TESTATE AMOEBAE (PROTOZOA: TUBULINEA) DIVERSITY IN MOOKAMBIKA WILDLIFE SANCTUARY, KARNATAKA, INDIA

ABSTRACT

Studies on testate amoebae in Karnataka state have been sporadic and no studies were done in Mookambika Wildlife sanctuary. Therefore, as part of the faunistic survey of Zoological Survey of India moss samples were collected from Mookambika wildlife sanctuary and has filled the lacuna of testate amoebae study in the sanctuary to form the baseline data.The study resulted 27 species of testate amoebae span over 7 families under the Phylum Tubulinea.The study highlights the importance of conducting more research on testate amoebae in different regions of Mookambika WLS.

Key words: Testate amoebae, Protozoa, Tubulinea, Karnataka, Western Ghats, India

1. INTRODUCTION

"Testate amoebae are unicellular protists characterised by a decay-resistant test" (Meisterfeld, 2002a, b). "They live in a variety of habitats where they are directly exposed to pollutants. They are abundant and diverse in mosses and associated with terrestrial mosses are increasingly used in ecological and ecotoxicological studies".(Smith et al., 2009) "The testate amoebae community is sensitive to changes in its environment like climate change, metal or gas pollution.Among microbial organisms, testate amoebae have been shown to be useful for bioindication of anthropogenic pollution in peatland and soil" (Gilbert and Mitchell, 2006).They are commonly found in wetland environments, mosses from barks of trees, on rocks etc. Their presence in mosses might depend on factors like moisture, pH and nutrient levels in the moss habitat.The distribution of mosses is diverse, spanning various habitats globally, such as forests, wetlands and tundra. Testate amoebae often live in the water-filled cavities of mosses or on their surfaces. "Their well-defined ecological preferences in relation to important ecological variables in different type of ecosystems have made them useful in biomonitoring" (Charman,1992; Mitchell *et al.*, 2008).

“Mookambika Wildlife Sanctuary is a protected wildlife sanctuary in the southern state of Karnataka in India (13°42' N74°39' E) . It derives its name from the presiding deity "Goddess Mookambika" of the popular KollurMookambika Temple. The sanctuary lies in the Western Ghats in Udupi and Shimoga districts and consists of an area of 370.37 km^2. The Sanctuary starts from Uttar Kannada district border on its north near Shiroor and runs along inter-district boundary till the head of Chakra dam Then turning west, it goes up to Halejaddu near Aloor and then turns north and goes back to the starting point Via Chittur, Areshirur and Ganganadu village limits” (Netalkar, 2010).

The present study is conducted as part of the scientific programme of Western Ghat Regional Centre of Zoological Survey of India, Kozhikode, Kerala to explore the diversity of testate amoebae in Mookambika wildlife sanctuary which is the first study on Testate amoebae in this sanctuary.

MATERIALS AND METHODS

The moss collection was done along the tree trunks, rocks and soil. Moss samples (100 g) were collected by quadrant sampling and transferred to a polythene bag by scraping the mosses from the rocks and trees from the survey conducted by Zoological Survey of India to various parts of Mookambika WLS during the years 2022 and 2023(Fig.1).The samples were brought back to the laboratory and a portion of sample (50g) was soaked in distilled water in a petri dish (Non-Flooded Petri dish method) as described by Foissner (1987,1992) and Mazei *et al*. (2011). The samples were then thoroughly stirred by adding distilled water in a petri dish. Subsequently, aqueous drops were extracted from the moss samples using a micropipette, placed on microslides, thoroughly examined under Leica DM 2000compound microscopeand the protozoans were isolated from the samples. The slidesare then air-dried for 24 hours and then mounted with DPX and permanent mounts were prepared for each sample and examined under the microscope for further identification. The slides with identified specimens were registered and deposited in the National Zoological collections of the Western Ghats Regional Centre, Zoological Survey of India, Kozhikode.

2. RESULTS AND DISCUSSION

The study resulted the exploration of 27 species of testate amoebae under the phylum Tubulinea belonging to 9 genera and under 7 families. All the species recorded here are new reports to Mookambika wildlife sanctuary as well as to Karnataka state. Although studies were done in other parts of Western Ghats no studies were made in Western ghats of

Karnataka and recently Bindu (2023) reported 91 species of testate amoebae from Kerala part of Western Ghats. This is the only consolidated study on testate amoebae of Western Ghats. In the present study the family Centropyxidae represents dominant percentage of species (52%) and the families with least percentage of species (4%) were Heleoperidae and Phryganellidae.

SYSTEMATIC ACCOUNT

The systematic account of the species recorded from the study area and their distribution in India is as follows as per the classification of Adl *et al*., 2019.

Phylum Tubulinea Smirnov *et al.,* 2005

Class Elardia Kang *et al.,* 2017

Order Arcellinida Kent, 1880

Family Arcellidae Ehrenberg, 1843

1. *Galeripora discoides* (Ehrenberg, 1871) Gonzalez-Miguens *et al.*, 2021
   Distribution: Arunachal Pradesh, Assam, Himachal Pradesh, Kerala, Maharashtra, Manipur, Meghalaya, Mizoram, Nagaland, Odisha, Punjab, Sikkim, Tamil Nadu, Telangana, Tripura, Uttar Pradesh, Uttarakhand, West Bengal
2. *Galeripora catinus* (Penard, 1890) Gonzalez-Miguens et al., 2021
   Distribution : Arunachal Pradesh, Assam, Himachal Pradesh, Mizoram, Odisha, Sikkim, Tamil Nadu, Uttarakhand

Family Netzeliidae Kosakyan *et al.*, 2016

3. *Cyclopyxis arcelloides* Deflandre, 1929
   Distribution : Andhra Pradesh, Arunachal Pradesh, Himachal Pradesh, Kerala, Manipur, Meghalaya, Mizoram, Odisha, Sikkim, Tamil Nadu, Uttar Pradesh, Uttarakhand, West Bengal, Chandigarh
4. *Cyclopyxis eurystoma* Deflandre, 1929
   Distribution : Arunachal Pradesh, Assam, Himachal Pradesh, Kerala, Maharashtra, Nagaland, Tamil Nadu, Telangana, Uttarakhand, West Bengal
   Incertae sedis Arcellinida
5. *Trigonopyxis arcula* Penard, 1912
   Distribution: Assam, Himachal Pradesh , Maharashtra, Manipur , Sikkim, West Bengal

Family Difflugiidae Wallich, 1864

6. *Difflugia corona* Wallich, 1864

   Distribution : Andhra Pradesh, Himachal Pradesh, Kerala, Tamil Nadu, Tripura, Uttar Pradesh, West Bengal

7. *Difflugia globulosa* Dujardin, 1837

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

8. *Difflugia oblonga* Ehrenberg, 1838

   Distribution : Assam, Himachal Pradesh, Meghalaya, Sikkim, Telangana, Uttar Pradesh, West Bengal

Family Centropyxidae Jung, 1942

9. *Centropyxis aculeata* (Ehrenberg, 1832) Stein, 1857

   Distribution : Andhra Pradesh, Arunachal Pradesh, Assam, Himachal Pradesh, Maharashtra, Manipur,Meghalaya, Mizoram, Nagaland, Orissa, Rajasthan,Sikkim, Tripura, West Bengal.

10. *Centropyxis aculeata grandis* Deflandre, 1929

    Distribution : Himachal Pradesh

11. *Centropyxis aerophila* Deflandre, 1929

    Distribution :Arunachal Pradesh, Andhra Pradesh, Assam, Chandigarh, Himachal Pradesh,Jammu & Kashmir, Manipur, Mizoram, Meghalaya,Nagaland, Sikkim,Tripura, Uttarakhand, West Bengal

12. *Centropyxis aerophilasphagnicola*Deflandre, 1929

    Distribution: Assam, Himachal Pradesh, Uttarakhand, Uttar Pradesh

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

    Distribution : Assam, Andhra Pradesh, Himachal Pradesh, Meghalaya, Orissa, Uttarakhand, West Bengal

14. *Centropyxis constricta* (Ehrenberg, 1841) Penard, 1890

    Distribution : Andhra Pradesh, Assam, Chandigarh, Himachal Pradesh, Meghalaya,Mizoram, Sikkim, Tripura, Uttarakhand, West Bengal

15. *Centropyxis ecornis* Leidy, 1879

    Distribution :Andhra Pradesh, Arunachal Pradesh, Assam, Himachal Pradesh, Manipur, Maharashtra, Meghalaya, Mizoram, Nagaland, Sikkim,Uttarakhand, West Bengal.

16. *Centropyxis elongata* (Penard, 1890) Thomas, 1959

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

17. *Centropyxis laevigata* Penard, 1890

    Distribution : Assam,Himachal Pradesh, Maharashtra, Mizoram,Sikkim, West Bengal

18. *Centropyxis minuta* Deflandre, 1929

    Distribution :Andhra Pradesh, Arunachal Pradesh, Assam, Himachal Pradesh, Kerala, Maharashtra, Manipur, Meghalaya, Mizoram, Nagaland, Odisha, Sikkim, Tamil Nadu, Tripura, Uttar Pradesh, Uttarakhand, Jammu & Kashmir

19. *Centropyxis orbicularis* Deflandre, 1929

    Distribution : Andhra Pradesh, Assam, Himachal Pradesh, Kerala, Maharashtra, Sikkim, Uttar Pradesh

20. *Centropyxis platystoma* Penard, 1890

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

21. *Centropyxis spinosa* Cash, 1905

    Distribution : Andhra Pradesh, Arunachal Pradesh, Himachal Pradesh, Kerala, Maharashtra, Manipur, Meghalaya, Mizoram, Nagaland, Odisha, Sikkim, Tamil Nadu, Telangana, Uttar Pradesh, West Bengal

22. *Centropyxis sylvatica* (Deflandre, 1929) Bonnet and Thomas, 1955

    Distribution :Arunachal Pradesh, Assam, Himachal Pradesh, Jammu & Kashmir, Sikkim

Family Hyalospheniidae Schultze, 1977, emend. Kosakyan and Lara, 2012

23. *Nebela bohemica* Taranek, 1882

    Distribution : Arunachal Pradesh, Himachal Pradesh, Sikkim

24. *Padaungiella lageniformis* Penard, 1890

    Distribution: Himachal Pradesh, Uttarakhand

25. *Padaungiella wailesi* Deflandre, 1936

    Distribution : Himachal Pradesh, Uttarakhand

Family Heleoperidae Jung, 1942

26. *Heleopera rosea* Penard, 1890

Distribution : Assam, Arunachal Pradesh, Himachal Pradesh, Manipur, Nagaland, Sikkim.

Family Phryganellidae Jung, 1942

27. *Phryganella acropodia* Hopkinson, 1909

Distribution :Himachal Pradesh, Sikkim, Tamil Nadu, Telangana, Uttarakhand**4. CONCLUSION**

The distribution of testate amoebae in Mookambika WLS was found to be diverse, with different habitats supporting different species. The majority of species were found in wetland habitats followed by forest habitats. This indicates the importance of preserving these diverse habitats for the conservation of testate amoebae species. Eventhough this communication is only a part of the study under one phylum, Tubulinea the diversity is found to be high. This could be due to relatively undisturbed nature of the WLS which provides a suitable habitat for these microorganisms to thrive. Overall the study highlights the importance of conducting more research on testate amoebae in different regions of Mookambika WLS to better understand their distribution and ecological roles. This information can be valuable for conservation efforts and ecosystem management in these biodiverse regions.

Disclaimer (Artificial intelligence)

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

REFERENCES

Adl S. M., Simpson A. G. B., Lane C. E., Lukeš 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. e revised classification of eukaryotes. *Journal of Eukaryotic Microbiology*, **59**: 429-514.

Bindu. L. 2023.Diversity of testate amoebae (Amoebozoa and Cercozoa) in Western Ghats of Kerala with 8 new records to India, *Rec.zool.Surv. India*, **123**(2) :117-134.

Charman, D.J and Warner, B.G.1992. Relationship between testate amoebae (Protozoa : Rhizopoda)and microenvironmental parameters on a forested peatland in Northeastern Ontario. *Canadian Journal of Zoology*, **70** : 2474-2482.

Foissner, W. 1987. Soil Protozoa: Fundamental problems, ecological significance, adaptations in ciliates and testaceans, bioindicators and guide to the literature. *Progressive protistology*,**2**: 69-212.

Foissner, W. 1992. Estimating the species richness of soil protozoa using the 'non-flooded petri dish method' (J.J. Lee, and A.T. Soldo eds.).*Protocols in Protozoology* Allen Press, Lawrence, Kanas (pp. B-10.1-B-10.2).

Gilbert, D and Mitchell, E.A.D. 2006. Microbial diversity in sphagnum peatlands. In: Peatlands: *Evolution and records of Environmental and climatic changes*. Elsevier, Amsterdam. Pp. 289-320.

Mazei, Y.U., Blinokhvatov, Y.U. and Embulaeva, E. 2011. Specific features of the microspatial distribution of soil testate amoebae in theforests of the Middle Volga Region. *Arid Ecosystems*, **1**: 46-52.

Meisterfeld R. 2002a. Order Arcellinida. Lee J. J., Leedale G. F., Bradbury P. (eds.). *The Illustrated Guide to the Protozoa,* Society of Protozoologists, Lawrence, Kansas, USA. P. 827–860.

Meisterfeld R. 2002b. Testate amoebae with filopodia.Lee J. J., Leedale G. F., Bradbury P. (eds.). *The Illustrated Guide to the Protozoa*. Society of Protozoologists, Lawrence, Kansas, USA. P. 1054–1084.

Mitchell, E. A.D., Charman, D. J and Warner, B. G.2008. Testate amoebae analysis in ecological and paleoecological studies of wetlands; past, present and future. *Biodiversity and conservation*, **17**:2115-2137.

Netalkar, P.S.2010. Mookambika Wildlife Sanctuary, Kollur. Management Plan January 2010-December, 2015. Forest Department, Government of Karnataka, 74 pp.

Smith, H. G., Bobrov, A and Lara, E. 2009. Diversity and biogeography of testate amoebae. *Protist diversity and geographical distribution*, 95-109.

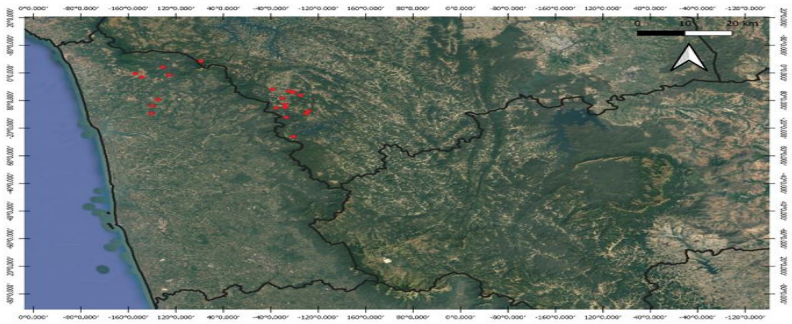

Fig.1 Collection localities in the Mookambika WLS

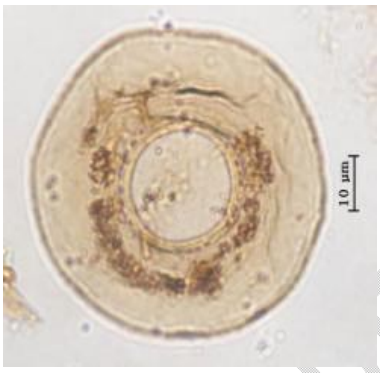

Image 1.*Galeripora discoides*(Ehrenberg, 1871) Gonzalez-Miguens *et al*., 2021

Image 2.*Galeripora catinus* (Penard, 1890)

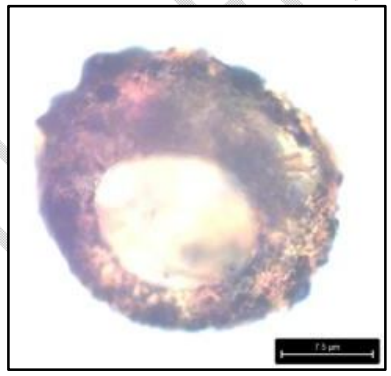

Image 3.*Cyclopyxis arcelloides* Deflandre, 1929

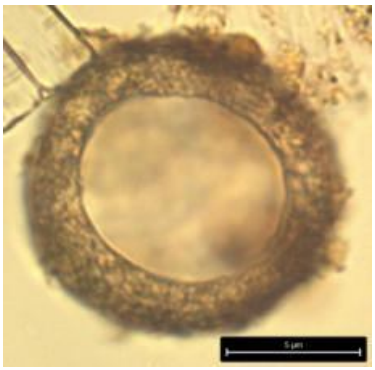

Image 4.*Cyclopyxis eurystoma* Deflandre, 1929

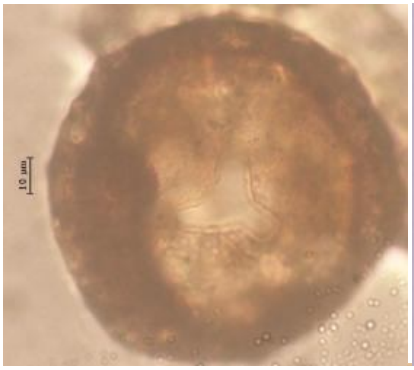

Image 5.*Trigonopyxis arcula* Penard, 1912

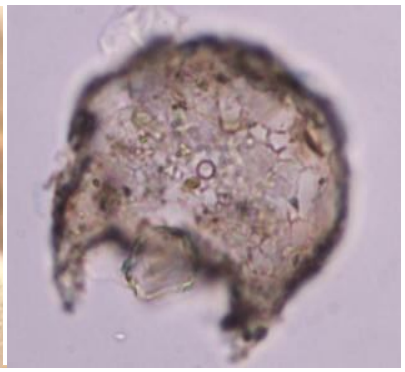

Image 6.*Difflugia corona* Wallich 1864

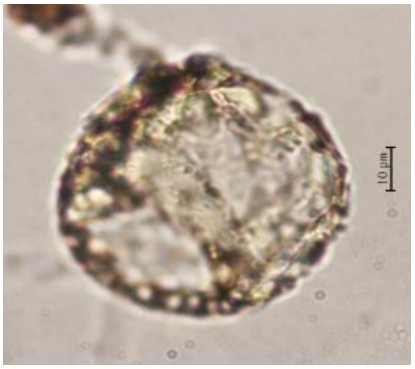

Image 7.*Difflugia globulosa* Dujardin, 1837

Image 8.*Difflugia oblonga* Ehrenberg, 1838

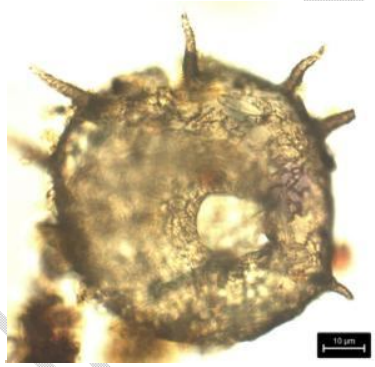

Image 9.*Centropyxis aculeata* Stein, 1857

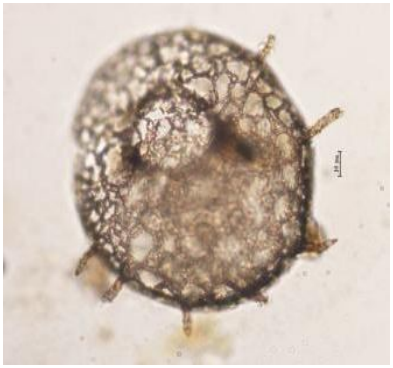

Image 10.*Centropyxis aculeata* grandis Deflandre, 1929

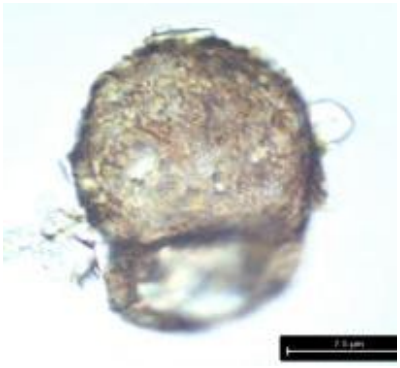

Image11.*Centropyxis aerophila* Deflandre, 1929

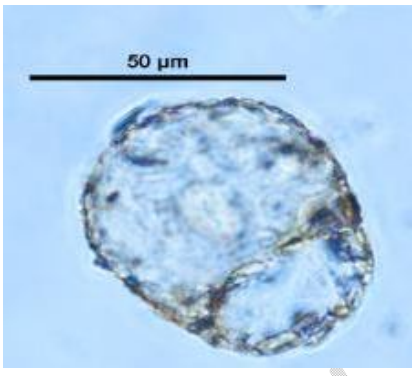

Image 12.*Centropyxis cassis* Deflandre, 1929

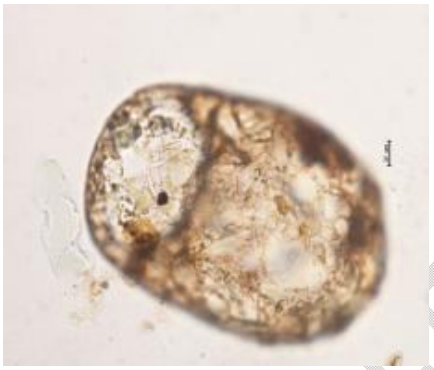

Image 13.*Centropyxis constricta* Penard, 1890

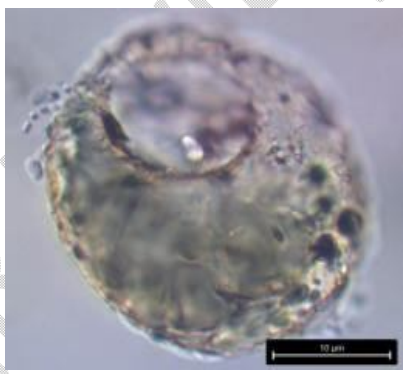

Image 14.*Centropyxis ecornis* Leidy, 1879

Image 15.*Centropyxis elongata* Thomas, 1959

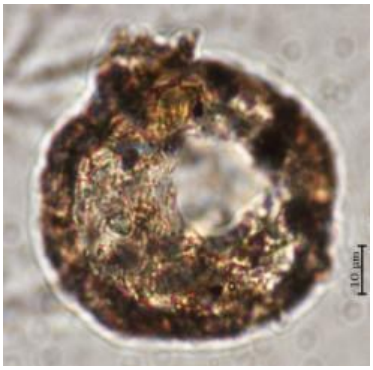

Image 16.*Centropyxis laevigata* Penard, 1890

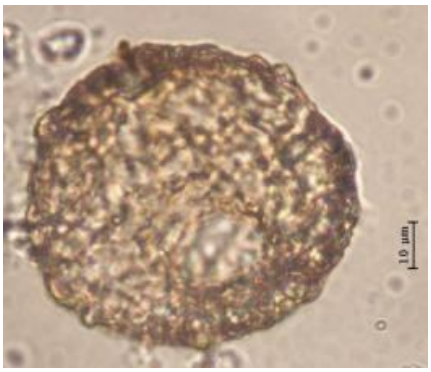

Image 17.*Centropyxis minuta* Deflandre, 1929

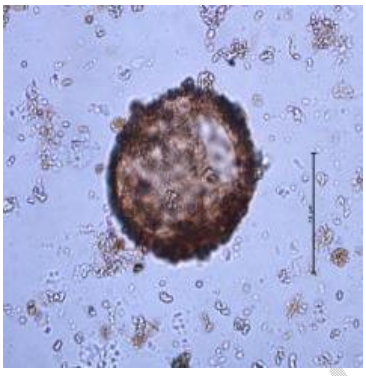

Image 18.*Centropyxis orbicularis* Deflandre, 1929

Image 19.*Centropyxis platystoma* Penard, 1890

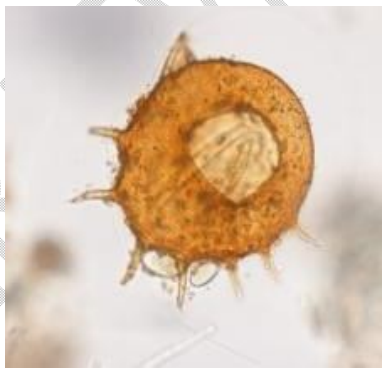

Image20.*Centropyxis spinosa* Cash, 1905

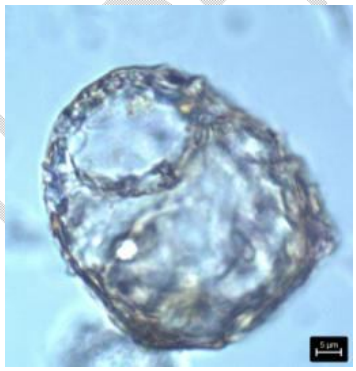

Image 21.*Centropyxis sylvatica* Bonnet and Thomas 1955

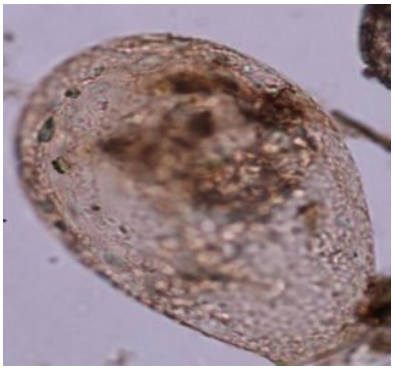

Image22.*Nebela bohemica* Taranek, 1882

Image23.*Padaungiella lageniformis* Penard, 1890

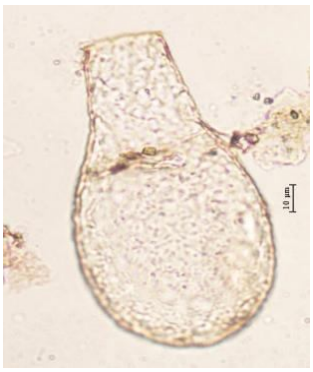

Image24.*Padaungiella wailesi* Deflandre, 1936

Image25.*Heleopera rosea* Penard, 1890

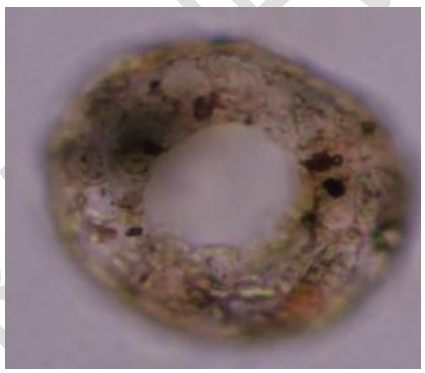

Image26.*Phryganella acropodia* Hopkinson, 1909