Special issue.
Extinct plants, evolution
and Earth's history

Birbal Sahni
centenary, 1991

# Ultrastructural studies in fossil plant cuticles

**Sergio Archangelsky**

División Palaeobotánica, Museo Argentino de Ciencias Naturales "B. Rivadavia", Av. A. Gallardo 470, Buenos Aires (1425), Argentina, CONICET

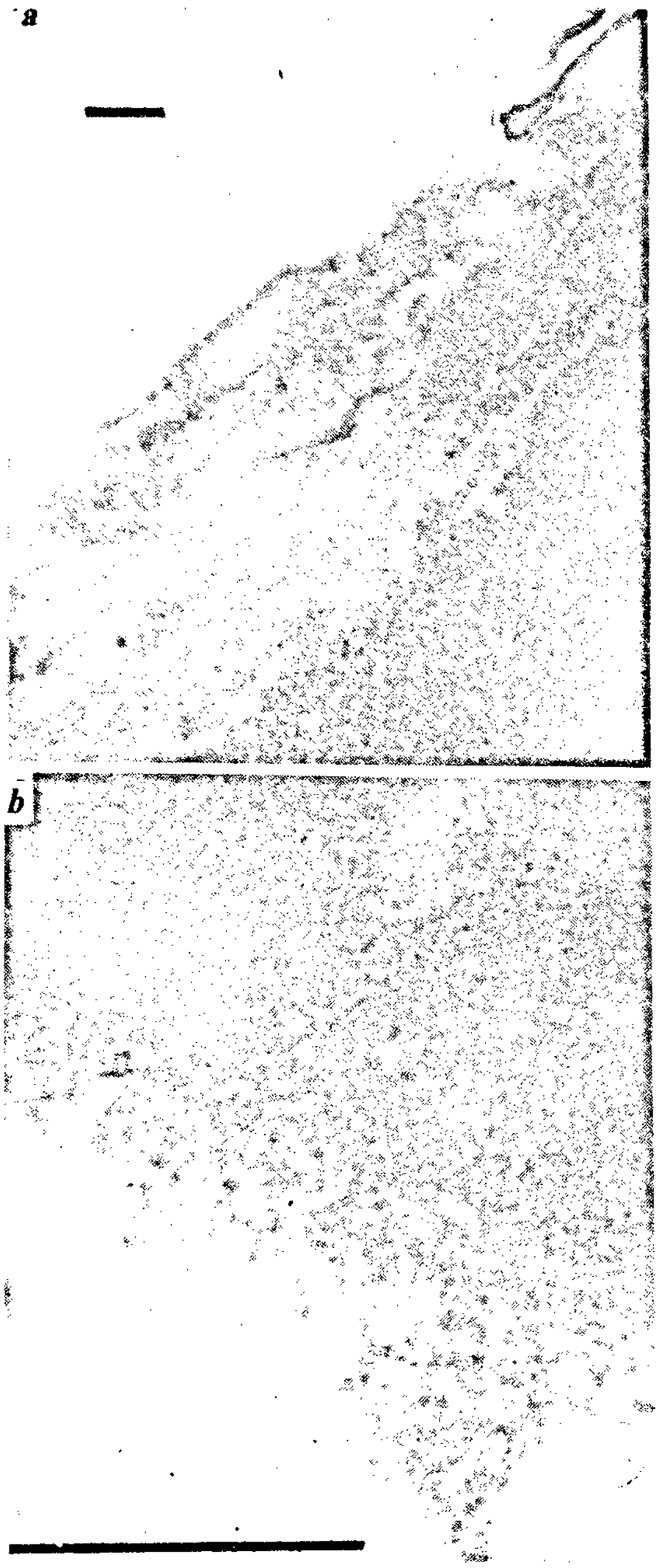
**Organization of epidermal layers and their variations in different groups are detailed through ultrastructural studies. Role of preservational potential and palaeoecological significance of fossil plant cuticles is discussed. Comparative analysis of living and fossil leaf cuticles help to understand the nature of and variations in stratification. Gymnosperm cuticles offer excellent possibilities for comparing fossil ultrastructures with extant analogues. These studies give a new dimension to palaeobotanical studies.**

THE study of fossil plants varies according to the mode in which they are preserved. Palaeobotanists often become specialized with a particular type of material (permineralizations, cuticles). There is no doubt that all fossils, including the poorly preserved, bring information that is used either in taxonomy or in other fields, not necessarily related to palaeontology: palaeoecology, biostratigraphy, palaeobiochemistry. On the other hand investigation techniques are constantly improving, allowing a deeper knowledge of plant tissues buried in clastic rocks. The way in which modern palaeobotanists look at a fossil plant is far more complicated than it was not so long ago, in the field or in laboratory, where new techniques are constantly being used to retrieve biological information. Cuticular analysis started in the last century and is one line of research in palaeobotany that is widely used in modern work. The membrane that protects some plant organs from drought or moisture stress may be recovered and chemically treated for microscopic observation. Several features are observed and measured; they reflect the organization of the epidermal layers in which there are sensitive areas related to gas exchange, liquid storage or protection from organic and inorganic injuries.

In Gondwana, studies on fossil plant cuticles started in the last century[1]. Birbal Sahni[2,3] took a step forward by studying the cuticle of some gymnosperms from India: Conifers and Glossopteridales. He demonstrated that several characters found in fossil cuticles can be used to differentiate taxa that otherwise look identical in their impression state. He opened the way to many distinguished palaeobotanists from India who are currently devoted to the study of fossil cuticles. Sahni's work is contemporaneous with that of R. Florin and T. M. Harris who during 1930–1970 published abundant information that substantially advanced our knowledge on living and fossil cuticular membranes.

The next step in the study of fossil plant cuticles showed the possibility to combine images obtained with light and scanning electron microscopy (SEM) while the ultrastructure of extant cuticular membranes was studied with transmission electron microscopy (TEM)[4].

Few attempts to use TEM with fossilized plant material have been made to date. Early references and illustrations of fossil tissue, including cuticles, have been presented by Niklas *et al.*,[5] on Miocene leaves from Oregon, USA. The ultrastructure and cytochemistry of these tissues, preserved in pyroclastic deposits, demonstrated that detailed research was possible with fossils. It was shown that chloroplasts and nuclei were preserved and that cell walls retained their cellulosic microfibrillar architecture. The cuticular membrane was only mentioned at that time. Niklas *et al.*[5] underscored that cellular ultrastructure may be preserved in the case of a mild fossilization process, suggesting that older volcanic ash deposits may yield such fossils. This proved to be the case with early Cretaceous leaf cuticles found in pyroclastic deposits in Patagonia[6–8]. The techniques used showed that the cuticle of *Ticoa* (a pinnate leaf probably of cycadean affinity) had several layers topographically equivalent to those found in extant plants. Moreover, cuticle stratification varied in the same leaf depending on its position (lower or upper side) or the development in specialized structures (stomata, trichomes). Basically two layers, named A (with two subunits, $A_1$ the outer and $A_2$ the inner) and B, were recognized in this fossil and referred to the cuticle proper and the cuticular layer of the cuticular membrane in living plants[6]. A stratified organization




ıre 1. *Frenelopsis* sp. Late Cretaceous, Spain. *a*, Peripheral zone . leaf cuticle showing translucent lamellae. Scale bar = 0.1 μm. nner surface of leaf cuticle at the base of an anticlinal flange wing an interface zone with the cell wall. Scale bar = 1 μm. From *A* block 94, Palaeobotany Division, Buenos Aires Natural History seum. Staining: $KMnO_4$, 1.5%, 5 minutes; uranyl acetate, 2%, ıinutes.

s found in the cuticle of *Tarphyderma*[7], a conifer m the same pyroclastic unit, suggesting that uctural details as seen with TEM provide a means relating structure and function. A third layer (layer that fills the majority of the cell lumina and nnants of possible epicuticular wax were also scribed. The excellent preservation of the cuticle rastructure was related to the palaeoecological ting, underscoring the pyroclastic nature of the posits[7]. This conifer also showed structural adaptons to xerophytic conditions.

Comparative studies of the leaf cuticle in fossil and living Ginkgoales[8] have shown a general similarity in their stratification (Holloway's type I structure[9]). An outer layer (peripheral zone) with lucent and opaque lamellae and an inner cuticle surface with fibrillae, probably of pectinaceous nature, were described. This distribution suggests that layer $A_1$ in *Ticoa* may be equivalent to the peripheral lamellate zone and layer B with the fibrillae or 'interface' zone with the cell wall.

Work in progress with conifer leaf cuticles (*Brachyphyllum*) from the same early Cretaceous in Patagonia sites shows a well-defined lipid polymer pattern in the IR spectra (infrared spectroscopy) and also some proportion of peptide material (C. Alvarez Ramis, personal communication, 1991).

Locality and age do not alter this general scheme of the ultrastructure of fossil cuticles. Late Cretaceous conifer leaf cuticles from Spain show the same polylamellate nature of the peripheral zone (Figure 1,*a*) and also the interface zone with the cell wall (Figure 1,*b*). Carboniferous pteridosperm foliage from Indiana, USA, have also peripheral lucent lamellae and internal fibrillae[8].

Ultrastructural studies of fossil plant cuticles revealed new data with regard to their potential for preservation. Diagenesis plays undoubtedly an important role in the slight alteration of the original organic material. Similar reactions to stains of cuticular membranes of living and fossil plants point to a very small alteration in chemical composition of this tissue. In such cases fossil membranes may be considered true mummifactions.

Gymnosperms offer excellent possibilities for comparing fossil ultrastructures with living analogues. Further studies will show. the kind of taxonomic perspectives and palaeoecological significance that fossilized cuticular membranes have. The recognition of ultrastructural characters is a strong means of evaluating the degree of diagenesis that plant tissues included in different kinds of clastic rocks underwent. This new dimension gives a further impulse for palaeobotanical studies taking into account the abundance of fossil cuticles in strata of different ages and varied palaeoecological settings.

1. Zeiller, R., *Bull. Soc. Géol. France*, 1896, Sér. III, **24**.
2. Sahni, B., *Rec. Geol. Surv. India*, 1923, **54**.
3. Sahni, B., *Palaeontol. Indica*, 1928, **11**.
4. Cutler, D. F., Alvin, K. L. and Price, C. E., *The Plant Cuticle*, 1982, The Linnean Society of London.
5. Niklas, K. J., Brown, Jr., R. M., Santos, R. and Vian, B., *Proc. Natl. Acad. Sci. USA*, 1978, **75**.
6. Archangelsky, S., Taylor, T. N. and Kurmann, M. H., *Bot. J. Linn. Soc.*, 1986, **92**.
7. Archangelsky, S. and Taylor, T. N., *Am. J. Bot.*, 1986, **73**.
8. Taylor, W. A., Taylor, T. N. and Archangelsky, S., *Rev. Palaeobot. Palynol.*, 1989, **59**.
9. Holloway, P. J. in *The Plant Cuticle* (eds. Cutler, D. F., Alvin, K. L. and Price, C. E.), 1982