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# ГІДРОЕКОЛОГІЧНІ ДОСЛІДЖЕННЯ

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UDK 574

A. Farooqui

## FRESH WATER THECAMOEBIANS: ECOLOGICAL INDICATORS OF SEASONALLY INDUCED HYDROLOGICAL CHANGES IN LAKES

*Birbal Sahni Institute of Palaeobotany, India*

The data available for thecamoebians as environmental indicators in ecological perspective is scarce from India. The sediment collected from five lakes in the outskirts of Lucknow city, India was studied for thecamoebian response to winter (November) and summer (May) climatic conditions. About 11 species were recovered from these lakes and could be segregated into Type-I and Type-II. During winters Type-I shows dominance of *Centropyxis aculeata*, *C. arcelloides*, *Arcella vulgaris*, *A. discoides*, *Diffflugia oblonga*, *Diffflugia grame* which are adapted to high dissolved oxygen (DO), normal pH and low salinity in November along with the aquatic weed *Lemna* in its exponential phase of growth. The above thecamoebians succumb to hydrological changes during May when the air temperature increases to about 46° Celsius, pH and salinity also increase and DO of water decreases to as low as 5 mg/l. Subsequently, the growth of *Lemna* ceases during this period and the weed detritus settles in the lake. Under such conditions the dominance of Type-II comprised of *Diffflugia protaeiformis*, *Trigonopyxis arcula*, *Amphitrema flavum*, *A. stenostoma* and *Trinema* spp. increase in number indicating its tolerance and adaptability. It is suggested that these species are detritivorous and DO plays a major role in defining the susceptibility and tolerance of *Centropyxis aculeata* and *Amphitrema stenostoma*. While Type-I are the indicators of high DO, the Type-II are useful microbes that help in cleaning the aquatic detritus essential for maintaining the trophic level of natural lakes.

*Keywords: ecology, lake, thecamoebians, Lucknow, India.*

А. Фарооки

*Институт палеоботаники Бирбал Сахни, Индия*

### АМЕБЫ СЕМЕЙСТВА THECAMOEBIDAE, ЖИВУЩИЕ В ПРЕСНОЙ ВОДЕ, КАК ЭКОЛОГИЧЕСКИЕ ИНДИКАТОРЫ ПРИ ИЗУЧЕНИИ СЕЗОННЫХ ГИДРОЛОГИЧЕСКИХ ИЗМЕНЕНИЙ ОЗЕР

Исследования амеб семейства *Thecamoebidae* в качестве индикаторов окружающей среды до сих пор не получали широкого распространения в Индии. Данная работа является одной из первых, посвященных этому подходу. В качестве исходных данных взяты пробы осадков пяти озер, расположенных вблизи города Лакнау (Индия). Целью изучения стала активность амеб, живущих в отобранных образцах, в зимнее и летнее время (ноябрь и май). В ходе работ было установлено 11 видов амеб, которые условно разделили на два типа – 1-й и 2-й. К представителям 1-го типа относятся *Centropyxis aculeata*, *C. arcelloides*, *Arcella vulgaris*, *A. discoides*, *Diffflugia oblonga*, *Diffflugia grame*, а также водоросли *Lemna*, к представителям 2-го типа – *Diffflugia protaeiformis*, *Trigonopyxis arcula*, *Amphitrema flavum*, *A. stenostoma* и *Trinema*. Результаты исследований показали, что амебы 1-го типа могут служить индикаторами уровня растворенного кислорода, а амебы 2-го типа – использоваться для борьбы с детритами и соответственно для поддержания трофического уровня естественных озер.

*Ключевые слова: экология, озера, амебы семейства Thecamoebidae, Лакнау, Индия.*

А. Фарооки

*Институт палеоботаники Бирбал Сахни, Индия*

### АМЕБИ РОДИНИ THECAMOEBIDAE, ЯКІ МЕШКАЮТЬ У ПРІСНІЙ ВОДІ, ЯК ЕКОЛОГІЧНІ ІНДИКАТОРИ ПРИ ВИВЧЕННІ СЕЗОННИХ ГІДРОЛОГІЧНИХ ЗМІН ОЗЕР

Дослідження амеб родини *Thecamoebidae* як індикаторів навколишнього середовища до цього часу не отримали широкого розповсюдження в Індії. Дана робота є однією з перших,

присвячених цьому підходу. Як вихідні дані прийнято проби осадів п'яти озер, які розташовані поблизу міста Лакнау (Індія). Метою дослідження стала активність амеб, які знаходилися в одібраних зразках, у зимовий та літній час (листопад та травень). У ході роботи було встановлено 11 видів амеб, які умовно поділили на два типи – 1-й та 2-й. До представників 1-го типу належать *Centropyxis aculeata*, *C. arcelloides*, *Arcella vulgaris*, *A. discoides*, *Diffflugia oblonga*, *Diffflugia grame*, а також водорості *Lemna*, до представників 2-го типу – *Diffflugia protaeiformis*, *Trigonopyxis arcula*, *Amphitrema flavum*, *A. stenostoma* та *Trinema*. Результати досліджень показали, що амеби 1-го типу можуть слугувати індикаторами рівня розчиненого кисню, а амеби 2-го типу – використовуватися для боротьби з детритами і відповідно для підтримання трофічного рівня природних озер.

*Ключові слова:* екологія, озера, амеби родини *Thecamoebidae*, Лакнау, Індія.

Thecamoebians (testate amoeba) are a diverse group of testate rhizopods living in aquatic to moist habitats and occur commonly worldwide in a variety of freshwater, slightly brackish environments and salt marshes (Medioli and Scott, 1988; Patterson and Kumar, 2002; Charman et al., 2002). Studies of testate amoebae are reported from Canada, Europe, Africa and China etc. (Kumar and Dalby, 1998; Yang, Shen, 2005; Lahr and Lopes, 2006), South America (Vucetich, 1978), Brazil (Lansac-Toha et al., 2001). Environmental tolerances of testate amoeba in different regions have been studied (Booth and Zygmunt 2005). Very few records of past and present testate amoebae are available from India (Rao, H.S.M. 1928; Nair, & Mukherjee, 1968; Mishra and Saksena, 1990; Das and Chattopadhyay, 2003; Farooqui & Gaur, 2007). The knowledge of testate amoeba ecology is limited from India where the fresh water ecosystems are seasonally subjected to limnological changes induced by extreme conditions of temperature, humidity and precipitation in the central and north-central part. The present work documents the seasonal fluctuation of thecamoebians responding to dissolved oxygen, pH and salinity in Lake Ecosystem.

#### DESCRIPTION OF THE STUDY SITE

The study area is located in nearby Lucknow city which falls in the north-central alluvial plains of India (Fig. 1). The area is subjected to three extreme seasonal conditions such as summer, rainy and winter characterized by maximum 46°C in summers and minimum 6°C in winters. The average atmospheric temperature in rainy seasons remain ~28°C. All the five lakes were shallow with water level not exceeding more than a meter in the centre covering an area of about 300 to 400 square meters in November (average atmospheric temperature- 18°C). However, the lakes shrank to about half a diameter in May (average atm. temp. 34°C and low precipitation-100-150mm). During July and August (average atm. temp. 28°C ) due to maximum precipitation (350-400mm) the lake area increases and the nutrients drain into it from nearby areas (sodic and agriculture land

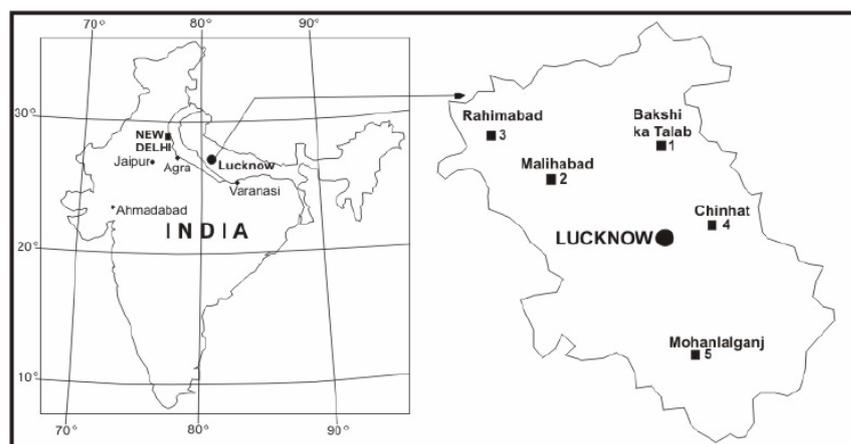


Fig.1: Location Map of lucknow and nearby studied lakes (1-5)

including anthropogenic input). During the rainy season (July-Aug-September) begins the onset of exponential growth phase of *Lemna* (duckweed) which continues until January showing its maximum cover in the lake surface during November and December. From January onwards, probably, due to exhaustion of lake nutrients the propagation of *Lemna* fronds start declining. Gradually, the brownish *Lemna* fronds appear on the surface of the lake and by February, March all the detritus settles in the lake bed. Subsequently, the summer season sets in and the increase in atmospheric temperature results into rapid evaporation of lake water.

### MATERIALS AND METHOD

The sediment-water interface samples were collected from the periphery of 5 lakes by Veen-Van grab sampler and stored in polythene bottles. The aqueous soil solution was analyzed for pH, salinity and dissolved oxygen (DO) within 40 minutes of collection by portable 'Thermo-Orion, U.S.A' electrode analyzer at standardized 25° Celsius temperature. The results given in figures are the average value of 6 samples from each lake. Testate amoebae were isolated from soil samples in laboratory. Samples were boiled in distilled water for 10 minutes and passed through 250 mesh size. Thereafter, the samples were treated in series with glacial and anhydrous acetic acid in order to reduce the opacity of the thecamoebians to study it under high power transmitted light microscope (Olympus BX-52). The material was subsequently caught in the 600 mesh size and was stored in glycerol. Slides were prepared, and all testate amoebae were identified and counted in the processed 10g samples. The results of the thecamoebian count is the average value of 6 samples from each lake.

### RESULTS

In both the seasons the pH in all the water bodies ranged between 7 and 8 except in lake-3 which increased to 9 during May (Fig.2). The salinity ranged between 0.1 to 0.4 ppt in November and between 0.3 to 1 in May. The dissolved oxygen in water was low in May which ranged between 5 and 6 mg/l. However, the dissolved oxygen remained high during November which ranged between 7-11 mg/l.

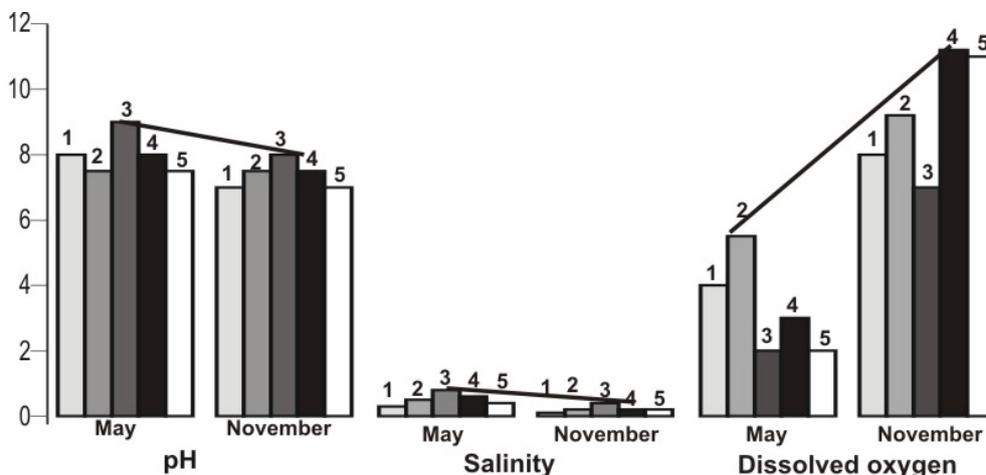


Fig.2- Physico-chemical properties of aqueous soil solution in 1-5 Lakes

Two types of thecamoebians were identified on the basis of percentage count in summers and winters. These are Type-I comprising of dominant species in winters (November) and Type-II which show comparative dominance in summers (May).

Type-I thecamoebians show dominance in the total count comprising *Centropyxis aculeata* (max. 42 % & min. 31 % in Lake [L]-5 & L-2, respectively) followed by *Arcella discoidea*, *Diffugia gramen*, *D.oblonga*, *A. vulgaris* and *Centropyxis arcelloides* (Fig.3). However, in May the Type-II species dominated comprising 30.7% of *Amphitrema*

*stenostoma* ( max.34 % and min. 26% in L -1,2 and L-5, respectively) followed by *A. flavum*, *Diffflugia protaeformis*, *Trigonopyxis arcula* and *Trinema* type. Thus, the percentage of the average total count of thecamoebians in all the lakes in May shows highest percentage of *Amphitrema stenostoma* (30.7%), followed by *A. flavum* (18.8%), *Trigonopyxis arcula* type (10.1%), *Trinema* and *Centropyxis aculeata* (5.1 & 5.2%), *Diffflugia oblonga*, *Centropyxis arcelloides*, *D. gramen*, *A. discoides* and *A. vulgaris* (4.4, 4.1, 3.8 and 2.5, respectively). However, the percentage of the average total count in November was highest for *Centropyxis aculeata* (35.9) followed by *Diffflugia gramen* (11.1%), *Arcella vulgaris* (10.4%), *A. discoides* (10.7), *D. oblonga* (8.9%) and *Centropyxis arcelloides* (8.2%). The standard deviation is highest for *Centropyxis aculeata* by  $\pm 3.7$  in November and highest for *Amphitrema stenostoma* by  $\pm 6.4$ . Results indicate that these two species are very sensitive and indicator species of seasonally induced hydrological fluctuations in shallow lake system.

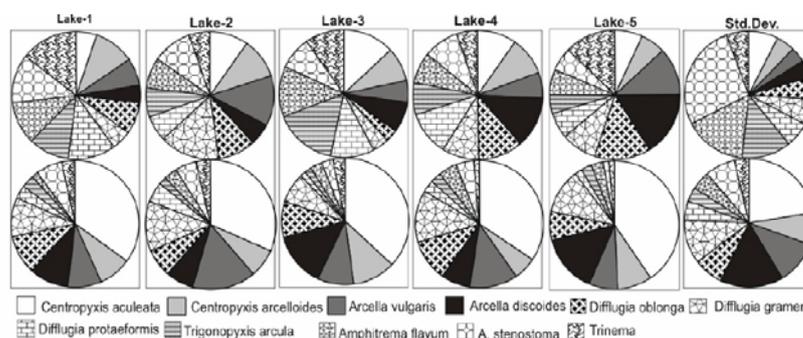


Fig.3- Pie Diagram showing percentage of average total count (6 samples per lake) and standard deviation ( between 5 lakes) of thecamoebians in May (top row) and November (bottom row)

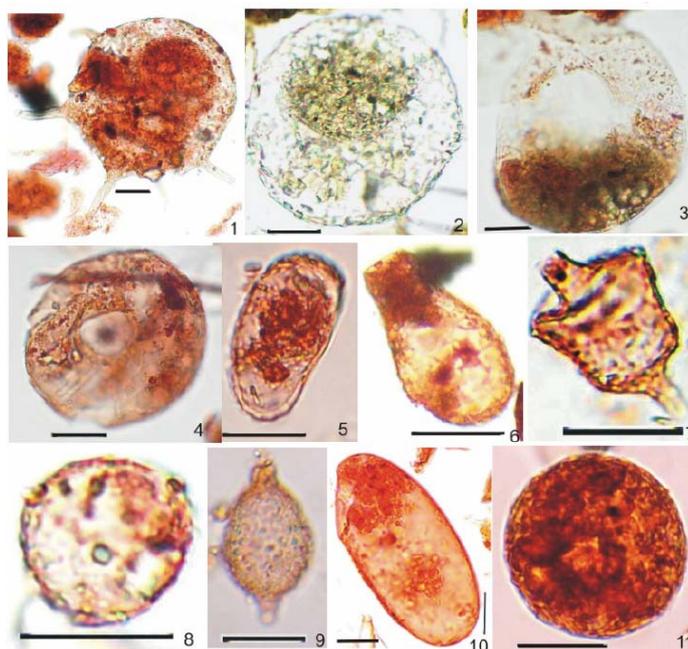


Fig. 4. Light Microscopic photomicrographs. All scale = 20  $\mu$ m:

- 1 – *Centropyxis aculeata* (Ehrenberg); 2 – *Centropyxis arcelloides* (Penard);  
 3 – *A. vulgaris* (Ehrenberg); 4 – *A. discoides* (Ehrenberg); 5 – *Trinema enchelys* type (Ehrenberg);  
 6 – *Diffflugia oblonga* (Ehrenberg); 7 – *D. protaeiformis* (Lamarck); 8 – *Diffflugia gramen* (Leclerc);  
 9 – *Amphitrema stenostoma* (Nüsslin); 10 – *A. flavum* (Archer); 11 – *Trigonopyxis arcula* (Penard)

All the lakes show similar results with respect to general increase in Type-I species in November and Type-II species in May. However, Lake-3 shows highest pH (9) with lowest DO (5 mg/l) and lake-3 and 4 shows high salinity (0.8 to 1 ppt) in May as compared to other lakes. Despite the change in the abiotic factors within the studied 5 lakes, the trend in thecamoebian dominance of Type-I and II species did not change significantly in both the seasons. While pH and salinity varied in both seasons by  $\pm 0.6$  and  $\pm 0.3$ , respectively, the DO in water show wide variation of  $\pm 2.1$  Standard Deviation. Probably, the parameters like DO play a major role in defining the susceptibility and tolerance of *Centropyxis aculeata* and *Amphitrema stenostoma* (as compared to other species) along with pH towards alkalinity and high salinity in the present study. Therefore, it is apparent that Type-I thecamoebians in general show adaptability to aquatic ecosystem having normal to high DO but are sensitive to low DO and succumb to adverse summer conditions. On the other hand, Type-II species are adapted to low DO and are detritus loving enabling the natural ecosystems to get rid of organic detritus.

## DISCUSSION

The aim of the study was to present the relationships between species composition of thecamoebians and seasonally induced hydrological conditions in lakes mostly subjected to extreme climatic conditions. The study shows that testate amoebae respond to main habitat parameters, i.e. pH, salinity and dissolved oxygen (DO). Particular amoebae species occurrence may be more strongly correlated with DO than with the pH and salinity, that would suggest that DO has important influence on the formation of amoebae communities. In the analysed case, the physico-chemical properties of the lake water shows increase in pH and salinity during summer season because the salts are drained from agricultural land and nearby municipal waste mainly during the rainy season. During this period due to increased nutrient and shallow depth in the lake, the propagation of *Lemna* fronds dominate. Profuse propagation of *Lemna* fronds and low temperature during winter months perhaps resulted into oxygenation of the water and therefore, high Dissolved Oxygen (DO) was recorded during the exponential growth phase during winters. As the atmospheric temperature increases to maximum of 46°C in summer season, high rate of evaporation from the lake surface is expected which is likely to leave behind salts and therefore, increase in salinity is obvious. The increase in pH may have been due to municipal wastes, washing and bathing activity that generally add detergents in the water body. The *Lemna* fronds decline propagation and settle in the lake bed during summers perhaps due to stressed conditions of high pH and salinity coupled with nutrient exhaustion. The degradation of the weed detritus and other organic waste input in the lakes may have increased the Biological Oxygen Demand (BOD) which resulted into low DO during summers. As compared to all the lakes the pH and salinity was highest in lake-3 which may be due to vast area covered by sodic land in the vicinity. It was observed that even during November the pH was 7.5 and salinity was 0.4 ppt in some of the studied lakes as compared to in May, but the DO of lake water showed a drastic change both in November and May. Therefore, it is suggested that the dominance of Type-I and Type-II thecamoebians are sensitive to increase and decrease in DO but may have adaptability to fluctuating pH and salinity which may help in biomonitoring trophic status of the lakes.

It has been reported earlier that *Diffflugia protaeiformis*, is often abundant in pH of 6.5–7.5, and in higher pH and highly contaminated lakes in Canada (Reinhardt et al., 1998; Kumar and Patterson, 2000). Dominance of *D. protaeiformis* strains in an assemblage has generally been related to either polluted or stressed environments in northern Ontario and Italy (Asioli et al., 1996). Centropyxids are known to be opportunistic, and they dominate stressed environments. The present study shows that all the lakes are under stressed conditions in most months of the year and therefore, high percentage of *Centropyxis* species is likely to be present but interestingly, it has been observed here that these are highly sensitive to DO irrespective of either pH or salinity and are indirectly sensitive to high atmospheric temperature inducing hydrological changes in the water body.

Species of *Amphitrema*, *D. protaeiformis*, *Trigonopyxis* and *Trinema* increase in May but were also recorded in low number in November. It is suggested that these are detritivorous thecamoebians and therefore, show its dominance when the weed detritus settles in the lake during May. *Trigonopyxis arcuata*, is a taxon indicative of relatively dry conditions and are known to be good indicators of soil moisture levels in soils and acidity in lakes (Charman et al., 2000; Mieczan, 2007). In the present study its abundance in dry season and sub-optimal conditions of high salinity and low DO indicate that *Trigonopyxis* is tolerant to stressed conditions which may be either acidic or alkaline /saline. Earlier records also show that the fluctuation in its population is attributed to the moisture content of their habitat (Smith, 1982; Mitchell et al., 1999). Other factors like pH (Costan and Planas, 1986; Ellison, 1995), eutrophication (Schonborn, 1962), temperature (Medioli and Scott, 1988), light, oxygen and food availability (Charman et al., 2000) control the testacean community structure.

Our results indicate that change in abiotic factors particularly pH, salinity and DO induced by extreme climatic conditions in tropics and sub-tropics play a more important role in defining thecamoebian community in an ecosystem. It is interesting to monitor quantitatively and qualitatively the susceptibility and tolerances of individual testate species in the geographic space like north-central part of India where extreme seasonality induced changes in the abiotic factors of the aquatic ecosystem. Thus, testate amoebae have several strengths relevant for monitoring and studying high resolution palaeoecology and paleoenvironment to infer hydroperiods, climate and temperature variability.

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