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Fossil cutin of *Johnstonia coriacea* (Corystospermaceae, Upper Triassic, Cacheuta, Argentina)

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ABSTRACT

For the first time, a cutin polymer was obtained from specimens of *Johnstonia coriacea* (Corystospermales, Corystospermaceae) from the Upper Triassic of Cacheuta, Argentina. These are preserved as fossilized cuticles i.e., naturally macerated compressions under anoxic conditions. Laboratory oxidation reactions were used to obtain the cuticle, which after additional, long-term oxidation yielded the cutin polymer. Cutin, cuticles, and fossilized cuticles, were analyzed by semi-quantitative Fourier transform infrared (FTIR) spectroscopy. Cutin IR spectrum was mainly characterized by intense peaks of aliphatic stretching bands at 3000–2600 cm^{-1}, ester C=O groups centered at 1730–1715 cm^{-1}, and aromatic C=C absorption bands at 1645–1640 cm^{-1}. Values of semi-quantitative, IR-derived ratios of cutin were lower than those of the cuticle. The only exception was the notably higher C=O/C=C ratio found in cutin, which is supportive of the presence of ester C=O groups. CH_{al}/C=O value (0.8) of *J. coriacea* compares with those recorded for *Lycopersicon esculentum* (0.8; extant) and *Macroneuropteris sheuchzeri* (0.9; Pennsylvanian), which indicates a similar cross-linking degree of the monomers characterizing the cutin of both extant and fossil taxa.

1. Introduction

Johnstonia Walkom, 1925 is a leaf fossil-genus assigned to the Family Corystospermaceae Thomas (1933), typically found in the Triassic of Gondwana. Walkom (1925) proposed the name *Johnstonia* for a group of relatively small, forked fronds from the Mesozoic of Tasmania (Australia), which can be easily identified by the absence of pinnules, having an entire, slightly lobed or pinnatifid laminar margin, and taeniopteroid venation (e.g., Frenguelli, 1943; Retallack, 1977; Petriella, 1979, 1981, 1985; Stipanicic et al., 1995). However, other authors (e.g., Townrow, 1957; Bonetti, 1966; Archangelsky, 1968) synonymized *Johnstonia* with *Dicroidium* (Gothan, 1912), an iconic Permian-Triassic frond-like leaf that inhabited all Gondwanan continents (Anderson and Anderson, 1983).

Although *J. coriacea* (Johnston, 1887) Walkom, 1925 is one the most common species of the genus, its cuticular morphology is known from a limited number of specimens only (e.g., Jain and Delevoryas, 1967; Archangelsky, 1968; Retallack, 1977). The general chemical composition of the *J. coriacea* cuticle has been studied in the context of coalification characteristics and chemotaxonomy (D'Angelo, 2006; D'Angelo et al., 2011). However, the macromolecular constituents (monomers) of *J. coriacea* cuticle are completely unknown.

In this contribution we report the first data on the resistant nature of a highly aliphatic cutin macropolymer in foliar remains of *J. coriacea* (Upper Triassic, Cacheuta, Argentina).

2. Materials and methods

The *J. coriacea* specimens originated from Upper Triassic strata of the lower Cacheuta Formation (Trinchera La Mary locality, Cacheuta, Mendoza, Argentina) (Fig. 1A–C). Their macro- and micro-morphological features and chemical preservation characteristics are excellent, in agreement with lower vitrinite reflectance values (Ro% = 0.61, sd = 0.07, n = 40) of coal samples from the associated (unnamed) coal seam (Fig. 1B).

The specimens that were freed from the rock matrix (Fig. 2A) using HF (48%) are fossilized cuticles (i.e., naturally macerated compressions under anoxic conditions; see Zodrow and Mastalerz, 2009). These were laboratory-oxidized (using 4–6 g of $KClO_3$ dissolved in 150 mL non-fuming HNO_3) to obtain (1) a bleached cuticular material after one-day treatment, and (2) a beige cuticular material after 60-day treatment at room temperature (see Schulze, 1855). For simplicity, (1) and (2) are

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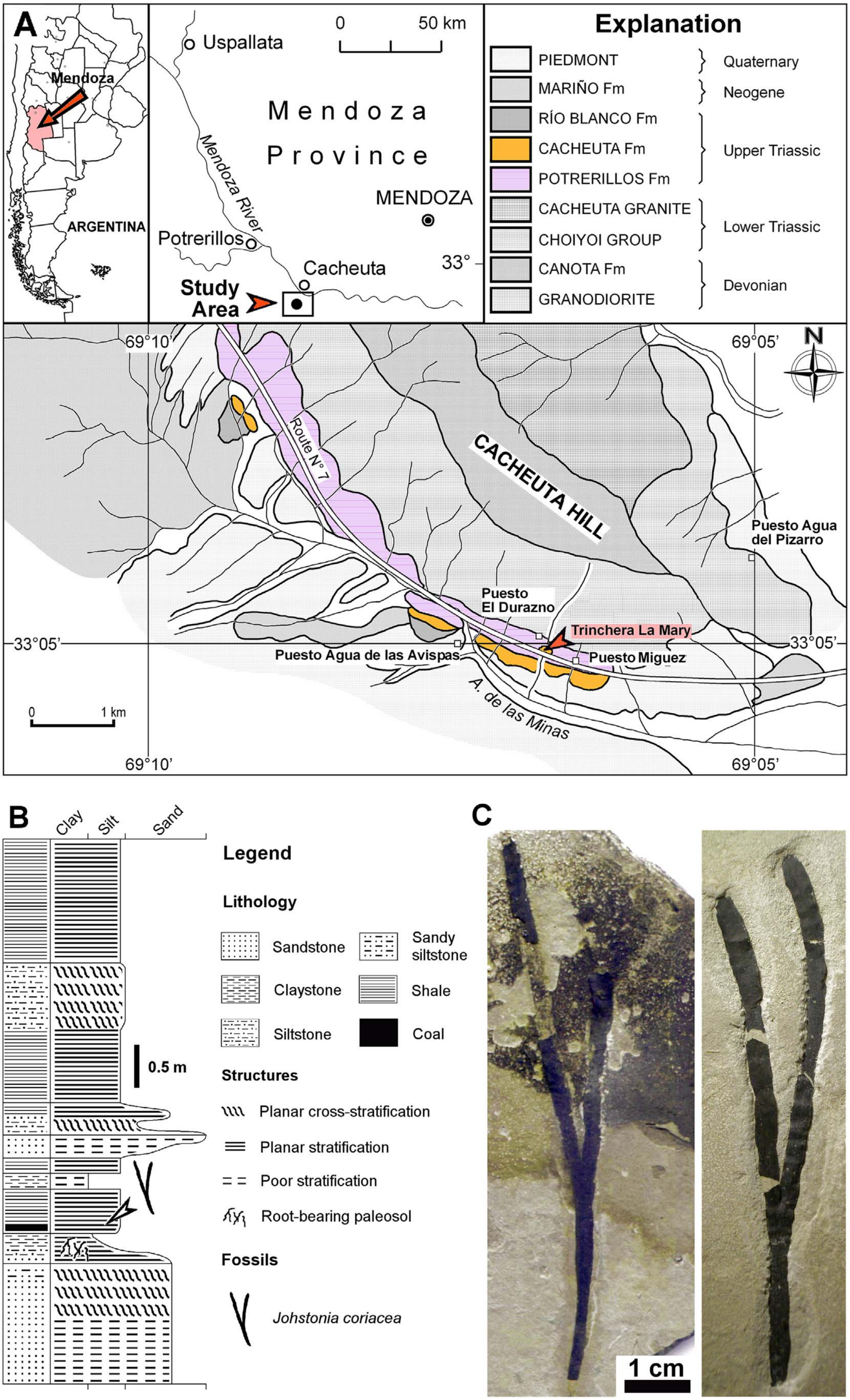

Fig. 1. (A) Location map and geologic sketch of the sampling area. (B) Stratigraphic section of Trinchera La Mary locality, Cacheuta, Mendoza, Argentina. (C) Hand specimens.

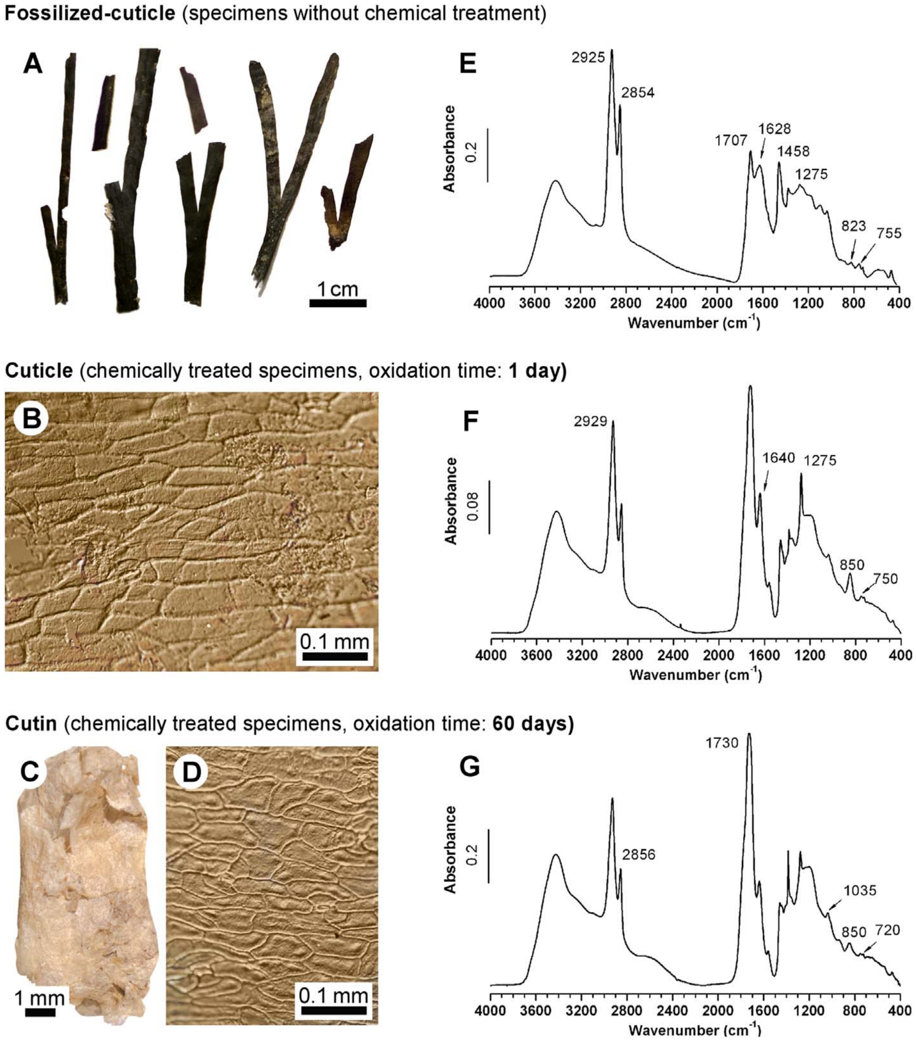

Fig. 2. *Johnstonia coriacea.* (A) HF-released fossilized cuticles. (B) Microscopic structure of cuticle after oxidizing (A) for 1 day. (C) General view of cutin obtained after oxidizing (A) for 60 days. (D) Microscopic structure of cutin. (E) IR spectrum of fossilized cuticle. (F) IR spectrum of cuticle. (G) IR spectrum of cutin. (B) and (D) Nomarski phase-contrast microscopy.

hereafter termed cuticle and cutin, respectively.

Changes in functional groups (chemical structure) were studied via semi-quantitative Fourier transform infrared (FTIR) spectroscopy. Samples for the solid-state FTIR spectra were prepared by the KBr-pellet technique, and analyzed on a Nicolet Thermo-Electron 6700 spectrometer, accumulating 256 scans at a resolution of 4 cm^{-1} wavenumber (for further details see D'Angelo, 2006 and Zodrow et al., 2009). Semi-quantitative FTIR analyses included the applications of Fourier self-deconvolution, area integration methods, and the calculation of area ratios (e.g., D'Angelo et al., 2010; and Zodrow et al., 2009).

3. Results and discussions

Salient results of oxidation procedure are summarized as follows:

(i) Micromorphological features: The cuticle (Fig. 2B) showed excellent epidermal details, e.g., anticlinal walls and stomatal complexes, though papillae were not found. After a total of 60 days of oxidation, beige cutin formed (Fig. 2C), which hardly showed any micromorphological changes/damages (Fig. 2D) when compared to day 1, particularly concerning the stomatal structure.

(ii) Qualitative IR information: The fossilized cuticle spectrum (Fig. 2E) exhibited prominent peaks assigned to aliphatic C-H stretching bands (3000–2800 cm^{-1} region), carbonyl groups of carboxylic acids (peaks centered at 1707 cm^{-1}), and aromatic carbon groups (C = C peaks at 1628 cm^{-1}). Noted is the presence of the 1275 cm^{-1} band generally assigned to phenols. Small peaks at 823 and 755 cm^{-1} were assigned to aromatic C-H out-of-plane bending (CH_{ar}) vibrations. The latter was assigned to the contribution of vitrinitic matter (Lyons et al., 1992, 1995; Zodrow and Mastalerz, 2002), which was, for the most part, eliminated by oxidation. IR characteristics of the cuticle (Fig. 2F) and the cutin (Fig. 2G) were very similar to each other. They showed intense C = O peaks (centered at approximately 1730 cm^{-1}) indicating aliphatic ester structures. Absorptions at 1640 cm^{-1} can be assigned to the stretching of C = C bonds. Medium to weak intensity peaks at 1035 cm^{-1} region were assigned to C-OH in alcohols and C-O-C in aliphatic ethers. These functional groups are usually encountered in the cutin structure of extant plants (e.g., Villena et al., 2000; Benítez et al., 2004; Heredia-Guerrero et al., 2014).

(iii) Semi-quantitative, IR-derived data: The fossilized cuticle specimen showed the highest values of CH_{al}/Ox, C = C contribution, CH_{ar}/CH_{al}, and $CH_{al}/C{=}O$ (Table 1), implying the highest contents of aliphatics and aromatics, as well as the lowest contents of carbonyl groups. At the same time, the fossilized cuticle had the lowest values of CH_2/CH_3, C = O/C = C, and C = O contribution of the available dataset. The latter implies branched and short polymethylenic chains with lower contents of carbonyls (mainly from carboxylic acids).

Table 1
Semi-quantitative FTIR data of the sample types of *Johnstonia coriacea*: fossilized cuticle, cuticle, and cutin.

Sample type	Remarks	CH_2/CH_3	CH_{al}/Ox	$C=O/C=C$	$C=O$ cont	$C=C$ cont	$CH_{al}/C=O$	CH_{ar}/CH_{al}
Fossilized cuticle	Without chemical treatment	5.0	1.46	3.0	0.1	0.06	9.8	0.01
Cuticle	Chemically treated specimen, oxidation time: 1 day	7.1	0.49	6.4	0.4	0.06	1.3	n.d.
Cutin	Chemically treated specimen, oxidation time: 60 days	5.7	0.38	12	0.4	0.04	0.8	n.d.

CH_2/CH_3 = Relating to length and branching degree of aliphatic hydrocarbon side groups (side chains attached to macromolecular structure).
CH_{al}/Ox = Aliphatic C-H stretching/oxygen-containing compounds.
$C=O/C=C$ = Carbonyl/aromatic carbon ratio.
$C=O$ cont = Carbonyl contribution.
$C=C$ cont = Aromatic carbon contribution.
$CH_{al}/C=O$ = Aliphatic C-H stretching/carbonyl contribution.
n.d. = not detected.
Further details regarding the definitions of IR-derived ratios can be found in D'Angelo et al. (2010, 2012).

Cutin is differentiated from the cuticle mainly by the considerably higher value of $C=O/C=C$ ratio (Table 1). Values of other IR-derived ratios determined in cutin i.e., CH_2/CH_3, CH_{al}/Ox, $C=C$ contribution, and $CH_{al}/C=O$ were lower than those found in cuticles. $C=O$ contribution (mainly from aliphatic esters) was the same in both cutin and cuticle.

It should be noted that, to the best of our knowledge, there are no published semi-quantitative FTIR data for cutin of South American fossil plants. This lack of information prevented us from performing more comprehensive comparisons. However, our previously reported IR data (D'Angelo et al., 2013) on *Macroneuropteris scheuchzeri* (Pennsylvanian seed fern, Canada) indicated that after 11 days of oxidation, cutin showed similar functional groups (i.e., similar structure; Table 2) to those obtained for cutin of *J. coriacea*.

Carbonyl groups in cuticle and cutin are usually associated with the presence of ester bonds, which play an important role in the cross-linking of different hydroxy fatty acids that forms the biopolymers (van Bergen et al., 2004). $CH_{al}/C=O$ is considered an indicator for cross-linking degree of a polymeric structure. Lower values indicate higher $C=O$ content and higher cross-linking (Benítez et al., 2004).

Benítez et al. (2004) provided semi-quantitative IR ratios $CH_{al}/C=O$ for cutin isolated from extant *Lycopersicon esculentum*: younger fruits: 0.92, and ripe fruits: 0.72. Our data of $CH_{al}/C=O$ for cutin obtained from cuticles of *M. scheuchzeri* is 0.9 (D'Angelo et al., 2013). The 0.8 value (Table 1) for *J. coriacea* cutin compares well with cutin $CH_{al}/C=O$ ratios for both *L. esculentum* (extant) and *M. scheuchzeri* (Pennsylvanian). Similar $CH_{al}/C=O$ ratios suggest similarities in the cross-linking degree of polymeric structures of both extant and fossil taxa.

IR spectra of cutin and cuticle of *J. coriacea*, *M. scheuchzeri*, *Clivia miniata*, and *L. esculentum* share prominent aliphatic structures in the 3000–2800 cm^{-1} region, which is the most common structural group in the cutin biopolyester (e.g., Benítez et al., 2004; Koch and Ensikat, 2008; Villena et al., 2000; Heredia-Guerrero et al., 2014, and others). Peaks of weak to medium intensity are located at about 1167, 1113, 1073, 1044, and 964 cm^{-1} and are assigned to different C-O and O-H deformations in secondary alcohols (Table 2). They could be indicative of cellulose (Fengel, 1993), and were previously recorded in *M. scheuchzeri* (Lyons et al., 1992, 1995). Cutin IR spectra of *L. esculentum* and *M. scheuchzeri* showed similarities regarding C-O and O-H deformations (Table 2). However, cutin of *J. coriacea* exhibited only two weak and medium intensity peaks at 1035 cm^{-1} and 850 cm^{-1}. Relatively low substituted aromatic rings in cutin spectra of *M. scheuchzeri* and *J. coriacea* are indicated by weak absorptions recorded at 750 cm^{-1}. Cuticles of both *J. coriacea* and *M. scheuchzeri* did not show peaks at 1167 cm^{-1}, 1113 cm^{-1}, and 1073 cm^{-1}, which were present in cuticles of *C. miniata*.

4. Conclusions

(1) Highly aliphatic, oxidation-resistant, cutin obtained from the cuticle of *J. coriacea* is reported for the first time.
(2) Comparisons with cutin IR-derived ratios of *L. esculentum* (extant) and *M. scheuchzeri* (Pennsylvanian) indicate a similar degree of cross-linking (very likely ester bonds) of the polymeric structure, suggesting similarities in the chemical structures of both extant and fossil taxa.

Overall, the presented IR information for *J. coriacea* is yet the most positive support for the preservation of 220 Ma fossil cutin. It is suggested that this geomacropolymer is likely related to the resistant biomacropolymer making up the cuticle of the once living plants. If confirmed, the high resistance of the ancient biopolymer could be associated with a physiological adaptation to surviving extreme climatic conditions (e.g., hot and dry environments) as those proposed for Late Triassic times in southern Gondwana.

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Table 2
Comparison of main FTIR peaks (1800–800 cm^{-1} range) obtained from cutin and cuticle for some taxa available in the literature: *Clivia miniata* (extant), *Lycopersicon esculentum* (extant), *Macroneuropteris scheuchzeri* (Pennsylvanian), and *Johnstonia coriacea* (Triassic).

Taxon	Sample ID[a]	Peaks in the region 1800–900 cm^{-1} [b]								Reference
J. coriacea	Cutin	1730 (s)	1640–1540 (v)	1459 (m)	–	–	–	1035 (w)	850 (m)	This study (Fig. 2F)
J. coriacea	Cuticle	1726 (s)	1640–1540 (v)	1458 (m)	–	–	–	1035 (w)	850 (m)	This study (Fig. 2E)
M. scheuchzeri	Cutin	1730 (s)	1640–1540 (v)	1491 (w)	1170 (w)	1111 (m)	–	1025 (m)	840 (m)	D'Angelo et al. (2013, Fig. 2C1)
M. scheuchzeri	Cuticle	1714 (s)	1640–1540 (v)	1454 (m)	–	–	–	~1040 (vw)	838 (m)	D'Angelo et al. (2013, Fig. 2B1)
C. miniata	Cuticle	1732 (s)	1650–1500 (v)	1470 (m)	1167 (w)	1113 (w)	1073 (w)	1044 (w)	964 (w)	Villena et al. (2000), Fig. 1A
L. esculentum	Cutin	1730 (s)	1630–1550 (v)	1470 (m)	~1150 (m)	~1100 (w)	~1050 (w)	~1020 (w)	900–800 (w)	Benítez et al. (2004), Fig. 2

[a] As identified in the original reference.
[b] Intensity: s = strong, m = medium, w = weak, vw = very weak, v = variable.

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