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Thecamoebian communities as proxies of seasonality in Lake Sadatal in the Ganga-Yamuna Plains of North India

Anjum Farooqui, Arun Kumar, and Graeme T. Swindles

ABSTRACT

Thecamoebians are testate protists that occur in a variety of freshwater habitats and brackish environments. They have been successfully used as proxies for a variety of environmental and climatic parameters in limnological and paleolimnological studies. The perennial Lake Sadatal is situated near the small town of Mallanwan (Latitude 27° 3' 0" North and Longitude 80° 9' 0" East) in the Ganga-Yamuna Plains of North India. Lake Sadatal is a shallow remnant of a past oxbow lake left by the meandering Ganga River and its tributaries (maximum depth ~1.5 m during summer and ~3.0 m during July-August monsoon season). The soil around this region is saline, sodium rich, and the maximum soil alkalinity is pH 10. This region shows strong seasonality, and average atmospheric temperatures during winter (December-March) range between 7–20°C and during summer (April–June) range between 21- 45°C (2005–2007). Taxonomically diverse and mixed thecamoebians were recovered from Lake Sadatal showing distinct summer and winter communities for three years. Centropyxids and Arcellenids dominate the low humidity, low precipitation cooler months (October-March) whereas *Amphitrema* spp. and *Difflugia oblonga* "*triangularis*" dominate summer and the high precipitation, high humidity monsoon months (April-September). Dominance of *Amphitrema* spp. is related to abundance of aquatic weed *Lemna* detritus at the lake bottom during summer. Total concentrations of thecamoebians were higher during summer monsoon months than winter.

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KEYWORDS: Acid resistant thecamoebians; seasonality; lake sediments; Ganga-Yamuna Plains; India

INTRODUCTION

Thecamoebians are an artificial polyphyletic group of protozoans also known as agglutinated rhizopods or testate amoebae, and Arcellaceans are a supergroup within thecamoebians (Loeblich and Tappan, 1964). These are unicellular testate (shelled) forms that occur widely in a variety of freshwater habitats (Medioli and Scott, 1983, 1988) and also in marginally brackish environments (Charman et al., 2000; Patterson and Kumar, 2002). They have been widely reported from lake sediments from the tropics to the Arctic region (see references in Patterson and Kumar, 2002; Boudreau et al., 2005), peat bogs (Woodland et al., 1998; Charman et al., 2000; Swindles et al. 2010), and salt marshes (Charman et al., 2002; Roe et al., 2002; Riveiros et al., 2007). Their pre-Quaternary geological record is quite scanty but their oldest forms are reported from the Neoproterozoic (Porter and Knoll, 2000; Porter et al. 2003). Despite a long geological history thecamoebian lineages have shown minimal evolution through time (van Hengstum et al., 2007; Kumar et al., 2011).

Published thecamoebian studies are mainly from the lakes and bogs of higher latitudes in Europe and North America, but they have also been studied from the Arctic region (Beyens et al. 1990; Beyens and Chardez, 1995). There are relatively fewer thecamoebian studies from the tropical regions. Some of them are lakes in Java, Sumatra and Irian Jaya in Indonesia (Hoogenraad and Groot, 1940, 1946; van Oye, 1949; Dalby et al., 2000); lakes in Malaysia (Sudzuki, 1979); brackish water lakes and ponds of nineteenth century Mumbai, India (Carter, 1856, 1864); Bronze Age archaeological site in Porbandar, west coast of India (Farooqui and Gaur, 2007); Lake Cocococha, Peru (Haman and Kohl, 1994), Sokoto River in Nigeria (Green, 1963), wetlands of central Brazil (Green,1975), small lakes and ponds of Barbados, (Roe and Patterson, 2006) and a dam in Colombia (Escobar and Martinez, 2008). Most of these studies except Roe and Patterson (2006) are either descriptive works or simply reports of thecamoebian occurrence. Environmental and paleoenvironmental analysis of the collected data has largely been ignored. Patterson and Kumar (2000a, 2002) provide comprehensive reviews of environmental and paleoecological utility of lacustrine thecamoebians in higher latitudes. Medioli et al. (2003) present a useful bibliography of thecamoebian literature. According to Roe and Patterson (2006), "thecamoebian species can be correlated to a variety of environmental and climatic parameters, including metal and organic pollutant contamination, substrate type, salinity, levels of organics, oxygen concentration, water temperatures, water table fluctuations, humification, changes in intertidal flooding and land use change."

Thecamoebians are proving to be useful proxies for a variety of new environmental and climatic parameters also, for example, water quality indicators in the Greater Toronto area (Roe et al., 2010). Neville et al. (2010a, and in press) show that they can also be used to monitor seasonal environmental changes and ecosystem health in oil sand reclamation wetlands in Northern Alberta. Lakes within different vegetation zones of Alberta also show corresponding biogeographic variations in thecamoebian assemblages (Neville et al. 2010b).

LAKE SADATAL

The perennial Lake Sadatal is situated near the small town Mallanwan (Latitude 27° 3' 0" North and Longitude 80° 9' 0" East) in the Ganga-Yamuna plains of north India (Figure 1). There are several lakes and ponds in and around Mallanwan, and most of them are small ephemeral ponds that dry up during summer but Lake Sadatal is a perennial lake located on the eastern edge of the town (Figures 1 and 2). These lakes and ponds are remnants of past oxbow lakes left by the meandering river Ganga in this region. They are usually shallow, and their maximum depth reaches up to 3 m during the Monsoon season. Photographs of the natural surroundings of Lake Sadatal are shown in Figure 3.

The anthropogenic impact has caused severe environmental stress in Lake Sadatal. Solid biological and chemical waste generated from the town is dumped into this lake. The soil around this region is very saline, sodic, and soil alkalinity can rise up to pH 10. Pore spaces of the wet soil of this region contain varying amounts of calcium, magnesium, sodium, and potassium ions and the anions of chloride, sulphate, and bicarbonate. Small quantities of various other cations and anions are also present.

This region of India shows strong seasonality. The average atmospheric temperature during winters (December to March) ranges between 7- 20° C and during summers (April-June) between 21-45°C. Humidity is high during monsoon months from July to January covering both the south-west

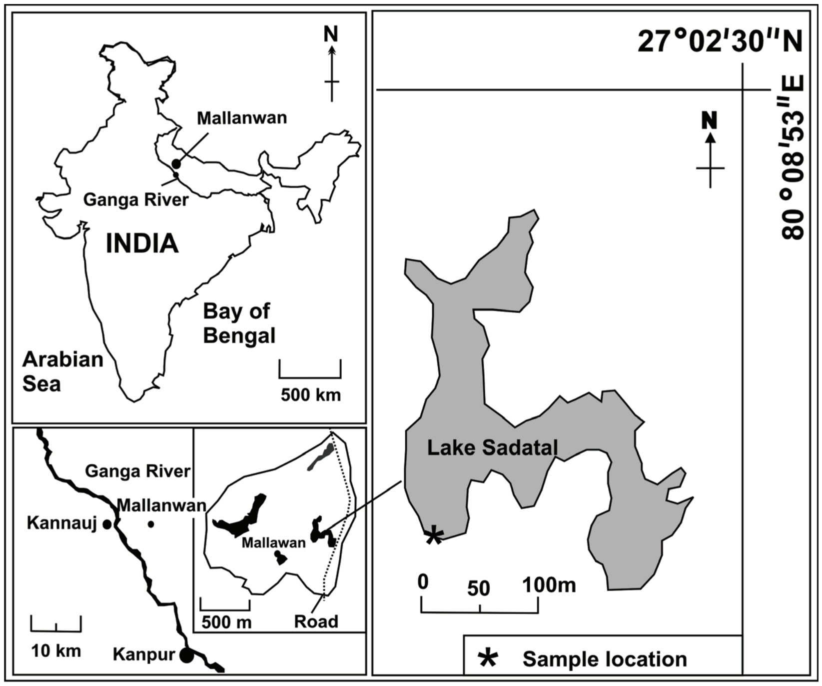


FIGURE 1. Location of Lake Sadatal in India.

summer monsoons and the north-east winter monsoons. Maximum rain fall in July is due to south-west monsoon. The average atmospheric temperature in February is 16°C followed by moderate 21°C in October and highest 45°C in May. The water temperature also varies in a similar pattern but does not exceed 32°C.

The pH of the aqueous sediment is alkaline throughout the year, which varies between a high of 9.02 during May to a low of 8.58 in February. The dissolved oxygen (DO) is minimum in May (4.58) and maximum (10.8) in October. Thus, the amount of dissolved oxygen in aqueous soil solution is dependent and inversely proportional to the temperature of water induced by seasonal temperature change. The warmer the water, the less dissolved oxygen was recorded. All the parameters like electrical conductivity (EC), total dissolved solids (TDS), salinity, sodium, potassium, magnesium, iron, copper, and lead show lowest values in October but gradually increase in February and become highest in May (Table 1).

The physico-chemical properties of the water vary with the season that controls growth of *Lemna* fronds. Its growth begins during the rainy season (July/August) and reaches its peak in October when it almost covers the entire lake surface but in February the *Lemna* fronds begin to degrade and settle at the lake bottom. By May very few *Lemna* fronds remain on the lake surface due to increased atmospheric temperature and aridity.

The chemistry of lake water shows seasonally induced chemical changes. The lowest values of nutrients like sodium, potassium, magnesium, and iron and other elements like copper and lead (probably derived from pollution) were recorded in October. This was probably due to absorption of these elements by *Lemna* from the lake water during its exponential growth phase. There is a slight increase in all these elements along with the salinity, conductivity, and total dissolved solids in February probably due to leaching of these nutrients by *Lemna* fronds and also due to drainage of nutrients from the surrounding land during the winter monsoon. The highest values of these elements were recorded in May due to addition of *Lemna* detritus settled at the lake bottom.

MATERIALS AND METHOD

The sediment-water interface samples were collected from only one location using the Van Veen grab sampler (Eijelkamp, Netherlands; capacity 180 cm^3) from the periphery of the Lake Sadatal (Figures 1 and 2). This sample location was selected because of its minimal proximity to the nearby human habitation. This study is based on 18 samples of which 12 were collected during 2005 one sample in each month and the remaining

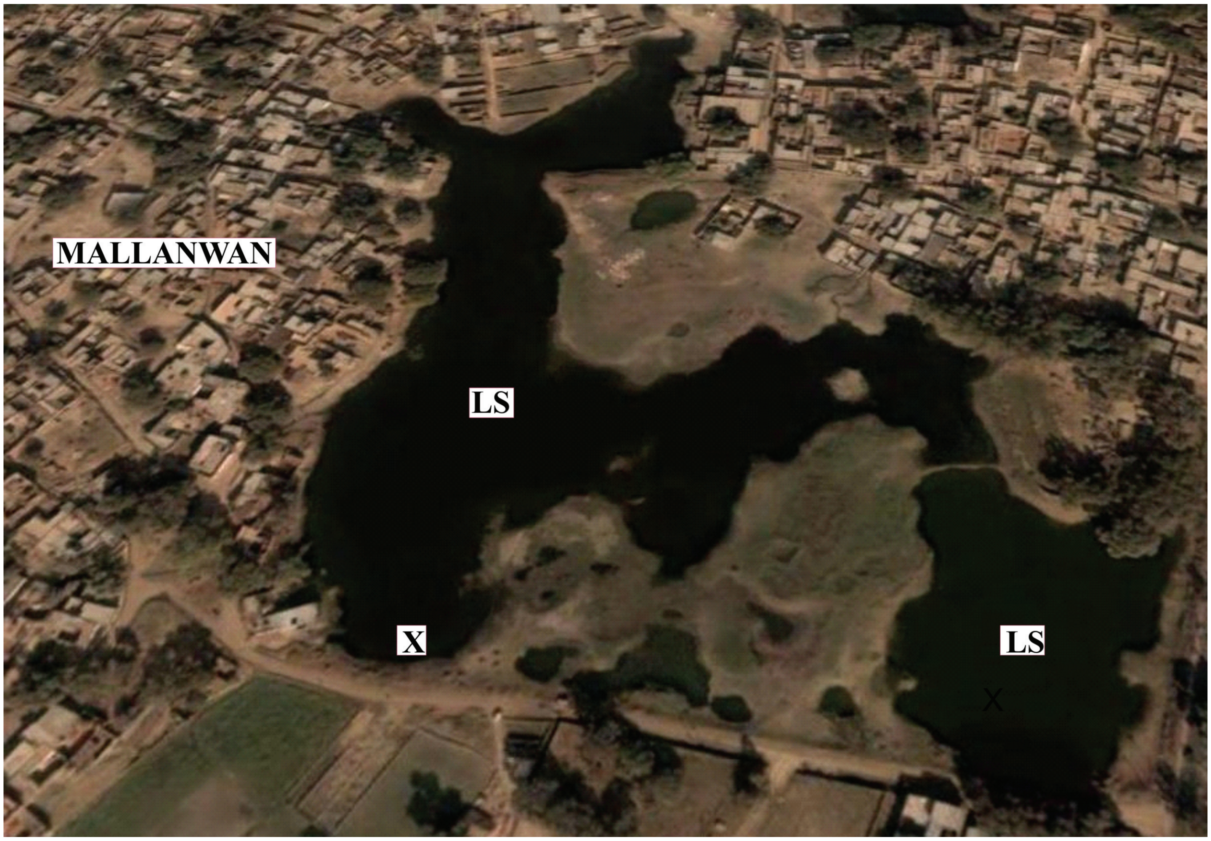


FIGURE 2. Google Map showing location of Lake Sadatal (LS = Lake sadatal; X = sample location).

six were collected in February, May, and December of 2006 and 2007. These months were chosen because of extreme weather conditions, maximum testacean counts, and significant compositional changes observed during preliminary studies carried out every month in the first year (2005) of this study. Thecamoebian counts in each sample are presented in Table 2.

Initial microscopic examination of the samples using standard micropalaeontological method (Patterson and Kumar, 2002) failed to provide any meaningful information because of smaller size, and most specimens remained dark to very dark due to clay and organic particles sticking on the tests. Thus, the palynological preparation method was used to study them on glass slides using reflected light. Five cm^3 of sediment sample was boiled in 50 ml of 5% KOH mixed with distilled water for 10 minutes. It was sieved through 150 mesh size (~105 µm) sieve to remove larger macroscopic material. Each sample was then acetolysed using acetic acid (Erdtman, 1943) and centrifuged at 1000 rpm for 5 minutes. Due to acetolysis opaque thecamoebian specimens became transparent and free from clay, and organic particles thus were easier to study under transmitted light. But due to this process we could not determine the proportion of living thecamoebians (with protoplasm) compared to dead ones. The samples were then sieved through 600 mesh size (~20 µm) to remove fine particles that often stick to the tests hindering the clarity and identification. The >20 µm size fraction was collected for this study. Before mounting on slides the residues were stained with Safranin for morphological clarity as suggested by Tolonen (1986) not to observe presence of protoplasm in the specimens.

This method of sample preparation is normally not used by micropaleontologists who study Holocene lacustrine thecamoebians where a much simpler method using water is preferred so that delicate fossils or sub-fossils are not damaged or totally destroyed (Patterson and Kumar 2000a, b, 2002). However, acid- resistant thecamoebians have been reported from the palynological slides of Holocene sediments from the fjords of Vancouver Island, Canada (Kumar and Patterson, 2002), Cretaceous sediments of Saudi Arabia (Kumar, 2011), and Permian sediments of Himalayas in India (Kumar et al. 2011). It is true that there must be

FIGURE 3. Photograph showing the lake and its surroundings.

some information loss using palynological preparations for thecamoebian studies because only autogenous proteinaceous tests are acid resistant, and most other types will not survive harsh chemical and mechanical (centrifuging at high rpm) treatment. However, rich and diverse forms of thecamoebians were recovered from all sediment-water interface samples from Lake Sadatal using this method. This method also shows a new way to study thecamoebians from pre-Quaternary soft and hard sediments. Two slides of each sample were prepared and all the specimens were counted. Percentage abundances of each taxa are presented in Figures 4 and 5. The nomenclature and identification of thecamoebians is after Tolonen (1986) and Kumar and Dalby (1998).

The physical and chemical data about sediment samples were measured in the field and in laboratory. For this purpose 10g of dried sample (at 50º C) was dissolved in 100 ml of de-ionized water and kept overnight after rigorous shaking for one hour. Parameters like pH, dissolved oxygen (DO),

TABLE 1. Abiotic characteristics of surface sediments (sediment-water interface) in Lake Sadatal during 2005.

PARAMETERS	Jan	Feb	March	April	May	June	July	Aug	Sept	Oct	Nov	Dec
pH	7.2	7.0	7.0	7.0	8.4	8.0	8.0	8.0	7.6	7.0	7.0	7.0
Salinity ppt	0.9	1.4	1.5	1.5	1.8	1.9	1.8	1.6	0.9	0.7	1.0	0.6
EC, mS/cm	.432	.628	.701	.714	1.67	1.84	1.70	.843	.532	.242	.689	.172
TDS, mg/L	643	889	899	1011	1236	1432	1289	954	789	345	634	532
AverageAtmospheric Temperature, °C	15.5	21.8	24	30	31.3	31.1	29.4	29.4	29.4	26.6	20.9	16.6
Temp. Minimum	7.6	13.6	15.9	20.5	24.9	25.6	25.3	25.2	24.5	20	13.7	9.8
Temp. Maximum	25.8	31.4	33.5	39.3	38.7	38.1	34.6	34.7	35.2	34.4	29.5	25.4
Dissolved oxygen in Water (mg/L)	8.6	7.8	7.0	6.5	4.6	3.2	4.0	5.7	4.9	6.2	9.5	9.0
Sodium, (mg/g)	89.2	107.8	112	134	174	157.3	184.5	197.2	178.2	98	84	97.4
Potassium, (mg/g)	67	80.84	72	98	112	89	102	123	97.3	45.9	57.3	47.8
Magnesium (mg/g)	32.1	24.3	43	44	48	49.7	46	32	22	25	11	23.8
Iron (mg/g)	.46	.59	.51	.84	.89	.93	.87	.65	.57	.32	.43	.37
Copper (mg/g)	.34	.22	.29	.42	.67	.94	.86	.91	.75	.67	.42	.21
Lead (mg/g)	.10	.13.4	.21.	.23	.26	.32	.28	.10	.9	.9	.12	.8

TABLE 2a. Numerical distribution of thecamoebians in 18 samples from Lake Sadatal (numbers within parenthesis are percentage values).

2005	Total Count	*Centropyxis aculeata"discoides"*	*C. aculeata "spinosa'*	*C.aculeata"aculeata"*	*C. sylvatica type*	*Cyclopyxis kahlii*	*Arcella vulgaris*	*Arcella discoides*	*Arcella c.f. discoides var. pseudovulgaris*	*Difflugia oblonga "oblonga"*	*Difflugia oblonga"glans'*	*D. urens*	*D. gramen*	*D.pulex*	*D. gramen*
Jan	65	7 (10.8)	8 (12.3)	18 (27.7)	1 (1.5)	2 (3.1)	2 (3.1)	2 (3.1)	1 (1.5)	6 (9.2)	2 (3.1)	1(1.5)	2 (3.1)	0	2 (3.1)
Feb	61	2 (3.3)	6(9.8)	22 (36.1)	0	3 (4.9)	3 (4.9)	2 (3.3)	0	4 (6.6)	2 (3.3)	1 (1.6)	2 (3.3)	0	2 (3.3)
Mar	49	2 (4.1)	2 (4.1)	9 (18.4)	0	1 (2.0)	1 (2.0)	-6.1	0	2 (4.1)	3 (6.1)	0	1(2.0)	4	1(2.0)
Apr	52	3 (5.8)	2(3.8)	9 (17.3)	0	1 (1.9)	1 (1.9)	1(1.9)	0	2 (3.8)	3 (5.8)	0	1(1.9)	4	1(1.9)
May	83	1 (1.2)	2 (2.4)	4 (4.8)	0	2 (2.4)	0	0	0	0	0	2 (2.4)	0	9	0
June	101	2 (2.0)	2 (2.0)	3 (3.0)	0	0	0	1(1.0)	0	0	0	1(1.0)	0	11	0
July	90	2 (2.2)	0	2 (2.2)	0	0	0	0	0	0	0	1(1.1)	0	13	0
Aug	83	1 (1.2)	0	4 (4.8)	0	0	0	0	0	0	0	0	0	7	0
Sept	75	4 (5.3)	2 (2.7)	4 (5.3)	0	0	0	0	0	2 (2.7)	0	0	0	0	0
Oct	65	7 (10.8)	8 (12.3)	16 (24.6)	1 (1.5)	2 (3.1)	2 (3.1)	0	1 (1.5)	8 (12.3)	2 (3.1)	1(1.5)	2 (3.1)	0	2 (3.1)
Nov	70	8(11.4)	6 (8.6)	20 (28.6)	0	3 (4.3)	3 (4.3)	2 (2.9)	0	7 (10.0)	1(1.4)	1(1.4)	1 (1.4)	0	1 (1.4)
Dec	74	10 (13.5)	2 (2.7)	17 (23.0)	0	1 (1.4)	1 (1.4)	3 (4.1)	1 (1.4)	11 (14.9)	2 (2.7)	0	1 (1.4)	4	1 (1.4)
2006															
Feb	62	3 (4.8)	4 (6.5)	18 (29.0)	0	2 (3.2)	3 (4.8)	2 (3.2)	0	3 (4.8)	2 (3.2)	1(1.6)	3 (4.8)	2 (3.2)	3 (4.8)
May	94	1 (1.1)	0	2 (2.1)	0	4 (4.3)	1 (1.1)	0	0	1 (1.1)	0	0	0	4 (4.3)	0
Oct	91	7 (7.7)	3 (3.3)	15 (16.5)	1 (1.1)	6 (6.6)	2 (2.2)	2 (2.2)	1 (1.1)	9 (9.9)	5 (5.5)	3 (3.3)	2 (2.2)	2 (2.2)	2 (2.2)
2007															
Feb	91	2 (2.2)	7 (7.7)	24 (26.4)	1 (1.1)	3 (3.3)	4 (4.4)	2 (2.2)	2 (2.2)	8 (8.8)	4 (4.4)	2 (2.2)	5 (5.5)	1(1.1)	5 (5.5)
May	108	2 (1.9)	2 (1.9)	3 (2.8)	0	0	0	1 (0.9)	0	0	0	1 (0.9)	0	11(10.2)	0
Nov	80	5 (6.3)	7 (8.8)	16 (20.0)	1 (1.3)	4 (5.0)	4 (5.0)	3 (3.8)	2 (2.5)	5 (6.3)	2 (2.5)	2 (2.5)	2 (2.5)	1(1.3)	2 (2.5)

salinity, electrical conductivity, and total dissolved solids were measured using 'Orion-5 Star' (Thermo-Orion, Scientific Equipment, USA) at standardized 25°C. The sodium, potassium, and calcium in the acid-digested (nitric and perchloric acid) and air dried samples were analyzed using Flame photometer (ELICO-C1-360). Magnesium, iron, copper, and lead were analyzed using Atomic Absorption Spectrophotometer (Varian Spectra AA-200).

STATISTICAL ANALYSIS OF DATA

Q-mode cluster analysis based on Ward's method (Ward, 1963) was carried out to examine community groups in the species data using the PAST software (Hammer et al., 2001). Gradient

TABLE 2b (continued).

2005	*D.pulex*	*D. pristis type*	*D. oblonga 'triangularis'*	*D.oblonga 'spinosa'*	*D. protaeformis*	*Trigonopyxis arcula*	*Nebela carinata*	*N. tincta*	*N. militaris*	*N. collaris-parvula-tincta group*	*Euglypha tuberculata*	*Amphitrema flavum*	*A. wrightianum*	*A stenostoma*	*Corythion-Trinema type*
Jan	0	0	1(1.5)	1(1.5)	2(3.1)	2(3.1)	1(1.5)	0	0	2(3.1)	0	1(1.5)	1(1.5)	2(3.1)	0
Feb	0	1(1.6)	2 (3.30	1(1.6)	1(1.6)	3(4.9)	0	0	0	1(1.6)	0	1(1.6)	0	4(6.6)	0
Mar	4	2 (4.1)	5 (10.20	4 (8.2)	0	2(4.1)	0	0	0	0	1(2.0)	2(4.1)	0	4(8.2)	1(2.0)
Apr	4	2 (3.8)	5 (9.60	4(7.7)	0	2(3.8)	0	0	1(1.9)	0	3(5.8)	3(5.8)	0	4(7.7)	1(1.9)
May	9	0	7 (8.4)	2 (2.4)	4(4.8)	1(1.2)	2(2.4)	1(1.2)	2(2.4)	0	3(3.6)	6(7.2)	11(13.3)	24(28.9)	0
June	11	0	21(20.8)	8 (7.9)	3(3.0)	0	2(2.0)	1(1.1)	3(3.0)	0	4(4.0)	3(3.0)	8 (7.9)	21(20.8)	7(6.9)
July	13	0	15 (16.7)	7(7.8)	6(6.7)	0	2(2.2)	0	2(2.2)	0	2(2.2)	2(2.2)	9(10.0)	19(21.1)	8(8.9)
Aug	7	0	14 (16.9)	2 (2.4)	4(4.8)	0	1(1.2)	0	2(2.4)	0	1(1.2)	5(6.0)	10 (12.0)	28(33.7)	4(4.8)
Sept	0	0	12(16.0)	292.7	3(4.0)	2(2.7)	1(1.3)	0	1(1.3)	0	0	3(4.0)	8(10.7)	25 (33.3)	6(8.0)
Oct	0	0	1(1.5)	4(6.2)	2(3.1)	2(3.1)	1(1.5)	0	0	0	0	2(3.1)	1(1.5)	2(3.1)	0
Nov	0	1(1.4)	2(2.9)	3(4.3)	1(1.4)	3(4.3)	0	0	0	1(1.4)	0	3(4.30	0	4(5.7)	0
Dec	4	2 (2.7)	5 (6.8)	4(5.4)	0	2(2.7)	0	0	0	1(1.4)	0	2(2.7)	0	4(5.4)	1(1.4)
2006															
Feb	2 (3.2)	1 (1.6)	2 (3.2)	2(3.2)	1(1.6)	2(3.2)	0	0	0	2 (2.2)	2(3.2)	2(3.2)	1(1.6)	3(4.8)	1(1.6)
May	4 (4.3)	0	12 (12.8)	14(14.9)	3(3.2)	3 (3.2)	3 (3.2)	2(2.1)	1(1.1)	0	2(2.1)	8(8.50	11(11.7)	21(22.3)	1(1.1)
Oct	2 (2.2)	1(1.1)	5 (5.5)	7(7.7)	2(2.2)	3(3.3)	1(1.1)	1(1.1)	0	0	1(1.1)	3(3.3)	4(4.4)	5(5.5)	0
2007															
Feb	1(1.1)	0	4 (4.4)	5 (5.5)	3 (3.3)	4(4.4)	1(1.1)	1(1.1)	1(1.1)	0	1(1.1)	2(2.2)	1(1.1)	3(3.3)	0
May	11(10.2)	0	21(19.4)	8(7.4)	6 (5.6)	1(0.9)	2(1.9)	1(0.9)	3(2.8)	2(1.9)	4(3.7)	4(3.7)	8(7.4)	21(19.4)	7(6.5)
Nov	1(1.3)	1(1.3)	1 (1.3)	4 (5.0)	2(2.5)	2(2.5)	1(1.3)	0	1(1.3)	1(1.3)	1(1.3)	3(3.8)	2(2.5)	5(6.3)	2(2.5)

lengths in the data were examined using Detrended Correspondence Analysis (DCA). Redundancy Analysis (RDA) was then used to examine the relationships between thecamoebian taxa and environmental variables in the 2005 data. The analyses were carried out using Canoco version 4.5 (ter Braak, 2002).

RESULTS AND DISCUSSION

Since thecamoebians inhabit the sediment-water interface they are highly susceptible to environmental and climatic response. Cluster analysis shows two major groups in the data; one characterized by samples taken from the late spring, summer,early fall and one characterized by samples taken from the late fall, winter, and early spring (Figure 6). DCA analysis showed that the gradient lengths are short, so linear-based ordination methods (e.g., RDA) are most suitable. RDA axes one (Eigenvalue = 0.797) and two (Eigenvalue = 0.082) explain 87.9% of the species-environment relationship (Figure 7). It is evident that a seasonal gradient is present across axis one as most samples from winter and fall are negatively correlated with it. The analysis illustrates that minimum, maxi-

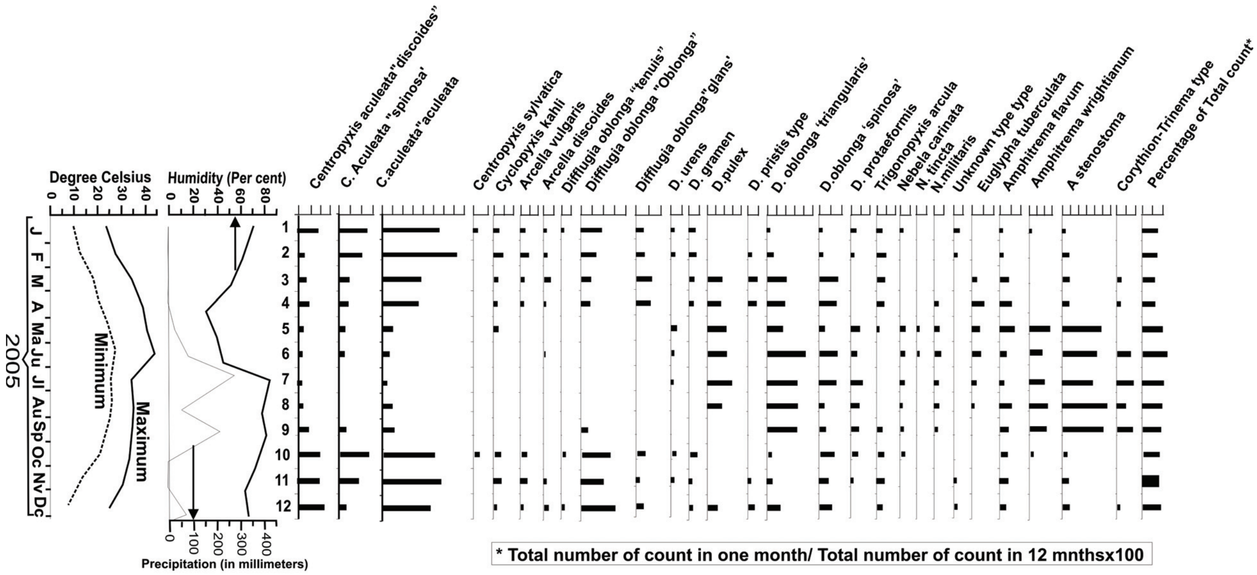


FIGURE 4. Annual percentage spectrum of thecamoebians in sediment-water interface samples from Lake Sadatal.

mum, and average temperature, pH, Cu, Na, K, Fe, Mg, electrical conductivity, total dissolved solids, and salinity are all strongly correlated with axis one. These variables vary significantly in relation to the seasonal changes. The three strongest controls appear to be Na, pH, and minimum temperature. Dissolved oxygen (DO) and Pb are not responding to this primary gradient and instead are correlated with axis two. Two clear groups of taxa and samples can be seen on the RDA diagram reflecting the seasonal variability of the assemblages. The samples on the right-hand side of axis 1 (in the direction of most of the environmental variables) are mostly from the spring and summer, and those on the left are mostly from fall and winter (Figure 7).

This analysis demonstrates clear relationships between thecamoebian species and their intraspecific "strains" to seasonal variations in the region, and to environmental factors in the Lake Sadatal. This lake is environmentally highly stressed because of extreme variations in seasonal temperature and humidity, biotic (seasonal variations in *Lemna* cover) and abiotic environmental variations, and high levels of anthropogenic dissolved chemicals (Table 1).

Percentages of thecamoebian taxa and "strains" recorded every month for the year of 2005 are shown in Figure 4, likewise similar data is presented for the months of February, May, and December for the years 2005, 2006, and 2007 (Figure 5). Some of the significant observations are as follows;

1. Highest percentages of *Centropyxis aculeata* and its strains *'aculeata' , 'discoides,'* and *'spinosa'* are during winter months when maximum temperatures are ~ 25°C and relative humidity is low.
2. *Arcella vulgaris* and *Arcella discoides* occur only during winter months and follow the same trend as centropixids.
3. Highest percentages of *Amphitrema stenostoma* and *Amphitrema wrightianum* are during summer months when maximum temperature can reach ~ 45°C, and relative humidity is high due to following monsoon season.
4. *Difflugia oblonga "triangularis"* is a significant form that occurs throughout the year but its proportion declines during cold winter months and is highest during summer and monsoon season.
5. *Nebella* spp. do not occur during winter months.
6. Distribution of difflugids is throughout the year. *D. oblonga* and its three "strains" like '*glans,*' '*oblonga,*' and '*tenuis*' are absent from summer months. Their distribution is opposite of the distribution of *Difflugia oblonga "triangularis."*
7. The total concentration of thecamoebians was highest during summers particularly during the monsoon months when precipitation and humidity are very high.
8. Few difflugids, *Nebella* spp., and *Euglypha*

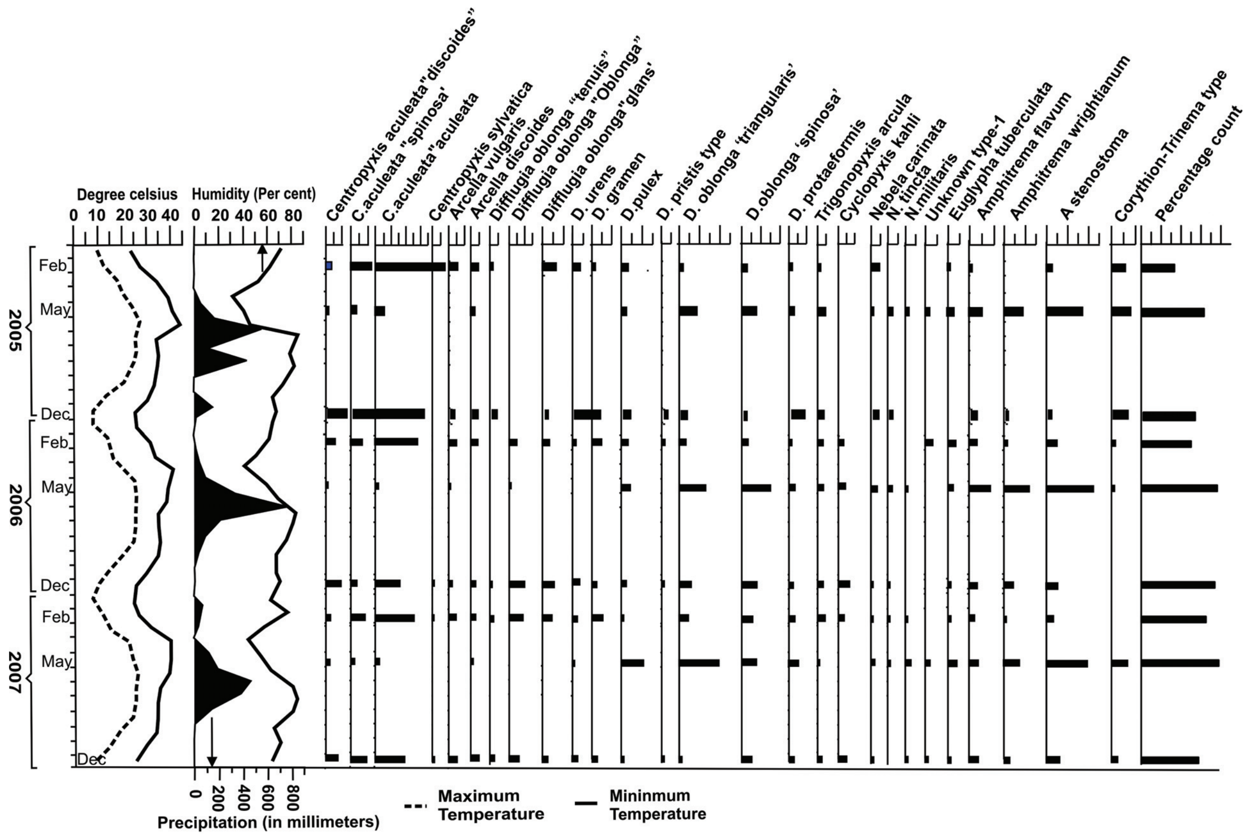


FIGURE 5. Percentage spectrum of thecamoebians in three subsequent years 2005-2007 (February, May and December) Each division = 5%.

tuberculata survive eutrophic conditions developed during pre-monsoon summer months (April-mid June) and could be saprobes on organic detritus of *Lemna* fronds that settle down in the lake bed. During these months the lake level falls by almost 50%. Thus, these species tend to survive in eutrophic conditions, low water level, high temperature, high salinity, and very low dissolved oxygen (DO).

9. Low thecamoebian counts during winter months are due to dryness that causes a decline in centropyxids.
10. Hydrological characteristics of thecamoebian habitat like running versus standing water is very important and can mask the effects of other physico-chemical variables of the lake. Thus they are sensitive indicators of hydrological conditions in both lakes and peatlands, primarily the depth of the water table. Our results illustrate a drastic change in testate amoebae community and their morphometric response to seasonally induced lake's hydrological changes.

Thecamoebians in Chemically Stressed Lacustrine Environment

Lacustrine thecamoebian assemblages are well-known indicators of pollution levels (Collins et al., 1990; Asioli et al., 1996; Patterson et al., 1996). Their species and intraspecific "strains" have also been successfully used as proxies to heavy metal pollution resulting from mine tailings in northern Ontario, Canada (Reinhardt et al., 1998), long- and short-term changes in lake bottom acidity (Kumar and Patterson, 2000), and contaminant indicators (Patterson and Kumar, 2000 a and b).

There are several thecamoebian species and "strains" in Sadatal, which were also commonly reported from chemically polluted lakes of Canada and Italy. The significant forms indicating stressed environments in Sadatal are *Arcella vulgaris, Centropyxis aculeata* and its "strains" like "*aculeata,*" "*discoides,*" and "*spinosa,*" and they dominate the thecamoebian assemblage (Figures 4 and 5). Several difflugid forms along with other taxa are also present in the lake but their proportional representation usually is < 5%. This lake is perennially under high level of environmental stress but the

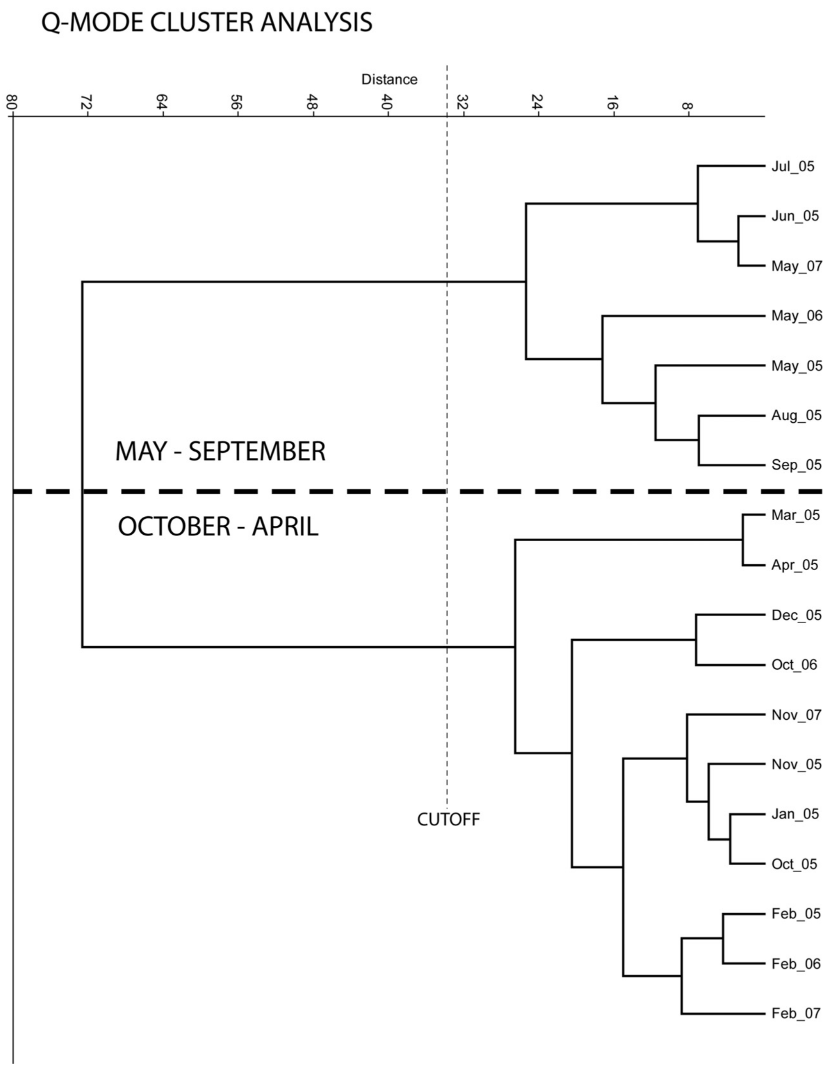


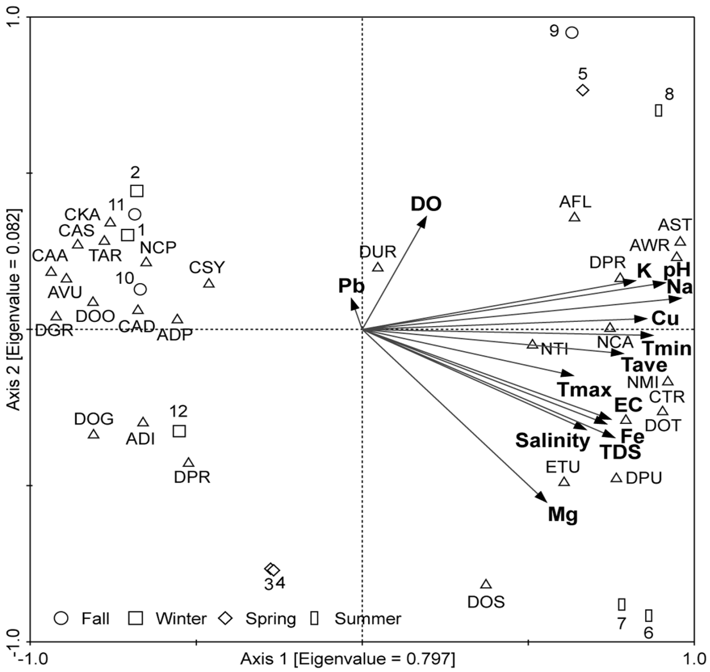
FIGURE 6. Q-mode cluster analysis dendrogram (Ward's method) of the 2005-2007 data .

stress reduces during monsoon season (June-September) when the lake level rises. During these months *A. vulgaris* almost disappears, and Centropyxids become a proportional minority, and Difflugids become a dominant group. This situation becomes reverse during rest of the year (Figures 4 and 5).

Thecamoebian Assemblages in Response to Seasonality

Holocene thecamoebian faunas in association with pollen successions have been used as proxies for record of climate change in Canadian lakes (McCarthy et al., 1995; Boudreau et al., 2005). In a similar study of Holocene thecamoebian faunas and succession of pollen assemblages from Swan Lake were used as indicators of land-use change and settlement history around environmentally sensitive Oak Ridge Moraine watershed in southern Ontario, Canada (Patterson et al., 2002). Dallimore et al. (2000) studied Holocene environmental of thermokarst lakes in the Northwest Territories, Canada using thecamoebians as paleolimnological indicators. Peatlands have been studied for decades to decipher Holocene climate change especially in the northern hemisphere. Both plant macrofossils and microfossils have been used. Thecamoebian faunas are also being successfully used for this purpose (Swindles et al., 2010).

Thecamoebian response to seasonal changes in the tropics has never been reported before. However, Neville et al. (2010a) show the use of thecamoebians to monitor seasonal environmental changes in oil sand reclamation wetlands in Northern Alberta, Canada. In Lake Sadatal Centropyxids and Arcellinids dominate the low humidity, low pre-



Code	Taxon
CAD	Centropyxis aculeata "discoides"
CAS	C. aculeata "spinosa"
CAA	C. aculeata "aculeata"
CSY	C. sylvatica type
CKA	Cyclopyxis kahlii
AVU	Arcella vulgaris
ADI	A. discoides
ADP	A. c.f. discoides var. pseudovulgaris
DOO	Difflugia oblonga "oblonga"
DOG	D. oblonga "glans"
DUR	D. urens
DGR	D. gramen
DPU	D. pulex
DPR	D. pristis type
DOT	D. oblonga 'triangularis'
DOS	D. oblonga 'spinosa'
DPR	D. protaeformis
TAR	Trigonopyxis arcula
NCA	Nebela carinata
NTI	N. tincta
NMI	N. militaris
NCP	N. collaris-parvula-tincta group
ETU	Euglypha tuberculata
AFL	Amphitrema flavum
AWR	A. wrightianum
AST	A. stenostoma
CTR	Corythion-Trinema type

FIGURE 7. RDA triplot showing taxa, environmental variables and sample data for 2005. Sample codes 1-12 correspond to the month from January-December. The environmental variables include pH, salinity (ppt), electrical conductivity (mS/cm), total dissolved solids (mg/l), average atmospheric temperature (°C), minimum temperature (°C), maximum temperature (°C), dissolved oxygen in Water (mg/l), Na (mg/g), K (mg/g), Mg (mg/g), Fe (mg/g), Cu (mg/g), Pb (mg/g).

cipitation cooler months (October-March) whereas *Amphitrema* spp. and Difflugids summer, high precipitation, and high humidity months (April-September). Dominance of *Amphitrema* spp. may be related to abundance of aquatic weed *Lemna* detritus at the lake bottom during summer (Figure 4 and 5). Total thecamoebian counts are higher during summer than winter.

CONCLUSIONS

1. Thecamoebian species and "strains" are reported from perennial Lake Sadatal situated near Mallanwan in the Ganga-Yamuna Plains of North India.
2. Lake Sadatal is a shallow (maximum depth ~1.5 m during summer and ~3.0 m during July-August monsoon season) remnant of a past oxbow lake left by meandering Ganga River and its tributaries.
3. This region shows strong seasonal changes resulting in very high variation in the average summer and winter temperature, and humidity.
4. Taxonomically diverse and mixed thecamoebians that are known to occur in lakes and peatlands in different parts of the world were recovered from Lake Sadatal showing distinct summer and winter communities for three years (2005-2007).
5. A strong seasonal control on the thecamoebians present is illustrated through cluster and redundancy analysis (RDA). RDA shows the strong control of minimum, maximum, and average temperature, pH, Cu, Na, K, Fe, Mg, electrical conductivity, total dissolved solids, and salinity on the thecamoebian communities.
6. Centropyxids and Arcellenids dominated the low humidity, low precipitation cooler months (October-March) whereas *Amphitrema* spp. and *Difflugia oblonga "triangularis"* dominate summer and high precipitation, high humidity monsoon months (April-September).
7. Dominance of *Amphitrema* spp. is most likely related to abundance of aquatic weed *Lemna* detritus at the lake bottom during summer.
8. Total counts of thecamoebians were higher during summer than winter.

Thecamoebian Taxa Recovered in This Study and Their Short Descriptions

Since this study is based on acid resistant thecamoebians from a tropical lake with peculiar physical and chemical environment a short description is deemed necessary for the forms recovered. The present forms appear to be sturdier than their counterparts from the temperate regions. There is a general trend also in diminishing size of these taxa in comparison to the ones known from the northern hemisphere but over all morphological characters are common.

Phylum: PROTOZOA Goldfuss, 1818
Subphylum: SARCODINA Schmarda, 1871
Class: RHIZOPODA von Siebold, 1845
Subclass: LOBOSA Carpenter, 1861
Order: ARCELLINIDA Kent, 1880
Family: CENTROPYXIDAE Jung, 1942

***Centropyxis* Stein, 1859**

***Centropyxis aculeata* "*aculeata*"** Reinhardt et al., 1998 (Figure 8-1): Test diameter 70-80 µm, circular, ovoid, or discoid; aperture 20-25 µm, eccentric, circular or ovoidal, often with a lobate border; spine length not exceeding more than 10 µm, slightly curved, and with a gentle curve at the tip; aperture rounded or slightly invaginated at 4-5 angular points; shell mostly membranous, reticulate, encrusted with moderate and even size foreign particles or sandy material.

***C. aculeata* "*spinosa*"** Reinhardt et al., 1998 (Figure 8-2): Test diameter 60-80 µm, ovoid, or discoid; aperture 25-30 µm, eccentric and circular, spines 8-11, are about 20 µm in length, straight, broad at base with sharply pointed tip. Tests are generally membranous, finely reticulate, encrusted with foreign particles, or sandy material. The size of the test and number of spines shows similarity with those recorded in Japan (Saitama Website 1999, 9 spines).

***C. aculeata*"*discoides*"** Reinhardt et al., 1998 (Figure 8-3): Test diameter 30-40 µm "doughnut" shaped, without spines. Test shape ovoid to oblong, pseudostome eccentric, circular measuring 20-25 µm, wall composed of either numerous alveoli arranged in layers or agglutinated mineral particles; aperture sub-terminal or occasionally central.

Centropyxis arcelloides Penard, 1902 (Figure 8-4): Test size 70-80 µm, circular or discoid, no spines, wall finely reticulate with adhering fine sand or organic particles. Aperture circular measuring 25-30 µm, which is about half the diameter of the shell in width. It occurs associated with the *Lemna* weed.

C. sylvatica Deflandre, 1929 (Figure 8-5): Test length varies between 35-40 µm, and width between 30-35 µm. The shell is oval and in ventral view almost circular with a sub-terminal aperture of diameter15-20 µm. The surface of the shell is almost smooth. This form is similar to *C. aerophila* and *C. aerophila* var. *sphagnicola*.

Family Arcellidae Ehrenberg, 1843

Arcella vulgaris Ehrenberg, 1830 (Figures 8-6, 7): Can be distinguished from *Centropyxis aculeata* "*discoides*" by absence of agglutinated particles. The test is hyaline and almost transparent measuring 40-55 µm, circular to slightly oval in dorso-ventral view, made of proteinaceous matter, surface smooth or punctuate occasionally with honeycomb like reticulation. Aperture central and circular, margin not well defined measuring 15-20 µm.

Arcella discoides Ehrenberg, 1843 (Figure 8-8): Test diameter 60-70 µm, circular in front view, diameter about three to four times the height; test appearance and body structure similar to *A. vulgaris*; but without a distinct border. Shell smooth, circular aperture with one-fourth size of the diameter, margin well defined with few small pores around the aperture. The whole of the shell surface appears to have few small pores, although they are less apparent on the basal collar.

Arcella artocrea type Leidy, 1876 (Figure 8-9): Test measures 50-55 µm with a distinct and well-defined border, surface with honey-comb like structure, pitted, or mammillated. The shell is brown, perfectly circular. The basal collar is usually prominent and smooth. The aperture is small (10 µm) circular and rimmed.

Family: Cyclopyxidae Schönborn, 1989

Cyclopyxis type Deflandre, 1929 (Figure 8-10): Test circular with organic matrix and reticulate surface. Pseudostome central, round, or slightly irregular, margin not very distinct, large (12-15 µm) and equal to the radius of the test.

Trigonopyxis type Leidy, 1879 (Figures 8-11, 12): The diameter of shell is 30-35 µm. Central aperture less than 10 um, circular, invaginated, or triangular, surrounded by a thin ring of organic cement. Shell spherical with broadly reticulated surface that becomes finer towards the pseudostome. Shell margin well defined and surface covered with inorganic particles of variable size and shape.

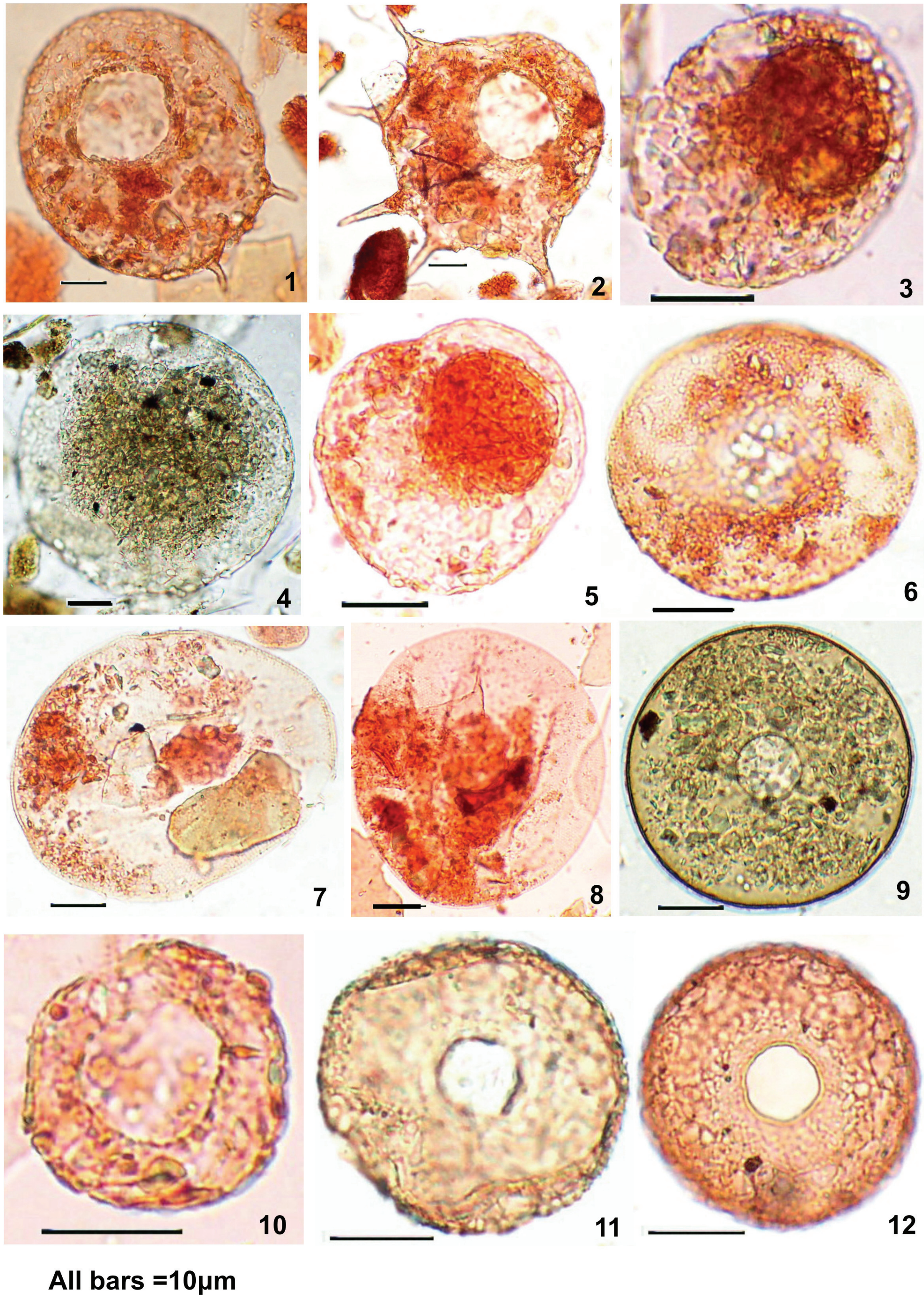


FIGURE 8. Photograph of described thecamoebian specimens, taken under high power light microscope (Olympus-BX2). 1. ***Centropyxis aculeata* "*aculeata*"** Reinhardt et al., 1998; 2 ***C. aculeata "spinosa"*** Reinhardt et al., 1998; 3. ***C. aculeata"discoides"*** Reinhardt et al., 1998; 4. ***Centropyxis arcelloides*** Penard, 1902; 5. ***C. sylvatica*** Deflandre, 1929; 6., 7. ***Arcella vulgaris*** Ehrenberg 1830; 8. ***Arcella discoides*** Ehrenberg, 1843; 9. ***Arcella artocrea*** type Leidy, 1876; 10. ***Cyclopyxis*** type Deflandre,1929; 11,12. ***Trigonopyxis*** type Leidy, 1879 .

Family Difflugidae Stein, 1859

Difflugia oblonga "oblonga" Reinhardt et al., 1998 (Figures 9-1, 2, 3): Test pyriform, flask-shaped, ovoid basal part, neck variable in length, or slightly flattened at the base, test composed of angular sand-grains and diatom frustules, 15-25 μm wide and 25-40 μm long. Aperture is terminal, measuring 10 μm in diameter, typically circular and lacking a collar.

Difflugia oblonga"glans" Reinhardt et al., 1998 (Figure 9-4): Test width measures 20-25 μm and length 40-50 μm, oblong with rounded fundus, wall composed of angular sand-grains or organic particles, aperture terminal, invaginated, and circular measuring about 10-15 μm.

Difflugia gramen Penard, 1902 (Figure 9-5): Test oblong, slightly tapered at apertural end, wall smooth with fine reticulate patterns having small agglutinated particles mainly of sand grains. Test width measures 20-25 μm and length 35-40 μm. Aperture measures 10-15 μm, terminal, with three to four petal-shaped lobes, delimited by a slightly raised irregular ridge. The present specimen revealed a very smooth texture.

Difflugia globulosa Dujardin, 1837 (Figure 9-6): Test egg-shaped, measuring 15-20 μm in width and 20-25 μm in length. Wall with agglutinated sand-grains and organic matter. Aperture wide measuring more than10 μm, margin well defined but no collar like structure.

Difflugia pulex type Penard, 1902 (Figure 9-7): Test oblong with a well-defined margin, measuring 25-30 μm. Aperture is terminal that ends in a very small neck (5 μm), margin of the neck is irregular.

Difflugia lobostoma type Leidy, 1879 (Figure 9-8): Test globose to ovoid measuring 25 μm in width and 30-35 μm in length. Margin is irregular densely covered by sand grains of irregular size. Aperture terminal, measuring less than 10 μm.

Difflugia pristis type Penard, 1902 (Figure 9-9): Test oval or oblong measuring 15-20 μm in width and 35-40 μm in length; wall smooth with fine reticulation. Small neck ending in a small (5 μm) pseudostome, lacking lobed collar.

Family: Hyalospheniidae Schulze, 1877

Nebela carinata Archer, 1867 (Figure 9-12): The shell is pyriform measures 20 μm in width at the posterior end and length is 24-30 μm, has slightly extended neck. The aperture is round and surrounded by a thin collar of organic cement.

Nebela sp. (Figure 9-13): The length of shell is 20-25 μm, and the width 15-20 μm. It is drop-like, laterally strongly flattened.

Nebela tincta type (Figure 9-14): Test flask-shaped, with a slight neck, test 25-30 μm long and 12-15 μm wide, pseudostome 5 μm wide.

Family: Trinematidae Hoogenraad et Groot, 1940

Certisella type (Figure 9-15): Test flask-shaped, elongated neck with terminal pseudostome. A frilled organic rim extends around the body as a collar, is prominent at the sub-terminal end. The base measures 10 μm and the length is 60-70 μm. The collar measures about 10-12 μm in diameter.

Corythion dubium Taranek, 1881 (Figure 9-16): Test length is 15 μm and the width 10 μm is ovoid and flattened. The subterminal aperture (2 μm) is circular to oval, invaginated. These specimens are very small.

Amphitrema wrightianum Archer, 1869 (Figure 9-17): Test smooth proteinaceous and with distinct collared apertures at both poles, which measure 1-1.5 μm. It is 20-25 μm long and 15-20 μm wide.

A. stenostoma Nusslin, 1884 (Figure 9-18): Test is 20-25 μm long and 10-12 μm wide having two pseudostomes at opposite ends; the size of the pseudostome is 2-3 μm, the anterior pseudostome is wider than posterior one that appears like a tail.

Amphitrema flavum Archer, 1869 (Figure 9-19, 20): Test linear or ovoid, symmetrical, compressed, composed of a smooth proteinaceous test with or without adherent extraneous particles. It measures 25-30 μm long and 15 μm wide. It has a circular aperture at both poles measuring about 10 μm.

?Amphitrema type (Figure 9-10, 11).

Figures 10-1, 2, and 3 are unidentified loricated bodies showing affinity with rhizopods with hyaline body of similar character but variable in size.

Figure 10-4 shows a similar inclusion inside the *Centropyxis* sp.

Figures 10- 8, 9, 10, 11, 12, and 13 present tests showing affinity with testate amoebae in shape and pseudostome like features suggesting the cyst formation of Centropyxids during unfavourable environmental conditions. With the onset of favourable winter conditions and oligotrophic state these perhaps regain vitality and grow.

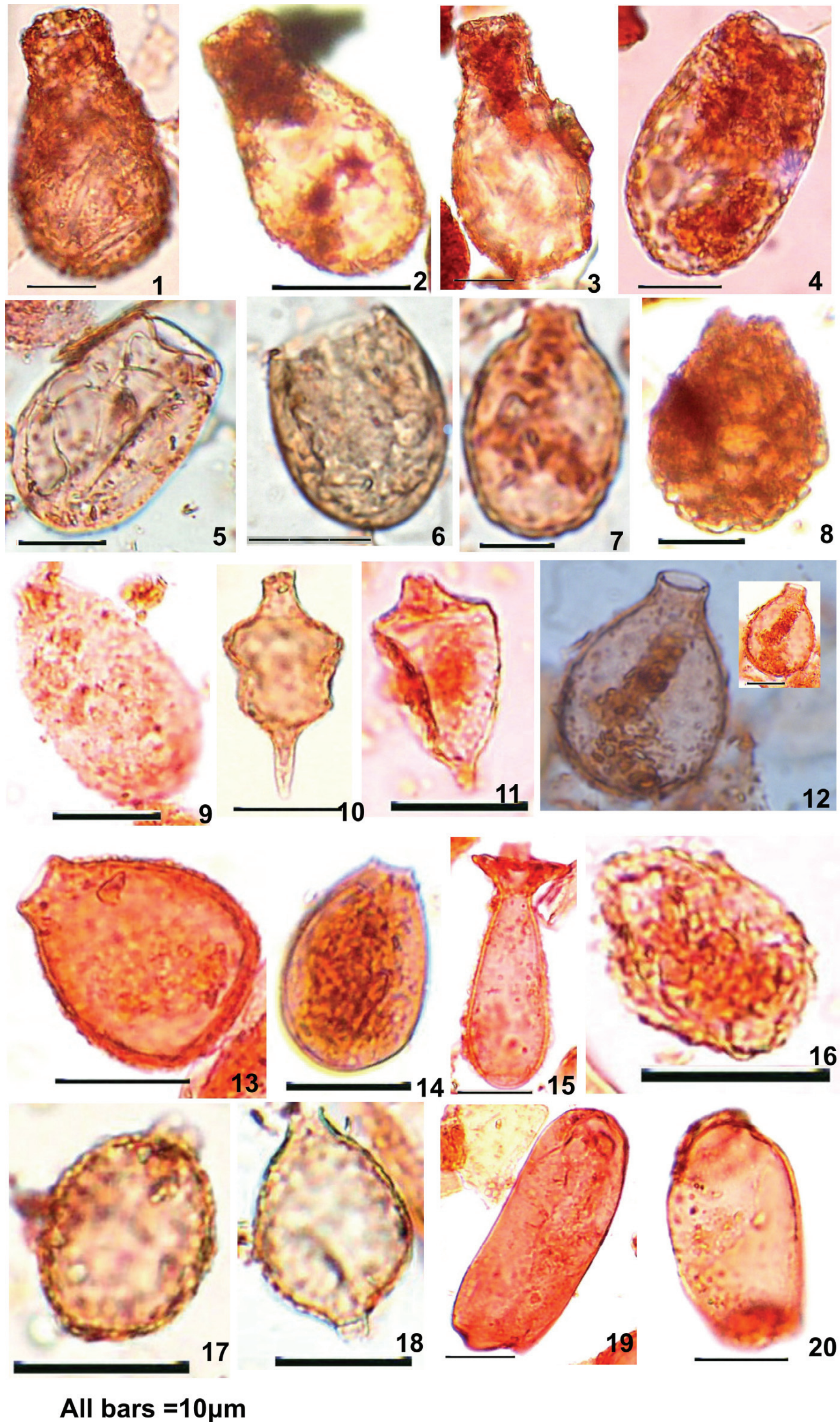


FIGURE 9. Photograph of described thecamoebian specimens, taken under high power light microscope (Olympus-BX2). 1, 2, 3: ***Difflugia oblonga "oblonga"*** Reinhardt et al., 1998; 4. ***Difflugia oblonga"glans'*** Reinhardt et al., 1998; 5. ***Difflugia gramen*** Penard, 1902; 6. ***Difflugia globulosa*** Dujardin, 1837; 7. ***Difflugia pulex*** type Penard, 1902; 8. ***Difflugia lobostoma*** type Leidy, 1879; 9. ***Difflugia pristis*** type Penard, 1902; 10, 11. ?***Amphitrema*** type; 12: ***Nebella carinata*** Archer, 1867; 13. ***Nebella sp.***; 14. ***Nebella tincta*** type; 15. ***Certisella*** type; 16. ***Corythion dubium*** Taranek, 1881; 17. ***Amphitrema wrightianum*** Archer, 1869; 18. ***Amphitrema stenostoma*** Nusslin, 1884; 19., 20. ***Amphitrema flavum*** Archer 1869.

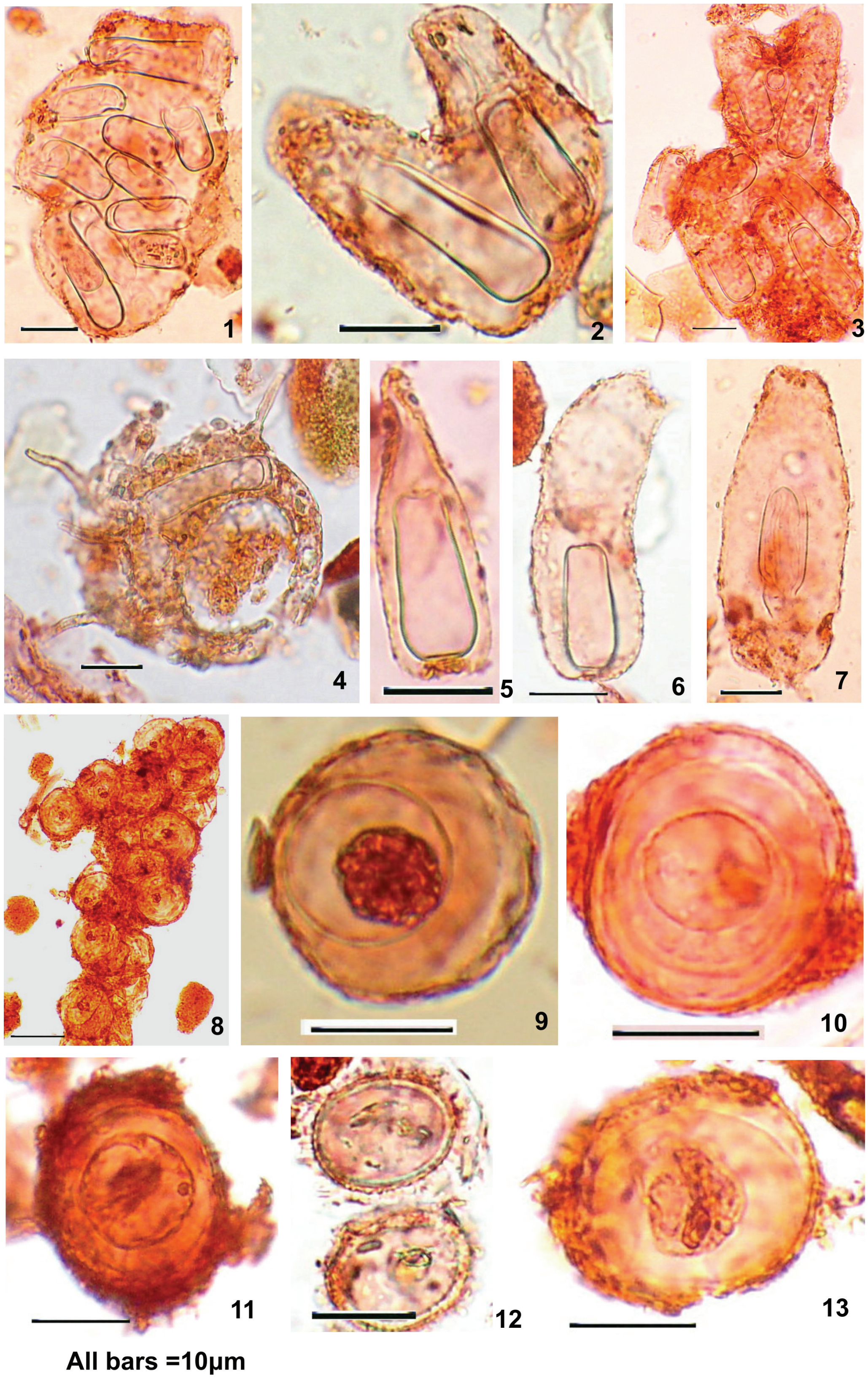


FIGURE 10. Photograph of described thecamoebian specimens, taken under high power light microscope (Olympus-BX2). 1-3. Unidentified Form A; 4. inclusion inside the *Centropyxis* sp., 5-7. *Loxophyllum elegans*; 8-13. ?testate amoebae cyst.

Family: Amphileptidae Bütschlii, 1889

Loxophyllum elegans Dujardin, 1841 Figures 10- 5, 6, and 7 ; This is a medium-sized, predatory ciliate, body flattened.

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