

ORIGINAL ARTICLE

**AN ASSESSMENT OF TESTATE AMOEBAS (PROTOZOA) ASSEMBLAGE  
BASED ON ALTITUDINAL GRADIENT IN PROTECTED AREAS OF WEST  
BENGAL**

**Aradhana Chaudhary<sup>1,2,\*</sup> and Jasmine Purushothaman<sup>2</sup>**

<sup>1</sup>Raja Peary Mohan College, Uttarpara, Hooghly, West Bengal, Pin- 712258, India

<sup>2</sup>Zoological Survey of India, M- Block, New Alipore, Kolkata – 700053, India

Email: <sup>1</sup>chaudharyaradhana@gmail.com

**Abstract:** Testate amoeba, the polyphyletic group comprising of amoeboid protozoans are enclosed in a distinctively unique covering called test (shell). They are the abundant, diverse and widely distributed microbial group which is used for ecological studies and acts as ecological health predictor organism. The testate amoeba diversity decreases with increasing latitude:  $\pm 300$  taxa in Arctic from a total of  $\pm 2000$ , and a decline in nebelid species richness were observed in the high southern latitudes. In this study we assessed the assemblage of the testate amoeba in the fresh water sample collected from the protected areas of West Bengal. Higher species richness was found at lower altitude within the selected protected areas for the study conducted. Altitude has a negative correlation with diversity of testate amoeba.

**Key words:** Testate amoeba, diversity, altitudinal gradient, protected areas, West Bengal.

Communicated: 9.8.2023

Revised: 2.12.2023

Accepted: 10.12.2023

## 1. INTRODUCTION

Testate amoebas are unicellular, single-celled protozoans having an exterior shell (the test). This polyphyletic group consists of amoeboid protozoans contained in a test (shell) that may be found in both freshwater (sediments) and terrestrial settings. The protist community assays a prominent role both in ecological health functioning and structuring [1] of the ecosystem which is reported across the globe, However, significant reporting about this community from West Bengal is scanty. These studies in many biotopes [2-4] across various countries provide the templates to assume the significance of testate amoeba in the past and present condition in those areas. Experimental studies across the globe have revealed its wide habitat spreading from terrestrial, wetlands and freshwater habitats, lake margins and even biofilms of sewage treatment plants [5-9]. Its rapid response to environmental changes makes them an ideal indicator of the health of the ecosystem. Its rate of reproduction depends on the availability of temperature, humidity, food and material to constructing tests [10-11]. Its shell morphology is affected by abiotic and biotic factors such as food source, temperature and insecticides [12-13]. Its sensitivity towards many environmental variables both at lower and higher latitudes were depicted in the studies of [13-17].

According to the 2021 report of the Indian state of Forest, the total geographical area of the West Bengal covered by forests (including the protected areas) is only 18.96%. The forest cover of West Bengal is spread across varying altitude range (from sea level to 4000 meters above sea level).

The altitudinal gradient has significant impact on the diversity and distribution of testate amoeba [5. 18-20]. In this study, an attempt was made to study the assemblage of the testate amoeba in the fresh water sample collected from different altitudes within the protected areas of Birbhum, Nadia and North 24 Parganas district of West Bengal.

## 2. MATERIALS AND METHODS

### A. STUDY AREA

The study was conducted on fresh water samples collected from different water bodies and several regions of protected areas belonging to dry deciduous forest at Birbhum district, tropical moist deciduous forest at Nadia district and tropical dry deciduous forest at North 24 Parganas district of West Bengal during December 2021-January 2023. As all of the three studied forests conserve a specific type of soil, floral and faunal diversity, they were selected for the present survey.

### B. SAMPLING

Samples were collected from GPS fixed locations (Garmin GPS 72H) and brought to laboratory. Aqueous drops were drawn from the sample, using micropipette, put on glass slides and examined under the light microscope. Testacids were isolated and then kept for air drying. After two or three washings in absolute alcohol, then the specimen was mounted in DPX [21-23]. The enumeration and identification using published guides and keys including [10-11, 15, 24-25] of these prepared permanent microscopic slides were carried out at 200 X to 400 X magnification with Olympus BX41 and Nikon Eclipse Ni-U microscopes. All the prepared permanent slides were deposited in the National Zoological Collections (NZC) of the Protozoology Section of Zoological Survey of India, Kolkata.

## 3. RESULTS AND DISCUSSION

A total of 57 species of testate amoeba was recorded during this study which belonged to 12 families, 13 genus and 2 orders. The altitude ranges from 20 to 230 feet in this study area where the highest species richness was recorded at 31.4 feet and 20 feet and least (3 species) at 230 feet. The genus *Arcella* Ehrenberg 1830, *Euglypha* Dujardin 1841, *Centropyxis* Stein 1857, *Trinema* Dujardin 1841 and *Diffugia* Leclerc 1815 are the most prominently found genus among the study areas and the also at the sites (altitude 20, 22, 23 and 31.4 feet) having maxing species diversity. The prominence of this genus at all forest type state there cosmopolitan and resilience nature in all environmental parameters. Most of the sites lying between scale 0 - 75 feet had the expressive number of testate amoeba diversity.

Based on the results we can see the dominance of Phylum Tubulinea in the study area considered as a whole (74%) and also in individual forest (dry deciduous 80%, tropical moist deciduous forest 67% and tropical Dry deciduous forest 52.5%). The Phylum Cercozoa in the study area considered has (26%) and also in individual forest has (dry deciduous 20%, tropical moist deciduous forest 33% and tropical Dry deciduous forest 47.5%) the following contribution respectively.

The diversity of testate amoeba in the study area belongs to 12 families of which Arcellidae, Diffugiidae, Trinematidae, Centropyxidae, Euglyphidae and Netzeiliidae are found in descending order of presence in the altitudinal gradient of 20-230 feet (Figure 2).

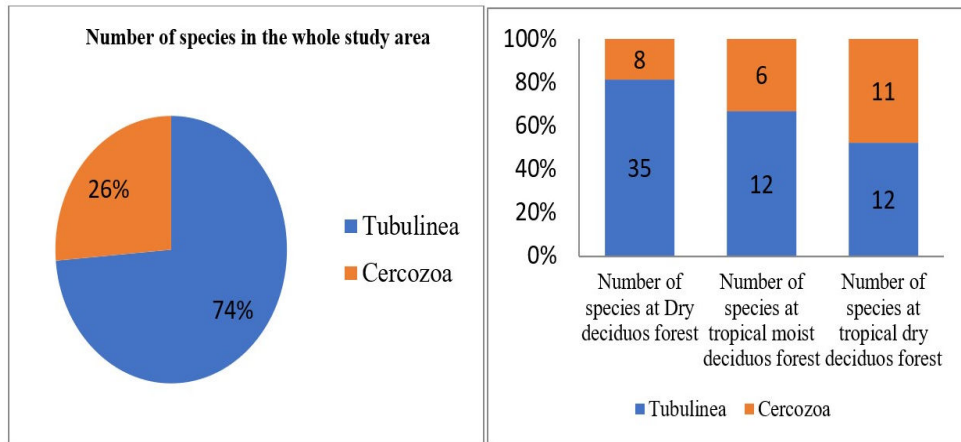


Figure 1. Diversity of testate amoeba (based on phylum) in the whole study area (left) and diversity of testate amoeba (based on phylum) in each of the three forests studied (right).

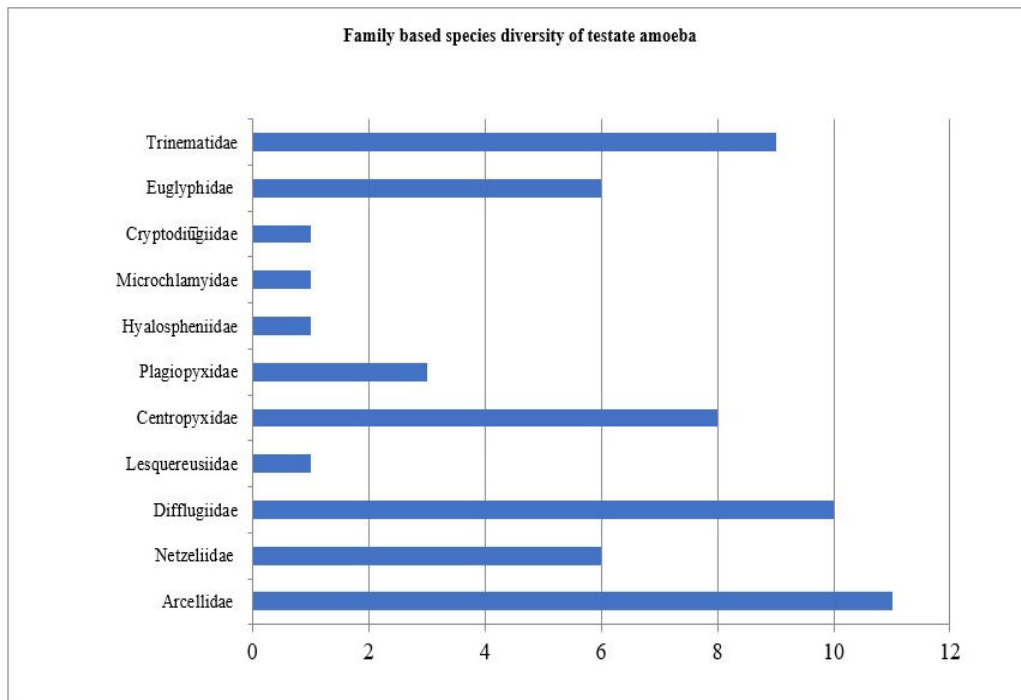
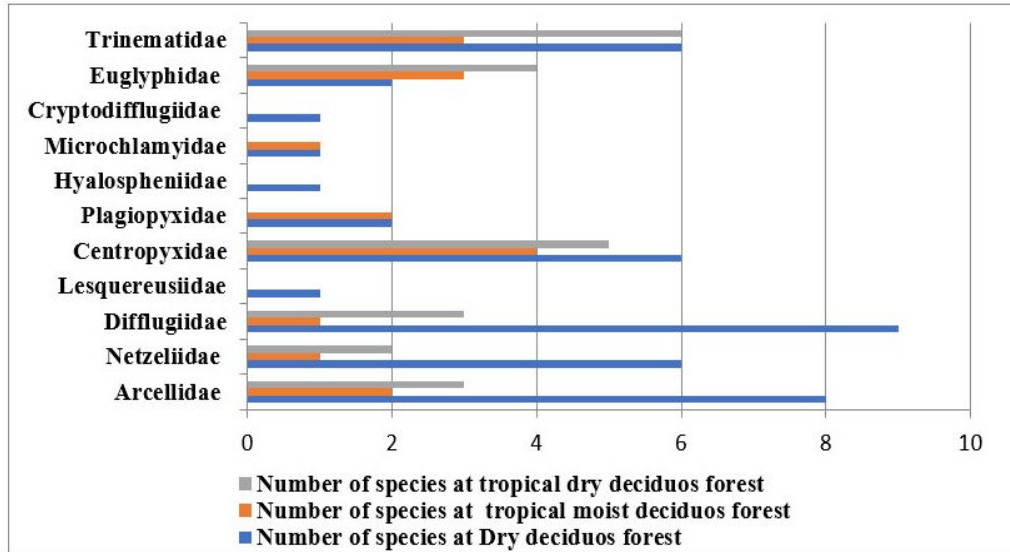
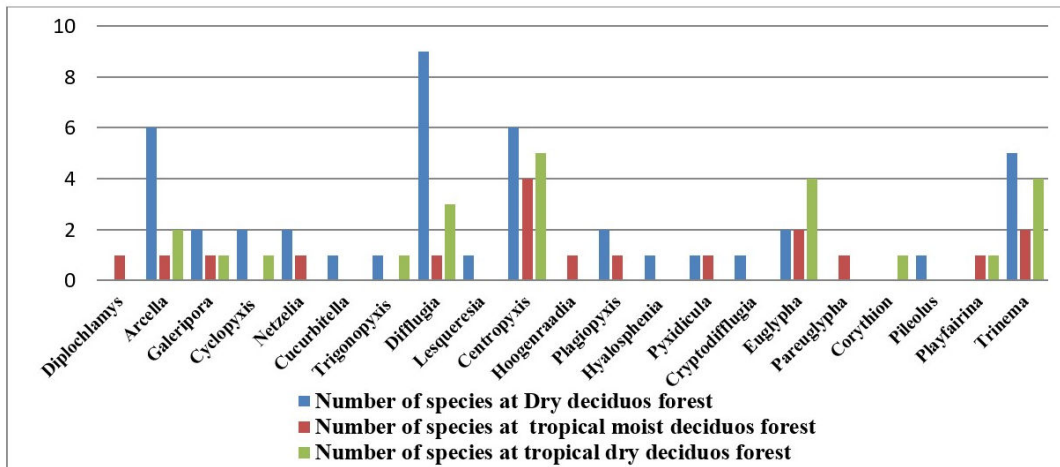


Figure 2: Family based diversity of testate amoeba across the altitude gradient of 20-230 feet.



**Figure 3: Family based diversity of testate amoeba in the three different forests.**

The family Difflugidae (9) has the highest species diversity in the Dry deciduous forest followed by Arcellidae, Trinematidae, Centropyxidae, Difflugidae, Netzeiliidae and Euglyphidae. The family Trinematidae (6) has the highest species diversity in the tropical drydeciduous forest followed by Centropyxidae, Euglyphidae, Difflugidae, Arcellidae and Netzeiliidae. The family Centropyxidae (4) has the highest species diversity in the tropical moist deciduous forest followed by Trinematidae, Euglyphidae, Arcellidae, Plagiopyxidae, Difflugidae and Netzeiliidae. The family Difflugidae has negative correlation with altitudinal gradient; Dry deciduous forest located in range below 250 feet has 9 species >tropical moist deciduous forest located in range below 50 feet has 3 species>tropical Dry deciduous forest located in range below 25 feet has 1 species.



**Figure 5: Genus based diversity of testate amoeba in the three different forests.**

The family Trinematidae is cosmopolitan in nature and found in all forests across the whole altitudinal gradient. It has negative correlation with altitudinal gradient; Dry deciduous forest located in range below 250 feet has 5 species > tropical moist deciduous forest located in range below 50

feet has 4 species > tropical Dry deciduous forest located in range below 25 feet has 2 species. The family Centropxyidae has negative correlation with altitudinal gradient; Dry deciduous forest located in range below 250 feet has 6 species > tropical moist deciduous forest located in range below 50 feet has 5 species > tropical Dry deciduous forest located in range below 25 feet has 4 species (Figure 4-6).

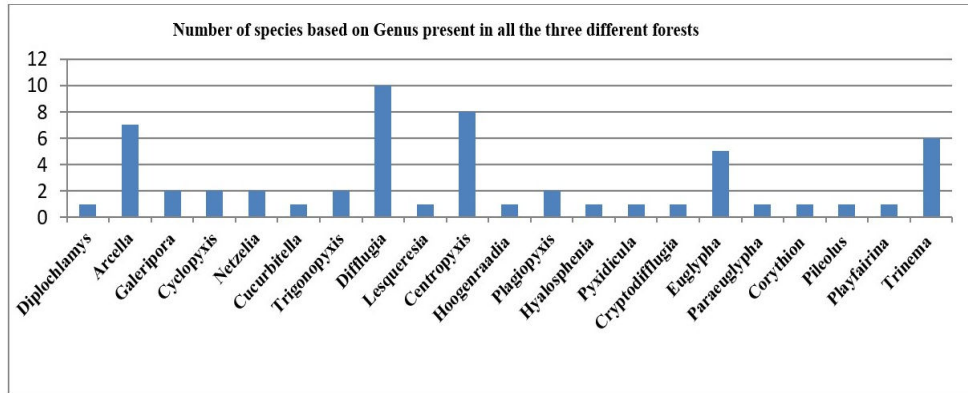


Figure 4: Genus based diversity of testate amoeba across the altitude gradient of 20-230 feet.

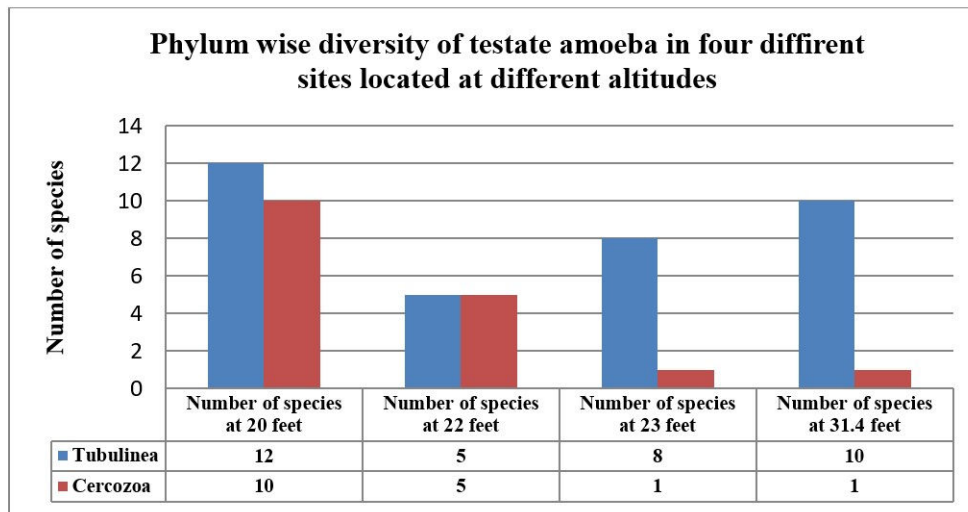
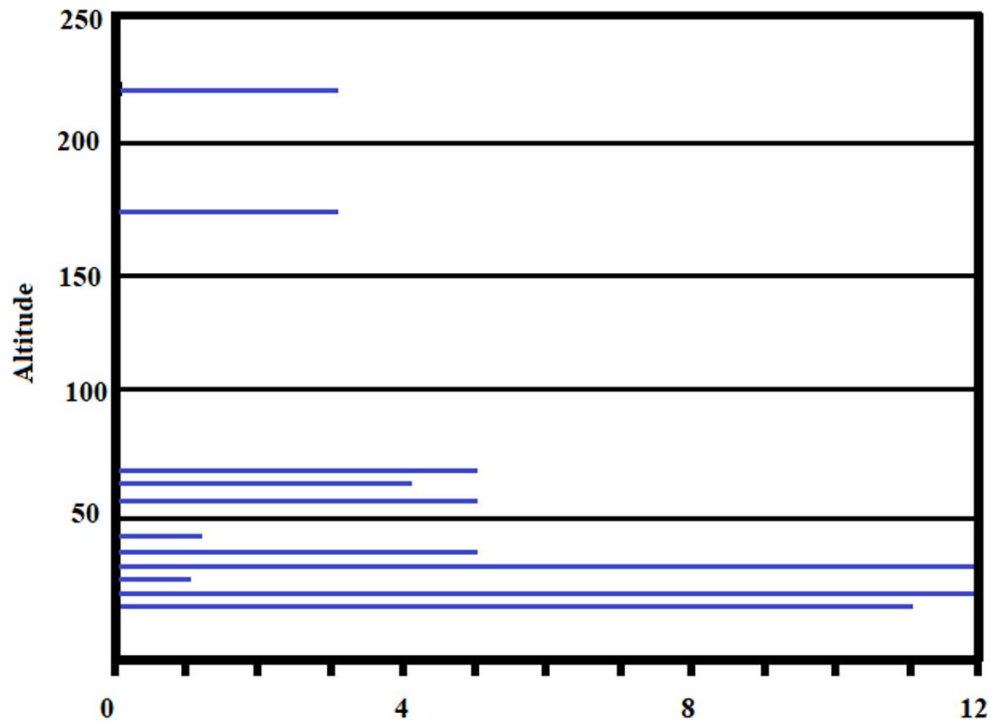


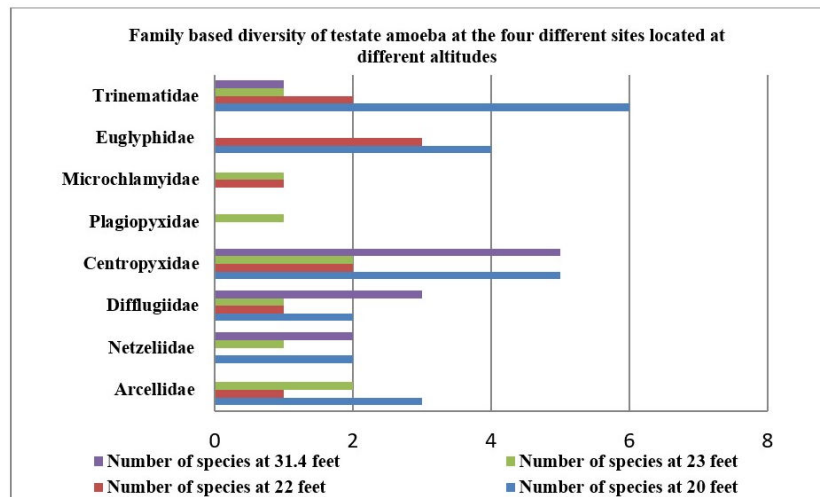
Figure 6: Phylum based diversity of testate amoeba situated at 20 ft, 22 ft, 23 ft and 31.4 feet altitude among the study areas situated in three different forests having maximum diversity.

All the study sites located at lower altitude, the Phylum Tubulinea has major number of species. The results show Phylum Tubulinea: Phylum Cercozoa found in the ratio of 3:1 in dry deciduous, 2:1 in tropical moist deciduous forest and 1:1 in tropical Dry deciduous forest. Among the 12 families found across the study areas, Arcellidae excels followed by Diffugiidae, Trinematidae, Centropxyidae, Euglyphidae and Netzeliidae. The diversity of testate amoeba has negative correlation with altitudinal gradient which is shown in Figure 7.



**Figure 7: Diversity of testate amoeba across the Altitudinal gradient of three forests**

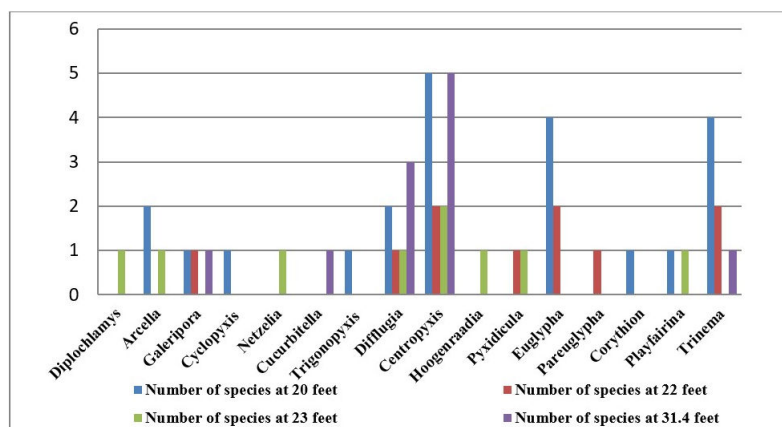
The four sites having the highest diversity of testate amoeba show Tubulinea as the dominant phylum (Figure 5 and 6). The four sites have diversity of testate amoeba belonging to 8 families (Figure 8) and 21 Genus (Figure 9). Along with altitudinal gradient the pH, temperature and moisture content in the forest areas impact the diversity of testate amoeba (Figure 9).



**Figure 8: Family based diversity of testate amoeba situated at 20 ft, 22 ft, 23 ft and 31.4 ft altitude among the study areas situated in three different forests having maximum diversity.**

The Genus *Centropyxis* (5), *Euglypha* (4), *Trinema* (4), *Diffugia* (3) and *Arcella* (2) have the highest number of species at the altitude of 20 feet in the decreasing order respectively. The species abundance is high for *Centropyxis discoides*, *Euglypha laevis* and *Trinema lineare* at this site. The Genus *Centropyxis*, *Euglypha* and *Trinema* have the highest number of species at the altitude of 22

feet. *Trinema lineare* and *Euglypha laevis* have species abundance at this site. *Diffflugia pulex* has the highest species abundance at altitude of 23 feet. The genus *Centropyxis* has highest number of species at the altitude of 31.4 feet.



**Figure 9: Genus based diversity of testate amoeba situated at 20 ft, 22 ft, 23 ft and 31.4 ft altitude among the study areas situated in three different forests having maximum diversity.**

The testate amoeba diversity decreases with increasing latitude:  $\pm 300$  taxa in Arctic [5] from a total of  $\pm 2000$ , and a decline in nebelid species richness were observed in the high southern latitudes [18-20]. A significant difference was found in the diversity and species abundance of testate amoeba at these study areas which were also located at various altitudes. Along with altitude gradient, other environmental parameters also play role in their diversity.

Testate amoeba, a highly important element of the microbial community plays several roles in soil and other freshwater habitats. A comprehensive survey of such a crucial living component in the soil, water, and moss habitats of West Bengal's protected areas is quite limited, allowing for a broad breadth of study to be done now and in the future.

#### 4. ACKNOWLEDGEMENTS

We thank the PCCF, West Bengal State Forest Department for necessary permissions for the sampling and all forest staff for their timely help to facilitate this research. We are grateful to the Director, Zoological Survey of India for the encouragement and providing the necessary facilities. This research work is done under the annual program of ZSI, Kolkata. We are also grateful to the sectional staff of Protozoology section, HQ, ZSI, Kolkata for the field sampling and processing at the laboratory.

#### 5. REFERENCES

1. Burger, J. and Gochfeld, M., "On developing bioindicators for human and ecological health. Environmental monitoring and assessment, *Journal of Eukaryotic Microbiology*", vol 66(1), (2001), pp 4-119.
2. Amesbury, M. J., Swindles, G. T., Bobrov, A., Charman, D. J., Holden, J., Lamentowicz, M. and Warner, B. G., "Development of a new pan-European testate amoeba transfer function

- for reconstructing peatland palaeohydrology, *Quaternary Science Reviews*, vol 152, (2016), pp 132-151.
3. Payne, R. J., "Testate amoeba response to acid deposition in a Scottish peatland", *Aquatic Ecology*, vol 44, (2010), pp 373-385.
  4. Roe, H. M. and Patterson, R. T., "Arcellacea (testate amoebae) as bio-indicators of road salt contamination in lakes", *Microbial Ecology*, vol 68, (2014), pp 299-313.
  5. Beyens L. and Chardez, D., "An annotated list of testate amoebae observed in the Arctic between the longitudes 27°E and 168°W", *Arch Protistenkd*, vol 146, (1995), pp 219-233
  6. Charman, D. J. and Hendon, D., "Long-term changes in soil water tables over the past 4500 years: relationships with climate and North Atlantic atmospheric circulation and sea surface temperature", *Clim Change*, vol 47, (2000), pp 45–59.
  7. Farooqui, A., Singh, H., Prasad, M. and Singh, V. K., "Morphometry and morphology of Testate amoebae from the Miocene sub Himalayan zone of Darjeeling, India", *Himalayan Geology*, vol 42(1), (2021), pp 137-154.
  8. Golemansky, V., "Interstitial testate amoebae (Rhizopoda: Testacea) from the Italian coast of the Mediterranean Sea", *Acta Protozoologica*, vol 37(3), (1998), pp 139-143.
  9. Qin, Y., Mitchell, E. A., Lamentowicz, M., Payne, R. J., Lara, E., Gu, Y. and Wang, H. Ecology of testate amoebae in peatlands of central China and development of a transfer function for paleohydrological reconstruction. *Journal of Paleolimnology*, vol 50, (2013), pp 319-330.
  10. Charman, D. J., "Biostratigraphic and palaeoenvironmental applications of testate amoebae", *Quaternary Science Reviews*, vol 20(16-17), (2001), pp 1753-1764.
  11. Ogden, G. G. and Hedley, R. H., "An atlas of freshwater testate amoebae", *Soil Science*, vol 130(3), (1980), p 176.
  12. Bobrov, A. and Mazei, Y., "Morphological variability of testate amoebae (Rhizopoda: Testacealobosea and Testaceafilosea) in natural populations", *Acta Protozoologica*, vol 43(2), (2004), pp 133-146.
  13. Heger, T. J., Pawlowski, J., Lara, E., Leander, B. S., Todorov, M., Golemansky, V. and Mitchell, E. A., "Comparing potential COI and SSU rDNA barcodes for assessing the diversity and phylogenetic relationships of cyphoderiid testate amoebae (Rhizaria: Euglyphida)", *Protist*, vol 162(1), (2011), pp 131-141.
  14. Lamentowicz, M., Bragazza, L., Buttler, A., Jassey, V. E. J. and Mitchell, E. A. D., "Seasonal patterns of testate amoeba diversity, community structure and species–environment relationships in four Sphagnum-dominated peatlands along a 1300 m altitudinal gradient in Switzerland", *Soil Biology and Biochemistry*, vol 67, (2013), pp 1-11.
  15. Mitchell, E. A., Charman, D. J. and Warner, B. G., "Testate amoebae analysis in ecological and paleoecological studies of wetlands: past, present and future", *Biodiversity and Conservation*, vol 17, (2008), pp 2115-2137.
  16. Mitchell, E. A., Lamentowicz, M., Payne, R. J. and Mazei, Y., "Effect of taxonomic resolution on ecological and palaeoecological inference—a test using testate amoeba water table depth transfer functions", *Quaternary Science Reviews*, vol 91, (2014), pp 62-69.
  17. Turner, T. E. and Swindles, G. T., "Ecology of testate amoebae in moorland with a complex fire history: implications for ecosystem monitoring and sustainable land management", *Protist*, vol 163(6), (2012), pp 844-855.
  18. Smith, H. G., "Distribution and ecology of the testate rhizopod fauna of the continental Antarctic zone", *Polar Biol*, vol 12, (1992), pp 629–634
  19. Smith, H. G. "Diversity of Antarctic terrestrial protozoa", *Biodivers Conserv*, vol 5, (1996), pp 1379–1394



20. Wilkinson, D. M., "A review of the biogeography of the protozoan genus *Nebela* in the southern temperate and Antarctic zones", *Area*, vol 26, (1994), pp 150–157.
21. Chattopadhyay, P. and Das, A. K., "Morphology, morphometry and ecology of moss dwelling testate amoebae (Protozoa: Rhizopoda) of North and North –East India", *Mem Zool Surv India*, vol 19(4), (2003), pp 1-113.
22. Das, A. K., Mondal, A. K and Sarkar, N. C., "Freeliving Protozoa", *Zool Surv India, State Fauna Series, 3: Fauna of West Bengal, Part 12*, (1993), pp 1-134.
23. Das, A. K., Mondal, A. K., Tiwari, D. N. and Sarkar, N. C., "Protozoa", *Zool Surv India, State Fauna Series, 4: Fauna of Meghalaya, Part 10*, (1995), pp 1-107.
24. Adl, S. M., Bass, D., Lane, C. E., Lukeš, J., Schoch, C. L., Smirnov, A. and Cárdenas, P., "Revisions to the classification, nomenclature, and diversity of eukaryotes", *Journal of Eukaryotic Microbiology*, vol 66(1), (2019), pp 4–119.
25. Hoogenraad, H. R. and Groot, A. A. de, "Observations on a special manner of feeding of a species of *Diffugia* (*Diffugia rubescens* Penard)", *Proc Ned Akad Wet*, vol 44, (1941), pp 3-14.

-----