Original Research Article

# Microfauna Hidden in Moss: First Report of Testate Amoebae from Bhimbandh Wildlife Sanctuary, Bihar, India


## ABSTRACT

Moss associated testate amoebae of Bhimbandh Wildlife Sanctuary in Bihar are yet to be reported from the literature. Therefore, this article serves as the initial attempt to document and evaluate the state of species richness of testate amoebae in this sanctuary. Testate amoebae belong to the single celled organisms classified under protist fauna characterized by developing complex covering of outer chitin shells; they are renowned as bio indicators owing to their high reactivity to any changes in the environment. In September 2024, moss samples were taken from several different sites from the sanctuary in a faunal survey to assess the diversity of testate amoebae in moss environments. Upon analysing these samples 17 species of testate amoebae span over 6 genera and 6 families were found during this preliminary investigation. All of these are new records for Bhimbandh Wildlife Sanctuary and establish moss as a critical habitat for these species.



**Keywords:** Testate Amoebae, Protozoa, Moss, Bhimbandh Wildlife Sanctuary, Pollution Indicator


## 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. This study documents for the first time this microfauna in Bhimbandh Wildlife Sanctuary, a part of the rich biodiversity of Bihar, India. The research examines moss samples from a variety of microhabitats to determine the diversity and ecological importance of testate amoebae, providing a new view of the biological heritage of the sanctuary's lesser-known organisms.

Bhimbandh Wildlife Sanctuary (BWS) is in the Munger district in Bihar, India and covers an area of $681.99^2$ kms. It is located within the geographical position of 24°50 'to 25°10 'north latitude and 86°15 'to 86°30 'eastern longitude. The task of creating this sanctuary was

initiated in the year 1976 and is considered as an important bio diverse region located in the Rajgir hills of Kharagpur range [1].


**Map. 1: Study area, Bhimbandh Wildlife Sanctuary (BWS) in Bihar (Source: SOI & Google Earth)**

BWS is popular for its diverse vegetation and wildlife, it is most famous for its hot springs and numerous tracts of ecosystems that host many species of mammals, birds and reptiles besides many groups of Insects. BWS has a significant role in wildlife protection and is a very crucial wildlife avenue that links other forests in Bihar. As a sanctuary, it has an inherent ecological value for both conservation and ecotourism purposes, which has been recognised by the Ministry of Environment, Forest and Climate Change, Government of India. The contrasting environmental conditions in India ranging from alpine to tropical forest provide a suitable background for the study of testate amoebae in different habitats throughout India. But scientific studies on testate amoebae in many wildlife sanctuaries of India are still limited or un explored. Majority of works have focused on areas of moderate or high altitude, for example the Western Ghats or the Himalayas, whereas tropical areas like those present in BWS, Bihar, remain poorly investigated in terms of testate amoebae diversity.

While the diversity of testate amoebae has been documented in various states and eco-regions across India, only a few preliminary studies have been conducted in Bihar [2] leaving significant gaps in understanding their distribution and ecological roles in the region, Particularly, their presence and diversity within the wildlife sanctuaries and national parks of

Bihar remain largely unexplored, highlighting the need for focused research in these ecologically significant areas.

Shelled protozoa specifically testate amoebae are the species that occupy large numbers of habitats. They are very susceptible to ecological alterations [3] and are therefore good biomarkers to ecological variations because of their short generation intervals [4,5,6]. These organisms are found in different parts of the world and from tropical to polar regions and from land to sea water environments. 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 [7]. 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 [8,9,10,11]. A diverse group of shelled protozoa (testate amoebae) are essential to ecosystem functioning, especially in soil and aquatic microhabitats. Because of their sensitivity to changes in the environment, these protists are also important as bioindicators and play an essential role in nutrient cycling and decomposition of organic material [12]. There has been a well documented association of testate amoebae with mosses, as moss microhabitats provide moist, nutrient rich environments in which they can survive and reproduce. Besides enriching the microfauna diversity, these moss layers also create microecosystems that support complex food webs, that way microbial communities are linked with higher trophic levels [13].

This study is the first to report the diversity of testate amoebae within BWS within the moss-dominated microhabitats. By documenting these protozoan species, this research enhances our understanding of testate amoebae distribution in tropical ecosystems and underscores their ecological roles within moss communities [3]. Furthermore, insights into their diversity contribute to the broader biogeographical understanding of protozoa across India's varied landscapes, highlighting the importance of conserving microhabitats in tropical sanctuaries. Therefore, this work is an attempt to carry out preliminary assessment of testate amoebae in BWS as they are ecologically important. Thus, the faunal diversity of the given area has been expanded by studying 17 species, 6 genera, and 6 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.

## 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 [14]. 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.

## MATERIAL AND METHODS

The moss samples for the present study were collected on 28$^{th}$ September 2024 as part of a faunal survey program within the BWS in Bihar. The samples were obtained from various biotopes like Tree, soil and wall (Map. 1; 25.01.109° N and 86.42.022° 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 [15]. 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.

## RESULTS AND DISCUSSION

The study yielded the following new records to Bhimbandh Wildlife Sanctuary, Bihar belonging to 17 species of testate amoebae span over 6 genera and 6 families during this preliminary investigation.

Systematic list of Testate Amoebae from BWS, Bihar: Findings from the present study (Plate 1) (Classification as per Adl et al., 2019) [16, 17]

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, Bihar

*Remarks*: Present record from BWS, 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, Bihar

*Remarks*: Present record from BWS, Bihar

**3. *Cyclopyxis kahli*** Deflandre, 1929

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

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

*Remarks*: Present record from BWS, Bihar

Family Phryganellidae Jung, 1942

**4. *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, Bihar

*Remarks:* Present record from BWS, Bihar

Family Difflugiidae Wallich, 1864

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

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

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, Bihar

Remarks: Present record from BWS, Bihar

Family Cryptodifflugiidae Jung, 1942

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, Bihar

Remarks: Present record from BWS, Bihar

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

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

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

*Remarks*: Present record from BWS, 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, Bihar

*Remarks*: Present record from BWS, Bihar

**9. *Centropyxis kurakchayensis*** Snegovaya and Alekperov, 2005

2005. *Centropyxis kurakchayensis* Snegovaya et Alekperov, *Protistology* 4(2) : 149-183.

*Material examined* :Mi.884, 2exs., 11. iv. 2019, Coll. Sheela. S, Tree moss, Singalila N.P, West Bengal

*Distribution* : India: West Bengal, Bihar

*Remarks*: Present record from BWS, Bihar

**10. *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, Bihar

*Remarks*: Present record from BWS, Bihar

Phylum Cercozoa Cavalier-Smith, 2018

Class Silicofilosea Adl et al., 2012

Order Euglyphida Cavalier-Smith, 1997

Family Euglyphidae Lara et al., 2007

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

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

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

*Remarks*: Present record from BWS, Bihar

**12. *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, Bihar

*Remarks*: Present record from BWS, Bihar

**13. *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, Bihar

*Remarks*: Present record from BWS, Bihar

Family Trinematidae Adl et al., 2012

**14. *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, Bihar

Remarks: Present record from BWS, Bihar

**15. *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, Bihar

*Remarks*: Present record from BWS, Bihar

**16. *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, Bihar

*Remarks*: Present record from BWS, Bihar

**17. *Trinema galeata*** Jung, 1942

1942. *Corythion constricta* Jung, *Archv für Protistenkdunde*, 95 (3): 253-356.

*Material examined* : Mi.1002, 4 exs., 18. x. 2019,Coll. Bindu. L, Rock moss, Raj Bhawan, Nainital

*Distribution* : India: Himachal Pradesh, Meghalaya, Bihar

*Remarks*: Present record from BWS, Bihar

## CONCLUDING REMARKS

Moss-associated testate amoebae are most helpful for studying changes to the environment because mosses tend to grow in wet or in moist habitats. They also adapt quickly to changes in their environment, and hence are ideal as pollution and other ecological changes as biomarkers. This makes their study invaluable especially in these un-investigated ecosystems like wildlife sanctuaries and national parks in Bihar. It is therefore critical to gain knowledge of the distribution and spacial heterogeneity of protozoans, especially testate amoebae, in these ecosystems due to rising effects of climate change and human activities. Commensurate with the responses they give to communities of these small organisms, they give prime data on major environmental issues, as such constituting significant importance in the field of biodiversity and ecosystems.

In the present study, it proposed to evaluate the health and overall states of ecosystems in Bihar and other regions through the species richness of testate amoebae. These organisms, which are now well documented to inhabit both terrestrial and aquatic environments, serve as baseline biomarkers, providing essential information for the assessments and inventory of ecosystems while also contribute new distributional records of species.

Therefore, on the basis of the current state of knowledge about testate amoebae present within the Bhimbandh Wildlife Sanctuary and other protected regions of Bihar, targeted, extensive site-specific research are required. Such inquiries should address their function on overseeing of ecological condition, equitable in areas that have felt the influence of human beings. Thus, the microhabitat-centered studies will contribute to improving our understanding of the ecological mechanisms, approximating efficiency of the protected area network, and providing directions for the sustainable use of these unique environments.

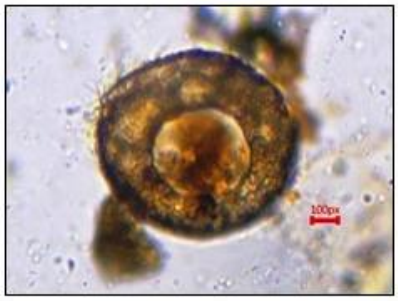


## REFERENCES

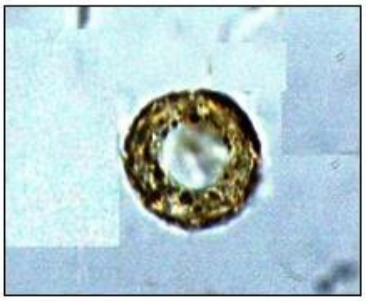
1. MoEF&CC (Comp.) 2021. National Parks and Wildlife Sanctuaries in India. Ministry of Environment, Forest and Climate Change, Government of India. Pg. 454.
2. Kumar, V.M. Sathish, Bindu. L, and Anil Kumar. 2024. "First Report on Moss-Dwelling Testate Amoebae from Bihar, India: Species Diversity and Ecological Significance". BIONATURE 44 (2):73-81. https://doi.org/10.56557/bn/2024/v44i22044
3. Smith, H.G., Bobrov, A., Lara, E. (2007). Diversity and biogeography of testate amoebae. In: Foissner, W., Hawksworth, D.L. (eds) Protist Diversity and Geographical Distribution. Topics in Biodiversity and Conservation, vol 8. Springer, Dordrecht. https://doi.org/10.1007/978-90-481-2801-3_8.
4. Ogden, C. G., & Hedley, R. H. (1980). An Atlas of Freshwater Testate Amoebae. British Museum (Natural History).
5. Mitchell , EAD and Gilbert , D . 2004 . Vertical micro-distribution and response to nitrogen deposition of testate amoebae in Sphagnum . Journal of Eukaryotic Microbiology , 51 : 485 – 495 .
6. Mitchell, Edward & Charman, Dan & Warner, Barry. (2008). Mitchell EAD, Charman DJ, Warner BG.. Testate amoebae analysis in ecological and paleoecological studies of wetlands: past, present and future. Biodivers Conserv 17: 2115-2137. Biodiversity and Conservation. 17. 10.1007/s10531-007-9221-3.
7. 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 Ecol. 53: 53-65.
8. Vincke S, Gremmen N, Beyens I, Van de Vijver B (2004a). The moss dwelling testaceans fauna of IIe de Possession. Polar Biology 27:753-766.
9. Mattheeussen R, Ledeganct P, Vincke S, Van de Vijver B, Nijs I, Beyens L (2005). Habitat selection of aquatic testate amoebae communities on Qeqertarsuq (Disko Island), West Greenland. Acta Protozoologica 44:253-263.
10. Bobrov, A., Charman, D. J and Warner, B. G. Ecology of Testate Amoebae (Protozoa: Rhizopoda) on Peatlands in Western Russia with Special Attention to Niche Separation in closely related taxa. Protist.1999; 150(2): 125-136.
11. Jassey, V. E., Signarbieux, C., Hättenschwiler, S., Bragazza, L., Buttler, A., Delarue, F and Mitchell, E. A. D. 2013. An unexpected role for mixotrophs in the response of peatland carbon cycling to climate warming. Scientific Reports.2013; 3: 23-54.

12. Ogden, C.G., Hedley, R.H. 1980. An atlas of freshwater testate amoebae: 95 plates. British Museum (Natural History) and Oxford University Press (London and Oxford). Pp. 222.
13. Mitchell EAD, Charman DJ, Warner BG. 2015. Testate amoebae analysis inecological and paleoecological studies of wetlands: past, present and future. Biodiversity and Conservation.17(2):211-230.
14. Bindu, L., Purushothaman, J., Ambili, A., Chaudhary, A. (2024). Checklist of Fauna of India:Testate Amoeba (Protozoa: Tubulinea; Cercozoa; Stramenopiles). Version 1.0. Zoological Survey India. DOI: https://doi.org/10.26515/Fauna/1/2023/Protista:Tubulinea; Cercozoa; Stramenopiles.
15. Foissner, W. Estimating the species richness of soil protozoa using non-flooded petridish method. In Protocols in Protozoology.1992; Lee, J.J and Soldo,A.T.(eds), Allen Press.
16. Adl S. M., Simpson A. G. B., Lane C. E., Lukes J., Bass D., Bowser S. S., Brown M. W., Burki F., Dunthorn M., Hampl V., Heiss A., Hoppenrath M., Lara E., Le Gall L., Lynn D. H., McManus H., Mitchell E. A. D., Mozley-Stanridge S. E., Parfrey L. W., Pawlowski J., Rueckert S., Shadwick L., Schoch C. L., Smirnov A., Spiegel F. W. (2012) The revised classification of eukaryotes. J. Eukaryot. Microbiol. 59: 429–493
17. Adl, S.M., Bass, D., Lane, C.E., Lukes, J., Schoch, C.L., Smirnov, A et al. Revisions to the classification, nomenclature, and diversity of eukaryotes. Journal of Eukaryotic Microbiology.2019; 66: 4–119.

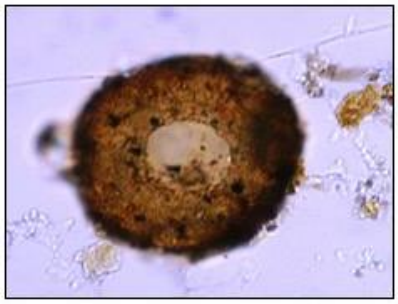
**Plate 1. Microphotograph of Testate Amoebae from BWS, Bihar**



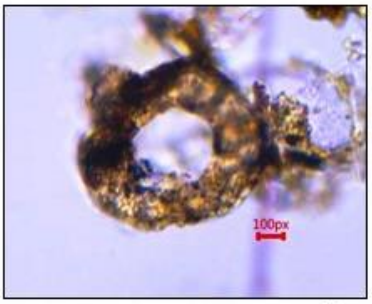
*1. Cyclopyxis arcelloides* **(Penard, 1902) Deflandre, 1929**



*2. Cyclopyxis eurystoma* **Deflandre, 1929**



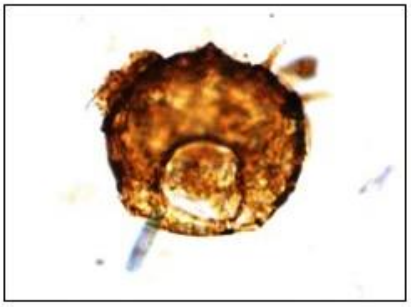
*3. Cyclopyxis kahli* **Deflandre, 1929**



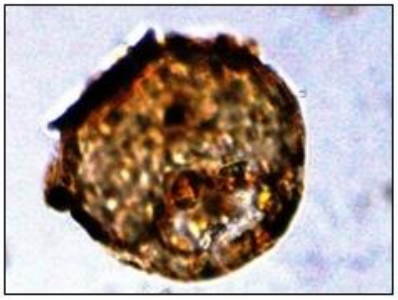
*4. Phryganella acropodia* **(Hertwig & Lesser, 1874) Hopkinson, 1909**

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

*6. Centropyxis aerophila Deflandre, 1929*

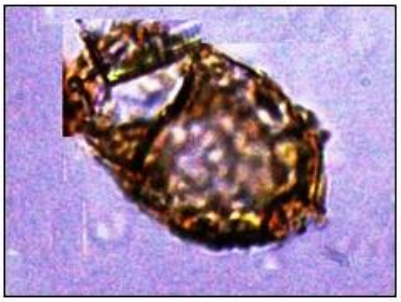


*7. Centropyxis aculeata (Ehrenberg, 1838) Stein, 1859*



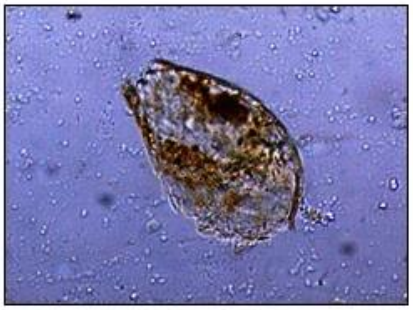
*8. Centropyxis ecornis (Ehrenberg, 1841)*

*9. Centropyxis kurakchayensis Snegovaya and Alekperov, 2005*



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

*11. Euglypha capsiosa Coûteaux, 1978*

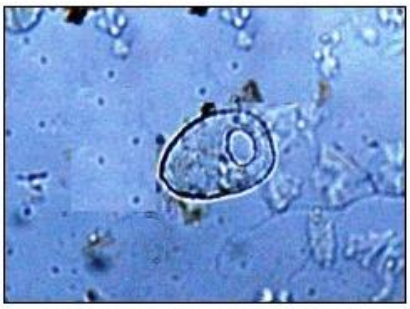


*12. Euglypha rotunda (Ehrenberg, 1845)*

*13. Euglypha laevis (Ehrenberg, 1845)*

*14. Trinema enchelys (Ehrenberg, 1838)*

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



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

*17. Trinema galeata* **Jung, 1942**