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Morphology of Indian populations of *Coleps elongatus* (Ehrenberg, 1830) Kahl, 1930 and *C. amphacanthus* Ehrenberg, 1833 (Ciliophora: Prostomatea: Prorodontida)

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Abstract

The present study reports the morphology of two population each of free-living colepid ciliate *Coleps elongatus* (Ehrenberg, 1830) Kahl, 1930 and *C. amphacanthus* Ehrenberg, 1833. The populations of *C. elongatus* were isolated from the protected area (Mahananda Wildlife Sanctuary) of West Bengal and a small natural pond (Senhati Jheel) in Kolkata. The populations of *C. amphacanthus* were isolated from the two natural ponds in Kolkata. Both species are new records to Indian fauna. Brief description based on the live observations, silver stained preparations, and 18S rDNA sequence is presented.

Keywords: Colepids, Free-Living, Ganga, Mahananda Wildlife Sanctuary, Protozoan, 18S rDNA

Introduction

The genus *Coleps* was established by Nitzsch in 1827 for small to medium size ciliates with the cylindrical or barrel-shaped body covered with armour plates made of calcium carbonate. They possess oral structure at the anterior end of the body, positioned apically with almost holotrichous ciliation (Lynn, 2008). Colepids are usually benthic, pelagic or marine psamnobiotic that play a crucial role in the management of the microbial communities.

Coleps hirtus (Müller, 1786) Nitzsch, 1827 is one of the most abundant species worldwide. It has been reported by many researchers from India along with *Coleps tesselatus* Kahl, 1930. The latter was reported from Chilika, Odisha by Das (1995). Since the description of *Coleps hirtus* (Müller, 1786) Nitzsch, 1827 two centuries ago the genus now possess 40 species. The present study briefly describes two populations each of *Coleps elongatus* (Ehrenberg, 1830) Kahl, 1930 and *Coleps amphacanthus* Ehrenberg, 1833 identified using modern techniques, i.e., live observations and silver staining. Both species are new records to Indian fauna.

Material and Methods

Water samples were collected from the Mahananda Wildlife Sanctuary, a protected area of North Bengal (26°55'44"N 88°26'57"E), and natural ponds in Kolkata (small natural pond-22°29'26"N 88°12'28"E and Senhati Jheel-22°29'46"N 88°19'44"E). Raw cultures were established at room temperature in Petri dishes and maintained by providing green algae *Chlorogonium elongatum* as food (Ammermann *et al.*, 1974). Staining was performed as described by Kamra and Sapra (1990) with some modifications. Live observations and photomicrography were performed using a stereo microscope (SZ2-ILST, Olympus) and bright-field microscope (CX 43, Olympus). In-vivo measurements were performed at 400-1000x and that of stained specimens at 1000x magnification. Classification is according to Lynn (2008).

The 18S rDNA sequence of only *Coleps amphacanthus* was generated. Several cells of *C. amphacanthus* were isolated from clonal culture and subsequently washed with sterilized distilled water to decrease the amount of contaminants. Genomic DNA was extracted using the DNeasy blood and tissue kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's instructions. The universal eukaryotic primers Euk A (5′-AAC CTG GTT GAT CCT GCC AG-3′) and reverse Euk B (5′-CAC TTG GAC GTC TTC CTA GT-3′) (Medlin *et al.*, 1988) were used for amplification of the 18S rDNA. Sequencing was performed at the Yaazh xenomics, Tamil Nadu, India.

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Results and Discussion

The SSU rRNA gene sequence of *C. amphacanthus* (1525bp; GC content – 45.6%) was deposited in GenBank with accession number MT271853.

Species of the genus *Coleps* are morphologically distinguished by their body size, shape, number of armour tiers, arrangements of the armoured plates, anterior and posterior spine number and the number of caudal cilia (Corliss 1979; Dragesco & Dragesco-Kernéis 1991; Foissner 1983; Foissner *et al*., 1994, 1999; Kahl 1930; Noland 1925; Obolkina 1995). Brief descriptions of both species are provided below:

Taxonomic Account

Phylum CILIOPHORA
Class PROSTOMATEA
Order PRORODONTIDA
Family COLEPIDAE
Genus ***Coleps*** Nitzsch, 1827

1. *Coleps elongatus* (Ehrenberg, 1830) Kahl, 1930 (Figure 1A–O; Table 1).

Two populations were studied, one from the protected area (Mahananda Wildlife Sanctuary) of West Bengal and another from the small natural freshwater pond (Senhati Jheel) in Kolkata.

Description: (*Mahananda Wildlife Sanctuary population*) (Figure 1A–H): Size in vivo 30-50 µm × 15-25 µm on average, usually about 45 µm × 25 µm, as calculated from some in vivo measurements (n=4) and the morphometric data in Table 1, adding 15% for preparation shrinkage (Kumar and Foissner 2016; Kumar *et al*., 2016). Body slender with length: width ratio about 2.0. Macronucleus, spherical, 7.0–10.0 µm across, below

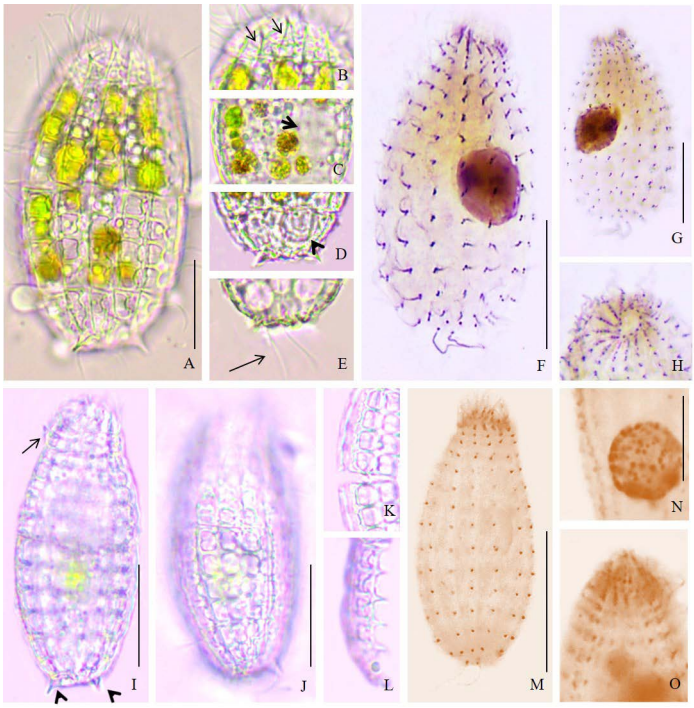

Figure 1. Photomicrography of *Coleps elongatus* Mahananda Wildlife Sanctuary population from live (A-E) and after protargol impregnation (F-H), Senhati Jheel population from live (I-L) and after protargol impregnation (M-O). **A.** Specimen showing typical barrel shaped body. **B.** Arrows showing anteriormost plates. **C.** Arrow indicating globular macronucleus. **D.** Arrowhead indicating contractile vacuole at the posterior body end. **E.** Arrow showing two distinct caudal cilia. **F, G.** Specimen showing ciliature on the dorsal and ventral surface. **H.** Oral ciliature. **I.** Specimen showing body shape, arrow indicates anterior spine and arrowheads point caudal spines. **J.** Specimen showing armour plate and 3rd caudal spine in different focus. **K.** *hirtus*-type of armour. **L.** pretzel-shaped window. **M.** Specimen showing dorsal ciliature. **N.** Macronucleus. **O.** Oral ciliature with perioral kineties. Scale bars: A, F, G, M- 15 µm; I, J- 20 µm; N- 10 µm.

Table 1. Morphometric data on *Coleps elongatus* (Ehrenberg, 1830) Kahl, 1930 (Mahananda WLS population-M, Senhati Jheel population-Sj)

Characteristic[a]	Pop	Mean	M	SD	SE	CV	Min	Max	n
Body, length	M	34.0	35.0	3.4	0.9	10.0	28.0	42.0	14
	Sj	40.7	40.0	3.5	1.0	8.7	36.0	50.0	13
Body, width	M	18.3	18.0	3.0	0.8	16.2	15.0	26.0	15
	Sj	21.6	20.0	4.1	1.1	18.7	17.0	0.0	13
Body length:width, ratio	M	1.8	1.9	0.2	0.1	12.7	1.3	2.2	14
	Sj	1.9	2.0	0.3	0.1	14.4	1.3	2.4	13
Anterior body end, width	M	8.4	8.0	1.1	0.3	12.8	7.0	10.0	10
	Sj	8.9	9.0	1.1	0.3	12.2	7.0	10.0	12
Macronuclear nodules, number	M	1.0	1.0	0.0	0.0	0.0	1.0	1.0	16
	Sj	1.0	1.0	0.0	0.0	0.0	1.0	1.0	10
Macronucleus, diameter	M	8.7	9.0	0.9	0.2	10.4	7.0	10.0	15
	Sj	7.8	7.5	1.5	0.6	18.8	6.0	10.0	6
Cirral rows, number	M	13.0	13.0	0.0	0.0	0.0	13.0	13.0	16
	Sj	13.0	13.0	0.0	0.0	0.0	13.0	13.0	8
Caudal cilia, number	M	2.0	2.0	0.0	0.0	0.0	2.0	2.0	10
	Sj	2.0	2.0	0.0	0.0	0.0	2.0	2.0	10

[a]Data based on mounted, protargol-impregnated, and randomly selected specimens from a clonal culture. 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.

midline towards posterior body end. Cortex without symbiotic algae. Contractile vacuole at posterior end of the body. Armour brownish, anterior end narrow with single spine, posterior end rounded with three distinct caudal spines. Six types of armour tiers with 13 windows: anterior secondary with 2, anterior main with 5, posterior main with 4, and posterior secondary with 2 windows. Windows pretzel-shaped or *hirtus*-type. Body midline strongly distinguished by the borders of anterior and posterior main plates. Thirteen transverse ciliary rows and about 15 meridionally arranged somatic kineties. On average, two distinct long caudal cilia.

The Senhati Jheel population (Figure 1I–O) is slightly larger (40.7 µm vs. 34 µm) then the Mahananda Wildlife Sanctuary population. Other characters were rather similar (for details refer Table 1). *Coleps elongatus* has been mentioned in two articles published from India however, their identification is doubtful.

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 numbers Pt 4276 (MWLS population) and Pt 5200 (Senhati Jheel population).

Occurrence and ecology: Cosmopolitan distribution, feeds on bacteria and flagellates.

2. *Coleps amphacanthus* Ehrenberg, 1833 (Figure 2A-P; Table 2).

Two populations isolated from ponds in Kolkata, i.e., (small natural pond and Senhati Jheel).

Description: (*small natural pond population*) (Figure 1A–I): Size in vivo 60-80 µm × 30-45 µm on average, usually about 75 µm × 45 µm, as calculated from some in vivo measurements (n=3) and the morphometric data in Table 1, adding 15% for preparation shrinkage (Kumar and Foissner 2016; Kumar *et al.*, 2016). Body broad, typical barrel shaped with length:width ratio about 1.5. Macronucleus, spherical, 11.0–17.0 µm across, below midline towards posterior body end. Cortex without symbiotic algae. Contractile vacuole at posterior end of the body. Cells appear dark at low magnification, anterior

end narrow with single spine, posterior end rounded with three distinct caudal spines. Six types of armour tiers with 18 windows: anterior secondary with 2, anterior main with 8, posterior main with 6, and posterior secondary with 2 windows. Windows *hirtus*-type. Eighteen meridionally arranged somatic kineties, 23 transverse ciliary rows. On average, seven distinct long caudal cilia.

The Senhati Jheel population (Figure 1J–P) was studied in live only and found to be similar the above mentioned population from small pond in Kolkata (for details refer Table 2 and Figure 2).

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 numbers Pt. 4917/2, 5082, 5166/2, 5212 (Small natural pond population) and Pt. 5200/1 (Senhati Jheel population).

Occurrence and ecology: Distribution, Europe and Asia; feeds on bacteria, flagellates and small ciliates.

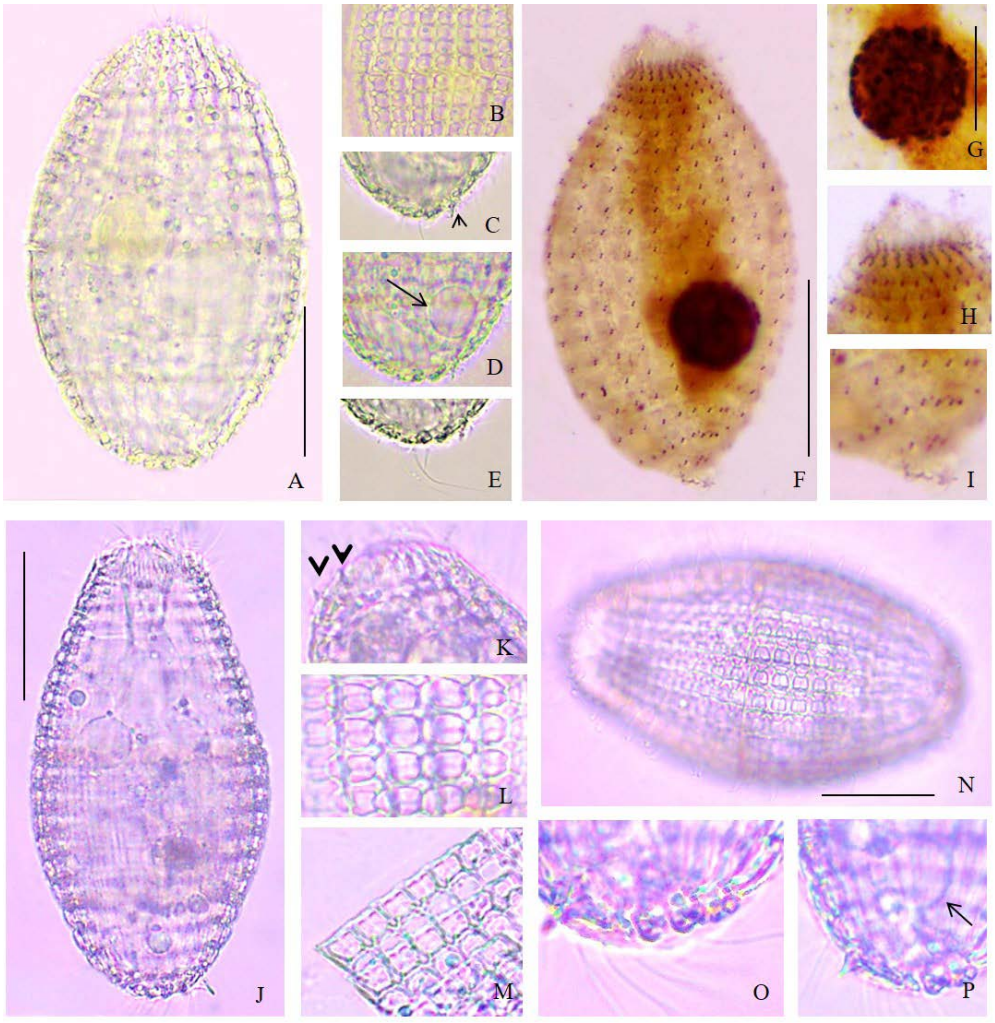

Figure 2. Photomicrography of *Coleps amphacanthus* small natural pond, Kolkata population from live (A-E) and after protargol impregnation (F-I), Senhati Jheel population from live (J-P). **A.** Specimen showing broad barrel shaped body. **B.** *hirtus*-type windows. **C.** Arrowhead showing posterior spine. **D.** Arrow indicating contractile vacuole. **E.** Specimen showing caudal cilia. **F.** Specimen showing body ciliature. **G.** Macronucleus. **H.** Oral ciliature. **I.** caudal cilia. **J.** Specimen showing typical barrel shaped body. **K.** Arrowheads showing two anterior spines. **L.** Specimens showing typical *hirtus*-type armour plates. **M.** Enlarged view of the ruptured specimen showing 8-shaped windows. **N.** Specimen showing body shape with armoured plates. **O.** Caudal cilia. **P.** Arrow indicating contractile vacuole. Scale bars: A, F, J, N- 25 µm; G- 15 µm.

Table 2. Morphometric data on *Coleps amphacanthus* Ehrenberg, 1833 (small natural pond, Kolkata population)

Characteristic[a]	Mean	M	SD	SE	CV	Min	Max	n
Body, length	62.5	63.0	2.5	0.8	4.1	56.0	66.0	11
Body, width	34.3	33.0	4.2	1.4	12.4	30.0	41.0	9
Body length: width, ratio	1.8	1.9	0.2	0.1	10.7	1.6	2.1	9
Anterior body end, width	10.8	11.0	1.1	0.4	10.5	9.0	13.0	10
Macronuclear nodules, number	1.0	1.0	0.0	0.0	0.0	1.0	1.0	11
Macronucleus, diameter	13.3	14.0	2.1	0.6	15.4	11.0	17.0	11
Cirral rows, number	18.0	18.0	0.0	0.0	0.0	18.0	18.0	9
Somatic kineties, number	23.3	23.5	0.8	0.3	3.5	22.0	24.0	6
Caudal cilia, number	6.5	6.0	1.9	0.6	29.2	4.0	9.0	10

[a]Data based on mounted, protargol-impregnated, and randomly selected specimens from clonal cultures. 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.

Molecular sequence comparison: The SSU rRNA gene sequence of C. amphacanthus (1525bp; GC content – 45.6%) was deposited in GenBank with accession number MT271853. The 18S rDNA sequence of the Kolkata population of *C. amphacanthus* shows difference with the available sequences of the Chinese population of *C. amphacanthus* by 97%. This indicates, along with the difference in the body size (i.e., the Kolkata population is considerably smaller, 63 × 34 µm vs. 90 × 45 µm in protargol preparation), that the Kolkata population could be a new species/subspecies. However, we do not perform any change and wait for further data.

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