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New Applications of FIB: a 3D Look into the Past throughout the Ultrastructure of Fossil Plant Cuticles

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Palaeobotany is a rapidly expanding branch of palaeontology. However, one of its main limitations so far has been the complexity of mechanical slicing and SEM/TEM imaging of fossil material [1].

A few years ago, we applied the unique capabilities of Focused Ion Beam (FIB) microscopy to section and image of Cretaceous fossil both spores and pollen grains from several taxonomic species [2], providing the botanical palaeontology community a valuable inside view with unprecedented spatial resolution.

We present here a similar study of the inner ultrastructure of fossil plant cuticles of conifers from the Early Jurassic of the Argentinian Patagonia.

As it is well known, every material respond in a different way to the ion beam; therefore, the methodology we developed for the previously cited palynological specimens could not be directly applied. In particular, we found out that the use of water vapour-enhanced milling (SCE, Selective Carbon Etching), which would normally speed up the milling process in organic materials, result in the formation of large columnar structures inside the trench (Figs 1a, b). X-ray analysis revealed them to be mostly composed of Ga, which rules out redeposition of milled material as an explanation. A detailed study was therefore carried out to determine the best experimental conditions.

Once the experimental parameters were optimised, several zones of the cuticle containing stomata were sectioned and observed by a combination of high current (I = 9.3 nA) milling and low current polishing (I = 0.93 nA as a first step, followed by I = 0.28 nA).

Results show (Figs. 2a,b) the presence of microlayers, cavities and a diversity of nanometer sized structures placed inside these cavities which never ever had been seen in the fossil plant record. This new sight into the ultrastructure features of fossil plant cuticles provides new data with further applications in taxonomy, climatic and environmental studies concerning both to extinct and extant plants.

References:

[1] J.R. Rowley and J.J. Skvarla, Review of Palaeobotany and Palynology **132** (2004) p. 237.
[2] U. Villanueva-Amadoz *et al.*, Grana **51** (2012) p. 1.

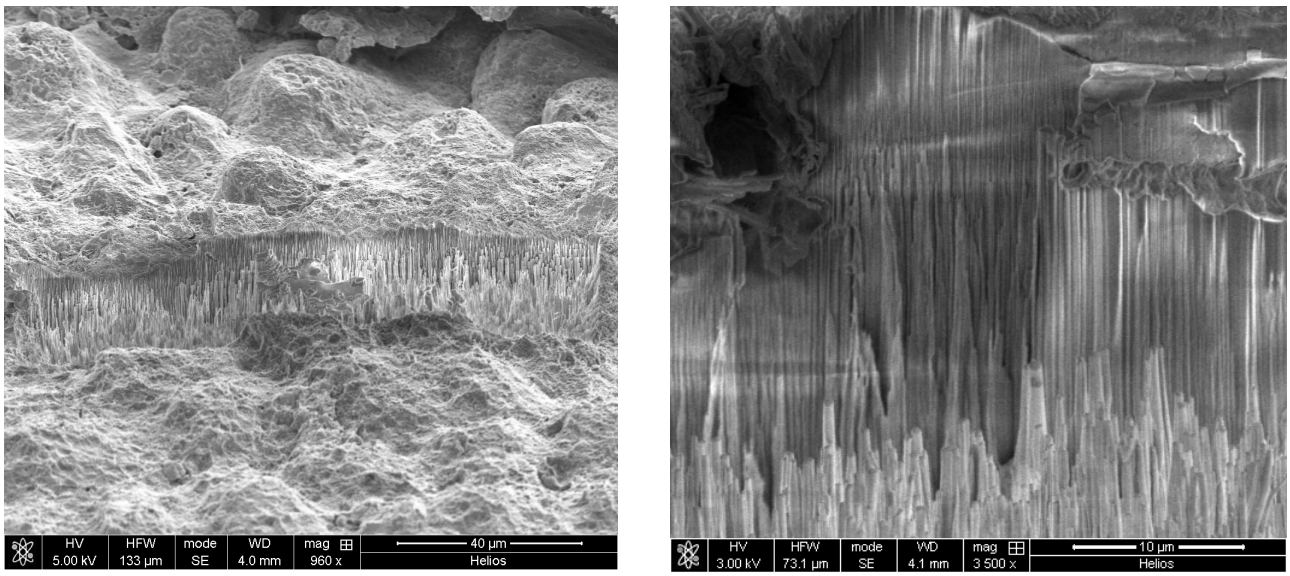

Figure 1. a) Large trench milled using SCE; b) columnar artefacts as deep as the trench are visible.

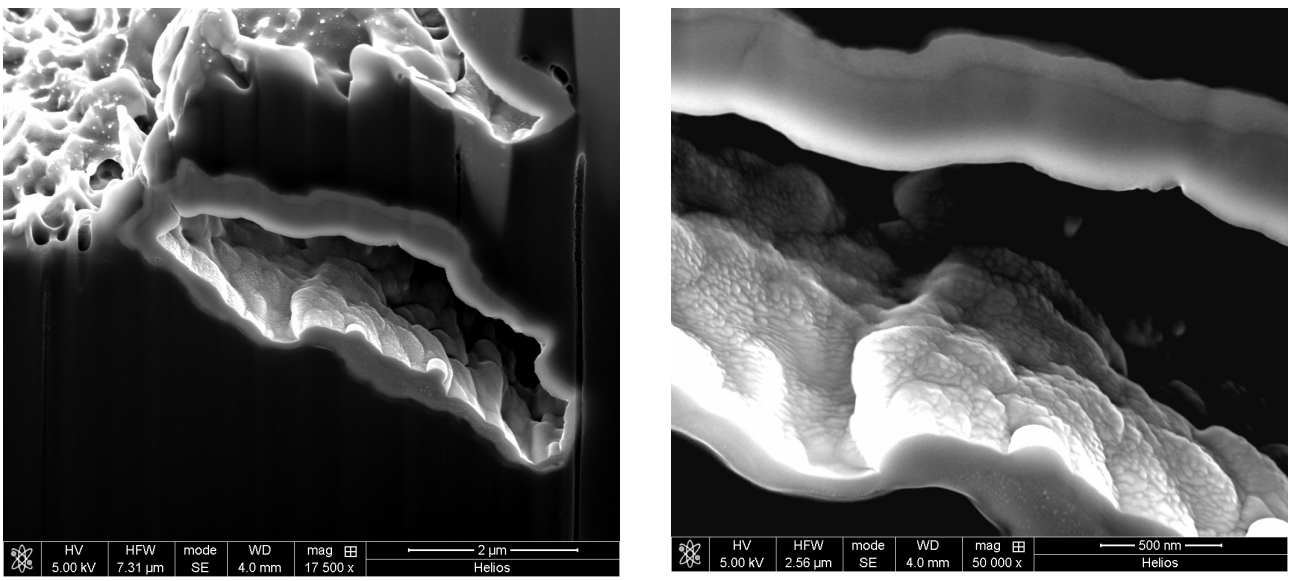

Figure 2. a) Low current polished cavity inside a fossil cuticle; b) highly magnified detail of the same cavity showing ultrastructure of both the layered section and the rough surface.

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