

Diversity Analysis of Moss-Inhabiting Amoebae from North and North-East India

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ABSTRACT

The present work is designed to study biodiversity of moss dwelling testate amoeba from 12 states of North and North-East India covering 6 biotopes. This study analyses the data, which were earlier published by Chattopadhyay and Das (2003), hypothesizing that occurrence of testate amoeba is dependent on its microhabitat i.e. biotope and moss genus. In the present analysis 5 biotopes namely soil, wall, rock, tree and tree-associate were considered. Different similarity measures (Sørensen's similarity index and Bray-Curtis similarity matrix) reveal that testate amoebae of soil, rock and wall biotopes are closely similar, while rock and tree-associate biotopes exhibit minimum similarity. It is found that ~13% of testate amoeba species were recorded from five biotopes whereas 36% were registered from single biotope. It is further analysed that 63.2% species of testaceans can colonise a wide range of moss genera. This shows that association of testate amoeba and moss genera are not specific but random. Diversity index is highest in rock biotope, while evenness measure is highest in tree-associates and minimum in rock biotope. The rock biotope forms a separate cluster in the multivariate analysis that shows maximum similarity level (73.5%) between soil and wall. It can be inferred from the present analysis that among 27 moss genera, Fissidens seems to be the most preferred habitat and the rock biotope provides ideal habitat condition for both moss genera and testaceans.

Key words: Biodiversity, Biotope, Bootstrap, Moss, Multivariate analysis, Testate amoebae.

INTRODUCTION

Testate amoebae (Super Class Rhizopoda; Class Lobosea and Filosea) are characterized by the presence of tests that cover the entire body except the pseudopodia. These amoebae inhabit the soil, moist places, and also in association with mosses. Mosses belong to the Division Bryophyta and Class Bryopsida and grow on soil, rock, wall and barks of trees in humid environment. In India, they are mostly found in Himalayas, Western Ghats and other hilly areas. They provide suitable microhabitat by providing insulation against heat, cold and wind, particularly for free living protozoa of which testate amoebae are most abundant. Reports on moss dwelling testate amoebae from India are very scanty (Penard 1907, Nair and Mukherjee 1968a, b). Chattopadhyay and Das (2003) studied the taxonomy and ecology of moss dwelling testaceans from North and North-East India from 12 states covering 6

biotopes and 7 biotic provinces. They collected testate amoebae from biotopes, viz., soil, rock, wall, tree moss, tree moss interspersed with lichen, and tree fern mosaic. In the present work, the last two biotopes are combined together as a single biotope, the fifth one. Chattopadhyay and Das (2003) had generated huge amount of data, which initiated the present authors to analyse the biodiversity of these moss dwelling testaceans.

Ecology of moss-inhabiting testate amoeba was studied in many parts of the globe considering different physical parameters and moss type (Bobrov et al. 1999 2002, Chatterjee et al. 2000, Finlay et al. 2001, Robinson et al. 2002, Mitchell et al. 2004, Kishaba and Mitchell 2005, Lamentowicz and Mitchell 2005). These studies were concentrated on the distribution pattern of testate amoeba in relation to climate, pH, depth of water, and moss species. Nguyen et al. (2004) suggested that testate amoeba might be considered as potential biomonitors for atmospheric pollution.

Based on the data of Chattopadhyay and Das (2003), the present work is designed to study the biodiversity of testate amoebae collected from 27 genera of moss inhabiting five biotopes. The data so far recorded by Chattopadhyay and Das (2003) is analysed using a number of diversity indices, species richness and multivariate analysis. The hypothesis of the present analysis is that occurrence of testate amoeba is dependent on its microhabitat i.e., biotope and moss genus.

MATERIALS AND METHODS

Present paper analysed the data, which were presented by Chattopadhyay and Das (2003). The authors have collected 118 moss samples pertaining to soil mosses, rock mosses, wall mosses, tree mosses, and tree associated mosses from 12 states of North and North-East India, namely, Jammu and Kashmir, Himachal Pradesh, Uttaranchal, Sikkim, West Bengal, Arunachal Pradesh, Assam, Nagaland, Manipur, Mizoram, Meghalaya and Tripura. They have also recorded the details of moss types, testacean species, and pH of the habitat. The samples were isolated from habitat by scraping with the help of a spatula. Quadrat sampling (1m^2) of mosses was done through 8 to 10 sub-samples (Chattopadhyay and Das 2003). The sub-samples were pooled and subsequently analysed for species richness. The present work analysed the above data to unravel the diversity of moss inhabiting testate amoebae from 5 biotopes. However, biotopes V (tree moss interspersed with lichen) and VI (tree moss and tree fern mosaic) of Chattopadhyay and Das (2003) are here combined together because of somewhat similar habitats and of sample size, and henceforth referred to as biotope Tree moss associates.

Analysis carried out with the help of different diversity measures and indices like, Simpson's diversity, Reciprocal Simpson's diversity, Shannon-Wiener diversity, Brillouin diversity (Krebs 1999) and evenness measures. Occurrence of 76 species under 20 genera in different biotopes was compared by means of Sørensen's index of similarity (Sørensen 1948) and Bray-Curtis measure (Bray and Curtis 1957). Cluster analysis also carried out to separate cluster of similar biotopes from the dissimilar groups. The output of cluster analysis consists of a dendrogram, which shows the relationships between all biotopes. Cluster analysis was done using MINITAB 13.1 programme. Jackknife method of Heltshe and Forrester (1983) is also carried

out to estimate species richness to comment on the sampling and on the observed number of species.

RESULTS AND DISCUSSION

The survey data on moss inhabiting testate amoebae by Chattopadhyay and Das (2003) were analysed variously to address the biodiversity of this group of protozoa.

The biotope-moss preference seems to be most important aspect with regard to the diversity of testate amoebae. In the present analysis 27 genera of mosses housed 76 species of testate amoebae under 20 genera (Table 1). The rock and wall shared 6 common genera of mosses, rock and soil 3 genera, soil and wall had three common genera, and rock-wall-soil shared two genera of mosses, while rock only provided habitat to 8 genera of mosses, and wall and soil were the habitat of 3 common moss genera. Moss genus *Homomaliium* was found only on rock and wall and the rocks harboured maximum 12 genera covering 28 species of testate amoebae, while the wall biotope sheltered 5 genera of testate amoebae of which three are common to both the biotopes. The moss genus *Fissidens* harboured 17 genera (44 species) of testate amoebae that were collected from rock biotope, but wall and soil had 5 and 3 genera of amoebae respectively, of which 3 are common to three biotopes. This shows that the association of testate amoebae and moss is not highly specific. However, Sørensen's similarity index (Table 2) reveals that the testate amoebae of the biotopes soil-rock and soil-wall were closely similar (0.67), while minimum similarity was found between rock and tree associated biotopes (0.32). Bray-Curtis measure of similarity matrix (Table 3) also exhibits similar trends to that of Sørensen's index. The results of other similarity indices like Canberra matrix, Horn index and Percent similarity matrix vary little, but minimum similarity remains same

Table 2. Sørensen's Similarity Index of testate amoebae from different biotopes

| | Soil | Rock | Wall | Tree | Tree-A |
|--------|-------|-------|-------|-------|--------|
| Soil | 1.0 | | | | |
| Rock | 0.667 | 1.0 | | | |
| Wall | 0.667 | 0.614 | 1.0 | | |
| Tree | 0.622 | 0.590 | 0.583 | 1.0 | |
| Tree-A | 0.560 | 0.370 | 0.500 | 0.571 | 1.0 |

Table 3. Similarity matrix (Bray-Curtis measure) of testate amoebae from 5 different biotopes

| | Soil | Rock | Wall | Tree | Tree-A |
|--------|------|------|------|------|--------|
| Soil | 1.0 | | | | |
| Rock | 0.67 | 1.0 | | | |
| Wall | 0.67 | 0.62 | 1.0 | | |
| Tree | 0.65 | 0.59 | 0.58 | 1.0 | |
| Tree-A | 0.56 | 0.37 | 0.50 | 0.57 | 1.0 |

between rock and tree associated biotopes. It can be inferred that the diversity pattern of testate amoebae is dependent neither on moss genus nor biotopes.

Charman (1997), who analysed the assemblage of testate amoebae from thirteen peatlands in New Zealand, concluded that among different hydrological factors, water table and soil moisture seemed to be the most dominant factors. Chatterjee et al. (2000) commented that similarity or dissimilarity of species structure of testate amoebae from various sites in Antarctica might be influenced by physical parameters. They remarked that although the assemblages of testate amoebae were not dependent upon any one single physical parameter, pH of water seems to be the most important one. However, the pH variations were also not in conformity with the similarity of amoebae in our study (Chattopadhyay and Das 2003). Testate amoebae are reported to be passively sensitive to temperature fluctuation (Charman and Warner 1992, Tolonen et al. 1994). Balik (1991) studied the effect of road traffic pollution on the communities of testate amoebae and commented that a decrease in abundance, species richness and species diversity of testaceans were related with the atmospheric pollution. Mitchell et al. (2004) inferred that the variations in species richness of testate amoebae communities in the moss, *H. splendens* from southeastern Alps of Italy, are influenced by moss tissue chemistry.

Among the 20 genera of amoebae collected by Chatterjee et al. (2000), *Centropyxis* was recorded from all 27 moss genera, followed by *Euglypha* from 25 moss genera and *Trinema* occurring on 20 moss genera. Three genera of amoebae, namely, *Cryptocorythion*, *Cyphoderia* and *Phryganella* are recorded from only one moss genus. Out of these 20 genera of amoebae, 7 genera covering 10 species were collected from all 5 biotopes (rock, wall, soil, tree and tree associates) inhabiting on 7 to 23 moss genera. There are three species of the genus

Centropyxis and 2 of genus *Euglypha* and 1 each of the genera *Assulina*, *Corythion*, *Cyclopyxis*, *Tracheleuglypha* and *Trinema*. *Assulina muscorum* is collected only from 7 moss genera, while the other 9 species, were recorded from more than 12 moss genera. It may be concluded that almost 13% (10 out of 76) of all testate amoebae exhibit no specific biotope choice. Percent occurrence of amoebae in five biotopes is calculated as follows: 13.2%, of amoebae prefer 5 biotopes, 13.2% for 4 biotopes, 17% for 3, 19.7% for 2 and 36.8% for a single biotope. This observation shows that 63.2% species of testate amoebae can colonise a wide range of moss genera as well as biotopes.

Bonnet (1973) recorded a total of 49 species of testate amoebae from 13 different moss species, while Mitchell et al. (2004), observed 25 species from a single moss species, *Hylocomium splendens*. The study of Bonnet (1973) has revealed that there is no specific moss preference shown by the amoebae. (Rather elemental chemistry of moss may be a factor in determining species abundance and moss specificity of these amoebae.) In the present observation maximum of 44 species of amoebae has been recorded from *Fissidens* sp. occurring on rock, wall and soil. It has been further observed that among these 44 testaceans 31 species occur exclusively on rock and 2 species found only on wall, while remaining 11 species occur among the rest 3 biotopes. Of these 31 testaceans occurring on rock, 6 species inhabit exclusively the *Fissidens* sp. It may be inferred that among the 27 moss genera, *Fissidens* seems to be the most preferred habitat for the amoebae. It is also noted that moss genus *Thuidium* harbours 7 species of testate amoebae, which occur exclusively in tree biotopes. On the other hand, *Cyphoderia ampulla* is collected only from the moss *Stereophyllum*, occurring in the rock biotope.

Previous studies have indicated that the numbers of amoeba species that colonise on the moss are remarkably variable. Nguyen et al. (2004) recorded 9 species of amoebae from *Tortula ruralis* growing on different kind of substrates. The number of amoeba species so far recorded from other parts of the World, ranges from 9 to 53, but this includes several moss species (Beyens and Chardez 1994, Beyens et al. 1986 1990, Todorv and Golemansky 1996 1999, Van Kerckvoorde et al. 2000). Bobrov (2003) studied the micro-range of soil testaceans and showed that the Morisita-Horn index of similarity of index differs between the top soil and enzymatic layer of litter.

The estimated species richness of Jackknife against the observed number 76 is 98.4 (Table 4). The richness

Table 4. Jackknife estimate of species richness of moss dwelling testate amoebae from 5 biotopes

| | |
|--|-------------|
| Observed Number of Species | 76 |
| Estimated Species Richness | 98.4 |
| Standard Deviation of Species Richness | 13.72 |
| 95% Confidence Limits | 60.3! 136.5 |
| No. of Unique Species | 28 |

indices show that the estimate between observed and expected is very wide at the species level.

According to Heltshel and Forrester (1983), Jackknife estimate of species richness tends to over-estimate due to a positive bias. However, Palmer (1990) considered this method as most accurate estimator. According to Krebs (1999), communities with large number of rare species and with low sampling number should not be analysed by Jackknife estimate. In the present study the sample size is large enough (118) to be analysed by this method. The unique species estimated in Jackknife is 28 (28.45%). The number of unique species for microorganisms is always expected to be higher than that of the macro-organism. This may be ascribed to the inherent difficulty in isolation and identification of microorganisms. Jana et al. (2006) have analysed insect fauna using Jackknife estimate and have justified its application in case of macro-organisms.

It is previously said that highest number of amoeba species (44) is recorded from the moss genus *Fissidens*, followed by *Thuidium* (42), and *Homomalium*

and *Entodon* 28 each. The minimum number of amoeba species is found in *Funaria* (3) and *Grinimia* (3).

Fifteen species of *Centropyxis* are recorded in all the 27 moss genera of which *C. minuta* is associated with 23 moss genera and all 5 biotopes, except in *Funaria*, *Stereophyllum*, *Brotherella* and *Enthostoneutans*. Among the 11 species of *Euglypha*, *E. tuberculata* inhabits 19 moss genera followed by *E. rotunda* (18). Thus *C. minuta* exhibits wider habitat preference among all amoeba species of the present study.

Result of Bootstrap analysis (Table 5) shows that among the five biotopes, Simpson's diversity index (1-D) and (1/D) is highest in rock followed by tree, wall, soil and tree associate biotopes. While this sequence is rock, soil, tree, wall and tree associate for Shannon-Wiener (H') and rock, tree, soil, wall and tree associate for Brillouin Diversity. Rock harbours 16 moss genera, where 65 amoeba species are associated. Thus, a higher diversity index for rock is obvious. The diversity index for tree associated biotope supports Routledge's (1980) contention. Pielou (1966) prefers Brillouin index to the other indices for finite and non-replaced population. As the present sampling follows Pielou's criteria, so Brillouin's index may be preferred to the other indices. The findings of Majumdar (2004) on the carrion flies, shows highest diversity value for a single site, where the sample sizes are equal, non replaced and finite.

The results of the four evenness measures (Table 6) are found highest in tree associate biotope and the minimum in rock biotope. Smith and Wilson's evenness measure as well as modified Nee et al. (1992) measure also shows maximum evenness for tree associates, while minimum in rock. The tree associate biotope harbours

Table 5. Bootstrap estimate of moss inhabiting testate amoebae from 5 biotopes.

| | Soil | Rock | Wall | Tree | Tree-A |
|---|--------------|---------------|--------------|--------------|--------------|
| No. of Genera | 13 | 19 | 13 | 12 | 9 |
| No. of Species | 34 | 65 | 32 | 40 | 16 |
| Simpson Diversity Measure (1-D) | 0.848 | 0.893 | 0.851 | 0.853 | 0.826 |
| | 0.756-0.907 | 0.85-0.925 | 0.758-0.909 | 0.795-0.897 | 0.675-0.917 |
| Reciprocal of Simpson Diversity Measure (1/D) | 5.886 | 8.489 | 5.928 | 6.102 | 4.702 |
| | 3.905-8.141 | 6.259-10.974 | 4.063-8.000 | 4.598-7.767 | 2.977-6.737 |
| Shannon-Wiener Diversity (H') | 2.897 | 3.477 | 2.862 | 2.884 | 2.422 |
| | 2.452-3.278 | 3.152-3.761 | 2.448-3.225 | 2.518-3.200 | 1.802-2.906 |
| Number of Equally Common Genera [N(1)] | 7.542 | 11.216 | 7.356 | 7.451 | 5.472 |
| | 5.585-9.554 | 9.056-12.415 | 5.580-9.204 | 5.827-9.080 | 3.747-7.252 |
| Brillouin Diversity (H) | 2.397 | 3.019 | 2.362 | 2.447 | 1.849 |
| | 2.037-2.696 | 2.749-3.251 | 2.029-2.640 | 2.149-2.700 | 1.424-2.193 |

Table 6. Evenness measures of moss inhabiting testate amoebae from 5 biotopes

| | Soil | Rock | Wall | Tree | Tree-A |
|----------------------------------|-------|-------|-------|-------|--------|
| Simpson Diversity Measure (1/D) | 0.517 | 0.497 | 0.547 | 0.585 | 0.677 |
| Camargo' Measure | 0.566 | 0.54 | 0.587 | 0.571 | 0.681 |
| Smith and Wilson Evar measure | 0.686 | 0.642 | 0.71 | 0.64 | 0.796 |
| Modified Nee et al. E(Q) measure | 0.219 | 0.211 | 227 | 0.212 | 0.249 |

only one moss genus (*Thuidium* sp.), which houses 16 species of amoeba. This may be the plausible reason of higher evenness index in this biotope, since there is no other alternative habitat choice for amoeba species.

The multivariate analysis considering amoeba species against biotopes shows four distinct clusters (Figure 1). The maximum similarity level (73.54) is observed between soil and wall biotopes. The rock biotope forms a separate cluster with a similarity of 46.34% with the other biotopes and it is substantiated by the results obtained through Bootstrap estimates of all diversity indices. The greater similarity between soil and wall biotopes may be due to the fact that both these biotopes provide similar habitat conditions to the moss genera and consequently to the amoeba species.

Some moss harbours a large number of amoeba species, while some has minimum number. This discrepancy in the distribution of amoeba species on moss genera is the reason for minimum evenness.

Moss requires minimum soil for their colonisation and growth unlike higher plants. Therefore, rock provides ideal habitat conditions for the moss species to grow. In the present study, among the five biotopes, rock harbours the maximum numbers of moss genera (16), followed by wall (10) and soil (7). The low number of moss genus in the soil biotope could be attributed to the stiff competition faced by them with the higher plants in which the latter always win.

Cluster analysis between amoeba and moss genera reveals 11 clusters with varying linkage distance (Figure 2). It is evident from this study that *Phryganella* and *Centropyxis* together form a distinct cluster. Likewise, minimum linkage distance is found among the 6 genera, *Trinema*, *Awerintzewia*, *Nebela*, *Heleopera*, *Euglypha*, *Assulina*. Similarly, *Cyphoderia*, *Plagiopyxis*, *Trachel-euglypha* and *Cryptocorythion* also form another distinct cluster showing minimum linkage distance. All these 11 clusters express a community relationship among the different genera of testate amoeba inhabiting different moss genera (Figure 2).

The existing literature survey reveals that no detail studies have been made on diversity measures on moss-inhabiting testate amoebae considering a number of biotopes. Use of modern analytical methodologies in present study is probably the first of its kind in India to unravel the diversity dynamics among the testate amoebae, moss, and the biotopes.

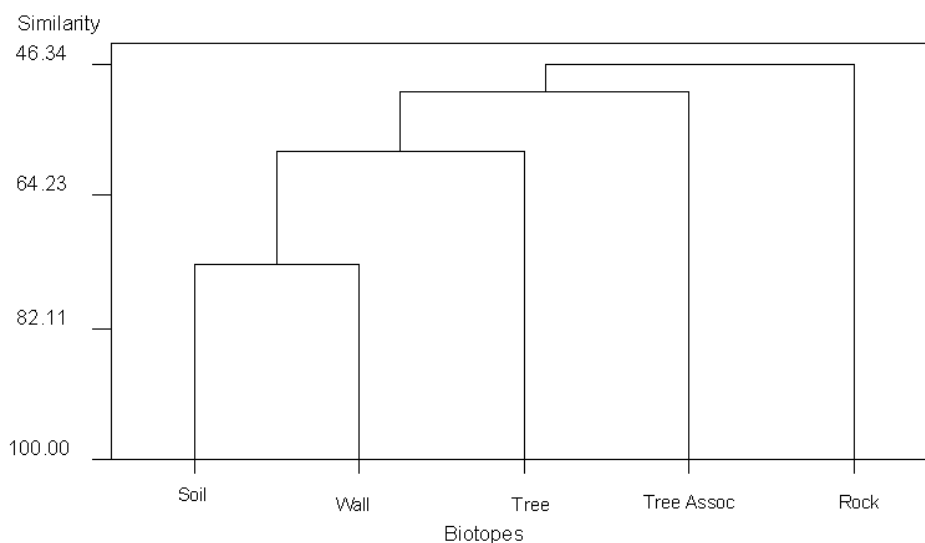


Figure 1. Dendrogram showing similarity between biotopes of moss-dwelling testate amoebae

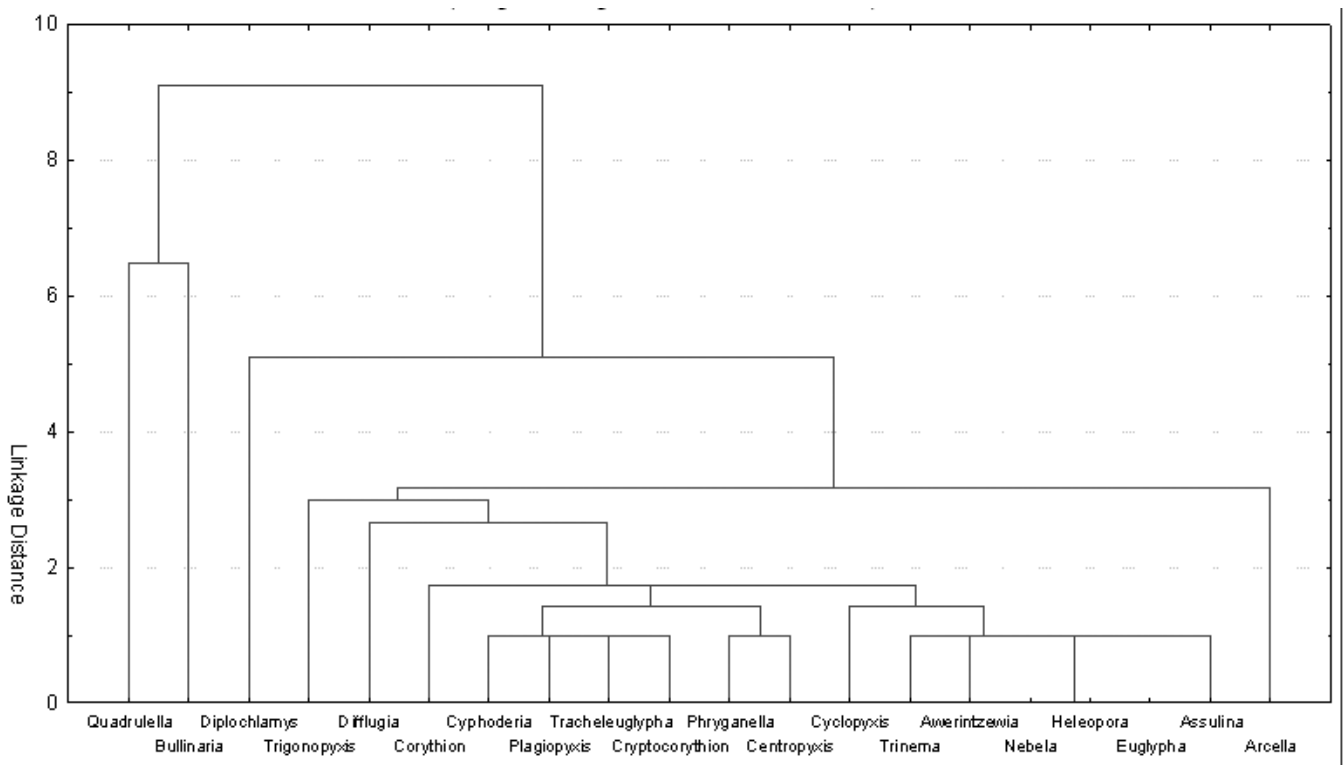


Figure 2. Cluster analysis of occurrence of different genera of testate amoebae on various mosses (Single linkage, Euclidean distance)

The inferences that could be drawn from the present analytical study are:

- The moss-inhabiting testate amoebae have exhibited no clear biotope preference.
- Some degree of preference for the moss genera (habitat) by the amoebae has been observed.
- No pH preference has been observed.

Quantitative analysis is important in studying diversity in any group of organisms. In case of testate amoeba, however, because of difficulties in collection, isolation, and identification, quantification becomes a serious problem.

The inference deduced from the present observations deviates from the proposed hypothesis on biotope dependency of the testate amoebae. However, partial support to the hypothesis of moss preference has been substantiated by the analysis. Further studies on this aspect should be undertaken for better community analysis of this interesting group of shelled protozoa.

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