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Two freshwater flagellates from the Mahananda Wildlife Sanctuary, West Bengal

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Abstract

During the study of the diversity of free living protozoan ciliates from the Mahananda Wildlife Sanctuary, West Bengal, two free-living flagellates were identified, namely *Rhabdomonas costata* (Korshikov) Pringsheim, 1951 and *Entosiphon sulcatum* Skvortzov and Noda 1969. Brief description, based on live observation and silver stained preparations has been provided for both species. The attempt to stain the specimens using the silver staining technique resulted in impregnation of the nuclear apparatus and flagella rendering easy morphological measurements and studying variations.

Keywords: Flagellates, Morphology, Protargol Staining, Protected Areas

Introduction

The diversity of free living flagellates has not been studied intensively from the country, though some reports exists (Ade, 2012; Hosmani, 1976; Hosmani and Bharati, 1983; Kamat and Frietas, 1976; Patel and Waghodekar, 1981; Prasad and Chaudhary, 1986; Reddy and chaturvedi, 2017; Satpati *et al*., 2013; Waghodekar and Patel, 1991). It is also evident that very few report exist on the diversity from the eastern India (Ratha *et al*., 2006; Roy and Pal, 2016; Shaji and Patel, 1991). Probable reason could be the lack of experts in the field and the difficulty in identification because of their small size. Further, reinvestigation is restricted by the lack of submission of permanent slides in the Indian repositories. Most of the articles on the diversity of flagellates show the identification based on live observations and mediocre quality photomicrographs which increases the chances of synonym. It is proposed that the diversity of flagellates from India should also be studied based on the integrated approach, i.e., detailed morphology based on live and stained preparations. Further, it may also be supplemented with molecular analyses.

The present investigation reports two free-living flagellates identified from the water samples collected from the Mahananda Wildlife Sanctuary, West Bengal. The detail observation based on live specimens has been presented along with photomicrographs of the silver stained specimens.

Material and Methods

Sampling and Sample Processing: Water (about 500 ml) was collected from the Mahananda Wildlife Sanctuary, West Bengal. For details on sampling sites refer descriptions of individual species. Water samples were immediately processed at room temperature 20°C by adding some squashed wheat kernels to promote bacterial growth after bringing them to laboratory. Live observations and photomicrography were performed using stereo zoom microscope (SZ2-ILST, Olympus) and bright field microscope (CX 43, Olympus). The protargol staining method described by Kamra and Sapra (1990) was used with some modification to reveal details of the flagella and nucleus. Counts and measurements of stained specimens were conducted at a magnification of 1000×.

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In vivo measurements were performed at magnifications of 40–1000×. Classification according to Cavalier-Smith (2016).

Results and Discussion

Two species of the free-living flagellates, i.e., *Rhabdomonas costata* (Korshikov) Pringsheim, 1951 and *Entosiphon sulcatum* Skvortzov and Noda 1969 identified from the water samples are new record to the Sanctuary. The water sample also showed the presence of six ciliate species (*Chilodonella* sp., *Coleps* sp., *Dileptus* sp., *Aspidisca* sp., *Paramecium* sp., and *Cyrtolophosis* sp.). The flagellate species belonged to 2 classes, 2 orders, and 2 families. The report provide details from the live observations and also silver staining of the specimens, the latter resulting in preparation of permanent slides which is being submitted at the National Zoological Collections of the Zoological Survey of India, Kolkata, India.

Brief descriptions on the flagellate species are presented

Phylum EUGLENOZOA Cavalier-Smith, 1981
Class PERANEMEA Cavalier-Smith, 1993
Order NATOMONADIDA Cavalier-Smith 2016
Family ASTASIIDAE *Saville* Kent, 1884
Genus ***Rhabdomonas*** Fresenius (Starmach 1983)

1. ***Rhabdomonas costata*** (Korshikov) Pringsheim, 1942 (Figure 1A-E)

Diagnosis of the Indian population (Data based on 7 specimens): Size about 20 × 10 µm in stained preparations; shape elliptical to narrowly elliptical, longitudinally extended, abdominal side convex, dorsal slightly curved. Nucleus slightly posterior of body midline, about 6-11 µm from anterior body end with diameter about 4-5 µm, nucleolus globular visible in stained preparation with diameter about 3-4 µm. Paramylon bodies of 2-5 µm length present throughout the cell. A row (sometime two rows) of ejectosomes present posterior to the base of flagellum. Two flagella emerging out from a prominent small groove at the anterior body end, one flagellum slightly shorter than the other.

Material deposited: A slide including protargol-impregnated specimens have been deposited at the National Zoological Collections of the Zoological Survey of India, Kolkata, India with the following accession numbers Pt. 4506 (specimens marked with black ink circles on the slide).

Occurrence and ecology: The species *Rhabdomonas costata* is rather common in both freshwater ecosystems. It has a rather cosmopolitan distribution with reports from Europe, Poland, Asia, and North America. The present study reports its presence from a small pond near Helay Khola watch tower, Lower Ghoramara Block, Sevok Beat, North Range, Mahananda Wildlife Sanctuary, West Bengal (26°50'671"N 88°26'293"E). When the sample was collected the specimens were in high abundance and possibly responsible for the slightly greenish colour of the water due to its blooming.

Infraphylum ENTOSIPHONA Cavalier-Smith 2016
Class ENTOSIPHONEA Cavalier-Smith 2016
Order ENTOSIPHONIDA Cavalier-Smith 2016
Family ENTOSIPHONIDAE Cavalier-Smith 2016
Genus ***Entosiphon*** Ritter von Stein, 1878.

2. ***Entosiphon sulcatum*** (Dujardin, 1841) Stein, 1878 (Figure 2A-F)

Diagnosis of the Indian population (Data based on 5 specimens): Size about 30 × 15 µm in stained preparations; shape ovoid, flattened dorsoventrally, slightly narrowed at the posterior body end, body with deep grooves. Nucleus near body midline, about 10-13 µm from anterior body end with diameter about 5-7 µm, nucleolus visible in stained preparation with irregular shape and length about 4-6 µm. Crystals and granules were observed on the surface in live observations. Cytoplasm with food vacuoles and lipid droplets. A vacuole present in the anterior 2/3 of the cell. Feeding organelles conspicuous extending from anterior to nearly posterior body end, conical in shape with capability of protruding and withdrawing. Two flagella emerging out from a prominent small groove at the anterior body end, one flagellum slightly shorter than the other. Some specimens observed with very short flagella.

Material deposited: A slide including protargol-impregnated specimens have been deposited at the National Zoological Collections of the Zoological Survey of India, Kolkata, India with the following accession numbers Pt. 4506/1 (specimens marked with black ink circles on the slide).

Occurrence and ecology: The species *Entosiphon sulcatum* is rather common in freshwater ecosystems and has a cosmopolitan distribution. The present study reports its presence from water sample collected from a small pond near Helay Khola watch tower, Lower Ghoramara Block, Sevok Beat, North Range, Mahananda Wildlife Sanctuary, West Bengal (26°50'671"N 88°26'293"E). It feed on bacteria and detritus.

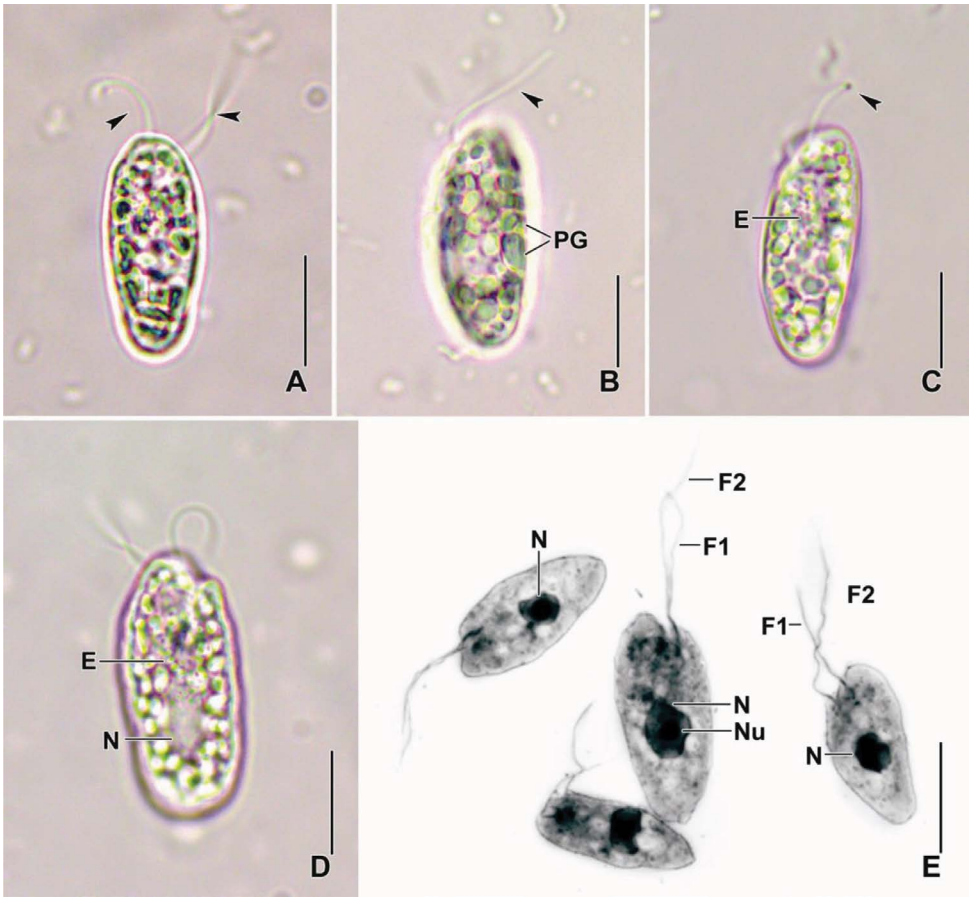

Figure 1. Photomicrographs of *Rhabdomonas costata* from live (A-D) and silver stained preparations (E). **A-D.** Specimens showing the body shape, paramylon bodies (B), nucleus position (D) and ejectosomes (C, D), Arrows point to the flagellum. **E.** Four specimens showing the body size variation, flagella length, and nucleus position. The nucleolus is visible in the stained preparations. E, ejectosomes; F1,2, flagellum 1, 2; N, nucleus; Nu, nucleolus; PG, paramylon bodies. Scale bars, 10 µm.

Table 1. Morphometric data on *Rhabdomonas costata* (upper line) and *Entosiphon sulcatum* (lower line)

Characteristic[a]	Mean	M	SD	SE	CV	Min	Max	n
Body, length	18.9	18.0	2.2	0.8	11.6	16.0	22.0	7
	27.6	27.0	1.9	0.9	7.1	25.0	30.0	5
Body, width	9.3	10.0	1.6	0.6	17.3	7.0	11.0	7
	14.6	15.0	1.7	0.7	11.5	13.0	17.0	5
Anterior body end to Nucleus, distance	8.6	9.0	1.7	0.6	20.0	6.0	11.0	7
	11.2	11.0	1.3	0.6	11.6	10.0	13.0	5
Nucleus Diameter	4.4	4.0	0.5	0.2	12.1	4.0	5.0	7
	5.8	5.5	1.0	0.5	16.7	5.0	7.0	4
Flagella 1, length	17.1	15.0	3.8	1.4	22.2	12.0	22.0	7
	14.3	14.0	7.8	3.9	55.1	7.0	22.0	4
Flagella 2, length	12.9	13.0	4.3	1.6	33.1	8.0	18.0	7
	14.0	14.0	0.8	0.4	5.8	13.0	15.0	4

[a]Data based on mounted, protargol-impregnated, and randomly selected specimens. Measurements in µm. CV – coefficient of variation in %, M – median, Max – maximum, Mean – arithmetic mean, Min – minimum, n – number of individuals investigated, SD – standard deviation, SE – standard error of arithmetic mean.

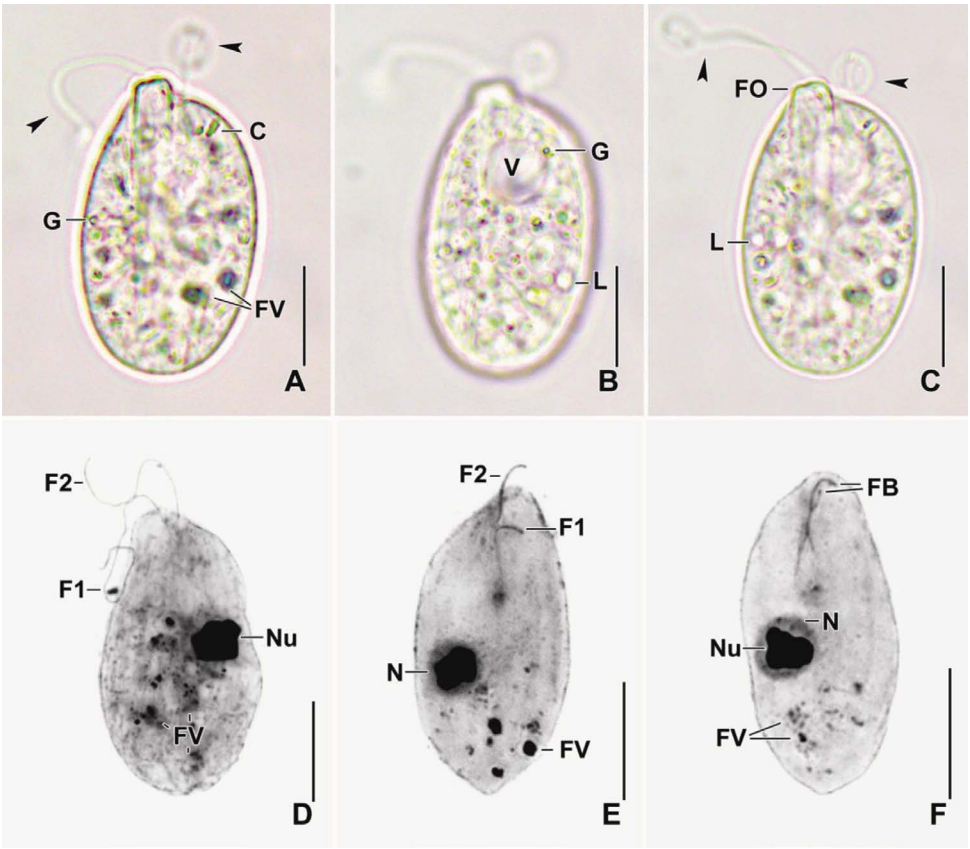

Figure 2. Photomicrographs of *Entosiphon sulcatum* from live (A-C) and silver stained preparations (D-F). **A-C.** Slightly pressed specimen due to cover slip pressure, showing the body shape, cytoplasmic crystals, lipid droplets, granules on the surface, food vacuoles and vacuole. Arrows point to the flagella. Note the protruding feeding organelles **D-F.** Specimens showing the body size variation, differences in the flagella length, and nucleus position. The irregular nucleolus and base of the flagellum is visible in the stained preparations. C, crystals; F1,2, flagellum 1, 2; FB, flagella base; FO, feeding organelle; FV, food vacuoles; G, granules; L, lipid droplet; N, nucleus; Nu, nucleolus; V, vacuole. Scale bars, 10 µm.

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