



# New Records of Two Ciliated Protists from India

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## Abstract

The diversity of free-living ciliated protists isolated from the soil samples collected from the Western Ghats, Kerala, were studied based on standard methods. Two ciliates were identified as new records to the Indian fauna, namely *Lembadion lucens* (Maskell, 1887) Kahl, 1931 and *Anteholosticha monilata* (Kahl, 1928) Berger, 2003. The present study provides a brief description of both species based on live observation and stained preparations along with photomicrographs.

**Keywords:** Ciliates, Morphology, Protargol Staining, Protists, Western Ghats

## Introduction

The Western Ghats, World Heritage Site in Nilgiri sub-cluster (10°09'N 77°03'E), India that includes biosphere reserves, national parks, and several wildlife sanctuaries which conserve a plethora of species of animals, plants, and microbes, some of which are endemic to the region (UNESCO, 2007). Till recently, reports have been published from this region describing species that are new to science as well as a new record to Indian fauna. The diversity of free-living ciliated protists have been studied by Kumar *et al.* (2010, 2015) with the description of three new species, i.e., *Anteholosticha angida* Kumar, Kamra and Sapra, 2010; *Bakuella nilgiri* Kumar, Kamra and Sapra, 2010; *Sterkiella tetracirrata* Kumar *et al.*, 2015 as well as new records to Indian fauna, i.e., *Caudiholosticha sylvatica* (Foissner, 1982) Berger, 2003; *Holostichides chardezi* Foissner, 1987; *Pseudourostyla franzi* Foissner, 1987; and *Pseudourostyla levis* Takahashi, 1973.

About 700 free-living ciliates have been reported thus far from India. It is expected that the actual diversity of free-living ciliates from India could be over 2,000 species when investigated using modern techniques, i.e., live observation and silver staining supplemented with molecular analyses. The present report attempts to catalogue the diversity of free-living protists using the standard staining techniques that resulted in the identification of two ciliates new to Indian fauna.

## Material and Methods

### Sampling and Sample Processing

Soil samples (about 100 gm each) collected from the Western Ghats, India, were air-dried for two weeks before rewetting, employing the non-flooded Petri dish culture (Foissner *et al.*, 2002). For details on sampling sites, refer to descriptions of individual species. Raw cultures were established at room temperature 20°C by adding some squashed wheat kernels or providing green algae as a food source. Live observations and photomicrography were performed using a stereo zoom microscope (SZ2-ILST, Olympus) and a bright-field microscope (CX 43, Olympus). The protargol staining method described by Kamra and Sapra (1990) was used with some modifications 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 40 ciliates have been recorded from the soil and water samples from the Western Ghats, India (Kumar *et al.*, 2010, 2015, 2016). The present report adds two species belonging to two families and two genera. The report indicates that a huge gap exists for the study of ciliates both in terms of the standard methodology used and the unexplored areas (freshwater, soil and marine

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forms) from the country. Brief reports on two species new records of Indian fauna are presented.

### Taxonomic Account

Phylum CILIOPHORA Doflein, 1901

Class OLIGOHYMENOPHOREA de Puytorac *et al.*, 1974

Order PENICULIDA Fauré-Fremiet in Corliss, 1956

Family LEMBADIONIDAE Jankowski in Corliss, 1979

Genus *Lembadion* Perty, 1849

#### 1. *Lembadion lucens* (Maskell, 1887) Kahl, 1931 (Figure 1A-D)

**Diagnosis of the Indian population** (Data based on 11 specimens): Size on average  $55 \times 30 \mu\text{m}$  in protargol preparations, about  $65 \times 35 \mu\text{m}$  in vivo; shape oval. Nuclear apparatus composed of one elongate or kidney-shaped or L-shaped macronuclear nodule with size about  $12 \times 8 \mu\text{m}$  located in posterior third of the body, about  $25 \mu\text{m}$  distant from anterior body end and single micronuclei close to the macronuclear nodule. Somatic infraciliature consist of 25-35 meridional kineties. Single contractile vacuole at cell half toward right cell margin. Six to 10 caudal cilia present at the posterior body end. Buccal field wide with two paroral membranes.

**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 accession number Pt. 3854 (11 specimens marked on the slide).

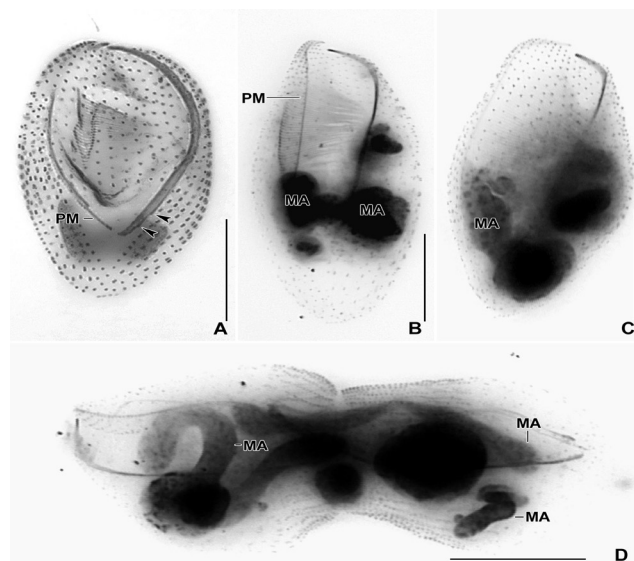
**Occurrence and ecology:** The species *Lembadion lucens* has been reported several times, since its original description, in both freshwater and terrestrial ecosystems (Maskell, 1887; Kahl, 1931; Dragesco & Dragesco-Kernéis 1986; Guinea *et al.*, 1990; Foissner *et al.*, 1994; Asadullayeva & Alekperov, 2007; Liu *et al.*, 2017). The present study reports its presence from the soil samples collected from the Western Ghats ( $11^{\circ}08'N$   $76^{\circ}28'E$ ), India. The soil sample was collected from the roots of a tall tree in the Silent Valley National Park. It feeds on flagellates, bacteria, and amoeba.

Class SPIROTRICHEA Bütschli, 1889

Order UROSTYLIDA Jankowski, 1979

Family UROSTYLIDAE Bütschli, 1889

Genus *Anteholosticha* Berger, 2003

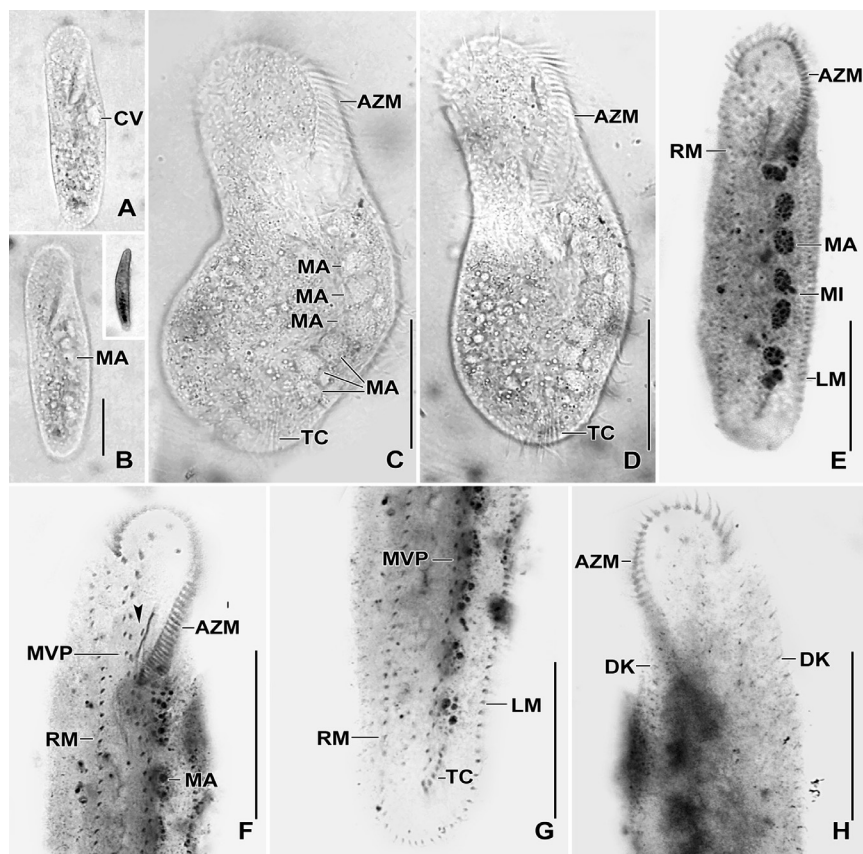


**Figure 1.** Photomicrographs of protargol impregnated specimens of *Lembadion lucens*. A-C. Specimens, showing the body shape, ciliary structures, and nuclear apparatus. Arrowheads in (A) point to the gradually shortened outer rows of adoral membranelles posteriorly. D. A divider. MA, macronuclear nodules; PM, paroral membrane. Scale bars  $20 \mu\text{m}$ .

#### 2. *Anteholosticha monilata* (Kahl, 1928) Berger, 2003 (Figure 2A-H)

**Diagnosis of the Indian population** (Data based on 4 specimens): Size about  $160 \times 40 \mu\text{m}$  in protargol preparations; shape elliptical with body ends rounded, dorsoventrally flattened. Nuclear apparatus composed of on average seven macronuclear nodules, about  $10 \times 7 \mu\text{m}$  in size mostly arranged in a row slightly left of mid-body and 2-4 micronuclei attached or near to macronuclear nodules. Cortex flexible. Contractile vacuole in mid-body near left cell margin. Buccal cavity wide. Adoral zone occupies about 32% of body length, composed of 47 membranelles on average. Cirri, on average, composed of three frontal, one buccal, two frontoterminal, 19 mid-ventral cirral pairs, two pretransverse ventral cirri, eight transverse cirri, one left and one right marginal cirral rows composed of 46 and 44 cirri, respectively. Caudal cirri absent. Six dorsal kinety rows.

**Material deposited:** A slide containing protargol-impregnated specimens have been deposited at the National Zoological Collections of the Zoological Survey



**Figure 2.** Photomicrographs of live (A-D) and protargol impregnated (E-H) specimens of *Anteholosticha monilata* Indian population. A-D. Specimens from live, showing body shape, position of contractile vacuole, nuclear apparatus arranged in a row, and ciliature. Inset in 9B) shows the lateral view of a specimen. E-G. Specimens showing ciliature on the ventral surface. Arrowhead in (F) points to the buccal cirrus. H. A specimen, showing ciliature on the dorsal surface. AZM, adoral zone of membranelles; CV, contractile vacuole; DK, dorsal kineties; LM, left marginal row; MA, macronuclear nodules; MI, micronuclei; MVP, mid-ventral cirral pairs; RM, right marginal row; TC, transverse cirri. Scale bars 50  $\mu$ m.

of India, Kolkata, India with the accession number Pt. 4298 (4 specimens marked on the slide).

**Occurrence and ecology:** The species *Anteholosticha monilata* is rather common in freshwater though rare in terrestrial habitats. It has been recorded from Austria, Czechoslovakia, France, Germany, Moldavia, Norway, Spain, Belgium, Cameroon, USA (for further details, refer Berger (2006)). The present study reports its presence from soil samples collected from the Parambikulam Wildlife Sanctuary, Sangam Range, Palakkad district, Western

Ghats (10°26'29.2"N 76°46'29.3"E), India. It feeds on flagellates, bacteria, amoeba, diatoms, and small ciliates.

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