



Chemometric study of structural groups in medullosalean foliage (Carboniferous, fossil Lagerstätte, Canada): Chemotaxonomic implications



José A. D'Angelo ^{a,b,*}, Erwin L. Zodrow ^b

^a IANIGLA-CCT-CONICET-MENDOZA and Área de Química, FCEN, Universidad Nacional de Cuyo, M5502JMA Mendoza, Argentina

^b Palaeobotanical Laboratory, Cape Breton University, Sydney, Nova Scotia B1P 6L2, Canada

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ABSTRACT

A larger chemometric study (data derived from Fourier transform infrared spectroscopy-FTIR interpreted by principal component analysis) is undertaken involving species (numbers bracketed) from the four medullosalean families Neurodopteridaceae (7), Potonieaceae (1), Cyclopteridaceae (1), and Alethopteridaceae (1). Samples originated from the fossil Lagerstätte ("medullosalean forest") in the Late Pennsylvanian of Sydney Coalfield, Nova Scotia, Canada, where they are preserved as compressions or fossilized-cuticles. The study aims at demonstrating how the level of objectivity for medullosalean fossil-plant determinations can be raised through inquiring (i) if the chemistry of isolated foliage supports the specific classification based on morphologies alone, (ii) if chemistry of different preservation states hampers the application of chemometrics, and (iii) if taxonomic assignment of poorly preserved specimens can be assisted by chemometrics. Given the similar thermal history, direct chemical comparisons amongst taxa having the same preservation state were possible. The chemometric approach resulted into a two principal-component model (80% cumulative-explained variance) that provided information on the relative contributions of aromatic structures, oxygen-containing, and aliphatic groups. These functional groups enabled statistical distinction amongst most of the morphology-defined species, and families, irrespective of the two preservation states. We concluded that results, overall, support the aims of the study, with implications to solve some palaeobotanical difficulties related to biostratigraphy and palaeobiogeography.

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1. Introduction

Fragmentary fern-like foliage represents one of the most common and widespread types of plant fossils preserved in early-middle Carboniferous times. Recognition by [Oliver and Scott \(1904\)](#) that some fern-like foliage represents seed-bearing plants (gymnosperms: order Medullosales, now extinct), thus establishing a whole new plant group, represents a major revolution in palaeobotany ([Mosbrugger, 1989](#); [Taylor et al., 2009](#)). Medullosalean foliage corresponds to a grouping of many genera belonging to various families (e.g., [Cleal and Shute, 2012](#); [Laveine, 1997](#)). Foliar species are distinguished based on character sets that include frond architecture, pinnule, and cuticular morphologies (e.g., summaries: [Cleal and Zodrow, 1989](#); [Zodrow and Cleal, 1988, 1998](#)). These sets are not equally known for each which makes taxial identification difficult and, sometimes, ambiguous. This situation may lead to confusing medullosalean taxonomy or duplications

of names, and besides these character sets, though contributory, are by themselves insufficient for approaching the concept of a more natural species (e.g., [Zodrow and Cleal, 1998](#)). Suggested is the addition of parameters to the character set derivable from the fossil-plant chemistry, and involvement of chemotaxonomic hypotheses for determining chemical similarities/differences amongst morphologically-based taxa (e.g., [Lockheart et al., 2000](#); [Nguyen Tu et al., 2007](#)). However, chemotaxonomic hypotheses are very much circumscribed by diagenetic intervention and the limitations it imposes, i.e., what is actually preserved vs. original plant composition ([Niklas, 1976](#); [Niklas and Gensel, 1977](#))?

Because of "mild fossilization conditions", well-preserved medullosalean type of foliage from a Late Pennsylvanian fossil Lagerstätte of the Sydney Coalfield (Canada), have yielded useful chemical data, i.e., related to lipid-derived compounds such as n-alkanes, n-alkanols, and fatty acids. During the last 20 years, many Canadian seed-fern taxa have been chemically characterized (e.g., [D'Angelo et al., 2010, 2012, 2013](#); [Lyons et al., 1995](#); [Stoyko et al., 2013](#); [Zodrow and Mastalerz, 2001, 2002, 2007, 2009](#); [Zodrow et al., 2007, 2009, 2010, 2012, 2013, 2014](#)).

We present results of a larger chemometric study that involves ten foliar taxa from the four families of the order Medullosales. This

* Corresponding author at: IANIGLA-CCT-CONICET-MENDOZA and Área de Química, FCEN, Universidad Nacional de Cuyo, M5502JMA Mendoza, Argentina. Fax: +54 261 5244201.

E-mail address: joseadangelo@yahoo.com (J.A. D'Angelo).

Table 1
Sample forms used.

| Form | Description |
|--------------------|--|
| Compression | Conceptualized by an analogue model of the anatomy of an extant leaf: vitrinite (mesophyll) + cuticle (biomacropolymer) = compression (Zodrow et al., 2009). |
| Fossilized-cuticle | Cuticle, where coalified layer (vitrinite) is no longer preserved, only the cuticle itself (summary Zodrow and Mastalerz, 2009). |
| Vitrain | Bright and shiny banded bituminous coal. |

approach essentially identifies taxon-specific functional-group ratios, which when combined with morphological data is bound to achieve a more objective level of taxonomic determinations than otherwise would be possible with morphological data alone. Thus, IR (infrared) data as additional taxonomic parameters find application with solving “problematic” groups, not necessarily confined to medullosaleans, and with several interrelated areas such as biostratigraphy, palaeobiogeography, and evolution.

2. Definitions

2.1. Functional groups and molecular structure

As it is well-established, Fourier transform infrared (FTIR) spectroscopy allows for both qualitative and semi-quantitative analyses of specific groups of atoms or bonds i.e., functional groups within molecules (e.g., Colthup et al., 1990; Shurvell, 2002). FTIR-derived information helps, but does not in itself determine complex

molecular structures of organic compounds (or mixture of them) making up the sample under study. Semi-quantitative FTIR analysis is based on the peak-area integration used to calculate areal ratios, which do not represent absolute contents of functional groups. The rationale for using area ratios, and not individual peak areas, is for normalization and elimination of the spectral background. The reader is referred to Table 1 (Appendix A, supplementary data) for a list of the most important functional groups present in generalized molecular fragments and the meaning of semi quantitative area ratios derived from FTIR spectra. See Section 4.2 for further details.

2.2. Chemometrics

Chemometrics is a well-established discipline, where multivariate-statistical methods are applied to complex chemical-data sets to generate new insights for interpretation (e.g., Kvalheim et al., 1985; Lockheart et al., 2000). We use the statistical methods of PCA (principal component analysis) in two interrelated ways. First, as a complexity-reducing tool of the set of IR-derived data (functional groups or chemical structures) when subjectively accepting the number of components that cumulatively account for ca. 80% variance (Izenman, 2008; Johnson and Wichern, 2008; Jolliffe, 2002; Kaiser, 1960; Kendall, 1965). Secondly, as a method of evolving a set of data groupings which is facilitated by calculated component scores thereby revealing hidden patterns or trends that emphasize natural groupings. This identifies which variables most strongly influence those patterns. PCA was performed using STATISTICA® (StatSoft, 2012) on raw data (correlation matrices) consisting of the four variables, with 98 determinations each. See Section 5.2 for further details.

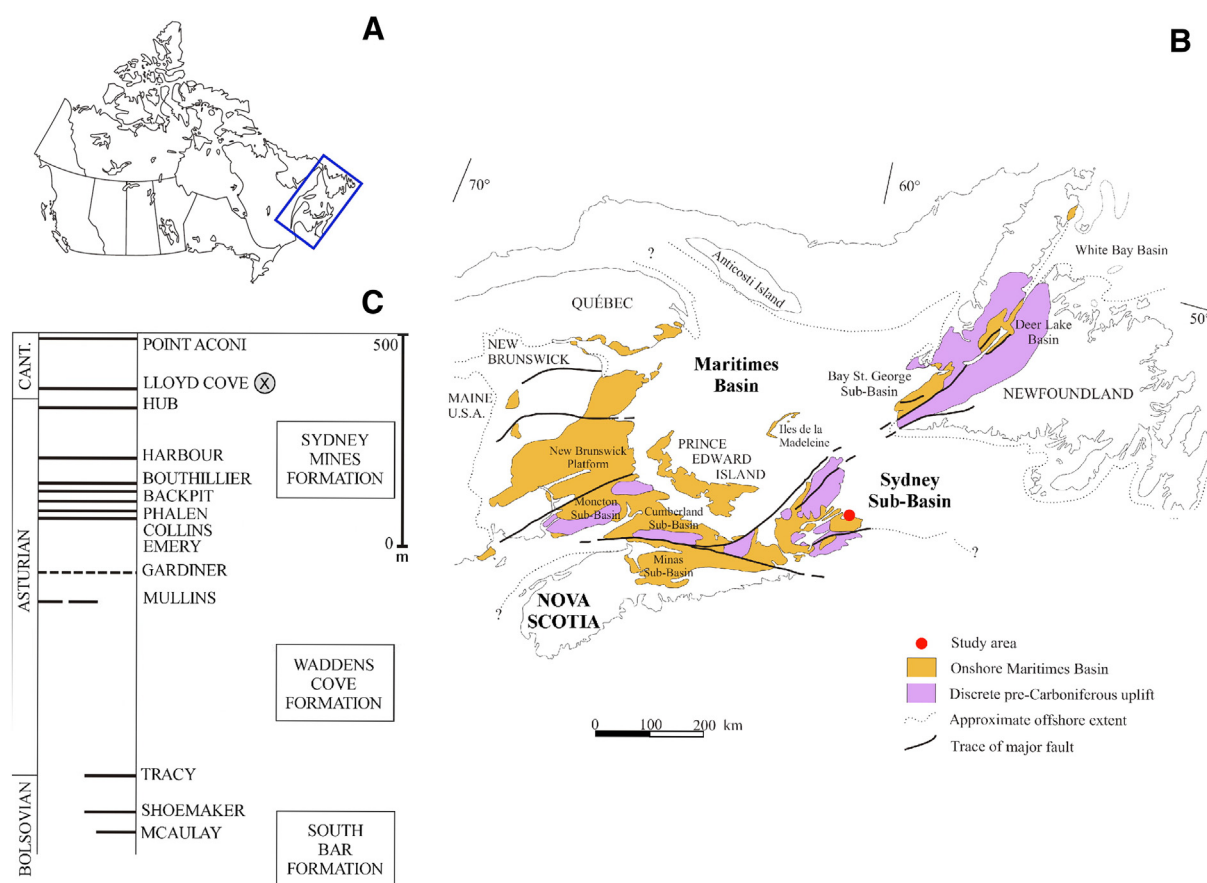


Fig. 1. Study location. (A) Canada. (B) Maritimes Basin with Sydney Coalfield (sub-basin), Nova Scotia. (C) Local coal stratigraphy. Sampled coal seam is marked (X), and the medullosalean forest occurs in the roof rocks of the Lloyd Cove Seam, basal Cantabrian (=CANT., Zodrow and Cleal, 1985).

2.3. Sample forms

At the Lloyd Cove sampling site (Fig. 1; see Section 3 for further details), foliage is preserved in two interrelated states (Zodrow and D'Angelo, 2013): as compression, a synonym is adpression (Shute and Cleal, 1987), and as fossilized-cuticle, being a state of naturally-macerated compression (Table 1). In the context of chemometric analysis, these are collectively referred to as sample forms. An additional form is coal (vitrain) samples, chemically not

treated, from the associate Lloyd Cove Seam for chemical comparison with the compression form.

2.4. Three taxonomic notes

With the exceptions of “*Odontopteris subcuneata*” Bunbury (1847, pl. 23, fig. 1A) with type locality, Sydney Coalfield, Canada, *Neuropteris* sp. A, and *Neuropteris* sp. B which require comments, all other species (Fig. 2, Table 2) are taxonomically straight forward

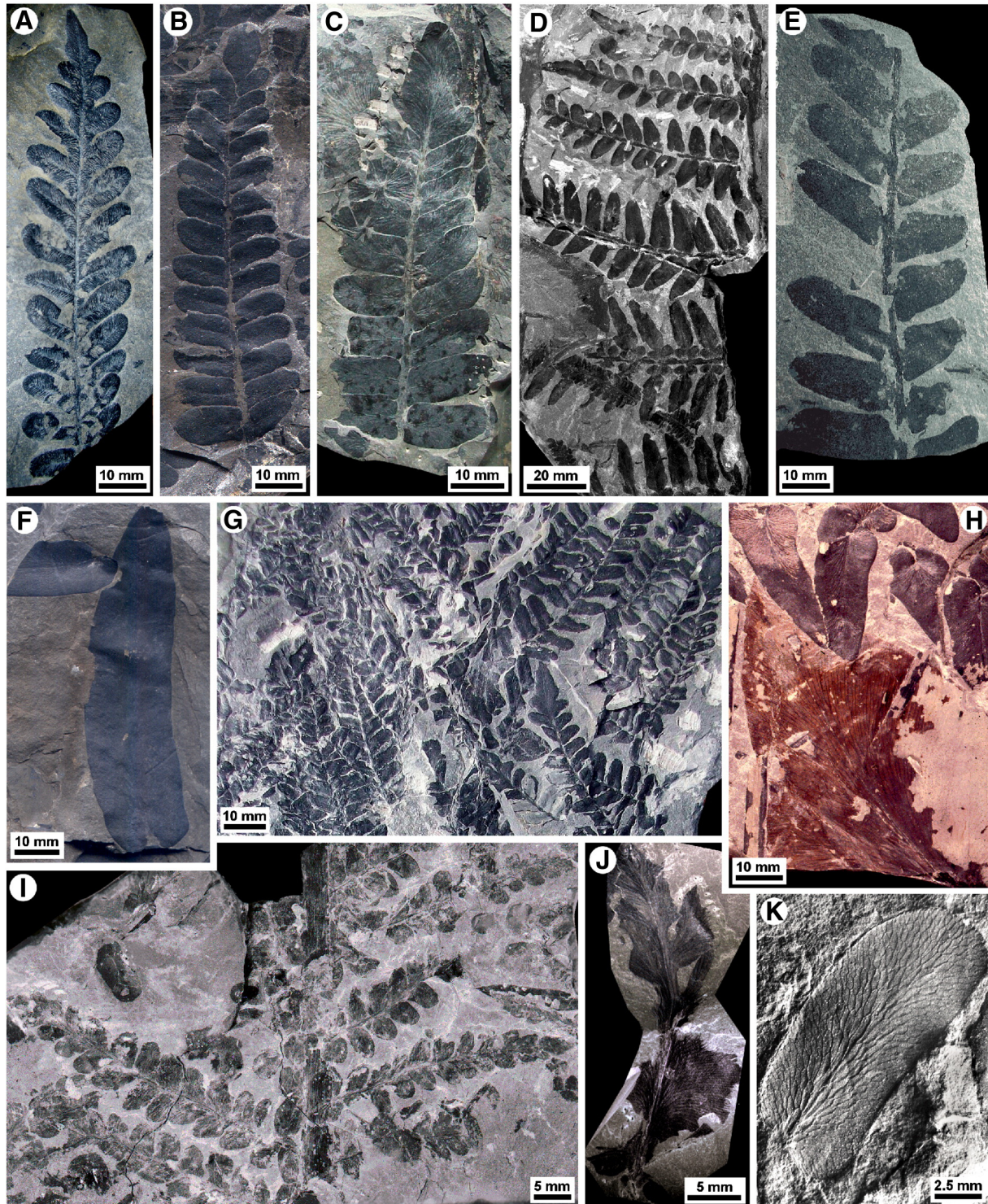


Fig. 2. Seed-fern species studied. (A) *Alethopteris pseudograndinioides*. (B) *Neuropteris ovata*. (C) *N. flexuosa*. (D) *Neuropteris* sp. A. (E) *Neuropteris* sp. B. (F) *Macroneuropteris scheuchzeri*. (G) *Laveineopteris rarinervis*. (H) *M. macrophylla*. (I) *Odontopteris cantabrigia*. (J) “*Odontopteris subcuneata*”. (K) *Linopteris obliqua*.

Table 2

Classification of the sample forms into four families of Medullosales^a, and number of specimens.

| No. | Family/morphospecies | Sample form (no. of samples ^b) |
|-----|---|--|
| 1 | Neurodopteridaceae | |
| 2 | <i>Neuropteris ovata</i> | Compression (8) |
| 2 | <i>N. flexuosa</i> | Compression (3) |
| 3 | <i>Neuropteris</i> sp. A | Fossilized-cuticle (4) |
| 4 | <i>Neuropteris</i> sp. B | Compression (3) |
| 5 | <i>Odontopteris cantabriga</i> | Fossilized-cuticle (5) |
| 6 | <i>Macroneuropteris macrophylla</i> | Compression (5) |
| 7 | <i>M. scheuchzeri</i> | Fossilized cuticle (7) |
| 7 | <i>M. s. ("O. subcuneata")</i> | Compression (21) |
| 8 | Potoniaceae | Fossilized-cuticle (8) |
| 8 | <i>Linopteris obliqua</i> | Compression (2) |
| 9 | Cyclopteridaceae | |
| 9 | <i>Laveineopteris rarinervis</i> | Compression (3) |
| 10 | Alethopteridaceae | Fossilized-cuticle (1) |
| 10 | <i>Alethopteris pseudograndinioides</i> | Compression (8) |
| | Total number of samples | 95 |

^a According to Cleal and Shute (2012).

^b Only entire or fragmentary pinnules were analyzed (i.e., no rachides were used).

(e.g., Cleal and Zodrow, 1989; Cleal et al., 1990; Cleal et al., 2007; Zodrow, 1985; Zodrow and McCandlish, 1980).

Neuropteris sp. A (Fig. 2D) is a 22-cm long apical frond segment that was originally assigned to *Neuropteris scheuchzeri* (Zodrow, 1985, Text-Fig. 4; Zodrow, 2003, Fig. 4). D'Angelo et al. (2012) referred to it simply as "compression", following discussions with J.P. Laveine (pers. comm. 2004, E.L. Zodrow) who considered that grouping the specimen with *N. scheuchzeri* would have unintended taxonomic consequences, not acceptable for neuropteroid classification. We regard that specimen specifically as indeterminate, although a rapidly changing pinnule morphology from odontopteroid to neuropteroid to tripartite occurred, a fully developed morphology for unquestionable assignment is not preserved. Reference to neuropteroids is nevertheless warranted.

Neuropteris sp. B (Fig. 2E) is a ten-cm long ultimate pinna without preserved apical pinnule. The specimen is specifically indeterminate, as the venation scheme is eroded beyond recognition, but the stalked, auriculate pinnules are neuropteroid-like in character.

"*O. subcuneata*" (Fig. 2J): Bunbury's (1847) holotype is a 6-cm long, incomplete ultimate? pinna with a fragmentary ultimate pinnule which he characterized by mentioning that "Different parts of the frond of the same Fern are often so unlike..." Bell (1938), by observing persistent physical association, suspected it to represent atypical

N. scheuchzeri (= *Macroneuropteris scheuchzeri*, Cleal et al., 1990). Bell's implied conspecificity is confirmed by independent studies since (e.g., Laveine, 1967, 1997; Kotasowa, 1973; Laveine and Belhis, 2007; Zodrow, 2003, 2014; Zodrow et al., 2014, and others). However, for "book-keeping" purposes in the chemometric analysis we retain Bunbury's name in quotation marks.

3. Materials

Sampling in an open-pit mining area of 200 m by 150 m was confined to the one-meter thick roof shale of the Lloyd Cove Seam (Cantabrian, Sydney Coalfield, Nova Scotia, Canada, Fig. 1). This is part of a fossil Lagerstätte of a medullosalean forest in the Canadian Carboniferous Maritimes (Zodrow, 2002). Measured vitrinite reflectance of Ro% = 0.65, or Bituminous, High Volatile B (Zodrow et al., 2009) of the Lloyd Cove Seam is indicative of lower-rank coalification conditions ("milder fossilization"). This, together with the sampling methodology employed, implies uniform fossilization conditions at large, but does not guarantee the absence of localized variation (e.g., D'Angelo et al., 2012).

HF (48%) was used for freeing the fossil samples from the rock matrix. Representative medullosalean specimens are documented in Fig. 2, while Table 2 indicates their morphologic-based taxonomic assignment according to a proposed classification of the order Medullosales (Cleal and Shute, 2012).

4. Methods

4.1. FTIR spectra

IR spectra were collected on a Nicolet Thermo-Electron 6700 spectrometer, equipped with a DTGS-Csl detector. The acquisition conditions included 256 scans per sample collected at a resolution of 4 cm⁻¹ over the wavenumber interval 4000 to 400 cm⁻¹. Used for analysis were 0.5 to 2.0 mg samples ground with 250 mg KBr pressed into a 10-mm diameter pellet.

4.2. Qualitative and semi-quantitative FTIR analysis

Qualitative FTIR analysis included assignments of salient IR bands as well as group and class of compounds (Table 3), which are applicable to all of the samples. Mathematical treatments (e.g., D'Angelo, 2004, 2006; D'Angelo and Marchevsky, 2004; Mastalerz and Bustin, 1993; Sobkowiak and Painter, 1992) applied to digitized spectra furnished the semi-quantitative IR ratios for interpretation and PCA input. Definitions, interpretations, and descriptive statistics of semi-quantitative IR-derived ratios are explained in Tables 4 and 5. More details and additional references

Table 3

Wavenumber ranges in which main functional groups and classes of compounds absorb.

| Range (cm ⁻¹) ^a | Group and class of compound | Assignment |
|--|---|--|
| 3450–3250 | Hydroxyl (–OH) in alcohols and phenols | O–H stretch |
| 3100–3000 | =CH in aromatic and unsaturated hydrocarbons | =C–H stretch |
| 2936–2913 | Methylene and methyl (CH ₃ –, CH ₂ –) in aliphatic compounds ^b | CH ₃ –, CH ₂ – antisymmetric stretch |
| 2864–2843 | CH ₃ –, CH ₂ – in aliphatic compounds | CH ₃ –, CH ₂ – symmetric stretch |
| 1724–1695 | Carbonyl (C=O) in carboxylic acids, ketones | C=O stretch |
| 1660–1650 | C=O in highly conjugated ketonic structures | C=O stretch |
| 1620–1498 | Benzene ring in aromatic compounds | C=C aromatic ring stretch |
| 1449–1435 | CH ₃ –, CH ₂ – in aliphatic compounds | CH ₃ antisymmetrical or CH ₂ scissor deformation |
| 1279–1250 | C–O in aromatic ethers | C–O stretch |
| 1040–1027 | C–O–C in aliphatic ethers, Si–O in silicates | C–O–C antisymmetric stretch, Si–O stretch |
| 900–700 | =CH in aromatic hydrocarbons | =C–H out-of-plane bending |

^a According to Colthup et al. (1990), D'Angelo and Zodrow (2011), D'Angelo et al. (2011), Petersen and Nytoft (2006) and Petersen et al. (2008).

^b It should be noted that peak at 2925 cm⁻¹ (obtained after deconvolution of aliphatic C–H stretching region, not shown) represents the contribution from CH₃ and CH₂ groups attached directly to aromatic rings (see Petersen and Nytoft, 2006).

Table 4
Descriptive statistics of semi-quantitative FTIR data relating to *Neuropteris ovata*, *N. flexuosa*, *Neuropteris* sp. A, *Neuropteris* sp. B, *Odontopteris cantabriga*, *Macroneuropteris macrophylla*, *M. scheuchzeri*, *M. scheuchzeri* (*O. subcuneata*), *Linopteris obliqua*, *Laveineopteris rarinervis*, *Alethopteris pseudograndinioides*, and associated coal (vitrain) samples from the Lloyd Cove Seam.

| Taxon/sample form | ID ^a | IR ratio | Mean | sd | Min | Max | N | Taxon/sample form | ID | IR ratio | Mean | sd | Min | Max | N |
|--------------------------------|-----------------|----------------------------------|------|------|-------|------|----|-------------------------------|----|----------------------------------|------|------|-------|------|---|
| Compressions (N = 67) | | | | | | | | Cyclopteridaceae | | | | | | | |
| Neurodopteridaceae | | | | | | | | <i>L. rarinervis</i> | R | CH ₂ /CH ₃ | 2.1 | 0.1 | 2 | 2.2 | 8 |
| <i>N. ovata</i> | O | C=O/C=C ^b | 1.7 | 0.2 | 1.5 | 1.9 | 8 | | | C=O/C=C | 0.02 | 0.01 | 0.01 | 0.02 | 8 |
| | | C=C cont ^d | 0.01 | 0.01 | 0.004 | 0.02 | 8 | | | C=C cont | 0.47 | 0.02 | 0.43 | 0.48 | 8 |
| | | 'A' factor ^e | 0.50 | 0.01 | 0.48 | 0.51 | 8 | | | 'A' factor | 0.51 | 0.01 | 0.5 | 0.54 | 8 |
| | | | 0.53 | 0.02 | 0.50 | 0.55 | 8 | Alethopteridaceae | | | | | | | |
| <i>N. flexuosa</i> | F | CH ₂ /CH ₃ | 1.8 | 0.1 | 1.7 | 1.8 | 3 | <i>A. pseudograndinioides</i> | P | CH ₂ /CH ₃ | 4.3 | 0.6 | 3.2 | 5.1 | 8 |
| | | C=O/C=C | 0.03 | 0 | 0.03 | 0.03 | 3 | | | C=O/C=C | 0.1 | 0.1 | 0.02 | 0.4 | 8 |
| | | C=C cont | 0.47 | 0.01 | 0.46 | 0.47 | 3 | | | C=C cont | 0.71 | 0.13 | 0.52 | 0.84 | 8 |
| | | 'A' factor | 0.56 | 0.01 | 0.55 | 0.56 | 3 | | | 'A' factor | 0.61 | 0.05 | 0.56 | 0.71 | 8 |
| <i>Neuropteris</i> sp. A | N | CH ₂ /CH ₃ | 2.7 | 0 | 2.7 | 2.7 | 3 | Fossilized-cuticles (N = 28) | | | | | | | |
| | | C=O/C=C | 0.04 | 0.01 | 0.03 | 0.05 | 3 | Neurodopteridaceae | | | | | | | |
| | | C=C cont | 0.50 | 0.03 | 0.48 | 0.53 | 3 | <i>N. flexuosa</i> | F | CH ₂ /CH ₃ | 3.0 | 0.2 | 2.8 | 3.2 | 4 |
| | | 'A' factor | 0.51 | 0.02 | 0.48 | 0.52 | 3 | | | C=O/C=C | 0.08 | 0.02 | 0.06 | 0.1 | 4 |
| <i>Neuropteris</i> sp. B | U | CH ₂ /CH ₃ | 1.5 | 0.1 | 1.4 | 1.7 | 5 | | | C=C cont | 0.30 | 0.05 | 0.22 | 0.34 | 4 |
| | | C=O/C=C | 0.01 | 0.01 | 0.004 | 0.02 | 5 | | | 'A' factor | 0.75 | 0.05 | 0.69 | 0.8 | 4 |
| | | C=C cont | 0.48 | 0.01 | 0.47 | 0.48 | 5 | <i>Neuropteris</i> sp. A | N | CH ₂ /CH ₃ | 2.9 | 0.5 | 2.3 | 3.5 | 5 |
| | | 'A' factor | 0.51 | 0.03 | 0.46 | 0.53 | 5 | | | C=O/C=C | 0.04 | 0.03 | 0.005 | 0.08 | 5 |
| <i>O. cantabriga</i> | C | CH ₂ /CH ₃ | 3.1 | – | – | – | 1 | | | C=C cont | 0.43 | 0.06 | 0.35 | 0.48 | 5 |
| | | C=O/C=C | 0.06 | – | – | – | 1 | <i>O. cantabriga</i> | C | CH ₂ /CH ₃ | 4.5 | 0.3 | 4.1 | 4.7 | 3 |
| | | C=C cont | 0.45 | – | – | – | 1 | | | C=O/C=C | 0.10 | 0.01 | 0.09 | 0.1 | 3 |
| | | 'A' factor | 0.55 | – | – | – | 1 | | | C=C cont | 0.29 | 0.04 | 0.26 | 0.34 | 3 |
| <i>M. macrophylla</i> | M | CH ₂ /CH ₃ | 1.5 | 0.2 | 1.3 | 1.6 | 5 | | | 'A' factor | 0.73 | 0.04 | 0.69 | 0.78 | 3 |
| | | C=O/C=C | 0.04 | 0.01 | 0.03 | 0.05 | 5 | <i>M. macrophylla</i> | M | CH ₂ /CH ₃ | 2.7 | 0.2 | 2.4 | 3 | 7 |
| | | C=C cont | 0.41 | 0.01 | 0.40 | 0.42 | 5 | | | C=O/C=C | 0.04 | 0.01 | 0.03 | 0.06 | 7 |
| | | 'A' factor | 0.60 | 0.01 | 0.58 | 0.61 | 5 | | | C=C cont | 0.44 | 0.03 | 0.39 | 0.49 | 7 |
| <i>M. scheuchzeri</i> | S | CH ₂ /CH ₃ | 2.4 | 0.3 | 2.0 | 2.8 | 21 | | | 'A' factor | 0.62 | 0.02 | 0.59 | 0.64 | 7 |
| | | C=O/C=C | 0.02 | 0.01 | 0.01 | 0.03 | 21 | <i>M. scheuchzeri</i> | S | CH ₂ /CH ₃ | 1.9 | 0.1 | 1.8 | 2 | 8 |
| | | C=C cont | 0.50 | 0.04 | 0.41 | 0.56 | 21 | | | C=O/C=C | 0.20 | 0.05 | 0.1 | 0.2 | 8 |
| | | 'A' factor | 0.52 | 0.03 | 0.47 | 0.57 | 21 | | | C=C cont | 0.27 | 0.02 | 0.24 | 0.3 | 8 |
| <i>M. s. ("O. subcuneata")</i> | B | CH ₂ /CH ₃ | 2.7 | 0 | 2.7 | 2.7 | 2 | | | 'A' factor | 0.66 | 0.03 | 0.62 | 0.71 | 8 |
| | | C=O/C=C | 0.01 | 0.01 | 0.01 | 0.02 | 2 | Potonieaceae | | | | | | | |
| | | C=C cont | 0.39 | 0 | 0.39 | 0.39 | 2 | <i>L. obliqua</i> | Q | CH ₂ /CH ₃ | 2.6 | – | – | – | 1 |
| | | 'A' factor | 0.55 | 0.05 | 0.52 | 0.59 | 2 | | | C=O/C=C | 0.2 | – | – | – | 1 |
| Potonieaceae | | | | | | | | | | C=C cont | 0.1 | – | – | – | 1 |
| <i>L. obliqua</i> | Q | CH ₂ /CH ₃ | 0.9 | 0.3 | 0.6 | 1.2 | 3 | | | 'A' factor | 0.77 | – | – | – | 1 |
| | | C=C cont | 0.50 | 0.03 | 0.46 | 0.53 | 3 | Coal (vitrain) (N = 3) | V | CH ₂ /CH ₃ | 0.9 | 0.1 | 0.8 | 1 | 3 |
| | | 'A' factor | 0.44 | 0.02 | 0.43 | 0.47 | 3 | | | C=O/C=C | 0.01 | 0.01 | 0.003 | 0.03 | 3 |
| | | C=O/C=C | 0.02 | 0.01 | 0.009 | 0.03 | 3 | | | C=C cont | 0.72 | 0.07 | 0.64 | 0.77 | 3 |
| | | | | | | | | | | 'A' factor | 0.39 | 0.03 | 0.35 | 0.41 | 3 |

^a Identification (see principal component analysis).

^b CH₂/CH₃ (band region: 3000–2800 cm^{−1}) = Methylene/methyl ratio. It is an estimate of the length and branching degree of the aliphatic side-chains (Lin and Ritz, 1993a,b; Petersen and Nytoft, 2006).

^c C=O/C=C (band-region ratio: (1700–1600)/(1600–1500)) = Carbonyl/aromatic carbon groups ratio. Relative contribution of C=O to aromatic carbon groups (D'Angelo, 2006).

^d C=C cont (band-region ratio: (~1600)/(1800–1600)) = Aromatic carbon contribution. Relative contribution of aromatic carbon groups (C=C) to combined contribution of oxygen-containing groups and C=C structures.

^e 'A' factor (band-region ratio: (3000 – 2800) / [(3000 – 2800) + (1600 – 1500)]) = Relative contribution of aliphatic C–H stretching bands to sum of aliphatic C–H stretching and aromatic carbon structures (Ganz and Kalkreuth, 1987).

can be found in Table 2 (Appendix A, supplementary data) and in D'Angelo and Zedrow (2011).

5. Results and discussion

5.1. FTIR data

Only representative FTIR spectra are shown in Figs. 3–5. Relevant IR information is found in three spectral zones: 3700–2600 cm^{−1}, 1800–1000 cm^{−1}, and 900–700 cm^{−1}. From a qualitative point of view, irrespective of the taxon, FTIR spectra of compressions (Figs. 3A–F and 4A–E) are visually similar to one another. Noted are the functional-group similarities between vitrain samples (Fig. 4F) and compressions. On the other hand, and considering fossilized-cuticles (Fig. 5A–F), similarities are also found amongst FTIR spectra of the different taxa. However, for a given taxon differences between compression and fossilized-cuticle exist (see delimited zones in Figs. 3–5).

Calculated are semi-quantitative IR-derived ratios (i.e., CH₂/CH₃, C=O/C=C, C=C cont, and 'A' factor; see Tables 1 and 2, Appendix A, supplementary data) for detailed comparisons of the structural groups amongst the different sample forms and amongst taxa. Salient points of IR-derived ratios are summarized as follows:

- Aliphatic structures (CH₂/CH₃ and 'A' factor) for fossilized-cuticles and vitrain are the highest and the lowest, respectively.
- C=O stretching bands corresponding to aliphatic esters are absent in all samples. Instead, C=O groups assigned to ketones and carboxylic acids (1724–1695 cm^{−1} region) are recorded for compressions and fossilized-cuticles, but not for vitrain samples (e.g., compare Fig. 4D with Fig. 4F). This is confirmed by C=O/C=C data for vitrain, which are amongst the lowest values in the dataset.
- C=O stretching in highly conjugated ketonic structures (1652 cm^{−1}) is present in only one spectrum of "*O. subcuneata*"

(compression, Fig. 3F), which could be indicative of an incipient stage of a fossilized-cuticle.

- (d) Compressions (IR ratios) are characterized by (1) relatively low contents of aliphatic compounds ('A' factor ratios ranging from 0.43 to 0.71) with (2) variable length and branching degree of the hydrocarbon side chains (range of CH_2/CH_3 values is 0.6–5.1), (3) very variable contents of oxygen-containing species (C=O/C=C range is 0.004–0.4), and (4) variable composition of aromatic compounds (C=C cont range is 0.39–0.84).
- (e) Fossilized-cuticles have (1) the highest contents of aliphatic compounds ('A' factor ratios range from 0.56 to 0.80) with (2) relative short and more branched hydrocarbon side chains (CH_2/CH_3 ratios range from 1.8 to 4.7), (3) very variable contents of oxygen-containing species (C=O/C=C ratios range from 0.005 to 0.2), and (4) variable composition of aromatic compounds (C=C cont ranges from 0.10 to 0.49).
- (f) Vitrain samples show a (1) relatively poor content of aliphatics (ranges for 'A' factor and CH_2/CH_3 ratios are from 0.35 to 0.41, and 0.8 to 1.0, respectively), as well as (2) oxygen-containing species (C=O/C=C ratios range from 0.003 to 0.3), whereas (3) values of aromatic-related compounds are amongst the highest in the entire data set (C=C cont ranges from 0.64 to 0.67).

The following sections detail, examine, and evaluate results of the chemometric approach (Section 2.2). This includes proposing a multivariate-chemotaxonomic model.

5.2. Multivariate model

Two principal components account for 80.24% of the variance (see Tables 3–5, Appendix A, supplementary data), and their loading plots and scores are shown in Figs. 6 and 7, respectively. The most important component, accounting for the largest variance (51.7%), has positive loadings on CH_2/CH_3 , 'A' factor and C=O/C=C , and a

moderate negative loading on C=C cont. Reflected in this component is the abundance of aliphatic and oxygen-containing functionalities vs. aromatic-carbon groups. Fossilized-cuticles of *Linopteris obliqua*, *M. scheuchzeri*, *Neuropteris flexuosa*, and *Odontopteris cantabrica* exhibit the most positive scores (Fig. 7A, x axis), reflecting their low content of aromatic carbon groups (C=C cont). This is clearly confirmed by the lowest values of C=C cont and the highest values of both 'A' factor and C=O/C=C obtained for these taxa. In contrast, fossilized-cuticles of *Macroneuropteris macrophylla* and *Neuropteris* sp. A have low positive scores (Fig. 7A, x axis) against PC 1, as a result of their lower values of aliphatic and oxygen-containing groups (see 'A' factor and C=O/C=C , Table 2, Appendix A, supplementary data).

With a few exceptions (e.g., *O. cantabrica*), compression specimens of *N. ovata*, *N. flexuosa*, *Neuropteris* sp. A, *Neuropteris* sp. B, "*O. subcuneata*", *M. scheuchzeri*, and *Laveineopteris rarinervis* have low to moderate negative scores against PC 1 (Fig. 7A, B) mainly because of their low values of 'A' factor and C=O/C=C . The lowest values for both aliphatic and oxygen-containing groups recorded for compressions of *L. obliqua* and vitrain samples explain their most negative scores against the first component.

The relatively least important component (28.6% explained variance) has a very high positive loading on C=C cont and a moderate positive loading on CH_2/CH_3 ratio. The latter reflects the abundance of longer and relatively unbranched polymethylenic chains attached to the aromatic carbon groups making up the macromolecular structure.

Compressions of *Alethopteris pseudograndinioides* are completely separate, exhibiting the most positive scores against PC 2 (Fig. 7A, y axis). The reason is that their composition is characterized mainly by longer and straight polymethylenic side chains attached to a macromolecule composed of relatively abundant aromatic structures (higher C=C cont). These likely have ester bridges (i.e., medium to low contents of C=O groups implying a lower cross-linking degree), and other oxygen-bearing moieties.

Compressions of *M. macrophylla* form a tight group by themselves, showing moderate negative scores against PC 2 (Fig. 7A, y axis), as a

Table 5

Descriptive statistics. Semi-quantitative FTIR data relating to the four families assigned to the Medullosales: Neurodopteridaceae, Potonieaceae, Cyclopteridaceae, and Alethopteridaceae, including compressions and fossilized-cuticles.

| Taxon/sample form | IR ratio | Mean | sd | Min | Max | N |
|-------------------------------------|---------------------------|------|------|-------|------|----|
| <i>Compressions (N = 67)</i> | | | | | | |
| Neurodopteridaceae | CH_2/CH_3 | 2.1 | 0.5 | 1.3 | 3.1 | 48 |
| | C=O/C=C | 0.02 | 0.01 | 0.004 | 0.06 | 48 |
| | C=C cont | 0.48 | 0.04 | 0.39 | 0.56 | 48 |
| | 'A' factor | 0.53 | 0.04 | 0.46 | 0.61 | 48 |
| Potonieaceae | CH_2/CH_3 | 0.9 | 0.3 | 0.6 | 1.2 | 3 |
| | C=O/C=C | 0.02 | 0.01 | 0.009 | 0.03 | 3 |
| | C=C cont | 0.50 | 0.03 | 0.46 | 0.53 | 3 |
| | 'A' factor | 0.44 | 0.02 | 0.43 | 0.47 | 3 |
| Cyclopteridaceae | CH_2/CH_3 | 2.1 | 0.1 | 2 | 2.2 | 8 |
| | C=O/C=C | 0.02 | 0.01 | 0.01 | 0.02 | 8 |
| | C=C cont | 0.47 | 0.02 | 0.43 | 0.48 | 8 |
| | 'A' factor | 0.51 | 0.01 | 0.5 | 0.54 | 8 |
| Alethopteridaceae | CH_2/CH_3 | 4.3 | 0.6 | 3.2 | 5.1 | 8 |
| | C=O/C=C | 0.1 | 0.1 | 0.02 | 0.4 | 8 |
| | C=C cont | 0.71 | 0.13 | 0.52 | 0.84 | 8 |
| | 'A' factor | 0.61 | 0.05 | 0.56 | 0.71 | 8 |
| <i>Fossilized-cuticles (N = 28)</i> | | | | | | |
| Neurodopteridaceae | CH_2/CH_3 | 2.7 | 0.8 | 1.8 | 4.7 | 27 |
| | C=O/C=C | 0.09 | 0.06 | 0.005 | 0.2 | 27 |
| | C=C cont | 0.35 | 0.09 | 0.22 | 0.49 | 27 |
| | 'A' factor | 0.66 | 0.06 | 0.56 | 0.8 | 27 |
| Potonieaceae | CH_2/CH_3 | 2.6 | – | – | – | 1 |
| | C=O/C=C | 0.2 | – | – | – | 1 |
| | C=C cont | 0.1 | – | – | – | 1 |
| | 'A' factor | 0.77 | – | – | – | 1 |

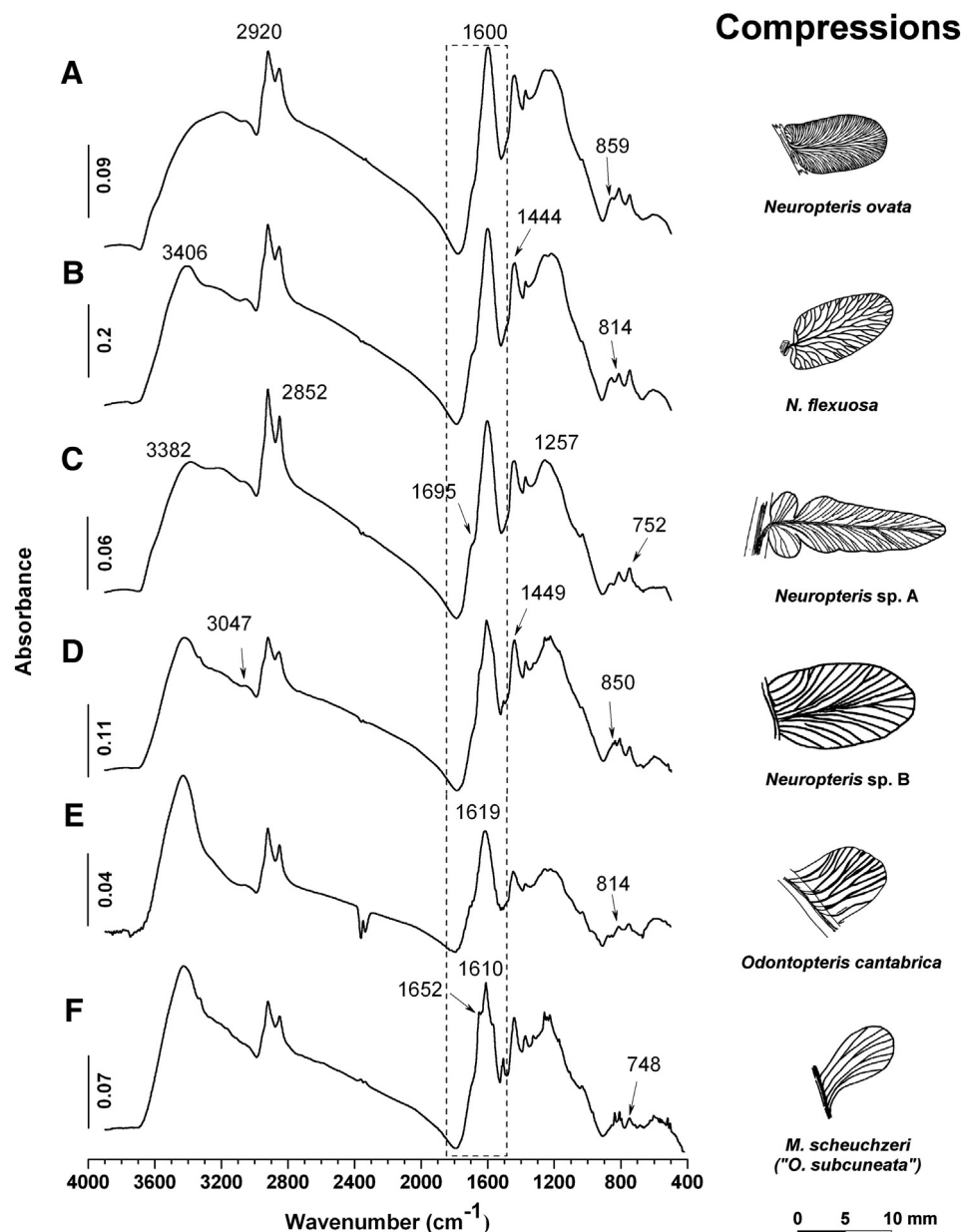


Fig. 3. FTIR spectra of compressions and pen sketches of corresponding typical pinnule morphology.

result of the C=C cont ratios, which are the lowest values amongst those recorded for compression samples.

5.3. Chemometrics, compression, fossilized-cuticle, and vitrain

The main chemical characteristics of the three forms (summarized in (a) to (f), Section 5.1) are reflected by the plot of scores (Fig. 7A, B). Fig. 8 shows a simplified plot where the groupings are indicated by delimiting ellipses (with no statistical significance) around the groups. Fossilized-cuticles are well-separated from vitrain and particularly from compressions, by having a different structure and composition as hypothesized in previous studies (D'Angelo, 2006; Zodrow and Mastalerz, 2001, 2009; Zodrow et al., 2009). However, a partial overlapping with data groupings for compressions is recorded (Fig. 8). From this (a) a compression-derived origin of fossilized-cuticles is inferred that is based on (b) a physicochemically-controlled process of preservation proceeding along a multidimensional continuum. This is reflected in the preservation variability frequently found in compressions, e.g. *Neuropteris* sp. A, even in a single pinnule (D'Angelo

et al., 2012). The preservation in this case ranges from the comparatively more coalified pinnules (denoted by N, see Fig. 7B) to less coalified pinnules or fossilized-cuticles (denoted by *N*, see Fig. 7B).

Fossilized-cuticles of *M. macrophylla* plot very close to some compression-preserved specimens assigned to *Neuropteris* sp. A, *O. cantabrica*, "*O. subcuneata*", and *M. scheuchzeri*. A lower degree of natural oxidation undergone by *M. macrophylla* specimens is evident when compared to the rest of fossilized-cuticles.

Vitrain-sample grouping is close to some compressions, especially *L. obliqua*, where the former records the most extreme values of the chemical ratios mentioned above, particularly aromatic compounds.

Fig. 9 is a simplified plot of groupings of medullosalean- and vitrain-derived data as indicated by delimiting ellipses (without statistical significance). Some compression groups (i.e., *A. pseudograndinioides*, *L. obliqua*, and *M. macrophylla*) are well separated from the other groups, while some other groups overlap (e.g., *M. scheuchzeri*–"*O. subcuneata*", *N. flexuosa*–*L. rarinervis*), indicating remarkable similarities regarding their chemical compositions.

5.4. Morphology-based taxonomy and the multivariate model

Amongst medullosalean pteridosperms, the neuropteroid group as classified to-date includes many morphogenera belonging to different families (e.g., see the name ‘*Neuropterid galaxy*’ of Laveine, 1997). Furthermore, many of the species very likely represent morphological variation within a frond, which in many cases remains unknown because of sample limitations. Thus, a list of morphotaxa unequivocally assigned to the neuropteroids is far from being achieved, even if the question is restricted to the most commonly occurring taxa.

Despite many research efforts, a clear circumscription of the neuropteroid groups based on available frond architecture (e.g., Kidston, 1923–1925; Laveine, 1967, 1997), and cuticular analysis (e.g., Barthel, 1961, 1962; Cleal and Zodrow, 1989; Zodrow and Cleal, 1993, 1998) alone seems to be inadequate. As stated by Laveine (1997), this may be an indication of “a lack of acuteness of the available characters, the systematic inadequacy of the term ‘Neuropterids’, or both”. Improving the taxonomy of the group clearly requires the use of new character sets, e.g., chemical data, complementing, not replacing, the currently employed morphological features for diagnostic determination.

Functional groups determined describe the composition of the preserved geomacromolecules, which reflect the composition of the

biomacromolecules making up the tissues of the once living plant (D'Angelo and Zodrow, 2014; unpublished results). Thus, for instance, the thick epidermis and mesophyll of *A. pseudograndinioides* (Cleal and Shute, 2012; Šimůnek, 1989; Zodrow, 2007) are in agreement with the combination of recorded functional groups.

Overall, inferred from the multivariate model is that clusters corresponding to fossilized-cuticle taxa show little or, in some cases, no overlapping at all. With a few exceptions, the same is true for clusters of compression-preserved taxa (Figs. 7A, B and 9). Thus, compression specimens of *N. flexuosa*, *N. ovata*, *Neuropteris* sp. A, *Neuropteris* sp. B, *M. scheuchzeri*, “*O. subcuneata*”, *O. cantabrica*, and *M. macrophylla* formed a tight group in the Neurodopteridaceae and in the Cyclopteridaceae (i.e., *L. rarinervis*). On the other hand, compressions of the Potoniaceae (i.e., *L. obliqua*) are chemically different from all of the other medullosaleans studied (Fig. 9). Fig. 10 shows a simplified plot of Fig. 7A, where the ellipses show approximate zones corresponding to the different morphofamilies under study. Tables 4 and 5 show the statistical information related to semi-quantitative FTIR data for the different taxa (and vitrain). Statistically significant delimitation of clusters is beyond the scope of this contribution, and is addressed in detail in D'Angelo and Zodrow (2014; unpublished results).

Summarizing, three out of four families assigned to the Medullosales, namely Alethopteridaceae, Neurodopteridaceae,

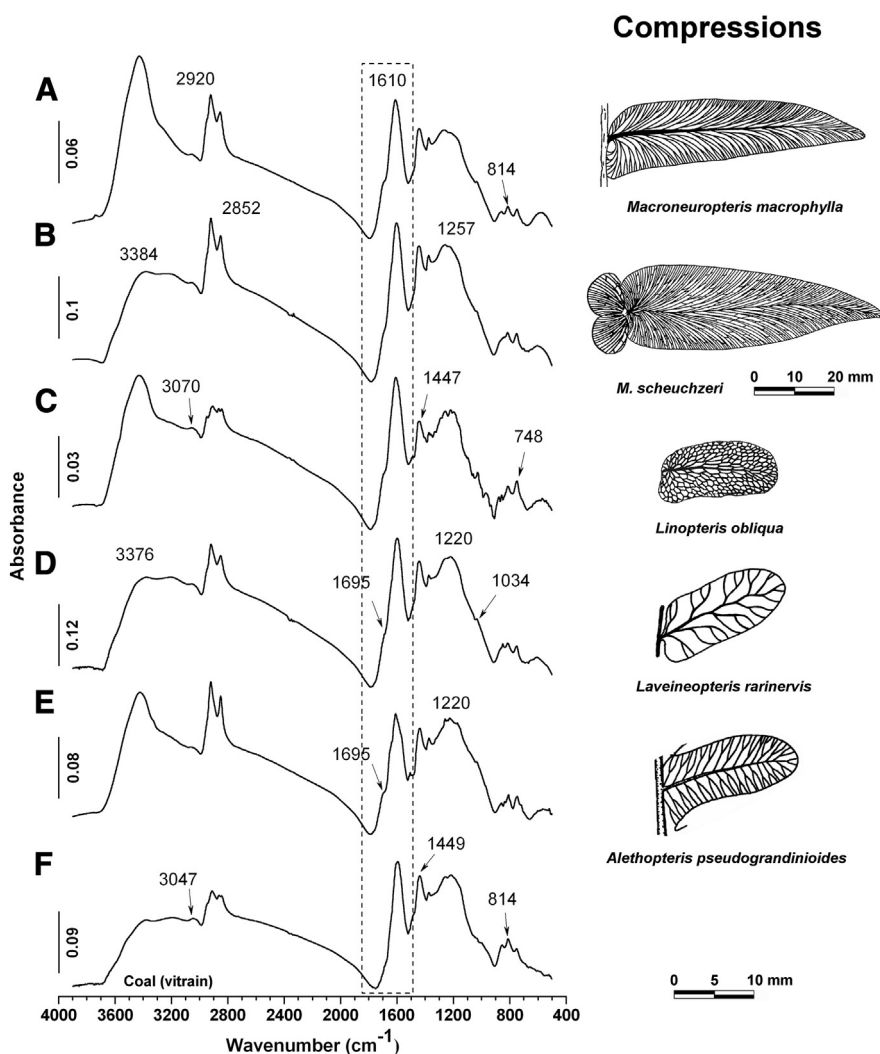


Fig. 4. FTIR spectra of compressions and coal (vitrain). Pen sketches corresponding to typical pinnule morphology.

Fossilized-cuticles

5.5. Implications of chemometric-assisted circumscription of taxa

As discussed in Section 5.4, the morphological delimitation of the medullosalean foliage, particularly the neuropteroid taxa, has been debated for a long time. Morphological variability and, in some cases limited morphological characters, e.g., due to poor preservation, have frequently precluded an indisputable taxonomic assignment of some neuropteroid specimens (see Laveine, 1997). Interestingly, these problems in morphology-based classifications are in agreement with some of the difficulties we found regarding a clear chemical differentiation of taxa. Thus, in some cases results of our chemometric analysis are not unequivocal i.e., some groups overlap. This is the case of compressions assigned to *M. scheuchzeri* and "*O. subcuneata*" (Fig. 9B) showing some overlapping in agreement with the conspecificity mentioned above (see Section 2.4). Furthermore, compression-preserved specimens of *M. scheuchzeri*, "*O. subcuneata*", *N. ovata*, *N. flexuosa*, *Neuropteris* sp. A, and *L. rarinervis*, share morphological (e.g., Cleal and Shute, 2012; Laveine, 1997) and chemical characteristics (Fig. 9). This proclivity to be in a continuum of characteristics indicates the subjectivity of the taxa differentiation. Thus, the grouping of these taxa under a single generic name (which should then be *Neuropteris* Brongniart more or less *sensu lato*) is not ruled out.

5.5.1. Specimens of difficult assignment

To assess further the utility of our chemometric approach for the identification of isolated medullosalean-like specimens, data for *Neuropteris* sp. A and for *Neuropteris* sp. B are included in the PCA. These specimens likely belong to the Neurodontopteridaceae (see Section 2.4). In respect to *Neuropteris* sp. A (accession no. 982GF-224: Zodrow, 1985; see also N and \bar{N} in Fig. 7B), the chemometric results bear this out by showing that some of the smaller and most naturally macerated pinnules (i.e., the less resembling compressions), are grouped with fossilized-cuticles of *M. macrophylla*. On the other hand, the larger and most coalified pinnules are grouped with compressions of *M. scheuchzeri*. Inferred is chemical affinity with *Macroneuropteris*, either with *M. macrophylla* or *M. scheuchzeri*, which in part supports Zodrow's (1985, 2003) assignment of the specimen 982GF-224 to *M. scheuchzeri*. The absence of pinnule "hair" negates *M. scheuchzeri* assignment (Zodrow, 2014). At the same time it illustrates that, in some cases, chemometric studies applied to specimens having an incomplete morphological character set cannot solve taxonomic uncertainties.

In respect to compression *Neuropteris* sp. B (denoted U, Fig. 7B), the chemometric results revealed similarities with those of *N. ovata* and

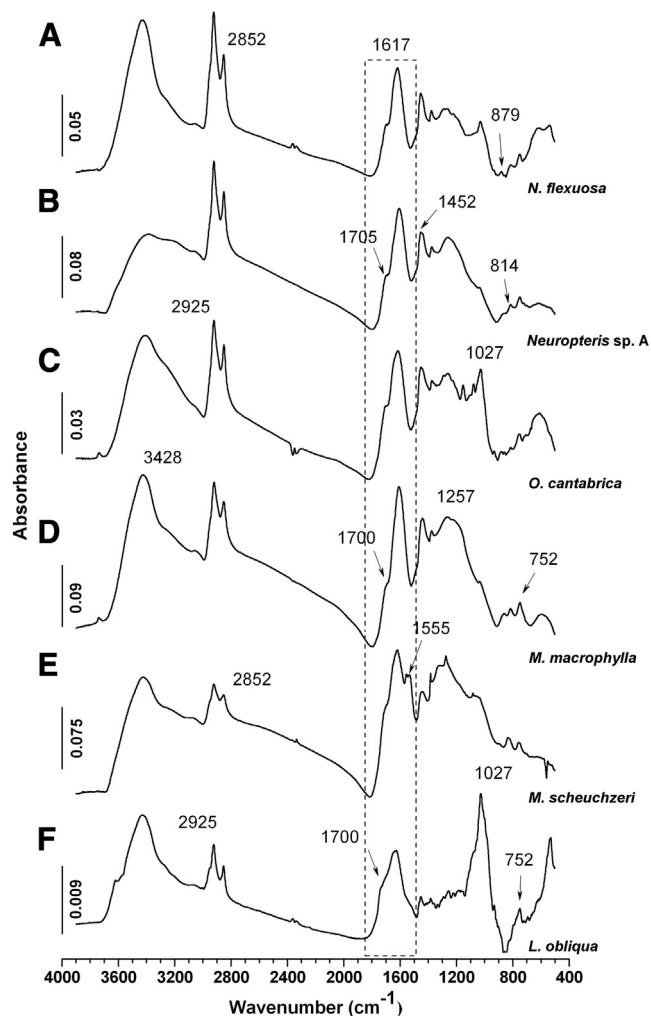


Fig. 5. FTIR spectra of fossilized-cuticles.

and Potonieaceae are clearly differentiated from each other. In marked contrast, specimens of the family Cyclopteridaceae (i.e., *L. rarinervis*) are clearly "included" in the group representing the Neurodontopteridaceae. Though suggestive, this result should be considered with caution because of the limited number of studied samples belonging to the Cyclopteridaceae (i.e., only compression-preserved specimens and no fossilized cuticles of *L. rarinervis* were available for study).

Interesting to note is that some of the biggest morphological similarities found amongst the medullosalean families are those shared by the Cyclopteridaceae and the Neurodontopteridaceae (Cleal and Shute, 2012), which is in agreement with the multivariate model. Considering our chemical data of the *L. rarinervis* foliage and the morphology-based counter arguments presented against the proposal to separate *Neuropteris* from *Laveineopteris* (e.g., Cleal et al., 1990; Laveine, 1997), we suggest the inclusion of *L. rarinervis* in the family Neurodontopteridaceae. Based on morphological and chemical arguments, speculating on the synonymy of *L. rarinervis* with some *Neuropteris* species (e.g., *N. ovata* and *N. flexuosa*) is not ruled out. However, maintaining the *status quo* as proposed by Cleal et al. (1990) may have some advantages for medullosalean biostratigraphy and palaeogeography.

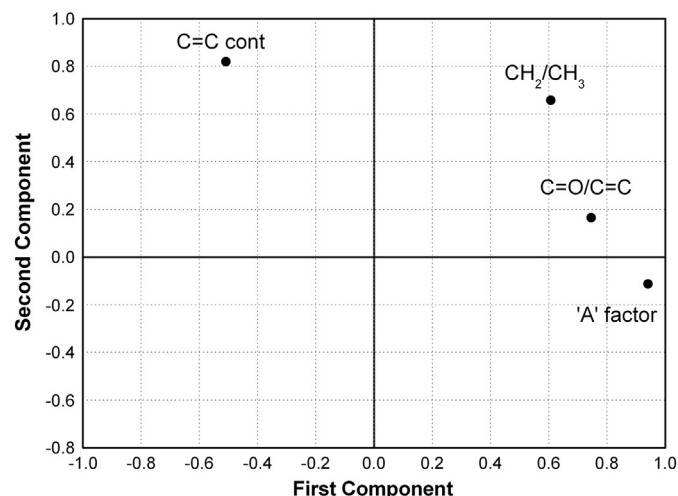


Fig. 6. PCA (principal component analysis): Plot of component loadings.

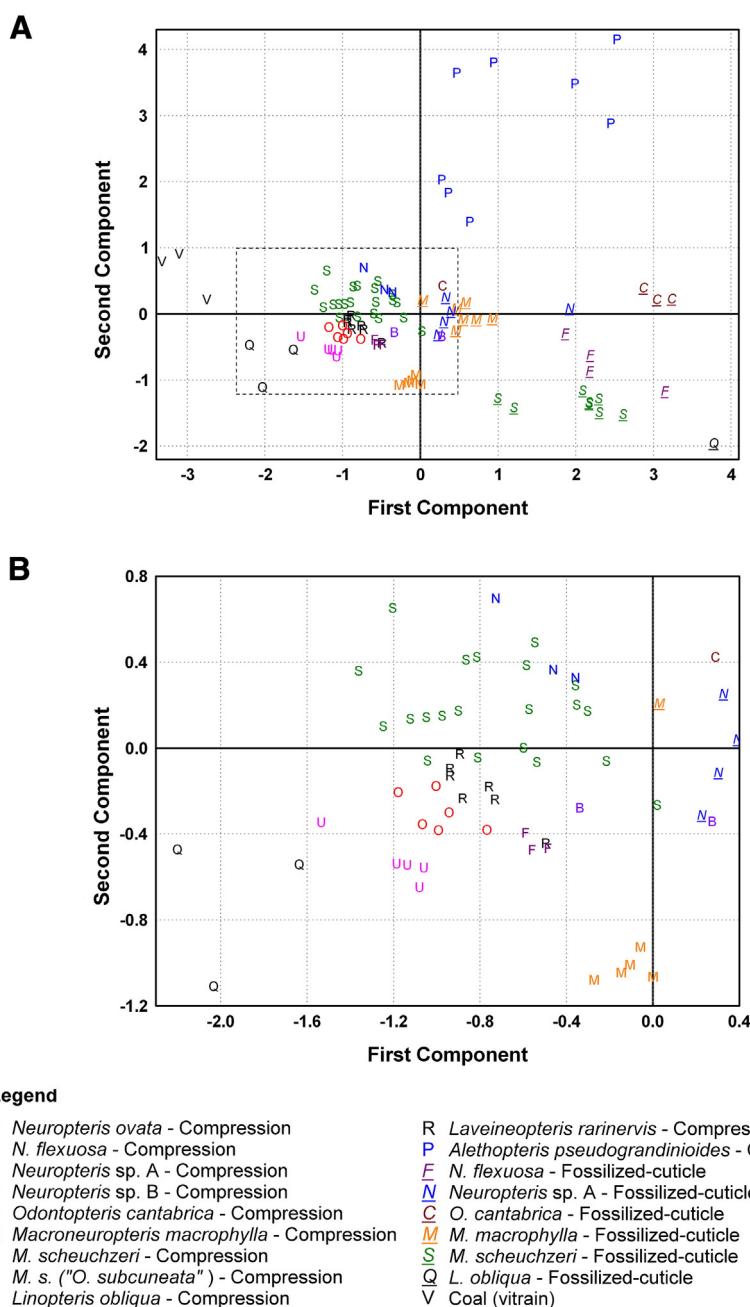


Fig. 7. PCA: Plot of component scores. (A) Full-scale plot. (B) Detail of the zone delimited in (A).

L. obliqua (Figs. 7A, B and 9) to infer 'neuropteroid' assignment, specifically to *N. ovata*.

In summary, Figs. 6, 7, and 9 leave little doubt about the usefulness of some FTIR-derived variables (i.e., CH_2/CH_3 , $\text{C}=\text{O}/\text{C}=\text{C}$, $\text{C}=\text{C}$ cont, and 'A' factor) for discriminating different neuropteroid and non-neuropteroid fossil taxa, including some specimens of difficult assignment.

6. Concluding remarks

This chemometric study of functional groups (chemical structure) involves compressed-fossilized-cuticles foliage from the four medullosalean families: Neurodopteridaceae, Potonieaceae, Cyclopteridaceae, and

Alethopteridaceae (Sydney Coalfield, Canada). New insights gained include:

- (i) Chemometrics supported the morphological classification, following literature, with few exceptions as explained, given isodiagenetic conditions and similar if not the same rank of coal (macerals). *A. pseudograndinioides* (Alethopteridaceae) is chemically differentiated from the 'true neuropteroid' taxa, by longer, polymethylenic chains, higher values of 'A' factor, $\text{C}=\text{C}$ cont, and $\text{C}=\text{O}/\text{C}=\text{C}$. This is consistent with the morphological separation of *Alethopteris* from 'neuropteroid' species. *L. rarinervis* (Cyclopteridaceae) specimens are not chemically different from those assigned to the family Neurodopteridaceae. We suggest the inclusion of *L. rarinervis* in the family Neurodopteridaceae,

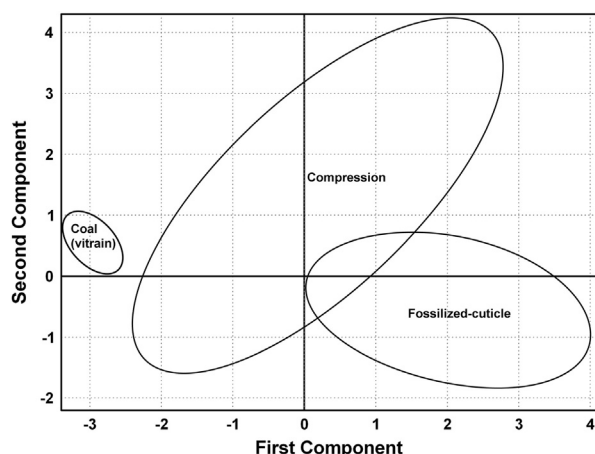


Fig. 8. Simplified plot of Fig. 7A showing groupings of compressions, fossilized-cuticles and coal (vitrain)-derived data, as approximately delimited by elliptical zones that do not have statistical meaning.

which is in agreement with the morphological features shared by *L. rarinervis* and some *Neuropteris* spp. According to our results, the synonymy of *L. rarinervis* with some *Neuropteris* species (e.g., *N. ovata* and *N. flexuosa*) is not ruled out.

- (ii) Given the conditions in (i), chemometric inferences about classification of medullosalean foliage are not hampered by the two fossil-preservation states (i.e., compression and fossilized-cuticle).
- (iii) Chemometrics *per se* is no taxonomic panacea for poorly preserved samples with insufficient morphological parameters.

In general, this study serves as an example of the application of chemometric techniques to achieve a more objective level of taxonomic assignments. In a future paper we will discuss chemometric models searching for the explanation of the variability in leaf morphology (including foliar anatomy and some architectural features) as a function of the chemical structure (IR-derived data). The likely relationships between chemotaxonomy and some paleoecological aspects (e.g., the relative metabolic cost and longevity of foliage) of the Pennsylvanian Medullosales will be also addressed there in.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.coal.2014.12.003>.

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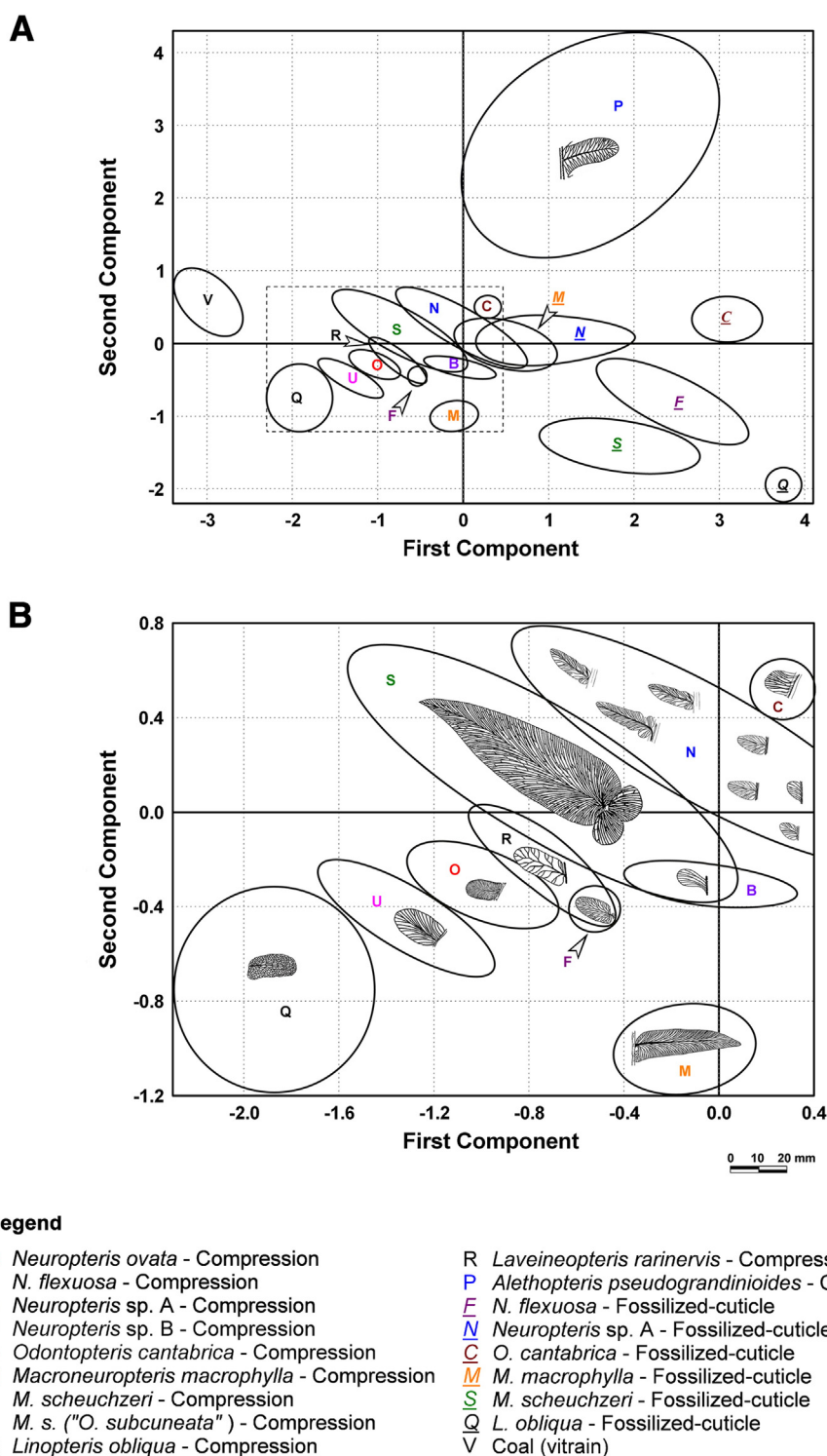


Fig. 9. Simplified plot of Fig. 7A and B. Pen sketches (compression forms only) are to scale in A and B. (A) Full plot indicating data groupings for different medulloslean taxa and coal (vitrain), where ellipses have no statistical meaning. (B) Detail of the zone delimited in A showing approximate zones corresponding to compressions (i.e., fossilized-cuticle groups are excluded for a better visualization).

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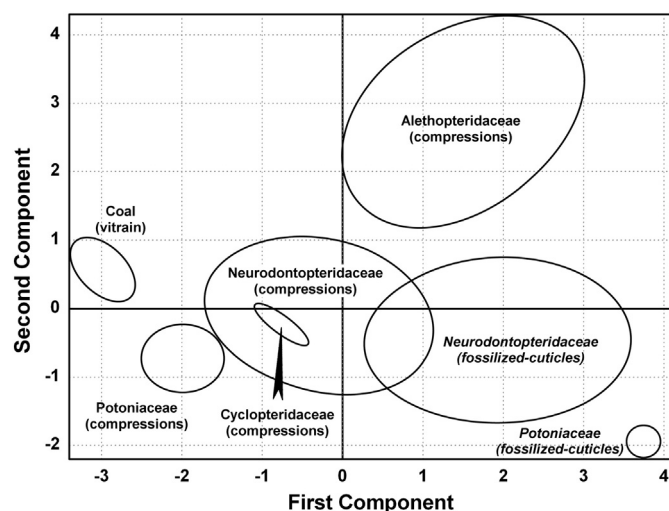


Fig. 10. Simplified plot of Fig. 7A. Ellipses indicate approximate zones corresponding to the four morphofamilies of the order Medullosales i.e., Alethopteridaceae, Neurodontopteridaceae, Potoniaceae, and Cyclopteridaceae, including compressions, fossilized-cuticles, and coal (vitrain). Cyclopteridaceae specimens clearly plot 'within' the group representing the Neurodontopteridaceae.

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