Journal of the Palaeontological Society of India
Volume 65(1), June 30, 2020: 81-89
ISSN 0552-9360

DISTRIBUTION OF TESTATE AMOEBAE (THECAMOEBIANS) IN A DESICCATING LAKE, INDIA

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ABSTRACT

Distinct assemblages of testate amoebae can be correlated to a variety of environmental and climatic parameters. Among these factors, the substrate type, trophic status, dissolved oxygen and water table fluctuations are crucial for the proliferation of testate amoebae. Most of the research on testate amoebae has long been focused on a variety of ecosystems at mid-high latitudes; there is little data from the tropics in comparison. The purpose of this research is to contribute to this insufficient database by documenting testate amoebae in lake associated with *Eichhornia crassipes* macrophyte affected by annual extreme conditions of desiccation during summers in northern part of India. Fifteen species of testate amoebae belonging to six genera were identified. *Centropyxis laevigata* are in abundance and are excellent indicators of lake ecosystem stressed by seasonal desiccation and expansion facilitated by excess evapotranspiration from aquatic macrophyte mat over the lake surface. *Arcella artocrea*, *A. vulgaris* from the sediment-water interface beneath the *Eichhornia* mat shows its hydrophilous nature and hence are the markers of permanently wet oligotrophic conditions. Testate amoebae are one of the detrivores that indicate the trophic status of aquatic ecosystem, climate induced ecology and therefore, have potentials for monitoring (paleo) environmental conditions. It is inferred *C. laevigata* is cosmopolitan and not restricted geographically but may be distinguished by its ecological preferences and the biotope in the high seasonality region.

Keywords: Testate amoebae, Bari lake, horizontal distribution, *Eichhornia crassipes*.

INTRODUCTION

Testate amoebae (Protozoa: Rhizopoda) are free-living heterotrophic protists which play a key role in functioning of the microbial loop (Gilbert and Mitchell, 2006). Testate amoebae can be correlated to a variety of environmental and climatic parameters (Scott and Medioli; 1983, Asioli *et al*., 1996; Patterson and Kumar, 2000; Booth and Jackson, 2003; Farooqui *et al*., 2012). The distribution of these vary in relation to key environmental factors, such as seasonality, moisture, pH, organic content and vegetation (Charman and Warner, 1992; Mazei and Tsyganov, 2007; Farooqui *et al*., 2012; Davidova and Vasilev, 2012). Heterotrophs largely dominate in the aphotic zone. Thus, the environment contains a great amount of organic matter, which is oxidized in the upper levels and reduced in the lower levels (of ecosystems) (Margalef, 1992). Such vertical differentiation is prominent in *Sphagnum* biotopes (Denisenkov, 2000). Testate amoebae are an important component of soil microbial communities and are increasingly used as ecological and palaeoecological studies (Booth, 2001; Farooqui and Gaur, 2007; Swindles *et al*., 2010; Kumar *et al*, 2011). Due to their short-generation time these are good indicators of short term seasonal environmental changes. Studies on testate amoebae from low latitudes include Brazil (Green, 1975), Peru (Haman and Kohl, 1994), Nigeria (Green, 1963), Java, Sumatra, Indonesia (van Oye, 1949; Dalby *et al*., 2000; Malaysia (Sudzuki, 1979) and the islands of Bombay (Carter, 1856, 1864). The response of testate amoebae in fresh water lake growing in association with *Lemna* (duckweed) and subjected to seasonal extremes in northern part of India has been documented (Farooqui *et al*., 2012). Here we document testate amoebae assemblage in association with *Eichhornia crassipes* under similar climatic and environmental conditions. The lake studied here is highly subjected to dessication during summers (enhanced by evapotranspiration due to *E. crassipes* mat on the water) and expands during rainy season. We explored the horizontal distribution patterns of testate amoebae in surface sediment covered by *Eichhornia crassipes* mat, down to a scale of few cms during summer season.

STUDY AREA

Bari Tal (Fig.1) is situated about 20 km east of the Lucknow city (India) between 26º 58′ 19″ Latitude and 80º 57 35″ Longitude in the vicinity of the Gohana Kala Village. The Bari Tal'(lake) is irregular in shape and quite large in dimension, measuring 100 m in length and 50 m in breadth at its widest. The lake is highly waterlogged and overgrown with *Eichhornia crassipes* (Figs. 2 and 3), a common noxious weed of ponds and lakes in the Central Ganga Plain, impeding the proliferation of other aquatic flora and fauna. During summer season the *Eichhornia crassipes* mat affixes the lake bottom due to low water level in the periphery which eventually dries off as the soil desiccates, but it remains free-floating in centre with water depth not exceeding 0.5-0.7m. The lake is largely fed by the subterranean water as well as by a stream on the western side. Most of the flat area on its west and south is under intensive agricultural practice. However, far-flung area beyond the cultivated land is marked by the presence of open *Acacia*-scrub vegetation. Presently, plantation of *Prosopis julifera* has been made about 400 m north of Gohana Kala Village in order to reclaim the wasteland under afforestation program.

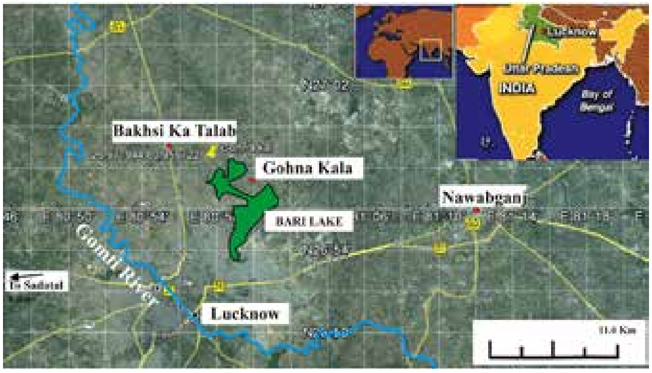


Fig. 1. Location map of the Study area.

CLIMATE

The climate of the region, in general, is humid and it is greatly influenced by the southwestern monsoon. The average minimum and maximum temperatures from November to February (winter season) is 7.6ºC and 21ºC respectively. The temperature occasionally drops to 0ºC during the coldest month of January. The average minimum and maximum temperatures from March to June (summer season) is 27ºC and 32.5ºC respectively. The temperature increased to 46ºC in the month of June during the time of sample collection. However, monsoon season commences in the mid-June and continues till mid-September which gradually lowers temperature to 30-32 ºC. The weather gets very warm and humid during July to September. The lake water expands in a fortnight with the onset of monsoon.

MATERIALS AND METHODS

A total number of 10 surface soil samples were picked up from the lake site at an interval of 100 m for a pollen analytical investigation. Ten gram of sample was boiled in 10% aqueous KOH solution to deflocculate the pollen/spores and other biological forms from the sediments. This is followed by the treatment of samples with 40% HF to dissolve the silica content. Thereafter, the samples were acetolysed (Erdtman, 1943) to remove the cytoplasmic content of pollen/spores and other biological forms to make them more discernible, while examining under microscope (Olympus BX-51). During the course of palynological investigation we have come across a large number of testate amoebae that have been categorized on the basis of lake moisture/water condition which varies from periphery (desiccated- Fig. 3) to the central part (50 cm water depth- Fig. 2) during the month of June. The identification of the testate amoebae is following Ogden and Hedley (1980), Leidy (1879) and Kumar and Dalby (1988).

The Shannon Diversity Index (SDI) was used to examine the diversity of each sample (Shannon, 1948). A two-way cluster analysis (using relative Euclidean distance) was carried out to discriminate groups of samples and species. Species data was Hellinger transformed (Legendre and Gallagher, 2001) and a Redundancy Analysis was carried out using R (R Core Team, 2012) to examine species-environmental variable relationships.

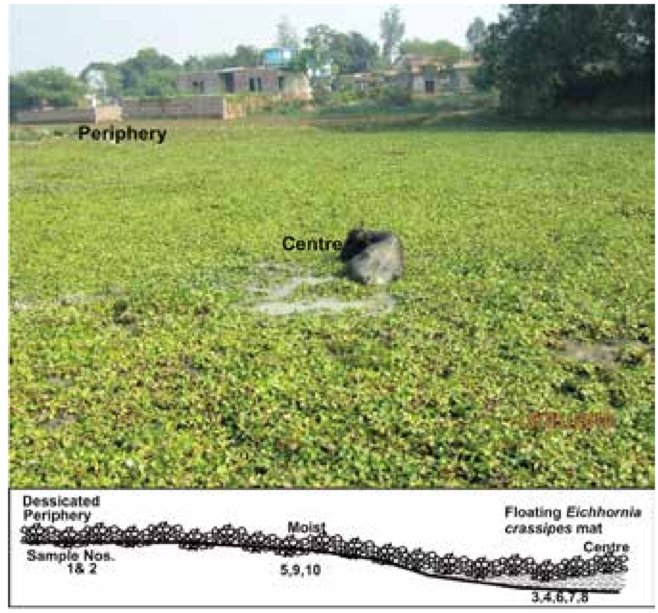


Fig. 2. The central part of the Bari Lake profusely covered by *Eichhornia crassipes*.

RESULTS

Testate amoebae (thecamoebian) results provide here a greater understanding of relationships between microenvironmental factors in a biotope. Within the limits of the shrinking/ desiccating lake ecosystem three types of testate amoebae community were identified in horizontal distribution. These are 1) xerophilous, 2) hygrophilous and 3) hydrophilous. Fifteen species belonging to six genera were identified (Plate I and II) which belong to five Families- Arcellidae, Centropyxidae, Trigonopyxidae, Hyalospheniidae and Difflugiidae.

Desiccated surface sediment of lake (*Xerophilous assemblage*)

The percentage of total count of testate amoebae species in sample numbers 1 and 2 (Fig. 4) show total 8 species with high values of cyst forms (45.1 & 43.2%, respectively) showing strong lineage to testate amoebae tests. Other forms recorded here constitute *A. megastoma* (2.4%), *A. vulgaris* (2.4%), *Centropyxis laevigata* (26.8%), *Centropyxis constricta* (4.9%). *Cyclopyxis arcelloides* (8.5%) and *Nebela* type (9.8%) species in Sample1. However, in Sample 2 the percentage of *A. megastoma* is 5.4%, *Centropyxis aerophila* 'sylvatica' (1.45%), *C. constricta* (5.4%), *C. laevigata* (21.6%), *Cyclopyxis arcelloides* ((5.4%), *Cyphoderia calceolus* (4.1%), *Nebela* type (9.8%) and cyst forms (43.2%).

EXPLANATION OF PLATE I

Plate 1 and 2. (Light Microscopic photomicrographs of testate amoebae: All scale = 10µm)

Fig. 1. ***Arcella vulgaris***, Fig. 2. ***A. discoides***, Fig. 3. ***A. megastoma***, Figs. 4-5. ***A. arenaria***, Fig. 6. ***A. catinus***, Fig. 7. ***A. artocrea***, Fig. 8. Enlarged pseudostome of ***A. artocrea***, Figs. 9-14. ***Centropyxis arcelloides***, Figs. 15-16. ***Centropyxis aerophila*** *'sylvatica*, Fig. 17. ***Centropyxis aerophila***, Fig. 18. ***C. constricta***, Fig. 19. ***Cyclopyxis arcelloide****,* Fig. 20. Incerta sp., agglutinated sphere of unknown affinity, Figs. 21-22. Unidentified ***Nebela*** type, Figs. 23-24. ***Cyphoderia calceolus***.

Journal of the Palaeontological Society of India
Volume 65(1), June 30, 2020

Plate I

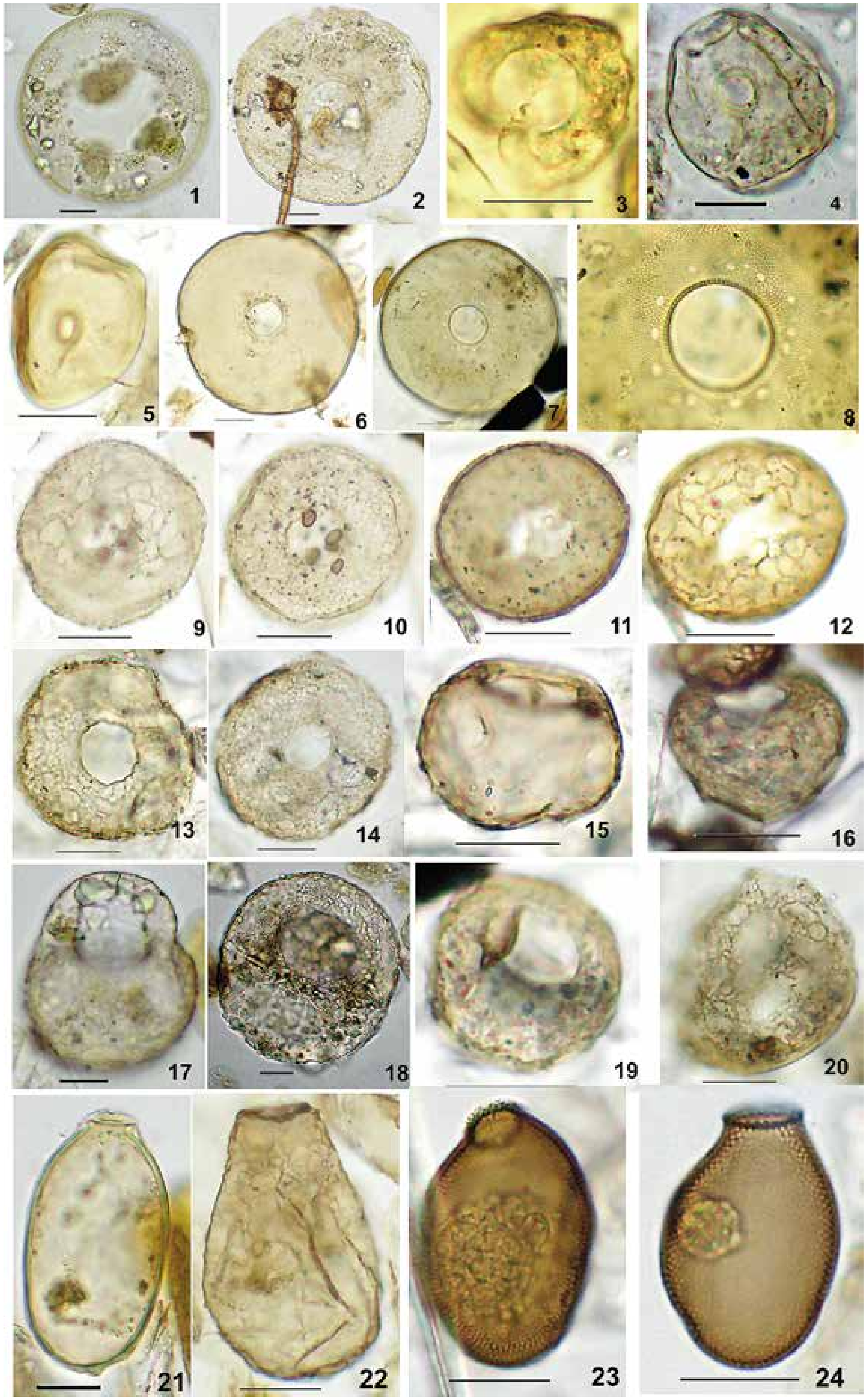


Fig. 3. The desiccated part of the Bari lake from where the trench samples are being retrieved.

Moist surface sediment of the lake (*Hygrophilous assemblage*)

The analysis of sediment samples 5, 9 and 10 (Fig. 4) show total 14 species with an average percentage constituting *Arcella arenaria* (1.1%), *A artocrea* (15.5%), *A. catinus* (6.9%), *A. discoides* (4.8%), *A. megastoma* (3.9%), *A. vulgaris* (2.7%), *C. aerophila* (2.5%), *Centropyxis aerophila* 'sylvatica' (1.0%), *C. arcelloides* (5.3%), *C. constricta* (1.1%), *Centropyxis laevigata* (19.9%), *Cyclopyxis arcelloides* (8.4%), *Cyphoderia calceolus* (1.6%) *Nebela* type (12.7%), Unidentified *Certisella* type (0.5%), Unidentified (1.3%) and Cyst forms (10.8%).

Sediment–water interface (Floating *Eichhornia crassipes* mat) (Hydrophilous)

The samples 3,4,6,7 and 8 contain high abundances of testate amoebae (Fig. 4). Total 16 species were identified with the average percentage as *Arcella arenaria* (2.0), *A. artocrea* (15.8%), *A. catinus* (4.0), *A. discoides* (5.6%), *A. megastoma* (8.9), *A. vulgaris* (6.1%), *C. aerophila* (2.4%), *C.aerophila* 'sylvatica'(1.5%), *Centropyxis arcelloides* (8.2%), *C. constricta* (3.7%), *C. laevigata*- about seven strains exhibiting morphological variations in the test (20.4%), *Cyclopyxis arcelloides* (6.8%), *Cyphoderia calceolus* (0.9), *Difflugia oblonga* type (0.4%), *Nebela* type (8%), Unidentified *Certisella* type (0.4%), Other unidentified forms (0.3%), testate amoebae Cysts (4.6%).

The cluster analysis shows three groups of data, samples 1-2, samples 3,9 and 10 and samples 4-8 which supports the qualitative description above (Fig.5). In the RDA, axis 1 explains 58% of the variance, whereas axis 2 explains 10% (Fig. 6). The RDA shows that the communities are driven primarily by changes in moisture, which cause changes in pH and Salinity. A permutation test (999 permutations) on individual terms shows that moisture ($p < 0.001$) and salinity ($p < 0.01$) are the most important controls on the distribution of testate amoebae.

DISCUSSION

Testate amoebae abundances in surface sediment samples show here a general increasing trend from desiccated lake periphery to central part of the lake where the maximum water depth is 0.5-0.7 m. It has been documented earlier that testate amoebae communities are determined by the moisture and temperature conditions available to them (Chiba and Kato, 1969). When the conditions are dry, many rhizopod amoebae can encyst (Heal, 1962; Chardez 1990), thus escaping the need for water during long/short periods of drought (Hingley, 1993). Construction of cases/tests may help them to survive brief dry periods, but most encysted until favorable moisture returns. Thus in the desiccating surface, it is likely to get high number of cyst forms/resting stages of testate amoebae. *Centropyxis* and *Arcella* are among the most common of the testate amoebae recorded in the present study which is also quite common among epiphytic bryophytes, lakes, bogs and aquatic macrophytes across the globe. These are commonly found in a fresh water lake ecosystem and survive the wet-dry changes where temperature and precipitation largely varies seasonally (Farooqui *et al*., 2012) and are found as very well preserved fossils in palynological slides as well (Farooqui and Naidu, 2010; Farooqui and Gaur, 2007; Kumar *et al*., 2011; Payne, 2012).

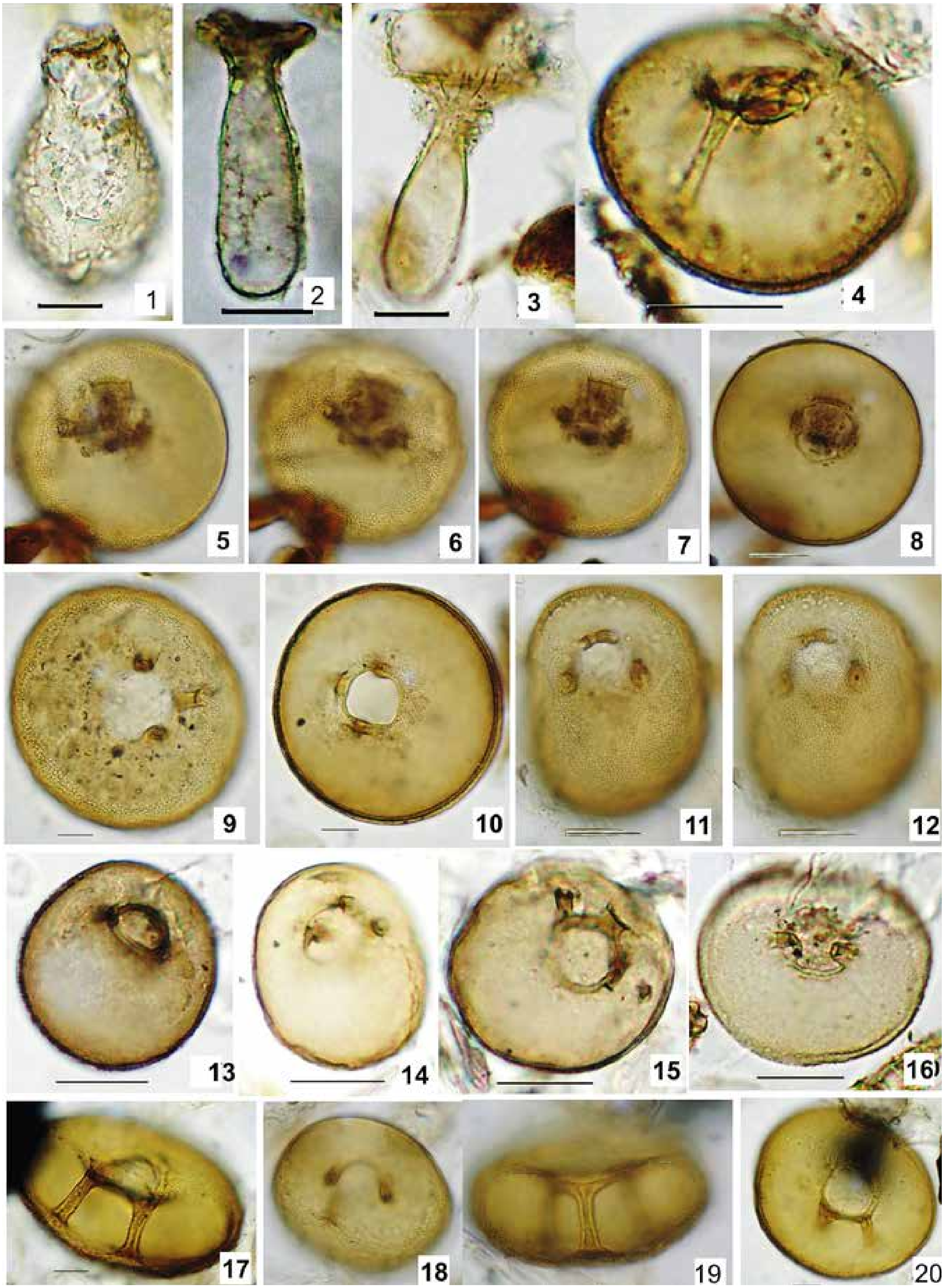
Our results, show high percentage of *Centropyxis laevigata*, *A. artocrea* along with the other species and low cyst forms in sediment-water interface which serve as good indicators of moist conditions that are infested by *Eichhornia crassipes* macrophyte (Wolverton and Mcdonald, 1978) in particular. *Centropyxis laevigata* are in abundance in the studied sediment which can be regarded as excellent indicators of lake sediment stressed by seasonal desiccation and expansion of lake facilitated by excess evapotranspiration from aquatic macrophyte mat over the lake surface. Similar description of *Centropyxis aculeata* is reported from ponds and ditches on wet moss and *Sphagnum* and is most common in swampy ground (Ogden and Hedley, 1980). *C. laevigata* are also found commonly in tundra landscapes of the arctic mainland and islands region and is regarded as cosmopolitan hygro-hydrophillic species (Bobrov and Wetterich, 2012). Thus, it is inferred that this species is although cosmopolitan and not restricted geographically but may be distinguished by its ecological preferences and the biotope in the region. It is also interesting that no *Centropyxis laevigata* were recorded from Sadatal lake sediments (Farooqui *et al*., 2012) existing about 10km from the studied Bari lake under similar climate and environmental conditions but in association with duckweed (Lemna).This lake however, did not shrink much during summer season but the water column reduced.

Arcella species generally occur in constantly wet conditions, low in nutrients, and in a pH range of 4-6, but also occur elsewhere (Patterson and Kumar, 2000). The abundance of *Arcella artocrea*, *A. vulgaris* from the sediment-water interface beneath the *Eichhornia crassipes* mat in the central part of lake shows its hydrophilous nature and hence are the markers of permanently wet conditions. The densities of testate amoebae

EXPLANATION OF PLATE II

Fig. 1. ***Difflugia oblonga*** type, Figs. 2-3. Unidentified ***Certisella*** type, Fig. 4. Cyst form of thecamoebians, Figs. 5-8. ***Centropyxis laevigata*** strain 1, Fig. 9. *C.* ***laevigata*** strain 2, Fig. 10. ***C. laevigata*** strain 3, Figs. 11-12. ***C. laevigata*** strain 4, Figs. 13-14. ***C. laevigata*** strain 5, Figs. 15-16 ***C. laevigata*** strain 6, Figs. 17-20. ***C. laevigata*** strain 7.

Plate II



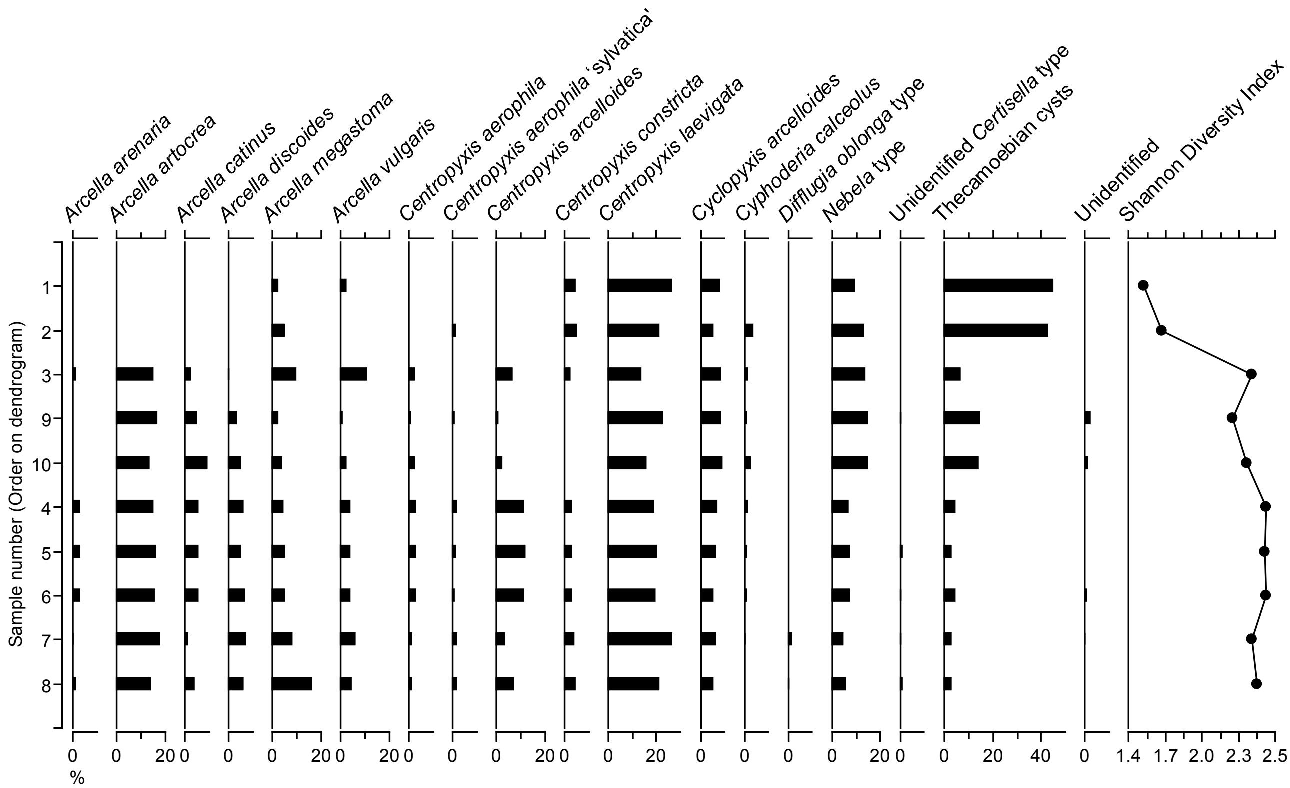


Fig. 4. Percentage occurrence and Shannon diversity index values of testate amoebae communities in surface samples of Bari Lake.

are considerably smaller in eutrophic lakes than in oligotrophic lakes (Schonborn, 1962). The principal factor involved is the oxygen content of the water overlying the sediment (Tolonen, 1966; Farooqui *et al.*, 2012). The profuse growth of *E. crassipes* tends to alter dissolved oxygen, nitrogen, phosphorous and other contaminants from the water (Reddy and Tucker, 1983). The complex structure provided by macrophytes provide suitable microhabitats for epiphytic zooplankton (Arora and Mehra, 2003) and testate amoebae are among these detrivores which help in self-cleaning of water bodies and maintain the trophic status. It is suggested that the *C. laevigata* in particular has potentials for monitoring (paleo) environmental conditions in a high seasonality region,

Table 1. Nature of substrate and Testate amoebae community in association with *Eichhornia crassipes* in Bari lake.

Testate amoebae (Thecamoebians) +, rare; ++, common; +++, Abundant; NR- not recorded	Ecological Substrate and Soil Moisture		
	Permanently wet 73, 72, 78, 62 & 58 %	Hygrophylic (Moist substrate) 43,41 &38 %	Xerophilic (Dry substrate) 19 & 21%
Arcella arenaria Greeff, 1866	+++	+	NR
A.artocrea Leidy, 1876	+++	+	NR
A. catinus Penard, 1890	+++	+	NR
A. discoides Ehrenberg, 1843	+++	+	NR
A. megastoma Penard, 1902	+++	+	+
A. vulgaris Ehrenberg, 1830	+++	+	+
Centropyxis aerophila Deflandre, 1929	+++	+	NR
C.aerophila 'sylvatica' Deflandre, 1923	+	+	++
Centropyxis arcelloides Penard, 1902	+++	++	NR
C. constricta (Ehrenberg, 1838) Deflandre, 1929	+++	++	++
C. laevigata Penard, 1890	+++	++	+
Cyclopyxis arcelloides (Penard 1902) Deflandre, 1929	+++	++	+
Cyphoderia calceolus Penard, 1899	+	+	++
Difflugia oblonga type	+	NR	NR
Nebela type	+	+	+
Certisella type	+	+	NR
Cysts	+	++	+++

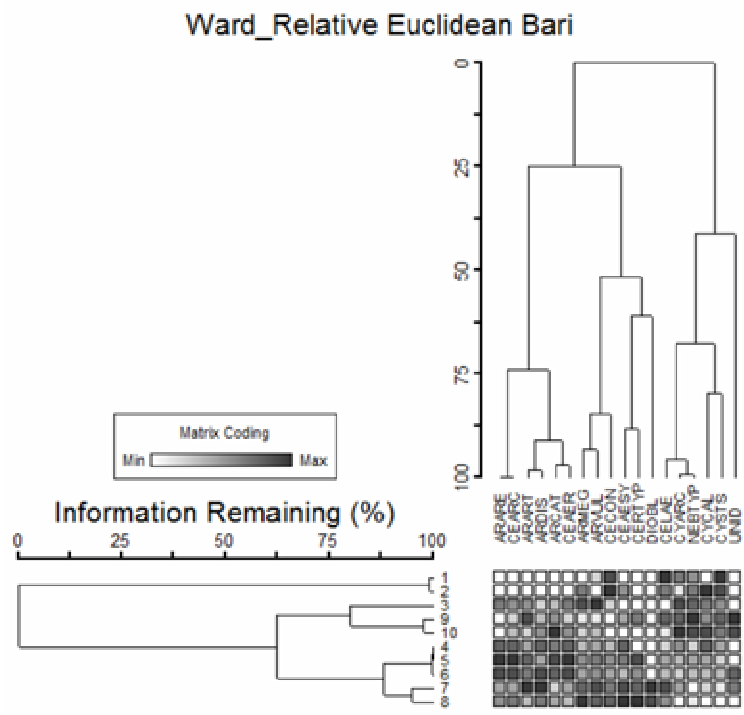


Fig. 5. A two-way Cluster analysis of the testate amoebae present in Bari lake.

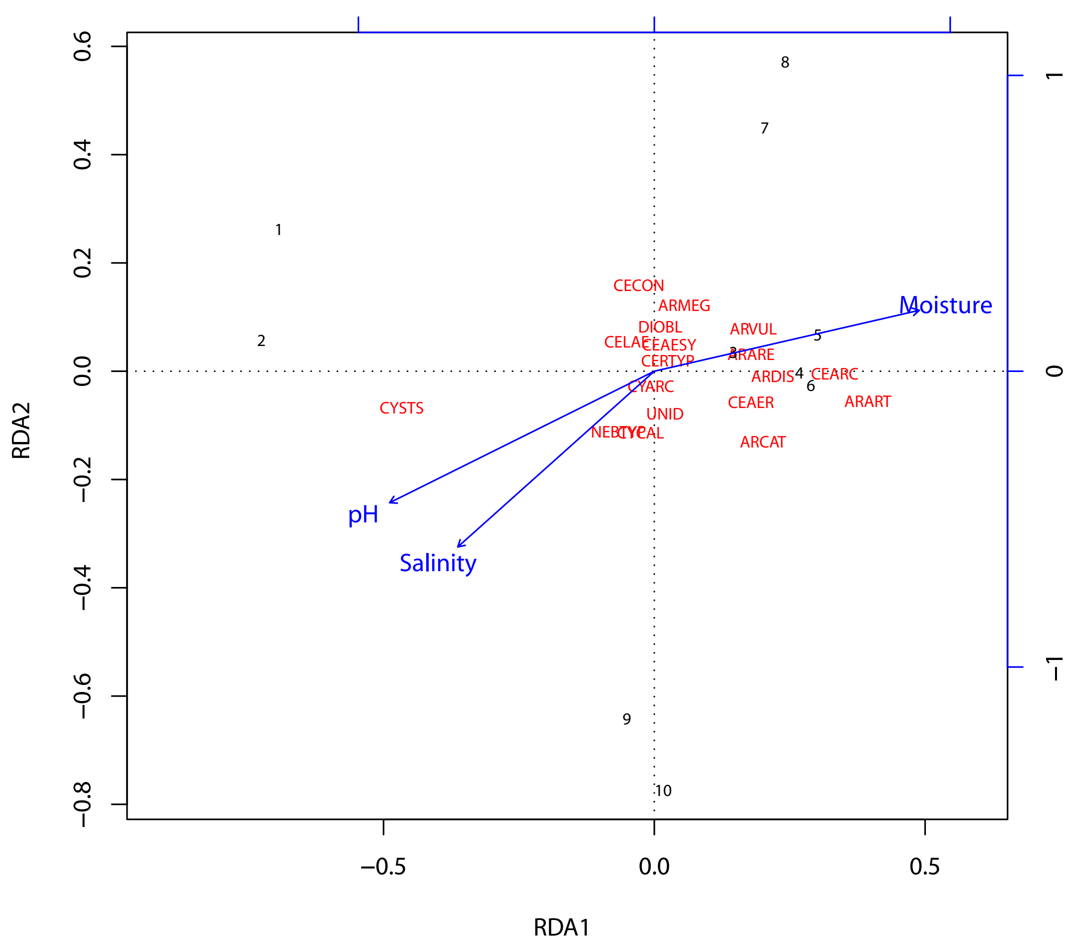


Fig. 6. Redundancy Analysis to examine species-environmental variable relationships.

CONCLUSIONS

The study reveals the variable horizontal gradient in testate amoebae community assemblage in response to moist/wet/dry conditions of the lake induced by seasonal extreme variations, primarily moisture in the substrate. The abundance of *Centropyxis laevigata* here indicates its hygro-hydrophillic nature. Whereas, *Arcella vulgaris* and *A. artocrea* occupy permanently wet oligotrophic conditions. Differences in other testate amoeba assemblages and its sensitivity to micro-habitat variation here facilitate the understanding of ecological characteristics of the exact spot where they lived. Therefore, they can be used to analyse small-scale gradients of (palaeo) climate induced functioning of lake/peatland ecosystems that may help in improving the high resolution precision of inferences drawn in palaeoecological studies.

ACKNOWLEDGEMENT

The authors thankful to the Director Birbal Sahni Institute of Palaeosciences for providing necessary facilities and co-operation to accomplish this work.

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Manuscript received : November 2017

Manuscript accepted : December 2019