

Original Research Article
**Diversity and Environmental Significance of
Moss-Dwelling Testate Amoebae in various
Biomes Around Nainital Lake, Uttarakhand**

ABSTRACT

The present research aims to record the diversity of testate amoebae occurring in different biomes around Nainital Lake, Uttarakhand, concerning distribution. Free-living, testate amoebae are single-celled protists with external shells and acting excellent bio-indicators of environmental changes. Collection of Tree, Rock and wall Moss samples was done at different locations around the Nainital Lake area from both the littoral zones and the terrestrial regions. The results show an impressive number of species of testate amoebae; 39 species of testate amoebae span over 11 genera and 8 families that all are new for the region and stress the role of some species in biomonitoring and evaluation of the condition of the environment in these peculiar biomes. This study not only increases researcher's knowledge on the testate amoebae species distribution in Nainital Lake but also contributes to the body of knowledge that would be useful for the ecological and environmental status of the area in the future. The outcomes of the research confirm the need for the conservation of the habitats in the area surrounding Nainital Lake to sustain the biodiversity and ecosystems of the region.

Keywords: Testate Amoebae, Protozoa, Moss, Nainital, Pollution Indicator

1. INTRODUCTION

Nainital is a hill station in the state of Uttarakhand located in the Kumaon region, often referred to as the "Lake District of India". It is well-known for its picturesque beauty, calm waters of lakes and prosperity in bio-diversity. Locationally, Nainital stands at about 2,084 meters above sea level and is located in the Kumaon Hills, a part of the lesser Himalayas.

The town is set around the Nainital Lake, a natural freshwater lake that covers approximately 48 hectares and with a maximum depth of 29 meters [1].

Geographically and climatically, the region is very peculiar with exciting flora and fauna and is especially important for ecological and environmental research. Various plant and animal life pertain to the habitats of Nainital which includes aquatic and terrestrial species [2]. This diversity is complemented by the presence of forests, alpine meadows, and various mosses and ferns, which creates suitable conditions for research on various microfauna, especially the testate amoebae.

Environmental conservation factors of Nainital are not restricted to its biological importance; it also has hydrological and climatological importance to the region. In actuality, the lake and its associated catchment area serve as a water supply for the water requirements of the town and nearby region, along with they control the micro-climate through evapotranspiration [3]. Based on these factors, Nainital is a significant natural laboratory for ecological and environmental studies concerning the need to conserve and manage Nainital's natural resources sustainably. Moss-dwelling testate amoebae, a unique group of protists characterized by shell-like tests, are pivotal indicators of environmental conditions and play significant roles in microbial food webs and nutrient cycling [4]. These microorganisms are particularly abundant and diverse in mosses, where they utilize the microhabitats provided by the dense mat of moss leaves and the associated microclimate [5]. The biotopes around Nainital Lake present a unique mosaic of habitats ranging from aquatic to terrestrial, creating ideal conditions for studying these amoebae. A comprehensive account of the moss-dwelling testate amoebae from this region remains sparse. So, this study aims to fill this gap by providing an account of the testate amoebae inhabiting various biotopes around Nainital Lake. The findings will contribute to the understanding of the biodiversity and ecology of testate amoebae in this region and offer insights into their potential as bioindicators for environmental monitoring and conservation efforts.

2. LITERATURE REVIEW

2.1. TESTATE AMOEBAE AS BIOINDICATORS

Testate amoebae, a diverse group within the phyla Tubulinea and Cercozoa, have shells called tests made of silicon oxide, chitin, or calcium carbonate [6,7]. Biochemical research has found that there are more than a thousand known species all over the world and the discovery is still added to the list day by day [8,9]. The available research on testate amoebae diversity in India has shown that the species of this group are diverse in various habitats, ranging from the freshwater ponds to the forest soils [10]. These organisms have essential functions in nutrient turnover and microbial networks of consumers, with their abundance and species richness varying with the local environment, the water content, acidity and nutrient concentrations [11].

Shelled protozoa such as testate amoebae can be met in various aquatic and terrestrial habitats and have been revealed to be useful in reflecting the conditions of an environment, especially in peatland, freshwater environments, and soils [12]. Due to their high degree sensitivity of moisture, pH, and other environmental changes, they can be used as an indicator of ecological changes [13]. Testate amoebae are of sensitivity to water quality, pollution and climate change parameters and is therefore used in paleo reconstructions of water characteristics as well as in the current ecological state [14]. The existence, absence and variety of testate amoebae can indicate alterations in the conditions of the habitat, for instance hydrological characteristics or in the pollution level. For example, some species are adapted to acidic conditions while others to near neutral conditions hence the use of the species to deduce changes in pH and moisture levels over the years [15].

Free-living amoebae are known to have significant applications as bioindicators to show changes in the environment, especially as regards to the pollution status of water and land. These organisms, which are usually microscopic and have shelllike structures made of organic materials are known to be very sensitive to changes in water chemistry, nutrients and organic pollution [4,15]. For example, some species of *Arcella* genus can live well in water with low nutrient concentration but are affected by polluted water due to high heavy metals and organic pollutants [11]. Furthermore, the *Diffugia* species exhibited changes in community about eutrophication and change of sediment related to anthropogenic influence [16]. Such findings depict testate amoebae as bioindicators that could effectively show environmental alterations caused by pollution, hence being relevant in monitoring ecosystems and helping in the conservation of ecosystems. Besides being helpful in presentday environmental analysis, testate amoebae are also helpful in paleoecology. Fossils preserve their physical bodies, allowing their remains can be used to help study past conditions and thus long term changes in the climate and its effects on the ecological systems [17].

2.2.GLOBAL DIVERSITY

The global distribution of testate amoebae is more than 675 species under 104 genera and 22 families including records from polar regions.

2.3. DIVERSITY IN INDIA

In India 209 species belong to 37 genera under two classes and two orders of testate amoebae were recorded.

2. MATERIAL AND METHODS

The moss samples for the present study were collected from various biotopes around Nainital Lake (Fig.1) as part of the survey of the Western Himalaya by the Zoological Survey of India in October 2019. The samples were obtained from various biotopes like rocks, trees and walls around the lake (29° 23' 02.94" N and 079° 27' 21.42" E., Alt. 1940 m.) by scraping with a spatula into polythene bags and brought to the laboratory for further processing. The processing of samples followed the non-flooded petri dish method outlined by Foissner [18]. Subsequently, prepared permanent slide mounts from each sample and examined them using compound microscopes equipped with a camera attachment for image capturing and species-level identification. All the registered permanent slides were deposited in the National Zoological collections of Marine Biology Regional Centre, Zoological Survey of India, Chennai.

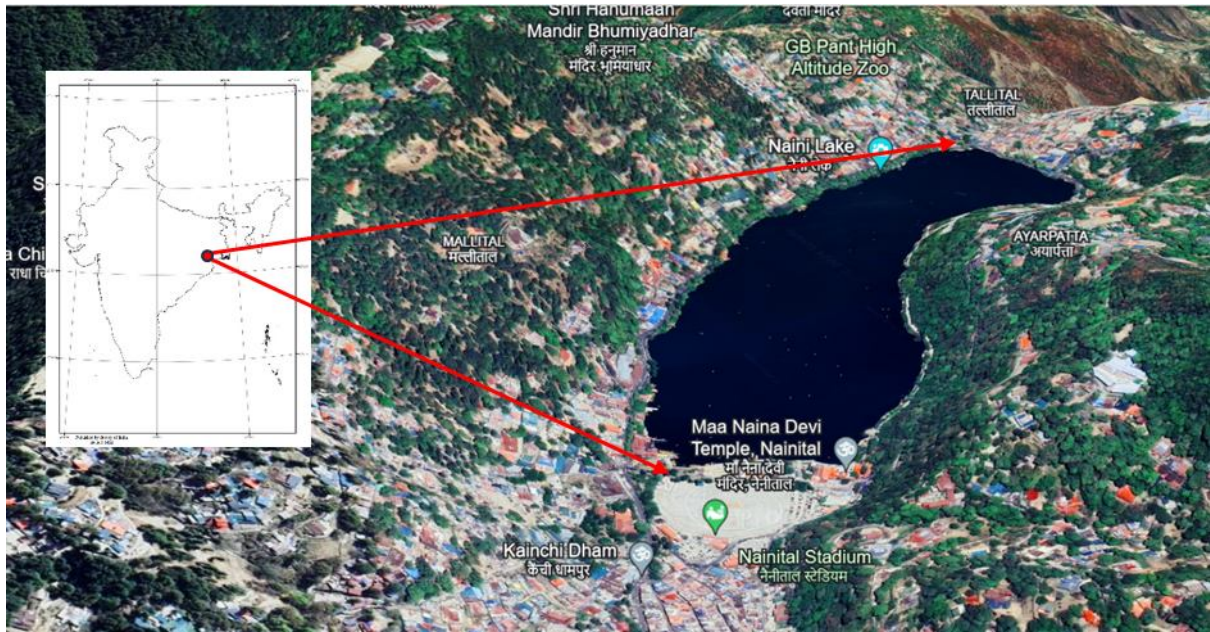


Figure 1: Map of Nainital Lake: The Study Area (Source: Modified from Google Earth & SOI)

3. RESULTS

The study yielded the records of 39 species of testate amoebae spanning over 11 genera and 8 families from various biotopes of mosses viz., tree, rock and wall (Fig.2). It is found that the maximum diversity of species was observed in Tree moss and the least diversity was in rock moss. Tree mosses hold most moisture content and the moisture regime of the habitat has a significant influence on the activity of the testate amoebae fauna and its population fluctuation [19].

Systematic list of Testate Amoebae around Nainital Lake: Findings from the present study (Classification as per Adlet *et al.*, 2019)[20]

Phylum Tubulinea Smirnov *et al.*, 2005

Class Elardia Kang *et al.*, 2017

Order Arcellinida Kent, 1880

Family Arcellidae Ehrenberg, 1843

1. *Galeripora catinus* (Penard, 1890) González-Miguéns *et al.*, 2021

Family Netzeiliidae Kosakyan *et al.*, 2016

2. *Cyclopyxis arcelloides* (Penard, 1902) Deflandre, 1929
3. *Cyclopyxis arcelloides gibbosa* Van Oye, 1949
4. *Cyclopyxis eurystoma* Deflandre, 1929
5. *Cyclopyxis kahli* Deflandre, 1929 tree, wall

Incertaesedis

6. *Trigonopyxis arcularia* Penard 1912

Family Diffugiidae Wallich, 1864

7. *Diffugiaglobulosa* (Dujardin, 1837) Penard, 1902
8. *Diffugiale vanderi* Playfair, 1918

Family Centropyxidae Jung, 1942

9. *Centropyxis aculeata* (Ehrenberg, 1838) Stein, 1859
10. *Centropyxis aculeata grandis* Deflandre, 1929
11. *Centropyxis aculeata oblonga* Deflandre, 1929

12. *Centropyxisaerophila* Deflandre, 1929
13. *Centropyxisaerophilasphagnicola* Deflandre, 1929
14. *Centropyxis cassis* (Wallich, 1864) Deflandre, 1929
15. *Centropyxisconstricta* (Ehrenberg, 1841) Penard, 1890
16. *Centropyxislaevigata* Penard, 1890
17. *Centropyxisminuta* Deflandre, 1929
18. *Centropyxis orbicularis* Deflandre, 1929
19. *Centropyxisplatystoma* (Penard, 1890) Deflandre, 1929

Incerta sedis Arcellinida

20. *Awerintzewiacyclostoma* Schouteden, 1906

Phylum Cercozoa Cavalier-Smith, 2018

Class Silicofilosea Adlet *et al.*, 2012

Order Euglyphida Cavalier-Smith, 1997

Family Assulinidae Lara *et al.*, 2007

21. *Assulinamuscorum* Greeff, 1888
22. *Assulinaseminulum* (Ehrenberg, 1848)

Family Euglyphidae Lara *et al.*, 2007

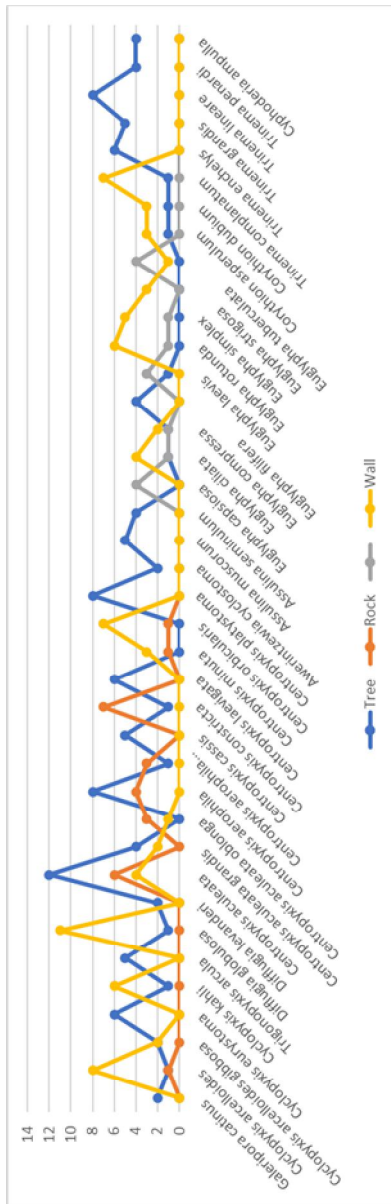
23. *Euglyphacapsiosa* Coûteaux, 1978
24. *Euglyphaciliata* (Ehrenberg, 1848)
25. *Euglyphacompressa* Carter, 1864
26. *Euglyphafilifera* Penard, 1890
27. *Euglyphalaevis* (Ehrenberg, 1845)
28. *Euglypha rotunda* (Ehrenberg, 1845)
29. *Euglypha simplex* Decloitre, 1965
30. *Euglyphastrigosa* (Ehrenberg, 1848) Leidy, 1878
31. *Euglyphatuberculata* Dujardin, 1841

Family Trinematidae Adlet *et al.*, 2012

32. *Corythionasperulum* Schonborn, 1988
33. *Corythiondubium* Taranek, 1871
34. *Trinema complanatum* Penard, 1890
35. *Trinemaenchelys* (Ehrenberg, 1838)
36. *Trinemapenardis* (Chardez, 1960) Golemansky, 1963
37. *Trinema lineare* Penard, 1890
38. *Trinemapenardi* Thomas & Chardez, 1958

Family Cyphoderiidae Saedeleer, 1934

39. *Cyphoderia ampulla* (Ehrenberg, 1840)



4. Conclusion

In conclusion, by showcasing the beauty of the location marked Lake and the nature around it, Nainital has undoubtedly region's economy. This popularity also comes with increased impacts to the extent that it is hard to sustain. The town's Naini Lake for both entertainment and domestic water highlights the necessity to manage the problem of water pollution quality by controlling the impact of the pollutants generated by modern agriculture.

The continued environmental impacts in Nainital are air pollution emission and industrial discharge, water pollution through sewage discharge, and soil pollution through improper waste are threats to wildlife and human beings. Such problems require

Fig. 2. Line Chart showing composition of dwellings from various biotopes around Nainital lake, Uttarakhand

by the Naini benefitted the environmental dependence on requirements and water urbanization and

due to vehicle untreated disposal which prompt and

efficient steps to combat pollution and introduce rational management practices.

In this connection, the presence of testate amoebae dwelling in mosses offers a good chance of observing changes concerning the state of the environment. These microorganisms are susceptible to the changes in their environment, so they are widely used in signaling pollution and ecological changes [21]. Field studies have demonstrated that species in the genera, *Arcella* and *Diffugia* perform to changes in water chemistry and nutrient concentrations [21,22,23], which is an indication of overall environmental conditions. In the present study, the abundance of *Centropyxisaculeata* in tree moss indicates higher level of organic pollution in the lake area which supports the studies of Olivia [24].

Further research should be conducted on testate amoebae to develop more precise site-specific research concerning the function of these organisms in ecological assessment, especially in disturbed habitats influenced by people. Therefore, by incorporating such microhabitat-specific studies, it will be possible to understand better the environmental processes of such delicate mountain ecosystems as Nainital Lake and improve the chances of their protection and further use.

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Option 1:

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REFERENCES

1. Joshi, J.C., Joshi, D.R and Dani, D.D. Kumaun Himalaya: A Geographic Perspective on Resource Development. The University of Michigan. GyanodayaPrakashan. 1983. 324 Pp.
2. Semwal, R.L. and Mehta, J.P. Ecology of forest fires in Chir pine (*Pinus roxburghii* Sarg.) forest of Garhwal Himalaya. 1996; *Current Science*, 70(6): 426-427.
3. Rajput, V., Jong, T.F. and NEGI, R. K. Hydrological study of Naini lake (Nainital), India. *J. Flora and Fauna*. 2009; 15(1): 83-86.
4. Mitchell, E. A. D., Charman, D. J., Warner, B. G and Wilmshurst, J. M. Testate amoebae as environmental and climate indicators in peatland studies. In: Mitchell, E. A. D and Charman, D. J. (Eds.), *Testate Amoebae in Peatlands: Past and Present. Developments in Earth Surface Processes*. 2008; 11: 1-30.
5. Baynes, L and Chardez, D. An annotated list of testate amoebae observed in the Arctic between the longitudes 27° E and 169° W. *Archiv für Protistenkunde*. 1995; 146(3-4): 219-233.
6. Lara, E., Heger, T. J., & Mitchell, E. A. D. Recent progress in the taxonomy of testate amoebae (order Arcellinida Kent, 1880) (Protista): new scales from Venezuela. *European Journal of Protistology*. 2007; 43(1): 37-52.
7. Kosakyan, A., Heger, T. J., Leander, B. S., Todorov, M., Mitchell, E. A. D and Lara, E. COI barcoding of neotropical freshwater testate amoebae (Arcellinida): diversity assessment and insights into the genetic structure of Arcellinida. *Protist*. 2016; 167(6): 523-536.
8. Bobrov, A., Mazei, Y and Lahr, D. J. G. 2017. The new family Paramphitrematidaenov.fam.

and two new species of amphitrematid testate amoebae (Arcellinida, Tubulicorythida) from Russia and Scotland. *Protistology*. 2017;11(3): 148-160.

9. Mitchell, E. A. D., Charman, D. J and Warner, B. G. Testate amoebae analysis in ecological and paleoecological studies of wetlands: past, present and future. *Biodiversity and Conservation*. 2015; 17(2): 211-230.

10. Bobrov, A., Todorov, M., Kosakyan, A and Mitchell, E. A. D. The diversity and distribution of testate amoebae in the Eastern Ghats of India. *European Journal of Protistology*. 2018; 64: 69-80.

11. Heger, T. J., Mitchell, E. A. D and Overpeck, J. T. Climatic and environmental controls on testate amoebae in tropical South America over the Last Glacial-Interglacial Transition. *Quaternary Science Reviews*. 2018; 188:161-175.

12. Charman, D. J. Biostratigraphic and Paleoecological Applications of Testate Amoebae. *Quaternary Science Reviews*. 2001; 20(16): 1753-1764.

13. Anna Simova, Martin Jirousek, Patricia Singh, Petra Hajkova and Michal Hajek. Ecology of testate amoebae along an environmental gradient from bogs to calcareous fens in East-Central Europe: development of transfer functions for palaeoenvironmental reconstructions, *Palaeogeography, Palaeoclimatology, Palaeoecology*. 2022; 60: 111-145, ISSN 0031-0182, <https://doi.org/10.1016/j.palaeo.111145>.

14. Bobrov, A., Charman, D. J and Warner, B. G. Ecology of Testate Amoebae (Protozoa: Rhizopoda) on Peatlands in Western Russia with Special Attention to Niche Separation in closely related taxa. *Protist*. 1999; 150(2): 125-136.

15. Jassey, V. E., Signarbieux, C., Hättenschwiler, S., Bragazza, L., Buttler, A., Delarue, F and Mitchell, E. A. D. 2013. An unexpected role for mixotrophs in the response of peatland carbon cycling to climate warming. *Scientific Reports*. 2013; 3: 23-54.

16. Wilkinson, D. M., Mitchell, E. A. D., Wilkinson, G. M. and Richards, K. The response of testate amoeba communities to changes in land use and vegetation over the past 500 years. *Journal of Biogeography*. 2019; 46(7): 1362-1375.

17. McCarthy, F. M. G., Collins, E. S., McAndrews, J. H and Kerr, H. A. A Comparison of Postglacial Arcellacean (Thecamoebian) and Pollen Succession in Atlantic Canada, illustrating the Potential of Arcellaceans for Paleoclimatic Reconstruction. *Journal of Paleontology*. 1995; 69(5): 980-993.

18. Foissner, W. Estimating the species richness of soil protozoa using non-flooded petridish method. *In Protocols in Protozoology*. 1992; Lee, J.J and Soldo, A.T. (eds), Allen Press.

19. Smith, H. G. Distribution and ecology of the testate rhizopod fauna in continental Antarctic zone. *Polar Biol*. 1992; 12: 629-634.

20. Adl, S.M., Bass, D., Lane, C.E., Lukes, J., Schoch, C.L., Smirnov, A et al. Revisions to the classification, nomenclature, and diversity of eukaryotes. *Journal of Eukaryotic*

Microbiology.2019; 66: 4–119.

21.Patterson, R.T. and Kumar, A. A review of current testate rhizopod (thecamoebian)research in Canada. *Palaeogeography, Palaeoclimatology, Palaeoecology*.2002;180 (1-3): 225-251. [https://doi.org/10.1016/S0031-0182\(01\)00430-8](https://doi.org/10.1016/S0031-0182(01)00430-8).

22.Yang, J., Zhang, W and Shen, Y. Response of testate amoebae (*Diffugia: oblonga*) to different levels of pollution in freshwater ecosystems. *Environmental Pollution*.2010; 158(2): 287- 294. <https://doi.org/10.1016/j.envpol.2009.08.026>

23.Mitchell, E. A. D and Gilbert, D.Vertical micro-distribution and response to nitrogen deposition of testate amoebae in Sphagnum. *Journal of Eukaryotic Microbiology*. 2004; 51(4): 480- 490. <https://doi.org/10.1111/j.1550-7408.2004.tb00400.x>

24.Olivia Kuuri-Riutta¹, MinnaValiranta, Eeva-StiinaTuittila.Literature review on testate amoebae as environmental indicators and as a functional part of the microbial community in northern peatlands, *Mires and Peat*.2022; 28:Article 28, 16 pp., <http://www.mires-and-peat.net/>, ISSN 1819-754X, International Mire Conservation Group and International Peatland Society, DOI: 10.19189/MaP.2022.OMB.StA.2412

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