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A study of functional groups of trichomes of *Odontopteris cantabrica*: Implications for molecular taxonomy (Seed fern, Carboniferous, Canada)

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ABSTRACT

This study investigates the nature of trichomes over a large tripinnate frond segment of *Odontopteris cantabrica* Wagner that is also the index fossil for the Cantabrian Substage (Kosmovian), Late Pennsylvanian age. One objective is methodological, to illustrate the application of micro-FTIR (Fourier transform infrared) spectroscopy to microstructures, and the other one is to document morphology and chemical make-up of trichomes. Significant results reported for the first time from a Carboniferous seed fern include the occurrence of three types of trichomes over the frond, their differentiated cells in terms of color, stomatiferous nature (anomocytic type), and functional-group abundances of trichomes and pinnule cuticles. The trichomes are characterized as being 'fatty', with lower aromaticity, yet distinct aromatic carbon. As inferred from the high CH_2/CH_3 ratio, aliphatic-chains were long and straight, and even less branched than in the pinnule cuticle. Although there are small chemical differences between trichomes and pinnule cuticles, their identity is hypothesized to be very close. In summary, the new information increases the stratigraphic significance of *O. cantabrica* as a robust index fossil, and supplies significant taxonomic data for separating species in this genus.

1. Introduction

Conspicuous on foliar and rachial surfaces of Carboniferous seed ferns are trichomes which have been documented and described by many authors, e.g., Oliver and Scott (1904); Schopf (1948); Barthel (1962); Baxter and Willhite (1969); Cleal and Zodrow (1989); Kerp and Krings (2003); Krings et al. (2003, 2004); Zodrow (2007); Cleal et al. (2007); Zodrow et al. (2014, 2017); to reference only a few sources. Descriptions of physiological functions of fossil trichomes in the palaeobotanical literature are usually limited to insect-defense mechanisms (cf. Zodrow et al., 2016). This contrasts with angiospermous trichomes and their known complex biochemical, and assimilatory/excretory functions, cf. Johnson (1975); Barthlott et al. (2017). Although not well documented, we assume that these functions apply also to the fossil trichomes.

Odontopterids from the Canadian Sydney Coalfield (Fig. 1) were first described by Zodrow (1985). Among them is *Odontopteris cantabrica*Wagner et al., 1969 (Fig. 2A, B) that is tripinnate and the largest known specimen of this species (Zodrow, 1985). The size and the preservation of it allowed us to use state-of-the-art micro-FTIR to demonstrate the applicability of the technique to study epicuticular microstructures of Carboniferous seed ferns. Initiated in particular is the study of trichomes and pinnule cuticles of *O. cantabrica* to test the chemical composition for a more comprehensive circumscription of the species. The obtained data could also be used in conjunction with previously established parameters to discriminate among the plethora of odontopterid species described in the literature. Ultimately we hope that the present study will stimulate trichome research in a wider sense for the classification of Carboniferous seed ferns.

2. Taxonomic note

The genus *Odontopteris* Brongniart is defined by pinnules that (a) are attached to a rachis with their entire width and (b) lack a midvein. The species *O. cantabrica* Wagner is differentiated from other odontopterids by elongate pinnules with a round apex, 3–8 mm long and 3–5 mm wide, and widely-spaced, subparallel veins that generally fork once or twice. Second-order rachides are 1–4.5 mm wide and strongly striate (Wagner et al., 1969). The identity between the studied specimen 980–281 (Fig. 2A and B) and Wagner's type species was proposed by Zodrow (1985), and subsequently confirmed by Cleal et al. (2007). Noted is the stratigraphic significance for *O. cantabrica* as a robust index fossil for the base of the Cantabrian Substage in non-marine facies (Cleal et al., 2003; Zodrow and Cleal, 1985).

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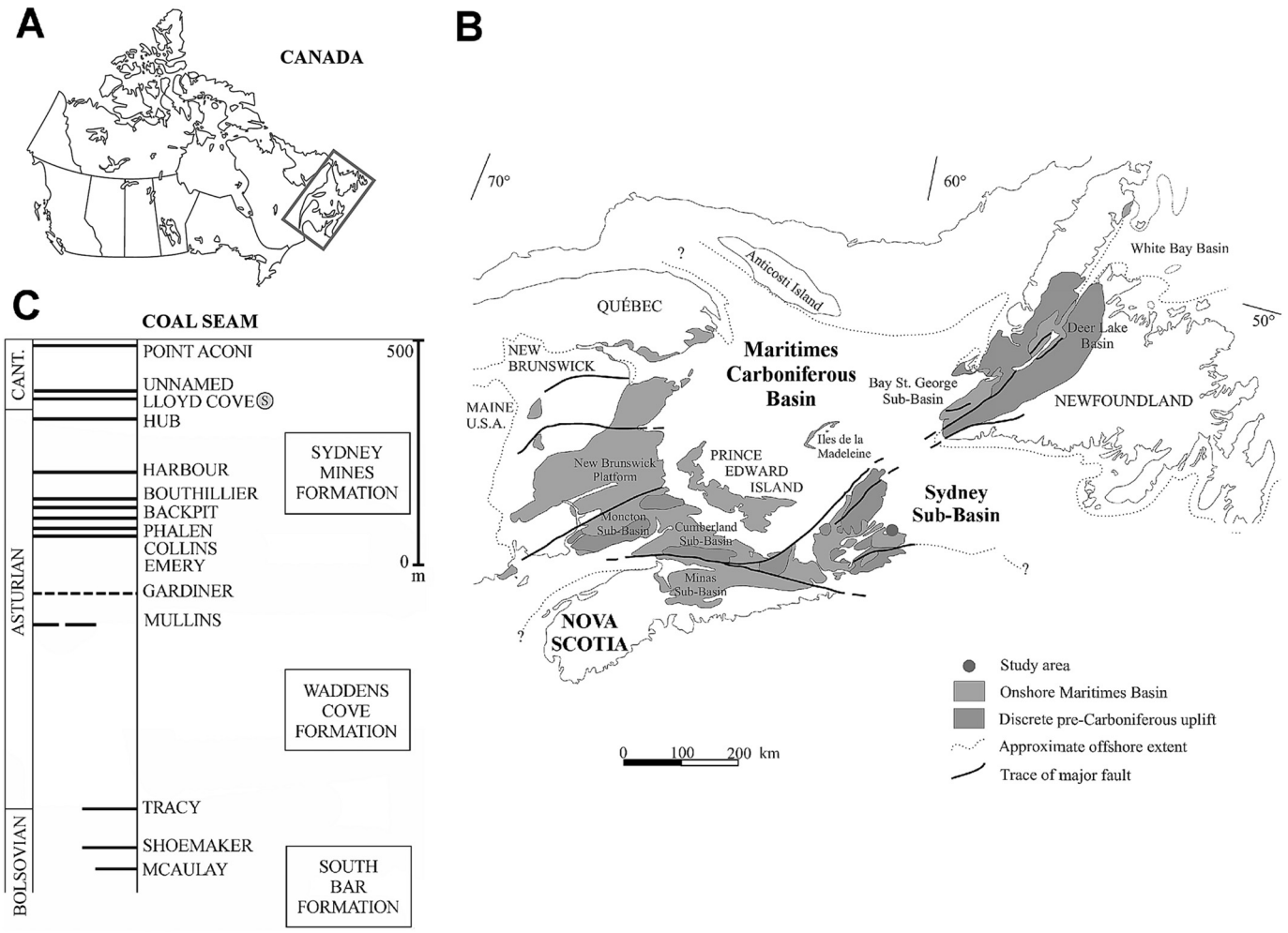

Fig. 1. Sample location. (A) Canada. (B) Maritimes Carboniferous Basin. (C) Coal lithostratigraphy and the Lloyd Cove Seam sample location (S).

3. Material, preservation and sampling methods

The 365-mm long *O. cantabrica* frond originated from the roof-shale section of the Lloyd Coal Seam that represents a relatively younger part of the on-shore portion of the submarine (post Tertiary) Sydney Coalfield (Bell, 1938; Hacquebard, 1984).

As is the case with all odontopterid specimens in the Sydney Coalfield, *O. cantabrica* was also naturally macerated (Zodrow and Mastalerz, 2009). This implies that the pinnules are actually preserved as cuticles (referred to in the text as pinnule cuticles), whereas the rachides remained coalified, to a certain degree. In terms of coal-rank assessment of associated sediments, vitrinite reflectance (R_o%) of the Lloyd Cove Seam section is 0.65%, suggesting that diagenetic influences were minimal, but not absent (Berner, 1980).

Areas sampled both for the purposes of FTIR analyses and the description and distribution of trichomes are marked in Fig. 2A as 1PUr (2 × 2 cm), 2PUr (11 × 5 cm), penultimate rachides, and 1APUr (2 × 3 cm), 2APUr (2 × 4 cm), antepenultimate rachis. Two to 6 h of HF (48% strength) digestion typically freed the sample material from the shaley matrix. Two sample sets of trichomes were prepared. One was for micro-FTIR, where from random pinnule cuticles (2PUr) small areas were cut each containing an organically attached and intact trichome, and from compression (2APUR) where entire trichomes were randomly collected. No further chemical treatment was applied to either preparation.

Random trichomes samples were also subjected to long-term maceration experiments of up to 11 days to test solubility in Schulze's oxidative solution (Schulze, 1855). In addition, some pinnule cuticles from which trichomes were removed were macerated for 15 min to eliminate bits of adhering coaly material, balancing this against the oxidative influence on the biomacromolecules (Werner-Zwanziger et al., 2009). Compression samples from 2PUr and 2APUr were also FTIR-analyzed, after removal of the trichomes, for purposes of comparison.

4. Analytical methods

The prepared samples were analyzed by two different solid-state FTIR methods, (i) and (ii). For (i), the micro-FTIR method was chosen to analyze the pinnule cuticles and the trichomes, and single trichomes, as