



## TESTATE AMOEBAE COMMUNITY IN PERMIAN SEDIMENTS OF INDIA

NEHA AGGARWAL\*, ANJUM FAROOQUI and NEERJA JHA

BIRBAL SAHNI INSTITUTE OF PALAEOSCIENCES, 53, UNIVERSITY ROAD, LUCKNOW-226007

\*Corresponding author e-mail: neha\_264840@yahoo.co.in

### ABSTRACT

Testate amoebae are protists which occur worldwide in fresh/ brackish water ecosystems. During the geological time period, five mass extinction events were globally witnessed, but about 96% of species extinction occurred during Permian-Triassic ( $\pm 252$  Ma) and Cretaceous-Tertiary ( $\pm 66$  Ma) boundary. Here, in the present investigation, an attempt has been made to explore the testate amoebae species in sediments from the northern and southern part of India deposited during the Permian Period. Twenty six species have been recorded which belong to the family Arcellidae (6 spp.), Centropyxidae (8 spp.), Trigonopyxidae (2 spp.), Diffugiidae (7 spp.) and one each to Amphitremidae, Hyalospheniidae, and Trinematidae. The morphology and morphometry were studied in fossil specimens and their extant counterparts recorded from sub-tropical lakes in India. The dominance of *Centropyxis*, *Arcella* and *Diffugia* species is in general recorded in the present day ecosystem subjected to varied stresses, like temperature, rainfall, pH, dissolved oxygen, heavy metal content, etc. It is inferred that the fossil specimens survived the similar adverse climatic conditions and thus could overcome the two major mass extinction events. Statistical analysis of shell morphometry reveals minimum evolution in terms of the ratio of shell diameter and shell aperture.

**Keywords:** Permian, testate amoebae, India, Himalayas, Godavari Graben.

### INTRODUCTION

Testate amoebae (thecamoebians) are “rhizopods” (Tolonen, 1986; Patterson *et al.*, 1985) frequently occurring in all aquatic or humid terrestrial environments (Murray, 2007). They also occupy freshwater to marginally brackish habitats (Farooqui and Naidu, 2010). These occupy the important position as detritivores in the food chain and have potentials for primary destruction of the cellulose (Ilham and Nataly, 2000). These eukaryotes are unicellular organisms differentiated by a decay resistant shell (agglutinated or autogenous) enclosing the cytoplasm which helps them to fossilize in the sediments. The autogenous bodies are made up of complex organic proteinaceous compounds. The foreign particles as xenosomes come from the ecosystem in which they live. It has the capability to encyst in the adverse ecological conditions and rejuvenate during the favourable periods. This characteristic feature of these eukaryotes perhaps enabled them to sustain the two significant mass extinction periods of Permian-Triassic and Cretaceous-Tertiary boundary (Berner, 2009; Mc Elwain and Punyasena, 2007).

The taxonomy and identification of testate amoebae are in general based on the morphology of the shell and shell aperture (Kumar and Patterson, 2000). Until now, the recorded fossil testate amoebae (Wightman *et al.*, 1994; Schönborn *et al.*, 1999; Porter and Knoll, 2000; Schmidt *et al.*, 2004, 2006; Kumar *et al.*, 2011; Singh *et al.*, 2015) advocates minute taphonomic bias, and the lack of noteworthy new species and insignificant changes in morphometry (Farooqui *et al.*, 2014) supports the existing hypothesis of minimum evolutionary trend in testate amoebae lineages through geologic time (Van Hengstum *et al.*, 2007). The objective of the present study was to explore the fossil and extant testate amoebae community to understand the sustenance and morphological evolutionary status of these protists evading the two major mass extinction events.

### PALYNOSTRATIGRAPHY OF THE SEDIMENTS

Manjir Formation is the major stratigraphic unit of the Chamba basin which represents the Tethyan realm in the northwest Himalayas. Studied section of the Manjir Formation is mainly composed of three nonpebbly units (NP-1, NP-2, and NP-3) which are separated by four pebbly units (P-1, P-2, P-3 and P-4). Studied samples (Fig. 1) were collected from non-pebbly horizons (dark grey to black shale/slate units) along the Khundi–Maral–Raula section along the Siul River (Farooqui *et al.*, 2010; Kumar *et al.*, 2011). On the basis of recovered palynomorphs (Plate I) dominated by monosaccates (*Barakarites*, *Plicatipollenites* and *Parasaccites*) along with some non-striate bisaccates (*Platysaccus*, *Scheuringipollenites*) and striate bisaccates (*Striatopodocarpites*, *Striatites*, *Faunipollenites*), the early Permian age has been assigned to these sediments (Pande *et al.*, 2004). Recovered testate amoebae from these samples belong to shallow-paralic environments during the early Permian ( $\pm 299$ -294 Ma) deglacial phase of the widespread late Carboniferous to the early Permian glaciation of Gondwana.

A 50 km long unbroken stretch of the Barakar Formation occurring between Lingala in the north west to Koyagudem in the south east on western margin of the Godavari Valley coalfield is named as Lingala-Koyagudem coal belt. Mamakannu and Kachinapalli areas of the Lingala-Koyagudem coal belt have been dealt for the present study. This coal belt covers the Talchir, Barakar, Barren Measures and Raniganj formations (entire Lower Gondwana succession). Testate amoebae recuperated here belongs to the late Permian age ( $\pm 270$ -250 Ma) as the age of the sediments has been defined on the basis of palynofloral studies by Aggarwal and Jha (2013). The abundance of striate bisaccates (*viz.*, *Faunipollenites*, *Striatopodocarpites*, *Crescentipollenites*, *Straisulcites*) along with stratigraphically

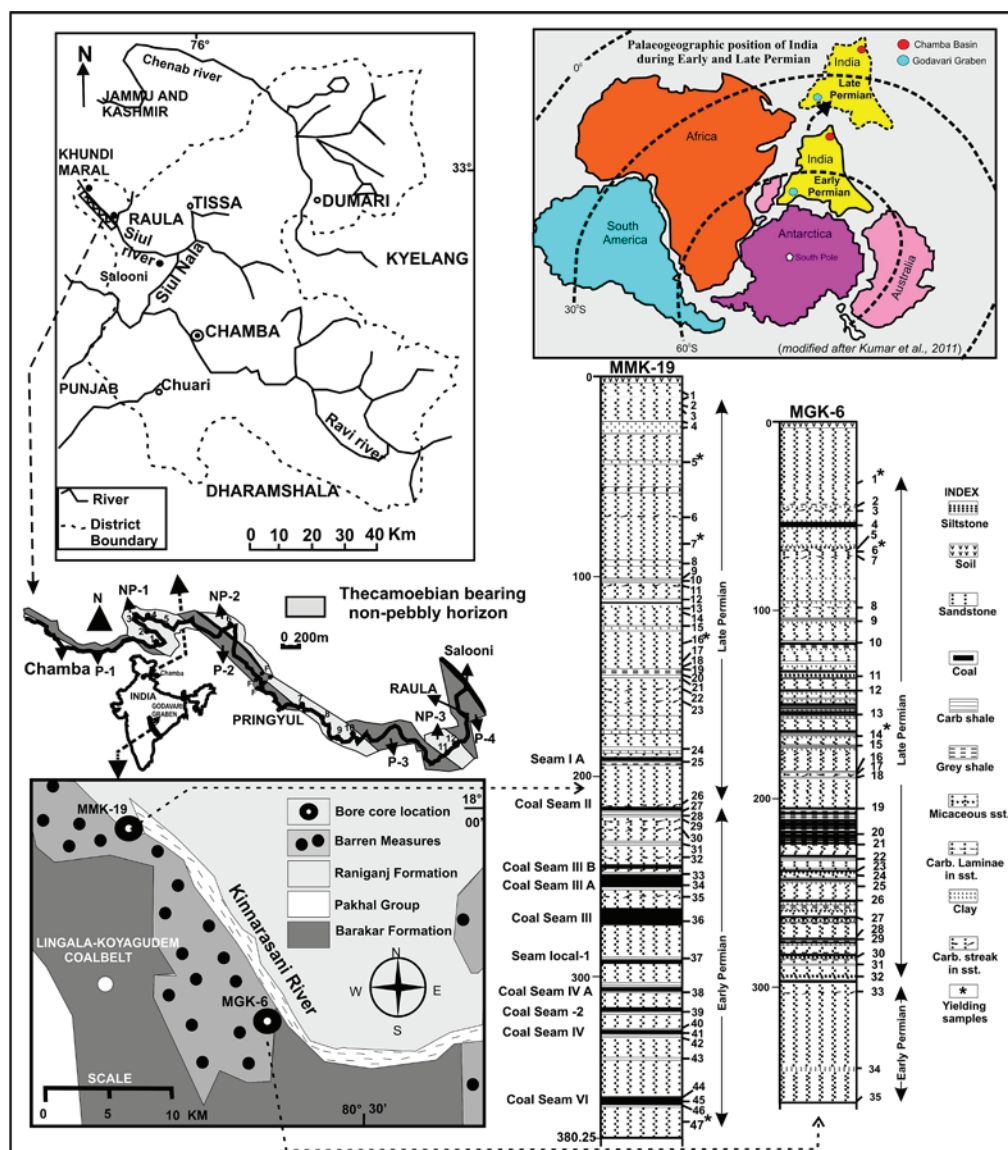


Fig. 1. Location map of the Khundi–Maral–Raula section in Chamba District and Mamakannu (MMK-19) and Kachinapalli (MGK-6) areas of the Lingala-Koyagudem coal belt of the Godavari Graben, the palaeolatitudinal position of India during the early and late Permian.

significant taxa viz., *Chordasporites*, *Klausipollenites*, *Falcisporites*, *Strotersporites*, *Lunatisporites*, *Hamiapollenites*, *Guttulapollenites*, *Densosporites*, *Playfordiaspora*, etc. in these sediments confirms the late Permian affinity.

## MATERIAL AND METHODS

The sediment was collected from the Khundi–Maral–Raula section along the Siul River in the Chamba Basin, northwest

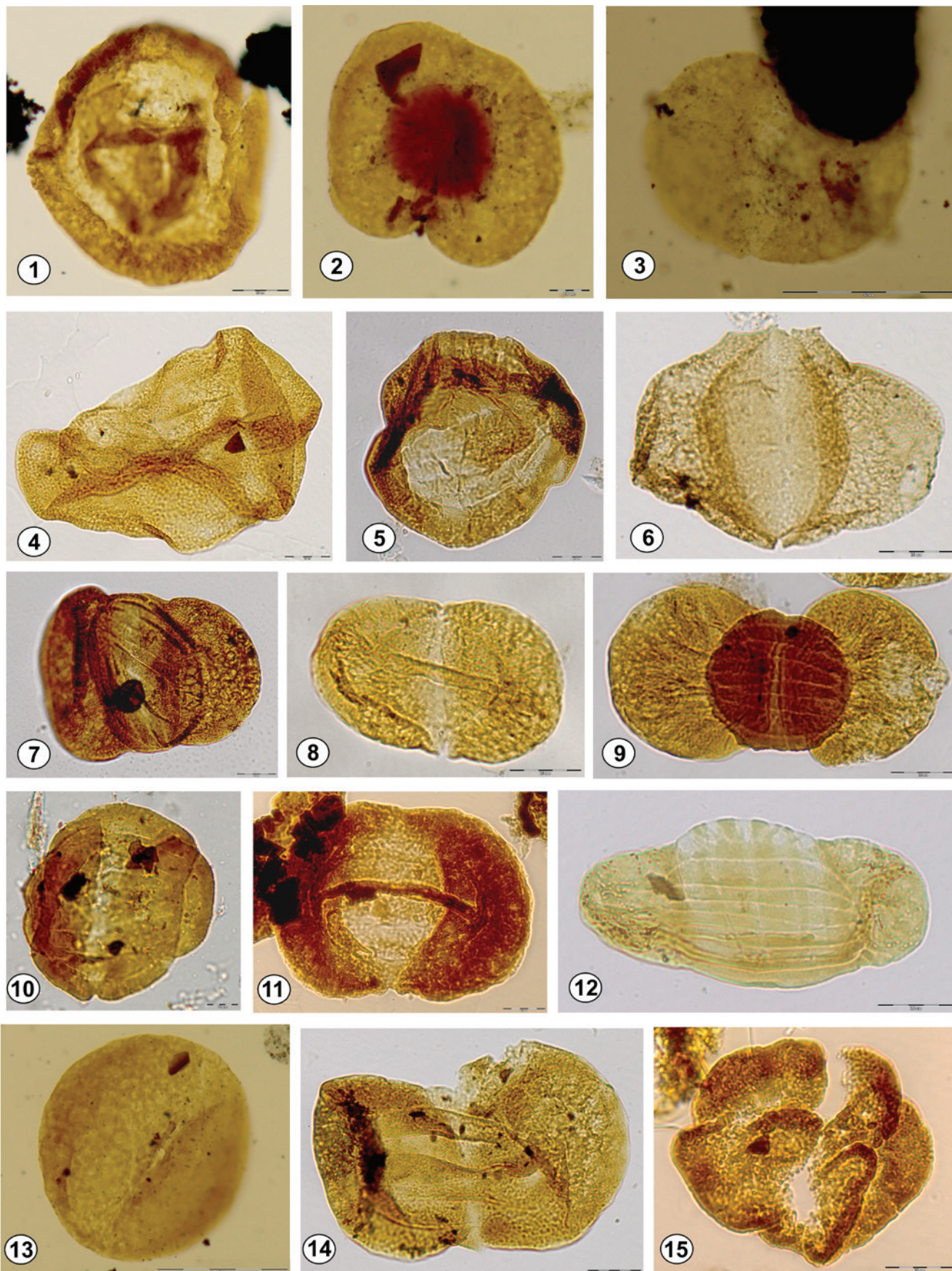
Himalaya. Alternate pebbly and non-pebbly horizons were observed in this section, and 12 samples (dark grey to black shale/slate units) were collected from the latter horizon which yielded testate amoebae. From the Godavari Graben, 47 samples from Mamakannu area (MMK-19) and 35 samples from Kachinapalli area (MGK-6) of Lingala-Koyagudem Coalbelt (Fig. 1) were collected. Four samples (sandstone, clay) from MMK-19 and three samples (sandstone, grey shale) from MGK-6 yielded testate amoebae. As the samples were hard sedimentary rock, it was processed through wet digestion. Five- ten grams of sample was first treated with 40% hydrofluoric acid (HF) to loosen the siliceous matrix. Acid digestion was essential for the recovery of testate amoebae from hard sedimentary rock. After thorough washing of water post, hydrofluoric acid organic residues were oxidized with  $\text{HNO}_3$  (nitric acid) for 2-5 days followed by 10% KOH treatment. Samples were sieved through 400 mesh (37  $\mu\text{m}$ ), and the residue was mounted on slides in the Canada balsam with the help of Polyvinyl Chloride (PVC). The scanning of slides and the photography were done under 40x with DP25

Olympus digital camera mounted on a BX62 microscope. The numbers given to fossil testate amoebae represent the specimen slide numbers deposited in the museum of the Birbal Sahni Institute of Palaeosciences, Lucknow, India. Fossilized forms of the testate amoebae have been compared with the extant forms (glycerinated specimens) at 40x magnification with an Olympus BX51 microscope. These fossil forms, belong to the family Arcellidae (6 spp.), Centropyxidae (8 spp.), Trigonopyxidae (2 spp.), Diffugiidae (7 spp.) and one each of Amphitremidae, Hyalospheniidae, and Trinematidae. The shell and pseudostome

## EXPLANATION OF PLATE I

Fig. 1. Stratigraphically significant Permian palynomorphs. *Barakarites* sp. B.S.I.P. Slide No. 12861, J45-4, Fig. 2. *Platysaccus* sp. B.S.I.P. Slide No. 12865, L66, 3. *Faunipollenites* sp. B.S.I.P. Slide No. 12865, L40-3, 4. *Densipollenites magnicarpus* B.S.I.P. Slide No. 14019, S30-2, 5. *Striomonosaccites circularis*, B.S.I.P. Slide No. 14020, M53- 4, Fig. 6. *Falcisporites zapfei*, B.S.I.P. Slide No. 13980, O57-2, 7. *Striapollenites obliquus*, B.S.I.P. Slide No. 14021, K41-2, Fig. 8. *Strotersporites* sp., B.S.I.P. Slide No. 14022, D55, Fig. 9. *Striatites varius*, B.S.I.P. Slide No. 14022, D55, Fig. 9. *Striatites varius*, B.S.I.P. Slide No. 14022, F33-4, Fig. 10. *Striasulcites Ovatus*, B.S.I.P. Slide No. 14023, F50-1, Fig. 11. *Strotersporites indicus*, B.S.I.P. Slide No. 13574, S49-2, Fig. 12. *Hamiapollenites insolitus*, B.S.I.P. Slide No. 14024, E52-2, Fig. 13. *Scheuringipollenites* sp. B.S.I.P. Slide No. 12865, N43, Fig. 14. *Lunatisporites pellucidus*, B.S.I.P. Slide No. 14025, J 60-3, Fig. 15. *Guttulapollenites hannonicus*, B.S.I.P. Slide No. 13577, MS1-1.







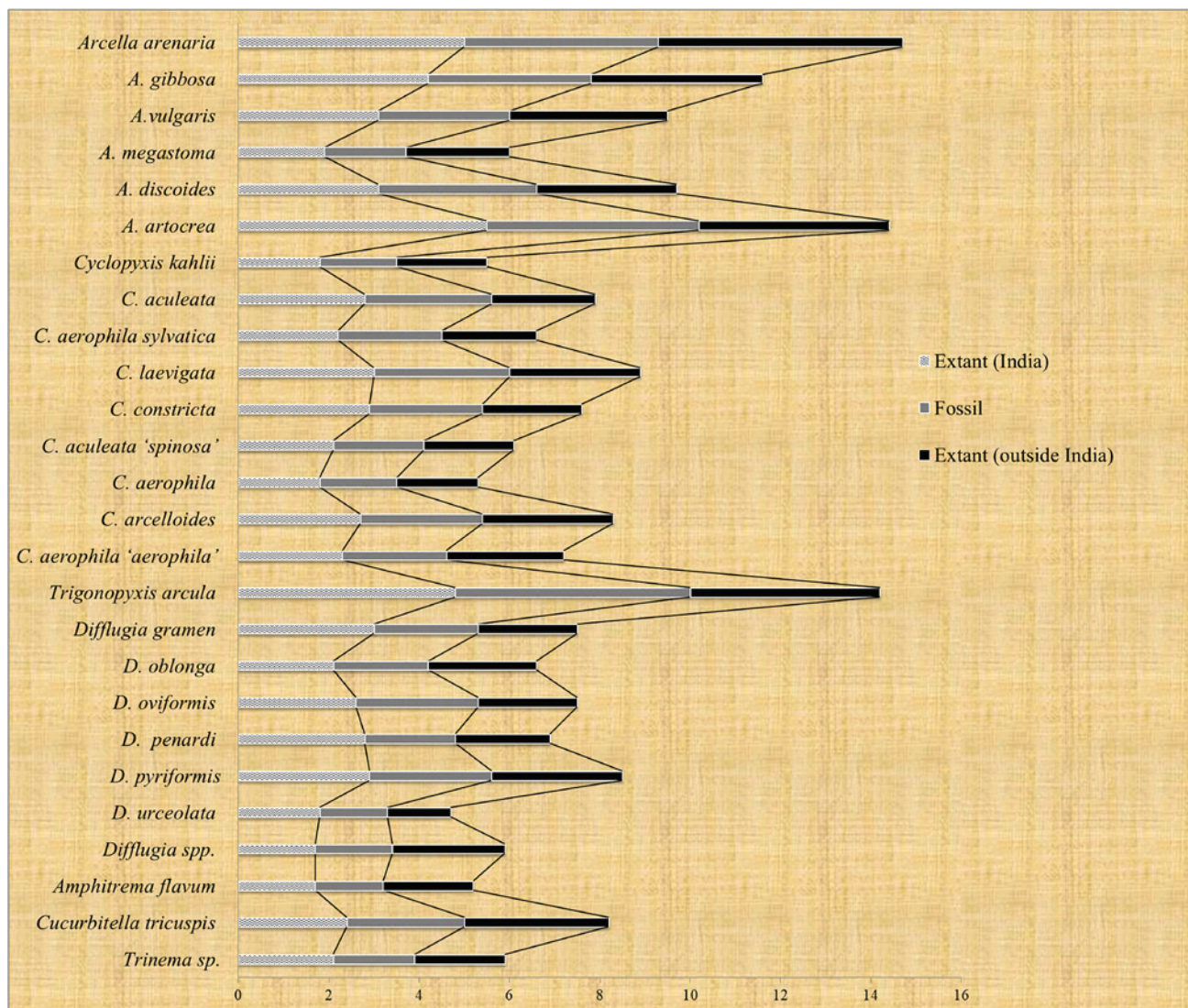


Fig. 2. Ratio of shell diameter and shell aperture is insignificant (Farooqui *et al.* 2014).

diameter and their ratio remain insignificant irrespective of its size depending on variability in the ecosystem in which they dwell (Foissner and Korganova, 2000; Farooqui *et al.*, 2014). Therefore these parameters were identified and the ratio of shell diameter (widest) and shell aperture (widest diameter) of the recovered fossil specimens have been compared with their extant counterparts from different modern ecosystems in India (Nawabganj Bird Sanctuary, Sadatal lake, and Bari Lake, Lucknow). The study was carried out following standard literature (Deflandre, 1929; Ehrenberg, 1930; Ogden and Hedley, 1980; Kumar and Dalby, 1998; Foissner and Korganova, 2000; Lee and Leedale, 2000; <http://www.arcella.nl/shelled-amoebae>).

A multivariate statistical analysis was applied to the ratio of shell diameter and shell aperture in the fossil and extant forms of testate amoebae using 'PAST' statistical software. R-mode hierarchical cluster analysis (Paired group and Euclidean distances as similarity index) was used to determine the testate amoebae association. (Hammer *et al.*, 2001).

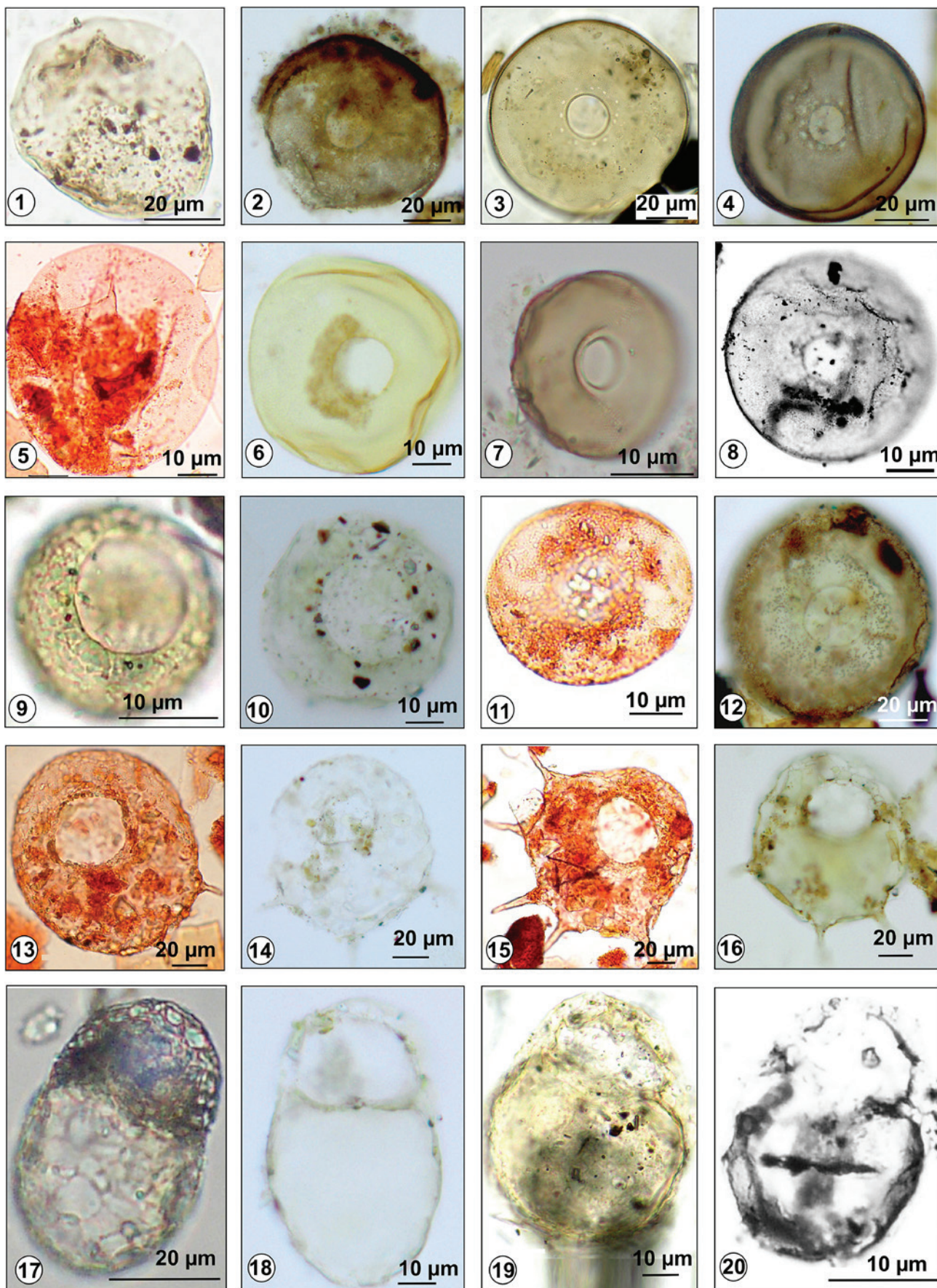
## RESULTS

The average ratio of shell diameter and aperture diameter in fossil and extant specimens recorded from India and its

### EXPLANATION OF PLATE II

Fig. 1. Extant-sadatal lake, Lucknow, Fig. 2. *Arcella arenaria*, B.S.I.P. slide no. 13581, Q66-4, Fig. 3. Extant-sadatal lake, Lucknow, Fig. 4. *Arcella artocrea*, B.S.I.P. slide no. 13576, R36, Fig. 5. Extant-sadatal lake, Lucknow, Fig. 6. *Arcella discoides*, B.S.I.P. slide no. 13850a, R59-3, Fig. 7. Extant-sadatal lake, Lucknow, Fig. 8. *A. gibbosa* B.S.I.P. slide no. 12861, P21-2, Fig. 9. Extant-sadatal lake, Lucknow, Fig. 10. *Arcella megastoma*, B.S.I.P. slide no. 13578, M39-3, Fig. 11. Extant-sadatal lake, Lucknow, Fig. 12. *Arcella vulgaris* B.S.I.P. slide no. 13577, Q61, Fig. 13. Extant-sadatal lake, Lucknow, Fig. 14. *Centropyxis aculeata*, B.S.I.P. slide no. 13576, Q46-2, Fig. 15. Extant-sadatal lake, Lucknow, Fig. 16. *Centropyxis aculeate* 'spinosa', B.S.I.P. slide no. 13850a, C37-3, Fig. 17. Extant-Nawabganj Bird Sanctuary, Lucknow, Fig. 18. *Centropyxis aerophila*, B.S.I.P. slide no. 13574, V70, Fig. 19. Extant-in association with bryophytes and lichens growing on Mango tree bark Malihabad, Lucknow, Fig. 20. *Centropyxis aerophila* 'aerophila' B.S.I.P. slide no. 13961, G33-2





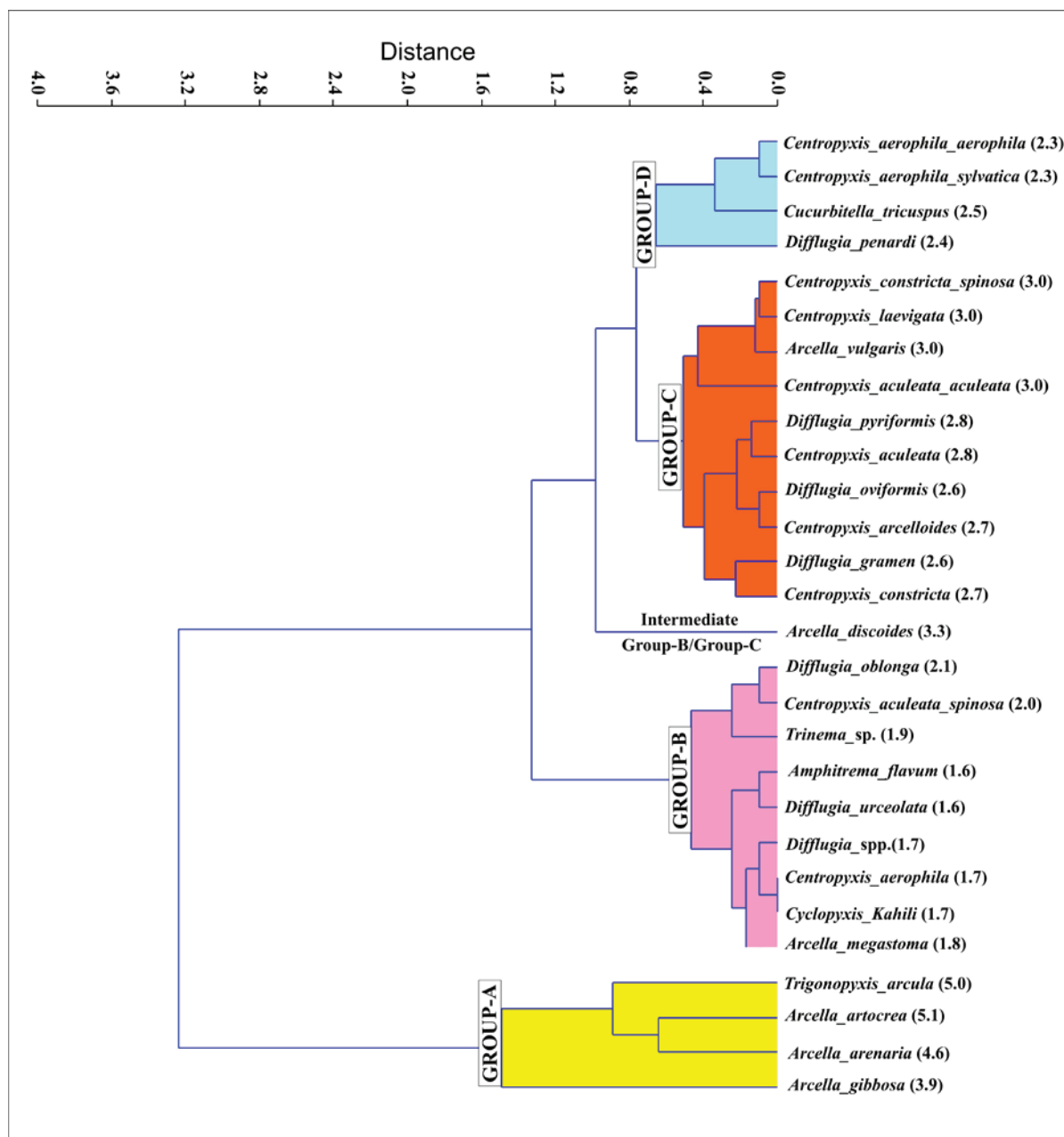


Fig. 3. Dendrogram classifications of testate amoebae (average ratio of shell diameter and an aperture diameter of fossil and extant form of India) produced by R-mode hierarchical cluster analysis (Paired group and Euclidean distances as similarity index).

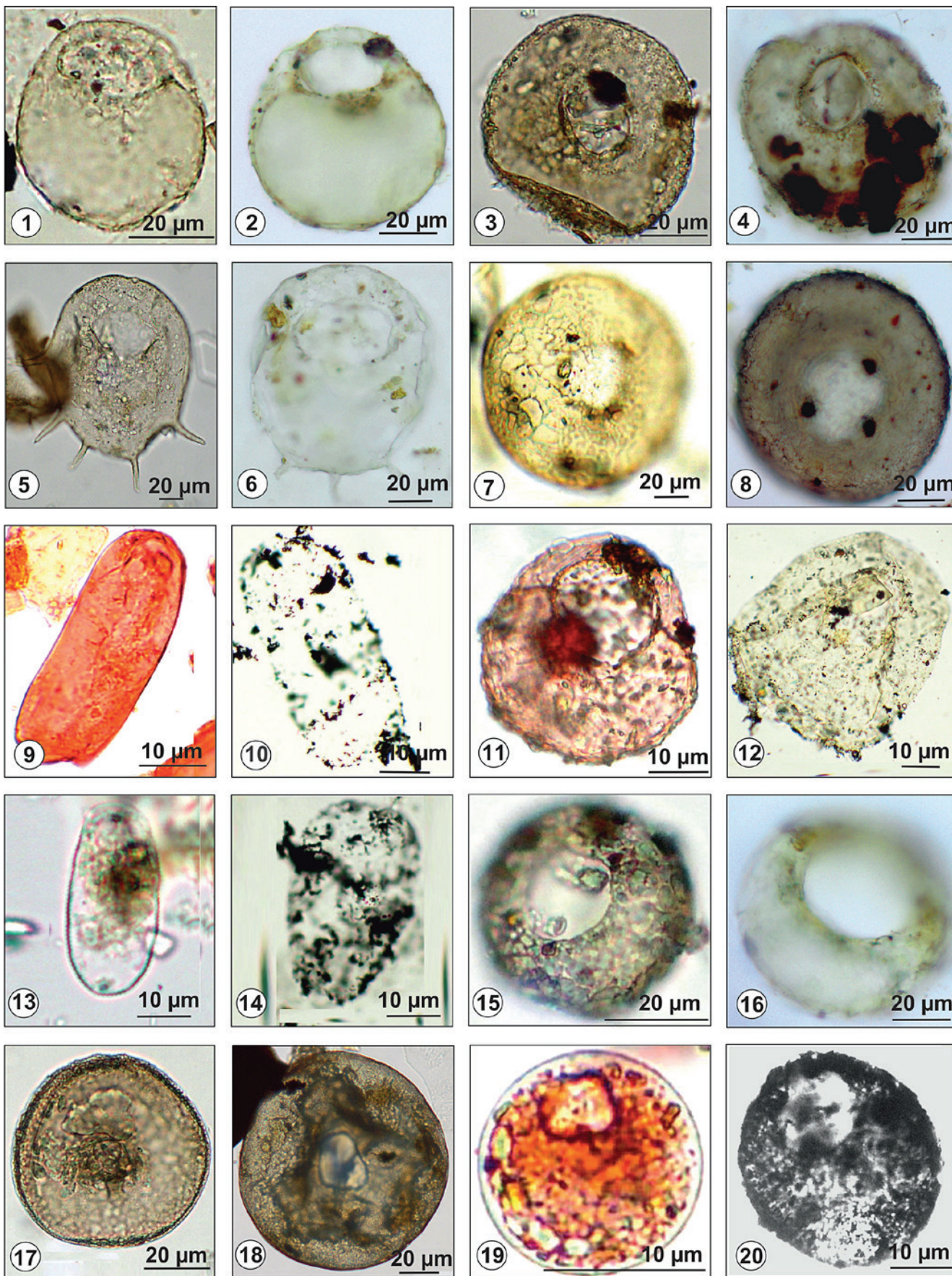
comparison with available records (Foissner and Korganova, 2000; Farooqui *et al.*, 2014) has been shown in Fig. 2. Total of four clusters (Group A-D) was identified (Fig. 3) displaying the average values of the ratio (shell/aperture diameter) within

the testate amoebae community. A comparative morphological and morphometrical study of fossil testate amoebae and their corresponding extant species recovered from sub-tropical freshwater wetlands show that most of the Permian species

### EXPLANATION OF PLATE III

Fig. 1. Extant Nawabganj Bird Sanctuary, Lucknow, Fig. 2. *Centropyxis aerophila* 'sylvatica' B.S.I.P. slide no. 13582a, J69, Fig. 3. Extant-Nawabganj Bird Sanctuary, Lucknow, Fig. 4. *Centropyxis arcelloides*, B.S.I.P. slide no. 13579a, D33-3, Fig. 5. Extant-Nawabganj Bird Sanctuary, Lucknow, Fig. 6. *Centropyxis constricta*, B.S.I.P. slide no. 13579a, M39, Fig. 7. Extant-Bari lake, Lucknow, Fig. 8. *Centropyxis laevigata*, B.S.I.P. slide no. 13850a, D51-3, Fig. 9. Extant-sadatal lake, Lucknow, Fig. 10. *Amphitrema flavum*, BSIP slide no. 13956, N22-5, Fig. 11. Extant-sadatal lake, Lucknow, Fig. 12. *Cucurbitella tricuspis*, BSIP slide no. 13961, H45-2, Fig. 13. Extant- Nawabganj Bird Sanctuary, Lucknow, Fig. 14. *Trinema* sp., BSIP slide no. 13960, Q38-1, Fig. 15. Extant-Nawabganj Bird Sanctuary, Lucknow, Fig. 16. *Cyclopyxis kahlia*, B.S.I.P. slide no. 13582a, U51-2, Fig. 17. Extant-Nawabganj Bird Sanctuary, Lucknow, Fig. 18. *Trigonopyxis arcula*, B.S.I.P. slide no. 13576, T46-2, Fig. 19. Recent, source – stream polluted with sewage (flowing throughout the year), Lucknow, Fig. 20. *Diffugia gramen*, BSIP slide no. 13966, J35-2.





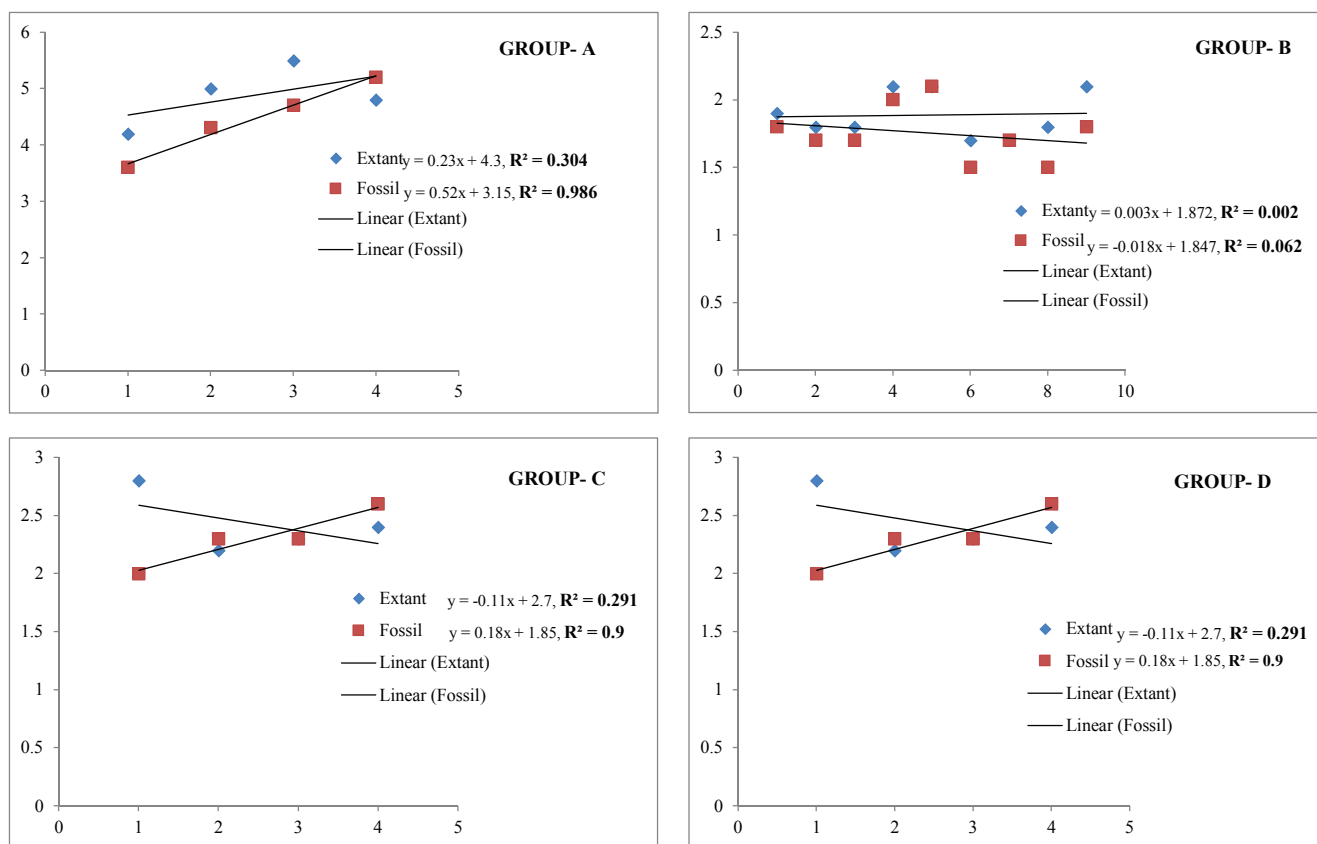
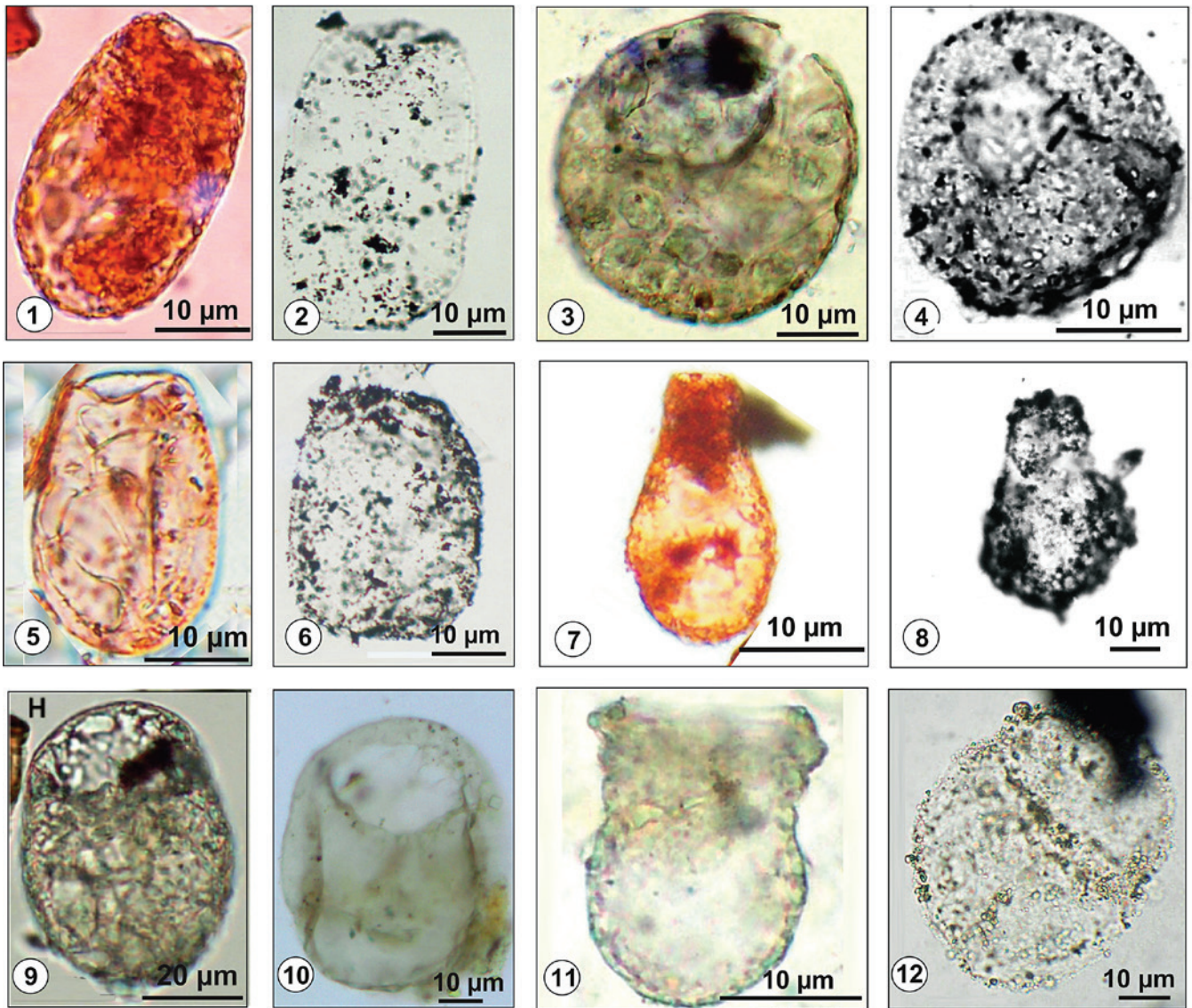


Fig. 4. Total four groups (A-D) have been identified on the basis of the average ratio of shell diameter and pseudostome diameter.

Table 1. Showing the recorded fossil testate amoebae until now.

Name of species	Early Permian	Late Permian	References
<b>Family: Arcellidae</b>			
<i>Arcella arenaria</i> Greff, 1866	+	+	Farooqui <i>et al.</i> , 2014
<i>Arcella artocrea</i> Leidy, 1876	+	+	Farooqui <i>et al.</i> , 2010, Farooqui <i>et al.</i> , 2014
<i>Arcella discoides</i> Ehrenberg, 1843	+	+	Farooqui <i>et al.</i> , 2010, Farooqui <i>et al.</i> , 2014
<i>Arcella megastoma</i> Penard, 1902	+	+	Farooqui <i>et al.</i> , 2014
<i>Arcella vulgaris</i> Ehrenberg, 1830	+	+	Kumar <i>et al.</i> , 2011, Farooqui <i>et al.</i> , 2010, Farooqui <i>et al.</i> , 2014
<i>Arcella gibbosa</i> Penard, 1890	+		
<b>Family: Centropyxidae</b>			
<i>Centropyxis aculeata</i> 'aculeata' Reinhardt <i>et al.</i> , 1998	+		Farooqui <i>et al.</i> , 2010, Kumar, 2011
<i>Centropyxis aculeate</i> 'oblonga'			Schönborn <i>et al.</i> , 1999
<i>Centropyxis aerophila</i> 'aerophila' Deflandre, 1929	+		Farooqui <i>et al.</i> , 2010, Singh <i>et al.</i> , 2015
<i>Centropyxis constricta</i> 'spinosa' Reinhardt <i>et al.</i> , 1997	+		Farooqui <i>et al.</i> , 2012
<i>Centropyxis hirsuta</i> Deflandre, 1929	+		Kumar <i>et al.</i> , 2011, Schmidt <i>et al.</i> , 2004; 2006
<i>Centropyxis arcelloides</i> Penard, 1902	+	+	Farooqui <i>et al.</i> , 2010, Farooqui <i>et al.</i> , 2014
<i>Centropyxis aerophila</i> Deflandre, 1929		+	Farooqui <i>et al.</i> , 2014
<i>Centropyxis aculeata</i> 'spinosa' Cash, 1905		+	Farooqui <i>et al.</i> , 2010, Singh <i>et al.</i> , 2015, Farooqui <i>et al.</i> , 2014
<i>Centropyxis constricta</i> (Ehrenberg, 1841) Penard, 1890		+	Farooqui <i>et al.</i> , 2014, Wightmann <i>et al.</i> , 1994
<i>Centropyxis laevigata</i> Penard, 1902		+	Farooqui <i>et al.</i> , 2014
<i>Centropyxis aerophila</i> var. <i>sylvatica</i> Deflandre, 1929		+	Farooqui <i>et al.</i> , 2014
<i>Centropyxis aculeata</i> Ehrenberg, 1838		+	Farooqui <i>et al.</i> , 2014, Singh <i>et al.</i> , 2015, Wightmann <i>et al.</i> , 1994





EXPLANATION OF PLATE IV

Fig. 1. Extant-sadatal lake, Lucknow, Fig. 2. *Diffflugia oblonga*, BSIP slide no. 13959, P13-4, Fig. 3. Extant-sadatal lake, Lucknow, Fig. 4. *Diffflugia oviformis*, BSIP slide no. 13957, S23-1, Fig. 5. Extant-sadatal lake, Lucknow, Fig. 6. *Diffflugia penardi*, BSIP slide no. 13960, F32-1, Fig. 7. Extant-sadatal lake, Lucknow, Fig. 8. *Diffflugia pyriformis*, BSIP slide no. 13962, G43, Fig. 9. Extant-Nawabganj Bird Sanctuary, Lucknow, Fig. 10. *Diffflugia* spp. BSIP slide no. 13850a, N55-3, Fig. 11. Extant-sadatal lake, Lucknow, Fig. 12. *Diffflugia urceolata*, BSIP slide no. 12861b, V40-1

<i>Centropyxis platystoma</i>			Singh <i>et al.</i> , 2015
<i>Centropyxis</i> sp.	+		Kumar <i>et al.</i> , 2011, Bassi <i>et al.</i> , 2008, Poinar <i>et al.</i> , 1993, Waggoner, 1996a, 1996b
<i>Centropyxis</i> stein, 1895			Bassi <i>et al.</i> , 2008, Singh <i>et al.</i> , 2015
<b>Family: Trigonopyxidae</b>			
<i>Cyclopyxis kahlili</i> Deflandre, 1929	+	+	Farooqui <i>et al.</i> , 2014
<i>Cyclopyxis eurystoma</i>			Singh <i>et al.</i> , 2015, Schönborn <i>et al.</i> , 1999
<i>Trigonopyxis arcula</i> Penard, 1912	+	+	Farooqui <i>et al.</i> , 2014
<b>Family: Difflogiidae</b>			
<i>Diffflugia gramen</i> Penard, 1923	+		Farooqui <i>et al.</i> , 2012
<i>Diffflugia oblonga</i> Ehrenberg, 1838	+		Farooqui <i>et al.</i> , 2010, Kumar, 2011, Hengstum <i>et al.</i> , 2007
<i>Diffflugia protaeiformis</i>			Hengstum <i>et al.</i> , 2007

<i>Diffflugia urens</i>			Hengstum <i>et al.</i> , 2007
<i>Diffflugia baukalabastron</i>			Hengstum <i>et al.</i> , 2007
<i>Diffflugia oviformis</i> Cash, 1909	+		Kumar <i>et al.</i> , 2011
<i>Diffflugia penardi</i> Hopkinson, 1909	+		Kumar <i>et al.</i> , 2011
<i>Diffflugia pyriformis</i> Ehrenberg, 1838	+		Medioli <i>et al.</i> , 1990b
<i>Diffflugia urceolata</i> Carter, 1864	+		Kumar <i>et al.</i> , 2011
<i>Diffflugia</i> spp. Lamarck, 1816		+	Farooqui <i>et al.</i> , 2014, Wightmann <i>et al.</i> , 1994, Bardley, 1931
<i>Diffflugia</i> (Leclerc, 1816)			Bassi <i>et al.</i> , 2008
<i>Lagenodiffflugia</i> sp.	+		Kumar <i>et al.</i> , 2011
<i>Lagenodiffflugia vas</i>			Hengstum <i>et al.</i> , 2007
<i>Pontigulasia compressa</i>			Hengstum <i>et al.</i> , 2007
<i>Pontigulasia</i> sp.			Bassi <i>et al.</i> , 2008
<b>Family: Plagiopyxidae</b>			
<i>Bullinularia indica</i> Penard, 1907		+	Farooqui <i>et al.</i> , 2014
<b>Family: Amphitremidae</b>			
<i>Amphitrema flavum</i> Archer, 1869	+		Farooqui <i>et al.</i> , 2010, Kumar, 2011
<b>Family: Halospheniidae</b>			
<i>Hyalosphenia baueri</i>			Schönborn <i>et al.</i> , 1999
<i>Cucurbitella tricuspis</i> (Carter) Reinhardt <i>et al.</i> , 1998	+		Kumar <i>et al.</i> , 2011, Singh <i>et al.</i> , 2015, Hengstum <i>et al.</i> , 2007
<b>Family: Trinematidae</b>			
<i>Trinema</i> sp.	+		Kumar <i>et al.</i> , 2011, Martin-González <i>et al.</i> , 2009
Incertae sedis	+	+	Kumar <i>et al.</i> , 2011
VSM			Porter and Knoll, 2000
<i>Lesquereusia spiralis</i>			Hengstum <i>et al.</i> , 2007
<i>Nebela collaris</i>			Wightman <i>et al.</i> , 1994
<i>Proteonima</i>			Crushman, 1930
<i>Urnulina</i>			Crushman, 1930
<i>Pseudarcella</i>			Crushman, 1930
<i>Quadrula</i>			Bardley, 1931
<i>Euglypha</i>			Bardley, 1931
<i>Tracheuglypha</i>			Frenguelli, 1933
<i>Arcellites</i>			Miner, 1935
<i>Silicoplacontina</i>			Kovary, 1956
<i>Prantlitina</i>			Vasicek and Ruzicka, 1957
<i>Cyphoderiid</i>			Waggoner, 1996
<i>Assulina</i>			Martin-González <i>et al.</i> , 2009
<i>Cyphoderia</i>			Martin-González <i>et al.</i> , 2009

recorded in the northern and peninsular part of India have existed since long and continue to exist in India till present.

**4.1 GROUP-A** It includes two families Trigonopyxidae (*Trigonopyxis arcula* Penard (Plate III, 17, 18) and Arcellidae (*Arcella artocrea* Leidy (Plate II, 3 and 4), *Arcella arenaria* Greeff (Plate II, 1, 2) and *Arcella gibbosa* Penard (Plate II, 7 and 8)). This group includes the highest range of average ratios (3.9-5.1) of their shell diameter and shell aperture. Among this highest ratio is in *A. artocrea* (5.1) and least in *A. gibbosa* (3.9). Shell of *Trigonopyxis arcula* is composed of agglutinated mineral particles while the shell of *Arcella* is organic in nature. *Arcella artocrea* is characterized by the mammilated or pitted shell surface with a distinct and firm border. *Arcella arenaria* is

distinguished by its mammilated shell surface and slight folds on its dorsal surface with a small circular aperture. Shell of *Arcella gibbosa* is characterized by its hemispherical/dome shape with the smooth apertural surface. Linear regression analysis (Fig. 4) shows insignificant variation in values calculated between fossil and extant forms recorded from India in the present study.

**4.2 GROUP-B** It includes six families Arcellidae (*Arcella megastoma* Penard (Plate II, 9 and 10)), Trigonopyxidae (*Cyclopyxis kahli* Deflandre (Plate III, 15 and 16)), Centropyxidae (*Centropyxis aerophila* Deflandre (Plate II, 17 and 18) and *Centropyxis aculeata* 'spinosa' Cash (Plate II, 15 and 16)), Diffflugidae (*Diffflugia* spp. (Figs. 6, 49 and 50)), *Diffflugia urceolata* Carter (Plate IV, 11, 12) and *Diffflugia oblonga*



(Plate IV, 1 and 2), Amphitremidae (*Amphitrema flavum* Archer (Plate III, 9 and 10) and Trinematidae (*Trinema* sp. (Plate III, 13 and 14). The species in this group show low values of average shell diameter and shell aperture ratio ranging between 1.6-2.1. The highest ratio (2.1) is in *Amphitrema flavum* and least (1.6) in *Diffflugia oblonga*. All the species in this group are agglutinated except *Arcella megastoma*, *Amphitrema flavum* and *Trinema* sp. are distinguished by the rigid organic body. In all the other species in this group, the xenozomes are discrete, uneven and embedded in the organic layer. *Centropyxis aerophila* has a semicircular/hemispherical, invaginated and sub-terminal pseudostome. *Centropyxis aculeata* 'spinosa' is characterized by its base of the spine is the extension of the theca. *Diffflugia* spp. is discriminated by its vase-shaped to urn shaped shell body made up of heterogenous mineral grains and other debris cemented together with organic material. *Cyclopyxis kahli* is composed of distinct xenozomes, and the invaginated pseudostome is in the ventral surface. *Amphitrema flavum* is characterized by having a circular aperture at both ends with a smooth proteinaceous test. *Trinema* test is made up of siliceous circular plates, and the aperture is circular, sub-terminal and invaginated. Linear regression analysis (Fig. 4) shows insignificant variation in values of ratios calculated between fossil and extant forms recorded from India in the present study.

**4.3 GROUP-C** It includes three families Arcellidae (*Arcella vulgaris* Ehrenberg (Plate II, 11 and 12), Centropyxidae (*Centropyxis constricta* Ehrenberg (Plate III, 5, 6), *Centropyxis aculeata* Ehrenberg (Plate II, 13 and 14), *Centropyxis arcelloides* Penard (Figs. 5, 23 and 24), *Centropyxis laevigata* Penard (Plate III, 7, 8) and Diffflugidae (*Diffflugia gramen* Penard (Plate III, 19 and 20), *Diffflugia oviformis* Cash and Hopkinson (Plate IV, 3 and 4), *Diffflugia pyriformis* Ehrenberg (Plate IV, 7, 8). All the species in this group are agglutinated except *Arcella vulgaris*. In this group, the values of shell diameter and shell aperture ratios range from 2.6-3.0. The details of the average ratios of all the species in this group are given in Fig. 3 while *Arcella vulgaris* is characterized by its hemispherical distinct dome-shaped test. *Centropyxis constricta* is characterized by its elliptical shell, *Centropyxis aculeata* is characterized by its cap-shape, ovoid-circular and usually with four or more lateral spines, *Centropyxis arcelloides* is distinguished by its circular, sub-centric, invaginated aperture, the shell of *Centropyxis laevigata* is flattened at the apertural end. The xenozomes are cemented with organic material. The pseudostome has ventral lips, recurved as well as invaginated where the ventral edge of the aperture is linked to the dorsal face by some plane pillars or bridges. *Diffflugia gramen* is characterized by its smooth wall with reticulation having tiny agglutinated particles mainly of sand particles on its shell. Shell of *Diffflugia oviformis* is more or less spherical, tapering slightly towards the aperture, composed of small siliceous elements which are unevenly arranged and cemented to construct an even surface. *Diffflugia pyriformis* is characterized by its pyriform shape and rough shell which is composed of quartz particles. Linear regression analysis (Fig. 4) shows insignificant variation in values of ratios calculated between fossil and extant forms recorded from India in the present study.

**4.4 GROUP-D** It includes three families Centropyxidae (*Centropyxis aerophila* 'aerophila' Deflandre (Plate II, 19 and 20), *Centropyxis aerophila* 'sylvatica' Deflandre (Plate III, 1, 2), Diffflugidae (*Diffflugia penardi* Cash and Hopkinson (Plate IV, 5, 6) and Halospheniidae (*Cucurbitella tricuspidis* Reinhardt

*et al.* (Carter) (Plate III, 11, 12). In this group, the values of shell diameter and shell aperture ratios range from 2.2-2.5. The details of the average ratios of all the species in this group are given in Fig. 3. All the species in this group have xenozomes cemented with organic material. *Centropyxis aerophila* 'sylvatica' is identified by its pseudostome which is generally semicircular and slightly concave posterior edge. *Cucurbitella tricuspidis* is characterized by its irregular shaped/more or less spherical body with crenulated aperture. *Diffflugia penardi* is characterized by its large pseudostome without a collar. Linear regression analysis (Fig. 4) shows insignificant variation in values of ratios calculated between fossil and extant forms recorded from India in the present study.

Except all these groups *Arcella discoides* (family Ehrenberg (Plate II, 5 and 6),) lies between Group-B and Group-C due to its little bit higher range of ratio (3.5) in the fossilized form. *Arcella discoides* is a nonagglutinated form of family Arcellidae and is distinguished by its reticulated body pattern.

## DISCUSSION

Environmental causes such as temperature, food source, moisture, pH, availability of extraneous material directly affect the morphology of testate amoebae (Bobrov *et al.*, 2004). Phenotypic variations in testate amoebae are also well documented in the literature (Bobrov and Mazei, 2004). Evolutionary studies recommended that phenotypic variability in response to the environmental changes may enhance the rate of adaptability when these disparities become genetic in environmental time scale (Logares *et al.*, 2008; Wund, 2012). *Diffflugia* spp. has been found highly prone to the phenotypic variations (Todorov and Golemansky, 2007). This interpretation has also been supported by Bobrov and Mazei (2004) in the biometric data analyses on 32 natural populations of 24 species which reveal significant morphological variability. The relationship between morphological features and environmental parameters of testate amoebae was first established by Schönborn (1962). Lahr *et al.* (2008) also divulged morphological, ecological and biometrical studies on 2210 *Centropyxis* individuals which suggest that morphospecies of *C. aculeata* and *C. discoides* are actually the same that exhibit a high polymorphism in the shell, aperture shape and number of spines. Testate amoebae morphology/morphometry have remained remarkably stable since long in the geological period (742 mya) (Foissner and Korganova, 2000; Farooqui *et al.*, 2014; Porter *et al.*, 2003).

The palaeogeographic position of India during the early Permian and late Permian was between 50°-70°S and 20°-45°S respectively (Kumar *et al.*, 2011). The northern part of India (Chamba Basin) would have been around 50°-55°S in the cooler region at this palaeolatitude (Fig. 1). While, as compared to other Indian Gondwana basins the Godavari Graben was very close to the South Pole during the late Permian as a result of which the variability in the climate has been recorded in the late Permian sedimentary sequences in this Graben. Most of the recovered testate amoebae specimens from the Permian Period in India are comparable to their extant forms (ratio of shell diameter and pseudostome diameter). Therefore, a slight change in shell size or ratio of shell diameter and pseudostome diameter may be due to environmental factors that prevailed in the ecosystem at the time of its existence. The recorded species are known to have a wide range of adaptability to different present day ecological conditions including Lake bathymetry, pollutants, etc. (Reinhardt

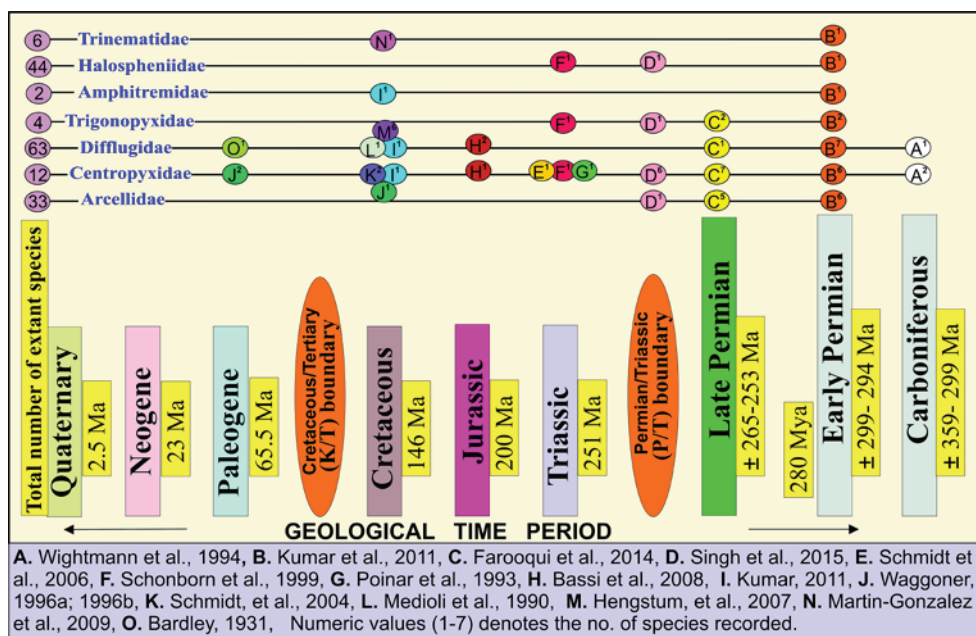


Fig. 5. Total number of fossil testate amoebae species recovered during different geological time.

et al., 1998; Farooqui et al., 2012). Being highly resistant and adaptability to survive such ecological variations could have perhaps enabled the protists to survive the two stressed events of mass extinction during the Permian-Triassic and Cretaceous-Tertiary boundaries. Some fossil testate amoebae species (Table 1) have been recorded until now from different geological periods (Fig. 5). The dearth of fossil global records is enormous which is attributed to either the processing methods of the samples or palynologists may have overlooked it.

The fossil and extant specimens recorded in the present study have been statistically grouped (A-D) according to the average ratio of shell diameter/shell aperture. In Group-A ratio of the studied specimens ranges between 5.5-3.6. In this group, the average ratio of extant *Arcella artocrea* is 5.5, and in fossil, it is 4.7 recorded in India. In extant *A. arenaria* the ratio is 5.0, and in fossil, it is 4.3. In extant *A. gibbosa* it is 4.2, and in fossil, it is 3.6. In extant *Tigonopyxis arcula* it is 4.8, and in fossil, it is 5.2. However, the average ratio recorded for these species until now from different parts of the world is 4.2, 5.4, 3.8 and 4.2 respectively (Foissner and Korganova, 2000; Lee and Leedale, 2000). The regression analysis reveals an insignificant difference in the ratio of this group and also shows morphological similarity. In Group-B ratio of the studied specimens ranges between 1.6-2.1. This group comprises of *Arcella megastoma*, *Cyclopyxis kahli*, *Centropyxis aerophila*, *Centropyxis aculeata* 'spinosa', *Diffflugia* spp., *Diffflugia urceolata* *Diffflugia oblonga*, *Amphitrema flavum*, and *Trinema* sp. The average ratio of extant and fossil specimens recorded in India of the Group -B species are 1.9 and 1.8; 1.8 and 1.7; 1.8 and 1.7; 2.1 and 2; 1.7 and 1.7; 1.8 and 1.5; 2.1 and 2.1; 1.7 and 1.5; 2.1 and 1.8 respectively. However, the average ratio recorded for these species until now from different parts of the world is 2.3, 2, 1.8, 2, 2.5, 1.4, 2.4, 2, 2 respectively (Foissner and Korganova, 2000; Lee and Leedale, 2000). The regression analysis reveals an insignificant difference in the ratio of this group. In Group-C ratio of the studied specimens ranges between 2.6-3.0. This group comprises of *A. vulgaris*, *Centropyxis constricta*, *C. constricta*,

'spinosa', *C. aculeata*, *C. arcelloides*, *C. laevigata*, *C. aculeata* 'aculeata', *Diffflugia gramen*, *D. oviformis*, and *D. pyriformis*. The average ratio of extant and fossil specimens recorded in India of the Group -C species are 3.1 and 2.9; 2.9 and 2.5; 3.1 and 3; 2.8 and 2.8; 2.7 and 2.7; 3 and 3; 3.4 and 2.7; 3 and 2.3; 2.6 and 2.7; 2.9 and 2.7 respectively. However, the average ratio recorded for these species until now from different parts of the world is 3.1, 3.5, 2.2, 2, 2.3, 2.9, 2.9, 2.9, 2.2, 2.2, and 2.9 respectively (<http://www.arcella.nl/shelled-amoebae>).

The regression analysis reveals an insignificant difference in the ratio of this group. However, *Arcella discoides* shows intermediate character of Group-B and Group-C, It is closer to group B in ratio

(3.1 and 3.5) than Group C showing lower range (2.6-3.0). This is attributed to varying environmental conditions which further needs thorough investigation in other specimens from contemporary similar ecosystems. In Group-D ratio of the studied specimens ranges between 2.2-2.5. In this group, the average ratio of extant *Diffflugia penardi* is 2.8, and in fossil, it is 2 recorded in India. In extant *Centropyxis aerophila* 'sylvatica' the ratio is 2.2, and in fossil specimen, it is 2.3. In extant *C. aerophila* 'aerophila' the ratio is 2.3, and in fossil specimen, it is 2.3. In extant *Cucurbitella tricuspis* the ratio is 2.4, and in fossil specimen, it is 2.6. The regression analysis reveals an insignificant difference in the ratio of this group. However, the average ratio recorded for these species until now from different parts of the world is 2.1, 2.1, 2.6 and 3.2 respectively. The available extant records of these specimens (Fig. 4) reveal the similar and statistically insignificant average ratio of shell diameter/shell aperture (Farooqui et al. 2014). However, the size variation in testate amoebae recorded from different environments has been established (Bobrov and Maize, 2004) but it is apparent through present study that the ratio of their shell size and aperture is statistically insignificant. Similar studies by Foissner and Korganova (2000) in *Centropyxis areophila* complex and other species reveal insignificant changes in the ratio. However, the size variation is observed depending on ecological parameters. It has been observed in the present study that most of the extant forms from India show comparatively larger size (Farooqui et al., 2012) than the contemporary specimens recorded during the Permian Period. It is inferred that the species recorded during the Permian Period show insignificant size variation depicting the similar type of oligotrophic conditions in which they were dwelling (Schönborn, 1962; Farooqui et al., 2014). Yablokov and Yusufov (1998) defined species as a system of close biotypes, genotypes, common habitat with common evolutionary fate. The dominance of asexual mode of the reproduction may be the reason for minimal evolution in testate amoebae. All the species recovered has a wide range of adaptation in Lower latitude and higher latitude (Escobar et al., 2008). The present



study is also supported by Wanner (1999), where no pronounced modifications in shell morphology, the shell size range of the testate amoebae was observed under exceptional experimental conditions in the laboratory. Therefore, it is inferred that the variation in the ratio of the shell aperture and shell diameter of a testate amoebae species is statistically insignificant although their size may vary (Fig. 4). Although, the size variation in testate amoebae may be effectively used as potential indicators of palaeoecological conditions the morphometry may be potentially used for identifying taxonomical and evolutionary trends in testate amoebae.

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