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New dimensions in palaeobotanical research: ultrastructural studies on plant fossils

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ABSTRACT

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In recent years palaeobiology has developed from a primarily descriptive science into a technically advanced and intellectually attractive field, with ramifications applicable to phylogeny, evolution, taphonomy and palaeoclimate. A combination of light microscopy (LM) with scanning electron microscopy (SEM) and transmission electron microscopy (TEM) is often used in the study of fossilised organs and organelles. Details of the surface microtopography, which can be seen only through SEM provide evidence of taphonomic processes and influences. Ultrastructural investigation of palynofossils can help resolve a number of problems concerning the interpretation of morphology, development and biology of fossil spores and pollen, and provide new significant data about their systematics. Studies of fine structure of fossil plant cuticles can be help determine the degree of cuticle preservation and organisation of structural components, and interpret the physical environment in which the plants lived and thrived. Ultrastructural studies of fossil plant cuticle may also reveal the presence of delicate structures such as infection pegs formed as a result of fungal infection or changes in the ultrastructure of the cuticle proper due to fungal infection. SEM study of Late Cretaceous fusainised angiosperm flowers has enabled their identification in terms of modern orders and families, thus greatly improving our knowledge of angiosperm evolution and radiation. SEM study of fossil charcoals (fusain) is also useful in understanding the past role of wildfire and ancient fire-prone plant communities.

Key-words—Plant fossils, Plant cuticles, Palynofossils, Fusainised fossils, Ultrastructure.

पुरावानस्पतिक खोज में नवीन आयाम- वनस्पति जीवाश्मों का परासंरचनात्मक अध्ययन

उषा बाजपेई

सारांश

हाल ही के वर्षों में पुराजीवविज्ञान प्राथमिक रुप से विवरणात्मक विज्ञान से एक तकनीकी रुप से उच्च व प्रतिभासंपन्न आकर्षक क्षेत्र में जातिवृत्त, विकास, जैवसादिकी एवं पुराजलवायु बहुशाखन सहित विकसित हुई है। जीवाश्मित अंग एवं कोशिकांग के अध्ययन में प्रायः क्रमवीक्षण इलेक्ट्रॉन सूक्ष्मदर्शिकी (एस ई एम) सहित प्रकाश सूक्ष्मदर्शिकी तथा संचरण इलेक्ट्रॉन सूक्ष्मदर्शिकी (टी ई एम) का संयोजन प्रयोग किया जाता है। पृष्ठीय सूक्ष्मस्थलाकृति के विवरण, जो कि केवल एस ई एम से ही देखे जा सकते हैं जैवसादिकीय प्रक्रमों और प्रभावों के प्रमाण प्रस्तुत करते हैं। परागाणुजीवाश्मों के परासंरचनात्मक शोध आकृतिविज्ञान, विकास और जीवाश्म बीजाणुओं व पराग के जीवविज्ञान की व्याख्या संबंधी बहुत-सी समस्याओं के विभेदन में सहायता कर सकते हैं, और इनके वर्गीकरण-विज्ञान के बारे में नवीन सार्थक आँकड़ा प्रदान करते हैं। जीवाश्म वनस्पति उपत्वचा की सूक्ष्म संरचना संरचनात्मक अवयवों का उपत्वचा परिरक्षण व संगठन के अंश निर्धारित करने तथा जिस भौतिक वातावरण में वनस्पति का निर्वाह व फूली-फली, की व्याख्या करने में मदद कर सकती है। जीवाश्म वनस्पति उपत्वचा के परासंरचनात्क अध्ययन कोमल संरचनाओं की विद्यमानता जैसे कवकी संक्रमण के परिणामतः संक्रमण खूँटियां गठित हुईं या कवकी संक्रमण के कारण स्वाभाविक रंग में उपत्वचा का परासंरचना में परिवर्तन भी व्यक्त कर सकते हैं। अंतिम क्रिटेशस फ्यूजेनीकृत आवृतबीजी पुष्पों के एस.ई.एम. अध्ययन से उनके आधुनिक गणों एवं कुलों के संबंध में आवृतबीजी विकास व विकिरण के हमारे ज्ञान की बृहत रूप से वृद्धि कर उनके अभिनिर्धारण योग्य बनाया है। वन्य-अग्नि व प्राचीन अग्नि-तत्पर वनस्पति समुदायों की गत भूमिका को समझने में जीवाश्म लकड़ी कोयला (फ्यूजेन) का भी एस.ई.एम. अध्ययन उपयोगी है।

संकेत-शब्द—वनस्पति जीवाश्म, जीवाश्म उपत्वचाएं, परागाणुजीवाश्म, फ्यूजेनीकृत जीवाश्म, परासंरचना।

Challenges in Indian Palaeobiology: Current Status, Recent Development and Future Directions
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INTRODUCTION

IN continuing quest to explore structure of an organism or its various organs and organelles at a very high resolution and to relate the structural organisation to functional significance, a variety of new tools is now available. The electron microscopes, which give a real-time magnification several hundred times of the light microscope, are now a relatively routine research tool in most areas of palaeobotany (Hill, 1990; Collinson, 1999). Digitally captured EM images can now be stored on a zip disk, on a compact disc or a pen-drive, printed on conventional laserjet printer or dye-sublimation printer (for photographic quality prints), and also shared with fellow researchers through the web.

The fossil record embodies a wealth of data on the origin and progressive evolution of the biosphere. Investigation of biological fossils is now a technically advanced and intellectually attractive field, with ramifications applicable to phylogeny, evolution, taphonomy and palaeoclimate. The electron microscopes have proved to be very potent tools for ultrastructural and micro-morphological studies undertaken to generate data towards this end. Often details which can be seen only through EM provide evidence of taphonomic processes (Collinson, 1999). For example, eroded micro-topography of a fossil or scratches on its surface may indicate transport in an abrasive context, or lack of lamellae in the exine of fossil gymnosperm pollen may indicate homogenisation during fossilization (Pl. 1). Electron probe microanalysis (EPMA) done on the SEM is a non-destructive technique used to map the distribution of elements present in a fossil. Analytical Transmission Electron Microscopy works on a similar principle and has the ability to derive chemical and crystallographic data from extremely small samples (Morgan, 1985). Specimens which may not be coated (e.g. type specimens) can be imaged using low vacuum SEM, though the resolution of this device is currently limited to x 500-1000 magnifications (Taylor, 1986).

Electron microscopy of Proterozoic fossils

Small fossilised cyanobacteria (single-celled - sometimes organised in colonies- prokaryotes, which lack internal organelles, a discrete nucleus and the histone proteins) have been extracted from Precambrian rocks, and studied with SEM and TEM. One may study the role of bacteria, with the help of electron microscopes, in conversion of structured mass to unstructured one and their capabilities to act with various minerals in the sediment (Bajpai *et al.*, 2001).

TEM and SEM analyses of acritarchs in Mesoproterozoic shales show promise for elucidating eukaryotic cell wall ultrastructure in ancient samples if the results could be verified from comparisons with modern analogues (Javaux *et al.*, 2003, 2004). There, however, are technical challenges in imaging modern microbes by conventional electron microscopy. Environmental scanning electron microscope (ESEM) allows high resolution imaging of uncoated delicate and hydrated samples. Using ESEM fresh microbial cultures can be imaged directly and then surface morphology can be compared with that of the fossil microbes in chert or shale. Using this tool, role of taphonomical factors in alterations in an organism, or an organ, or a layer, can be assessed at ultrastructural level with a very high degree of confidence. For example, some acritarchs possess a similar reticulate texture, which has been recognised as a taxonomic feature. Micro-morphological studies on the other hand suggest that this texture could be a result of digenetic processes, such as, compaction or desiccation, and hence may not be a taxonomic feature.

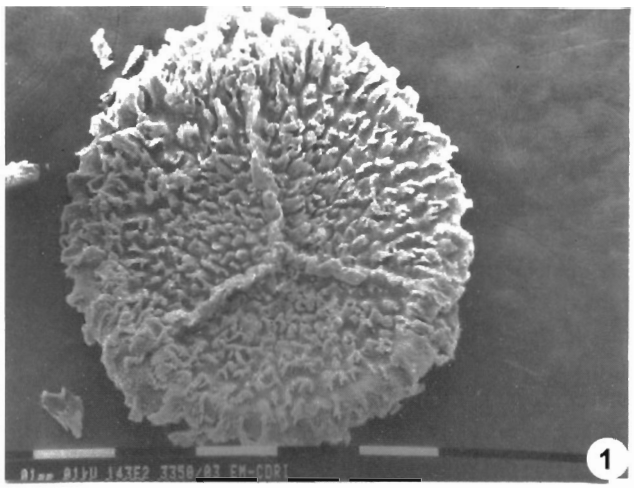
Investigation of 1500-1400 Ma fossils from Australia and broadly coeval rocks from China, with light and electron microscopes, has shown that these assemblages do include a diversity of eukaryotic remains (Javaux *et al.*, 2004). Ultrastructural studies on some Early Cambrian acritarchs have provided evidence of diverse wall ultrastructure within a single genus (e.g. in *Leiosphaeridia* and *Tasmanites)* and revealed the presence of at least four structural types of vesicle wall (Talyzina & Moczydlowska, 2000). From the available evidence it could be deduced that some of the Cambrian leiosphaerids were chlorophycean algae, probably belonging to the Order Chlorococcales.

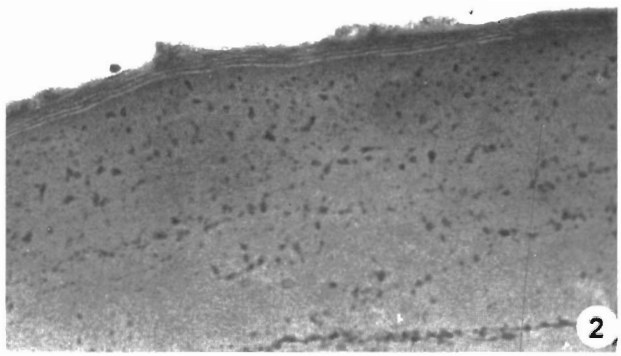
Ultrastructural studies of palynofossils

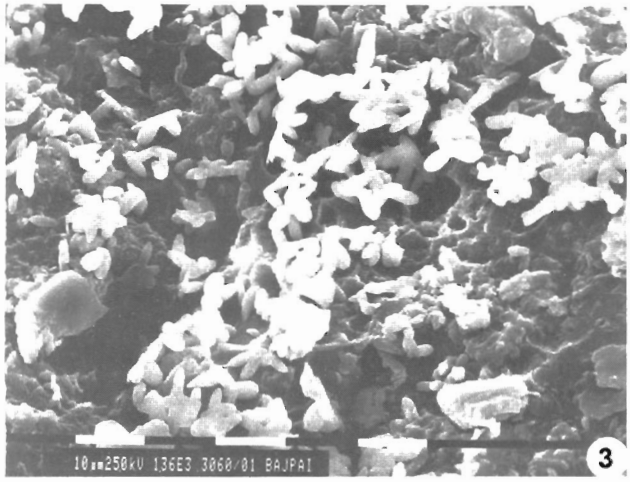
Geologists use palynological studies mainly to correlate strata and determine the relative age of a given bed, horizon, formation or stratigraphical sequence, or to reconstruct marine and freshwater communities to determine past climates, or to

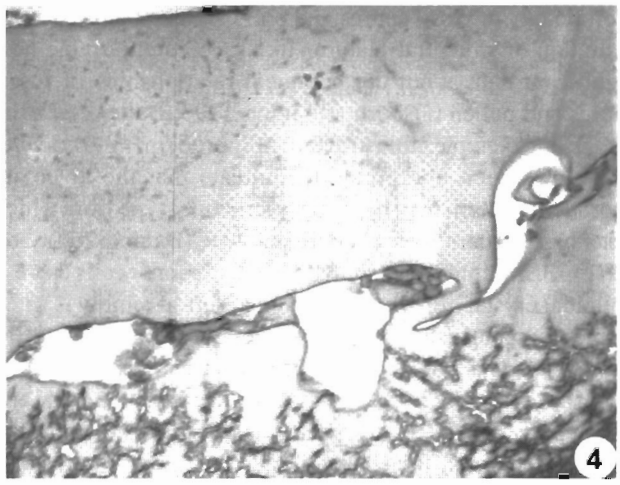
PLATE 1 →

1. The megaspore *Biharisporites ghoshii* showing trilete mark and spines with strong bases. x 1,160 (from Bajpai, 2003).
2. Outer zone of the cuticle in *Dicrodium gouldii* Retallack to show the nature and number of the lamellae, and remnant of wax deposit at the cuticle-air interface. x 28,000 (from Maheshwari & Bajpai, 1996).
3. A colony of bacteria on cuticle of a Late Permian *Glossopteris* leaf from Jharia Coalfield, India. x 8,000.
4. Infected cuticular membrane of *Thinnfeldia indica* Feistmantel showing disturbed cellular layer. x 21,000 (from Maheshwari & Bajpai, 1996a).
5. Cuticular membrane of *Dicroidium gouldii* showing fibrillar nature of middle and inner zones. In the inner zone the fibrillae acquire a “herring bone” pattern, a stress character. x 62,000 (from Maheshwari & Bajpai, 1996b).
6. Uninfected cuticular membrane of *Thinfeldia indica* showing amorphous nature. x 22,000 (from Maheshwari & Bajpai, 1996b).









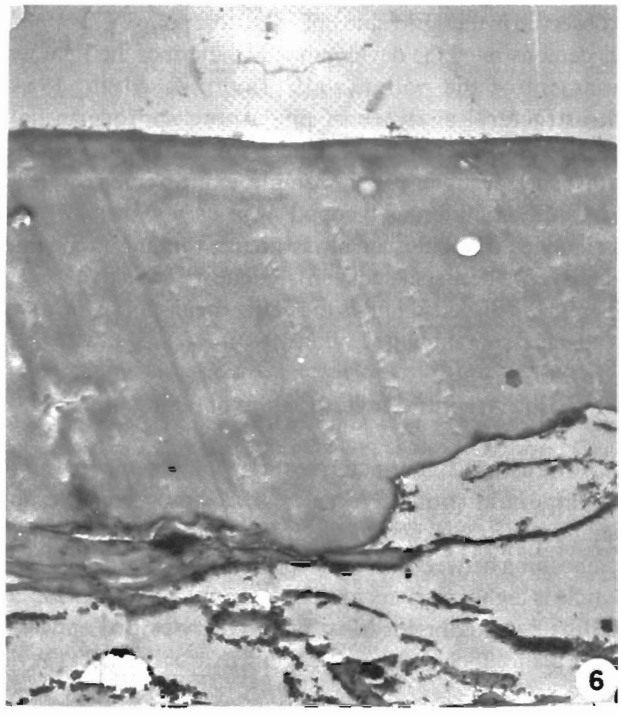


PLATE 1

examine the preservation of the particulate organic matter and palynofossils to understand the depositional environment of sediments. The investigation of ultrastructure of fossil spores and pollen provides information, hitherto unavailable, about the taxonomy, biology and evolution of past biota. Ultrastructural studies (both SEM and TEM) of spore-containing plant fragments from Late Ordovician rocks of Oman have shown that these represent earliest bona fide land plants of liverwort affinities.

A combination of LM with SEM and TEM is often most informative in the study of palynofossils. Ultrastructural investigation of fossil spores and pollen also reveals important proxy records of structure useful for deciding functional relationships by comparison with modern analogues. Supplementary data from ultrastructural studies can help to resolve a number of problems concerning the interpretation of morphology of fossil spores and pollen (Batten *et al.*, 1998), evolution of structural features like sacci, and provide new significant data about the botanical relationships of fossil spores and pollen (Osborn & Taylor, 1993; Batten & Dutta, 1997). For example, ultrastructure of sporoderm in *Trisaccites* pollen has been interpreted to indicate that the alveolate sporoderm pattern may be an early structural type from which other basic patterns of sporoderm evolved (Baldoni & Taylor, 1982). SEM study has shown that the spore walls have a colloidal crystal organisation, and that self-assembly is a key element in spore wall development (Hemsley *et al.*, 1996). SEM investigation has shown that in *Potoniea*, sporoderm initiation begins as a series of lamellae with the increase in the exine and differentiation between sexine and nexine the result of tapetal sporopollenin and lamellar exfoliation (Taylor, 1982). The presence of tapetal membranes in association with orbicules as observed in the TEM study of Mesozoic pollen *Classopollis* has demonstrated the developmental pattern of the final wall ornament of the pollen wall (Taylor & Alvin, 1984). Ultrastructural analyses of megaspore sporoderm have revealed that the exine structure can sometimes be correlated with dispersal strategy. For example, exine of megaspores of the Late Carboniferous lycopod *Mazocarpon* is made up of interconnected tubules, an ultrastructural organisation which is believed to be associated with a unique reproductive strategy wherein the dispersal unit is the entire megasporangium with its attached sporangium (Taylor, 1990).

Ultrastructural investigations of plant cuticles

The cuticle, a continuous extra-cellular membrane, plays the important protective role, and shows considerable variations in its thickness, degree of development and chemical composition between different species (Tegelaar *et al.*, 1991). Cuticle is highly resistant to chemical or physical degradation and is often fossilised. Most of the features that appear in extant plant cuticles also can be found in fossil cuticles (Taylor *et al.*, 1989). Cuticular analysis is an accepted method for identification of fossil leaves. Six basic morphological types of cuticle organisation in extant plants have been recognised on the basis of presence or absence and extent of fibrillar and lamellar components (Holloway, 1982). Studies of fine structure of fossil cuticles can be directed at determining the degree of cuticle preservation (Tegelaar *et al.*, 1993), organisation of structural components, and as a character in interpreting the physical environment in which the organisms lived and thrived. For example, the epidermis of xerophytic plants may consist of very thick, cutinised outer walls, deeply sunken stomata, and a dense layer of trichomes. The structure and function of these xeromorphic features may be studied with electron microscopes. It has been suggested, for example, that the curved and wavy lamellae of the Al layer of the subsidiary cell cuticle may be one of the xeromorphic features (Guignard *et al.*, 1998). Ultrastructural study of *Zostera kiewiensis* (see grass) from the Early Oligocene of Ukraine has shown that it was initially a land plant which had just begun to adapt to aquatic environments (Vickulin *et al.*, 1995). There, however, have been relatively few attempts at TEM investigation of fossil cuticles, may be because well preserved fossil cuticles are hard to obtain, or because of difficulties faced in staining and sectioning the fossil cuticle. But wherever such studies have been undertaken, results have been highly informative. A recent study of sun and shade leaves of the Jurassic leaf *Komlopteris nordenskioeldii* has revealed four distinguishable categories of cuticle, according to their thickness; sun upper, sun lower, shade upper and shade lower (Guignard *et al.*, 2001).

Cuticle ultrastructure may also be used as a taxonomic feature as the fossil cuticles are the most widespread unaltered plant remains that retain morphology (cellular pattern) diagnostic for the taxon (Maheshwari & Bajpai, 1996b). For example, Cheirolepidiaceae, a family of Mesozoic fossil conifers, considered to be (i) transitional between Voltziales and Taxodiaceae (Jung, 1968), or (ii) related to Araucariaceae on the basis of organisation of the ovule-producing organ (Krassilov, 1982) or (iii) related to Araucariaceae and Taxodiaceae on the basis of organisation of the foliar stomata (Clement-Westerhof & van Konijnenburg-van Cittert, 1991), has been found to show close similarity in cuticle ultrastructure to that in Taxodiaceae (de Seoane, 1998). A comparison of the ultrastructure of bennettitalean leaves (Barale & Baldoni, 1993; de Seoane, 1999, 2003) has led to the identification of 3 types, namely, (i) lamellate outer layer and alveolate or reticulate inner layer *(Dictyozamites, Otozamites* and *Zamites)*, (ii) alveolate or reticulate outer layer and lamellate-reticulate inner layer *(Pterophyllum* and *Ptilophyllum)*, and (iii) reticulate outer layer and lamellate inner layer *(Cycadolepis*, and bract of *Williamsonia)*.

Species of the fossil genera *Karkenia* and *Yimaia* are usually considered to be Mesozoic members of the Ginkgoales.

A recent study has shown that the megaspore membranes of *Ginkgo, Karkenia* and *Yimaia* differ considerably in their ultrastructure. Potential taxonomic significance of this find has to be carefully evaluated (Zhou *et al.*, 2002). Cuticle micromorphology of fossil *Gingko* leaves is quite similar to that of modern *Ginkgo* leaves, yet the chemistry is drastically altered (Collinson *et al.*, 1998). Ultrastructural studies of fossil plant cuticle may also reveal the presence of delicate structures such as infection pegs formed as a result of fungal infection (Archer & Cole, 1986) or changes in the ultrastructure of the cuticle proper due to fungal infection (Bajpai, 1997). In the cuticle of extant *Rhizophora* a cork-wart structure has been identified, which is interpreted as modification of stomata for exudation of excess salt to balance the physiology of the plant (Farooqui & Bajpai 1999). One of the most exciting examples of the importance of ultrastructural studies of fossil plants is the report of grana stacks, starch deposits, nuclei and plasmodesmata in a Miocene leaf (Niklas *et al.*, 1978).

Electron microscopy of fusainised plant fossils

Scanning electron microscopy has also been successfully used for morphological and anatomical analyses of pyritised (Poole & Llyod, 2000) and carbonified plant remains (Figueiral, 1999). It has been possible to decipher the nature of stomatal apparatus, and the presence of a complete annulus in completely carbonified plant remains from the Early Cretaceous (de Seoane, 2001). SEM study of Late Cretaceous fusainised (charcoalified) angiosperm flowers has enabled most of these small flowers to be identified in terms of modern orders and families, thus greatly improving our knowledge of angiosperm evolution and radiation, particularly in the Gondwanan realm (Taylor & Hickey, 1990; Mohr & Friis, 2000; Eklund, 2003). The flowers from Antarctica are three-dimensional, sometimes slightly flattened, and were obtained by wet sieving of unconsolidated sediment. Eleven different types have been recognised. SEM study of fossil charcoals (fusain) has provided valuable data about the past role of wildfire and ancient fire-prone plant communities. Charcoal is formed commonly under natural conditions when vegetal matter is heated in oxygen-depleted conditions during wildfire, and is abundant in the fossil record, archaeological sites and recent sediments.

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