***Original Research Article***

**First Record of Moss-Inhabiting Testate Amoebae from Jharkhand, India with Notes on Their Ecological Roles**

## ABSTRACT

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| Testate amoebae are protists with protective external shells, unicellular free-living and regarded as good bioindicators of environmental alteration. The occurrence of testate amoebae inhabiting the moss habitats was not recorded in the state of Jharkhand earlier. Therefore, this is the first description of species composition and diversity in the area. In this preliminary study, the samples of moss were taken from the surface of the soil and the wall in different sites of Argaghat in the Giridih district of Jharkhand. There were 12 different species of testate amoebae found based on analysis, which included 6 genera and 6 families. 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. Testate amoebae species are known to be ecologically sensitive and are very useful in monitoring the environment, as they can provide indications on the health and quality of their immediate habitats. In general, the results can substantiate the usefulness of testate amoebae as bioindicators, the study also represented an important source of base data that can be used in future to comprehend the distribution and richness of testate amoebae in India and can demonstrate their importance in monitoring the ecosystem in understudied regions. This preliminary diversity study of testate amoeba in Jharkhand warrants the need to carry-out more detailed and local-scale investigations focusing on their ecological importance, especially in anthropogenically impacted areas. This type of microhabitat-focused studies will enhance our knowledge of the functioning of the ecosystem and help us to apply more efficient conservation and sustainable management approach. |

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

## 1. INTRODUCTION

The worlds of microscopic organisms living in moss ecosystems are explored in this article. Testate amoebae, unicellular protozoans enclosed in protective shells are valuable bioindicators of environmental conditions (Kumar et al., 2024). Mosses belong to the Division ryophyta and Class Bryopsida and grow on soil, rock, wall and barks of trees in humid nvironment. In India, they are mostly found in Himalayas, Western Ghats and other hilly areas. They provide suitable microhabitat by providing insulation against heat, cold and wind, particularly for free living protozoa of which testate amoebae are most abundant. Reports on moss dwelling testate amoebae from India are very scanty (Jana et al., 2008). Jharkhand is an eastern Indian state that stretches approximately between 21°55′ N to 25°35′ N latitude and 83°20′ E to 88°02′ E longitude with an area of about 79,714 km². The landscape is part of the Chota Nagpur Plateau, which has rugged topography, ancient crystalline rocks belonging to the precambrian period and heavily dissected river valleys. Some of the major rivers, which flow out of this plateau area, are the Damodar, the Subarnarekha, the Koel and the Barakar. Most of Ranchi Plateau elevation is about 700 meters across the state, with the highest point being the hill at Parasnath in the district of Giridih, which stands at approximately 1,350 meters above sea level, bordered by Bihar in the north, Uttar Pradesh and Chhattisgarh in the west, Odisha in the south and West Bengal in the east. The state has a tropical monsoon climate, and forests occupy almost a third of the entire area According to the [1].

In this diverse terrain, mosses grow in different microhabitats; it is common to find them on tree trunks, bare rock surfaces, crumbling walls and in soil surfaces. These tiny plants although non-vascular, considerably contribute to the ecological niche of the environment by providing a stable habitat to other microscopic organisms, and as well as acting as a source of moisture retention, especially testate amoebae, which contact and stimulate the mossy micro-environs.

Testate amoebae are shelled protozoans and are found in almost every environment on earth. They are very good bioindicators due to their short life cycles that enable them to respond quickly to changes in the environment [2,3,4]. These microorganisms are found from the polar regions of the world to the tropics and exist in habitats ranging from terrestrial to aquatic ecosystems. These microorganisms are particularly numerous and diverse in *Sphagnum*-dominated moss habitats which exhibit diverging ecological preferences to environmental conditions [5]. Testate amoebae community engaged their survivorship highly influenced by the environmental factors and nutrients, moisture and temperature available in the mosses. The importance of moisture to testate amoebae is well known with some taxa and they are occurring most abundantly in wet environments. Studies in various environments have proved the significance of moisture on testate amoebae biology and ecology (Bindu and Chitra, 2025).

The testate amoebae are highly useful in ecological monitoring due to their sensitivity to the conditions of moisture availability, acidity (pH) and other aspects of habitat [6]. Their population pattern usually represents changes in water quality, pollution and climate variability. They therefore act as good tools for the reconstruction of past environmental conditions, together with the evaluation of the current health of the ecosystems [7].

The testate amoebae can be abundant and can be an active constituent of microbial food webs in Sphagnum bog ecosystems, where they participate in nutrient-cycling dynamics and potentially affect large-scale ecosystem processes [9, 10]. They are highly dependent on the accessibility of water to their microhabitat, the relationship between their abundance and community structure.

Conventionally, testate amoebae have been divided based on the structure of their pseudopodia- *Arcellinida* that develop wide, lobose extensions and *Euglyphida* which develop slender thread-like filose pseudopodia [11]. Their taxonomy has however, been changed with modern developments in molecular phylogenetics, and phylogenomics. The use of DNA-based analysis in the modern era revealed that testate amoeba is not a single evolutionary lineage. Rather, they contain several unrelated clades now assigned to the various eukaryotic groups, *Amoebozoa, Cercozoa* and *Stramenopiles* [12, 13].

However, in Jharkhand, studies on protozoan diversity, especially free-living protozoa such as testate amoebae have not been reported [14]. Therefore, some attempts have been made on this front. The first recorded account of the existence of testate amoebae that dwell in moss is reported from this study from different sites of Argaghat in the Giridih district of Jharkhand. There were 12 different species from 6 genera and 6 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.

**1.1 Global and Indian Diversity of Testate Amoebae**

These free-living testate amoebae have a wide geographical distribution, with around 1000 species identified, even in high polar latitudes. The diversity in the Indian context, where there are 218 species that belong to 37 genera, and they fall under two classes, and two orders [14]. This diversity concisely shows the ecological flexibility of testate amoebae and argues in favor of the role of testate amoebae as bioindicators because they occur in many habitats and ecotypes in India.

## 2. MATERIALS AND METHODS

The moss samples for the present study were collected from various biotopes of Argaghat, New Barganda, Giridih district of Jharkhand on 27th February 2025. The samples were obtained from soil and wall (24.19.671° N and 86.30.615° E., Alt.247m) 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 [15]. 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 AND DISCUSSION

The study yielded the following new records to the state of Bihar belonging to 12 species of testate amoebae span over 6 genera and 6 families during this, preliminary investigation.

Systematic list of Testate Amoebae from Jharkhand: Findings from the present study (Classification as per Adl *et al*., 2019) [13]

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 Netzeliidae 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 Jharkhand

1. *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 Jharkhand

Family Phryganellidae Jung, 1942

1. *Cyclopyxis kahli* Deflandre, 1929

1929. *Centropyxis (Cyclopyxis) kahli* Deflandre, Arch. Protistenkd., 67:371.

*Distribution*: India: Himachal Pradesh, Kerala, Tamil Nadu, Uttarakhand.

Remarks: Present record from Jharkhand.

Family Trigonopyxidae Loeblich & Tappan, 1964

1. *Trigonopyxis arcula* Penard 1912

1912. *Trigonopyxis arcula* Penard, Rev. Suisse Zool., 20 (1); 9 & 13, pl.1.

Distribution : India: Himachal Pradesh, Kerala, Tamil Nadu, Uttarakhand.

Remarks: Present record from Jharkhand.

Family Phryganellidae Jung, 1942

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

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

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

*Remarks:* Present record from Jharkhand.

Family Difflugiidae Wallich, 1864

1. *Difflugia globulosa* (Dujardin, 1837) Penard, 1902

1837. *Difflugia globosa* Dujardin, *Ann. Sci. nat. Zool*. (2) 8: 310, pl. 9.

1902. Difflugia globulosa Penard, *Faune Rhizopodique du Bassin de Leman. 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 Jharkhand.

Family Cryptodifflugiidae Jung, 1942

1. *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 Jharkhand.

1. *Centropyxis cassis* (Wallich, 1864) Deflandre, 1929

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

*Distribution:* India : Arunachal Pradesh, Assam, Himachal Pradesh, Kerala, Meghalaya, Odisha, Uttar Pradesh, Uttarakhand, West Bengal.

Remarks: Present record from Jharkhand.

1. *Centropyxis constricta (Ehrenberg, 1841) Deflandre, 1929.*

*1929. Centropyxis constricta Penard, Faune Rhizopodique du basin du Leman, Geneve, 32 :299.*

*Distribution:* India:Andhra Pradesh, Assam, Himachal Pradesh, Kerala. Manipur, Meghalaya, Mizoram, Nagaland, Odisha, Uttarpradesh, Uttarakhand, West Bengal, Chandigarh.

*Remarks:* Present record from Jharkhand.

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

1929. *Centropyxis platystoma* Defalndre, *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 Jharkhand.

Phylum Cercozoa Cavalier-Smith, 2018

Class Silicofilosea Adl *et al*., 2012

Order Euglyphida Cavalier-Smith, 1997

Family Euglyphidae Lara *et al*., 2007

1. *Euglypha laevis* (Ehrenberg, 1845)

1845. *Euglypha laevis* Ehrenberg, Ber. Akad., Berlin, p. 307.

1849. Euglypha laevis Perty, Mitth. nat. Ges. Bern., p. 163.

Distribution : India: Himachal Pradesh, Kerala, Maharashtra, Odisha, Telangana, Uttar Pradesh, Uttarakhand.

Remarks: Present record from Jharkhand.

1. *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 Jharkhand.

## 4. CONCLUSION

Testate amoebae found in mosses are important indicators for monitoring changes in the environment. They are found to be fast in responding to changes in the environmental conditions, making them effective in identifying pollution and other ecological imbalances. Therefore, studies on the distribution of the protozoa, in a variety of habitats in Jharkhand, including wetlands, forests as well as urban ecosystems are important in ecological studies.

With the rising effects of climate change and human manipulation on natural ecosystems, there is an immediate necessity to study these microorganisms in detail. These life forms, which exist in humid land and water communities, are very sensitive to environmental changes. It is such sensitivity that makes them effective bioindicators in the study of the integrity of the ecosystem and in the study of the occurrence of the hitherto unmentioned testate amoebae species diversity of Jharkhand.

To conclude, this preliminary diversity study of testate amoeba in Jharkhand warrants on a need to carry-out more detailed and local-scale investigations focusing on their ecological importance, especially in anthropogenically impacted areas. This type of microhabitat-focused study will enhance our knowledge of the functioning of the ecosystem and help us to apply more efficient conservation and sustainable management approaches.

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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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Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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