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# Analysis by Fourier transform infrared spectroscopy of *Johnstonia* (Corystospermales, Corystospermaceae) cuticles and compressions from the Triassic of Cacheuta, Mendoza, Argentina

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**Abstract.** Spectroscopic information (functional groups and semi-quantitative data) of corystosperm cuticles and compressions from the Triassic of Cacheuta, Mendoza, Argentina, is reported for the first time. Fossil leaves of *Johnstonia* spp. (Corystospermales, Corystospermaceae) were analyzed by Fourier transform infrared spectroscopy (FT-IR) in an attempt to identify spectroscopic patterns that would characterize these taxa. Infrared spectra obtained from cuticles and compressions of *Johnstonia* spp. showed a relatively rich aliphatic structure as well as hydroxyl, carbonyl and some other oxygen-containing functional groups. Semi-quantitative data derived from FT-IR spectra were statistically analyzed using one-way analysis of variance test (ANOVA). In the three taxa studied herein, one-way ANOVA revealed significant differences between cuticles and their corresponding compressions regarding the $CH_2/CH_3$ ratio ($p < 0.05$). Considering the FT-IR-derived ratios $CH_2/CH_3$, Al/Ox, Ox1/Ox2 and C-H/C=O, there were not significant differences ($p > 0.05$) between abaxial and adaxial surfaces in the cuticular samples of *Johnstonia coriacea* var. *coriacea* (Johnston) Walkom here studied. Infrared-derived ratios here considered ($CH_2/CH_3$, Al/Ox and Ar/Al) in compression samples did not differ significantly from one taxon to the other ($p > 0.05$). However, cuticular specimens of *Johnstonia* spp. showed statistical differences ($p < 0.05$) among the taxa studied, considering $CH_2/CH_3$, Ox1/Ox2 and C-H/C=O ratios. Although these results are suggestive of the likely application of FT-IR technique to the chemotaxonomic study of the Corystospermaceae, more data are needed before obtaining definitive conclusions.

**Resumen.** ANÁLISIS POR ESPECTROCOSPÍA DE INFRARROJO CON TRANSFORMADA DE FOURIER DE CUTÍCULAS Y COMPRESIONES DE *JOHNSTONIA* (CORYSTOSPERMALES CORYSTOSPERMACEAE) DEL TRIÁSICO DE CACHEUTA, MENDOZA, ARGENTINA. Por primera vez se da a conocer información espectroscópica (grupos funcionales y datos semicuantitativos) de cutículas y compresiones de corystospermas del Triásico de Cacheuta, Mendoza, Argentina. Utilizando espectroscopia infrarroja con transformada de Fourier (IR-TF), se analizaron hojas fósiles de *Johnstonia* spp. (Corystospermales, Corystospermaceae) en un intento por identificar patrones espectroscópicos que caracterizarían a estos taxones. Los espectros de infrarrojo obtenidos de cutículas y compresiones de *Johnstonia* spp. mostraron una estructura alifática relativamente rica como así también hidroxilos, carbonilos y otros grupos funcionales que contienen oxígeno. Los datos semi-cuantitativos derivados de espectros de IR-TF fueron analizados estadísticamente utilizando el test de análisis de la varianza de un factor (ANOVA). En los tres taxones aquí estudiados y considerando la relación $CH_2/CH_3$, ANOVA de un factor reveló diferencias significativas entre cutículas y sus correspondientes compresiones ($p < 0.05$). Respecto a las relaciones $CH_2/CH_3$, Al/Ox, Ox1/Ox2 y C-H/C=O, no hubo diferencias significativas ($p > 0.05$) entre las superficies abaxial y adaxial de las cutículas de *Johnstonia coriacea* var. *coriacea* (Johnston) Walkom aquí estudiadas. Las relaciones $CH_2/CH_3$, Al/Ox y Ar/Al consideradas en compresiones no difirieron significativamente de un taxón a otro ($p > 0.05$). Sin embargo, las cutículas de *Johnstonia* spp. mostraron diferencias estadísticas ($p < 0.05$) entre los taxones estudiados, considerando las relaciones $CH_2/CH_3$, Ox1/Ox2 y C-H/C=O. Aunque estos resultados parecen indicar la posible aplicación de la técnica de IR-TF al estudio quimiotaxonómico de las Corystospermaceae, se requieren más datos antes de obtener conclusiones definitivas.

**Key words.** Corystosperm cuticles. *Johnstonia*. FT-IR spectroscopy. Triassic. Cacheuta. Argentina.

**Palabras clave.** Cutículas de corystospermas. *Johnstonia*. Espectroscopia IR-TF. Triásico. Cacheuta. Argentina.

## Introduction

*Johnstonia* Walkom is a leaf form-genus assigned to the family Corystospermaceae Thomas (1933) and frequently found in Gondwana Triassic rocks. Walkom (1925) proposed *Johnstonia* for a group of Mesozoic fronds from Tasmania (Australia) and distinguished this genus from *Thinnfeldia* Ettingshausen by the dichotomous rachis, the continuous lamina and a distinct venation. As accepted by several authors, specimens assigned to *Johnstonia* possess the characteristic bifurcation of the Corystospermaceae and are easily distinguished from *Dicroidium* Gothan (1912) by the absence of pinnules, having an entire, slightly lobed or pinnatifid lamina margin and taeniopteroid venation (*e.g.*, Frenguelli, 1943; Retallack, 1977; Petriella, 1979, 1981, 1985; Stipanicic *et al.*, 1995;

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Gnaedinger and Herbst, 2001; Zamuner *et al.*, 2001). However, and based on similarities of the epidermal features preserved on cuticular remains, some authors consider *Johnstonia* as a junior synonym of *Dicroidium* (*e.g.*, Townrow, 1957; Bonetti, 1966; Archangelsky, 1968; Anderson and Anderson, 1983).

Though *Johnstonia* leaves have been found in many Triassic beds of Gondwana, no permineralized remains have been reported yet. Therefore, the anatomy of this taxon remains unknown and all the information available comes from compression / impression materials.

Although anatomically preserved fossils generally provide the most unambiguous information, compressions exhibit the greatest available amount of biochemical information. It is particularly the case of compressed remains that resulted from compaction in anaerobic environments where microbial destruction of plant tissues was very limited. These fossil-plant remains were subjected to different and complicated chemical (structural) changes. As a result, the most thermodynamically stable, saturated and aromatic hydrocarbon counterparts were accumulated (*e.g.*, Brassel *et al.*, 1983; Thomas, 1986).

Coalified compressions usually include the cuticular membranes or cuticles, provided lower maturation levels prevailed. These unique plant structures constitute a protective covering occurring as a thin, continuous layer on the surface of the epidermal cells of leaves, fruits and non-woody stems. Because of the unparalleled functions of the cuticle for exchange of gases and liquids, and as the necessary interface between plant and atmosphere, many biochemical investigations on fossil and extant gymnosperm and angiosperm cuticles have been undertaken (*e.g.*, Holloway, 1982; Nip *et al.*, 1986; Tegelaar *et al.*, 1989, 1991; Kerp, 1990; van Bergen *et al.*, 1994; Almendros *et al.*, 1999 and citations therein). Cuticles have been found to be composed of macromolecular constituents such as cutin, cutan or, most commonly, a mixture of them. Their monomeric units are substituted, long chain aliphatic acids containing many functional groups such as hydroxyl, ether, aldehyde, ketone, peroxide and unsaturated groups (Nip *et al.*, 1986; Tegelaar *et al.*, 1991; Lyons *et al.*, 1995; Mösle *et al.*, 1997, 1998; Collinson *et al.*, 1998).

Differences in the chemical makeup of cuticles are suggested by the well-known difficulties to prepare leaf cuticles of certain plant groups (*e.g.*, Carboniferous pteridosperms). This has been confirmed by Tegelaar *et al.* (1991) who reported that cutan was not ubiquitously present in all plant cuticles. Past variations in the chemical composition of plant cuticles may have influenced leaf preservation and could be used as the basis for chemotaxonomic studies.

Paleobiochemical analysis of compressed remains and their associated cuticles can offer valuable extra information not only for taxonomic but also for systematic purposes. A wide variety of chemical analysis techniques have been applied to the study of fossil-plant remains including pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS), liquid chromatography/mass spectrometry (LC/MS), $^{13}C$ nuclear magnetic resonance ($^{13}$CNMR), Fourier Transform Infrared Spectroscopy (FT-IR) and fluorescence spectra. Over the last decades, the development of rapid screening techniques has increased the interest in analyzing plant fossils. This is the case of FT-IR, a rapid technique, which requires very small amounts of sample (a few milligrams). This is especially valuable since each cuticle sample is limited in quantity. Recent studies, using mainly FT-IR, have focused in the chemical study (*e.g.*, determination of functional groups) of Pennsylvanian remains with chemotaxonomic purposes (Lyons *et al.*, 1995; Zodrow *et al.*, 2000, 2003; Zodrow and Mastalerz, 2001, 2002; Pšenicka *et al.*, 2005). Thus, FT-IR becomes a valuable tool to obtain chemical information, which, in addition to standard morphological and epidermal characters, could assist distinguishing among different taxa.

The aim of this contribution is to offer the first chemical study (FT-IR) of corystosperm remains (leaf cuticles and their associated compressions) assigned to *Johnstonia* Walkom from the Triassic of Cacheuta, Mendoza, Argentina, on which there is no previous organic chemical literature.

## Material and methods

### *Sampling site*

*Johnstonia* samples originated from a collecting site located near Quebrada del Durazno (33°04'74'' S, 69°07'18'' W, 1413 m above sea level) in the southern side of the Cacheuta hill, Mendoza, western Argentina. Fossils are preserved as compressions / impressions in gray pelites of the upper section of the Potrerillos Formation in the alternating psamites and pelites sedimentary facies. The latter is mainly composed by psamites and pelites (claystones, carbonaceous claystones and siltstones). Pelite colors vary from yellowish white to gray. They often contain organic, carbonaceous material, compressions of fossil plants and fish scales. From the information of lithofacies, Morel (1994) has interpreted this depositional environment as a floodplain of a fluvial system. Here, moderate energy flows alternated with relative quiescence events in which fine-grained sediments accumulated by settling.

According to the paleobotanical biozonation and the chronostratigraphic chart proposed by Spalletti *et al.* (1999) for the continental Triassic of Argentina, *Johnstonia* remains are located in the BNP biozone (*Yabeiella brackebuschiana - Scytophyllum neuburgianum -Rhexoxylon piatnizkyi*) of the Cortaderitian stage, which is early Late Triassic in age. The assemblage containing *Johnstonia* leaves suggests a parautochthonous taphocoenosis as indicated by the characteristics of preservation of the fossil remains. Herbaceous and shrub-like paleocommunities like these are interpreted as dominated by pteridosperms bearing *Johnstonia* leaves (Spalletti *et al.*, 1999).

The collecting site was selected because of the well-preserved cuticles yielded from compressions of *Johnstonia* and the relative abundance of this taxon, which is particularly important when chemical analyses and statistical studies require several samples.

Further details on the geologic, stratigraphic and paleofloristic contents of the Cacheuta hill Triassic sequences can be found in recent contributions (Morel, 1994; Morel and Povilauskas, 2002).

All specimens and FT-IR pellets are housed in the paleobotanical collection of Cátedra de Geoquímica (CGSL-Pb), Área de Química Analítica, Facultad de Química, Universidad Nacional de San Luis, Argentina.

### *Sample preparation*

In almost all the cases, compressions were relatively loosely attached to the rock. Thus, only a mechanical aid was needed to remove the required portion. In a few specimens, compressions were released from the rock surface using 24 M hydrofluoric acid (HF) for a few minutes. Each separated sample was split into two portions; one portion was retained without further treatment, whereas the other was chemically treated (maceration). The latter was carried out according to the standard procedure: compressions (pitch-black color) were immersed in Schulze's solution, prepared with 5 g potassium chlorate ($KClO_3$) dissolved in 150 ml of 16 M nitric acid ($HNO_3$), for a maximum of 1 h. Cuticles thus obtained (light-amber color) were treated in 1.3 M ammonium hydroxide solution ($NH_4OH$) and finally rinsed in distilled water to neutralize.

Specimens for FT-IR were prepared using the potassium bromide (KBr) pellet technique. A very small amount of the compression or cuticle (approximately 0.3 wt % of the mixture) was mixed with finely ground KBr to produce 13-mm diameter pellets.

### *Fourier transform infrared spectroscopy analysis*

Infrared spectra were collected on a Nicolet "Protégé 460" Spectrometer, equipped with a CsI beamsplitter, a DTGS-CsI detector and an Ever Glo-type source. The acquisition conditions were 4 $cm^{-1}$ resolution and 64 interferograms were co-added before Fourier transformation. Spectral band assignments were made according to Colthup *et al.* (1964), Painter *et al.* (1981), Wang and Griffith (1985) and Ingle and Crouch (1988).

Some area-integration methods (*e.g.*, Sobkowiak and Painter, 1992; D`Angelo, 2004; D`Angelo and Marchevsky, 2004) were applied in the following regions of FT-IR spectra to obtain semi-quantitative data: (a) 2800-3000 $cm^{-1}$ (aliphatic C-H stretching), (b) 1600-1800 $cm^{-1}$ and (c) 700-900 $cm^{-1}$ (aromatic C-H out-of-plane bending). Statistical analysis of results included one-way analysis of variance (ANOVA). This test was carried out to assess whether the different variables evaluated conducted to statistically different results.

## Systematic paleontology

### Johnstonia *and the taxonomy of the Corystospermaceae foliage*

The Corystospermaceae, a dominant component of most Gondwana Triassic palaeofloras, constitutes an independent order: the Corystospermales (Petriella, 1981). Thomas (1933) established the family Corystospermaceae for a relatively small group of reproductive structures (ovulate and pollen organs) from the Triassic of the upper Umkomaas Valley, Natal, South Africa. In the same contribution, Thomas (1933: 247) firstly suggested *Dicroidium*, "*Stenopteris*" (sic) and *Johnstonia* as the likely members of the Corystospermaceae foliage.

The leaf form-genus *Johnstonia* was proposed by Walkom (1925) for a group of Triassic fronds from Tasmania (Australia). According to this author, the dichotomous rachis, the continuous lamina and a distinct venation distinguish *Johnstonia* from *Thinnfeldia* leaves. In a classical contribution, Frenguelli (1943) recognized *Johnstonia* as an independent taxon, assigning to it some corystospermaceous leaves from several Triassic beds of Argentina. Since Gothan (1912) introduced the genus *Dicroidium*, several taxa have been proposed to describe the foliage of the Corystospermaceae. Usually based on external morphology with the aid, in some cases, of epidermal features preserved on cuticular remains, many taxa including genera, species, subspecies, formae and even varieties have been established and subsequently treated as synonyms.

To date, no general agreement has been achieved regarding the taxonomy of the Corystospermaceae foliage. Such a seriously inconsistent taxonomy is the result of both a poor knowledge of the entire plant and the use of a classification system generally based on pinnule morphology. Thus, there are currently in use two different proposals to identify the foliage of the Corystospermaceae. In the first, some authors, based on the similarities found in the epidermal features, consider that only the genus *Dicroidium* should be used (*e.g.*, Townrow, 1957; Bonetti, 1966; Archangelsky, 1968; Anderson and Anderson, 1983). In the second proposal, some others, considering more important the overall frond morphology (size and shape of lamina segments and venation patterns), recognize several form-genera: *Dicroidium*, *Diplasiophyllum*, *Johnstonia*, *Xylopteris* and *Zuberia* (*e.g.*, Frenguelli, 1943, 1944; Retallack, 1977; Petriella, 1979, 1981, 1985; Baldoni, 1980; Artabe, 1990; Stipanicic *et al.*, 1995; Gnaedinger and Herbst, 2001; Zamuner *et al.*, 2001).

Recently, more quantitative methods have been applied to the study of the Corystospermaceae foliage. In an attempt to avoid some of the taxonomic ambiguities produced by the overlap in pinnule shape, Boucher (1994) has employed a morphometric technique to assist delimiting foliage species of this group. In this study, only one genus, *Dicroidium*, is recognized and elliptic Fourier analysis followed by multivariate analysis (principal component analysis and cluster analysis) has been used as additional evidence supporting the species division. Only pinnate fronds have been included in this analysis (entire lamina, bipinnate and tripinnate fronds have not been considered).

As stated above, identification of genera, species and varieties may be subjective, the result being a seriously inconsistent taxonomy of the Corystospermaceae foliage. Only with the aim of clearly exhibiting information derived from FT-IR, can the corystosperm leaf remains studied in this contribution be identified according with the second proposal. Thus, *Johnstonia* specimens are classified using frond external morphology and the taxonomic guidelines given by Retallack (1977) and Petriella (1979) in an attempt to correlate chemical information and different morphotaxa (species and varieties). When appropriate for comparison purposes (and only as remarks), subspecies, formae and varieties proposed by some authors will be considered. A discussion regarding the advantages or disadvantages of using one proposal or the other to describe the Corystospermaceae foliage is beyond the scope of this contribution.

## Order CORYSTOSPERMALES Petriella, 1981
## Family CORYSTOSPERMACEAE Thomas, 1933

### Genus ***Johnstonia*** Walkom, 1925

**Type species.** *Johnstonia coriacea* (Johnston, 1887) Walkom, 1925.

### ***Johnstonia coriacea*** (Johnston, 1887) Walkom, 1925
Figures 1.C, E-G

1979. *Johnstonia coriacea* (Johnston) Walkom; Petriella, p. 98, lám. II, fig. 6, text-fig. 8.
1982. *Dicroidium coriaceum* var. *coriaceum* (Johnston) Townrow; Holmes, p. 6, fig. 3 B-D.
1983. *Dicroidium coriaceum* (Johnston) Townrow subspecies *coriaceum* Anderson and Anderson; p. 92, pl. 76 (1-11) and pl. 31 (1-6).

**Synonymy.** See Retallack, 1977, frames I 24, I 25.

**Referred material.** CGSL-Pb 285, 289, 334, 343, 351 and 412.

**Remarks.** Once forked specimens having leathery simple leaves and a continuous lamina are included in this species. The largest of the specimens here considered (CGSL-Pb 334, figure 1.G -left) shows forked leaves (dichotomy angle of 20-30º) estimated to be up to 15 cm in length and 0.3-0.5 cm in width. Specimens exhibit a narrow leaf blade with a lamina margin varying from entire (figures 1.C and G -left) to slightly lobed (figures 1.E-F and G -right). Venation (not clearly visible) shows a prominent mid rib. These characteristics agree with those given by Retallack (1977) and Petriella (1979). They prefer to include in this species those fronds with entire or very slightly wavy leaf margins. Retallack (1977, frames I 23 - I 25) recognized two varieties: var. *coriacea* and var. *obesa*. According to this author, *J. coriacea* var. *coriacea* is characterized (and easily distinguished from the second variety) by having a leaf blade no wider than 0.8 cm and a rachis below the fork shorter than that above the fork. The variety *obesa* has not been reported yet for the Triassic sequences of Argentina. Thus, the specimens here studied are assigned to *J. coriacea* var. *coriacea* (Johnston) Walkom. They are also similar to those specimens from the Molteno Formation (South Africa) described as *Dicroidium coriaceum* subsp. *coriaceum* (Anderson and Anderson, 1983: 92 pl. 76. 1-11 and pl. 31. 1-6).

### ***Johnstonia stelzneriana*** (Geinitz, 1876) Frenguelli, 1943
Figures 1.A-B, D

1979. *Johnstonia stelzneriana* (Geinitz) Frenguelli, Petriella, p. 98, lám. II, fig. 7, text-fig. 9.
1983. *Dicroidium crassinervis* (Geinitz) Anderson and Anderson forma *stelznerianum* (Geinitz) Anderson and Anderson; *e.g.*, p. 93, pl. 31 (7) and pl. 68 (4, 7-8).
1995. *Johnstonia stelzneriana* var. stelzneriana (Geinitz) Frenguelli; Ganuza *et al.*, p. 8, lám. I, fig. e.

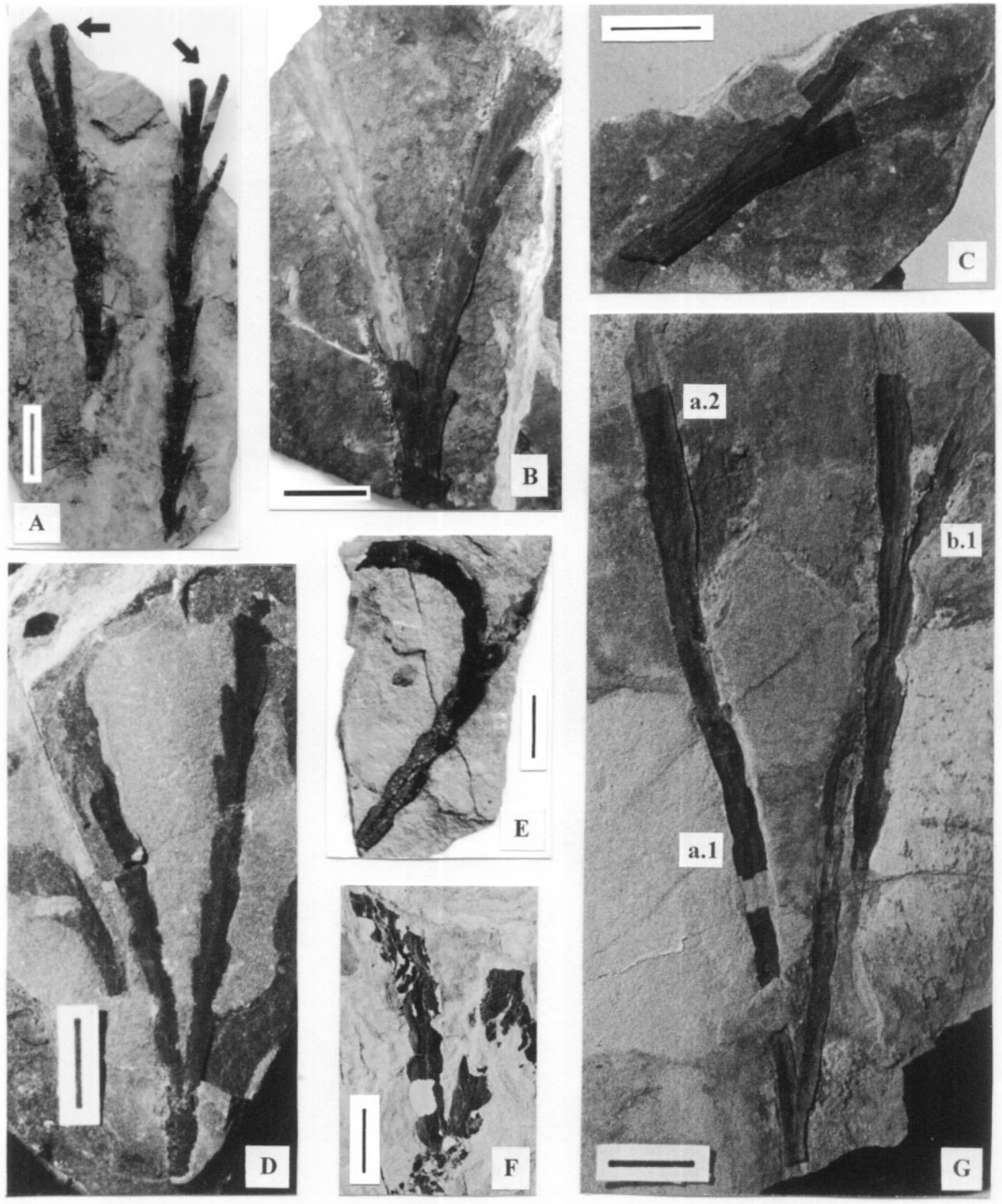



**Figure 1.** General view of the specimens / *Aspecto general de los ejemplares*. **A, B** and **D, *Johnstonia stelzneriana*** (Geinitz) Frenguelli; **A** and **B,** type / *tipo* var. ***stelzneriana*** (Geinitz) *Frenguelli*, **A** (CGSL-Pb 334-1) arrows indicate incomplete apices apparently forked for a second time / *las flechas indican los ápices incompletos aparentemente bifurcados por segunda vez*, **B** (CGSL-Pb 350); **D** type / *tipo* var. ***serrata*** Retallack (CGSL-Pb 341). **C, E-G, *Johnstonia coriacea*** var. ***coriacea*** (Johnston) Walkom; **C** (CGSL-Pb 351), **E** (CGSL-Pb 289), **F** (CGSL-Pb 285) and **G** (CGSL-Pb 334) two specimens partly superimposed with indication of sampling points for FT-IR analysis / *dos especímenes parcialmente superpuestos con indicación de los puntos de muestreo para el análisis por IR-TF*: a.1, (FT-IR specimen / *espécimen IR-TF)* 334-5; a.2, 334-6 and / *y* b.1, 334-3 . Scale bar / *escala*: 1 cm.

**Synonymy.** See Retallack, 1977, frames J 3, J 4.

**Referred material.** CGSL-Pb 328 b-L, 334-1, 341, 343 and 350.

**Remarks.** Specimens here assigned to *J. stelzneriana* (Geinitz) Frenguelli include simple leathery leaves, forking once (angle of dichotomy 20-30º) and estimated to be up to 12 - 14 cm in length and 0.4 - 0.5 cm in width. Leaf blades above the fork exhibit deeply

lobed margins. Some of these lobes give the leaf a pinnate appearance. Venation (not clearly visible) shows a mid rib and secondary veins emerging jointly at a very acute angle to the midvein. Each lobe has two - three secondary veins arising far below the lobe in which they end. These characteristics are in agreement with the natural variation of the species accepted by Retallack (1977) and Petriella (1979). The former author established two varieties: var. *serrata* and var. *stelzneriana* (Retallack 1977, frames J 2 - J 5). Though both varieties are pinnatifid, the var. *stelzneriana* is characterized by the well-incised, elongated and narrow lobes. In their comprehensive study of the *Dicroidium* flora, Anderson and Anderson (1983, *e.g.*, p. 93, pl. 31. 7 and pl. 68. 4, 7-8) considered the two varieties as *Dicroidium crassinervis* forma *stelznerianum*. Accepting the distinguishing features proposed by Retallack (1977), some of the specimens here studied can be assigned to *J. stelzneriana* (Geinitz) Frenguelli var. *serrata* Retallack (CGSL-Pb 328 b-L, CGSL-Pb 341 -figure 1.D-, and CGSL-Pb 343-1) while some others can be better regarded as *J. stelzneriana* var. stelzneriana (Geinitz) Frenguelli (CGSL-Pb 334-1 and CGSL-Pb 350 -figures 1.A-B, respectively). Specimen CGSL-Pb 334-1 (figure 1.A) is slightly different because of the aspect of its incomplete apices apparently forked for a second time with a more acute secondary fork angle of ≈ 17°. It should be noted that the counterpart of this specimen clearly shows the extension of the broken apices (see upper right corner of figure 1.A showing the compressed lamina loosely attached to the rock). Although there are only a few references in the literature, some authors have reported the presence of corystosperm leaf specimens (especially *Dicroidium* species) showing a secondary dichotomy (*e.g.*, Townrow, 1967: 464; Anderson and Anderson, 1983: 77, pl. 71).

## Results and discussion

Three corystosperm foliar taxa were analyzed using FT-IR technique: *Johnstonia coriacea* var. *coriacea* (Johnston) Walkom, *J. stelzneriana* var. *stelzneriana* (Geinitz) Frenguelli and *J. stelzneriana* (Geinitz) Frenguelli var. *serrata* Retallack. Structural information obtained from FT-IR spectra of *Johnstonia* leaves is presented for cuticles and their associated compressions. Only mature individuals were selected and each sample was separated from approximately the middle part of the leaf blade above the fork. For comparative purposes, apex and petiole samples of some specimens were also included. Cuticular samples of some specimens were compared in terms of abaxial and adaxial surfaces. Numbers identifying FT-IR pellets in the next sections follow the acquisition numbers given in "Systematic paleontology" section except for the abbreviation CGSL-Pb. The latter has been assigned only to hand specimens. It should be noted that FT-IR sample preparation resulted into some weight loss and the unpaired compression-cuticle for some specimens (see table 1).

### *Fourier transform infrared spectroscopy qualitative analysis*

Compression and cuticle FT-IR spectra of *Johnstonia* specimens are similar to one another, exhibiting the same general characteristics. Thus, only selected FT-IR spectra of compressions and cuticles (adaxial and abaxial or upper and lower -UC and LC, respectively-) are shown in figure 2. Peak assignments given here are applicable to both types of fossil remains.

A broad and intense band centered between 3400 and 3300 $cm^{-1}$ and generally attributed to H-bonded hydroxyl (OH) stretch is shown by both compression and cuticle FT-IR spectra (figures 2.A-D).

Distinct peaks, ascribed to aliphatic C-H stretching vibrations, are present in the region below 3000 $cm^{-1}$. These bands are assigned to asymmetric methylene ($CH_2$) stretch (2936-2916 $cm^{-1}$) and symmetric $CH_2$ stretch (2863-2843 $cm^{-1}$). Figures 2.A-D show that these bands are present in both cuticles and compressions.

Depending on the kind of sample (UC, LC or compression), absorption differences are recorded in the region 1800-1000 $cm^{-1}$ (figures 2.E-H).

Weak absorptions (shoulders) at ≈1700 $cm^{-1}$ due to carbonyl (C=O) stretching of carboxyl (COOH) and other C=O groups (*e.g.*, singly conjugated ketones) are exhibited by compressions. Cuticles (UC and LC) show prominent bands at higher wavenumbers (1716 $cm^{-1}$) which are more typical of acids.

A broad and intense peak is centered at ≈ 1620-1585 $cm^{-1}$ (compression samples) and at ≈1639-1632 $cm^{-1}$ (UC and LC samples). The intensity of this peak depends on the contribution of several structures: aromatic, olefinic, quinoid, ketone and, to some extent, conjugated carbonyl and some amide groups.

UC and LC show weak absorbances at 1554 $cm^{-1}$ (figures 2. G-H) in the range expected (1540-1610 $cm^{-1}$) for the asymmetric C-O stretch of ionized carboxylate groups. The associated symmetric stretch at ≈1400 $cm^{-1}$ is also present as a shoulder (figures 2.E-F). These bands are absent in the corresponding compressions.

Medium to low intensity bands assigned to aliphatic C-H deformations (alkyl C-H bending mode) are also present. They occur at 1452-1456 $cm^{-1}$ and represent $CH_2$ scissors deformation and / or methyl ($CH_3$) asymmetrical deformation (see figures 2.E-H).

**Table 1.** Infrared absorbance ratios of ***Johnstonia*** spp. cuticles and their associated compressions. Each value is the mean of three determinations. a Chemically treated cuticles: UC = Adaxial cuticle, LC = Abaxial cuticle, CT = non-separated cuticles; b St dev = Standard deviation; c Al / Ox = (2800-3000 $cm^{-1}$) / (1600-1800 $cm^{-1}$); d Ox1 / Ox2 = (1700-1800 $cm^{-1}$) / (1600-1700 $cm^{-1}$); e Ar / Al = (700-900 $cm^{-1}$) / (2800-3000 $cm^{-1}$). / *Relaciones de absorbancia de Infrarrojo de cutículas de* **Johnstonia** *spp. y de sus compresiones asociadas. Cada valor es la media de tres determinaciones. a Cutículas tratadas químicamente: UC = Cutícula adaxial, LC = Cutícula abaxial, CT = Cutículas no separadas; b St dev = Desviación estándar.*

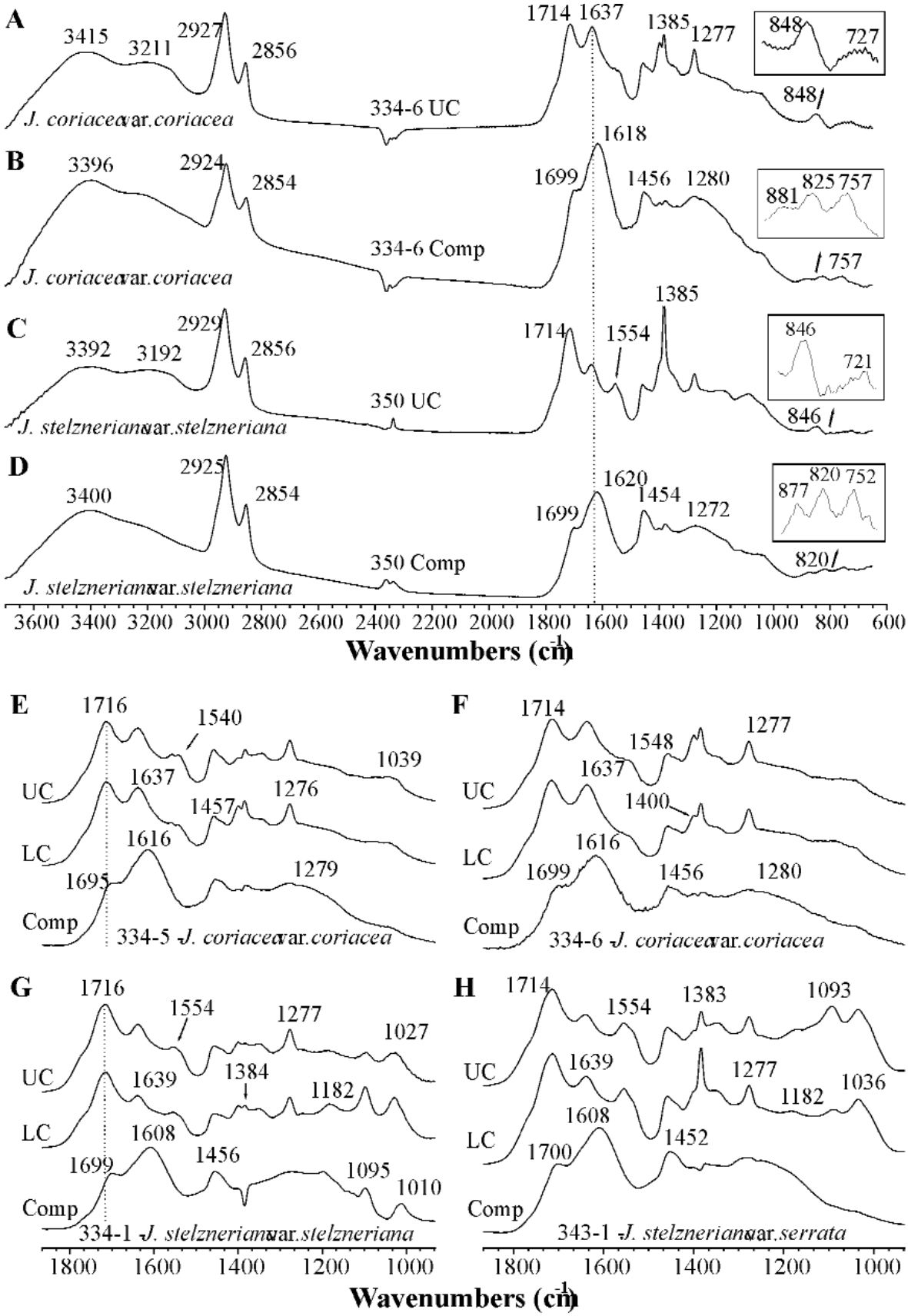
| Taxa and Sample number [a] | $CH_2$ / $CH_3$ | St dev [b] | Al / Ox [c] | St dev | Ox1 / Ox2 [d] | St dev |
|---|---|---|---|---|---|---|
| *Cuticles* | | | | | | |
| *Johnstonia coriacea* var. *coriacea* | | | | | | |
| 289 UC (petiole) | 8.4 | 0.2 | 0.87 | 0.02 | 7.1 | 0.1 |
| 334-3 CT | 7.2 | 0.1 | 0.95 | 0.02 | 6.5 | 0.1 |
| 334-5 UC | 6.7 | 0.1 | 1.1 | 0.02 | 2.3 | 0.05 |
| 334-5 LC | 7.6 | 0.2 | 0.78 | 0.02 | 2 | 0.04 |
| 334-6 UC (apex) | 6.1 | 0.1 | 0.84 | 0.02 | 1.7 | 0.03 |
| 334-6 LC | 7.1 | 0.1 | 0.58 | 0.01 | 1.7 | 0.03 |
| 343-2 UC | 6.5 | 0.1 | 1.07 | 0.02 | 5.5 | 0.1 |
| 343-2 LC | 9.1 | 0.2 | 0.95 | 0.02 | 5.3 | 0.1 |
| 351 UC | 9.6 | 0.2 | 0.9 | 0.02 | 5 | 0.1 |
| 351 LC | 8.1 | 0.2 | 0.77 | 0.02 | 2.1 | 0.04 |
| 412 CT | 5.8 | 0.1 | 0.96 | 0.02 | 4.9 | 0.1 |
| *J. stelzneriana* var. *serrata* | | | | | | |
| 328 b (L) CT | 11.1 | 0.2 | 0.63 | 0.01 | 8.4 | 0.2 |
| 341 CT | 10.2 | 0.2 | 0.79 | 0.02 | 7.2 | 0.1 |
| 343-1 UC | 12.1 | 0.2 | 1.09 | 0.02 | 7.7 | 0.2 |
| 343-1 LC | 12.4 | 0.2 | 1.13 | 0.02 | 6.2 | 0.1 |
| *J. stelzneriana* var. *stelzneriana* | | | | | | |
| 334-1 UC | 6.4 | 0.1 | 0.8 | 0.02 | 5.6 | 0.1 |
| 334-1 LC | 7.5 | 0.1 | 0.69 | 0.01 | 9 | 0.2 |
| 350 UC | 9.2 | 0.2 | 0.91 | 0.02 | 6.6 | 0.1 |
| 350 LC | 10.9 | 0.2 | 0.63 | 0.01 | 6.7 | 0.1 |
| Taxa and Sample number | $CH_2$ / $CH_3$ | St dev | Al / Ox | St dev | Ar / Al[e] | St dev |
| *Compressions* | | | | | | |
| *Johnstonia coriacea* var. *coriacea* | | | | | | |
| 285 | 3 | 0.06 | 0.56 | 0.01 | 0.084 | 0.002 |
| 289 (petiole) | 3.7 | 0.07 | 0.54 | 0.01 | 0.064 | 0.001 |
| 334-3 | 3.7 | 0.07 | 0.41 | 0.008 | 0.22 | 0.004 |
| 334-5 | 3.6 | 0.07 | 0.41 | 0.008 | 0.14 | 0.003 |
| 334-6 (apex) | 3.7 | 0.07 | 0.47 | 0.009 | 0.091 | 0.002 |
| 343-2 | 4.2 | 0.08 | 0.59 | 0.01 | 0.14 | 0.003 |
| 351 | 3.9 | 0.08 | 0.66 | 0.01 | 0.077 | 0.002 |
| 412 | 2.9 | 0.06 | 0.57 | 0.01 | 0.081 | 0.002 |
| *J. stelzneriana* var. *serrata* | | | | | | |
| 328 b (L) | 3.4 | 0.07 | 0.63 | 0.01 | 0.088 | 0.002 |
| 341 | 3.4 | 0.07 | 0.67 | 0.01 | 0.095 | 0.002 |
| 343-1 | 4.5 | 0.09 | 0.59 | 0.01 | 0.18 | 0.004 |
| *J. stelzneriana* var. *stelzneriana* | | | | | | |
| 334-1 | 3.3 | 0.07 | 0.58 | 0.01 | 0.12 | 0.002 |
| 334-4 (apex) | 3.3 | 0.07 | 0.41 | 0.008 | 0.2 | 0.004 |
| 350 | 4.2 | 0.08 | 0.82 | 0.02 | 0.052 | 0.001 |

Intense peaks at 1375-1382 $cm^{-1}$ could be attributed to $CH_3$ umbrella deformations but contribution of some mineral impurities should not be ruled out.

Bands at 1275-1270 $cm^{-1}$ could be assigned to methoxyphenolic, lignin-derived aromatic units (Durig *et al.*, 1988; Zodrow *et al.*, 2000).

A low intensity band at 1182 $cm^{-1}$ represents symmetric C-O-C vibrations. Bands at ≈1095 $cm^{-1}$ and 1035-1027$cm^{-1}$ are attributed to mineral-matter content (Si-O stretching of silicate impurities).

There are weak bands near 1010 $cm^{-1}$ (compression spectra) usually assigned to aromatic C-H in-


A
3415
3211
2927
2856
1714 1637
1385
1277
848
727
848
334-6 UC
J. coriacea var. coriacea
B
3396
2924
2854
1618
1699
1456
1280
881
825 757
757
334-6 Comp
J. coriacea var. coriacea
C
3392
3192
2929
2856
1714
1554
1385
846
721
846
350 UC
J. stelzneriana var. stelzneriana
D
3400
2925
2854
1620
1699
1454
1272
877
820 752
820
350 Comp
J. stelzneriana var. stelzneriana
3600 3400 3200 3000 2800 2600 2400 2200 2000 1800 1600 1400 1200 1000 800 600
Wavenumbers (cm-1)
E
1716
1540
1039
UC
1637
1276
1457
LC
1616
1695
1279
Comp
334-5 J. coriacea var. coriacea
F
1714
1548
1277
UC
1637
1400
LC
1616
1699
1456
1280
Comp
334-6 J. coriacea var. coriacea
G
1716
1554
1277
1027
UC
1639
1384
1182
LC
1608
1699
1456
1095
1010
Comp
334-1 J. stelzneriana var. stelzneriana
H
1714
1554
1383
1093
UC
1639
1277
1182
1036
LC
1608
1452
1700
Comp
343-1 J. stelzneriana var. serrata
1800 1600 1400 1200 1000
Wavenumbers (cm-1)

plane bending vibrations (on a benzene ring). These bands are absent in cuticle spectra.

Some low intensity bands occur in the region 700-900 $cm^{-1}$. In compression samples these bands occur at ≈755 $cm^{-1}$, ≈820 $cm^{-1}$ and ≈870 $cm^{-1}$. They are assigned to aromatic C-H out-of-plane bending vibrations (insets in figures 2.B and D). Cuticle samples rarely show aromatic bands in this region. Instead, there are some bands (at ≈720 $cm^{-1}$ and ≈850 $cm^{-1}$) representing $CH_2$ rocking vibrations (insets in figures 2.A and C).

### *Fourier transform infrared spectroscopy semi-quantitative analysis*

Some area-integration methods (*e.g.*, Sobkowiak and Painter, 1992; D`Angelo, 2004; D`Angelo and Marchevsky, 2004) were applied in the following regions of FT-IR spectra to obtain semi-quantitative data: (a) 2800-3000 $cm^{-1}$ (Al = aliphatic C-H stretching), (b) 1600-1800 $cm^{-1}$ (Ox = contribution of several groups - *e.g.*, aromatic, olefinic, ketone, conjugated carbonyl-) and (c) 700-900 $cm^{-1}$ (aromatic C-H out-of-plane bending). Among the corystosperm samples studied herein, aromatic C-H out-of-plane bending bands were detected in compression samples (they were absent in cuticle spectra). However, bands assigned to aromatic C-H out-of-plane bending vibrations and aliphatic C-H stretching vibrations can be used as a measure of the distribution of hydrogen.

Estimated areas were employed to calculate ratios of integration areas. Nevertheless, it should be noted that the ratios derived from FT-IR spectra do not represent absolute contents of functional groups. Procedures of band deconvolution (Fourier self-deconvolution) were applied in the C-H stretching region to obtain $CH_2/CH_3$ ratios (Mastalerz, personal communication). Infrared spectral regions such as aliphatic stretching, oxygen-containing groups and aromatic C-H out-of-plane bending offer bigger areas and subsequently smaller errors when applying the integration methods in semi-quantitative analysis. Therefore, FT-IR spectra of *Johnstonia* cuticles and compressions are semi-quantitatively described by the following variables (ratios of integration areas):

- Methylene / methyl ratio ($CH_2/CH_3$).
- 2800-3000 $cm^{-1}$ / 1600-1800 $cm^{-1}$ (Al/Ox).
- 2936-2916 $cm^{-1}$ / 1700-1800 $cm^{-1}$ (C-H/C=O) (calculated only in cuticle samples).
- 1700-1800 $cm^{-1}$ / 1600-1700 $cm^{-1}$ (Ox1/Ox2) (calculated only in cuticle samples).
- 700-900 $cm^{-1}$ / 2800-3000 $cm^{-1}$ (Ar/Al) (calculated only in compression samples).

Table 1 shows the infrared absorbance ratios obtained for *Johnstonia* spp. cuticles and their associated compressions. These ratios and some others may also be used for comparative purposes. They provide valuable information on both diagenetic changes and chemical composition of the fossil remains (cuticles and compressions). Thus, $CH_2/CH_3$ ratio can provide information on the amounts of alkyl structures, $C\text{-}H_{str}$ / $C{=}O_{str}$ ratio could indicate the average apparent length of the aliphatic chains, Ar/Al ratio is used as an indicator of aromaticity (diagenetic alteration) in organic matter and Al/Ox ratio could supply useful information about oxidation in organic matter (Pradier *et al.*, 1992; Lin and Ritz, 1993). These semi-quantitative variables could also be used to gain an insight into the possible chemotaxonomic implications of FT-IR technique (Zodrow *et al.*, 2000, 2003; Pšenicka *et al.*, 2005).

Estimate of the average apparent aliphatic chain length in cuticles were calculated. The ratio of the aliphatic (asymmetric) C-H to C=O absorptions found in molecules of simple esters is approximately 11.71. This indicates molecules with an aliphatic chain length of about 13 units (Mösle *et al.*, 1998). Using this relationship, an estimate of the average apparent aliphatic chain length can be obtained for the corystosperm cuticles. The values of the asymmetric C-H and C=O absorbances reveal some apparent aliphatic C-H / C=O ratios as shown in table 2 where lower mean values appear to be common for *J. stelzneriana* specimens. However, such an analysis should be interpreted with caution because C=O absorptions may occur in different regions of the spectrum as weak absorbances at 1554 $cm^{-1}$ (figures 2. G-H). The latter is in the range expected (1550-1610 cm-

**Figure 2.** Comparison of ***Johnstonia*** FT-IR spectra: cuticles and associated compressions of some selected specimens / *comparación de espectros de IR-TF de* **Johnstonia***: cutículas y compresiones asociadas de algunos especímenes seleccionados*. **A-D**, compression-cuticle pairs in the region 650-3700 $cm^{-1}$. Insets show details of the 700-900 $cm^{-1}$ region: $CH_2$ rocking bands (**A** and **C**) and aromatic C-H out-of-plane bending modes (**B** and **D**) / *pares compresión-cutícula en la región 650-3700 $cm^{-1}$. Los recuadros muestran detalles de la región 700-900 $cm^{-1}$: bandas de balanceo de $CH_2$ (**A** y **C**) y modos de flexión fuera del plano C-H aromáticos (**B** y **D**)*; **A** and **B**, ***Johnstonia coriacea*** var. ***coriacea*** (Johnston) Walkom (334-6); C and **D**, ***Johnstonia stelzneriana*** var. ***stelzneriana*** (Geinitz) Frenguelli (350). **E-H**, Peak details in the region 1000-1800 $cm^{-1}$: adaxial cuticle (UC), abaxial cuticle (LC) and compression (Comp) / *detalles de los picos en la región 1000-1800 $cm^{-1}$: cutícula adaxial (UC), cutícula abaxial (LC) y compresión (Comp)*; **E** and **F**, ***Johnstonia coriacea*** var. ***coriacea*** (Johnston) Walkom (334-5 and 334-6, respectively / *334-5 y 334-6, respectivamente)*; **G** and **H**, ***Johnstonia stelzneriana*** (Geinitz) Frenguelli; **G,** type / ***tipo*** var. ***stelzneriana*** (Geinitz) Frenguelli (334-1); **H**, type / *tipo* var. ***serrata*** Retallack (343-1).

**Table 2.** Infrared absorbance ratios of aliphatic (asymmetric) C-H and C=O groups ($C\text{-}H_{str}$ / $C{=}O_{str}$) and average apparent aliphatic chain lengths ($CH_2$ : C=O) in cuticles of ***Johnstonia*** spp. Each value is the mean of three determinations / *relaciones de absorbancia de Infrarojo de grupos C-H alifáticos (asimétricos) y C=O ($C\text{-}H_{str}$ / $C{=}O_{str}$) y longitudes de cadena alifática aparente promedio ($CH_2$: C=O) en cutículas de* **Johnstonia** *spp. Cada valor es la media de tres determinaciones.*

| Taxa and Sample number | $C\text{-}H_{str}$ / $C{=}O_{str}$ | St dev | $CH_2$ : C=O |
|---|---|---|---|
| *Johnstonia coriacea* var. *coriacea* | | | |
| 289 UC (petiole) | 1.98 | 0.04 | 23 |
| 334-3 CT | 2.29 | 0.05 | 27 |
| 334-5 UC | 3.01 | 0.06 | 35 |
| 334-5 LC | 2.38 | 0.05 | 28 |
| 334-6 UC (apex) | 2.59 | 0.05 | 30 |
| 334-6 LC (apex) | 1.95 | 0.04 | 23 |
| 343-2 UC | 2.15 | 0.04 | 25 |
| 343-2 LC | 1.90 | 0.04 | 22 |
| 351 UC | 2.11 | 0.04 | 25 |
| 351 LC | 2.36 | 0.05 | 28 |
| 412 CT | 2.20 | 0.04 | 26 |
| *J. stelzneriana* var. *serrata* | | | |
| 328 b (L) CT | 1.19 | 0.02 | 14 |
| 341 CT | 1.64 | 0.03 | 19 |
| 343-1 UC | 2.22 | 0.04 | 26 |
| 343-1 LC | 2.48 | 0.05 | 29 |
| *J. stelzneriana* var. *stelzneriana* | | | |
| 334-1 UC | 1.76 | 0.04 | 21 |
| 334-1 LC | 1.38 | 0.03 | 16 |
| 350 UC | 1.77 | 0.04 | 21 |
| 350 LC | 1.19 | 0.02 | 14 |

$^{-1}$) for the asymmetric C-O stretch of ionized carboxylate groups. Furthermore the associated symmetric stretch (range expected ca. 1400 $cm^{-1}$) is also present as a shoulder (figures 2. E-F). Thus, including additional C=O absorption would reduce the apparent average aliphatic chain lengths below those shown in table 2. Considering some C-H stretching contributed by non-ester material, estimates of the actual (average) aliphatic C-H / C=O ratio and the corresponding chain length could be even more decreased.

Mean values of C-H / C=O ratios obtained for *Johnstonia* specimens studied in this contribution are compared to some other available data from the literature (table 3), belonging to fossil and extant taxa. Fossil specimens are from different locations, geological ages and fine-grained enclosing lithologies. Mean values obtained for *J. stelzneriana* specimens are similar to those of *Frenelopsis* sp., *Abietites linkii* (Roemer) Dunker, *Ginkgo biloba* Linnaeus and *G. adiantoides* (Unger) Heer, while *J. coriacea* mean values are closer to *Ginkgo coviacea* Florin.

Although some relationships may be suggested by the apparent aliphatic C-H / C=O ratios between different species, the influence of taphonomic factors should not be ruled out. In order to assess the reliability of these C-H / C=O preliminary results and their possible systematic value more FT-IR data from many different taxa are needed.

## *Cuticles and compressions of* Johnstonia *spp.*

Qualitative information, derived from *Johnstonia* spp. FT-IR spectra of cuticles and compressions, shows major chemical structural features (functional groups) such as hydroxyl, aliphatic C-H, carbonyl and C-O functions (oxygen-containing groups). In the region below 1700 $cm^{-1}$, cuticular specimens show distinct bands. Here, simple and pyrolytically labile ester carbonyl groups are absent. Ketone and carboxylic acid groups, yielded by hydrolysis of simple esters and subsequent oxidation of the mid-chain alcohol produced, replace them.

Compressions assigned to *Johnstonia* spp. show FT-IR spectra exhibiting similar qualitative charac-

**Table 3.** Comparative list of aliphatic C-H:C=O ratios ($C\text{-}H_{str}$ / $C{=}O_{str}$) and average apparent aliphatic chain lengths ($CH_2$: C=O) in cuticles of ***Johnstonia*** spp. and some other taxa from literature (Mösle *et al.*, 1998); a Mean value (standard deviation) / *lista comparativa de relaciones C-H alifáticos: C=O ($C\text{-}H_{str}$ / $C{=}O_{str}$) y longitudes de cadena alifática aparente promedio ($CH_2$: C=O) en cutículas de* Johnstonia *spp. y en algunos otros taxones de la literatura (Mösle et al., 1998); a Valor medio (desviación estándar).*

| Taxa | Location | Age | Lithology | $C\text{-}H_{str}/C{=}O_{str}$ | $CH_2$:C=O |
|---|---|---|---|---|---|
| *J. coriacea* var. *coriacea* [a] | Cacheuta, | Upper Triassic | Pelite | 2.3 (0.3) | 27 |
| *J. stelzneriana* var. *serrata* [a] | Mendoza, | Upper Triassic | Pelite | 1.9 (0.5) | 22 |
| *J. stelzneriana* var. *stelzneriana* [a] | Argentina | Upper Triassic | Pelite | 1.5 (0.3) | 18 |
| *Frenelopsis* sp. | Central Spain | Lower Cretaceous | Carbonate | 1.27 | 15 |
| *Abietites linkii* | NW Germany | Lower Cretaceous | Coal | 1.14 | 14 |
| *Ginkgo coviacea* | China | Cretaceous | Marl | 2.37 | 28 |
| *G. adiantoides* | North Dakota, USA | Upper Cretaceous | Clay | 1.88 | 22 |
| *G. biloba* | England | Recent | – | 1.11 | 13 |

teristics. Irrespective of the taxa, there are two distinctive features found in FT-IR spectra of compressions (non-chemically treated materials) which are absent in cuticle spectra:
-a prominent band centered at about 1608-1620 $cm^{-1}$ and
-distinct aromatic C-H out-of-plane bands at ≈ 880, 820 and 750 $cm^{-1}$.

These regions likely represent the contribution of non-cuticular organic matter (*e.g.*, vitrinitic matter), and have been used to distinguish compressions from cuticles in other plant groups, for example Pennsylvanian seed ferns and true ferns (Lyons *et al.*, 1995; Zodrow and Mastalerz, 2002; Pšenicka *et al.*, 2005).

Semi-quantitative data obtained from FT-IR spectra of *Johnstonia* spp. reveal differences between cuticles and their corresponding compressions. $CH_2/CH_3$ values found in cuticles are 2 - 3 times higher than the values recorded for their compressions (table 1). In the three taxa studied herein and with 95% confidence, statistical test one-way ANOVA revealed that there were significant differences between cuticles and their corresponding compressions regarding the $CH_2/CH_3$ ratio (table 4). The most significant differences between cuticles and compressions ($CH_2/CH_3$ ratio) were recorded for *J.coriacea* var. *coriacea*, followed by *J. stelzneriana* var. *serrata* and *J. stelzneriana* var. *stelzneriana*.

Cuticles also exhibit higher Al/Ox ratios (2800-3000 $cm^{-1}$/1600-1800 $cm^{-1}$) in comparison with the same variable found in compressions. This is an expected result: chemical treatment of compression specimens removes organic matter (bands centered at 1608-1620 $cm^{-1}$ and aromatic C-H out-of-plane bending modes disappear). As the contribution of the bands at 1600-1800 $cm^{-1}$ diminishes with compression maceration, and considering little or no variation in the aliphatic contents, a higher Al/Ox ratio is obtained for cuticular specimens. In *J.coriacea* var. *coriacea*, one-way ANOVA revealed statistically different Al/Ox values ($p < 0.05$) between cuticles and compressions. However, in *J. stelzneriana* var. *serrata* and *J. stelzneriana* var. *stelzneriana* there were no significant differences between cuticles and compressions regarding Al/Ox values ($p > 0.05$, see table 4).

## *Comparison among different taxa*

As clearly shown by table 1, FT-IR-derived ratios ($CH_2/CH_3$, Al/Ox and Ar/Al) here considered in compression samples of *Johnstonia* spp. do not differ considerably from one taxon to the other. In fact, one-way ANOVA test revealed that there were no statistically significant differences among the three taxa here studied ($p > 0.05$, see table 5). Thus, the three taxa (compression specimens) considered herein seem to be characterized by the following mean values: $CH_2/CH_3 = 3.6 \pm 0.06$, Al/Ox = 0.6 ± 0.04 and Ar/Al = 0.11 ± 0.005.

Regarding cuticular specimens of *Johnstonia* spp., table 1 shows some differences in FT-IR-derived ratios among the taxa studied. Considering $CH_2/CH_3$ ratio, mean value recorded was 7.5 ± 1.2 (range: 5.8 - 9.6) in *J. coriacea* var. *coriacea* specimens. Higher values of the same variable could be characteristic of *J. stelzneriana* (there are some exceptions as for example specimens 334-1 UC and 334-1 LC, see table 1). Statistical test one-way ANOVA revealed that the three taxa studied herein were significantly different

**Table 4.** Results of one-way ANOVA (α = 0.05). Comparison between cuticles and compressions. / *Resultados de ANOVA de un factor (α = 0.05). Comparación entre cutículas y compresiones.*

| Variable | Taxa | Type | *N* | Mean | St dev | *F* | *p* |
|---|---|---|---|---|---|---|---|
| $CH_2$ / $CH_3$ | *J. coriacea* var. *coriacea* | Cuticle | 11 | 7.5 | 1.2 | 101.86 | $2.7 \times 10^{-9}$ |
| | | Compression | 11 | 3.5 | 0.4 | | |
| | *J. stelzneriana* var. *serrata* | Cuticle | 4 | 11 | 1 | 186.03 | $9.6 \times 10^{-6}$ |
| | | Compression | 4 | 3.6 | 0.6 | | |
| | *J. stelzneriana* var. *stelzneriana* | Cuticle | 4 | 8.5 | 2 | 22.93 | 0.003 |
| | | Compression | 4 | 3.7 | 0.4 | | |
| Al / Ox | *J. coriacea* var. *coriacea* | Cuticle | 11 | 0.89 | 0.15 | 37.23 | $5.8 \times 10^{-6}$ |
| | | Compression | 11 | 0.55 | 0.11 | | |
| | *J. stelzneriana* var. *serrata* | Cuticle | 4 | 0.91 | 0.24 | 5.82 | 0.052 |
| | | Compression | 4 | 0.61 | 0.05 | | |
| | *J. stelzneriana* var. *stelzneriana* | Cuticle | 4 | 0.76 | 0.12 | 2.13 | 0.195 |
| | | Compression | 4 | 0.61 | 0.17 | | |

**Table 5.** Results of one-way ANOVA (α = 0.05). Comparison among different taxa. * For each variable, mean values with common identification (a, b) are not significantly different ($p > 0.05$) / *resultados de ANOVA de un factor (α = 0.05). Comparación entre diferentes taxones. * Para cada variable, los valores medios con identificación común (a, b) no son significativamente diferentes (p > 0.05).*

| Variable | Taxa | *N* | Mean | * | St dev | *F* | *p* |
|---|---|---|---|---|---|---|---|
| | *Cuticles* | | | | | | |
| $CH_2$ / $CH_3$ | *J. coriacea* var. *coriacea* | 11 | 7.5 | a | 1.2 | 12.45 | 0.0005 |
| | *J. stelzneriana* var. *serrata* | 4 | 11 | b | 1 | | |
| | *J. stelzneriana* var. *stelzneriana* | 4 | 8.5 | a | 2 | | |
| Al / Ox | *J. coriacea* var. *coriacea* | 11 | 0.89 | a | 0.15 | 1.10 | 0.358 |
| | *J. stelzneriana* var. *serrata* | 4 | 0.91 | a | 0.24 | | |
| | *J. stelzneriana* var. *stelzneriana* | 4 | 0.76 | a | 0.12 | | |
| Ox1 / Ox2 | *J. coriacea* var. *coriacea* | 11 | 4 | a | 2.1 | 7.18 | 0.006 |
| | *J. stelzneriana* var. *serrata* | 4 | 7.4 | b | 0.9 | | |
| | *J. stelzneriana* var. *stelzneriana* | 4 | 7 | b | 1.4 | | |
| C-H / C=O | *J. coriacea* var. *coriacea* | 11 | 2.3 | a | 0.3 | 5.93 | 0.012 |
| | *J. stelzneriana* var. *serrata* | 4 | 1.9 | a,b | 0.6 | | |
| | *J. stelzneriana* var. *stelzneriana* | 4 | 1.5 | b | 0.3 | | |
| | *Compressions* | | | | | | |
| $CH_2$ / $CH_3$ | *J. coriacea* var. *coriacea* | 11 | 3.5 | a | 0.4 | 0.13 | 0.877 |
| | *J. stelzneriana* var. *serrata* | 4 | 3.6 | a | 0.6 | | |
| | *J. stelzneriana* var. *stelzneriana* | 4 | 3.7 | a | 0.4 | | |
| Al / Ox | *J. coriacea* var. *coriacea* | 11 | 0.5 | a | 0.1 | 0.66 | 0.531 |
| | *J. stelzneriana* var. *serrata* | 4 | 0.61 | a | 0.05 | | |
| | *J. stelzneriana* var. *stelzneriana* | 4 | 0.61 | a | 0.17 | | |
| Ar / Al | *J. coriacea* var. *coriacea* | 11 | 0.11 | a | 0.06 | 0.04 | 0.963 |
| | *J. stelzneriana* var. *serrata* | 4 | 0.12 | a | 0.04 | | |
| | *J. stelzneriana* var. *stelzneriana* | 4 | 0.11 | a | 0.07 | | |

($p < 0.05$, see table 5) considering $CH_2/CH_3$ ratio. There was a statistical difference between *J. coriacea* var. *coriacea* and *J. stelzneriana* var. *serrata* (the most significant difference recorded, $p = 6.4 \times 10^{-5}$). Similarly, *J. stelzneriana* var. *serrata* and *J. stelzneriana* var. *stelzneriana* presented significantly different $CH_2/CH_3$ ratios ($p = 0.0369$). However, no statistical differences were recorded between specimens of *J. coriacea* var. *coriacea* and *J. stelzneriana* var. *stelzneriana* ($p = 0.2438$).

Al/Ox ratio, recorded for cuticular specimens of *Johnstonia* spp., showed no considerable variations among the three taxa (mean value 0.85 ± 0.08). These results were confirmed by one-way ANOVA test, which revealed no statistically significant differences ($p > 0.05$, table 5).

Lower Ox1/Ox2 values (below ≈3) are shown by cuticular specimens of *J. coriacea* (see table 1 and compare peaks at 1714 and 1637 $cm^{-1}$ in figures 2.A and C). One-way ANOVA revealed that the three taxa studied herein were statistically different ($p < 0.05$, see table 5) considering Ox1/Ox2 ratio. There was a significant difference between *J. coriacea* var. *coriacea* and *J. stelzneriana* var. *serrata* ($p = 0.0089$). Similarly, specimens of *J. coriacea* var. *coriacea* and *J. stelzneriana* var. *stelzneriana* were statistically different ($p = 0.022$). However, there was not a significant difference between *J. stelzneriana* var. *serrata* and *J. stelzneriana* var. *stelzneriana* ($p = 0.65028$).

Regarding the C-H / C=O ratio, lower mean values of around 1.7 appear to be common for *J. stelzneriana* while *J. coriacea* specimens show higher mean values of around 2.3 (table 2). Statistical test one-way ANOVA revealed that the three taxa studied herein were significantly different ($p < 0.05$, see table 5) considering C-H / C=O ratio. A statistical difference was recorded between *J. coriacea* var. *coriacea* and *J. stelzneriana* var. *stelzneriana* ($p = 0.00148$). However, there were no significant differences between *J. coriacea* var. *coriacea* and *J. stelzneriana* var. *serrata* ($p = 0.12465$) and *J. stelzneriana* var. *serrata* and *J. stelzneriana* var. *stelzneriana* ($p = 0.3126$).

Although these preliminary results are suggestive they are not conclusive. More data from many other specimens belonging to the same taxa, and some others, are needed to confirm the use of these FT-IR data for chemotaxonomic purposes.

## *Comparison between UC and LC*

Infrared-derived ratios ($CH_2/CH_3$, Al/Ox,

Ox1/Ox2 and C-H / C=O) here considered in cuticular samples of *Johnstonia coriacea* var. *coriacea* do not differ considerably between abaxial and adaxial surfaces (see table 6). With 95% confidence one-way ANOVA test revealed that there were not statistically significant differences between UC and LC. Similar values recorded for $CH_2/CH_3$ and C-H / C=O ratios suggest similarities in alkyl content of adaxial and abaxial surfaces.

Considering Ox1/Ox2 ratio, no statistical differences between UC and LC were revealed by one-way ANOVA test in the specimens of *Johnstonia coriacea* var. *coriacea* here considered ($p > 0.05$, see table 6).

Al/Ox ratio could provide some information regarding oxidation in organic matter. Adaxial and abaxial cuticles did not show considerable differences in Al/Ox ratio. One-way ANOVA revealed that the specimens of *Johnstonia coriacea* var. *coriacea* studied herein were not significantly different ($p > 0.05$, see table 6) considering Al/Ox ratio. This result could indicate that adaxial and abaxial surfaces have the same response to maceration (chemical treatment), suggesting similar constituents.

It should be noted that, among the specimens of *Johnstonia coriacea* var. *coriacea* studied herein, $CH_2/CH_3$ ratio presented the main similarities between UC and LC, followed by C-H / C=O ratio (table 6). Because of insufficient sample amounts, UC and LC of J. *stelzneriana* specimens could not be statistically analyzed. However, similar trends could be observed in the variables described above for *Johnstonia coriacea* var. *coriacea*.

## *Apex, middle part of leaf blade and petiole*

Leaf specimens of *Johnstonia* spp. are usually fragmentary and the middle part of the leaf blade above the fork is more frequently preserved than petiole and apex. Although the number of specimens was insufficient to perform a statistical analysis, compressions and cuticles obtained from the apex and petiole of some *J. coriacea* var. *coriacea* specimens were also included. These comparative determinations can be useful for detecting possible variability in cutinization along the length of a single frond. Table 1 shows very similar values obtained for all of the variables studied in some samples. Specimens 334-5 and 334-6, representing the middle part of leaf blade and the apex of the same leaf respectively, (see FT-IR sampling points a.1 and a.2 in figure 1.G) exhibit no considerable differences in the variables determined. This tendency is recorded not only in cuticles but also in the associated compressions. In the case of cuticular specimens, apex samples (334-6 UC and 334-6 LC) show slightly lower values in the variables considered ($CH_2/CH_3$, Al/Ox, Ox1/Ox2 and CH/C=O) if compared to the corresponding values obtained for the middle part of the same leaf (334-5 UC and 334-5 LC, see tables 1 and 2). However, these differences are not considered as substantial. Similarly, the values obtained for specimen 289, representing the petiole of the frond, are also within the accepted variation range (an exception could be the Ox1/Ox2 ratio found in specimen 289 UC). Regarding compression specimens, FT-IR variables ($CH_2/CH_3$, Al/Ox and Ar/Al) do not show considerable differences when petiole, middle part or apex are compared. An exception could be a lower Ar/Al ratio (aromaticity factor) recorded for specimen 289 (petiole) likely reflecting a lower diagenetic alteration rather than chemical variations along the length of a single leaf.

Because of the insufficient sample amount, only one compression specimen of *J. stelzneriana* var. *stelzneriana* (334-4), representing the leaf apex of this variety was included. It shows a higher Ar/Al ratio than the sample taken from the middle part of the same leaf (334-1). Since the other two variables studied ($CH_2/CH_3$, Al/Ox) yield similar results in both apex and middle part, a higher aromaticity factor in the apex specimen is considered only as a higher degree of cutinization level.

These results suggest that FT-IR determinations can be meaningfully performed using different parts of the leaf such as petiole, middle part or apex, which is advantageous in the case of fragmentary specimens. However, some variability can be detected in the Ar/Al levels along the length of a single leaf for some compression specimens. These differences likely reflect varying degree of cutinization levels (diagenetic alteration) of the coalified mesophyll in the leaves rather than interspecific variation. Using leaf fragments from the middle part of the leaf blade (the leaf part most frequently preserved in fragmentary specimens) could be of some help to avoid variations in the FT-IR variables.

**Table 6.** Results of one-way ANOVA ($\alpha = 0.05$). Comparison between adaxial and abaxial surfaces (UC and LC, respectively) obtained from ***J. coriacea*** var. ***coriacea*** specimens / *resultados de ANOVA de un factor ($\alpha = 0.05$). Comparación entre las superficies adaxial y abaxial (UC y LC, respectivamente) obtenidas de especímenes de* **J. coriacea** *var.* **coriacea**.

| Variable | Type | *N* | Mean | St dev | *F* | *p* |
|---|---|---|---|---|---|---|
| $CH_2$ / $CH_3$ | UC | 5 | 7.5 | 1.2 | 0.42 | 0.536 |
| | LC | 4 | 8 | 0.4 | | |
| Al / Ox | UC | 5 | 0.96 | 1 | 4.38 | 0.075 |
| | LC | 4 | 0.77 | 0.59 | | |
| Ox1 / Ox2 | UC | 5 | 4.3 | 2 | 1.29 | 0.294 |
| | LC | 4 | 2.8 | 0.4 | | |
| C-H / C=O | UC | 5 | 2.4 | 0.15 | 0.84 | 0.390 |
| | LC | 4 | 2.1 | 0.11 | | |

*Comparison with selected fossil plants from the literature*

Several fossil-plant groups from the Carboniferous (Pennsylvanian) of the Northern Hemisphere have been analyzed to date using FT-IR technique: Medullosales, Cordaitales, Marattiales and sphenopterids (Lyons *et al.*, 1995; Zodrow *et al.*, 2000, 2003; Zodrow and Mastalerz, 2001, 2002; Pšenicka *et al.*, 2005).

After a detailed analysis of FT-IR spectra of *Johnstonia* spp. (cuticles and compressions), individually distinctive chemical signatures are revealed. However, specimens of *Johnstonia* spp. share some spectroscopic patterns with some Paleozoic taxa as shown by the literature. For comparative purposes, some specimens of these groups (including cuticles, naturally macerated cuticles -NMC- and compressions) have been selected from the literature. Table 7 shows some available FT-IR semi-quantitative data and functional groups information for some Paleozoic taxa. Some functional groups such as oxygen-containing groups are present in cuticles, NMC and compressions of all taxa including the Corystospermales represented here by *Johnstonia* Walkom. However, semi-quantitative variables such as $CH_2/CH_3$ and Al/Ox in cuticles seem to provide the most useful information. With a few exceptions, $CH_2/CH_3$ values above 8 and Al/Ox around 0.8 could be distinguishing chemical features of *Johnstonia*. Thus, in principle, these variables can be used to chemically characterize and to differentiate cuticular remains of different plant groups. Nevertheless, these results should be considered with caution until more data are available. Since there are no previous studies on the chemistry of any Triassic fossil-plant group from the Southern Hemisphere, and until more data derived from FT-IR are available, direct comparisons are currently impossible.

## Conclusions

Three corystosperm taxa were analyzed using FT-IR technique: *Johnstonia coriacea* var. *coriacea*, *J. stelzneriana* var. *stelzneriana* and *J. stelzneriana* var. *serrata*. Some general conclusions may be arrived at regarding the chemical composition (functional groups) and semi-quantitative values obtained for the FT-IR-derived variables here studied:

Cuticular and compression specimens of *Johnstonia* spp. showed the following structural information: (a) hydroxyl groups (3100-3700 $cm^{-1}$ range); (b) a relatively rich aliphatic structure (aliphatic C-H stretching modes in the 2800-3000 $cm^{-1}$ range); (c) prominent bands in the 1600-1800 $cm^{-1}$ region (contribution of several structures such as: aromatic, olefinic, ketone, conjugated carbonyl).

- In the three taxa studied herein and with 95% confidence, statistical test one-way ANOVA revealed that there were significant differences between cuticles and their corresponding compressions regarding the $CH_2/CH_3$ ratio.

Among the three taxa studied, FT-IR-derived ratios here considered ($CH_2/CH_3$, Al/Ox and Ar/Al) in compression samples of *Johnstonia* spp. did not differ significantly from one taxon to the other as evidenced by one-way ANOVA test ($p > 0.05$). Regarding cuticular specimens of *Johnstonia* spp., statistical test one-way ANOVA showed that the three taxa studied herein were significantly different ($p < 0.05$) considering $CH_2/CH_3$, Ox1/Ox2 and C-H/C=O ratios. However, statistical test of Al/Ox ratios revealed no significant differences ($p > 0.05$) among the cuticular specimens of *Johnstonia* spp. here studied.

With 95% confidence and considering the FT-IR-derived ratios $CH_2/CH_3$, Al/Ox, Ox1/Ox2 and C-H/C=O, one-way ANOVA revealed that there were not significant differences between abaxial and adaxial surfaces in the cuticular samples of *Johnstonia coriacea* var. *coriacea* here considered.

Among the specimens of *Johnstonia* spp. (cuticles and compressions) analyzed herein, no considerable variations in FT-IR-derived ratios were recorded along the length of the leaf.

Until more data are available, these preliminary, semi-quantitative, FT-IR results should be considered with caution. The chemical composition of cuticles and their associated compressions may contribute to the systematics of problematic plant groups such as the Corystospermaceae. However, more data from many other specimens, belonging to the same taxa and some others from several Triassic beds, are needed to confirm the results presented herein and to evaluate their potential usefulness in chemotaxonomy. The use of some other analytical techniques (*e.g.*, LC/MS, Py-GC/MS) will promote a better understanding of the chemical composition of fossil cuticles and their associated compressions.

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**Table 7.** Comparison of some available FT-IR semi-quantitative data and functional groups obtained from chemically treated cuticles, compressions and naturally macerated cuticles for some Carboniferous and Triassic taxa. a Mean value; b Mean value and standard deviation; c Peaks in the region 1600-1800 $cm^{-1}$, s = shoulder. / *Comparación de algunos datos semi-cuantitativos de IR-TF disponibles y grupos funcionales obtenidos a partir de cutículas tratadas químicamente, compresiones y cutículas maceradas naturalmente para algunos taxones carboníferos y triásicos. a Valor medio; b Valor medio y desviación estándar; c Picos en la región 1600-1800 $cm^{-1}$, s = hombro.*

| Taxa | $CH_2/CH_3$ | Al/Ox | 1600-1800 $cm^{-1}$ [c] | References |
|---|---|---|---|---|
| *Cuticles* | | | | |
| Medullosales | | | | |
| *Macroneuropteris scheuchzeri* (Hoffmann) Cleal, Shute and Zodrow | – | – | 1715, 1638 | Lyons *et al.*, 1995 |
| *Neuropteris ovata* Hoffmann *in* Keferstein var. *simonii* (Bertrand) Cleal and Zodrow | – | – | 1715, 1638 | |
| *Alethopteris ambigua* Lesquereux pars, nov. *emend.* Zodrow and Cleal | – | – | 1715, 1638 (s) | |
| *A. ambigua* | 11 | 0.34 | 1708, 1638 | Zodrow & Mastalerz, 2001 |
| *Odontopteris schlotheimii* (Sclotheim) Brongniart | 8.5 | 0.25 | 1718, 1638 | |
| *Neuropteris flexuosa* Sternberg [a] | 2.6 | 0.24 | 1710 (s), 1633 | |
| Cordaitales [a] | | | | |
| *Cordaites principalis* (Germar) Geinitz | 3.3 | 0.61 | 1710, 1632 | Zodrow *et al.*, 2000, 2003 |
| *C. borassifolius* (Sternberg) Unger | 1.9 | 0.1 | 1721, 1634 | |
| Marattiales | | | | |
| *Pecopteris (Asterotheca) nyranensis* Nemejc | 3.7 | 0.09 | 1716, 1632 | Pšenicka *et al.*, 2005 |
| *P. aspidiodes* Sternberg | 2.8 | 0.08 | 1716, 1640 | |
| *P. polypodioides* Sternberg [a] | 2.1 | 0.06 | 1716, 1632 | |
| *P. (Asterotheca) miltonii* Artis | 2.3 | 0.08 | 1716, 1632 | |
| Sphenopterids | | | | |
| *Eusphenopteris neuropteroides* (Boulay) Novik | 1.9 | – | 1630 | Zodrow & Mastalerz, 2002 |
| *Oligocarpia brongniartii* Stur | 8 | – | 1717, 1633 | |
| Corystospermales [b] | | | | |
| *Johnstonia coriacea* var. *coriacea* (Johnston) Walkom | 7.5 ± 1.2 | 0.89 ± 0.15 | 1714, 1637 | This study |
| *J. stelzneriana* (Geinitz) Frenguelli var. *serrata* Retallack | 11 ± 1 | 0.91 ± 0.24 | 1714, 1639 | |
| *J. stelzneriana* var. *stelzneriana* (Geinitz) Frenguelli | 8.5 ± 2 | 0.76 ± 0.12 | 1716, 1639 | |

| Taxa | $CH_2/CH_3$ | Al/Ox | 1600-1800 $cm^{-1}$ [c] | Ar/Al | References |
|---|---|---|---|---|---|
| *Compressions and naturally macerated cuticles (NMC)* | | | | | |
| Medullosales | | | | | |
| *Macroneuropteris scheuchzeri* (Hoffmann) Cleal, Shute and Zodrow | – | – | 1701, 1610 | 1.24 | Lyons *et al.*, 1995 |
| *Neuropteris ovata* Hoffmann *in* Keferstein var. *simonii* (Bertrand) Cleal and Zodrow | – | – | 1610 | 0.2 | |
| *Alethopteris ambigua* Lesquereux pars, nov. *emend.* Zodrow and Cleal | – | – | 1701 (s),1610 (s) | | |
| *A. ambigua* -NMC | 16.5 | 1.28 | 1710 (s), 1620 | 0.029 | Zodrow & Mastalerz, 2001 |
| *Odontopteris schlotheimii* (Sclotheim) Brongniart -NMC | 4.3 | 1.3 | 1710 (s), 1630 | 0.024 | |
| *Neuropteris flexuosa* Sternberg - NMC [a] | 2.6 | 1.39 | 1710 (s), 1638 | 0.015 | |
| Cordaitales [a] | | | | | |
| *Cordaites principalis* (Germar) Geinitz | 2.3 | – | 1608-1618 | 0.49 | Zodrow *et al.*, 2000, 2003 |
| *C. borassifolius* (Sternberg) Unger | 1.8 | – | 1710 (s), 1612 | 0.13 | |
| Marattiales | | | | | |
| *Pecopteris (Asterotheca) nyranensis* Nemejc | 0.9 | 0.17 | 1628 | – | Pšenicka *et al.*, 2005 |
| *P. aspidiodes* Sternberg | 1.8 | 0.28 | 1708 (s), 1624 | – | |
| *P. polypodioides* Sternberg [a] | 1.6 | 0.28 | 1623 | – | |
| *P. (Asterotheca) miltonii* Artis | 1.9 | 0.32 | 1611, 1628 | – | |
| Sphenopterids | | | | | |
| *Eusphenopteris neuropteroides* (Boulay) Novik | 3.2 | – | 1703, 1604 | – | Zodrow & Mastalerz, 2002 |
| *E. neuropteroides* - NMC [a] | 3.2 | – | 1706, 1637 | | |
| *Oligocarpia brongniartii* Stur - NMC | 3 | – | 1622 | – | |

**Table 7.** (continuation)

| | | | | | |
|---|---|---|---|---|---|
| Corystospermales [b] | | | | | |
| *Johnstonia coriacea* var. *coriacea* (Johnston) Walkom | 3.5 ± 0.4 | 0.55 ± 0.11 | 1699 (s), 1616 | 0.11 ± 0.06 | This study |
| *J. stelzneriana* (Geinitz) Frenguelli var. *serrata* Retallack | 3.6 ± 0.6 | 0.61 ± 0.05 | 1700 (s), 1608 | 0.12 ± 0.04 | |
| *J. stelzneriana* var. *stelzneriana* (Geinitz) Frenguelli | 3.7 ± 0.4 | 0.6 ± 0.2 | 1699 (s), 1620 | 0.11 ± 0.07 | |

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