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First Report on Moss-Dwelling Testate Amoebae from Bihar, India: Species Diversity and Ecological Significance

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Authors' contributions

This work was carried out in collaboration among all authors. Author VMSK conducted the field survey for obtaining the moss samples for the study, identified the specimens and Authors BL and AK helped in finalizing the article. All authors read and approved the final manuscript.

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ABSTRACT

Testate amoebae dwelling on moss at Bihar state have not been reported earlier in any literature. Consequently, this study seeks to establish an account of the testate amoebae species and their diversity first time from Bihar. Testate amoebae are the free-living, single-celled protists with external shells and are considered to be excellent bioindicators for the environmental changes. Samples of moss were taken from soil and walls in different parts of the Bahadurpur Housing Colony area of Patna during this preliminary investigation. The study results showed presence of 14 species of testate amoebae belonging to 7 genera and 7 families. All of these species are new record from the Bihar state and this study has shown the ecological significance of moss habitats. As some species are defined as ecological priority and are useful for biomonitoring, which gives considerable information on the state of the environment of the given region. In addition to these,

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this study augments the existing knowledge on the distribution and species richness of testate amoebae in India and the general scientific information necessary for future ecological surveys and conservation strategies. Thus, the results have proved the usefulness of testate amoebae as bioindicators and their applicability in the assessment of the state of the environment of the area.

Keywords: *Testate amoebae; protozoa; moss; Bihar; pollution indicator.*

1. INTRODUCTION

Bihar is one of the Indian states located in the eastern part of the country with co-ordinate of 24°20'10", 27°31'15" N latitude and 83° 19'50", 88°17'40" E longitude respectively. The state occupies a geographical area of 94,163 sq km and mainly consists of the fertile alluvial plains of the Ganges and its affluents: Gandak, Son and Kosi. Most of the landscape in the state is defined by broad plains that dominate the central and southern areas. Notwithstanding that fact, the northern area is close to the spectacular Himalayan Mountains, which creates greater variation in the terrain and higher altitudes with colder temperatures. In a variety of habitats, mosses are richly found. They are found on various surfaces, which includes tree bark, uncovered rocks, old walls, and even in the soil. These non-vascular micro-organisms contribute importantly to the prosperity of their environment, stabilizing moisture and providing shelter for a range of microorganisms, including testate amoebae.

Shelled protozoa specifically testate amoebae are the species that occupy large numbers of habitats. They are very susceptible to ecological alterations [1] and are therefore good biomarkers to ecological variations because of their short generation intervals [2,3]. These organisms are found in different parts of the world and from tropical to polar regions and from land to sea water environments. These species are most diverse and numerous in Sphagnum mosses in which they exhibit differential ecological affinities in different ecosystems and in relation to various environmental factors [4]. Due to their ability to respond to changes in moisture, pH, and other factors in the environment they are used as indicators of ecological change [5]. Testate amoebae also depend on the water quality, pollution, and climate change parameters and therefore are applied for paleo- reconstructions of past water conditions and contemporary ecological status [6]. Fluctuations in the number and kinds of testate amoebae indicate the changes in the habitat conditions such as variation in hydrophysical properties or in

pollution. For instance, some species are known to be well suited to acidic environments while others are well suited to near neutral conditions, and thus the researchers are able to deduce changes in pH and moisture levels over time [7].

In the Sphagnum biotopes, number and density of testate amoebae is high and they become involved in microbial loop and influence nutrient cycling and the processes in the ecosystem [8]. This means that their activity and population depend with the moisture available in the environment where they live [9]. Traditionally, testate amoebae have been divided into two primary groups based on pseudopodial morphology: of these, the Arcellinida possesses lobose pseudopodia while the Euglyphida possesses filose pseudopodia [10]. However, in the past ten years the molecular phylogenetic and phylogenomic techniques that utilize large DNA data have brought a taxonomic revolution in the testate amoebae. It is now understood that they represent a polyphyletic group, consisting of at least three unrelated taxonomic groups of unicellular eukaryotes: In the group Amoebozoa, Stramenopiles, and Cercozoa [11,12].

However, Bihar, as one of the most biodiverse states in India, studies on protozoan diversity, especially free-living protozoa such as testate amoebae have not been reported [13]. Therefore, some attempts have been made on this front. The first recorded account of the existence of testate amoebae that dwells in moss was reported from this study in Bihar particularly in Bahadurpur Housing Colony of Patna. There were 14 different species from 7 genera and 7 families of protozoans evidenced in the study, proving that there may be significant diversity of unexplored protozoan potential in the moss ecosystems of the state.

Therefore, this work is an attempt to carry out preliminary assessment of testate amoebae in Bihar as they are ecologically important. Thus, the faunal diversity of the given area has been expanded by studying 46 specimens that belong to 14 species, 7 genera, and 7 families. Furthermore, given the high level of pollution in

Bihar, it should also be mentioned that testate amoebae are considered to be sensitive organisms that can reflect the changes in the environment that is why they can be used as bioindicators of the ecological state of the area. Future work on the protozoan taxonomy could be very useful in monitoring and management of the environment in the state.

1.1 Global and Indian Diversity of Testate Amoebae

Free living amoebae also show a high degree of world distribution with 675 plus species belonging to 104 genera and 22 families and with records from the polar areas. In India this diversity is reflected in the distribution of 209 species belonging to 37 genera classified under two classes and two orders [13]. This wide dispersal underlines the versatility of testate amoebae and these organisms' role as bioindicators, as they are found in polar ice and other places in India as well as other environments.

2. MATERIALS AND METHODS

The moss samples for the present study were collected from various biotopes of Bahadurpur Housing Colony area of Patna on 14th August 2024. The samples were obtained from various biotopes like soil and wall (25.35.100° N and 85.10.041° E., Alt.) 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 [14]. All samples were air-dried and stored in plastic bags until investigated. The specimens were reactivated from resting cysts by the non-flooded Petri dish method. Samples (10–50 g) were placed in the petri dishes and were saturated with distilled water and the cultures were regularly inspected. Subsequently, permanent slide mounts were prepared from each sample and examined using Labomed (Lx 400) microscopes equipped with a Sony CMOS camera attachment for image capturing and species-level identification. All the registered permanent slides were deposited in the National Zoological collections of Gangetic Plains Regional Centre, Zoological Survey of India, Patna.

3. RESULTS

The study yielded the following new records to the state of Bihar belonging to 14 species of

testate amoebae span over 7 genera and 7 families from soil and walls in different parts of the Bahadurpur Housing Colony area of Patna during this, preliminary investigation.

Systematic list of Testate Amoebae from Bihar: Findings from the present study (Plates 1, 2 & 3) (Classification as per Adl et al.,) [12]

Domain Amorphea Adl et al., 2012
 Supergroup Amoebozoa Lühe, 1913, sensu Cavalier-Smith, 1998
 Phylum Tubulinea Smirnov et al., 2005
 Class Elardia Kang et al., 2017
 Order Arcellinida Kent, 1880
 Family Netzeiliidae Kosakyan et al., 2016

1. *Cyclopyxis arcelloides* (Penard, 1902) Deflandre, 1929

1902. *Centropyxis arcelloides* Penard, *Faune Rhizopodique du bassin du Léman, Geneve*, p. 309.

1929. *Centropyxis (Cyclopyxis) arcelloides* Deflandre, *Arch. Protistenkd.*, 67, p.367.

Distribution: India: Andhra Pradesh, Arunachal Pradesh, Himachal Pradesh, Kerala, Manipur, Meghalaya, Mizoram, Odisha, Sikkim, Tamil Nadu, Uttar Pradesh, Uttarakhand, West Bengal, Chandigarh, Punjab.

Remarks: Present record from Bihar

2. *Cyclopyxis eurystoma* Deflandre, 1929

1929. *Centropyxis (Cyclopyxis) eurystoma* Deflandre, *Arch. Protistenkd.*, 67: 370.

Distribution: India: Arunachal Pradesh, Assam, Himachal Pradesh, Kerala, Maharashtra, Nagaland, Tamil Nadu, Telangana, Uttarakhand, West Bengal, Punjab

Remarks: Present record from Bihar

Family Phryganellidae Jung, 1942

3. *Phryganella acropodia* (Hertwig & Lesser, 1874)

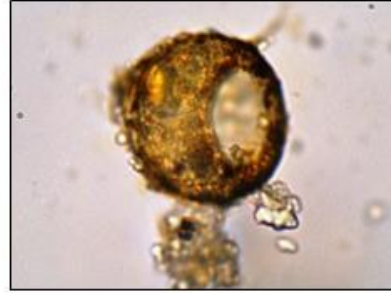
1909. *Phryganella acropodia* Hopkinson, *The British Freshwater Rhizopoda and Heliozoa*, 2: 74, pl.20, Figs.13-14.

Distribution: India: Himachal Pradesh, Sikkim, Tamil Nadu, Telangana, Uttarakhand, Punjab.

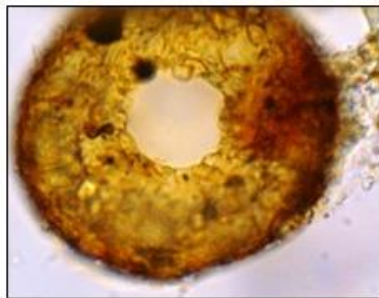
Plate 1



1. *Cyclopyxis arcelloides*
(Penard, 1902) Deflandre, 1929



2. *Cyclopyxis eurystoma*
Deflandre, 1929



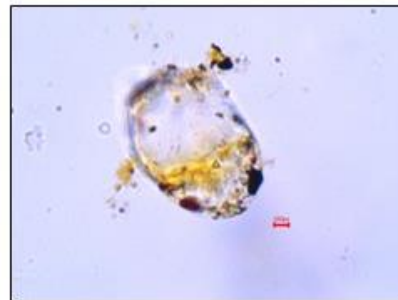
3. *Phryganella acropodia*
(Hertwig & Lesser, 1874)
Hopkinson, 1909



4. *Diffugia globulosa*
(Dujardin, 1837) Penard, 1902

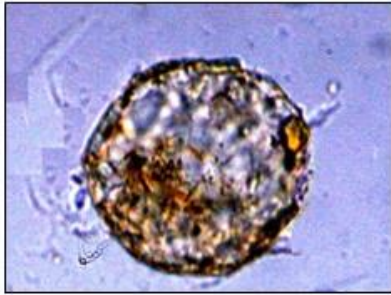


5. *Cryptodiffugia oviformis*
Penard, 1902

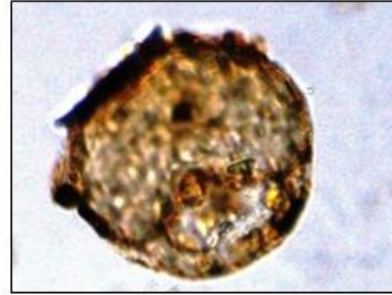


6. *Centropyxis aerophila*
Deflandre, 1929

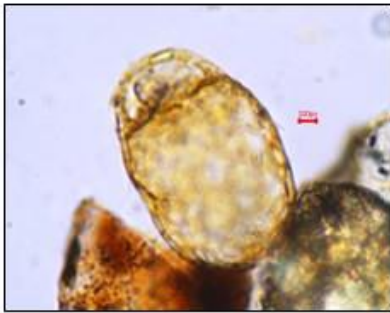
Plate 2



7. *Centropyxis aculeata*
(Ehrenberg, 1838) Stein, 1859



8. *Centropyxis ecornis*
(Ehrenberg, 1841)



9. *Centropyxis platystoma*
(Penard, 1890) Deflandre, 1929



10. *Euglypha capsiosa*
Coûteaux, 1978

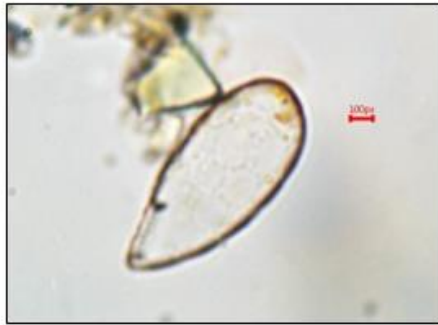


11. *Euglypha rotunda*
(Ehrenberg, 1845)



12. *Trinema enchelys* (Ehrenberg,
1838)

Plate 3



13. *Trinema lineare*
Penard, 1890



14. *Trinema complanatum*
Penard, 1890

Plates 1-3. Microphotographs of *Testate amoeba* species

Remarks: Present record from Bihar

Family Difflogiidae Wallich, 1864

4. *Difflogia globulosa* (Dujardin, 1837)
Penard, 1902

1837. *Difflogia globulosa* Dujardin, *Ann. Sci. nat. Zool.* (2) 8: 310, pl. 9. Fig. 1.

1902. *Difflogia globulosa* Penard, *Faune Rhizopodique du Bassin de Lemans. Geneve: Kundig*, pp.714.

Distribution: India: Andhra Pradesh, Assam, Himachal Pradesh, Meghalaya, Odisha, Rajasthan, Tamil Nadu, Uttar Pradesh, West Bengal, Punjab.

Remarks: Present record from Bihar
Family Cryptodifflogiidae Jung, 1942

5. *Cryptodifflogia oviformis* Penard, 1902

1902. *Cryptodifflogia oviformis* Penard. *Mem. Soc. Phys. Hist. Nat. Geneve. T.31. No.2:1-230.*

Distribution: India: Kerala

Remarks: Present record from Bihar

Family Centropyxidae Jung, 1942

6. *Centropyxis aerophila* Deflandre, 1929

1929. *Centropyxis aerophila* Deflandre *Arch. Protistenkd.*, 67:330.

Distribution: India: Andhra Pradesh, Arunachal Pradesh, Himachal Pradesh, Kerala, Manipur,

Meghalaya, Mizoram, Nagaland, Odisha, Sikkim, Tamil Nadu, Telangana, Tripura, Uttar Pradesh, Uttarakhand, West Bengal, Chandigarh, Punjab.

Remarks: Present record from Bihar

7. *Centropyxis aculeata* (Ehrenberg, 1838)
Stein, 1859

1958. *Centropyxis aculeata minima* Van Oye, *Etude sur les Rhizopodes des marais du-ouest d'Uvira (Congo-belge), Hydrobiologia*, 10:85-137.

Remarks: Present record from Bihar

8. *Centropyxis ecornis* (Ehrenberg, 1841)

1841. *Arcella ecornis* Ehrenberg, *Abh. Akad. Wiss. Berlin*, p. 368.

1879. *Centropyxis ecornis* Leidy, *Freshwater Rhizopods of North America*, pl.30, Figs. 20-24.

Distribution: India: Andhra Pradesh, Arunachal Pradesh, Assam, Himachal Pradesh, Kerala, Maharashtra, Meghalaya, Nagaland, Odisha, Sikkim, Tamil Nadu, Telangana, Uttar Pradesh, Uttarakhand, West Bengal, Punjab.

Remarks: Present record from Bihar

9. *Centropyxis platystoma* (Penard, 1890)
Deflandre, 1929

1929. *Centropyxis platystoma* Deflandre, *Arch. Protistenkd.*, 67: 338.

Distribution: India: Andhra Pradesh, Arunachal Pradesh, Assam, Himachal Pradesh, Kerala, Manipur, Meghalaya, Mizoram, Nagaland, Odisha, Sikkim, Tamil Nadu, Tripura, Uttar Pradesh, West Bengal, Chandigarh, Punjab.

Remarks: Present record from Bihar
Phylum Cercozoa Cavalier-Smith, 2018
Class Silicofilosea Adl et al., 2012
Order Euglyphida Cavalier-Smith, 1997
Family Euglyphidae Lara et al., 2007

10. *Euglypha capsiosa* Coûteaux, 1978

1978. *Euglypha capsiosa* Coûteaux, *Journal of protozoology*, 25: 50A

Distribution: India: Kerala, Tamil Nadu

Remarks: Present record from Bihar

11. *Euglypha rotunda* (Ehrenberg, 1845)

1911. *Euglypha rotunda* Wailes and Penard, *Proc. R. Irish Acad.*, 31: 60-62.

Distribution: India: Himachal Pradesh, Kerala, Maharashtra, Odisha, Telangana, Tripura, Uttarpradesh, Uttarakhand, Punjab

Remarks: Present record from Bihar

Family Trinematidae Adl et al., 2012

12. *Trinema enchelys* (Ehrenberg, 1838)

1890. *Trinema lineare* Penard, *Mem. Soc. Geneve*, 31: 187, pl. 11. Figs. 5-17.

1915. *Trinema lineare* Cash, Wailes and Hopkinson, *Ray. Soc. Publ. London*, 3: 91, pl. 47, Figs. 11-21.

Distribution: India: Andhra Pradesh, Assam, Himachal Pradesh, Kerala, Maharashtra, Odisha, Sikkim, West Bengal

Remarks: Present record from Bihar

13. *Trinema lineare* Penard, 1890

1890. *Trinema lineare* Penard, *Mem. Soc. Geneve*, 31: 187, pl. 11. Figs. 5-17.

1915. *Trinema lineare* Cash, Wailes and Hopkinson, *Ray. Soc. Publ. London*, 3: 91, pl. 47, Figs. 11-21.

Distribution: India: Andhra Pradesh, Arunachal Pradesh, Assam, Himachal Pradesh, Manipur, Meghalaya, Mizoram, Nagaland, Odisha, Tripura, Uttarpradesh, Uttarakhand, Chandigarh, Punjab
Remarks: Present record from Bihar.

14. *Trinema complanatum* Penard, 1890

1890. *Trinema complanatum* Penard, *Mem. Soc. Geneve*, 31: 187, pl.10, Figs. 1-4.

Distribution: India: Andhra Pradesh, Arunachal Pradesh, Himachal Pradesh, Kerala, Meghalaya, Mizoram, Nagaland, Sikkim, Uttar Pradesh, Uttarakhand, Punjab.

Remarks: Present record from Bihar.

4. CONCLUSION

Testate amoebae dwells in mosses are particularly valuable in terms of tracking changes in the environment. Because of the speed at which they record changes in their surroundings, they are helpful indicators of pollution and other ecological changes. Also importantly, the analytical study on the distribution of protozoans especially testate amoebae in Bihar is important in less explored ecosystem such as wetland, forest and urban ecosystem. Climate change and the effects of human activities on ecosystems require that these microorganisms are studied in further detail. The study conducted on protozoan systems may help in understanding the aspects of both biodiversity and ecosystems, because their community responses are typical for major environmental concerns. The present study is significant for the assessment of the health and stability of ecosystems in Bihar and beyond by analysing the diversity of testate amoebae. Proven to inhabit both the moist and the aquatic habitats, due to their ability to respond to environmental changes, they are acting the most basic biomarkers for the assessment of ecosystems by reporting new species distributional records.

In terms of ecological and environmental assemblages of urban spaces, Bahadurpur Housing Colony of Patna can be proposed as an interesting site for analysis. The climate of the zone is also similar to the climate of other areas of Patna and characterized by subtropical climate with hot summers and monsoon and relatively mild winter. It has conventional city's park like spaces along with residential and commercial layout of land in the housing colony. But with

rising urbanisation, there are some problems in this regard including shrinking greenery, water stagnation in rainy season and air pollution caused by vehicles. On these grounds, Bahadurpur Housing Colony is a perspective site to analyze the effects of urbanization upon bio equilibrium and environmental stability in Patna.

In conclusion, the diversity of testate amoebae in Bihar warrants detailed investigation, with site-specific studies exploring their role in assessing ecological health, particularly in habitats impacted by human activity. These microhabitat-focused investigations will advance our understanding of ecological processes and improve efforts to protect and sustainably manage these environments.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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/Protista:Tubulinea;Cercozoa;
Stramenopiles

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