Phloem sap in Cretaceous ambers as abundant double emulsions preserving organic and inorganic residues

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Fossilized remains preserved in amber provide abundant data on the paleobiota surrounding the resinproducing plants, but relatively scarcer information about the resinous sources themselves. Here, dark pseudoinclusions in kidney-shaped amber pieces from the Early Cretaceous (Albian) amber from Spain are studied. This type of fossilized remain, abundant in Cretaceous ambers, was first interpreted as fossilized vacuole-bearing microorganisms, but later regarded as artifactual and probably secreted by the resinous trees, although their origin remained unclear. Using complementary microscopy (light, electron, confocal), spectroscopy (infrared, micro-Raman), mass spectrometry and elemental analysis techniques, we demonstrate that the pseudoinclusions correspond to droplets of phloem sap containing amber spheroids and preserving both organic and inorganic residues consistent with degraded components from the original sap. The amber pieces containing pseudoinclusions are fossilized, resin-in-sap-in-resin double emulsions, showing banding patterns with differential content of resin-in-sap emulsion droplets. Our findings represent the first time fossilized phloem sap, 105 million years old, has been recognized and characterized, and open new lines of paleontological research with taxonomic, taphonomic, physiological and ecological implications.

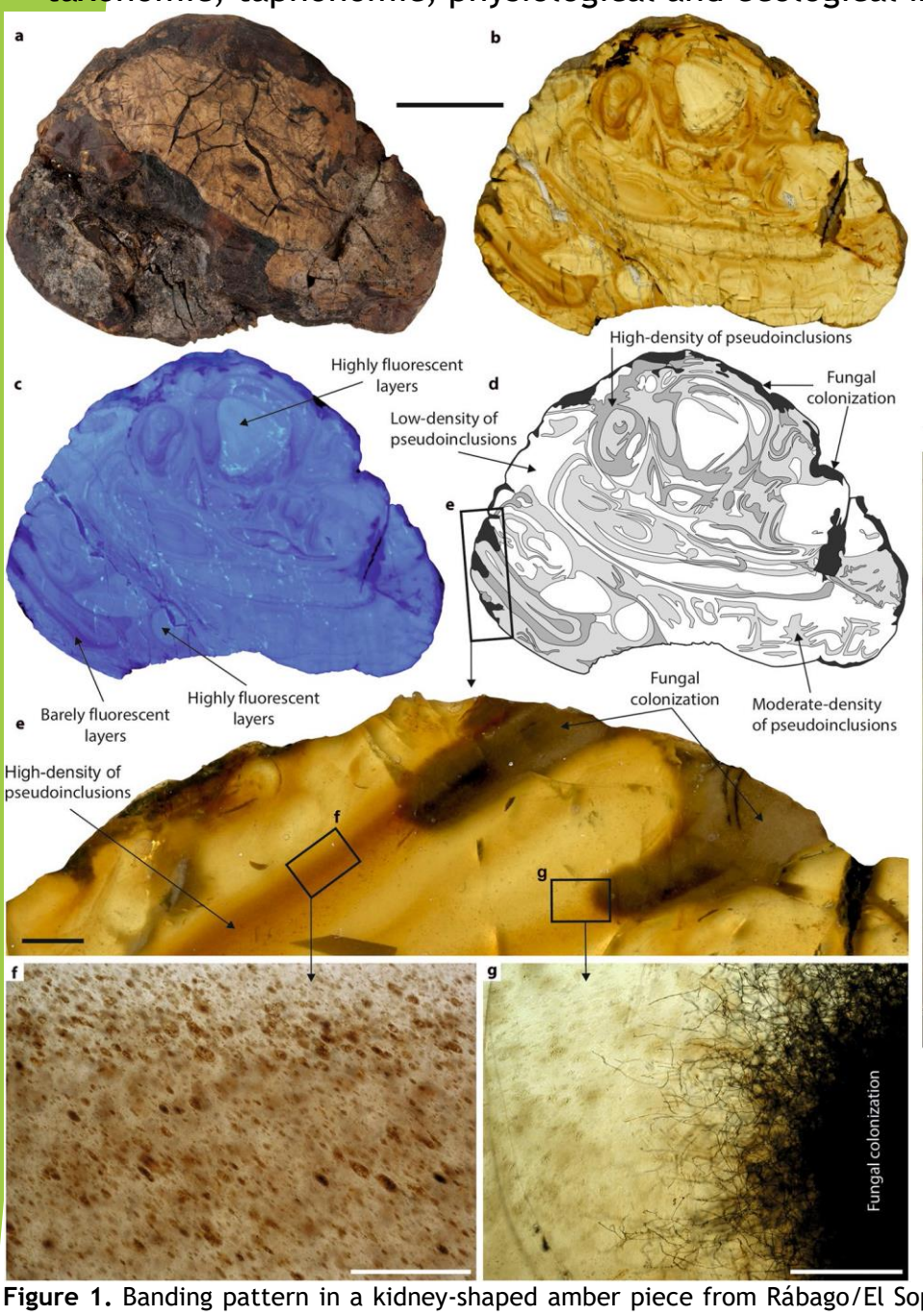

Figure 1. Banding pattern in a kidney-shaped amber piece from Rábago/El Soplao amber and fungal colonization. (**a**) Surface appearance of the piece. (**b**) Polished section of the same piece (1 mm thick; preparation 18067). Note the areas where the layers with high and low density of pseudoinclusions are evident. (**c**) Long-wave ultraviolet light image of the same section. The darker (less fluorescent) layers are the richest in pseudoinclusions and thus richest in fossilized phloem sap. (**d**) Drawing of the same amber piece, showing layers with low, moderate, and high concentration of pseudoinclusions (white, light grey and dark grey, respectively) and fungal colonization (black). (**e**) Magnified inset in (**d**), showing how fungal colonization took place preferentially along resin layers richest in pseudoinclusions (richest in fossilized phloem sap). (**f**) Detail of pseudoinclusion-rich amber layer shown in (**e**). (**g**) Front of advance of a fungal colonization (mycelium) shown in (**e**). Scale bars: 3 cm (**a–d**), 2 mm (**e**), 0.5 mm (**f–g**). Illustration created using CorelDRAW Graphics Suite X8 (www.coreldraw.com).

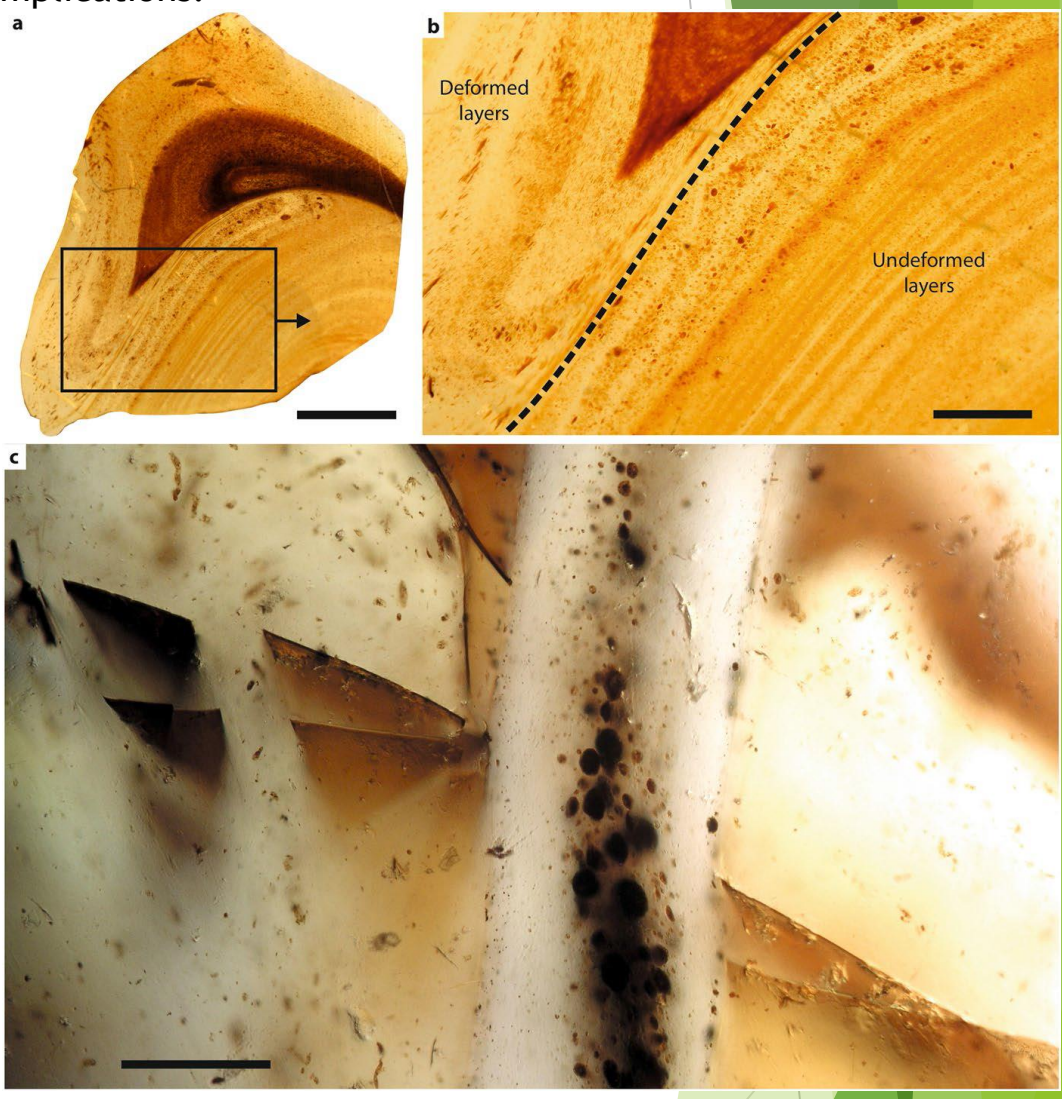

Figure 2. Layers of pseudoinclusions in kidney-shaped amber pieces. (**a**) Amber section where deformed and undeformed layers are found together (from preparation 18068). (**b**) Detail of the contact between deformed and undeformed amber layers in (**a**), showing deformed and undeformed pseudoinclusions, respectively. (**c**) Layer rich in pseudoinclusions (at the middle of the photograph) cutting off previous layers (or resin inputs) (from preparation 18070). Scale bars: 3 mm (**a**), 1 mm (**b**), 300 µm (**c**). Illustration created using CorelDRAW Graphics Suite X8 (www.coreldraw.com).

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