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## **Note sur la découverte d'ambre du Carbonifère associé à la fougère à graines *Linopteris obliqua*, terrain houiller de Sydney, Nouvelle-Écosse, Canada**

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Résumé de l'article

Nous rapportons une découverte d'ambre dans les roches sédimentaires carbonifères du terrain houiller de Sydney, en Nouvelle-Écosse, Canada. L'ambre se manifeste sous forme de gouttelettes ainsi que de figures linéaires, et il a une teinte variant du brun pâle au mauve foncé. L'ambre a été découvert in situ dans de la siltite au-dessus du filon de charbon du Pennsylvanien moyen Hub, où il est associé à des pinnules abondamment falsifiées de la fougère à graines *Linopteris obliqua*. Les spécimens d'ambre ont été analysés par spectrométrie infrarouge et leurs caractéristiques spectrochimiques ont été comparées à celles d'autres ambres fossiles. Cette découverte élargit non seulement l'inventaire de l'ambre à une période remontant à environ 300 millions d'années, mais documente également la capacité des fougères à graines carbonifères d'utiliser des mécanismes biosynthétiques pour produire des exsudats résineux.

# Note on the discovery of Carboniferous amber associated with the seed fern *Linopteris obliqua*, Sydney Coalfield, Nova Scotia, Canada

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## ABSTRACT

We report on a discovery of amber from the Carboniferous sedimentary rocks of the Sydney Coalfield, Nova Scotia, Canada. The amber occurs in the form of droplets and as a linear feature and ranges in colour from light brown to dark purple. The amber was found in situ in siltstone above the Middle Pennsylvanian Hub coal seam, where it was associated with abundantly abscised pinnules of the seed fern *Linopteris obliqua*. The amber specimens were analyzed by infrared spectrometry and their spectrochemical characteristics were compared with those of other fossil ambers. This discovery not only expands the inventory of amber to as old as ~300 million years, but also documents that Carboniferous seed ferns were able to utilize biosynthetic mechanisms to produce resinous exudates.

## RÉSUMÉ

Nous rapportons une découverte d'ambre dans les roches sédimentaires Carbonifères du terrain houiller de Sydney, en Nouvelle-Écosse, Canada. L'ambre se manifeste sous forme de gouttelettes ainsi que de figures linéaires, et il a une teinte variant du brun pâle au mauve foncé. L'ambre a été découvert in situ dans de la siltite au-dessus du filon de charbon du Pennsylvanien moyen Hub, où il est associé à des pinnules abondamment falsifiées de la fougère à graines *Linopteris obliqua*. Les spécimens d'ambre ont été analysés par spectrométrie infrarouge et leurs caractéristiques spectrochimiques ont été comparées à celles d'autres ambres fossiles. Cette découverte élargit non seulement l'inventaire de l'ambre à une période remontant à environ 300 millions d'années, mais documente également la capacité des fougères à graines Carbonifères d'utiliser des mécanismes biosynthétiques pour produire des exsudats résineux.

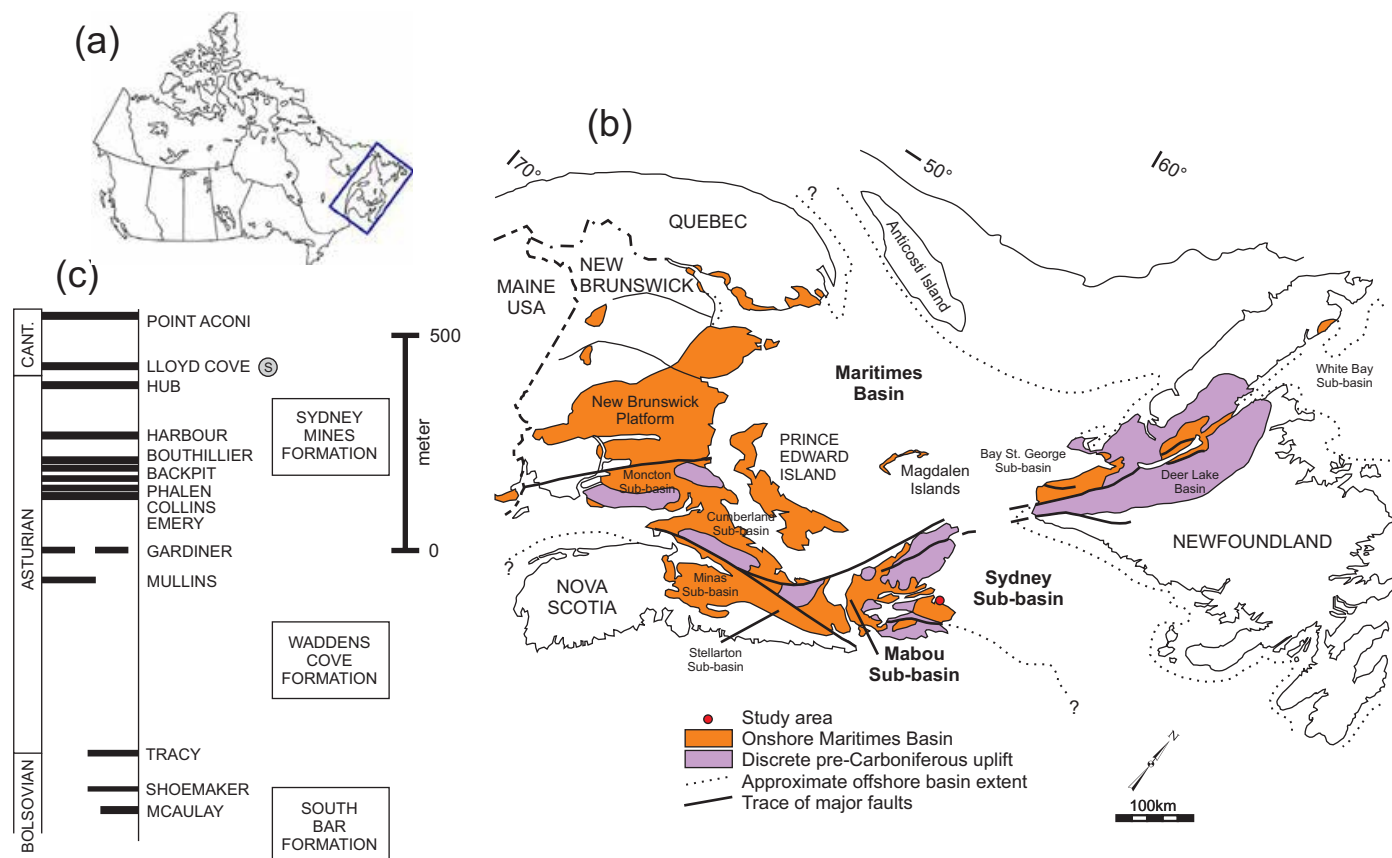
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## INTRODUCTION

Fossil resin, or amber, has been known since prehistoric times and used in a variety of applications, such as varnish, paint binder, adhesive, and jewelry (Lucas and Harris 1962). Resins can be defined as lipid soluble mixtures of volatile and non-volatile terpenoid and/or phenolic secondary compounds that are secreted either within or on the surface of plants (Langenheim 2003). Fossilization of resins and their preservation in the fossil record as amber provide important sources of information in relation to chemotaxonomy, geochemical processes, and provenance studies (e.g., Anderson *et al.* 1992; Broughton 1972; Lyons *et al.* 2009). Most fossil resins come from Cretaceous to Neogene sediments, and they are mostly products of conifers and angiosperms (Langenheim 2003).

Amber in Canada is known mainly from the western provinces, notably from Alberta, where it occurs in Late Cretaceous and, to a lesser extent, in Paleocene formations. In these rocks it was dominantly sourced from conifers, likely from members of the family Cupressaceae (McKellar and Wolfe 2010). Although mostly recovered from bituminous coal seams, amber is also found in organic-rich shale beds overlying those coals in Alberta.

In this paper we report the discovery of amber from Carboniferous (Middle Pennsylvanian) sedimentary rock from the Sydney Coalfield, Nova Scotia, Canada (Fig. 1). To our knowledge, it is the first discovery of amber in association with a seed fern. We compare the spectrochemical characteristics of the amber with other selected fossil ambers.



**Figure 1.** Location map and coal stratigraphy. (a) Regional. (b) Carboniferous basins and sub-basins in Atlantic Canada. (c) Coal stratigraphy. Amber samples (S) described in this study occurred approximately 5 m above the Upper Asturian Hub coal seam of the Sydney Sub-basin. Cant. = Cantabrian age.

## MATERIAL AND METHODS

### Material

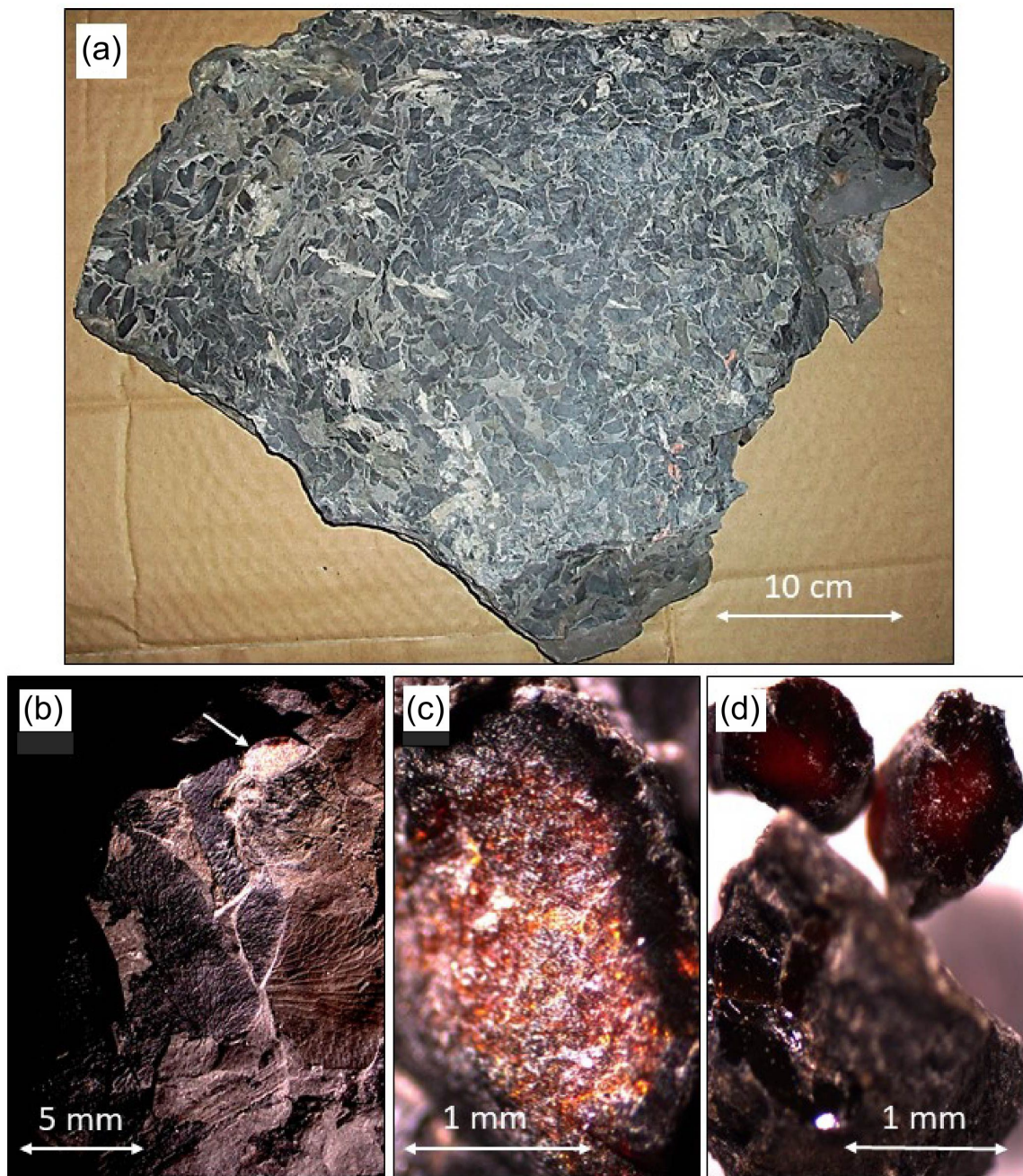
In 2008, a fossiliferous in-situ siltstone block was recovered above the upper Asturian Hub coal seam, Sydney Coalfield, in a developing open pit. The block measures 30 × 40 × 7 cm (Fig. 2a) and weighs 22 kg. Its accession number in the Palaeontological Collection of Cape Breton University, Sydney, Nova Scotia, Canada is 08-10/15-9. The block was systematically deconstructed, and a careful record of the process was kept. The megascopic analysis showed that abscised foliage of the tree fern *Linopteris obliqua* (Bunbury 1847) Zeiller 1899 (Zodrow and McCandlish 1978; Zodrow *et al.* 2007) was the overwhelmingly dominant compression fossil present, numbering in the thousands. Interestingly, Bunbury (1847, p. 427) commented on the abundance of this species in the Sydney Coalfield and its absence in American and European collections.

Two fragmentary amber samples were found. Sample 8-10/15-9 13 is a droplet 3 mm across and weighing ca. 5 mg (Fig. 2b, arrow). Enlarged 30 times (Fig. 2c), it shows micrometre-scale brownish-red grains under reflected light and no inclusions. The droplet is encased in a dark-coloured

rim, ca. 130 µm wide, that appears to have a different composition than the surrounding shaly matrix. The other amber sample is a composite specimen, 08-10/15-9 2, consisting of three fragments, each 3–4 mm in size (Fig. 2d). In contrast with the previous sample, these fragments are translucent, darkish in colour, and inclusion-free. The spatial relationship on these fragments in the block is unknown.

### Methods

After megascopic observations and careful isolation of the amber, the samples were analyzed by the Fourier transform infrared spectroscopy (FTIR) technique. This technique has long been proven helpful in investigating the source of amber, especially of Cretaceous and younger age (Langenheim and Beck 1965). For FTIR analysis, both amber samples (8-10/15-9 13 and 08-10/15-9 2) were analyzed on a Nicolet 6700 instrument equipped with a DTGS detector. Three FTIR spectra were obtained from the lighter coloured amber droplet (8-10/15-9 13), and three spectra from the darker coloured fragments (08-10/15-9 2, one spectrum from each fragment). For these analyses, a small amount of amber was mixed with finely ground KBr (the resin accounting for ca. 2 wt.% of the mixture) to form



**Figure 2.** Documentation of amber in the Carboniferous, Sydney Coalfield, Nova Scotia, Canada. (a) amber-bearing block showing abundant compression pinnules of *Linopteris obliqua* (Bunbury 1847) Zeiller 1899 (accession number 08-10/15-9 13). (b) Bright in situ droplet, arrowed, in association with compression pinnules (sample 8-10/15-9 13). (c) The bright granular amber droplet itself. (d) The dark, dense, and transparent amber pieces (sample 08-10/15-9 2).

a KBr pellet. Spectra were obtained in reflectance mode at a resolution of  $4\text{ cm}^{-1}$  and 400 scans were collected per analysis. The IR signal was recorded in the region of 400 to  $4000\text{ cm}^{-1}$  wavenumber. IR bands were identified by comparison with published assignments (e.g., Painter *et al.* 1981; Wang and Griffith 1985; Goodarzi and McFarlane 1991; McFarlane *et al.* 1993). The following three ratios of the integration-band areas (Table 1) were calculated:  $\text{CH}_2/\text{CH}_3$ ,  $\text{Ox}_1$ , and  $\text{Al}/1035$ . The  $\text{CH}_2/\text{CH}_3$  ratio was calculated in the 2800–

$3000\text{ cm}^{-1}$  aliphatic stretching region after band deconvolution, and two bands ( $\text{CH}_2$  at  $2925\text{ cm}^{-1}$  and  $\text{CH}_3$  at  $2960\text{ cm}^{-1}$ ) were used in calculation, following our previous procedures (e.g., Lis *et al.* 2005).  $\text{Ox}_1$  is a ratio of the  $\text{C}=\text{O}$  +  $\text{C}=\text{C}$  bands in the  $1500\text{--}1800\text{ cm}^{-1}$  region to the bands in the aliphatic stretching region ( $2800\text{--}3000\text{ cm}^{-1}$ ), and  $\text{Al}/1035$  is a ratio of the bands in the aliphatic stretching region ( $2800\text{--}3000\text{ cm}^{-1}$ ) to the band with the peak at  $1035\text{ cm}^{-1}$ .

**Table 1.** FTIR derived ratios of the amber samples.

| Analysis                  | CH <sub>2</sub> /CH <sub>3</sub> | Ox <sub>1</sub> | Al/1035     |
|---------------------------|----------------------------------|-----------------|-------------|
| 8-10/15-9-13 analysis 1   | 1.36                             | 0.18            | 0.40        |
| 8-10/15-9-13 analysis 2   | 1.18                             | 1.09            | 3.12        |
| 8-10/15-9-13 analysis 3   | 1.50                             | 0.39            | 1.60        |
| <b>Average</b>            | <b>1.35</b>                      | <b>0.56</b>     | <b>1.71</b> |
| 08-10/15-9 2 - fragment 1 | 1.27                             | 0.54            | 0.38        |
| 08-10/15-9 2 - fragment 2 | 1.32                             | 0.53            | 0.07        |
| 08-10/15-9 2 - fragment 3 | 1.42                             | 0.77            | 0.88        |
| <b>Average</b>            | <b>1.34</b>                      | <b>0.61</b>     | <b>0.44</b> |

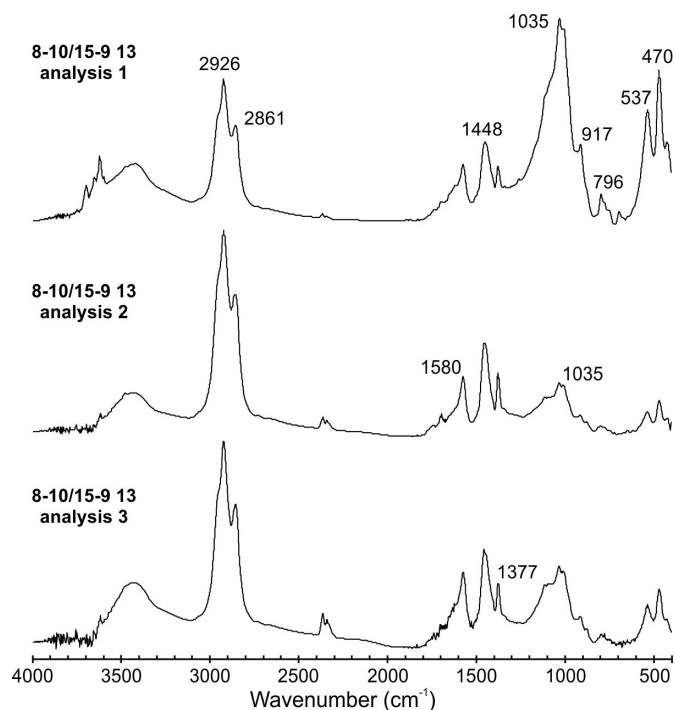
CH<sub>2</sub>/CH<sub>3</sub> was calculated after band deconvolution in the 2800–3000 cm<sup>-1</sup> region; Ox<sub>1</sub> is a ratio of the bands in 1500–1800/2800–3000 cm<sup>-1</sup> and Al/1035 uses intergration areas of 2800–3000 cm<sup>-1</sup> bands.

## RESULTS

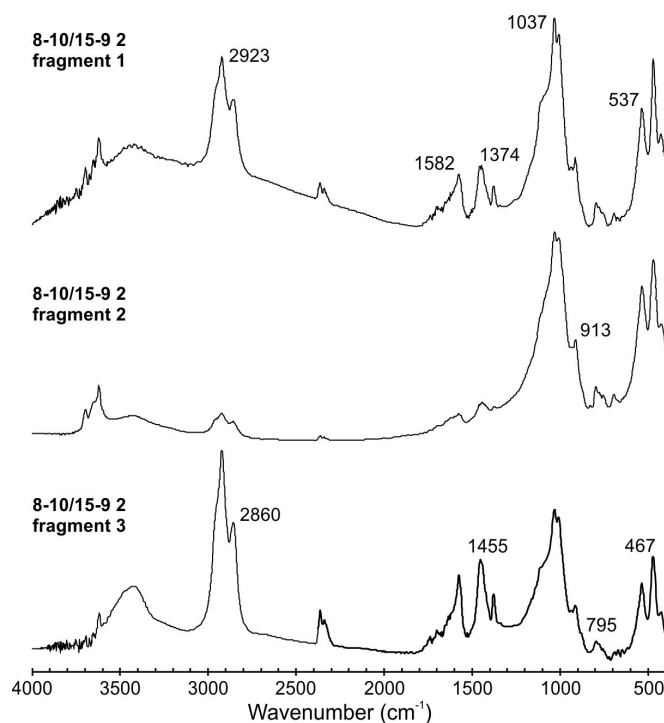
The FTIR spectra of both amber samples are dominated by aliphatic hydrogen bands in the stretching 2800–3000 cm<sup>-1</sup> region and the 1300–1500 cm<sup>-1</sup> aliphatic bending region, and have a broad band with the peak at ~1035 cm<sup>-1</sup> (Figs. 3–4). The latter band could come from  $\nu(\text{SO})$  undissociated sulphonate groups that were detected in some resins (Edwards *et al.* 2000); but more likely it represents C–O stretching bands (like those in lignin). Oxygenated groups and aromatic carbon bands in the 1500–1800 cm<sup>-1</sup> region are distinct, but their intensity is not large relative to aliphatic hydrogen bands. Small bands in the 700–900 cm<sup>-1</sup> region also occur, as do two prominent bands at 537 and 470 cm<sup>-1</sup> of an unassigned nature. It is interesting that bands representing exomethylene (CH<sub>2</sub>) groups in diterpenoid form with peaks at ~3082, 1644, and 887 cm<sup>-1</sup>, characteristic of many fossilized resins (Streibl *et al.* 1976; Poinar and Mastalerz 2000) were not recorded in the amber studied here (Figs. 3–4).

Qualitative comparison between spectra of the two amber samples (8-10/15-9 13 and 08-10/15-9 2) reveal close similarities between the functional-group distribution and their relative proportions (Figs. 3–4), and the difference between the two samples are not more distinct than within-sample differences. While three splits of sample 8-10/15-9 13 are very similar (Fig. 3), in sample 08-10/15-9 2 composed of three fragments (Fig. 4), one fragment has very reduced aliphatic bands (both in 2800–3000 and 1300–1500 cm<sup>-1</sup>) and the band with the peak at ~1037 cm<sup>-1</sup> was dominant; it is likely that this fragment has significant lignin content.

This functional-group similarity between the two amber samples is also supported by semiquantitative ratios (Table 1). CH<sub>2</sub>/CH<sub>3</sub> ratios range from 1.18 to 1.50 between individual analyses, with a very similar average values of 1.34 and 1.35 for the two samples, suggest similarity in the length and branching of aliphatic chains (e.g., Lin and Ritz



**Figure 3.** FTIR spectra of the amber droplet shown in Figure 2c. The three spectra represent three splits of sample 8-10/15-9 13.



**Figure 4.** FTIR spectra of three fragments of sample 08-10/15-9 2 (Fig. 2d).

1993). Ratio  $Ox_1$  is dependent on the relationship between the oxygenated plus aromatic carbon groups and aliphatic-hydrogen bands and usually reflects the oxidation level of the organic matter:  $CH_2$  and  $CH_3$  are known to be consumed during oxidation, with  $C=O$  groups being formed (Kister *et al.* 1988; Vasallo *et al.* 1991; Pradier *et al.* 1992). Following this interpretation, darker amber 08-10/15 9 2 would be on average slightly more oxidized compared to amber 8-10/15-9 13, as reflected by a higher  $Ox_1$  value of 0.61 compared to 0.56 in the latter (Table 1). In their study of the amber from Dominican Republic, Poinar and Mastalerz (2000) noted that the increase in oxidation/weathering was associated with a change from lighter to darker colour, an observation that seems to be supported in the present study. The main difference between the two amber samples is in the Al/1035 ratio, which is greater for the 8-10/15-9 13 sample, mainly because of significantly smaller absorbance of the bands with the peak at  $\sim 1035$ – $1037\text{ cm}^{-1}$  (likely coming from lignin) relative to the aliphatic stretching bands in this sample.

## DISCUSSION AND CONCLUSIONS

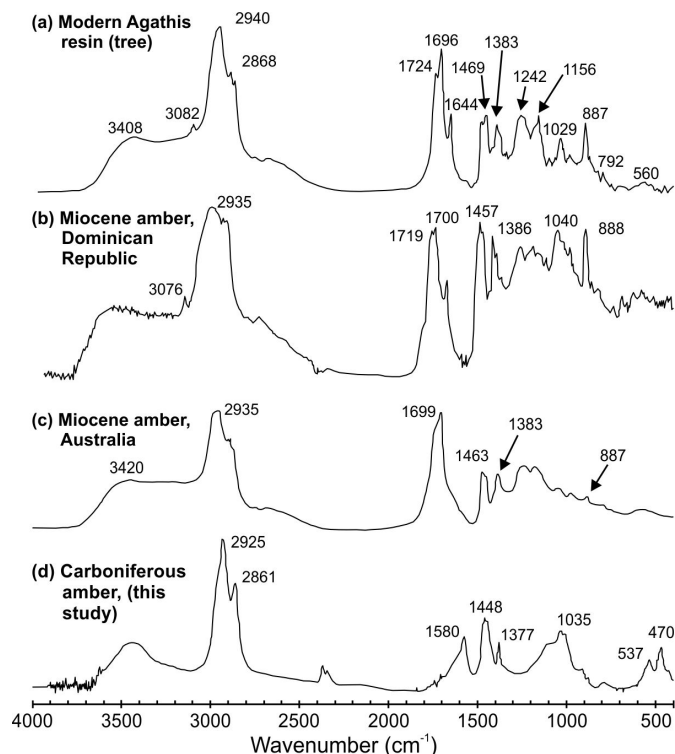
Our discovery of Carboniferous amber expands the inventory of amber to as old as  $\sim 300$  million years, and thus beyond that documented from Desmoinesian coal seams in Illinois (Bray and Anderson 2009). As those authors concluded, such early occurrences of amber demonstrate that pre-conifer gymnosperms were able to utilize the biosynthetic mechanisms to produce resins well before the onset of angiosperms, which are considered to be one of the main group of resin producers (Langenheim 2003). In fact, the development and utilization of these mechanisms by Carboniferous plants is indicated by the occurrence of maceral resinite (fossil resin) in Carboniferous coals, and by the presence of medullosalean seed-fern resin in Pennsylvanian coal balls (C. Eble, written communication 2022). Crelling and Kruege (1998) separated resinite from the Pennsylvanian Herrin Coal in Illinois and argued that its properties differed from those of Cretaceous and younger resins. The petrographic properties of Carboniferous resinites (Crelling 1995) are, however, similar to those of younger resins, and older resins likely served similar botanical functions to those of younger plants for example, sealing and protecting wounds and repelling insects (Langenheim 2003). In the Carboniferous sedimentary rocks, however, the resins are of small, typically microscopic size and, unlike the younger resins, megascopic “amber-size” occurrences are extremely rare (e.g., Grimaldi 2009). The very rare and small occurrences of amber and resinite in Carboniferous sedimentary rocks suggest that only limited types of plants then had the ability to utilize biosynthetic mechanisms to produce resins.

Identification of the parent plants of ambers is often impossible because ambers often occur isolated and in dissociation from the source plant (Langenheim 2003).

Therefore, documenting the association of the studied amber with an arborescent seed fern is an important aspect of this study. It is well known that resins are produced by almost all modern conifers and many angiosperms. So far, the earliest evidence of fossil resin comes from the Late Carboniferous with arborescent cordaitaleans (early relatives of conifers) or from seed ferns suggested as source plants (Langenheim 2003). In this study, the occurrence of the amber in association with huge quantities of abscised medullosalean seed-fern foliage *Linopteris obliqua* indicates that seed ferns were also able to utilize the biosynthetic mechanisms to produce resin exudates.

In their py-GC/MS study of Carboniferous amber from an Illinois Basin coal seam  $\sim 320$  million years old, Bray and Anderson (2009) suggested that the amber had Class I (polylabdanoid) characteristics, similar to that produced by a wide variety of modern species. In contrast, Crelling and Kruege (1998), in their study of resinite from a different Illinois Basin coal seam revealed predominantly aliphatic character (straight chain hydrocarbons), with only minor alkylbenzenes and phenols, characteristic of a cross-linked aliphatic biogeopolymer. They further concluded that these Carboniferous resinites differed from typical Cretaceous or younger resinites in their chemistry: the Cretaceous resinites were composed mainly of aromatized and unsaturated cadinanes, small aromatic molecules and isoprenoid hydrocarbons, characteristic of polycadinane structure. In the present study, we analyzed the chemistry of functional groups and it is therefore difficult to make direct comparison with the py-GC-MS compound data of Bray and Anderson (2009) or Crelling and Kruege (1998). However, the comparison of functional-group distribution of the studied amber to the younger ambers and modern resin from the other areas (Fig. 5) reveal two main differences: (1) the absence of oxygenated group bands around  $1700\text{ cm}^{-1}$  in the Carboniferous amber studied; this band is very prominent in Miocene ambers and in modern *Agathis* resin (Fig. 5), as well as in Cretaceous ambers from Canada (McKellar and Wolfe 2010); and (2) the presence of a distinct  $1580\text{ cm}^{-1}$  band in the Carboniferous amber; this band, likely represents aromatic carbon and does not occur in the spectra of other ambers. Van Bergen *et al.* (1995) also mentioned the unusual chemistry of Carboniferous resin rodlets, dominated by (alkyl)napthalenes, alkylbenzenes, and phenols.

For now it is difficult to conclude whether these differences are related to the original chemistry of the resin or are the result of more advanced maturation processes for the Carboniferous resin. Clifford and Hatcher (1995a, b) found that, with increased maturation from lignite to subbituminous coal, fossil resins showed depletion in exomethylene groups and increase in alkylnapthalenes. The coals associated with the amber studied are of high volatile bituminous rank ( $R_o$  % 0.64–0.66 %), and the reduction in oxygenated groups and the absence of exomethylene groups, at least to some extent, may be related to their more advanced maturation.



**Figure 5.** Comparison of FTIR spectra of the amber studied here with selected younger resins.

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