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New Records of Testate Amoebae from the Vikramshila Gangetic Dolphin Sanctuary, Bihar, India

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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Short Research Article

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ABSTRACT

The occurrence of testate amoebae from the moss habitats was not recorded in the Vikramshila Gangetic Dolphin Sanctuary, Bihar earlier. Therefore, this is the first report of the presence of testate amoebae diversity in the Vikramshila Gangetic Dolphin Sanctuary. Testate amoebae are protists with protective external shells, unicellular free-living and regarded as good bioindicators of environmental alteration. In this preliminary study, the samples of moss were taken out on the banks of the soil around Vikramshila Gangetic Dolphin Sanctuary. There were 9 different species of testate amoebae found based on analysis which included 4 genera and 4 families. 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 demonstrating their importance in monitoring the ecosystem in understudied regions, especially the only designated Dolphin sanctuary in India.

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Keywords: Moss; pollution indicator; protozoa; testate amoebae; Vikramshila; gangetic dolphin sanctuary.

1. INTRODUCTION

The Vikramshila Gangetic Dolphin Sanctuary (VGDS) in the central Ganga at Bhagalpur, Bihar, is a 50–60-km riverine protected stretch categorized by a mosaic of mid-channel bars, rocky outcrops, marginal wetlands, and variable flow regimes that create extensive hard and soft substrates for periphyton and bryophyte growth, the foundation for diverse microbial assemblages (Roy et al. 2013). Within VGDS itself, original floristic work documents a rich algal flora-including cyanobacteria and diatoms-colonizing stones and macrophytes along the sanctuary's banks and islands, underscoring the sanctuary's capacity to support complex microbial biofilms in lotic habitats (Das & Maurya 2015). Complementing this, a year-round zooplankton survey inside VGDS reported 35 species spanning bacterivores, detritivores, herbivores, and carnivores, indicating efficient microbial–metazoan coupling and bottom-up support from microbial production in the water column and on benthic surfaces (Roy et al. 2013). Bacteriological analyses at Bhagalpur shared with the VGDS, positively indicated high faecal coliform loads and risk to human health, indicating strong anthropogenic forcing on microbial water quality in this Ganga corridor (Bilgrami & Kumar 1998). In spite of this evidence of microbial habitat diversity in VGDS, there is an apparent gap: no published, site-specific survey has so far been performed to document moss-associated testate amoeba (TA) in the sanctuary to our knowledge.

This is notable because TA, the shelled protists that thrive in bryophyte mats and periphyton; are sensitive bioindicators of moisture, pH, nutrients, and pollution, and they integrate hydro-ecological variability at spatial scales relevant to sanctuary management. Importantly, very recent original studies from within Bihar demonstrate that bryophyte-associated TA are present and diverse in the wider region. An innovative survey in the Nagi Bird Sanctuary (Ramsar-listed, Jamui district, c. 100 km south of Bhagalpur) identified 16 species of TA on moss substrates, with new state records, under non-flooded Petri dish conditions and curated voucher slides to confirm regional habitat suitability and methodological viability (Sathish Kumar et al. 2025). Likewise, a separate TA assemblage of mosses was obtained on rocky and forested substrates within the Bhimbandh Wildlife Sanctuary (Munger district) in the first report, which confirms that bryophyte cushions on rocky and forested substrates in south-eastern Bihar harbour diagnosable TA communities (Sathish Kumar et al. 2024). Together, these original records from neighbouring protected areas strongly imply that riparian moss patches on VGDS boulder fields, bridge pilings, and shaded bank faces are likely to harbour TA assemblages responsive to riverine hydrology and pollution gradients, but they have simply not been inventoried yet.

Broader Ganga-basin microbiome research further motivates such work in VGDS. Shotgun metagenomics at major confluences and along upper-Ganga sediments reveals taxonomically and functionally rich microbiota (bacteria, archaea, viruses) with genes linked to carbohydrate-active enzymes, antimicrobial biosynthesis, and resistance, and shows that community composition shifts with allochthonous inputs and hydrological context (Reddy et al. 2019; Rajput et al. 2022). Within such a dynamic microbial setting, moss-hosted TA offer a tractable sentinel for integrating episodic disturbances (e.g., monsoon pulses, wastewater influx) with local microhabitat conditions along the VGDS banks. Given that VGDS already evidences periphytic algae, zooplankton feeding guilds linked to microbial production, and documented bacteriological pressures at Bhagalpur, establishing a standardized TA monitoring program on bryophyte substrates would fill a critical microbial bioindicator gap for this dolphin sanctuary (Das & Maurya 2015; Roy et al. 2013; Bilgrami & Kumar 1998).

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 (Adl et al 2012). 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 TA is not a single evolutionary lineage. Rather, they contain several unrelated clades now assigned to the various eukaryotic groups, *Amoebozoa, Cercozoa* and *Stramenopiles* (Adl et al, 2019; Bindu et al, 2024).

However, VGDS as the only Dolphin sanctuary in India, studies on protozoan diversity, especially

free-living protozoa such as TA have not been reported. Therefore, some attempts have been made on this front. The first recorded account of the existence of TA belonging to 9 different species from 4 genera and 4 families of protozoans evidenced in the study, proving that there may be significant diversity of unexplored protozoan probability in the moss ecosystems of this unique sanctuary dedicated to the endangered Gangetic river dolphin protection.

1.1 Global and Indian Diversity of Testate Amoebae

Free-living TA have widespread 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 (Bindu et al, 2024). This diversity concisely shows the ecological flexibility of TA and argues in favor of the role of TA 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 the soil banks of VGDS of on 23rd July 2025. The moss samples were obtained from soil (25.23.4903° N and 87.08.487° E) 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, 1992). 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 VGDS belonging to 9 species of TA span over 4 genera and 4 families during this, preliminary investigation.

Systematic list of TA VGDS: Findings from the present study (Classification as per Adl *et al*., 2019)

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 aplanata* (Penard, 1911) Deflandre, 1929

1929. *Cyclopyxis aplanata* Deflandre, Archiv fur Protistologie, 67: 322-375.

Distribution: India: Uttarakhand, Bihar

Remarks: Present record from VGDS.

2. *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, Bihar, Himachal Pradesh, Kerala, Manipur, Meghalaya, Mizoram, Odisha, Sikkim, Tamil Nadu, Uttar Pradesh, Uttarakhand, West Bengal, Chandigarh.*

Remarks: Present record from VGDS

3. *Cyclopyxis eurystoma* Deflandre, 1929

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

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

Remarks: Present record from VGDS

Family Difflugiidae Wallich, 1864

4. *Difflugia globularis* (Wallich, 1864) Chardez, 1956

1864. *Difflugia globularis* Wallich, Ann. Mag. Nat. Hist., 3 (13): 215-245.

Distribution: India: Kerala, Tamil Nadu

Remarks: Present record from VGDS.

5. *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, Bihar, Himachal Pradesh, Meghalaya, Odisha, Rajasthan, Tamil Nadu, Uttar Pradesh, West Bengal, Punjab.

Remarks: Present record from VGDS.

Family Centropyxidae Jung, 1942

6. *Centropyxis aerophila* Deflandre, 1929

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

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

Remarks: Present record from VGDS.

7. 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, Bihar, Kerala. Manipur, Meghalaya, Mizoram, Nagaland, Odisha, Uttarpradesh, Uttarakhand, West Bengal, Chandigarh.

Remarks: Present record from VGDS.

Phylum Cercozoa Cavalier-Smith, 2018
Class Silicofilosea Adl *et al*., 2012
Order Euglyphida Cavalier-Smith, 1997
Family Euglyphidae Lara *et al*., 2007

8. *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: Bihar, Himachal Pradesh, Kerala, Maharashtra, Odisha, Telangana, Uttar Pradesh, Uttarakhand.

Remarks: Present record from VGDS.

9. *Euglypha rotunda* (Ehrenberg, 1845)

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

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

Remarks: Present record from VGDS.

4. CONCLUSION

TA 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 VGDS are important in ecological studies.

TA can be used as sensitive and early warning signals of environmental disruption towards pollution control and management of the ecosystem in the VGDS. They respond to variations in water quality and nutrient load and habitat conditions in a very rapid manner, giving a fine scale measure of ecological health, supplementing traditional chemical measures. By observing the change in the community structure and abundance of TA enables conservation managers to identify the existence of the low-trophic effects of agricultural runoff, industrial effluents or even the change in hydrology long before the manifestation of stress in the ecosystem. The general focus of the presence of these protozoan bioindicators in long term monitoring is therefore proactive management, which will help in checking the water quality, the biodiversity and the protection of the keystone

animal species and the overall well-being of ecosystem within the sanctuary.

With the increasing effect of climate change and human alteration on the natural ecosystems, there is an urgent need to study these microorganisms in detail. These life forms, which exist in humid land and water communities, are very sensitive to the 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 TA species diversity of VGDS.

To conclude, this preliminary diversity study of TA in VGDS warrants 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 studies will enhance our knowledge of the functioning of the ecosystem and help us to apply more efficient conservation, and sustainable management approach.

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

Author has declared that no competing interests exist.

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