~~NOVEL RECORDS OF~~ TESTATE AMOEBAE (PROTOZOA: TUBULINEA) DIVERSITYIN~~FROM~~ MOOKAMBIKA WILDLIFE SANCTUARY, KARNATAKA, INDIA

ABSTRACT

Although lot of studies were done on testate amoebae in various parts of Western Ghats, so far no records from Karnataka part of Western Ghats and from Mookambika WLS as well. Accordingly, as part of the study programme of Zoological Survey of India, Kozhikode, Kerala a study to document the moss inhabitant testate diversity has been carried out. 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

Comment [AC1]: Abstract must be changed. Kindly check other similar papers for an idea.

Comment [AC2]: Don't repeat words from the title as "title' already acts as a key word.

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 (Smith *et al.*, 2009) and associated with terrestrial mosses are increasingly used in ecological and ecotoxicological studies. 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' N,74°39' E). It derives its name from the presiding deity "Goddess Mookambika" of the popular KollurMookambika Temple. The sanctuary lies ~~i~~along~~n~~ the ~~Western Ghats~~ Western Ghats Mountainin 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 MookambikaWLS which is the first study on t~~T~~estate amoebae in this sanctuary.~~The study resulted the records of 27 species of testate amoebae under 9 genera and 7 families. All the species are new records from the sanctuary as well as from Karnataka state~~.

Comment [AC3]: If using a abbreviation then need to mention before and this is not a very recognised abbreviation. SO better write the full form.

Comment [AC4]: This is the main result of the present study so why this sentence is in the introduction. This must be a part of "Results"

2. MATERIALS AND METHODS

The moss collection was done along the tree trunks, rocks and soils of Mookambika wildlife sanctuary, Karnataka. Moss samples (100 g) were collected by quadrant sampling methods and transferred to a polythene bag by scraping the mosses from the rocks and trees. These samples were collected during~~from~~ the survey conducted by Zoological Survey of India into~~o~~various parts of Mookambika~~WLS~~wildlife sanctuary during ~~the years~~ 2022 and 2023.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.~~,~~The samples are then thoroughly examined under a light microscope and the protozoans were isolated from the samples. After this the samples are ~~They are then~~ air-dried for 24 hours and then mounted with DPX for ~~.~~ making ~~10-15~~ permanent slides ~~mounts were prepared for each sample and~~which are then examined under the microscope for further identification. For species-level identification and further reference, photographs were taken with a Leica DM 2000 compound microscope with

Comment [AC5]: Mention which microscope model was used.

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LAS software version 3.6.0. 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.

3. 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 (~~Images are attached as~~ Annexure-1). All the species recorded here are new reports to MookambikaWLS 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 (Fig.1).

Comment [AC7]: Why this plate/fig is considered as a Annexure. I think this must be a part of the main paper since the aim of the paper is to understand the diversity.

Comment [AC8]: Full and check throughout the MS

SYSTEMATIC ACCOUNT

Comment [AC9]: I think it will be better when you mention different species after the distribution the ecological affinity of that species may be included so that this study may be used further to understand the utility of testate amoebae in ecological studies as mentioned in the "introduction"

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

Family Netzeliidae Kosakyan*et al.*, 2016

3. *Cyclopyxisarcelloides*Deflandre, 1929

Distribution :~~India: -~~Andhra Pradesh, Arunachal Pradesh, Himachal Pradesh, Kerala, Manipur, Meghalaya, Mizoram, Odisha, Sikkim, Tamil Nadu, Uttar Pradesh, Uttarakhand, West Bengal, Chandigarh

4. *Cyclopyxiseurystoma*Deflandre, 1929

   Distribution :~~India: -~~Arunachal Pradesh, Assam, Himachal Pradesh, Kerala, Maharashtra, Nagaland, Tamil Nadu, Telangana, Uttarakhand, West Bengal

   *Incertaesedis*Arcellinida

5. *Trigonopyxisarcula*Penard, 1912

   Distribution: ~~India : -~~Assam, Himachal Pradesh , Maharashtra, Manipur , Sikkim, West Bengal

   Family Difflugiidae Wallich, 1864

6. *Difflugia corona* Wallich, 1864

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

7. *Difflugiaglobulosa* Dujardin, 1837

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

8. *Difflugia oblonga* Ehrenberg, 1838

   Distribution :~~India: -~~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. *Centropyxisaerophila*Deflandre, 1929

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

12. *Centropyxisaerophilasphagnicola*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. *Centropyxisconstricta* (Ehrenberg, 1841) Penard, 1890

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

15. *Centropyxisecornis* Leidy, 1879

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

16. *Centropyxiselongata* (Penard, 1890) Thomas, 1959

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

17. *Centropyxis laevigata*Penard, 1890

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

18. *Centropyxisminuta*Deflandre, 1929

    Distribution : India :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. *Centropyxisplatystoma*Penard, 1890

    Distribution : India : 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 : India : 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. *Nebelabohemica*Taranek, 1882

    Distribution : Arunachal Pradesh, Himachal Pradesh, Sikkim

24. *Padaungiellalageniformis*Penard, 1890

    Distribution: Himachal Pradesh, Uttarakhand

25. *Padaungiellawailesi*Deflandre, 1936

    Distribution : India : 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.

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.

Comment [AC10]: Several references are missing, all the author citations of the 27 species, systematic account must be included in the reference section.

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.

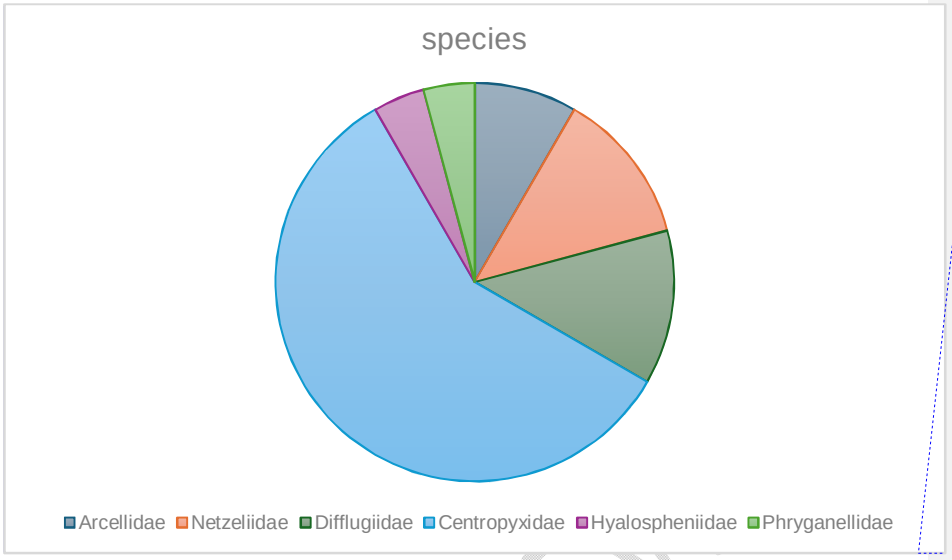
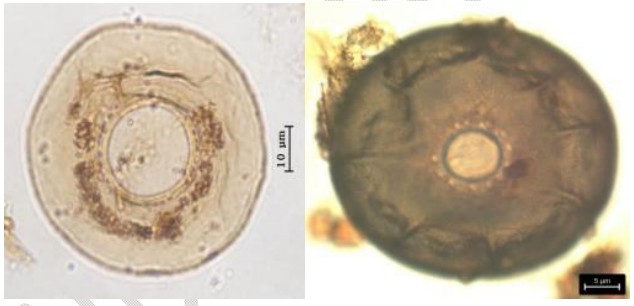
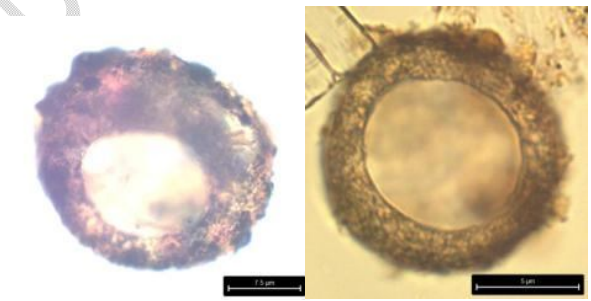


Fig.1.Distribution of species under various families

Annexure-1



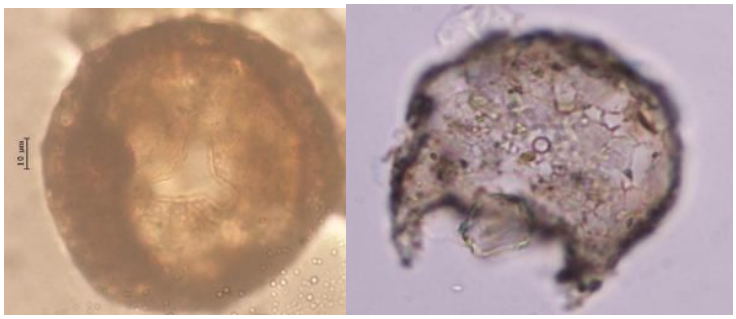
Galeriporadiscoides(Ehrenberg, 1871)*Galeripora catinus* (Penard, 1890)
Gonzalez-Miguens*et al*., 2021



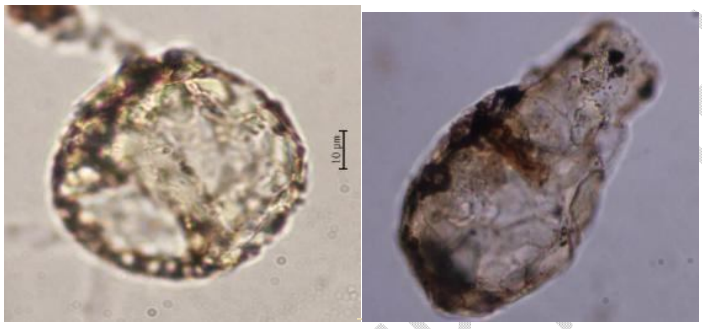
*Cyclopyxisarcelloides*Deflandre, 1929*Cyclopyxiseurystoma*Deflandre, 1929

Comment [AC11]: In the MS it was mentioned 7 families identified but in the current figure it shows only six families

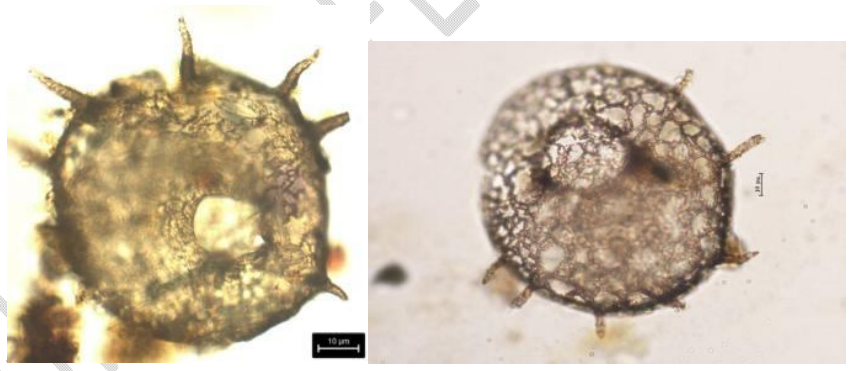
Comment [AC12]: Comments:
The photographs of the species must be arranged in a plate like format with visible scale in each species, the photographs must also have a numbering system. This plate/figure detail captions must be added at the end. The current form is not acceptable as this is submitted in a well reputed journal and to increase the quality of the paper this is needed. Check other published papers on the distribution of species.
Arrange the orientation of the species and be confirmed that those are not stretched.



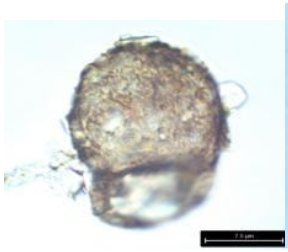
*Trigonopyxisarcula*Penard, 1912*Difflugia corona* Wallich 1864

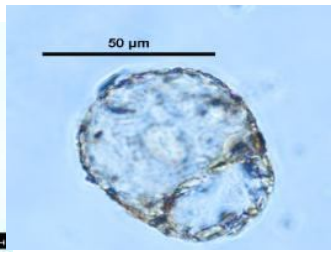


*Difflugiaglobulosa*Dujardin, 1837*Difflugia oblonga* Ehrenberg, 1838

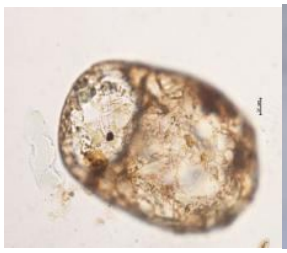


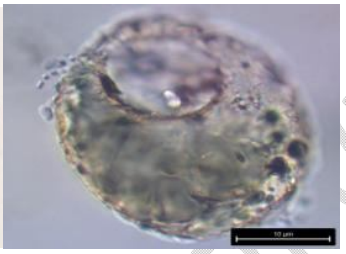
Centropyxis aculeata Stein, 1857*Centropyxis aculeata* grandis Deflandre, 1929



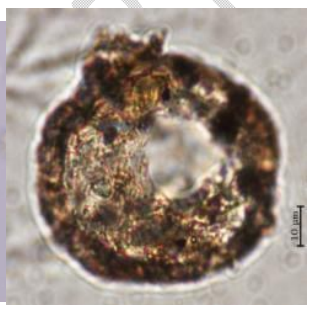


*Centropyxisaerophila*Deflandre, 1929*Centropyxis cassis*Deflandre, 1929

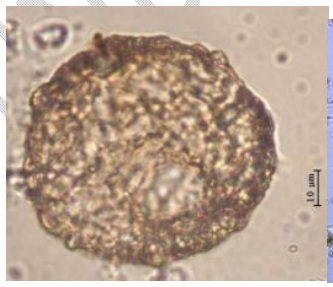


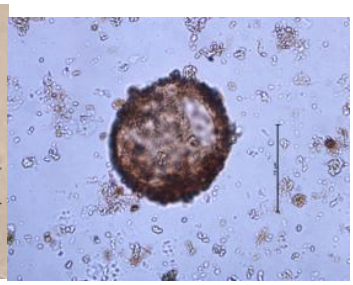


*Centropyxisconstricta*Penard, 1890 *Centropyxisecornis* Leidy, 1879

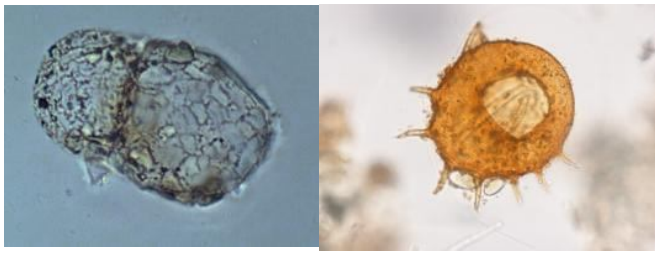


Centropyxiselongata Thomas, 1959*Centropyxis laevigata*Penard, 1890

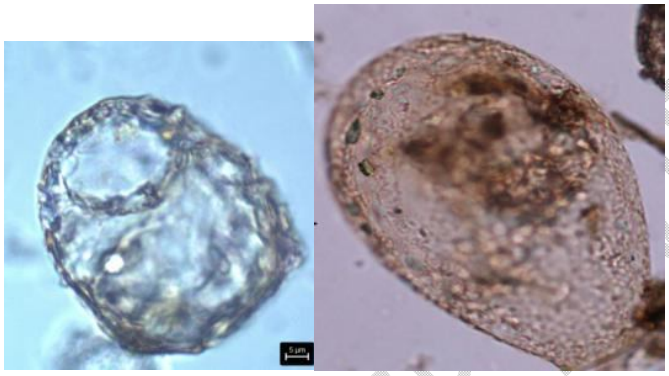




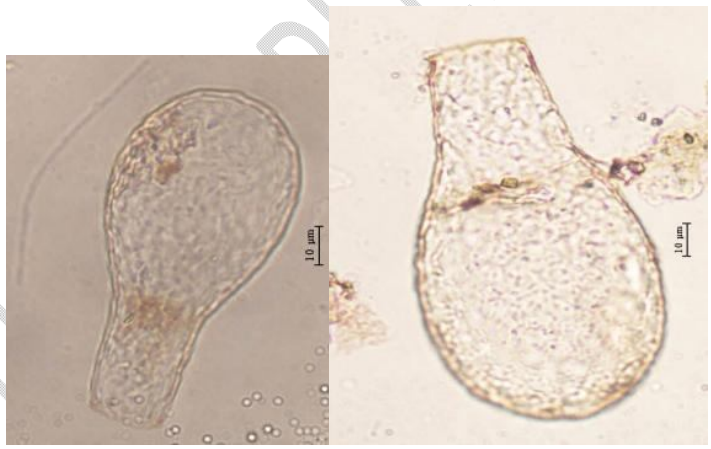
*Centropyxisminuta*Deflandre, 1929*Centropyxis orbicularis*Deflandre, 1929



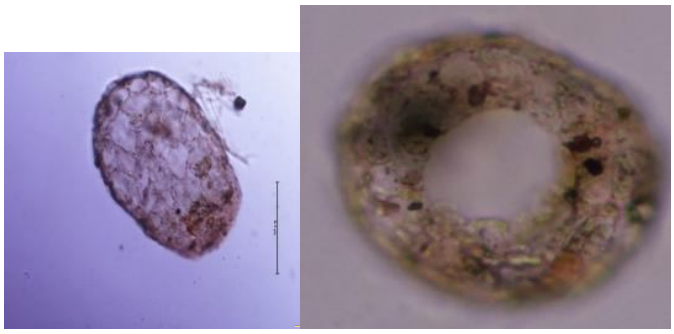
*Centropyxisplatystoma*Penard, 1890*Centropyxis spinosa* Cash, 1905



Centropyxis sylvatica Bonnet and Thomas 1955*NebelabohemicaT*aranek, 1882



PadaungiellalageniformisPenard, 1890 *Padaungiellawailesi*Deflandre, 1936



*Heleopera rosea*Penard, 1890*Phryganella acropodia* Hopkinson, 1909