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Research Paper

Extended distributional range of testate amoebae of the genus *Difflugia*Leclerc, 1815 (Arcellinida: Difflugiidae) to India

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Abstract: *Difflugia* Leclerc, 1815 (Arcellinida: Difflugiidae), one of the testate amoeba genus is the most species genus with almost 300 reported species worldwide with 36 records from India. In the present communication five species of the genus *Difflugia* are reported as novel records to India from moss habitats *viz*., *Difflugia ampla* Rampi, 1950, *Difflugia ampullula* Playfair, 1918, *Difflugia bryophila* (Penard, 1902) Jung, 1942, *Difflugia lacustris* (Penard, 1899) Ogden, 1983 and *Difflugia ventricosa* Deflandre, 1926 which were collected from Agastyamala BR, Kerala part and Singalila NP, West Bengal. A detailed description of all the species recorded is provided based on the specimens studied.
Keywords: Protozoa; Testate amoebae; Moss; Agastyamala BR; Singalila NP

Introduction:

Difflugia Leclerc, 1815 is the oldest genus of testate amoebae with the type species D. proteiformis Lamarck, 1816. Members of the genus have spherical to elongated agglutinated test with a single terminal pseudostome usually round, oval, lobed or toothed (never slit like) and sometimes with a collar or necklace (Ogden and Meisterfeld, 1989). The test is composed of mineral particles called xenosomes that are assembled on sheet like organic cement and they acquire xenosomes from the surroundings. These select and arrange these xenosomes according to their size and shape inorder to construct a shell with a morphology that is unique to a particular species; the nucleus is ovular or vesicular.
Difflugia is the most speciose genus of the order Arcellinida with almost 300 nominal species and 200 subspecific and intrasubspecific (Ogden, 1983; Meisterfeld and Mitchell, 2008). The size of the *Difflugia* species ranges from 15µm to more than 500µm. *Difflugia* genus inhabit in diverse habitats. Many species are common in freshwater sediments or between water plants and in sphagnum mosses and lakes of different trophic status have characteristic dominant *Difflugia* species.
In India intensive studies on testate amoebae initiated only recently. Pioneer studies on moss associated testate amoebae was by Chattopadhyay and Das (2003) from North and North-East India and reported only 3 species of *Difflugia*.

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Although testate amoebae species were reported in some state fauna series of Zoological Survey of India (Das *et. al*., 2000, 2005, 2006; Bindu and Saha, 2012; Bindu and Ruby, 2015) only 36 species of *Difflugia* have been reported from India and the exact diversity of these important environmental bio-indicators have not been recorded so far and it should be stressed that the already recorded diversity of testate amoebae may be far behind the actual number and may be well revised in the light of future research. The present communication reports the extended distribution of 5 more species of *Difflugia* Leclerc, 1815 to the Indian subcontinent from moss habitats of Singalila NP, Darjeeling, West Bengal and Agastyamala BR, Tamil Nadu, India.

Materials and Methods:

The moss samples for the present study were collected during the survey to Agastyamala BR and Singalila NP as part of the annual programme of Zoological Survey of India. Moss samples (100-200gms) from tree barks, rocks and soil were collected by scraping the upper surface by quadrant sampling ($1m^2$) and brought to the laboratory in polythene envelops. The samples were cultured and processed in the lab with non-flooded petri dish method as described by Foissner (1987, 1992) and examined under the compound microscope Nikon 50i for species level identification. Permanent slides were prepared for the identified specimens and deposited in the National Zoological collections of the Marine Biology Regional Centre, Zoological Survey of India, Chennai, Tamil Nadu, India.

Results:

Systematic position

Phylum Amoebozoa Luhe, 1913
Subphylum Lobosa Carpenter, 1861
Class Tubulinea Smirnov *et. al*. 2005
Order Arcellinida Kent, 1880
Suborder Difflugina Meisterfeld, 2002
Family Difflugiidae Wallich, 1864

Genus *Difflugia* Leclerc, 1815

Difflugia genus is characterized with ovoid elongate, agglutinated test in frontal view and somewhat pyriform in lateral view; posterior border rounded and flanks converging in perceptively towards the aperture, aperture usually oval or more or less rounded, lobed or toothed, sometimes with a collar or necklace, test made up of siliceous particles. The nucleus is usually ovular, but in larger species vesicular.

Difflugia ampla Rampi, 1950

Material examined: Slide No. Mi-725,2 examples; Date of collection, 16.i. 2019, (N 8.7599° and E 77.1169°) tree moss, Agastyamala Biosphere reserve, India

Description of species

The species is characterized with a broad test and widely rounded posterior part with sides almost regularly narrowing towards the apertural region; test is mostly colourless as live and mostly yellowish when empty; test covered with mineral grains. Pseudostome round and devoid of external differentiations; the oral aperture is small in size when compared to the size of the entire shell ; length of the shell ranges from 145±148.47µm and breadth is 122±123.46µm and diameter of the oral aperture is 43±45µm (Fig.1).

Difflugia ampullula Playfair, 1918

Material examined**:** Slide Nos**.** Mi-816 and Mi-874;3exs., Date of collection, 11.iv.2019(N 27. 0600° and E 88.0200°) tree moss, Singalila NP, Darjeeling, West Bengal, India.

Description of species

The species is specific with broad test and simultaneously converging convexly towards the circular pseudostome which has slightly emerged collar. The shell is composed of medium sized xenosomes which are connected together with organic cement; length of the shell ranges from 93

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± 94.76 µm and diameter of the oral aperture is 22 ± 23.93µm (Fig.2).

Difflugia bryophila (Penard, 1902) Jung, 1942

Material examined: Slide No. Mi-810; 3exs., 11.iv.2019(N 27. 0600° and E 88.0200°) tree moss, Singalila NP, Darjeeling, West Bengal; Mi-796; 2exs., Date of collection, 19.i. 2019 (N 8. 949933° and E 77. 180500°) Agastyamala BR, India.

Description of species

Shell colourless, agglutinated, oblong and elongated; sides of the shell gradually narrowing towards the aperture; shell consists of various sizes of quartz materials and some diatom frustules or xenosomes and siliceous crystals; aperture terminal, circular and surrounded by quartz particles of small pieces; length of the shell ranges from 130±132.79 and diameter of oral aperture is 26±27.31 (Fig.3).

Difflugia lacustris (Penard, 1899) Ogden, 1983

Material examined**:** Slide No.Mi-894, 2exs; Date of collection, 11.iv. 2019; (N 27. 0600° and E 88.0200°) tree moss, Singalila NP, Darjeeling, West Bengal.

Description of species

Shell composed of a mixture of flattish pieces of quartz fragments of diatoms and some small siliceous plates; shell is transparent, colourless and elongated ovoid with a long and thin neck that makes about half of the shell length; mostly the neck has an encrustation of thicker quartz particles just anterior to the junction with the body. Small isolations of organic cement in the form of small rings are sometimes seen. Aperture circular and surrounded by small pieces of quartz particles. This species closely resembles *D. Bryophila* (Fig.4).

Difflugia ventricosa Deflandre, 1926

Material examined**:** Slide No.Mi 839; 3 examples, Date of collection, 11.iv. 2019, (N 27. 0600° and E 88.0200°) rock moss, Singalila NP, Darjeeling, West Bengal.

Description of species

Test brown in colour and elongated and slightly broadening from the oral aperture towards the fundus and at the end turning into a spike; test is covered with irregularly shaped sand grains and diatom frustules; aperture is rounded and encrusted with small sized mineral grains; length of the shell ranges from 117± 119. 43 µm and diameter of oral aperture is 20.1 µm ± 22.77µm (Fig.5). But here size is slightly smaller than the already reported species.

Discussion:

The present communication reports the extended distributional range of five species of testate amoebae under the genus *Difflugia* to Indian testate fauna from Agastyamala BR and Singalila National Park and testate amoebae studies were not done in these areas previously. *Difflugia* Leclerc, 1815 is the oldest and abundant genus of testate amoebae. The abundance of species under the genus *Difflugia* may be due to combination of inadequate descriptions and the lack of good diagnostic features (Yuri Mazei and Alan Warren, 2012). There is difficulty with several groups of individuals which shared common features and thus some of the individuals are designated as varieties. Even slight variations in the shell shape have resulted in the establishment of new forms or species regardless of the range of variability that individual *Difflugia* taxon exhibits. Intensive studies on testate amoebae in India initiated only in the 2nd half of the 20th century (Nair *et. al*., 1971; Das *et. al*., 1993, 1995, 2006; Piyali and Das, 2003; Bindu, 2013, 2019). Perusal of literature revealed that 36 species under the genus *Difflugia* have been reported from India (Table-1). This figure could have come up far above if thorough and

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intensive studies have been made. It is also observed from the literature that no studies have been performed in many states of India and should be made in a priority manner to figure out the exact diversity of these bio indicators in India.

Table: 1. Distribution of *Difflugia* Leclerc, 1815 species along different states of India.

Species	Different states of India															
Family Difflugiidae	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. *Difflugia avellana* Penard, 1890					+				+						+	
2. *Difflugia acuminata* Ehrenberg, 1838	+	+				+		+	+		+			+		+
3. *Difflugia acutissima* Deflandre, 1931																+
4. *Difflugia amphora* Leidy, 1874		+														
5. *Difflugia amphoralis* Hopkinson, 1958									+							+
6. *Difflugia arcula* Leidy, 1879					+										+	+
7. *Difflugia bacillariarum* Perty, 1958																+
8. *Difflugia binucleata* Penard, 1902						+							+			
9. *Difflugia brevicolla* Cash, 1909																+
10. *Difflugia capreolata* Penard, 1902																+
11. *Difflugia corona* Wallich, 1864	+	+				+							+	+	+	+
12. *Difflugia curvicaulis* Penard, 1899																+
13. *Difflugia carinata* Van Oye, 1956		+														
14. *Difflugia difficilis* Thomas, 1954		+														+
15. *Difflugia elegans*															+	+

Penard, 1890																
16. *Dfflugia geosphaira* Ogden, 1991					+								+			
17. *Difflugia globularis* Wallich, 1909																+
18. *Difflugia globulosa* Dujardin, 1837		+				+			+		+				+	+
19. *Difflugia globulus* Ehrenberg, 1990																+
20. *Difflugia gramen* Penard, 1902		+														+
21. *Difflugia kabylica* Gauthier-Lievre&Thomas, 1958		+														
22. *Difflugia lebes* Penard, 1899					+	+										
23. *Difflugia lobostoma* Leidy, 1879		+	+	+	+	+		+	+	+	+			+	+	+
24. *Difflugia lucida* Penard, 1890					+	+				+		+			+	
25. *Difflugia lithophila* Penard, 1902				+		+	+		+					+	+	+
26. *Difflugia lismorensis* Playfair, 1917																+
27. *Difflugia muriculata* Gauthier-Liever&Thomas, 1958																+
28. *Difflugia muriformis*Gauthier-Liever&Thomas, 1958					+											+
29. *Difflugia oblonga* Ehrenberg, 1838	+				+				+			+	+			+
30. *Difflugia oblonga* var. *muscicola* Ehrenberg,1838			+	+	+										+	+

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31. *Difflugia oviformis* Cash & Hopkinson, 1909						+										
32. *Difflugia penardi* Cash & Hopkinson, 1909		+														
33. *Difflugia pyriformis* Perty, 1848		+							+		+	+			+	+
34. *Difflugia rubescens* Penard, 1902					+											
35. *Difflugia tuberculata* Wallich, 1864	+							+	+							+
36. *Difflugia urceolata* Carter, 1864	+				+								+			+

Explanation remarks : 1-Assam, 2-Andhra Pradesh, 3-Arunachal Pradesh, 4-Chandigarh,5-Himachal Pradesh, 6- Kerala,7-Maharashtra,8-Manipur, 9-Meghalaya, 10-Nagaland,11-Rajasthan, 12-Sikkim,13-Tamil Nadu,14-Tripura, 15-Uttar Pradesh, 16-West Bengal

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Plate-1

Fig.1 *Difflugia ampla* Rampi, 1950

Fig. 2 *Difflugia ampullula* Playfair, 1918

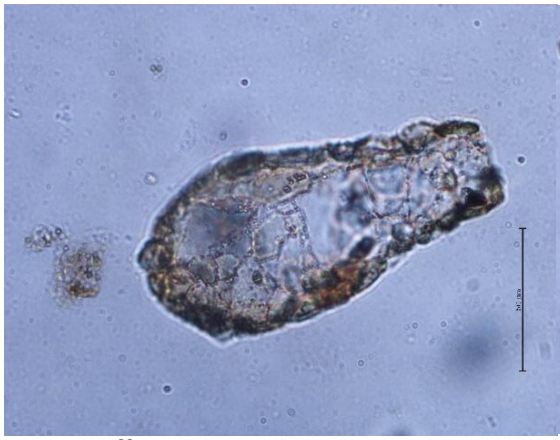

Fig.3 *Difflugia bryophila* (Penard, 1902) Jung,1942

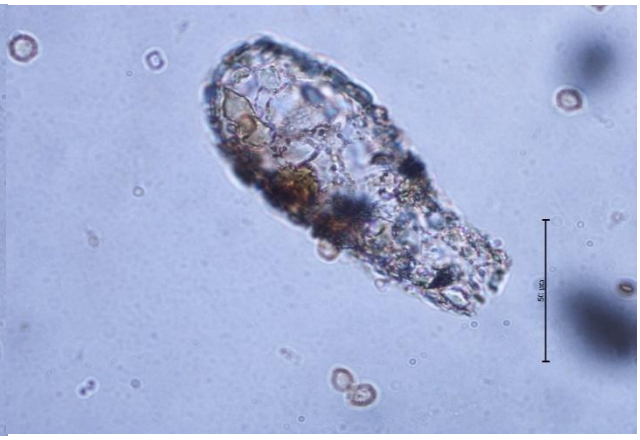

Fig.4 *Difflugia lacustris* (Penard,1899) Ogden,1983

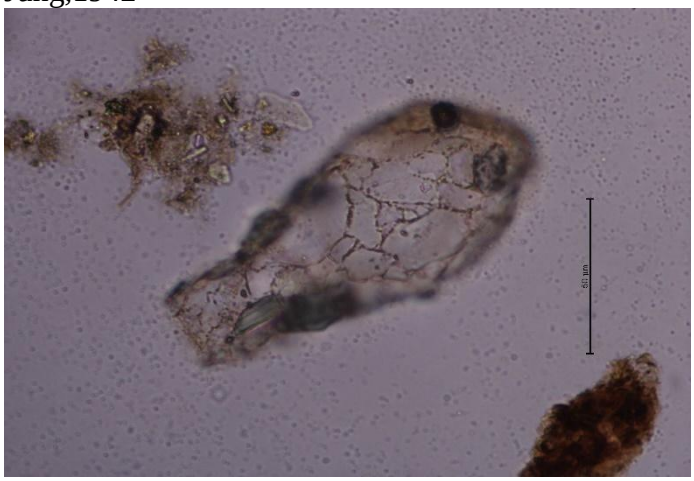

Fig.5 *Difflugia ventricosa* Deflandre, 1926