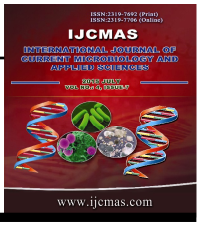

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Original Research Article

Oldest Record of Freshwater Diatom Frustules in Tests of Permian thecamoebians: Faithfulness of Sedimentary Record

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ABSTRACT

Keywords

Thecamoebian, diatom, Permian, Chamba basin, Chang-La lake, India

The first record of fresh water diatom frustules with the tests of thecamoebians recovered from Permian sediments (±251-299 Ma) Chamba basin, Himachal Pradesh and Godavari sub-basin, Andhra Pradesh, India. Four fossil specimens such as *Centropyxis aculeata*, *C. arcelloides* and *Arcella vulgaris* and *Arcella excavata* have been found and its tests/shells show diatom frustules as xenosomes resembling the extant *Nitzschia* type. Interestingly, the lakes from warmer tropical areas do not show diatoms as xenosomes in the tests but *Centropyxis aculeata* and *C. arcelloides* recorded from Naini lake, Nainital (temperate climate) form agglutinated shell with diatom frustules. On the other hand, *Arcella vulgaris* forms autogenous shell and while feeding on diatoms, the frustules remained inside the shell in the extant specimens recorded from Chang-La lake, ladakh (temperate climate). The palynostratigraphy records show that Permian sediments were deposited in comparatively cooler climate. It is inferred (1) the association of the camoebian shells with diatom frustules is related to cooler climate, (2) the dissolution of diatom silica in sediments reduces in cooler climate, therefore the less silicified palaeozoic diatoms got chance to leave impression in tests of the camoebians and (3) the study provides clue to existence of diatoms prior to Permian-Triassic boundary.

Introduction

The origin of diatom is still an unsolved mystery and on the basis of molecular data the record of diatoms goes back to average 240 Ma ago. The proposed monophyletic origin of diatoms by Round and Crawford (1981) is between the Proterozoic (Precambrian) and the Late Jurassic (c. 650-140 Ma). However, different workers have largely discussed the clock of diatoms (Medlin, 2009). It was concluded that the ancestral diatom developed in shallow-water benthic environment with algal mucilage masses. Thecamoebians too are very often found in association with the mucilaginous masses of algal origin. Diatoms are eukaryotic algae having characteristic silicified cell walls known as frustules (Sims *et al.*, 2006). Harwood and Gersonde (1990) and Gersonde and Hardwood (1990) recorded the oldest known fossil diatoms from Early Jurassic (± 174–201 Ma) and Albian (upper part of Lower Cretaceous ± 100–113 Ma). Earlier reports by Zurzolo and Bowler (2001) and Medlin *et al.* (2000) indicate that diatoms may have arisen around 280 Ma (Sakmarian) following an endosymbiotic event between a red eukaryotic algae and a heterotrophic flagella related to the oomycetes.

The fossil recovered in Cretaceous suggest that the overlapping parts appeared very early in the evolution of the pigmented heterokonts (Medlin *et al.*, 1997a). However, until now, the phylogenetic study places the diatoms within the pigmented heterokont algal lineages (Bhattacharya *et al.*, 1992; Leipe *et al.*, 1994; Medlin *et al.*, 1997a). On the basis of molecular data an average age of the earliest possible diatoms goes back to 240 Ma ago (Kooistra and Medlin; 1996, Medlin *et al.*, 1997b).

It is the faithfulness of the studied Permian sediment that allowed us to record the thecamoebian in association with the diatoms which is rather rare because the dissolution of rock matrix involves acid treatment due to which most of the siliceous forms become obscure.

The present work illustrates a clue to the existence of diatoms prior to Permian-Triassic boundary between ~ 299-251Ma (Early to Late Permian) in association with thecamoebian shells/tests as xenosomes in cooler climate. This association of thecamoebians and diatoms in relation to cooler climatic conditions have been explored with the extant thecamoebians from tropical (Farooqui *et al.*, 2012, 2014) and temperate (present study) region in India. The extant thecamoebian community and its association with diatoms were explored in freshwater lakes both from tropical and temperate areas.

Stratigraphy and age of the specimens

Thecamoebians such as *Centropyxis aculeata*, *C. arcelloides* have been recovered from Manjir formation, a major stratigraphic unit of Chamba basin (Himanchal Pradesh) representing the Tethyan realm of northwest Himalaya (Figure 1). Pande *et al.* (2004) assigned Early Permian age to Manjir Formation with the record of palynomarkers comprising chiefly monosaccates (*Parasaccites*, *Plicatipollenites*), non-striate disaccates (*Scheuringipollenites*, *Platysaccus*) and striate disaccates (*Striatopodocarpites*, *Faunipollenites* and *Striatites*). Thecamoebian reported here lived in shallow-marine environments during the Early Permian deglacial phase of the widespread Late Carboniferous–Early Permian glaciation of Gondwana. The samples studied were collected along the Bhalsu-sunu kothi-khundi Maral section along the Siul River (Farooqui *et al.*, 2010; Kumar *et al.*, 2011). In this section, diatoms were recovered in association with thecamoebians from dark grey to black shale/slate (±299–294 Ma) in sample number NP-3 (12) (Figure 1).

Arcella vulgaris, characterized by its autogenous proteinaceous test was recovered from Godavari Graben located in the Indian peninsula (Figure 2). The entire Lower Gondwana succession comprises Talchir, Barakar, Barren Measures and Raniganj formations. Barakar and Raniganj are the coal bearing horizons of Early and Late Permian age, respectively. The specimen reported here is from borecore MGK-6 (sample number 6) from Kachinapalli block of Lingala-Koyagudem coalbelt of Godavari sub-basin (Figure 2). Late Permian age (±265–253 Ma) has been assigned on the basis of dominance of striate disaccates (*Striatopodocarpites*, *Faunipollenites*, *Striasulcites*) and presence of some stratigraphically significant taxa *Klausipollenites, Guttulapollenites, Lunatisporites, Strotersporites, etc.* Thecamoebians recorded from this section and its comparative morphostasis with extant specimens found in fresh water lakes of India have been published (Farooqui *et al.*, 2014).

Position and climate of India during the permian

Palaeolatitudinal position of India during Permian was between 20^0-45^0S (Smith *et al.*, 1981). Glaciation was widespread during the Late Carboniferous–Early Permian and sequences of marine and nonmarine sediments were common in Permo-Carboniferous deposits. Widespread distribution of glacial sediments occurred in South America, Africa, Madagascar, Arabia, India, Antarctica and Australia. During the Early Permian the location of the Chamba Basin (Manjir Formation) was in a very cold region at that time (Figure 3).

Although, the overall climate in Indian Gondwana region during Late Permian time has been interpreted as warm and humid (Tiwari and Tripathi, 1987) in Godavari Graben, a mild cool oscillation during Late Permian time (Raniganj Formation) has also been reported based on palynological study (Srivastava and Jha, 1992, 1998; Jha and Aggarwal, 2010; Aggarwal and Jha, 2013). The studied specimens from the Late Permian (±265-253Ma) sequence of Godavari Graben provide evidences of cool temperate climate. Moreover, the position of Godavari Graben was very nearer to South pole as compared to other Gondwana basins of India. Hence, the variability in climatic conditions is recorded in sedimentary sequences deposited during Late Permian in India.

Basic characteristics of Thecamoebian/ testate amoeba

Thecamoebians are microscopic Rhizopods having rigid, sac like shell/test inside which the amoeba resides. The taxonomy is generally based on the morphology of the shell. Two types of shells are distinguished i.e., autogenous and xenogenous. Thecamoebians secrete either a calcite test, a siliceous test, or make a test from foreign material such as diatom frustules, organic matter, or mineral grains (Warner, 1990). These inhabit a variety of moist-wet environments (Warner, 1990). The autogenous tests are made up of complex organic proteinaceous compounds. The foreign particles as xenosomes come from the sediment upon which they live.

The size ranges from 40 to 300 microns and dwell in varied habitat from fresh water, brackish coastal waters, algal mass, moss cushions etc. Some species with agglutinated shells produce their own elements, called idiosomes. These elements can be siliceous or calcareous. Xenosomes and idiosomes are bounded together by organic cement.

Climate of Nainital (Naini lake), and Laddakh (Chang-La lake)

Naini lake, Nainital is at an altitude of 2084 meters above mean sea level and at Latitude 29º 38' N and Longitude 79 º 45' E in the foothills of the outer Himalayas. Surrounded by mountains, the pear- shaped valley is filled with water that forms an eye-shaped lake acting as a sink for nearby drainage. Nainital has temperate summers with maximum temperature as 27º C and minimum 5º C in winters. The temperature during winters may remain even 1-2º C during most of the time. The surrounding hilly ranges are covered by coniferous forest and the annual mean precipitation is reported to be ~120 cm. The water in the lake is alkaline in nature (pH 8.4-9.3). Enormous nutrient flow from nearby areas contributes to eutrophic conditions (Sharma and Pant, 1985). The upwelling and internal recycling of nutrients in the lake results into proliferation of phytoplanktons. The glacial lake in Chang-La, Ladakh (Jammu and Kashmir, India) is the third highest motorable road in the world situated at an elevation of 5360m above mean sea level. The climate is typical Alpine desert in the region.

Materials and Methods

A 10g of fossil rock sample retrieved from Chamba Basin, Himachal Pradesh and Kachinapalli area of Lingala-Koyagudem coalbelt, Godavari sub-basin, was initially treated with 30% Hydrofluoric acid (HF) for 2 days followed by nitric acid for 2–5 days and later by 10% alkali (KOH) treatment (Kumar *et al.,* 2011). Samples were sieved through 400 mesh (37µm) and the residue was mounted on slides with Canada balsam-polyvinyl chloride medium and observed under Olympus BX 51TF. The specimen slides have been deposited in the BSIP Museum. The recent sediments were collected from Naini Lake, Nainital and Chang-La lake, Ladakh (low and high altitude, respectively; temperate climatic conditions), India and were acetolysed following Erdtman (1943). The samples were treated with warm 10% KOH for 10minutes and sieved through 150mesh. The filtrate was kept overnight to decant the supernatant. The samples were then treated with 30 % HF only for a day and then centrifuged to decant the excess HF. The samples were then acetolyzed using glacial acetic acid and subsequently treated with anhydrous acetic acid and sulphuric acid (9:1) following Erdtman (1943). The extant specimens illustrated here were photographed in glycerinated medium under light Microscope (Olympus, BX-51). The identification was carried out according to Deflandre (1929), Foissner and Kornagova (2000), Lee *et al.* (2000), Ogden and Hedley (1980).

Results and Discussion

The extant specimen *Arcella discoides* recorded from sediment-water interface of glacial lake in Chang-La, Ladakh shows a single diatom frustule inside the shell (Plate 1, Figure A). The *Arcella* species is autogenous and makes its own organic shell therefore the diatom frustule retained inside after it was engulfed as food. The pennate diatom frustule resembles the extant *Nitzschia* type but because of obscure morphological features it is difficult to identify. Another specimen is *Arcella excavata* (Plate 1, Figure B) found in Chang La lake showing two frustules of pennate diatoms. These two forms are although un-acetolysed, yet it is difficult to identify the morphology of diatom. The extant specimens retrieved in sediments from Naini Lake at sediment-water interface showed dominance of *Centropyxis aculeata* (Plate 1, Figure C & D) and *C. arcelloides* (Plate 1, Figure E &F). The most common thecamoebians (95 per cent of the total count) from Naini Lake at sediment-water interface is dominated by *Centropyxis aculeata* (48%), *C. arcelloides* (23%) and *Difflugia pulex* (24%). Out of these about 23% of *Centropyxis aculeata* and *C. arcelloides* show shells with diatom frustules as xenosomes. The common diatom species are of *Nitzschia* and *Cymbella*. In most of the tests, fragments of diatom frustules are arranged a bit sparsely in haphazard position (Plate 1, Figures D).

Fossil specimens found from Early Permian sediment shows resemblance with its extant form *Centropyxis arcelloides* (Plate 2,

Figure G-I) showing three diatom frustules as xenosomes on the shell/test. The xenosomes are cemented with proteinaceous organic material. *Centropyxis* is the genus that forms agglutinated shell constituting various tiny materials amongst which it lives. The specimen here shows diatom frustules as xenosomes used in the formation of its shell. Plate 2, Figure J & K show several minute fragments of diatom frustules that constitute the shell/test of thecamoebia resembling the extant *Centropyxis aculeata*.

The Late Permian thecamoebian identified with diatom frustules is *Arcella vulgaris* (Plate 3, Figure L-O). These form an autogenous test of pseudo-chitin (proteinaceous organic matter). Two pennate diatoms are observed inside its shell. Although the detail morphology could not be understood, the outline of the specimen allowed us to identify as pennate diatom.

The biochronology of diatoms is still debatable because of lack of evidences that could not be recorded from older sediments prior to Permian-Triassic boundary. Until now no direct evidence of diatoms is available prior to Jurassic but the estimates based on the evolutionary trend of ribosomal genes it is suggested that diatoms originated near the Permian-Triassic boundary (~240 Ma) (Kooistra *et al.*, 2003). Recently, molecular data indicates that the diatom lineage evolved sometime near the Devonian- Carboniferous boundary (~354 Ma) (Brown and Sorhannus, 2010). The preservation of opaline silica frustules in original depositional settings has several limitations. Loss of diatom evidences in sedimentary deposits is high due to dissolution of silica or by the absence of a bio-mineralized structure in the early forms (Sims *et al.*, 2006). The diagenetic remineralization of opaline frustules into quartz in older continental sediments, or lack of preservation of representative diatom-bearing older geologic units may have destroyed the evidences. Preservation of diatom-bearing sedimentary deposits requires optimal conditions (Benson *et al.*, 2013). Therefore, the type and quality of diatom preservation in the fossil sites and its fruitful retrieval as fossil is the key component. Mineral transformation also alters the structure of the diatom frustules (Williams *et al.*, 1985). In extreme cases, the frustules appear only as a relic image or are completely obliterated in a groundmass of amorphous biogenic silica (Mustoe, 2005). Even when the frustule remains intact in the silicified host, the matrix cannot be disaggregated to free the frustules. Similar difficulty arises where diatoms may get encased with some other forms. In such cases, the evidence of diatom frustules becomes limited.

Here we present the faithfulness of the geologic unit of Permian period providing a clue to the existence of diatoms with the well preserved thecamoebian tests/shell. Thecamoebians have long been proved as useful proxy for a variety of past environmental and climatic parameters. The oldest record (± 742 Ma) of vas- shaped thecamoebians is from Neoproterozoic (Porter *et al.*, 2003). However, Phanerozoic (±541 Ma) record of thecamoebians is reported in organic residues from Nova Scotia, Canada (Scott *et al.*, 2003). The Palaeozoic thecamoebian findings are from the Carboniferous (Vasicek and Ruzicka, 1957, Wightman *et al.*, 1994, Wolf, 1995) and Early Permian (± 299-294 Ma) from 'Manjir Formation' of the Himalayas of India (Farooqui *et al.*, 2010, Kumar *et al.*, 2011) and Late Permian thecamoebians from Godavari sub-basin (Farooqui *et al.*, 2014). These microbes are known to have shown minimal evolution in the geological past (Farooqui *et al.*, 2014). The shells of the fossil thecamoebian specimens recorded until now from India and elsewhere, rarely show association with diatoms as xenosomes or diatoms encased in the shell. The present communication records *Centropyxis aculeata* and *Centropyxis arcelloides* shell showing diatom frustules as xenosomes as these are agglutinated forms. However, *Arcella discoides* and *Arcella vulgaris* forms autogenous proteinaceous shells and therefore, the diatom frustules are seen encased inside the shell. This could have been possible while feeding on diatoms.

Similar study from India conducted on the Deccan lacustrine intertrappean sediments recorded diatom frustules within the rhizopods (thecamoeba). All the diatoms recorded were pennate forms encased in the shell but the detailed morphology was not identifiable (Singh *et al.*, 2007). The present records of thecamoebians in association with Permian sediments is however not completely identifiable but resembles the extant *Nitszchia* type of diatom.

Several impressions of diatom frustule fragments appear to form the xenosomes of *Centropyxis aculeata* and *C. arcelloides* shell in Early Permian sediments. However, only *Arcella* species were recorded from Late Permian which shows morphological similarity with the diatom frustules encased inside the shell which could not be identified taxonomically.

The obscure morphological features of diatoms in association with the thecamoebians could be for two reasons (1) since the diatoms form the diet of thecamoebians it is likely that the morphological features are deteriorated (2) In the case, where diatoms form xenosomes of shell it becomes difficult to disaggregate them from the shell. The older sediments bound with siliceous matrix are to be dissolved in Hydrofluoric acid followed by nitric acid before any fossil specimen is retrieved. During this process, most of the morphological features become obscure but the impressions of diatoms are clearly visible. This was confirmed with the acetolysis process carried out in modern lake sediments to record the extant thacamoebian forms and its association with diatoms. In extant forms, it has been seen that the sediment deposited in warm areas of India do not show diatom frustules in shells of thecamoebians (Farooqui *et al.,* 2010, Kumar *et al.*, 2011). However, present study reveals that the modern lake sediments from colder temperate areas in India show diatom frustules either encased in autogenous thecamoebian shell or as xenosomes in agglutinated shells of thecamoebian.

The present study reveals that although, both tropical and temperate lake sediments have fairly good percentage of diatoms but, its preservation in association with the shells of thecamoebians is rarest in warmer conditions and more prominent in thecamoebians retrieved from sediments deposited in cooler environment.

The demise of biogenic silica factories during Permian time was an event associated with much warmer oceanic and continental climatic conditions but the position of India during this period was in the cool temperate zone close to the southern pole (Antarctica) and therefore, it is likely that the availability of nutrients and cold- oxygen-rich environment supported diatom community and either had a chance to contribute as xenosomes in thecamoebian shells or encased inside the autogenous shell. Therefore, the lack of diatom record from Palaeozoic could be 1) diatoms were probably less silicified and could have escaped preservation and 2) loss of specimens during retrieval while digestion of siliceous rock matrix.

Figure.1 Map showing location of sample collection from Bhalsu-sunu kothi-khundi Maral Section in Chamba District, Himachal Pradesh, Manjir Formation- Early Permian (NP3 12)

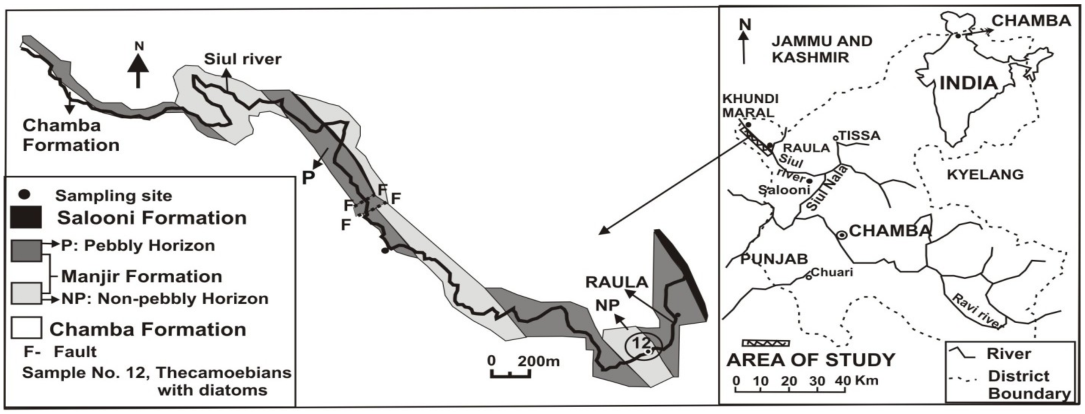


Figure.2 Map showing location of sample collection from Lingala-Koyagudem coalbelt of Godavari Graben, Andhra Pradesh: Late Permian (Borecore MGK-6)

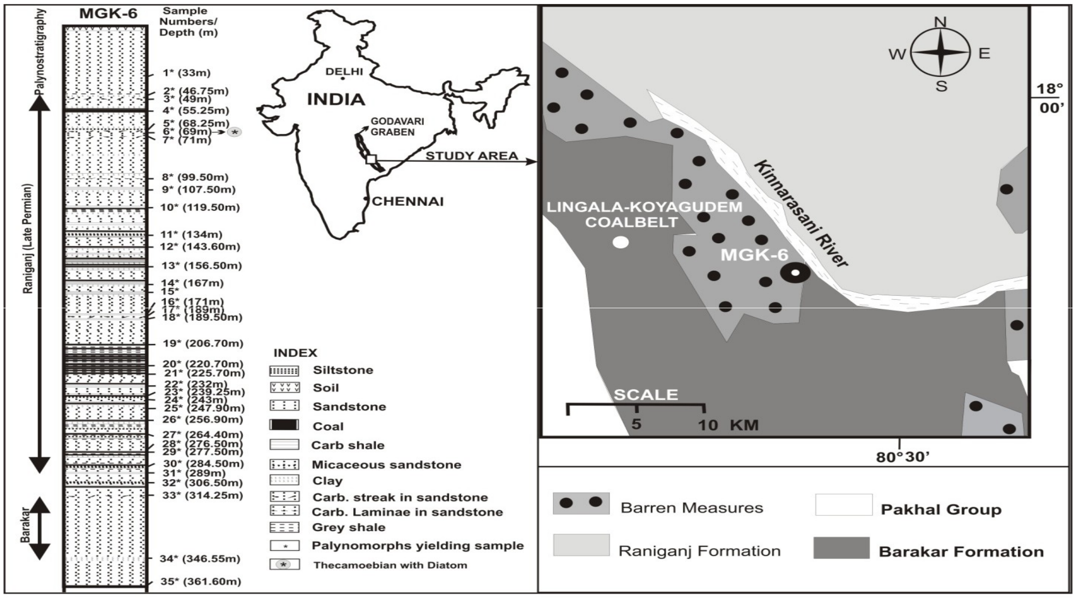


Figure.3 The global position of the land mass and location of India in cool temperate zone during Permian period. (Modified after, Smith *et al.*, 1981)

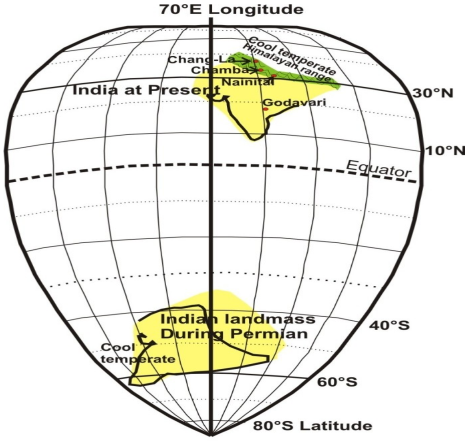


Plate.1 Extant specimen from Chang- La Lake: A-*Arcella discoides* aboral view showing pennate diatom frustules (arrow mark);B- *Arcella excavata* showing two diatom frustules; Extant specimen from Naini Lake: C & D- *Centropyxis aculeata* showing diatom frustule impressions (acetolysed). E. *Centropyxis arcelloides* showing diatom frustules (arrow mark); F. *Centropyxis arcelloides* showing frustule of *Cymbella* species.

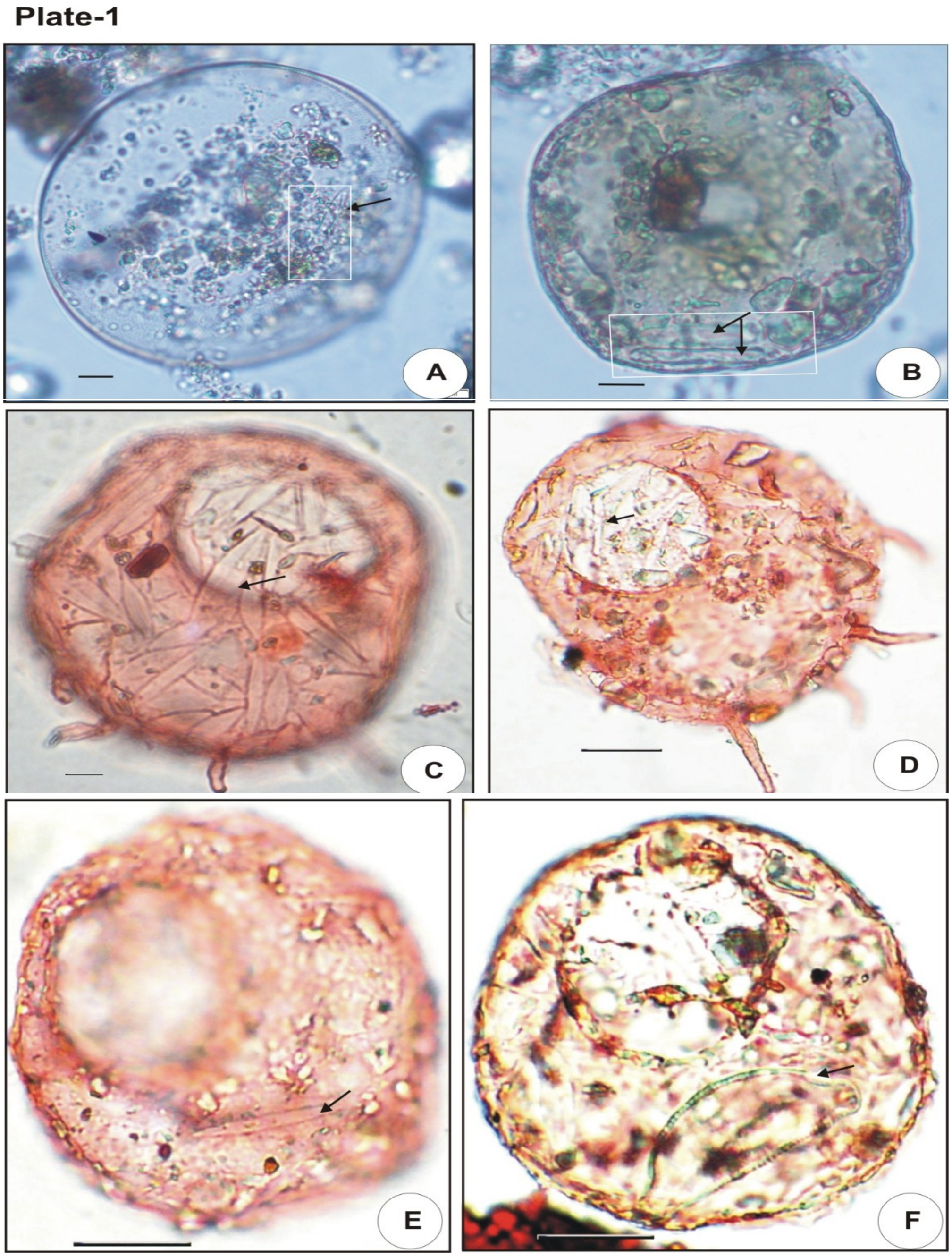


Plate.2 Fossil Specimen from Early Permian sediment (BSIP slide no. 13964, G25/3 from Bhalsu-sunu kothi-khundi Maral Section in Chamba District, Himachal Pradesh); G- location (a) of the fossil specimen under low power along with the Permian palynomorph. H- An enlarged view to show the pennate diatom frustules as xenosomes on the shell/test of *Centropyxis arcelloides*. I. An enhanced structure of the diatom as xenosomes on the shell. J (BSIP slide no. 12865A, G18 & K (BSIP slide no. 12865A, U38/3)- *Centropyxis aculeata* showing numerous fragments of diatom frustules as xenosomes in the shell

Plate-2

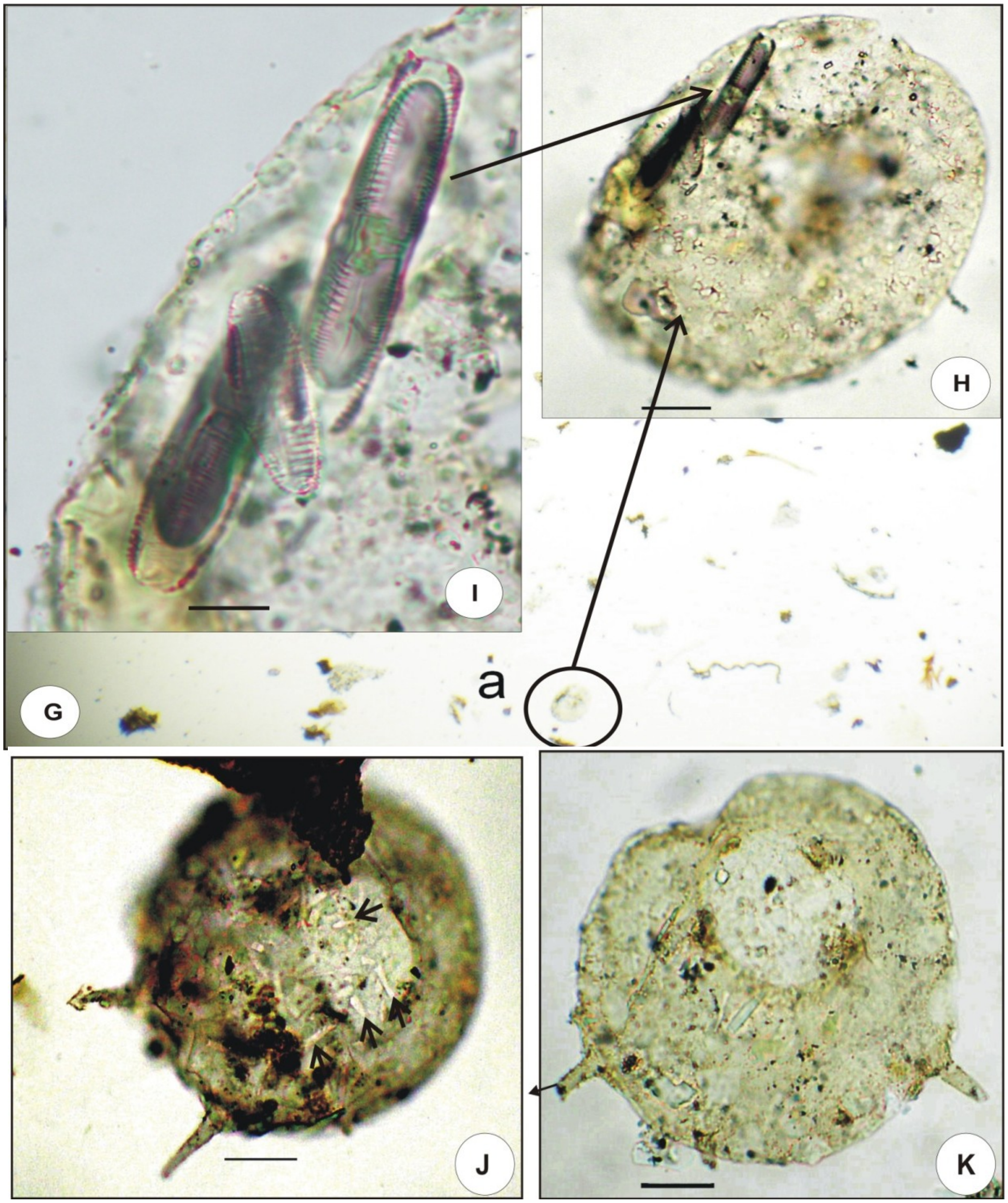
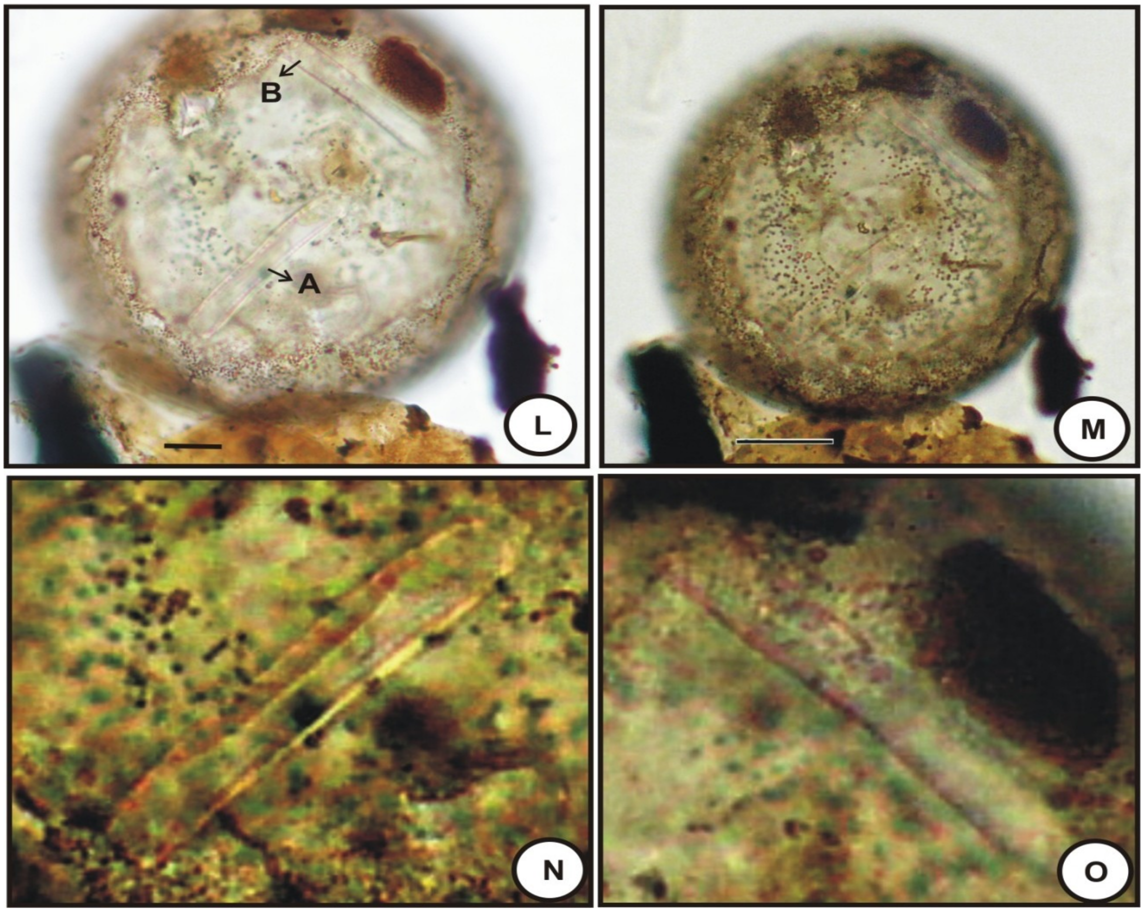


Plate.3 Fossil specimens from Late Permian (BSIP slide no. 13577, Q61 Lingala-Koyagudem coalbelt of Godavari Graben, Andhra Pradesh): L- *Arcella vulgaris* (aboral view) showing two pennate diatom frustules (A & B); M- *Arcella vulgaris* (Oral view) same specimen; N & O enlarged view of diatom

Plate-3



The present work illustrates the impressions of diatom frustules bound by organic cement in the tests of extant thecamoebians even after the acetolysis process (involving 40% Hydrofluoric acid). The fossil specimens were also retrieved after acid treatment and therefore the impressions of diatom frustules are apparent. This provides clue to the existence of diatoms during the Permian period because the xenosomes that form the shells comprise of the material available in the habitat in which thecamoebian lives in. The 'faithfulness' of the sedimentary record from Permian period allows us to report the first evidence of freshwater diatoms in thecamoebian shells.

Since many thecamoebian tests are autogenous and are made of acid-resistant proteinaceous material, they occur in the palynological preparations of fossil sediments. It has been observed that dilute usage of Hydrofluoric acid (20–30%) for 1–2 days do not dissolve the silica content fully. Under such conditions, the samples treated with HF reveal the outline of diatoms as well as observed in extant specimens. This processing (with dilute HF) is necessary for the older sediments in order to remove the siliceous matrix gently and retrieve fossil specimens, particularly siliceous components. Until now, due to dissolution of silica matrix in rocks, it is quite possible that the less silicified Palaeozoic diatom records have been lost during the process or overlooked. It is suggested that careful observation of xenosomes e.g., diatom frustules agglutinated to tests of thecamoebians in palynological slides could potentially lead to new discoveries of microfossils from Phanerozoic sediments. It has been reported that during the Palaeozoic era due to limited resource of silica the Radiolarians and the diatoms were less silicified. Since silica recrystallizes under pressure, any older diatom fossils may have been destroyed (Kaminski *et al.*, 2010). Diatom dissolution in lacustrine and marine systems has shown that pH, temperature, salinity and ionic strength are all important factors (Ryves *et al.*, 2006). Until now the absence of Palaeozoic diatom records could be the consequence of sampling, processing or preservational bias. However, the ultimate causes of these trends are still uncertain, and may be partially attributable to observer biases.

The present record reveals the possible clue to the existence of diatoms during the Permian period. This age period falls between ~251- 299 Ma which covers the Late to Early Permian, respectively. As the thecamoebians survived Permian-Triassic Boundary mass extinction, it is inferred that the less silicified diatoms too survived this event.

Until now the 'faithfulness' of sedimentary records depicting the existence of diatom prior to permo-triassic boundary has not been assessed. Here we studied the archival material with the extant source thecamoebian communities unraveling the environmental conditions during Permian favouring the existence/survival of both thecamoebians and diatoms together. Thus, the present study provides a first clue to the existence of diatoms during Permian period (~251- 299 Ma).

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