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ULTRASTRUCTURE OF A LOWER EOCENE LEAF SURFACE IMPRESSION IN AMBER, VASTAN LIGNITE MINE, GUJARAT

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ABSTRACT

Scanning Electron Microscopy of fossil leaf surfaces imprinted on amber nodules illustrate the potential of this technique in understanding the fine resolution details at the cell level including the density distributions of stomata and trichomes. Isolated trichomes are commonly found in certain ambers but are here described in a spatial cuticular context. Stomatal distribution and density are also recorded.

About 15 fragments of an unidentified fossil leaf from the Vastan Lignite Mine, near Surat were studied and provide morpho-structural details of the cuticular surface, mode of preservation and an interpretation of palaeoecological conditions and depositional palaeoenvironments. Using multi-imaging techniques it has been possible to obtain three-dimensional images of body fossils as well as surface impressions of plant and insect remains from the same material.

Keywords: Ultrastructure, leaf surface, Lower Eocene, Amber, Vastan Lignite Mine, Gujarat

INTRODUCTION

The Vastan Lignite Mine, situated about 30 kms in a north-easterly direction from Surat has yielded several interesting biotic remains (Rose *et al*., 2006; Bajpai *et al*., 2005; Rana *et al*., 2004; Rana *et al*., 2005) including those in amber nodules (Alimohammadian, *et al*., 2005, Alimohammadian, 2006) from the upper coal seam which is being actively mined. Other older coal seams in this open cast mine also yield amber of a clearer and better quality but so far no biotic elements have been reported from these. Scanning Electron Microscopy of fossil leaf surfaces imprinted on amber nodules illustrate the potential of this technique in understanding the fine resolution details at the cell level including the density distributions of stomata and trichomes. Isolated trichomes are commonly found in certain ambers but are here described in a spatial cuticular context. Stomatal distribution and density are also recorded.

About 15 fragments of an unidentified fossil leaf from the Vastan Lignite Mine, near Surat were studied and provide morpho-structural details of the cuticular surface, mode of preservation and an interpretation of palaeoecological conditions and depositional palaeoenvironments. Using multi-imaging techniques it has been possible to obtain three-dimensional images of body fossils as well as surface impressions of plant and insect remains from the same material. During two field seasons between November 2004 and in early 2006, two of the present co-authors (Sahni and H. A.) were part of a team that collected amber nodules from the upper coal seam (Fig. 1). The material was picked and sorted for insect, spider and plant remains and the present leaf impression is part of this collection. Most of the nodules range up to 5 cm in length and are found bedded in specific layers in the upper coal (Fig. 2a).

Resins when fossilized and polymerized, turn to amber which often has inclusions of biotic and abiotic material, very finely preserved, (Grimaldi *et al*., 2000; Poinar 2004; Rikkinen and Poinar, 2000). The study of the ultrastructural characteristics of the preserved fossils can reveal anatomical details not readily available by other means of preservation (Martinez-Delelos *et al*., 2004; Nguyen Duy-Jacquemin and Azhar, 2004; Nagel, 1997). These amber nodules are commonly found in coals and lignites and occur from the Permian onwards (Alimohammadian *et al*., 2005). Amber is a natural fossilized resin, essentially an amorphous substance which is very brittle and breaks with a conchoidal fracture and oxidizes when exposed to air. It is much harder than recent resin (about 2-3 on Moh's scale of hardness), is insoluble in water and normally occurs in the characteristic amber colour, varying from yellow to deep orange. "Amberization" is a continuous process very similar to coalification and requires an environment for its preservation which would limit oxidation and sub-aerial exposure

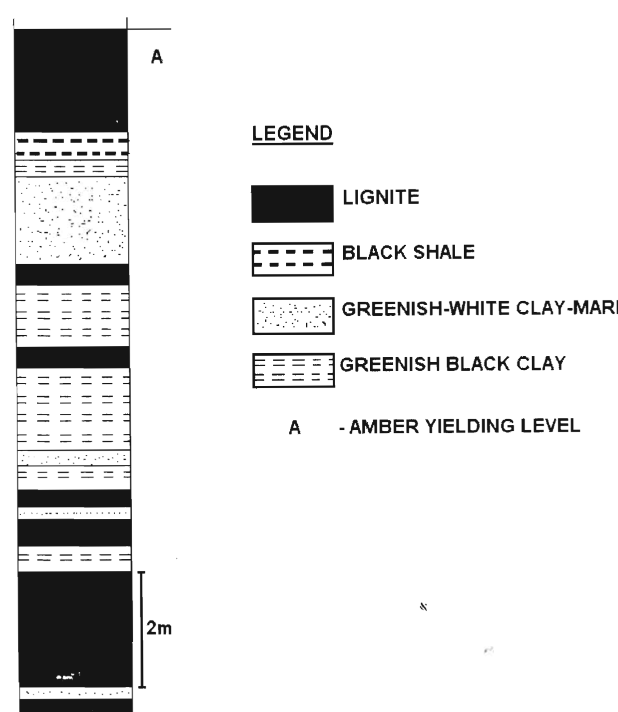

Fig. 1. Lithological section showing amber level in upper coal seam in the Vastan Mine, Gujarat.

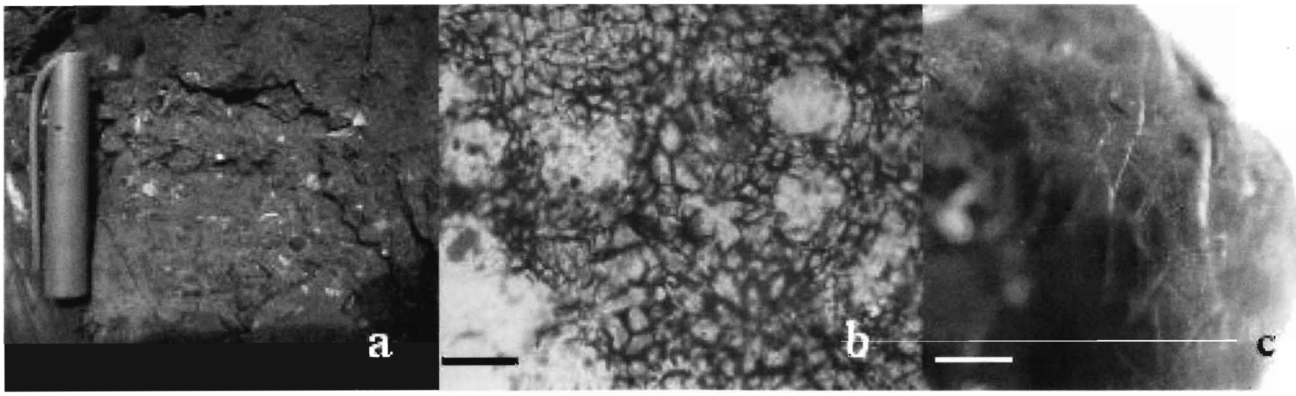

Fig. 2. a. Distribution of amber nodules in upper coal seam. b. Light microscope digital image of one of the presently described fragments of fossil leaf surface in amber, Bar length = 5 um, c. Light microscope image of embedded leaf in amber showing fine microstructure Bar length = 400um (Alimohammadian *et al.*, 2005).

for considerable lengths of time. Most of the worlds amber deposits therefore occur in association with coal deposits (Grimaldi *et al.*, 2000; Stankiewiez *et al.*, 1998).

To identify taxonomic sources of amber, plant remnants composing lignites are sometimes helpful. However, wood or leaf impressions on amber are not uniquely diagnostic of plant genera (Grimaldi *et al.*, 2000). Studies using C^{13} NMR analysis (Lambert 1990; Lambert *et al.*, 1996) diagnose the species of Araucariaceae as a source of all Cretaceous ambers. Chemical identification of amber using biotic inclusions such as stamens, petals and whole flowers has also been undertaken (Langenheim 1969; Hueber and Langenheim 1986; Langenheim, 2003). The presence of trichomes in the presently observed leaf impressions is significant because trichomes are fairly common in certain types of amber (Poinar, 1992.)

GEOLOGICAL AND PALAEONTOLOGICAL SETTINGS

Amber did not become abundant in the worldwide fossil record before the Early Cretaceous, but is found to occur especially in tropical and subtropical forests (Gomez *et al.*, 2002). Based on biostratigraphic evidence, the age of the Vastan lignite is reported as Ypresian (Sahni *et al.*, 2004; Rana, *et al.*, 2005). Early Eocene coal and lignites represent a significant feature of the Western margin of Indian plate prior to the India-Asia collision both in the India and Pakistan (Alimohammadian, *et al.*, 2005).

In the Vastan lignite mines, as in the other observed Palaeocene and Lower Eocene coal mines of Gujarat, amber occurs as nodules ranging upto 4 to 5 cms, in carbonaceous shales, lignites or coal. The carbonaceous shales are usually plant bearing and may or may not contain associated fossil bone material. At Vastan, in the Lower Coal seam, amber is associated with fossil vertebrates and megafloral remains. These include plant fossils, leaves, wood fragments and seeds. The dominant palaeoenvironment that favour the production and preservation of amber nodules is in peat bog or swamp marshland and poorly oxygenated conditions.

Fig. 1 shows the lithology giving the position of the amber layer in the Vastan Lignite Mine.

METHODOLOGY

Preservation of amber is problematic because upon exposure to air, the moisture in cracks evaporates rendering it opaque. The procedure for proper storing of amber is to keep it wet from the time of collection through laboratory preparations.

Amber pieces were cleaned and sorted under a stereo light microscope and each fragmented surface was observed for biotic elements. During this process several hundred biotic elements mostly insect and spider remains were recovered along with inclusions (fluid and gas) that are not biogenic (Berncer and Landis, 1987; Horibe and Craig, 1987; Hopfenberg *et al.*, 1988). To scan a piece for inclusions, opposite sides have to be ground and polished using a high grade emery paper 4/0 grade or polished on a glass plate with aluminium hydroxide. In a previous study, (Alimohammadian *et al.*, 2006) this technique was applied to insect body fossils in amber using SEM methods with SEI, BEI, compositional and topographical approaches with good results. Another commonly used technique is Laser Scanning Microscopy for biotic inclusions giving 3-D images.

The present specimens representing leaf impressions embedded in amber were obtained by sorting under a stereo light microscope. Surface exposure of woody tissue is fairly common and SEM imaging has revealed fine-scaled anatomical

EXPLANATION OF PLATE I

a. Low magnification SEM micrograph of the major part of preserved cuticular surface of amber preserved fragment with mesh and vein cells,
b. Enlarged view showing veinlets with parallely oriented cells,
c. Cuticular surface showing the well preserved structure of trichomes. It should be noted that trichomes are commonly found in some amber deposits such as the Baltic Amber,
d. Cell structure showing the presence of stomata and cell boundaries,
e. Close up of stomatal structure with guard cells and trichomes,
f. Overview of mesh and vein cells with stomata-bearing surface. Bar scales as given.

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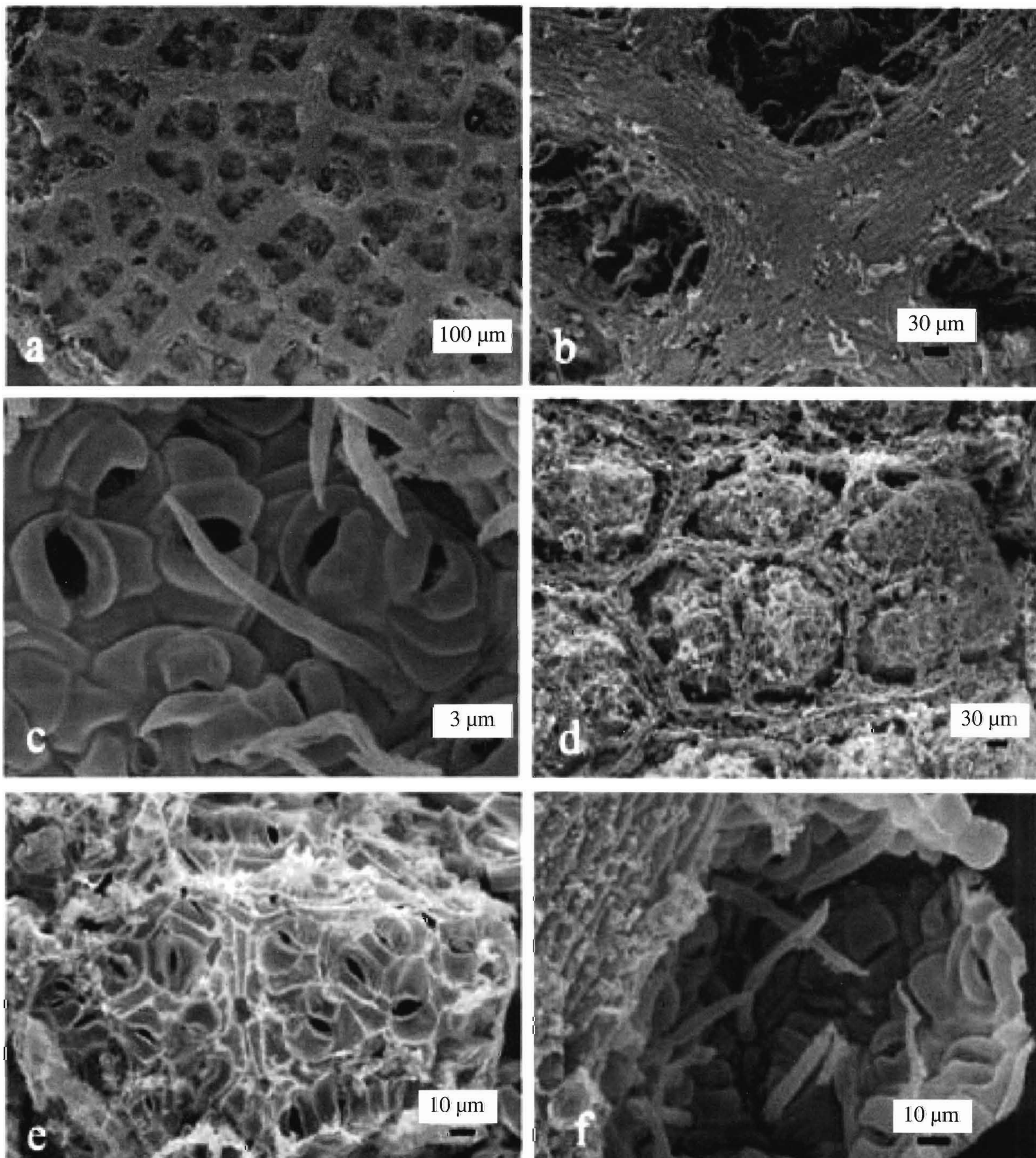
Plate I



SAHNI, SINGH, BAJPAI, AGARWAL, ALIMOHAMMADIAN AND SARKAR details.

Cuticular leaf surfaces are less common but these have been reported by Alimohammadian *et al.*, 2005, earlier. The illustrated specimen also shows cell structure and degraded veins (Pl. I, fig. c).

The present specimens were photographed digitally as well as by conventional light microscopy and were also examined by scanning electron microscopy using a model LEO 430,at 15 KV, at varying magnifications, at the Birbal Sahni Institute of Palaeobotany, Lucknow. About eight millimetric sized fragments each clearly showing surface structure, were examined.

SYSTEMATIC AND STRUCTURAL MORPHOLOGY

The cuticular surface structure of the leaf fragment preserved in amber was examined by light microscopy (Fig. 2b) as well as by scanning electron microscopy (Pl. I). Although the process of amberization has taken place as revealed by ongoing FT-IR and GC-MS analyses of these ambers by one of us (Sarkar), this has not affected the exceptional preservation of the fossil leaf surface. Cuticular structure can be observed at the micrometer scale in light microscopy (Fig. 2b) but it lacks the details of electron microscopy.

Pl. I, fig. a shows a low magnification view of several cells. The mesh cells are variable in shape, largely quadrangular and thin-walled. The vein cells are well preserved forming meshes about 12 to 20 cells in thickness with varying thicknesses for secondary, tertiary and higher order veinlets. Pl. I, fig. b clearly shows a quadri-junctional position with parallely oriented vein cells. The cells of the higher order veinlets are found to truncate at their intersection with lower order veinlets which are about 100 to 150um in thickness. Areoles are well developed, irregular in position, varying in size having a rounded to rectangular shape.

Pl. I, fig. c demonstrates trichome structure with a clearly demarcated circular basal cell located on the epidermis. The trichomes vary in size but are about 30 to 50 um in length. The shaft is cylindrical, ornamented by faint parallel striations. The apical region is acutely pointed and differentiated in this respect from the main shaft.

The structure of the stomata is illustrated in Pl. I, fig. 8c-f. There is considerable density variation in a spatial context. The stomata are randomly oriented, anomocytic in position and not sunken. They do not appear to have a specific orientation though there seems to be a marked density difference between cell and veinlet areas. The guard cells are semi-circular and separated from their counterparts by a narrow but deep furrow. The stomatal openings are oval in shape and commonly flanked by trichomes (Agarwal and Tewari, 2002).

Pl. I, fig. e shows-
Type of Stomata- Anomocytic
Stomatal Aperture (Slit)- L 8- 14μm x B 6- 8μm
Guard Cell- L 14- 21μm x B 7- 11μm
Stomatal Index- 17.1- 19.6
Trichome Base- L 6- 10μm x B 4- 6μm
Width of Trichome Base Wall- 1- 2μm
Trichome Base Index- 3.8
Size of Epidermal Cells- L 8- 15μm x B 6- 7μm
Stomatal Frequency (Variable)- 833- 900/ Sq. mm.

REMARKS

Amber deposits with well-preserved inclusions of microbes, nematode parasites, insect, spider and floral remains provide invaluable data about organisms that are not usually preserved by other means of fossilization (Poinar, 1992; Alimohammadian, 2005). They therefore provide insight into the geological history, distribution and evolution of a large terrestrial biotic component with an otherwise poor fossil record. Plant remains have been very well studied not only for megaflora and associated animal remains (Nel *et al.*, 1999; Bajpai and Ambwani, 2004; Gobulic and Seong-Joo, 1999; Harley, 2006; Heinrichs *et al.*, 2006; Herendeen *et al.*, 1994; Kim *et al.*, 2003; Tiwari and Agarwal, 2001) but also for pollen remains (De Franceschi *et al.*, 2000). The Vastan amber is confined to specific levels in the top and bottom coals which have been shown to be derived from a forested cover (Singh and Singh, 2000). The degree of alignment of the amber nodules suggests that though the source of the amber producing trees was not far from the depositional site some reworking of the amber including re-deposition has taken place (Fig. 2). The coals in which the amber nodules are embedded have pyritised cylindrical woody structures as also the presence of pyrite lenses suggesting euxinic conditions. Associated with the coals are inter-bedded carbonaceous shale and molluscan bearing black shale bands with extremely well preserved marine bivalves and gastropods. The sedimentary deposits are typical of backshore lagoonal environments (Sahni *et al.*, 2004) with dense forests analogous to the present day Sunderbans of the Hoogly Delta.

CONCLUSIONS

The present ultrastructure study of a cuticular surface of a leaf from Vastan Mines has clearly demonstrated the potential of electron microscopy in illustrating very fine structural details at the cell level. Such a fine resolution is not common in other modes of preservation. However great care has to be taken in the conservation of amber material as the external surface is susceptible to oxidation when kept exposed in air. In spite of these drawbacks, it is convenient to work out the morphological and surface details of organisms of animals and plants. In fact, stomatal density and trichome distribution can be specifically worked out. Further discovery of known taxa will help in providing a better idea of evolutionary transformations through time at the micro and ultrastructural levels.

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