© NATIONAL INSTITUTE OF ECOLOGY, NEW DELHI

# Assessment of Testate Amoeba (Protozoa) assemblage in Bibhutibhushan Wildlife Sanctuary, West Bengal

# ARADHANA CHAUDHARY<sup>1, 2,3</sup>AND JASMINE PURUSHOTHAMAN<sup>2,\*</sup>

<sup>1</sup>Raja Peary Mohan College, Uttarpara, Hooghly, 712258, West Bengal, India <sup>2</sup>Zoological Survey of India, M- Block, New Alipore, Kolkata, 700053, India <sup>3</sup>Department of Zoology, University of Calcutta, 35 Ballygunge Circular Road, Kolkata, 700019, India Email: chaudharyaradhana@gmail.com; jasbose@gmail.com \*Corresponding author

#### ABSTRACT

In this study we assessed the assemblage of the testate amoeba in the fresh water sample collected from the Bibhutibhushan Wildlife Sanctuary, West Bengal, India during January 2023. Twenty three species belonging to 10 Genera, 6 Families, and 2 orders are recorded from this sanctuary where 57 percent belongs to Phylum Tubulinea and rest 43 percentage belongs to Phylum Cercozoa. This evergreen forest showed a significant diversity and serves as a repository for many testaceans which indicates the fertile quotient of alluvial soil found in this region.

Key words: Alluvial soil, Bibhutibhushan Wildlife Sanctuary, Testate amoeba, Assemblage

# **INTRODUCTION**

Testate amoeba (TA) is the extensive found unicellular protozoans enclosed in shell which can withstand a wide range of environmental circumstances (Charman et al. 2000, Medioli and Scott 1983, Smith et al. 2008) and has a distinctive shape. Currently they are recognized as an assemblage of three unrelated groups Amoebozoa, Stramenopiles and Cercozoa (Adl et al. 2019). In many terrestrial ecosystems, especially tropical forests, testate amoebae are important bacterial consumers (Krashevska et al. 2007, 2017) and comprises as a very vital member of the microbial community. The Bibhutibhushan Wildlife Sanctuary (WLS) is fed by most fertile soil type (alluvial) and hence encompasses thick dense evergreen forest type with high biodiversity. The diversity and distribution of testate amoeba is still poorly studied in protected areas of West Bengal.

This study is the first attempt to analyze the diversity and distribution of TA in Bibhutibhushan Wildlife Sanctuary, West Bengal, India since the review of literature revealed that no work has been done so far from this area. The free-living protists are a major component of the Earth's biodiversity and play a crucial role in its ecological health. Understanding their diversity will disclose the information of its indicator value in multiple aspects. The detailed analysis of testate amoeba from fresh water bodies of Bibhutibhushan Wildlife Sanctuary (WLS) are represented in this research work.

## **MATERIALS AND METHODS**

### Study area

Bibhutibhushan Wildlife Sanctuary (WLS) lies on the banks of Ichamati River of North 24 Parganas Forest Division in the North 24 Parganas\_District\_of West Bengal State. This wildlife sanctuary is situated at geographical location of 23.1861775°N 88.7620868°E covering an area of 0.68 km<sup>2</sup>. It was established in 1980 under the name of 'Parmadan' by introducing chital in this area and was renamed as Bibhutibhushan WLS in 1995. It has large population of deer, birds, rabbits, langur, monkey, numerous species of birds and more than 200 floral species. The tall trees such as *Dalbergia sisso* Roxb., Morus alba Linnaeus, Terminalia arjuna (Roxb.) Wight & Arn., Trewia nudiflora Linnaeus, Bombax ceiba Linnaeus, Albizzia lebbek (L.) Benth forms the mainstay in the forest while the undergrowth is thick and dense comprising of mostly ferns, tall grasses, and arum bushes which border along the river and also the core area of the sanctuary.

Since no previous research has been done from the Bibhutibhushan Wildlife Sanctuary in West Bengal, this study is the first attempt to examine the richness and distribution of TA in this location. The high-diversity of free-living protists makes it an important component of Earth's biodiversity which is still unidentified in major forests in West Bengal. More intense studies will enable to formulate concrete information on their indicator value in several aspects available. This study paper represents the comprehensive investigation of testate amoeba from fresh water bodies of Bibhutibhushan Wildlife Sanctuary (BWLS).

## Sampling

The study was conducted on fresh water samples collected from different water bodies and several regions of Bibhutibhushan Wildlife Sanctuary, West Bengal, India from 2<sup>nd</sup> to 8<sup>th</sup>January 2023. The sample was collected from GPS fixed locations (Garmin GPS 72H) and brought to the laboratory. Thereafter aqueous drops were drawn from the sample using a micropipette, kept on glass micro slides, thoroughly 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 (Das et al. 1993, 1995, Chattopadyay and Das 2003). The enumeration and identification of these prepared permanent microscopic slides using published guides and keys Ogden and Hedley 1980, Hoogenraad and de Groot 1942, Charman et al. 2000, Mitchell et al. 2016, Adl et al. 2019) were carried out at 200X to 400X magnification with Olympus BX41 and Nikon Eclipse Ni-U microscopes. All the prepared permanent slides (Pt. 5623 to Pt. 5632) were deposited in the National Zoological Collections (NZC) of the Protozoology Section of Zoological

Survey of India, Kolkata.

# RESULTS

The faunistic survey to Bibhutibhushan Wildlife Sanctuary, West Bengal, India resulted in 23 species belonging to 10 Genera, 6 Families, and 2 orders. Among these, 13 species were described under Phylum Tubulinea and remaining 10 species belongs to the Phylum Cercozoa. *Euglypha* Dujardin, 1841, *Centropyxis* Stein, 1857 and *Trinema* Dujardin 1841 were found to be the dominant genera in the study area.

## DISCUSSION

At present, the protected areas and forests of West Bengal together constitute around 18.96% of the total geographical area of the state which is very low in comparison to the national average (Anonymous 2021). A resilient ecosystem has a healthy microbial loop of which testate amoeba is one key player that helps in the nutrient recycling processes. The testate amoeba communities are highly responsive to large scale changes in land use such as deforestation and subsequent watershed management such as fertilizer and pesticides use (Patterson et al. 2002, Scott and Medioli 1983). They are sensitive to water chemistry and other micro-environmental gradients. They respond to soil pollution (Kandeker et al. 1992, Wanner and Dunger 2001, 2002) and atmospheric pollution (Balik 1991, Nguyen-Viet et al. 2004). This polyphyletic group is characterized by a decayresistant and morphologically distinctive test (Beyens et al. 1986) which forms a functional group of

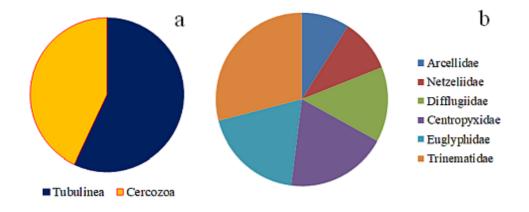


Figure 1. Representation of different Phylum (a) and families (b) of Testate amoeba

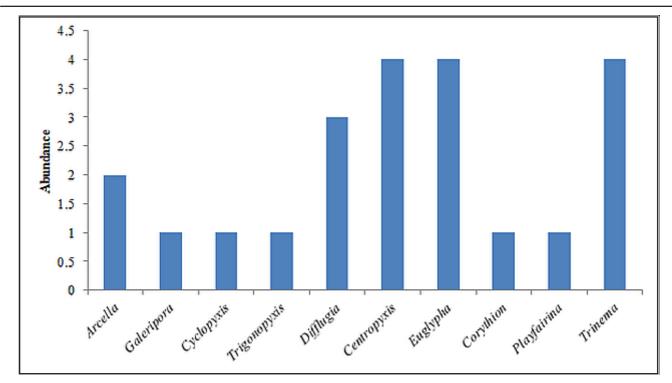


Figure 2. Representation of the Genus of testate amoeba

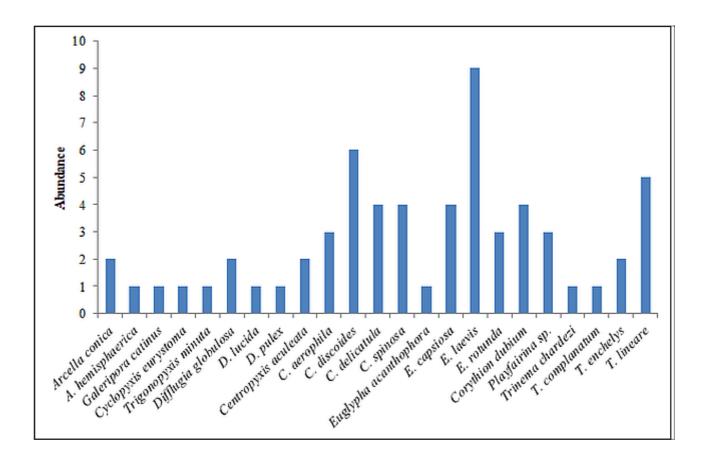


Figure 3. Representation of the species of testate amoeba

organisms with broadly similar ecologies (Smith et al. 2008). It is an important component of aquatic, semi-aquatic and terrestrial ecosystems and plays a significant biogeochemical and ecological role in both terrestrial and freshwater ecosystems (Mitchell et al. 2008, Yang et al. 2006).

Testate amoeba, a very vital member of the microbial community has got numerous roles in the soil and other freshwater ecosystems. A detailed study of this important component in the soil, water and moss habitats of protected areas of West Bengal is very limited thereby enabling a wide scope of study to be done at present and also further. This study is the first attempt to assess the assemblage of the testate amoeba in Bibhutibhushan Wildlife Sanctuary, West Bengal which resulted in recording 23 species belonging to 10 Genera, 6 Families, and 2 orders. Among these, 13 species belong to Phylum Tubulinea and remaining 10 to Phylum Cercozoa. Euglypha (Dujardin, 1841), Centropyxis (Stein, 1857) and Trinema (Dujardin 1841) were found to be the dominant genera in the study area.

Seasonality has an impact on TA communities due to changes in temperature (Lamentowicz et al. 2013a), light abundance (Marcisz et al. 2014a) and nutritional conditions (Mitchell 2004, Payne and Mitchell 2007, Mieczan 2007, 2010, Elliott et al. 2012, Jassey et al. 2013, Song et al. 2018). Hence further, the seasonal studies will enable to construct a strong repository of the testate amoeba diversity and distribution and decode their utility as reliable indicators in diverse biotopes.

### CONCLUSION

The dominant genus in the study area was *Euglypha* (Dujardin, 1841), *Centropyxis* (Stein, 1857) and *Trinema* (Dujardin 1841). This sanctuary was established to conserve the ecological balance of the forests in and along the Ichamati river. This study is the first attempt to analyze the diversity and distribution of TA in Bibhutibhushan Wildlife Sanctuary, West Bengal which resulted in recording 23 species belonging to 10 Genera, 6 Families, and 2 orders. This study lays the foundation for importance of study of testate amoeba in this sanctuary and further opens the need for analysis of various biotopes and microhabitats seasonally to record the testate amoeba diversity in this sanctuary

having evergreen forest lying in the alluvial soil type. ACKNOWLEDGEMENT

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 Director, Zoological Survey of India for the encouragement and providing the necessary facilities under the annual programme of ZSI, Kolkota. We are also grateful to the sectional staff of Protozoology section, HQ, ZSI, Kolkata for the field sampling and processing at the laboratory.

Authors' contribution: Both the authors contributed equally

**Conflict of interest**: Authors declare no conflict of interest

### REFERENCES

- Adl, S.M., Bass, D., Lane, C.E., Lukeš, J., Schoch, C.L., Smirnov, A. and Cárdenas, P. 2019.
- Revisions to the classification, nomenclature, and diversity of eukaryotes. Journal of Eukaryotic Microbiology, 66(1), 4-119. https://doi.org/10.1111/jeu.12691
- Adl, S.M., Simpson, A.G., Lane, C.E., Lukeš, J., Bass, D., Bowser, S.S. and Heiss, A. 2012. The revised classification of eukaryotes. Journal of Eukaryotic Microbiology, 59(5), 429-514. https://doi.org/10.1111/j.1550-7408.2012.00644. x
- Amacker, N., Mitchell, E.A.D., Ferrari, B.J.D. and Chèvre, N. 2018. Development of a new ecotoxicological assay using the testate amoeba *Euglypha rotunda* (Rhizaria; Euglyphida) and assessment of the impact of the herbicide S-metolachlor, Chemosphere, 201, 351-360. https:// doi.org/10.1016/j.chemosphere.2018.03.001
- Anonymous. 2021. Indian State of Forest Report 2021. FSI, Dehradun. https://fsi.nic.in/forest-report-2021-details
- Balik, V. 1991. The effect of the road traffic pollution on the communities of testate amoebae (Rhizopoda, Testacea) in Warsaw (Poland). Acta Protozoologica, 30, 5-11.
- Bindu, L. and Ruby, G. 2016. Protozoa. Zoological Survey of India, State Fauna Series 4: Fauna of Uttarpradesh, Part 1. ZSI, Kolkata. 107 pages.
- Beyens, L., Chardez, D., De, L.R., De, B.P. and Jacques, E. 1986b.Testate amoebae populations from moss and lichen habitats in the Arctic. Polar Biology, 5, 165-173. https:// doi.org/10.1007/BF00441696
- Chattopadyay, P. and Das, A.K. 2003. Morphology, Morphometry and ecology of moss dwelling testate amoebae (Protozoa: Rhizopoda) of North and North-East India. Memoiers of Zoological Survey of India, 19(4), 1-

113.

- Charman, D.J. 2001. Biostratigraphic and palaeoenvironmental applications of testate amoebae. Quaternary Science Reviews, 20, 1753–1764. https://doi.org/10.1016/S0277-3791(01)00036-1
- Chiba, Y. and Kato, M. 1969. Testacean community in the bryophytes collected in the Mt. Kurikoma district. Ecological Reviews, 17, 123-130.
- Das, A.K., Mondal, A.K. and Sarkar, N.C. 1993. Freeliving Protozoa. Zoological Survey of India, State Fauna Series, 3: Fauna of West Bengal, Part 12. ZSI, Kolkata. 134 pages.
- Das, A.K., Mondal, A.K., Tiwari, D.N. and Sarkar, N.C. 1995.
  Protozoa. Zoological Survey of India, State Fauna Series,
  4: Fauna of Meghalaya, Part 10. ZSI, Kolkata. 107 pages.
- Das, A.K., Tiwari, D.N. and Sarkar, N.C. 2000.Protozoa.Zoological Survey of India, State Fauna Series, 7: Fauna of Tripura, Part 4. ZSI, Kolkata. 52 pages.
- Das, A.K. and Nair, K.N. 1987. Protozoa. Pp. 323-340. In: Zoological Survey of India, State Fauna Series, 1: Fauna of Orissa, Part 1. ZSI, Kolkata.
- Elliott, S. M., Roe, H. M., & Patterson, R. T. (2012). Testate amoebae as indicators of hydroseral change: an 8500 year record from Mer Bleue Bog, eastern Ontario, Canada. Quaternary International, 268, 128-144. https://doi.org/ 10.1016/j.quaint.2011.08.020
- Ferreira, F., Leipnitiz, I., Leão, C.J. and Hansen, M.A.F. 2006. Tecamebas em sedimentos do rio Tramandaí e da lagoa do Passo, planície costeira norte do Estado do Rio Grande do Sul, Brasil. GAEA, 2, 66-74.
- Foissner, W. 1987.Soil protozoa: Fundamental problems, ecological significance, adaptation in ciliates and testaceans, bioindicators, and guide to the literature. Progress in Protistology, 2, 69-212. https://ia904708.us.archive.org/0/ items/foissner-1987/Foissner%201987.pdf
- Gilbert, D. and Mitchell, E.A.D. 2006.Microbial diversity in Sphagnum peatlands. pp. 287-319. In: Martini, I.P., Martinez-Cortizas, A. and Chesworth, W. (Eds.) Peatlands: Evolution and Records of Environmental and Climate Changes. Elsevier, Amesterdam.
- Golemanskym, V. 1967. Étude sur la faune de rhizopodes (Sarcodina, Rhizopoda) des mousses epiphytes etterricoles en Bulgarie. Bulletine de l'Institut de ZoologyetMusium de Sofia, 24, 103-119.
- Golemansky, V. and Todorov, M., 2004. Shell morphology, biometry and distribution of some marine interstitial testate amoebae (Sarcodina: Rhizopoda). Acta Protozoology, 43, 147-162.
- Hoogenraad, H.R. and Groot, A.A.D. 1941. Observations on a special manner of feeding of a species of *Difflugia* (*D. rubescens* Penard). Proceedings Nederlandse Akademie van Wetenschappen, 44, 217-228.
- Krashevska, V., Bonkowski, M., Maraun, M. and Scheu, S. 2007. Testate amoebae (protista) of an elevational gradient in the tropical mountain rain forest of Ecuador. Pedobiologia, 51(4), 319-331. https://doi.org/10.1016/ j.pedobi.2007.05.005
- Krashevska, V., Sandmann, D., Marian, F., Maraun, M. and Scheu, S. 2017. Leaf litter chemistry drives the structure

and composition of soil testate amoeba communities in a tropical montane rainforest of the Ecuadorian Andes. Microbial Ecology, 74, 681-690. https://doi.org/10.1007/s00248-017-0980-4

- Lamentowicz, M., Bragazza, L., Buttler, A., Jassey, V.E.J. and Mitchell, E.A.D. 2013. 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, 67, 1-11. https://doi.org/10.1016/ j.soilbio.2013.08.002
- Marcisz, K., Lamentowicz, £., S<sup>3</sup>owińska, S., S<sup>3</sup>owiński, M., Muszak, W. and Lamentowicz, M. 2014. Seasonal changes in *Sphagnum* peatland testate amoeba communities along a hydrological gradient. European Journal of Protistology, 50(5), 445-455. https://doi.org/10.1016/j.ejop.2014.07.001
- Mazei, Y.U., Blinokhvatov, Y.U. and Embulaeva, E. 2011. Specific features of the microspatial distribution of soil testate amoebae in the forests of the Middle Volga Region. Arid Ecosystems, 1, 46-52. https://doi.org/10.1134/ S2079096111010069
- Mattheeussen, R., Ledeganck, P., Vincke, S., van de Vijver, B., Nijs, I. and Beyens, L. 2005. Habitat selection of aquatic testate amoebae communities on Qeqertarsuaq (Disko Island), West Greenland. Acta Protozoology, 44, 253-263.
- Medioli, F.S. and Scott, D.B. 1983. Holocene Arcellacea (Thecamoebians) from Eastern Canada. Cushman Foundation Editorial, Washington, DC, 63 pages
- Mieczan, T. 2006. Species diversity of protozoa (Rhizopoda, Ciliata) on mosses of *Sphagnum* genus in restoration areas of the Poleski National Park. ActaAgrophysica, 7, 453-459. http://www.old.acta-grophysica.org/artykuly/ acta agrophysica/ActaAgr 133 2006 7 2 453.pdf
- Mieczan, T. 2007. Seasonal patterns of testate amoebae and ciliates in three peatbogs: relationship to bacteria and flagellates (Poleski National Park, Eastern Poland). Ecohydrology & Hydrobiology, 7(1), 79-88. https:// doi.org/10.1016/S1642-3593(07)70191-X
- Mieczan, T. 2010. Vertical MicroZonation of Testate Amoebae and Ciliates in Peatland Waters in Relation to Potential Food Resources and Grazing Pressure. International Review of Hydrobiology, 95(1), 86-102. https://doi.org/ 10.1002/iroh.200911188
- Mitchell, E.A.D., Bragazza, L. and Gerdol, R. 2004. Testate amoebae (Protista) communitie in *Hylocomium splendens* (Hedw.) B.S.G. (Bryophyta): Relationships with altitude, and moss elemental chemistry. Protist, 155, 423-436. https:// /doi.org/10.1078/1434461042650334
- Mitchell, E.A., Payne, R.J. and Lamentowicz, M. 2008. Potential implications of differential preservation of testate amoeba shells for paleoenvironmental reconstruction in peatlands. Journal of Paleolimnology, 40, 603-618. https:// /doi.org/10.1007/s10933-007-9185-z
- Nair, K.N. and Mukherjee, R.N. 1968a. On a new species of testacean rhizopods (Protozoa : Euglyphidae) from India. Journal of Zoological Society of India, 20, 124-127.
- Nair, K.N. and Mukherjee, R.N. 1968b. On some testacean rhizopods (Protozoa : Sarcodina) of the ground and tree

mosses from Calcutta and its environs. Proceedings of National Academy of Science, India, 38(B) III & IV, 185-192.

- Nguyen-Viet, H., Bernard, N., Mitchell, E.A.D., Cortet, J., Badot, P.-M., and Gilbert, D. 2007. Relationship between testate amoeba (protist) communities and atmospheric heavy metals accumulated in *Barbula indica* (Bryophyta) in Vietnam. Microbial Ecology, 53, 53-65. https://doi.org/ 10.1007/s00248-006-9108-y
- Ogden C.G. and Hedley R.H. 1980. An Atlas of Freshwater Testate Amoebae. Oxford University Press, London. 236 pages
- Patterson, R.T. and Kumar, A. 2002. A review of current testate rhizopod (thecamoebian) research in Canada. Palaeogeography, Palaeoclimatology, Palaeoecology, 180(1-3), 225-251. https://doi.org/10.1016/S0031-0182(01)00430-8
- Smith, H.G., Bobrov, A. and Lara, E., 2008. Diversity and biogeography of testate amoebae. Biodiversity and Conservation, 17, 329-343. https://doi.org/10.1007/

Todorov, M., Golemansky, V. and Blagovest, T. 2008. Diversity and biotopic distribution of testate amoebae (Protozoa: Arcellinida and Euglyphida) in the Batak Reservoir (Southern Bulgaria). Acta Zoologica Bulgaria, 60, 115-124.

s10531-007-9260-9

- Song, L., Li, H., Wang, K., Yan, X. and Wu, D. 2018. Seasonal dynamics in the community structure and trophic structure of testate amoebae inhabiting the Sanjiang peatlands, Northeast China. European Journal of Protistology, 63, 51-61. https://doi.org/10.1016/j.ejop.2018.01.005
- Wanner, M. and Xylander, W.E. 2005. Biodiversity development of terrestrial testate amoebae: is there any succession at all? Biology and Fertility of Soils, 41, 428-438. https://doi.org/10.1007/s00374-005-0850-y
- Yang, J., Smith, H.G., Sherratt, T.N. and Wilkinson, D.M. 2010. Is there a size limit for cosmopolitan distribution in freeliving microorganisms? A biogeographical analysis of testate amoebae from polar areas. Microbial Ecology, 59, 635-645. https://doi.org/10.1007/s00248-009-9615-8

Received: 8th July 2023 Accepted:29th September 2023