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 are excellent environmental bioindicators, and 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 resulted in 16 testate amoebae species from 6 genera and 6 families. This research expands the 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 wetlands.

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. According to recorded statistics, 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 has 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, 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 both sites above. The conversion of the Nagi River into the Nagi Dam paved the way for the man-made wetland, Nagi Bird Sanctuary (24°49'N 86°24'E) spread across 205.8 hectares. Different environmental surfaces including, the bark of trees and rocks and old walls, soil, and rocks support the widespread growth of mosses. The importance of mosses in Indian Ramsar wetlands must 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. Nonvascular 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 environmental ecological changes [2,3]. Testate amoebae reside in every worldwide zone from the tropical to polar regions,and also occur 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 with other environmental factors making 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 indicate 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 examiningspecies that thrive in acidic and neutral conditions [7].

Testate amoebae are present in large quantities and high densities in Sphagnum biotopes and play essential roles in the microbial loop, nutrient cycling, and ecosystem processes [8]. Environmental moisture dramatically 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 taxonomic status of the taxa. This molecular study shows that testate amoebae comprise a polyphyletic group of at least three unicellular eukaryotic taxonomic groups of Amoebozoa, Stramenopiles, and Cercozoa [11,12].

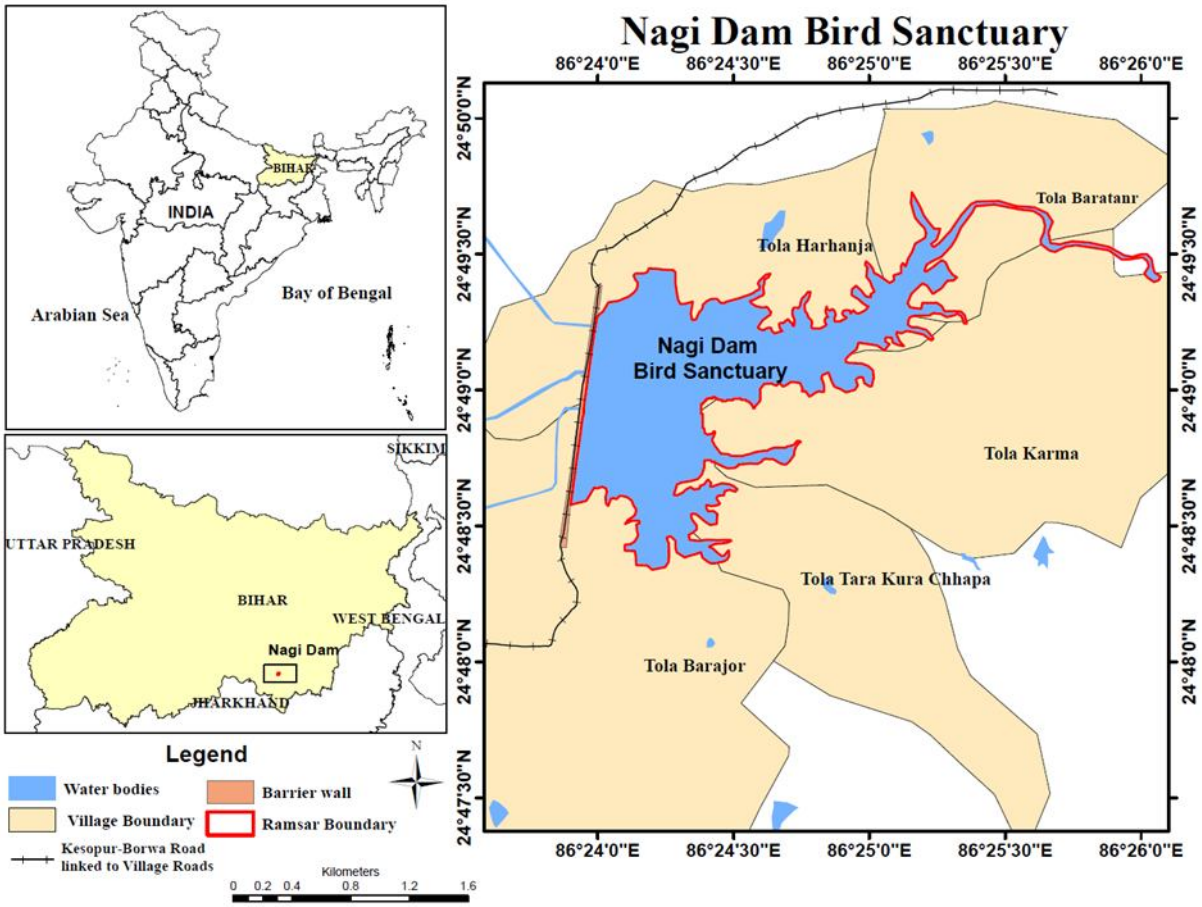
On the other hand, despite being one of the most biodiverse regions of India, research on protozoan diversity, particularly free-living protozoan species like testate amoebae, remains unexplored mainlywith many gap areas [13], with only a few recent studies reported in the literature in the Bihar state [14,15]. Notably, no research have 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, this study presents 16 species, belonging to 6 genera, and 6 families. Furthermore, given the high level of pollution in Bihar, it is important to mention that researchers consider testate amoebae as sensitive organisms capable of reflecting environmental changes, which is why they serve as bioindicators of the ecological state. Future work on the protozoan taxonomy could be very useful in monitoring and managing 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 that are classified under two classes and two orders [13]. Novel record of 16 testate amoebae species from six genera and six 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 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 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 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

During this preliminary investigation, the study yielded the following 16 species of testate amoebae span over 6 genera and 6 familiesfrom 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 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, Bihar, Himachal Pradesh, Kerala, Manipur, Meghalaya, Mizoram, Odisha, Sikkim, Tamil Nadu, Uttar Pradesh, Uttarakhand, West Bengal, Chandigarh, Punjab.

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

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

Distribution: India: Uttarakhand, Punjab

Remarks: New record from Bihar

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, Punjab

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

1929. Centropyxis (Cyclopyxis) kahli Deflandre, 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 Difflugiidae Wallich, 1864

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

Family Centropyxidae Jung, 1942

7. ***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

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

1929. *Centropyxis platystoma* 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 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: 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. ***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, Punjab

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

1871. Difflugia strigosa Ehrenberg, Nacthrag zur Übersicht der organischen Atmosphärilien. Abhandlungen der Königliche Akademie der Wissenshaften zu Berlin (1871), p. 233-275, Pl. 3.

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

Family Trinematidae Adl et al., 2012

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.

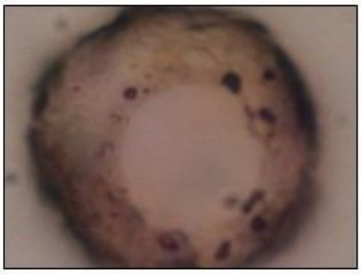
Distribution: India: Andhra Pradesh, Arunachal Pradesh, Assam, Bihar, Himachal Pradesh, Manipur, Meghalaya, Mizoram, Nagaland, Odisha, Tripura, Uttarpradesh, Uttarakhand, Chandigarh, Punjab

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

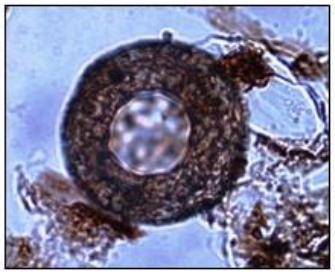
1890. *Trinema complanatum* Penard, *Mem. Soc. Geneve*, 31: 187, pl.10, figs. 1-4.

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

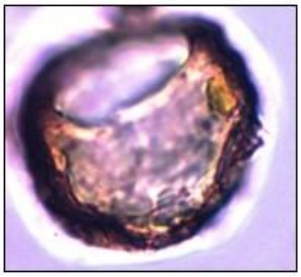
Plate 1



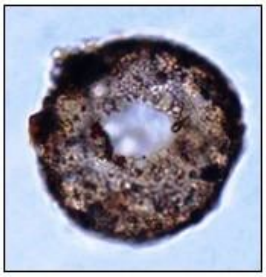
1. Cyclopyxis arcelloides
(Penard, 1902) Deflandre, 1929



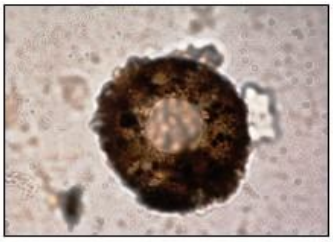
2. Cyclopyxis aplanata
(Penard, 1911) Deflandre, 1929



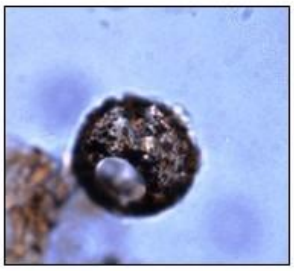
3. Cyclopyxis eurystoma
Deflandre, 1929



4. Cyclopyxis kahli
Deflandre, 1929

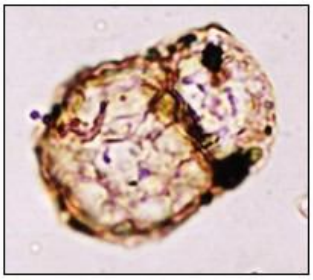


5. Phryganella acropodia
(Hertwig & Lesser, 1874)

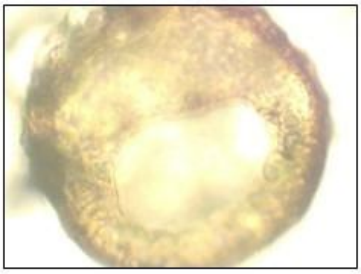


6. Difflugia globulosa
(Dujardin, 1837) Penard, 1902

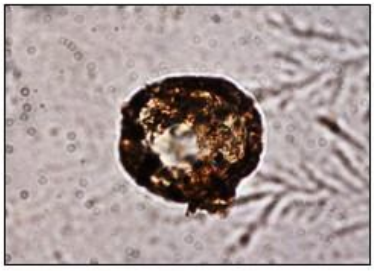
Plate 2



7. Centropyxis aerophila
Deflandre, 1929



8. Centropyxis ecornis
(Ehrenberg, 1841)



9. Centropyxis laevigata
Penard, 1890

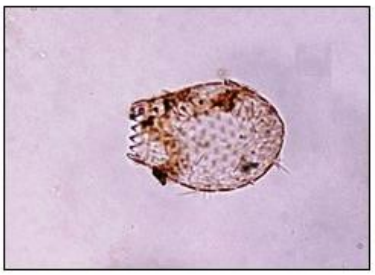
10. Centropyxis platystoma
(Penard, 1890) Deflandre, 1929

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. DISCUSSION

Testate amoebae as single-celled protists spread across different ecosystems where they reside within wildlife sanctuaries (WLS), national parks and wetlands. Research findings demonstrate that testate amoebae hold diverse populations in these habitats and establish them as critical indicators to monitor environmental well-being. Very recent findings in the Bhimbandh,WLS reported records of this microfauna within the sanctuary ecosystem [14,15]. Similarly reports of 28 previously undocumented testate amoeba species in Shenthuriney WLS, Kerala [17]. The testate amoebae hold a crucial position in wetland ecosystems while earning status as sensitive bioindicator organisms. Studies in the Nainital Lake region of Uttarakhand recently identified 39 testate amoeba species across 11 genera and 8 families which had never been documented in that area thus demonstrating their value as environmental condition monitors and highlighting the importance of habitat protection [18].The collection of these investigations establishes the crucial value of testate amoebae in bioindication studies. Scientific researchers utilize these organisms as cohort indicators because of their capability to respond to ecosystem changes which allows them to assess ecological health and track pollution levels. The assessment of testate amoebae communities enables scientists to understand habitat ecological conditions and detect pre-ecological degradation symptoms.

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 investigation, 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 completed on mosses and their testate amoebal associations. This substantial knowledge gap emphasizes the critical need for further investigations to understand the ecological processes of these important habitats fully. Testate amoebae, since they respond sensitivelyto changes in hydrological and nutrient regimes, are key to understanding 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 essential insights into wetland ecosystem dynamics and help the conservation activities. Furthermore, such investigations may be helpful intracking trends in wetland health, assuring the careful and continued management, and protection of these valuable ecosystems.

5. CONCLUSION

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. The present article outcome is based on a preliminarystudy from the Nagi Bird Sanctuary. The presence of 16 testate amoebae species from 6 genera and 6 families was confirmed through this study,since only limited research has been carried out to date, particularly in India's Ramsar sites, there is an urgent need for larger-scale research to tap into the full capabilities of these ecosystemsand to ensure the ecological integrity of these ecosystems for future generations.

Disclaimer (Artificial intelligence)

Option 1:

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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