*Original Research Article*

# Pioneering Study of Testate Amoebae in Nagi Bird Sanctuary, Bihar: A Contribution to Ramsar Site Microfauna


## ABSTRACT

No previous literature has documented testate amoebae living on moss in the Nagi Bird Sanctuary, a designated Ramsar site in Bihar. Therefore, this investigation became the first study to explore testate amoebae species diversity in this important wetland. Testate amoebae function as excellent environmental bioindicators, as they exist as single-celled microorganisms with external shells and are free-living. The present study is based on the moss samples collected from the Nagi Bird Sanctuary. The presence of 16 testate amoebae species from 6 genera and 6 families was confirmed through this study. This research expands existing understanding about testate amoeba species and distribution patterns at Ramsar sites in India as well as general ecological knowledge for future surveys and conservation efforts, especially for wetland habitats.



*Keywords: Testate Amoebae, Protozoa, Moss, Nagi Bird Sanctuary, Ramsar site*




## 1. INTRODUCTION

In February 2025 with the addition of Sakkarakottai&Therthangal Bird Sanctuaries, Tamil Nadu; Khecheopalri wetland, Sikkim &Udhwa Lake, Jharkhand brought the total number of Ramsar sites to 89 in India. This expansion has made India the leading Ramsar site holder in Asia and the third largest worldwide according to reported statistics. The United Kingdom ranks first worldwide with 175 sites while Mexico holds the second position with 144 sites. Within Indian states, Tamil Nadu holds the highest number of Ramsar sites in India with its present total of 20 sites (http://timesofindia.indiatimes.com; Accessed 03 February 2025).

Two Indian wetlands namely Nagi Bird Sanctuary and Nakti Bird Sanctuary received their Ramsar Convention Wetlands of International Importance status in 2024 during the commemoration of World Environment Day. The Jhajha forest range of Jamui district in Bihar hosts two artificial reservoirs that serve as the above both sites. The conversion of Nagi River into the Nagi Dam paved way for the man-made wetland, Nagi Bird Sanctuary (24°49'N 86°24'E) spread across 205.8 hectares. Different environmental surfaces including bark of trees and rocks as well as old walls, soil and rocks support the widespread growth of mosses. The importance of mosses in Indian Ramsar wetlands necessarily required to be studied by considering their contribution to ecological well-being through biodiversity support and soil moisture regulation as well as their function in nutrient cycling. The non-vascular micro-organisms help to maintain environmental well-being by ensuring water stability while offering living conditions to testate amoebae and various other microorganisms.

Shelled protozoa, specifically testate amoebae exist in multiple ecosystems while demonstrating high sensitivity to environmental changes [1]. Their quick generation cycle allows testate amoebae to serve as effective indicators of ecological changes in the environment [2,3]. Testate amoebae reside in every worldwide zone from the tropical to polar regions while also occurring in both terrestrial and marine environments. The Sphagnum mosses contain numerous testate amoebae populations that reveal different ecological preferences based on their environment and surrounding factors [4]. These organisms demonstrate sensitivity to moisture levels and pH conditions together with other environmental factors which makes them strong indicators of ecological modifications [5]. Testate amoebae populations react to water quality and pollution levels and climate change thus enabling their application in both past water condition reconstruction and modern ecological assessment [6]. Testate amoebae population numbers and species diversity serve as indicators to identify habitat modifications that occur due to hydrophysical transformations and pollution events. Research use specific test specimens to monitor present-day pH fluctuations and moisture changes through the examination of species that thrive in acidic and neutral conditions [7].

Testate amoebae are present in large quantities and high densities in Sphagnum biotopes and play important roles in the microbial loop, nutrient cycling, and ecosystem processes [8]. Environmental moisture greatly influences the dynamics and activity of populations of testate amoebae [9]. Traditionally, two major classes, namely Arcellinida characterized by lobose pseudopodia and Euglyphida characterized by filose pseudopodia have been identified as classes of testate amoebae [10]. Recent breakthroughs in DNA-based taxonomy of testate amoebae, which have had a secondary impact from molecular phylogenetics, have shown the actual taxonomic status of the taxa. This molecular study shows that testate amoebae comprise a polyphyletic group of at least three unrelated unicellular eukaryotic taxonomic groups of Amoebozoa, Stramenopiles, and Cercozoa [11,12].

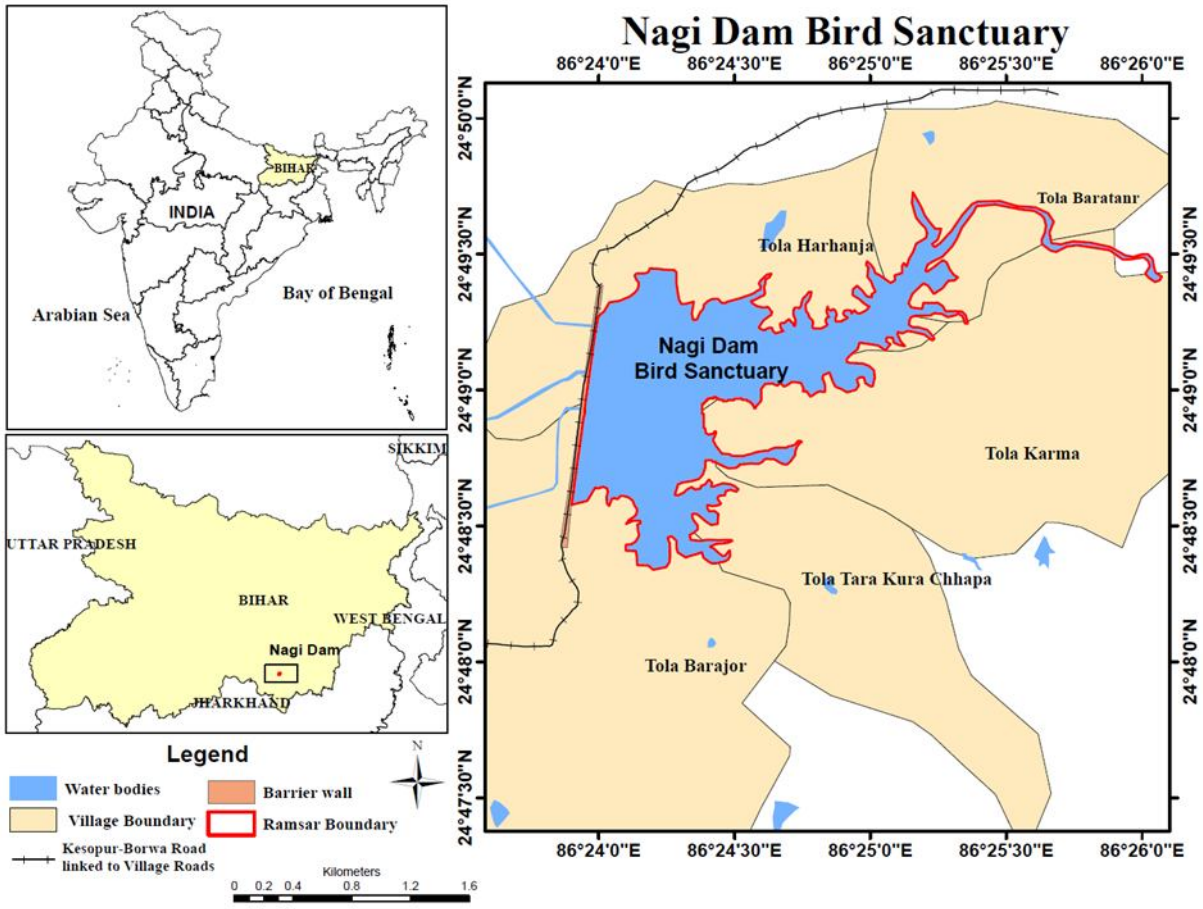
On the other hand, despite being one of the most biodiverse regions of India, research on protozoan diversity, particularly free-living protozoan species like testate amoebae, remains largely unexplored with many gap areas [13], with only a few recent studies reported in literature in the Bihar state [14,15]. Notably, no research has been conducted on the Nagi Bird Sanctuary, a designated Ramsar wetland in Bihar. This study represents the first preliminary survey of testate amoebae associated with mosses in one of Bihar's designated wetlands. Thus, 16 species, belonging to 6 genera, and 6 families are presented from this study. 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, especially in the wetlands, most urgently on all the 89 designated ramsar sites in India.

### 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]. Novel record of 16 testate amoebae species from 6 genera and 6 families were reported from Nagi Bird Sanctuary, a designated Ramsar wetland in Bihar, through this study with two more new records to the state of Bihar in addition to the species reported earlier [14,15].

## 2. MATERIAL AND METHODS

The moss samples for the present study were collected from Nagi Bird Sanctuary, a designated Ramsar wetland in Bihar on 3rd January 2025. The samples were obtained from the site (Map. 1; 24.82.488° N and 86.39.836° 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 [16]. 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.




**Map. 1. Study site, Nagi bird sanctuary, a designated Ramsar Convention Wetland.**

## 3. RESULTS AND DISCUSSION

The study yielded the following 16 species of testate amoebae span over 6 genera and 6 families from Nagi Bird Sanctuary during this preliminary investigation, with two new records to the state of Bihar.

Systematic list of Testate Amoebae fromNagi Bird Sanctuary, Bihar: Findings from the present study (Plate 1, 2 & 3) (Classification as per Adl et al., 2019) [12]

Domain AmorpheaAdl 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 NetzeliidaeKosakyan et al., 2016

1. ***Cyclopyxisarcelloides***(Penard, 1902) Deflandre, 1929

1902. *Centropyxisarcelloides*Penard, *FauneRhizopodique 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, Punjab.

2. ***Cyclopyxisaplanata*** (Penard, 1911) Deflandre, 1929

1929. CyclopyxisaplanataDeflandre, Archiv fur Protistologie, 67: 322-375.

Distribution: India: Uttarakhand, Punjab

Remarks: New record from Bihar

3. ***Cyclopyxiseurystoma***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, Punjab

4. ***Cyclopyxiskahli****Deflandre, 1929*

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

*Distribution :*India: Bihar, Himachal Pradesh, Kerala, Tamil Nadu, Uttarakhand, Punjab

Family Phryganellidae Jung, 1942

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

1909. *Phryganella acropodia* Hopkinson, *The British Freshwater Rhizopoda andHeliozoa*, 2: 74, pl.20, figs.13-14.

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

Family DifflugiidaeWallich, 1864

6. ***Difflugiaglobulosa*** (Dujardin, 1837) Penard, 1902

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

1902. *Difflugia*globulosaPenard, *FauneRhizopodique 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

Family Centropyxidae Jung, 1942

7. ***Centropyxisaerophila***Deflandre, 1929

1929. *Centropyxisaerophila*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

*8.* ***Centropyxisecornis***(Ehrenberg, 1841)

1841. *Arcellaecornis* Ehrenberg, *Abh. Akad. Wiss. Berlin*, p. 368.

1879. *Centropyxisecornis* Leidy, *Freshwater Rhizopods of North America*, pl.30, figs.20-24.

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

*9.* ***Centropyxis laevigata***Penard, 1890

1890. *Centropyxis laevigata*Penard, *Mem. Soc. Phys., Geneve*, 31(2): 151.

1929. *Centropyxis laevigata*Deflandre, *Arch. Protistenkd.*, 67: 356.

*Distribution*: India: Arunachal Pradesh, Assam, Himachal Pradesh, Kerala, Maharashtra, Mizoram, Sikkim, Tamil Nadu, Uttar Pradesh, Uttarakhand, West Bengal, Punjab

*Remarks*: New record from Bihar

10. ***Centropyxisplatystoma*** (Penard, 1890) Deflandre, 1929

1929. *Centropyxisplatystoma*Defalndre, *Arch. Protistenkd.*, 67:338.

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

Phylum Cercozoa Cavalier-Smith, 2018

Class SilicofiloseaAdl et al., 2012

Order Euglyphida Cavalier-Smith, 1997

Family Euglyphidae Lara et al., 2007

11. ***Euglyphacapsiosa***Coûteaux, 1978

1978. *Euglyphacapsiosa*Couteaux, *Journal of protozoology*, 25: 50A

*Distribution* : India: Bihar, Kerala, Tamil Nadu

12. ***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.

*13.* ***Euglyphalaevis***(Ehrenberg, 1845)

1845. *Euglyphalaevis* Ehrenberg, *Ber. Akad., Berlin*, p. 307.

1849. *Euglyphalaevis*Perty, *Mitth. nat. Ges. Bern*., p. 163

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

14. ***Euglyphastrigosa (***Ehrenberg, 1848)

*1871. Difflugia strigosa* Ehrenberg, NacthragzurÜbersicht der organischenAtmosphärilien. Abhandlungen der Königliche Akademie der Wissenshaftenzu Berlin (1871), p. 233-275, Pl. 3.

*Distribution*: India: Bihar, Himachal Pradesh, Kerala, Maharashtra, Uttarakhand, Punjab

Family TrinematidaeAdl et al., 2012

15. ***Trinemalineare***Penard, 1890

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

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

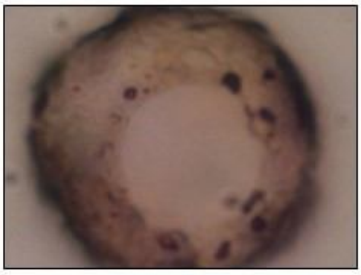
*Distribution*: India: Andhra Pradesh, Arunachal Pradesh, Assam, Bihar, Himachal Pradesh, Manipur, Meghalaya, Mizoram, Nagaland, Odisha, Tripura, Uttarpradesh, Uttarakhand, Chandigarh, Punjab

16. ***Trinemacomplanatum***Penard, 1890

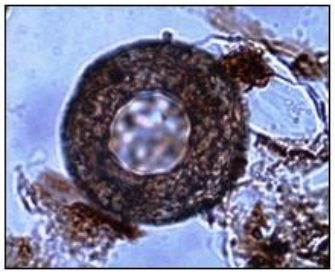
1890. *Trinemacomplanatum*Penard, *Mem. Soc. Geneve*, 31: 187, pl.10, figs. 1-4.

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

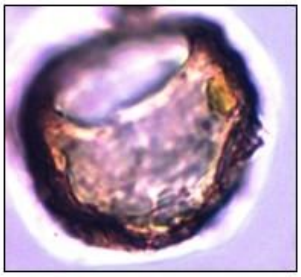
# Plate 1



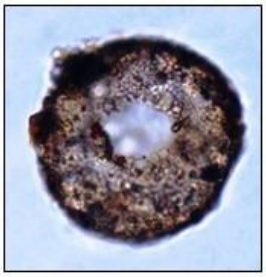
*1. Cyclopyxis arcelloides*
**(Penard, 1902) Deflandre, 1929**



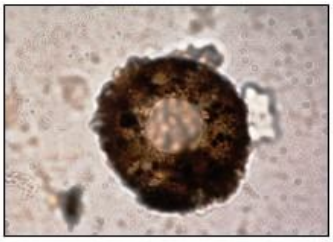
*2. Cyclopyxis aplanata*
**(Penard, 1911) Deflandre, 1929**



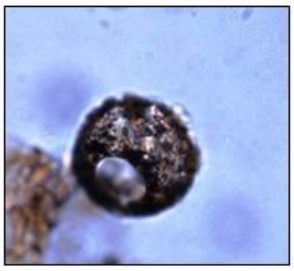
*3. Cyclopyxis eurystoma*
**Deflandre, 1929**



*4. Cyclopyxis kahli*
**Deflandre, 1929**

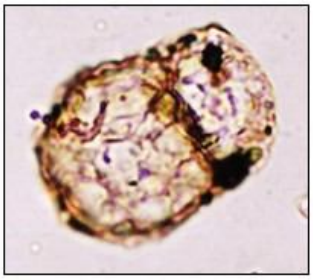


*5. Phryganella acropodia*
**(Hertwig & Lesser, 1874)**

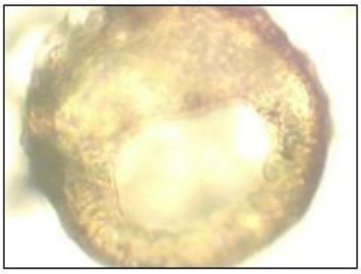


*6. Difflugia globulosa*
**(Dujardin, 1837) Penard, 1902**

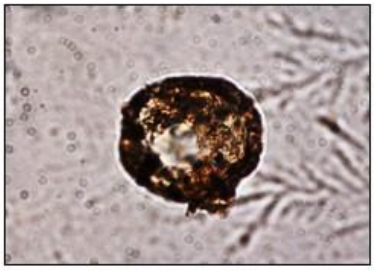
# Plate 2



*7. Centropyxis aerophila*
**Deflandre, 1929**



*8. Centropyxis ecornis*
**(Ehrenberg, 1841)**



*9. Centropyxis laevigata*
**Penard, 1890**

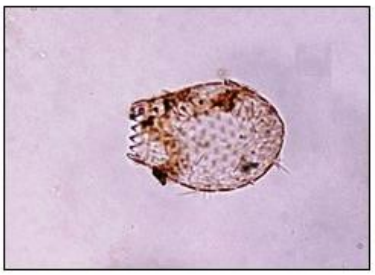
*10. Centropyxis platystoma*
**(Penard, 1890) Deflandre, 1929**

*11. Euglypha capsiosa*
**Coûteaux, 1978**

*12. Euglypha rotunda*
**(Ehrenberg, 1845)**

# Plate 3

*13. Euglypha laevis*
**(Ehrenberg, 1845)**



*14. Euglypha strigosa*
**(Ehrenberg, 1848)**

*15. Trinema lineare*
**Penard, 1890**

*16. Trinema complanatum*
**Penard, 1890**

## 4. CONCLUSION

Ramsar wetlands are of global importance, as ecosystems that support high levels of biodiversity, providing a wide range of ecological services, including water quality purification, carbon sequestration and climate regulation. Despite its broad implications, moss diversity in these wetlands remains under investigated, particularly in light of their ecological importance and their interactions with microbial communities. Mosses within Ramsar wetlands provide microhabitats for multiple microorganisms, among these microfauna such as testate amoebae, that are representative of environmental changes and valuable bioindicators of ecosystem health. India is home to 89 designated Ramsar wetlands, but a few studies have explored their biodiversity, and scant research has been done on mosses and their testate amoebal associations. This substantial knowledge gap emphasizes the critical need for further investigations to fully understand the ecological processes of these important habitats. Testate amoebae, since they respond with sensitivity to changes in hydrological and nutrient regimes, are key to understand the effects of climate change, pollution and habitat disruption on wetland communities. Research investigating the distributions and diversity of testate amoebae in mosses in Ramsar wetlands can provide important insights into wetland ecosystem dynamics and can help the conservation activities. Furthermore, such investigations may be useful for tracking trends in wetland health, assuring the careful and continued management and protection of these valuable ecosystems.

In conclusion, integrating moss diversity studies with testate amoebae assessments in Ramsar wetlands provides a robust framework for biodiversity conservation, ecological monitoring, and climate change mitigation. Since only limited research has been carried out to date, most particularly in India's Ramsar sites, there is an urgent need for larger scale research to tap into the full capabilities of these ecosystems, and to ensure the ecological integrity of these ecosystems for future generations.



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