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A new transfer technique to extract and process thin and fragmented fossil cuticle using polyester overlays

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Abstract

Extraction and chemical processing of fragmented cuticles from fossil leaves are facilitated by a new non-destructive technique. Transfer of fossil cuticle onto polyester overlays renders whole-leaf maceration unnecessary. This technique is completely safe and extremely easy to apply for the preparation of fragile and fragmented cuticles, while preserving the fossil specimen and reducing chemical processing time.

Keywords: Paleobotany; fossil leaves; cuticle; sample preparation

1. Introduction

Stomatal analyses of fossil leaf cuticles are used for taxonomic purposes (Harris, 1964, 1969; Dilcher, 1974; Upchurch, 1995; Wang et al., 2005), as a proxy for palaeoatmospheric CO_2 levels (Van der Burgh et al., 1993; McElwain and Chaloner, 1995; Royer, 2001), and have potential for paleoelevation reconstruction (McElwain, 2004). Leaves of Early Miocene *Quercus pseudolyrata* and *Platanus occidentalis* were collected from lacustrine and fluvial facies of the Bonta Formation in the northern Sierra Nevada (California, USA) in order to estimate paleoelevation from fossil leaf stomata. The fossils consist of thinly cutinized whole and partial leaves, preserved as coalified compressions in a sandy siltstone. In specimens where the abaxial leaf surfaces, displaying the stomata, faced upwards in the rock matrix, it was possible to directly perform stomatal counts under an epifluorescence microscope. However, for most of the studied fossils, these direct measurements were not possible, because (1) rock matrix adhered to the cuticle, obscuring stomatal and epidermal cell detail, or (2) the adaxial surface, lacking stomata, was facing upwards on the rock specimen. The cuticle could have been separated from the rock by acid maceration (Harris, 1926; Kerp, 1990; Wellman and Axe, 1999), but this process is destructive and can take weeks of processing time. Furthermore, the cuticle on most of the fossils studied was highly fragmented, and extraction from the rock matrix would have resulted in pieces too small to obtain useful morphological information.

Transfer of the cuticle fragments from selected areas of the specimen onto another surface, suitable for chemical processing, has several distinct advantages over whole-

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leaf maceration. First, the technique is less destructive, preserving the overall physiognomy and venation detail of the fossil leaf specimen for future analysis. Secondly, the transfer removes all cuticle fragments from a chosen area of fossil leaf irrespective of how thin the original coalified compression is. Because the transfer retains the *in situ* position of small cuticle fragments, which would be scattered after maceration, cuticle morphology in highly fragmented specimens can be studied in a larger field of view. This is particularly important for obtaining stomatal density and index data for paleoatmospheric CO_2 reconstructions and paleoelevation studies, as high stomatal variability across individual leaf surfaces (Poole et al., 1996) makes it advantageous to standardize the exact area of leaf surface analysed for all fossils. Moreover, because only the adhering matrix has to be removed, it is much more efficient than dissolving the entire fossil matrix in terms of both processing time and the quantity of chemicals used.

In this paper, a new cuticle transfer technique is described in detail. The new method enables extremely fast, simple, and cost effective transfer of highly fragmented and delicate fossil leaf cuticle from rock specimens onto pressure-sensitive polyester overlays. We compare its effectiveness against several conventional cuticle transfer methods (Dilcher, 1974) including (1) cellulose acetate peels (Kräusel, 1950; Stewart and Taylor, 1965), (2) nail polish peels, (3) collodion peels and (4) cellophane tape transfers, using Miocene *Quercus pseudolyrata* and *Platanus occidentalis* leaves as case studies together with information from literature and technical data sheets.

2. Materials and methods

Polyester overlays, commonly used as overlaminates for laboratory glassware labels (manufactured by Brady Worldwide Inc., #60323; supplied by Lab Safety Supply Inc., catalogue #17134), were cut into ~ 10 cm^2 pieces. If the adaxial leaf surface was exposed on the fossil, the adhesive side was pressed firmly onto the fossil leaf surface and pulled off with most of the cuticle adhering to the polyester (Fig. 1). Alternatively, if the abaxial surface was exposed on the fossil, the cuticle was first pulled off using cellophane tape (Highland Invisible Tape 6200). The cuticle on the tape was then transferred to the polyester overlay by firmly pressing the adhesive sides of the cellophane tape and overlay together and carefully removing the tape.

For the acetone/cellulose acetate method, several doses of acetone were applied onto part of the fossil specimens with a pipette. A square (5 cm^2) of cellulose acetate was cut out from the sheet and pressed on the specimen. After a few minutes the acetate was peeled off from the specimen and allowed to dry completely, with and without the use of a weight to prevent curling.

The transferred cuticles were treated with ~ 15% HCl for 1 h to remove the carbonate matrix, rinsed in tap water, then treated with ~ 10% HF for an hour to remove the silicate matrix, and again rinsed in tap water. The cuticles were mounted in water on a slide, and analysed using a Leica DMLB epifluorescence microscope. Images were digitally captured using a SPOT camera and SPOT image analysis software. Six to twelve serial images were obtained at different focus

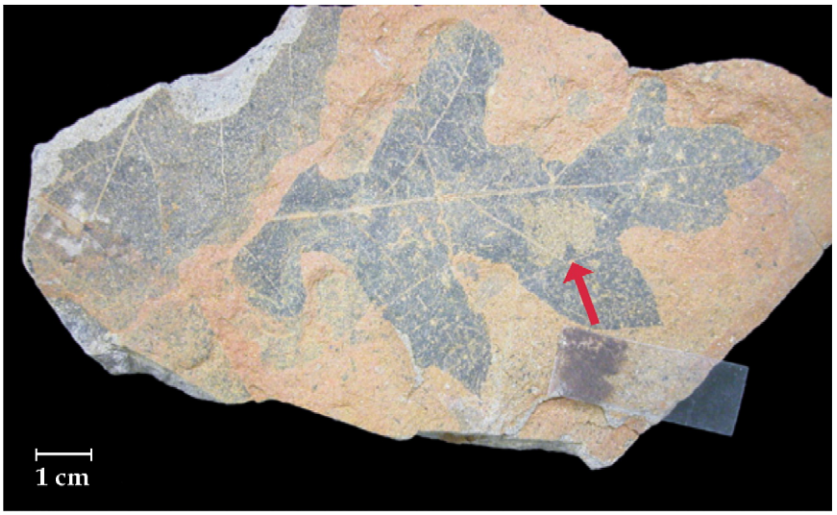

Fig. 1. Fossil *Quercus pseudolyrata* leaf after cuticle transposition onto polyester overlay, and overlay with adhering cuticle. Red arrow indicates the area of cuticle removal.