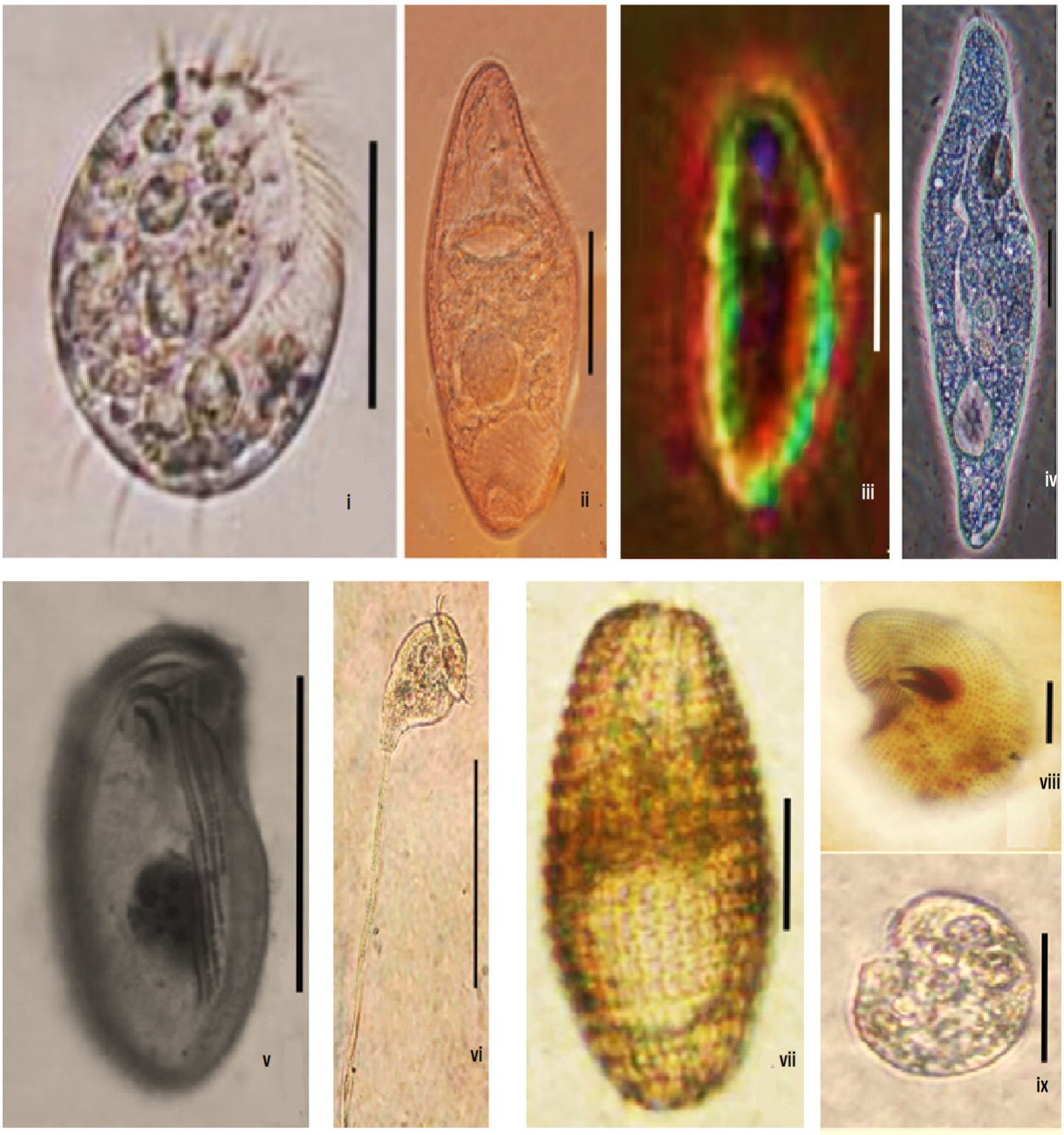

Figure 2 i) Blepharismasinosum ii)Chilodonellasp .iii)Paramecium multimicronucleatum iv)Cyclidium sp. v) Vorticella sp. vi)Coleps sp. vii)Colpoda magna viii& ix)Colpoda sp.

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