

Ten new records of Protozoan Ciliates (Protozoa : Ciliophora) from India

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Abstract

In an attempt to catalogue the diversity of free living protozoan ciliates from the Flamingo Bird Sanctuary, Mumbai, and surrounding areas, 10 species were identified as new record to Indian fauna. Brief description, based on live observation and protargol stained preparations has been provided for the species i.e., *Anteholosticha intermedia* (Bergh, 1889) Berger, 2006, *Rigidosticha italiensis* Bharti *et al.* 2016, *Monomicrocaryon balladyna* (Song and Wilbert, 1989) Foissner, 2016, *Urosoma karinae* (Ehrenberg, 1833) Berger, 1999, *Diophrys oligothrix* Borror, 1965, *Cyrtolophosis muscicola* Stokes, 1885, *Leptopharynx costatus* Mermod, 1914, *Rimaleptus mucronatus* (Penard, 1922) Vďačný, *et al.*, 2011, *Dileptus beersi* Jones, 1956 and *Pseudomonilicaryon falciforme* (Kahl, 1931) Vďačný and Foissner, 2012.

Keywords: Flamingo Bird Sanctuary, Morphology, Mumbai, Protargol Staining

Introduction

About 500 free living protozoan ciliates have been reported thus far from India. Most of these species has been described based on live observation. A detailed monograph on protozoan ciliates was published by Bhatia (1936) though the observations were mainly based on the live forms, he described not only the new records but also new species, most of which are valid till date. Thereafter, periodic publications were made e.g., Ganapati and Rao (1958), Mahajan and Nair (1965), Rao and Ganapati (1968), Das *et al.* (1993, 2000, 2003), Kalavati and Raman (2008). The present standard in ciliate taxonomy employs an integrated approach, i.e., live observations and silver stained preparation for visualizing surface structure as followed in Gupta *et al.* (2003), Kalavati and Raman (2008), Kamra *et al.* (2008), Kamra and Kumar (2010), Kumar *et al.* (2010, 2015), Singh and Kamra (2013, 2014), Bharti *et al.* (2018). The data obtained using modern techniques of staining is often supplemented by molecular analyses using ribosomal and mitochondrial genes thus reducing the chances of synonymy and further helps in identification of cryptic species (Singh and Kamra 2013, 2014; Kumar *et al.* 2015; Bharti *et al.* 2018).

The present report is an example where an attempt to catalogue the diversity of free living protozoans using the standard staining techniques resulted in identification of ten ciliates new to Indian fauna. It is expected that the actual diversity of free living ciliates from India could reach over 2000 species if the modern techniques are employed, i.e., silver staining supplemented with molecular analyses.

Material and Methods

Sampling and sample processing: Water (about 200 ml) and soil samples (about 150 gm) were collected from the Flamingo Bird Sanctuary, Mumbai and surrounding areas. For details on sampling sites refer descriptions of individual species. Water samples were immediately processed after bring them to Laboratory whereas soil samples were air dried for two weeks before rewetting employing the Non-flooded Petri dish culture (Foissner *et al.*, 2002). Raw cultures were established at room temperature 20°C by adding some squashed wheat kernels or providing green algae as food source. Live observations and photomicrography were performed using stereo zoom microscope (SZ2-ILST, Olympus) and bright field

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microscope (CX 43, Olympus). The protargol staining method described by Kamra and Sapra (1990) was used with some modification to reveal the infraciliature. Counts and measurements of stained specimens were conducted at a magnification of 1000×. In vivo measurements were performed at magnifications of 40–1000×.

Results and Discussion

Over 30 ciliates were recorded from the soil and water samples out of which ten species were identified as new record to Indian fauna. The species belonged to 4 classes, 5 orders, and 8 families. The report indicates that the unrecorded diversity of ciliates from India would be much more than previous report of about 500 species. Since a huge gap exists for the study of ciliates both in terms of methodology used and the unexplored areas (fresh water, soil and marine forms) from the country.

Brief reports on ten species new record to Indian fauna are presented:

Phylum CILIOPHORA Doflein, 1901

Class SPIROTRICHEA Bütschli, 1889

Order UROSTYLIDA Jankowski, 1979

Family UROSTYLIDAE Bütschli, 1889

Genus *Anteholosticha* Berger, 2003

1. *Anteholosticha intermedia* (Bergh, 1889) Berger, 2006 (Figure 1A)

Diagnosis of the Indian population (Data based on 11 specimens): Size about 110 × 30 µm in protargol preparations; shape elliptical with body ends rounded, dorsoventrally flattened. Nuclear apparatus composed of about 33 macronuclear nodules mostly arranged at left mid-body. Contractile vacuole in mid-body. Cortex flexible with yellowish granules arranged in rows of 2-4 granules throughout and near cell margins. Buccal cavity wide. Adoral zone occupies about 39% of body length, composed of 31 membranelles. Cirri, on average, composed of three frontal, four buccal, two frontoterminal, fifteen mid-ventral cirral pairs, two pretransverse ventral cirri, six transverse cirri, one left and one right marginal cirral rows composed of 40 cirri each. Caudal cirri absent. Three bipolar dorsal kinety rows.

Material deposited: Two slides 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. 3935 (12 specimens marked on the slide) and Pt. 4197/3 (6 specimens marked on the slide).

Occurrence and ecology: The species *Anteholosticha intermedia* is rather common in both freshwater and terrestrial ecosystems. It has been recorded from Denmark, Austria, Italy, Antarctica, USA, Norway, Germany, China, Korea, Hungary, Slovakia, Japan, Canada, New Zealand, France, Scotland, Poland, Costa Rica, Brazil, England, Spain, and Caspian Sea (Berger, 2006). The present study reports its presence from water and soil samples collected from the Flamingo Bird Sanctuary (19°05'37"N 72°35'38"E and 19°05'37"N 72°35'42"E). The soil sample was collected from a Bird watching area which contained grassland, small shrubs and trees; water sample was collected from small puddles slightly distant from the Bird watching area. It feeds on flagellates, bacteria, amoeba and small ciliates.

Family RIGIDOTRICHIDAE Foissner and Stoeck, 2006
Genus *Rigidosticha* Bharti, Kumar and La Terza, 2016

2. *Rigidosticha italiensis* Bharti, Kumar and La Terza, 2016 (Figure 1B)

Diagnosis of the Indian population (Data based on 5 specimens): Size about 210 × 110 µm in protargol preparations; shape ellipsoidal, dorsoventrally flattened. Nuclear apparatus composed of two macronuclear nodules and one or two micronuclei attached at various positions. Cortex rigid without any specific granules. Adoral zone occupies about 40% of body length, composed of about 50 membranelles. Cirri, on average, composed of three frontal, one buccal, one parabuccal, two frontoterminal, 20 cirral pairs, and four transverse cirri. One left and one right marginal cirral rows on average composed of 34 and 37 cirri, respectively. Eight to ten dorsal kineties; three or four caudal cirri.

Material deposited: One slide with protargol-impregnated specimens have been deposited at the National Zoological Collections of the Zoological Survey of India, Kolkata, India with the following accession number Pt. 4278 (7 specimens marked on the slide).

Occurrence and ecology: Thus far, the species *Rigidosticha italiensis* has been recorded only from Italy (Bharti *et al.* 2016). The present study reports its presence from soil sample collected from the Flamingo Bird Sanctuary

(19°05'46"N 72°56'01"E). The soil was collected from the butterfly park which had heavy tree vegetation and grass land. It feeds on flagellates, bacteria, naked and testate amoebae and ciliates.

Order SPORADOTRICHIDA Fauré-Fremiet, 1961

Family OXYTRICHIDAE Ehrenberg, 1838

Genus *Monomicrocaryon* Foissner, 2016

3. *Monomicrocaryon balladyna* (Song & Wilbert, 1989) Foissner, 2016 (Figure 1C)

Diagnosis of the Indian population (Data based on 4 specimens): Size about 45 × 20 µm in protargol preparations; shape elongate ellipsoidal to ellipsoidal sometimes ovate, dorsoventrally flattened. Contractile vacuole in midbody towards left cell margin. Cortex flexible, granules colourless and scattered. Nuclear apparatus composed of two macronuclear nodules; invariably one micronuclei arranged between nodules. Movement with jerk, static for a while and then crawling on soil surface. Adoral zone occupies about 42% of body length, composed of about 18 membranelles. Cirri, on average, composed of three frontal, one buccal, four frontoventral, three postoral, two pretransverse, and five transverse cirri, one left and one right marginal cirral row composed of nine and seven cirri respectively. Four or five dorsal kineties; three caudal cirri.

Material deposited: One slide with protargol-impregnated specimens have been deposited at the National Zoological Collections of the Zoological Survey of India, Kolkata, India with the following accession number Pt. 4012/9 (4 specimens marked on the slide).

Occurrence and ecology: This species has been recorded from Germany, Czechoslovakia, Yugoslavia, and USA. The present study reports its presence from soil sample collected from the Flamingo Bird Sanctuary (19°06'40"N 72°59'49"E). The sampling site was having grassland and trees. It feeds mainly of bacteria and flagellates.

Family OXYTRICHIDAE Ehrenberg, 1838

Genus *Urosoma* Kowalewskiego, 1882

4. *Urosoma karinae* (Ehrenberg, 1833) Berger, 1999 (Figure 1D)

Diagnosis of the Indian population (Data based on 5 specimens): Size about 90 × 65 µm in protargol

preparations; shape elongate ellipsoidal, dorsoventrally flattened, body ends rounded; flexible. Nuclear apparatus composed of two macronuclear nodules and two micronuclei attached to nodules at various positions. Adoral zone occupies about 27% of body length, composed of 21 membranelles. Cirri, on average, composed of three frontal, one buccal, four frontoventral, three postoral, and four transverse cirri. One left and one right marginal cirral row composed of about 24 and 32 cirri respectively. Four dorsal kineties, three caudal cirri at the posterior end of dorsal kineties 1, 2, and 3.

Material deposited: Two slides 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. 3984/2 (5 specimens marked on the slide) and Pt. 4014/4 (3 specimens marked on the slide).

Occurrence and ecology: This species has been recorded from Germany, Czechoslovakia, Yugoslavia, and USA. The present study reports its presence from soil samples collected from the Flamingo Bird Sanctuary (19°06'31"N 72°57'45"E and 19°05'46"N 72°56'25"E). The soil samples were collected near a waste management sites. It feeds mainly of bacteria and flagellates.

Order EUPLOTIDA Small and Lynn, 1985

Family URONYCHIIDAE Jankowski, 1979

Genus *Diophrys* Dujardin, 1841

5. *Diophrys oligothrix* Borror, 1965 (Figure 1E)

Diagnosis of the Indian population (Data based on 4 specimens): Size about 50 × 30 µm in protargol preparations; shape ovoid with concavity at the posterior body end. Nuclear apparatus composed of two parts of macronuclear nodules arranged in C-shape pattern, one or two micronuclei attached to nodules at various positions. Adoral zone occupies about 55% of body length, composed of about 24 membranelles. On average composed of seven frontoventral and five very prominent transverse cirri. Left marginal cirral row composed of two cirri. Four dorsal kineties, three caudal cirri.

Material deposited: One slide with protargol-impregnated specimens have been deposited at the National Zoological Collections of the Zoological Survey of India, Kolkata,

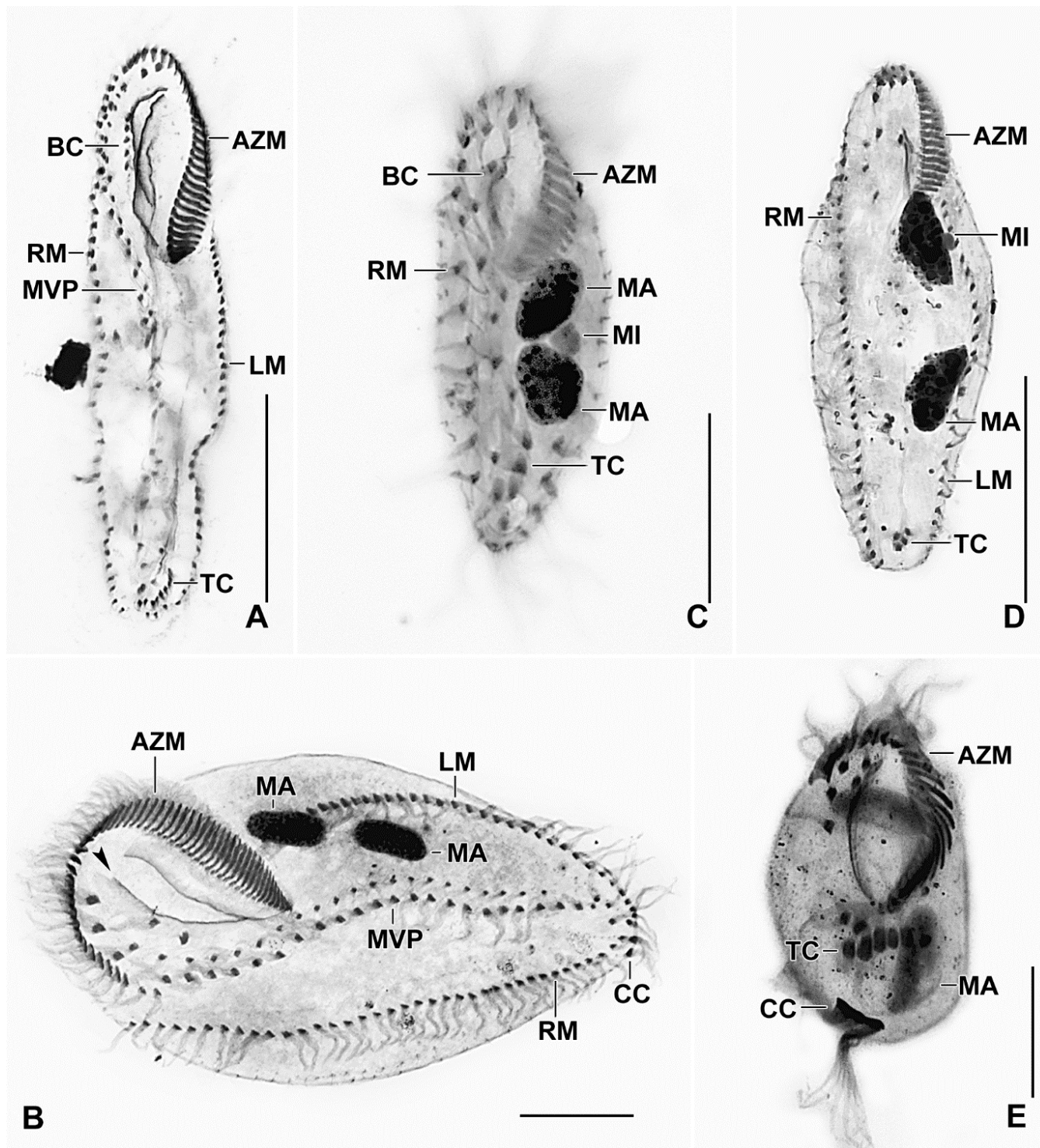


Figure 1. Photomicrographs of protargol impregnated specimens. **A.** *Anteholosticha intermedia*. **B.** *Rigidosticha italiensis*, arrowhead marks the paroral membrane. **C.** *Monomicrocaryon balladyna*. **D.** *Urosoma karinae*. **E.** *Diophrys oligothrix*. AZM, adoral zone of membranelles; BC, buccal cirrus; CC, caudal cirri; LM, left marginal row; MA, macronuclear nodules; MI, micronuclei; MVP, midventral cirral pairs; RM, right marginal row; TC, transverse cirri. Scale bars 40 μ m (A, B, D), 20 μ m (C, E).

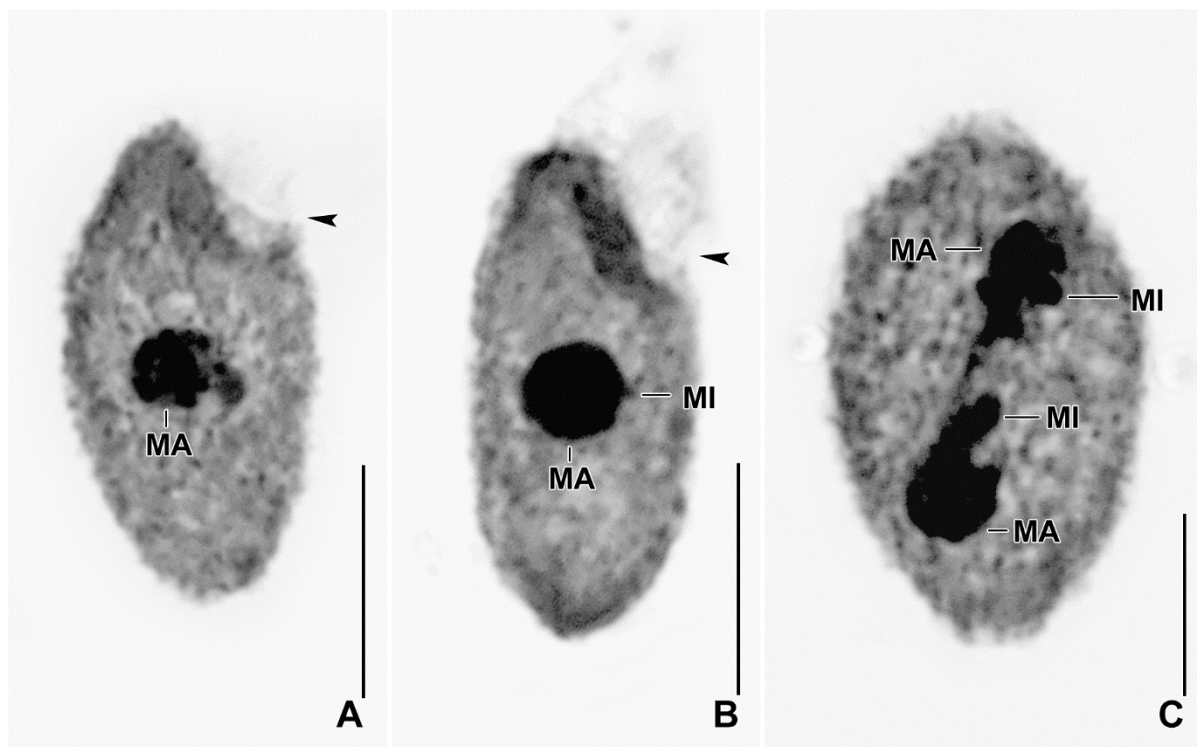


Figure 2. Photomicrographs of protargol impregnated specimens of *Cyrtolophosis muscicola* Indian population. A, B. Vegetative specimens, arrowheads mark the oral concavity. C. A specimen in divisional stage. MA, macronuclear nodules; MI, micronuclei. Scale bars 10 μ m.

India with the following accession numbers Pt. 3938 (5 specimens marked on the slide).

Occurrence and ecology: This species has been recorded from America, Antarctic, Europe, China and Korea. The present study reports its presence from the water sample collected from the urban area in Vashi, Navi Mumbai, i.e., surroundings of Flamingo Bird Sanctuary (19°04'34"N 72°59'02"E). It feeds mainly of bacteria.

Class COLPODEA Small & Lynn, 1981
Order CYRTOLOPHOSIDIDA Foissner, 1978
Family CYRTOLOPHOSIDIDAE Stokes, 1888
Genus *Cyrtolophosis* Stokes, 1885

6. *Cyrtolophosis muscicola* Stokes, 1885 (Figure 2A-C)

Diagnosis of the Indian population (Data based on 5 specimens): Size about 20 \times 10 μ m in protargol preparations; shape ovoid, oral aperture with a prominent shallow groove occupying the anterior third of the cell. Nuclear apparatus composed of one spherical and centrally positioned macronuclear nodule and one or two

micronuclei attached to macronuclear nodule. About 15 kinety rows, anterior tuft of cilia present.

Material deposited: One 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. 4012/2 (11 specimens).

Occurrence and ecology: *Cyrtolophosis muscicola* has a cosmopolitan distribution, it has been reported from five biogeographic regions, i.e., Holarctis, Palaeotropis, Australis, Neotropis, and Archinotis. The present study reports its presence from soil sample collected from the Flamingo Bird Sanctuary (19°06'40"N 72°59'49"E). The sampling site was having a small ponds, grassland, trees and mangrove. It feeds mainly on bacteria.

Class NASSOPHOREA Small & Lynn, 1981
Order MICROTHORACIDA Jankowski, 1967
Family LEPTOPHARYNIDAE Kahl, 1926
Genus *Leptopharynx* Stokes, 1885

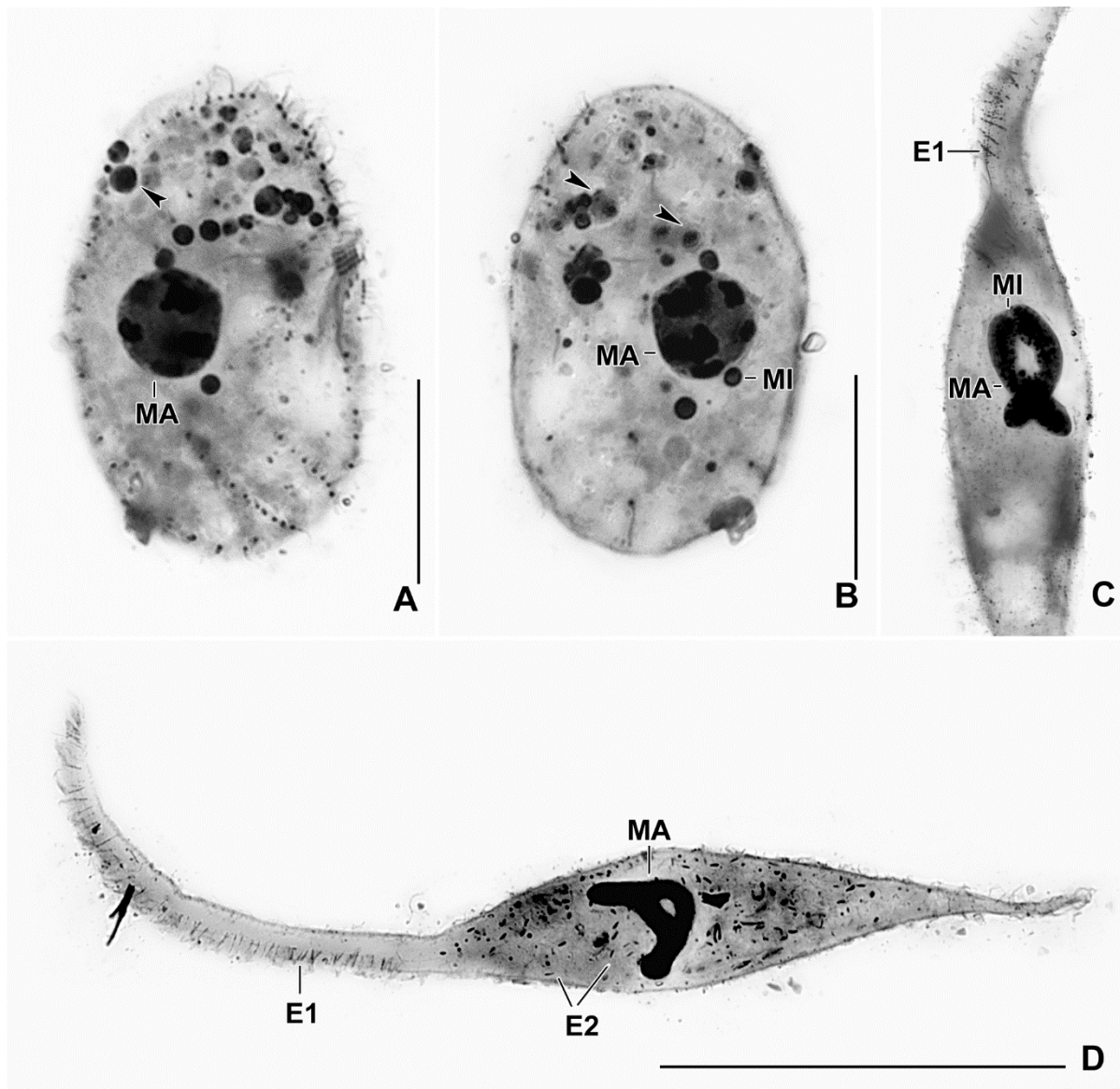


Figure 3. Photomicrographs of protargol impregnated specimens. A, B. *Leptopharynx costatus*, ventral (A) and dorsal (B) views, arrowheads point to the food vacuoles. C, D. *Rimaleptus mucronatus*. E1, type 1 extrusomes; E2, type 2 extrusomes; MA, macronuclear nodules; MI, micronuclei. Scale bars 15 μ m (A, B), 100 μ m (D).

7. *Leptopharynx costatus* Mermod, 1914 (Figure 3A, B)

Diagnosis of the Indian population (Data based on 5 specimens): Size about 30 \times 20 μ m in protargol preparations; shape broadly ellipsoidal with slightly blunt towards left of the ventral side. Nuclear apparatus composed of one spherical and centrally positioned macronuclear nodule and one or two micronuclei near to macronuclear nodule. Oral apparatus near anterior end of body, oral basket composed of nematodesmata bundles.

Two to three adoral membranelles. About nine somatic and three preoral kineties.

Material deposited: Three slides 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. 4015/4 (7 specimens marked on the slide), Pt. 4014/5 (2 specimens marked on the slide), and Pt. 4198/3 (3 specimens marked on the slide).

Occurrence and ecology: Thus far, the species *Leptopharynx costatus* has been recorded from Germany, Mexico, Austria, Brazil and Florida. The present study reports its presence from soil samples collected from the Flamingo Bird Sanctuary (19°06'37"N 72°59'42"E; 19°05'46"N 72°56'01"E; and 19°06'37"N 72°59'38"E). The samples were collected from the terrestrial site with heavy vegetation, grassland inside the butterfly park, and from area with vegetation including grassland, trees and mangrove. It feeds on bacteria.

Subclass RHYNCHOSTOMATIA Jankowski, 1980

Order DILEPTIDA Jankowski, 1978

Family DIMACROCARYONIDAE Vďačný, Orsi, Bourland, Shimano, Epstein and Foissner, 2011

Genus *Rimaleptus* Foissner, 1984

8. *Rimaleptus mucronatus* (Penard, 1922) Vďačný, Orsi, Bourland, Shimano, Epstein and

Foissner, 2011 (Figure 3C, D)

Diagnosis of the Indian population (Data based on 5 specimens): Size about 300 × 35 µm in protargol preparations; shape narrowly dileptid with distinct tail, proboscis about 40% of body length. Nuclear apparatus composed of two oblong macronuclear nodules with a micronucleus in between. Two types of extrusomes attached to proboscis oral bulge: type I rod-shaped to slightly ovate, usually 5 × 0.5 µm; type II oblong, 2 × 0.5 µm long. On average 20 ciliary rows.

Material deposited: Two slides 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. 4015/3 (7 specimens marked on the slide) and Pt. 4198/2 (4 specimens marked on the slide).

Occurrence and ecology: *Rimaleptus mucronatus* has a cosmopolitan distribution. It has been recorded from Benin, Kenya, Zanzibar, Venezuela, St. Vincent Island, Australia, Austria, Slovakia, Saudi Arabia, China, Namibia, USA, Switzerland, France, England, Spain, Bulgaria, Turkey, and Canada (Vďačný and Foissner, 2012). The present study reports its presence from soil samples collected from the Flamingo Bird Sanctuary (19°06'37"N 72°59'42"E and 19°06'37"N 72°59'38"E). The samples were collected from site having grassland, trees

and mangrove in surroundings. It feeds on flagellates, and bacteria.

Family DILEPTIDAE Jankowski, 1980

Genus *Dileptus* Dujardin, 1841

9. *Dileptus beersi* Jones, 1956 (Figure 4A, B)

Diagnosis of the Indian population (Data based on 4 specimens): Size about 350 × 40 µm in protargol preparations; shape narrowly dileptid with distinct tail, proboscis about 1/3 of body length. Nuclear apparatus composed of more than 200 macronuclear nodules and several micronuclei. Two types of extrusomes attached to proboscis oral bulge: type I rod-shaped to slightly ovate, usually 7 × 0.7 µm; type II oblong, 3 × 0.5 µm long. On average 35 ciliary rows.

Material deposited: One 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 number Pt. 4014/6 (7 specimens marked on the slide).

Occurrence and ecology: Thus far, the species has been recorded from North Carolina (USA), Venezuela, and Savannah (Vďačný & Foissner, 2012). The present study reports its presence from soil sample collected from the Flamingo Bird Sanctuary (19°05'46"N 72°56'01"E). The soil was collected from the butterfly park which had heavy tree vegetation and grass land. It feeds on flagellates and small ciliates.

Family DILEPTIDAE Jankowski, 1980

Genus *Pseudomonilicaryon* Foissner, 1997

10. *Pseudomonilicaryon falciforme* (Kahl, 1931) Vďačný & Foissner, 2012 (Figure 4C, D)

Diagnosis of the Indian population (Data based on 4 specimens): Size about 350 × 35 µm in protargol preparations; shape narrowly to cylindroidally dileptid with acute posterior end, proboscis about 1/3 of body length. Nuclear apparatus moniliform, composed of 30 globular to oblong macronuclear nodules with about 15 globular micronucleus. Two types of extrusomes attached to proboscis oral bulge: type I rod-shaped to narrowly ovate, usually 6 × 0.5 µm; type II ovate, 3 × 0.5 µm long. On average 40 ciliary rows.

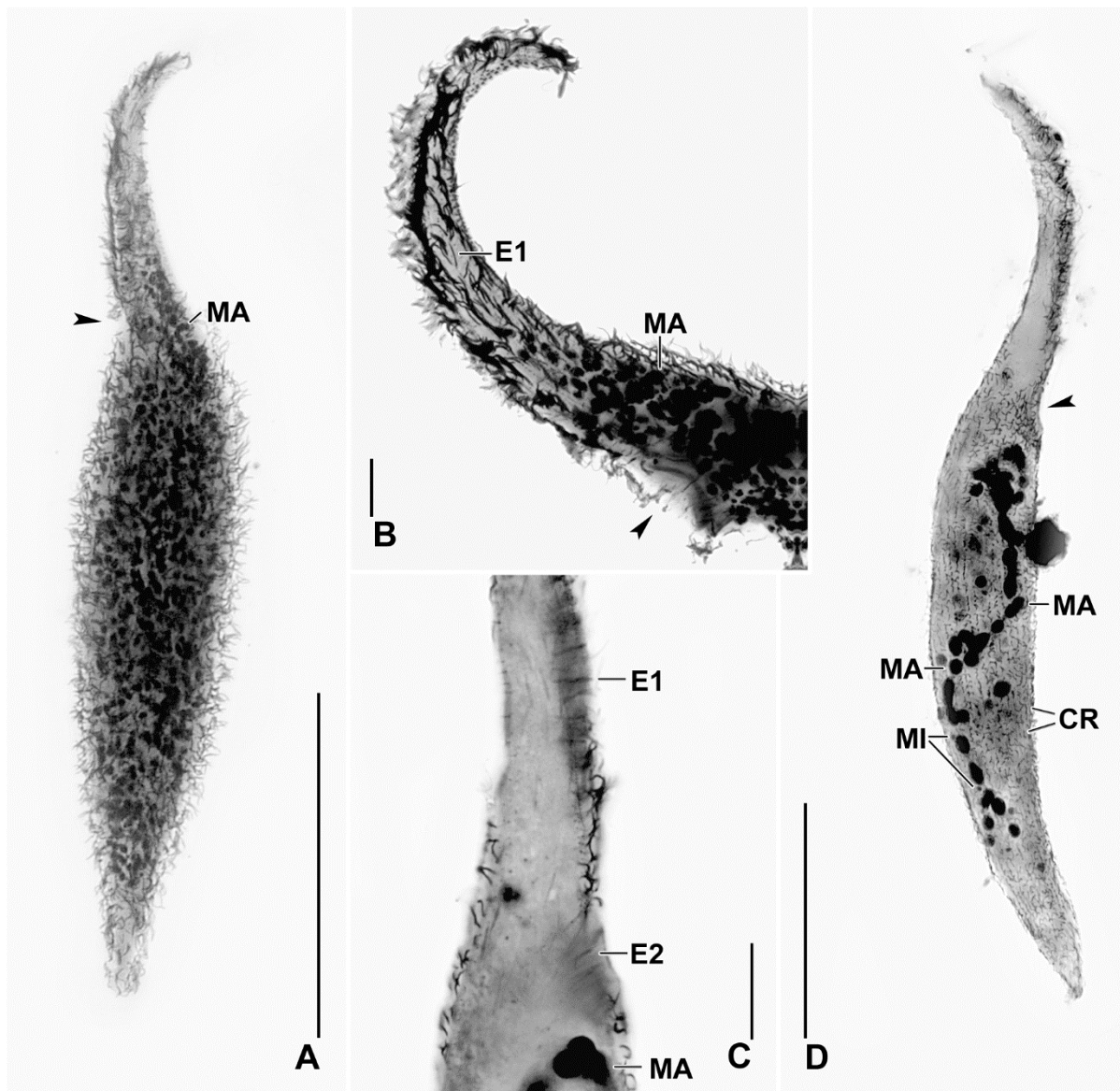


Figure 4. Photomicrographs of protargol impregnated specimens. **A, B.** *Dileptus beersi*. Arrowheads point to the oral opening. **C, D.** *Pseudomonilicaryon falciforme*. Arrowhead points to the oral opening. E1, type 1 extrusomes; E2, type 2 extrusomes; MA, macronuclear nodules; MI, micronuclei. Scale bars 80 μm (A, D), 15 μm (B, C).

Material deposited: Two slides with 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. 4012/5 (5 specimens marked on the slide) and Pt. 4013/5 (1 specimen marked on the slide).

Occurrence and ecology: Thus far, *Pseudomonilicaryon falciforme* has been recorded from Germany, Austria, Hawaii, Tibetan Plateau, and China (Vďačný & Foissner, 2012). The present study report its presence from the soil samples collected from the Flamingo Bird Sanctuary

(19°06'40"N 72°59'49"E and 19°05'50"N 72°56'25"E). The samples were collected from the terrestrial site with heavy vegetation and grassland inside the butterfly park and from grassland, trees and mangrove. It feeds on flagellates, bacteria, naked and testate amoebae, and ciliates.

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