(*Pi9*) from *O. minuta*[16], bacterial blight resistance from *O. longistaminata*[17] and *O. rufipogon*[18], and rice tungro disease resistance from *O. rufipogon* showed broad-spectrum resistance to different lineages of the rice diseases[2]. The breakdown of resistance in modern, high-yielding varieties after a few years of cultivation was attributed to fast-changing pathogens. Introgression of such novel genes from wild species is considered a better approach for introducing stability in resistance against diseases/pests.

The present findings suggest that rice gene pools can be broadened to improve several traits, including yield and disease/insect pest resistance by introgressing the novel genetic variations from diverse primary and secondary gene pools through wide hybridization. The present investigation also indicates that for introgression of multiple genes of agronomic importance from closely related wild species into cultivated rice, a better approach would be one or two backcrosses followed by selective intermating in segregating generations to break the undesirable linkages for superior recombinants. Such a strategy may be useful for introgressing complex traits such as yield from wild rice. Considering its yield superiority over national checks with resistance/tolerance to multiple diseases, the introgression line C 11-A-41 (IET 15358) released as variety Dhanrasi in 2002 for cultivation in irrigated shallow lowlands of Andhra Pradesh, Tamil Nadu and Karnataka and in rainfed lowlands of Maharashtra, is an example utilizing wild rice for improvement in yield and multiple disease resistance.


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# Arcellaceans and pollen/spores of a late Harappan settlement near Porbandar, west coast of India: Implications for palaeoecology and environmental monitoring

**Anjum Farooqui[1,*] and A. S. Gaur[2]**

[1]Birbal Sahni Institute of Palaeobotany, 53, University Road, Lucknow 226 007, India
[2]National Institute of Oceanography, Dona Paula, Goa 403 004, India

**Archaeological sites have been a storehouse of information on various aspects of the past, including climatic conditions and hydrological characteristics in a particular time bracket. Recent excavation near Porbandar has brought to light a late Bronze Age settlement close to Porbandar creek. The archaeological artefacts have close similarities with those of other Harappan settlements in the Saurashtra region. Soil samples were collected and analysed for pollen content. The samples show shallow riverine depositional environment, which was influenced by relative sea-level rise and fall corres-**

*For correspondence. (e-mail: afarooqui_2000@yahoo.com)

**ponding to warm and dry climatic periods. The area was devoid of vegetation except anthropogenic *Sesbania* plantation in the middle of late Holocene. This communication also elucidates the role of Arcellaceans (testate amoebae) for analysing palaeoecological and climatic hydroperiods during the human settlement (early to mid 2nd millennium BC) around Porbandar region.**

**Keywords:** Arcellaceans, climate, ecology, late Harappan, pollen/spores, Saurashtra coast, sea-level changes.

CLIMATIC conditions have been largely responsible for the dawn and devolution of any civilization across the world. Major civilizations of the world came into existence during the mid-Holocene period (6000–4000 yrs BP), and this period was marked with moderate climatic conditions[1,2]. However, climate has changed the coastal hydrology since then, and people have adopted to such changes and migrated suitably for survival. Archaeological sites have been the storehouse of several proxies to determine the past climatic conditions, such as raw materials for construction of houses, use of tools, remains of grains, etc. However, pollen/spore remains[3] and Arcellaceans are the direct proxies for the climatic and hydrological conditions in a particular region and time[4], especially in the coastal wetlands[5].

Studies of testate amoebae till date emphasize species from Europe, Africa and China[6–8], South America[9], Brazil[10,11] and from Priyadarshini lake in Antarctica[4]. However, no data about testate amoeba are available from India. This communication reports testate amoebae from India in sediments deposited during the late Holocene. Coastal wetlands and riverine systems are vulnerable to frequent hydrological changes induced by climate and sea-level fluctuations. The observations here bring to light the morphology, ecology and distribution of Arcellaceans useful for palaeoecological studies in coastal sediments.

The archaeological site is located adjacent to the Porbandar creek on the western side (Figure 1). Presently, the site is under cultivation. Further west of the excavated site, a few small limestone cliffs are noticeable indicating a higher sea level in the past. There is a well-defined depression around the site towards the creek, which is presently banked by high metal road. According to a local tradition, near the present creek there was a 'Juna Dhakka' (ancient jetty), which indicates that the site was close to a jetty in the past. Location of the study area clearly favours the utilization of the creek for maritime activities during the Bronze Age around Porbandar.

Four locations were selected for excavation to assess the settlement extension and total occupational deposits. Trenches I to III have an average occupational deposit of 60 cm, whereas the fourth trench dug close to the Porbandar creek has a deposit of 103 cm. The important artefacts are described below. Sediments samples for pollen analysis were collected from all trenches with a 10 cm regular interval. Structural activities of settlement include a few post-holes (Figure 2) and a stone structure built of small irregular limestone pieces. These evidences indicate that thatched-roof houses were in use at this site.

A large number of pottery has been recovered during the excavation. The important ceramic assemblages are red ware, black ware, buff ware and grey ware. Bowls of different sizes are the major attraction of the excavation besides a large number of shards of jars, lids, basins and other pots. The shape, size and paintings are similar to the pottery reported from Bet Dwarka[12], Rojdi[13], Lothal[14] and Kuntasi[15]. The important paintings on the potshards are roundels, wavy lines, cross lines and thick bands on the rim portions.

Other important artefacts include four stone tools, including a blade, point, fluted core and flake. Three of them are

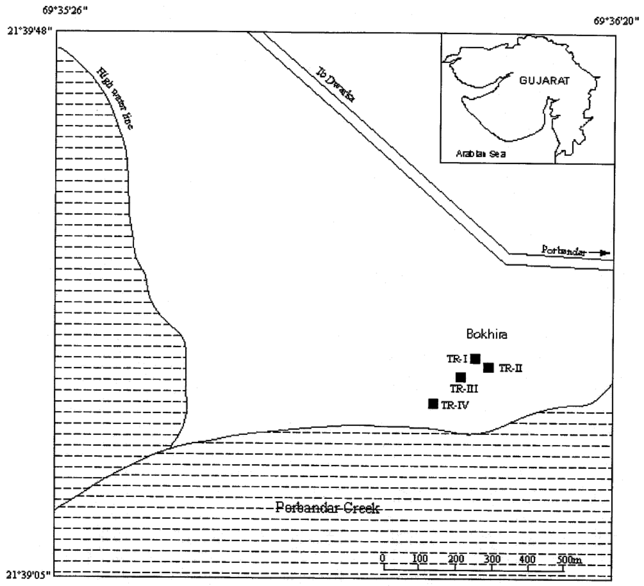


**Figure 1.** Location map of the study site.

**Figure 2.** Excavation site near Porbandar.

made of milky quartz and one is of crystal. Four terracotta beads, two balls, three sling balls besides one copper finger-ring and two terracotta artefacts are some of the important findings from the excavation.

For palynological study the air-dried samples were treated with 10% potassium hydroxide (KOH) for 5 min in a sand bath. The sediment was sieved (mesh No. 400 with pore size 0.037 mm) and filtrate acetolized following[16]. During this process the samples were treated with glacial acetic acid followed by treatment with a mixture of anhydrous acetic acid and sulphuric acid (9 : 1 ratio) and centrifuged (2000 rpm) to decant the supernatant. The samples were finally washed with glacial acetic acid followed by distilled water and centrifuged to decant the supernatant. The centrifuged sample was then stored in glycerine and distilled water (1 : 1 ratio) for study under light microscope (Olympus, BX-52). The shell morphometry of testate amoebae recorded here was studied while analysing palynological slides, unlike the normal method of studying Arcellaceans in the sediment under stereomicroscope after staining with Bengal Rose[8]. Thus, all the shells recorded here are acid-resistant (acetolysis) and of organic matrix. The photoplates shown are digital photomicrographs and the scale bar is 10 μm. The pH of the aqueous soil solution was measured using a pH paper (Merck).

Majority of testate amoebae are cosmopolitan and inhabit mainly freshwater pools, moist mosses and rarely soil litter, and more than 130 taxa of the genus have been described till now[17,18]. *Arcella* are strictly a freshwater dwellers and feed on algal mass. They produce a decay-resistant wall, or shell that protects the cell from desiccation. The shell may be proteinaceous, siliceous or calcareous and may incorporate extraneous materials such as fungal hyphae, diatoms and mineral grains[19–21]. It is a good indicator of a healthy environment, or one which is in the process of natural remediation. These are called ecophenotypes as they change their gross morphology depending upon the environmental conditions in which they are found[5].

They form an agglutinated test (microscopic shell) from tiny grains of sand or other usable materials[22], while some are exclusively with organic cement material[8]. Test is sub-spherical to spherical, or oval to elliptic in apertural view. Circular aperture is of variable size rimmed with lobopodium or pseudopodia in varying numbers and highly invaginated. The different types measured from 20 to 100 μm in size. In *Centropyxis*, a number of lobes are present lining the rimmed aperture. Scanty data about their morphology, biometry, distribution and ecological preferences for many of them exist in the literature[23]. However, morphometry related to environmental changes is scanty.

Presence of *Arcella* and *Centropyxis* was recorded at 40 cm depth in trench I, associated with amorphous organic matter (MOA), pseudoschizae and chytrids (Table 1). However, sediments at 30 cm depth show occurrence of toxic algae *Chatonella marina* and *Botryococcus* degraded mass. At 20 cm, pollen grains of *Sesbania* indicate its anthropogenic plantation. Few pollen grains of Cheno/Ams and Vescicular Arbuscular Mycorrhiza (VAM : soil fungi) suggest weak climatic hydroperiods and run-off from land. At 10 cm, low percentage spores of *Tilletia*, *Thecaphora* and VAM fungi indicate slow influx of freshwater from agricultural land. Significant variation in pH of the aqueous soil solution was observed (Table 1). Low and high pH values correspond to the occurrence of *Arcella* species and *C. marina*. Results show dominance of photosynthesizing algae and aquatic plant tissue that grew under weak fluvio-lacustrine ecosystem. Increase in pH by 9.2–10 was perhaps due to marine incursion, which is indicated by the appearance of *C. marina* and disappearance of freshwater *Arcella vulgaris* and *Centropyxis* spp.

The testate amoebae *Arcella* (Types I–III) and *Centropyxis* were recorded from trench II at 40 cm depth (Table 2). Pollen/spore assemblage throughout in the section does not show any marked change. However, at 20 cm depth *Arcella excavata* (Type IV) and Type VIII were recorded, where pH of the aqueous soil solution was 7.2. Rare

**Table 1.** Palynological record in soil sediments from trench I, Bokhira, Porbandar, Gujarat

| Depth (cm) | Testate amoebae | Algae | Pollen/spores | pH | Ecosystem | Climate/sea-level fluctuation |
|---|---|---|---|---|---|---|
| 10 | Nil | Spirogyra | Poaceae, Cheno/Ams, Chytrids, Tilletia, VAM fungi | 9.2 | Lotic | Dry and arid<br>No marine incursion |
| 20 | Nil | MOA | Sesbania, Cheno/Ams, VAM fungi | 10 | Fluvio-lacustrine (lotic) | |
| 30 | Nil | *Botryococcus* and *Chatonella marina* | Cheno/Ams, Chytrid fungi | 9.8 | Fluvio-lacustrine with marine incursion | Wet and humid<br>Relative rise in sea level |
| 40 | *Arcella* (Types I–III) and *Centropyxis* (Type IV) | MOA | Pseudoschizae | 6.2 | Fluvio-lacustrine (lotic) | Wet and humid<br>No marine incursion |

**Table 2.** Palynological record in soil sediments from trench II

| Depth (cm) | Testate amoebae | Algae | Pollen/spores | pH | Ecosystem | Climate/sea-level fluctuation |
|---|---|---|---|---|---|---|
| 10 | Nil | Spirogyra | Poaceae, Cheno/Ams, Chytrids, VAM fungi | 8.0 | Lotic | Dry and arid<br>No marine incursion |
| 20 | *Arcella excavata* and Type VIII | MOA | Sesbania, Cheno/Ams, Pseudoschizae, VAM fungi | 7.2 | Swampy | Moderate<br>No marine incursion |
| 30 | Nil | *Botryococcus* and *C. marina* | Cheno/Ams, Chytrids, Trilete fern spores | 10 | Fluvio-lacustrine with marine incursion | Wet and humid<br>Relative rise in sea level |
| 40 | *Arcella* (Types I–III) and *Centropyxis* (Type IV) | MOA | Chytrid mycelium and spores, VAM fungi | 6.4 | Fluvio-lacustrine (lotic) | Wet and humid<br>No marine incursion |

**Table 3.** Palynological record in soil sediments from trench III

| Depth (cm) | Testate amoebae | Algae | Pollen/spores | pH | Ecosystem | Climate/sea-level fluctuation |
|---|---|---|---|---|---|---|
| 10 | Nil | MOA | Poaceae, Cheno/Ams, Pseudoschizae, VAM fungi | 9.0 | Swampy | Dry and arid<br>No marine incursion |
| 20 | Types V–VII and IX | MOA and *C. marina* | Cheno/Ams | 9.0 | Swampy | Moderate<br>Marine incursion |
| 30 | Type IX | *Botryococcus* and *C. marina* | Cheno/Ams | 8.4 | Fluvio-lacustrine with marine incursion | Wet and humid<br>Relative rise in sea level |
| 40 | *Arcella* (Types I–III) and *Centropyxis* (Type IV) | MOA | Pseudoschizae, Poaceae, Chytrids, VAM fungi | 6.8 | Fluvio-lacustrine (lotic) | Wet and humid<br>No marine incursion |

occurrence of Annelid microworms and some unidentified bodies having lineage to other protists were also recorded at this depth. No Arcellaceans were recorded at 10 cm depth.

No Arcellacean was recorded at 10 cm depth from trench III, which is similar to the results in trenches I and II (Table 3). However, at 20 and 30 cm depth, Arcellaceans (Types V, VI, VII and IX) along with *C. marina* were recorded. Pollen spore assemblage is the same as in other trenches. Results suggest freshwater–brackish water ecosystem of low energy and stabilized environmental conditions. The pH of the aqueous soil solution was high. At 30 cm depth, terrestrial fungal spores of *Tilletia* are few. Parts of insects and egg shells along with freshwater *Arcella* (Type IX) and young colonies of *Botryococcus* were recorded. Presence of *C. marina* in few numbers was recorded. Average pH of the soil solution was 8.4. At 40 cm depth, MOA derived from other forms of algae shows abundance, suggesting limnic conditions and nutrient transport from land. Only stray pollen of Chenopodiaceae was recorded. Aquatic fungal spore and hyphae along with Pseudoschizae (algal cyst) suggest shallow freshwater ecosystem. Presence of *Arcella* (Types I–III) and *Centropyxis* was recorded in good percentage.

The pollen/spore assemblage is similar from 10 to 30 cm depth in trench IV (Table 4). Increased MOA is recorded and low percentage of black unidentified debris (BUD). However, high percentage of BUD from 40 to 60 cm depth indicates more clastic influx, suggesting high-energy fluvial system. Terrestrial pollen/spores are low in number but aquatic fungi dominate, suggesting its association with algal growth or other aquatic plants. The average pH of the soil solution in the entire section was acidic (5.2–5.8). At 20 cm depth, rich MOA is light coloured to colourless, showing heterogeneous black inclusions. From 30 to 60 cm depth BUD is of comparatively bigger size, suggesting its transport from land in the fluvial ecosystem. Abundant fungal spores belonging to Chytridiales suggest high productivity of *in situ* aquatic plants. Algal cysts of unknown origin were also observed. Results suggest clastic input from land under high water energy conditions. But, no *Arcella* or *Chatonella* species

**Table 4.** Palynological record in soil sediments from trench IV

| Depth (cm) | Testate amoebae | Algae | Pollen/spores | pH | Ecosystem | Climate/sea-level fluctuation |
|---|---|---|---|---|---|---|
| 10 | Nil | Dominance of MOA derived from algal mass | Poaceae, Cheno/Ams, Chytrids, VAM fungi | 5.2 to 5.8 | Swampy, recharged during climatic hydroperiods | Dry and arid No marine incursion |
| 20 | | | Sesbania, Pseudoschizae | | | Moderate No marine incursion |
| 30 | | | Cheno/Ams | | | Wet and humid |
| 40 | | High percentage of | Poaceae, | | | No marine incursion |
| 50 | | black oxidized debris | Cheno/Ams, | | Fluvio-lacustrine | |
| 60 | | | VAM fungi, Pseudoschizae | | | |

were recorded throughout in the trench, suggesting that although the substrate was moist the pH did not support its existence.

Palynological analysis reveals a poor assemblage of pollen/spores in all the trenches (Tables 1–4), except low pollen percentage of *Sesbania grandiflora* (short tree), which was recorded at 20 to 30 cm depth in trenches I, II and IV. The results indicate that since the edaphic and climatic conditions did not support natural vegetation, the area was used for *Sesbania* cultivation as this short tree has economic and medicinal value[24] and is generally used as fodder[25] and to increase soil fertility by fixing nitrogen. An outstanding feature is its tolerance to both saline and alkaline soil conditions[26]. Low pollen count of *Sesbania* in the studied sediment is because it is bird-pollinated[27].

Freshwater green algae like *Spirogyra* and *Botryococcus* were also recorded as shown in Tables 1–4. Presence of Pseudoschizae and photosynthesizing algae indicates shallow fluvio-lacustrine to swampy ecosystem recharged by seasonal influx of freshwater. Presence of soil fungi (VAM fungi) indicates its transport from land during seasonal hydroperiods. Chytrid fungi that are generally saprobes on algal and aquatic plants and Arcellaceans also inhabit the sapropele among aquatic plants[23].

Some of the testate amoebae (Arcellaceans) are exclusively made up of organic cement material[8]. During palynological study, amoebic tests showed morphometric changes in succession through a sedimentary section. These were readily stained by saffranin, which suggests that the recorded testaceans in the present study are made up of organic matrix which is acid-resistant (acetolysis) and therefore, easily identified in palynological slides. On the basis of gross morphology, apertural size and structure, we define nine types in the present study. While *Arcella vulgaris* (Types I–III) and *Centropyxis* (Type IV) are confined to normal pH (i.e. 6.2 to 6.8), *A. excavata* (Type V) is confined to slightly higher pH (7.2 to 8). Arcellaceans described under Types VI, VII, VIII and IX are confined to even higher pH, extending above 9.0. The gross morphology, however, changes with increase in pH indicated by decreased apertural size and small-to-obscure lobopodium in contrast to types I to IV, where the aperture is bigger in size and the lobopodium or pseudopodium shows distinct morphology. The shell morphology shows similar depressed reticulated matrix, except that the grains are more compact (Figure 3). Apertural size, and number and shape of the lobopodium or pseudopodium vary among the different types (Figures 3 and 4). These are inconsistent features in Arcellaceans, which can be effectively used for monitoring climate and sea-level-induced palaeoecology in coastal wetlands. Species-specific environmental tolerance of testate amoebae (Arcellaceans) from fossil data has been reported earlier[21,28,29].

During palynostratigraphical analysis it was observed that relative rise in sea level increased the salinity of the ecosystem, replacing different forms of Arcellaceans. It is suggested that either these forms (types) are modifications in adverse ecological conditions, or different species which are specific to higher pH and dry substrate. In either case the successive morphometric changes of Arcellaceans recorded in palynological slides from a sedimentary section serve as good biomarkers of marine incursion in freshwater ecosystems in coastal areas. The most variable character is the aperture diameter, which is attributed to changes in microhabitat[30]. Earlier records show that fluctuation in their population is attributed to moisture content of their habitat[31]. Other factors like pH[32,33], eutrophication[34], temperature[35], light, oxygen and food availability[36] control the testacean community structure. Above all, the short generation time of Arcellaceans makes them useful indicators of environmental and palaeo-environmental changes[5]. These occur abundantly in Holocene lacustrine sediments and have been successfully used to reconstruct Pleistocene–Holocene lacustrine palaeoenvironments[5,37]. Sensitivity of Arcellaceans to variation in hydrological characteristics of the habitat type (running vs standing water) and hetero- or homothermic ecosystems is yet another special feature[38], which makes them potential biomarkers of palaeohydroperiods, climate and temperature variability.

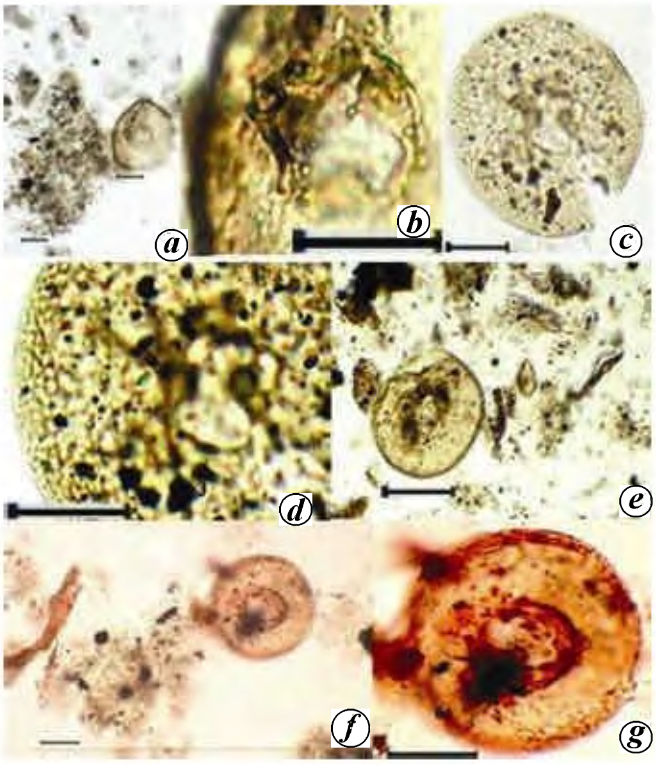


**Figure 3.** Light microscopic photomicrographs of testate amoeba (Arcellacean) species. ***a***, *Arcella* (Type I) along with amorphous organic matter (MOA) derived from algal mass and aquatic plants; ***b***, Enlarged view of the shell showing fine reticulated depressions. Aperture size is 10 μm and four short, slightly lobate pseudopodium are arranged in an irregular crown; ***c***, *Arcella* (Type II) showing firm circular shell body made up of organic matrix and many irregular inclusions; ***d***, Apertural size is less than 10 μm, lobopodium 2–3 and is dumble-shaped in an enlarged apertural view; ***e***, *Arcella* (Type III) associated with scattered MOA showing firm circular shell body measuring only 20 μm with 2–3 lobopodium, and the aperture is only 5 μm; ***f***, *Arcella* (Type IV) showing association with MOA and the shell body is stained with saffranin; ***g***, Lobopodium is continuous and 5 to 6 in number forming a crown and the aperture is more than 10 μm. The size of the body is 35 μm (Scale: 10 μm).

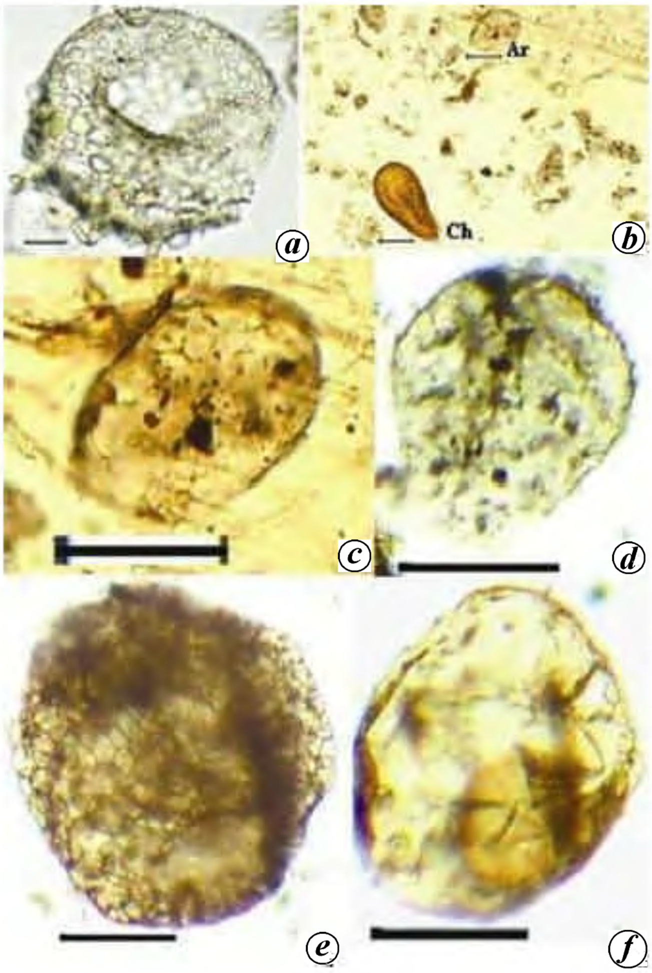


**Figure 4.** ***a***, *Centropyxis* species (Type V) is subspherical to spherical. Circular in apertural view, five conical spines inserted from the mid-line to the abapertural end surface of the test show aggregated particles which are acid-resistant (acetolysis process) organic matrix that take the stain of saffranin; highly areolar. Aperture measures 20 to 25 μm, circular, rimmed with fine denticulate lobes. Highly variable in size (70–120 μm). ***b***, Amorphous organic matter, *Chatonella marina* (Ch), Arcellacean (Ar) (Type VI); ***c***, Enlarged view (Type VI) showing elliptical shelled test with surface reticulate depressions and particle aggregates. Aperture is about 1 μm and obscure. Small lobate lobopodium forming dumble shape as in Type II; ***d***, Testate amoebae (Type VII) showing small depressions in shell outline with obscure aperture; ***e***, Testate amoebae (Type VIII) aboral view showing irregular test outline forming a reticulate organic matrix adhering particle aggregates. Aperture is small and dumble-shaped; ***f***, Testate amoebae (Type IX) showing sculptured test with small triangular, highly obscured aperture.

Presence of *C. marina* has been reported earlier[39] from a river mouth in the Malabar coast. It is mostly confined to river mouths where salinity is liable to increase due to marine incursions[40]. Therefore, the present study indicates a riverine system and its nearness to the shoreline which was influenced by marine incursion only once during the late Holocene corresponding to wet and humid climate. It is well known that from 3000 to 2000 BC, a cooling trend occurred. This cooling caused a large drop in sea level and the emergence of many islands (Bahamas) and coastal areas that are still above sea level today[41]. Retreat of sea level and dry/arid climate could have been one of the reasons for the decline of the Harappan civilization. Thereafter, a short warming trend took place from 2000 to 1500 BC, followed once again by colder conditions. The marine incursion with warm and humid climate observed in the present study, thus corresponds to this period of late Holocene. The presence of photosynthesizing algae – *Botryococcus* and *Ankistrodesmus* – (which grows in brackish water–freshwater ecosystem) during the spring season after winter hydroperiods suggests the dominance of the northeast monsoon in the middle of late Holocene coupled with relative rise in sea level. Successive changes in the type and morphometry of Arcellacean assemblage throughout the sedimentary section show sensitivity to pH and substrate moisture, indicating ecological changes induced by sea-level rise and climate in the past. Testate amoebae, therefore, have several strengths as environmental indicators during palynological analysis relevant to both monitoring and palaeoecological applications, especially in the less vegetated coastal areas.

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