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SUPPLEMENTARY INFORMATION

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Molecular signatures of fossil leaves provide unexpected new evidence for extinct plant relationships

Vivi Vajda[1]*, Milda Pucetaite[2], Stephen McLoughlin[1], Anders Engdahl[3], Jimmy Heimdal[3] and Per Uvdal[3,4]*

[1]Department of Palaeobiology, Swedish Museum of Natural History, Stockholm, Sweden. [2]Department of General Physics and Spectroscopy, Vilnius University, Vilnius, Lithuania. [3]MAX-IV laboratory, Lund University, Lund, Sweden. [4]Chemical Physics, Department of Chemistry, Lund University, Lund, Sweden.
*e-mail: vivi.vajda@nrm.se; per.uvdal@chemphys.lu.se

Leaf database

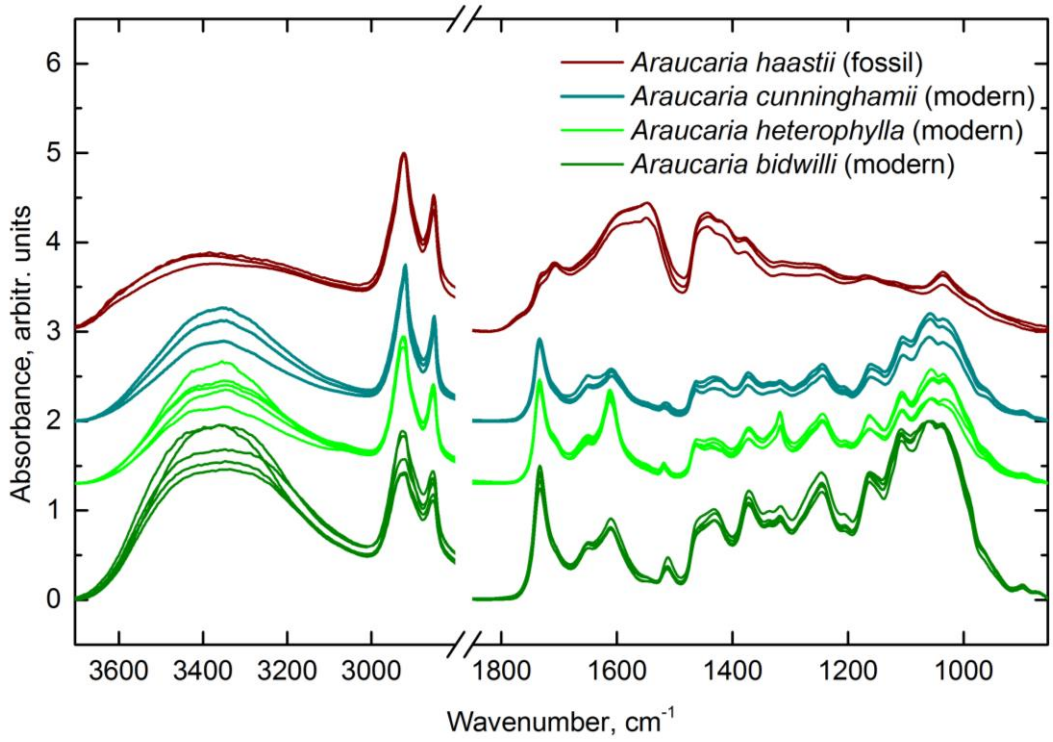
Supplementary Table 1. List of fossil and modern plant cuticles analysed by means of FTIR spectroscopy in this study. Each taxon is followed by the locality where the fossil was found, geological epoch and approximate age in million years of the sediments hosting the fossil cuticle according to Pole 1997[1]; Cookson & Duigan 1951[2], Bose 1975[3], McLoughlin *et al*. 2002[4] , Jansson *et al*. 2008[5,6,7], Lundblad 1950[8], Harris 1932[9], Harris 1935[10]. The fossils range in coalification rank from lignite B (*Agathis yallournensis*: Miocene of the Gippsland Basin, Australia)[11] to high volatile C bituminous (*Ctenis laxa*: Early Jurassic of the Vomb Trough, southern Sweden)[12]. All samples of extant plants were collected in the Botanical garden at Lund University, Sweden.

Species name	Locality	Age	Age in million years (ca.)
FOSSIL			
Podocarpus alwyniae	Otago, New Zealand	Miocene[1]	23–16
Retrophyllum vulcanense	Otago, New Zealand	Miocene[1]	23–16
Agathis yallournensis	Gippsland Basin, Victoria, Australia	Miocene[2]	23–16
Araucaria haastii	Shag Point, New Zealand	Late Cretaceous[3]	70
Otozamites boolensis	Gippsland Basin, Victoria, Australia	Early Cretaceous[4]	130
Allocladus helgei	Clarence-Moreton Basin, Queensland, Australia	Early Jurassic[5, 6, 7]	180
Nilssonia brevis	Skåne, Sweden	Early Jurassic[8]	180
Ctenis laxa	Skåne, Sweden	Early Jurassic[8]	200
Czekanowskia nathorstii	Scoresby Sound, East Greenland	Earliest Jurassic[9]	200
Ginkgo minuta	Scoresby Sound, East Greenland	Latest Triassic[9]	205
Ginkgo obovatus	Scoresby Sound, East Greenland	Latest Triassic[9]	205
Pterophyllum schenkii	Scoresby Sound, East Greenland	Latest Triassic[10]	205
Ptilozamites nilssonii	Scoresby Sound, East Greenland	Latest Triassic[10]	205
MODERN			
Ginkgo biloba	Botanical garden, Lund, Sweden	Anthropocene	0
Cycas zeylanica	Botanical garden, Lund, Sweden	Anthropocene	0
Araucaria bidwillii	Botanical garden, Lund, Sweden	Anthropocene	0
Araucaria cunninghamii	Botanical garden, Lund, Sweden	Anthropocene	0
Araucaria heterophylla	Botanical garden, Lund, Sweden	Anthropocene	0
Agathis robusta	Botanical garden, Lund, Sweden	Anthropocene	0
Wollemia nobilis	Botanical garden, Lund, Sweden	Anthropocene	0

Spectral analysis

Assignments of the modern plant cuticle IR spectral bands to molecular signals follow Heredia-Guerrero *et al.* [13] and Villena *et al.* [14]. Briefly, a broad band at 3700–3000 cm^{-1} is assigned to vibrations of a hydroxyl functional group. Sharp bands at 2960–2850 cm^{-1} are assigned to stretching vibrations of CH_2 and CH_3 functional groups in aliphatic material. A band at 1734 cm^{-1} is assigned to C=O vibration in esters (cutin, lipids). Bands in the 1650–1500 cm^{-1} spectral region are due to cyclic (aromatic, phenolic) compounds in the plants' cuticles. Bands due to deformation vibrations of CH_2 and CH_3 appear in the 1490–1400 cm^{-1} spectral region. Bands in the 1200–970 cm^{-1} region are due to CO, C-O-C, δ(OH) vibrations in polysaccharides.

For the fossil samples, a broad band at 3700–3000 cm^{-1} is assigned to vibrations of a hydroxyl functional group. The relative intensity of this band in spectra of fossil samples is considerably lower suggesting markedly lower water content. Sharp bands at 2960–2850 cm^{-1} are assigned to stretching vibrations of CH_2 and CH_3 functional groups in aliphatic material. A band at 1734 cm^{-1} is assigned to C=O vibration in esters. The relative intensity of this band in spectra of the fossil samples is diminished or absent in comparison to the spectra of the modern plants. Instead, a band at 1710 cm^{-1}, assigned to carboxylic acid/ketone C=O vibration appears. Bands in the 1650–1500 cm^{-1} spectral region are due to cyclic (aromatic, phenolic) compounds in the plants' cuticles. The relative intensities of these bands are much higher in the spectra of the fossils. This suggests stability of the compounds containing these functional groups. Bands due to deformation vibrations of CH_2 and CH_3 appear in the 1490–1400 cm^{-1} spectral region. Spectral bands, assigned to vibrations of polysaccharides in the spectra of the modern samples, are absent from the spectra of the fossils. A spectral band at approximately 1250 cm^{-1} could be assigned to OH deformation of phenolic compounds, whereas broad bands at 1174 and 1034 cm^{-1} are due to C-O-C vibrations (ester). Broad spectral bands at 1250, 1174 and 1035 cm^{-1} remain unassigned; C-O vibrations are normally present in this spectral region. Spectral bands at 3620, 3653, 3670, 3698, 1115, 1034, 1008 and 913 cm^{-1} (shoulder at 939 cm^{-1}) are assigned to the mineral kaolinite.

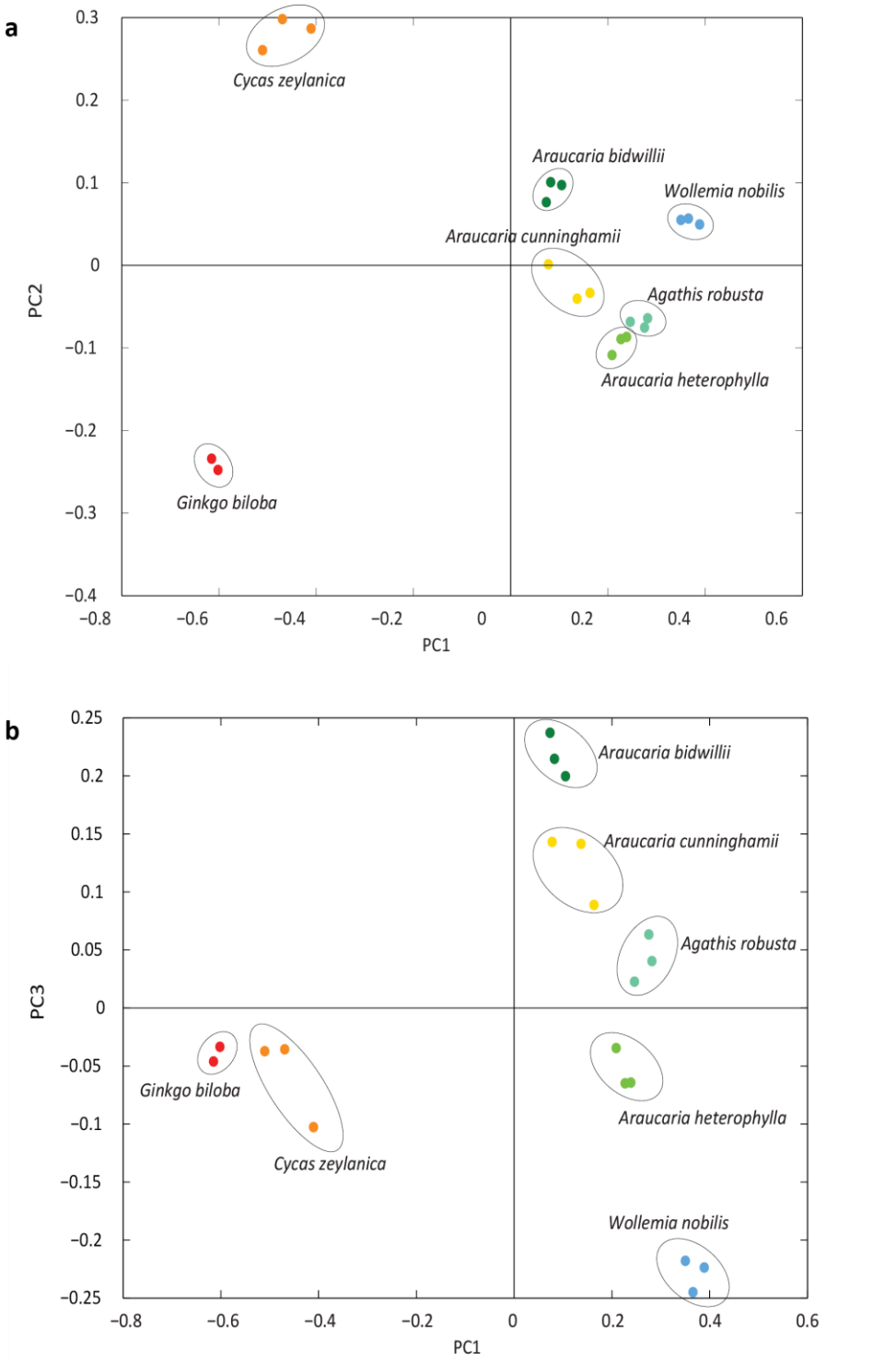


Supplementary Figure 1. IR absorption spectra of modern (*Araucaria bidwilli*, *Araucaria heterophylla* and *Araucaria cunninghamii*) and fossil (*Araucaria haastii*) araucariacean conifer cuticles. Note the diminished content of water (3700–3000 cm^{-1}) and absence of spectral bands assigned to polysaccharides in the spectral region 1200–970 cm^{-1} in the spectra of fossil samples. The ester band at 1734 cm^{-1} in the spectra of the modern plants is replaced by carboxylic acid/ketone band at 1710 cm^{-1} in the spectra of the fossils. The spectra have been shifted for clarity.

PCA analysis

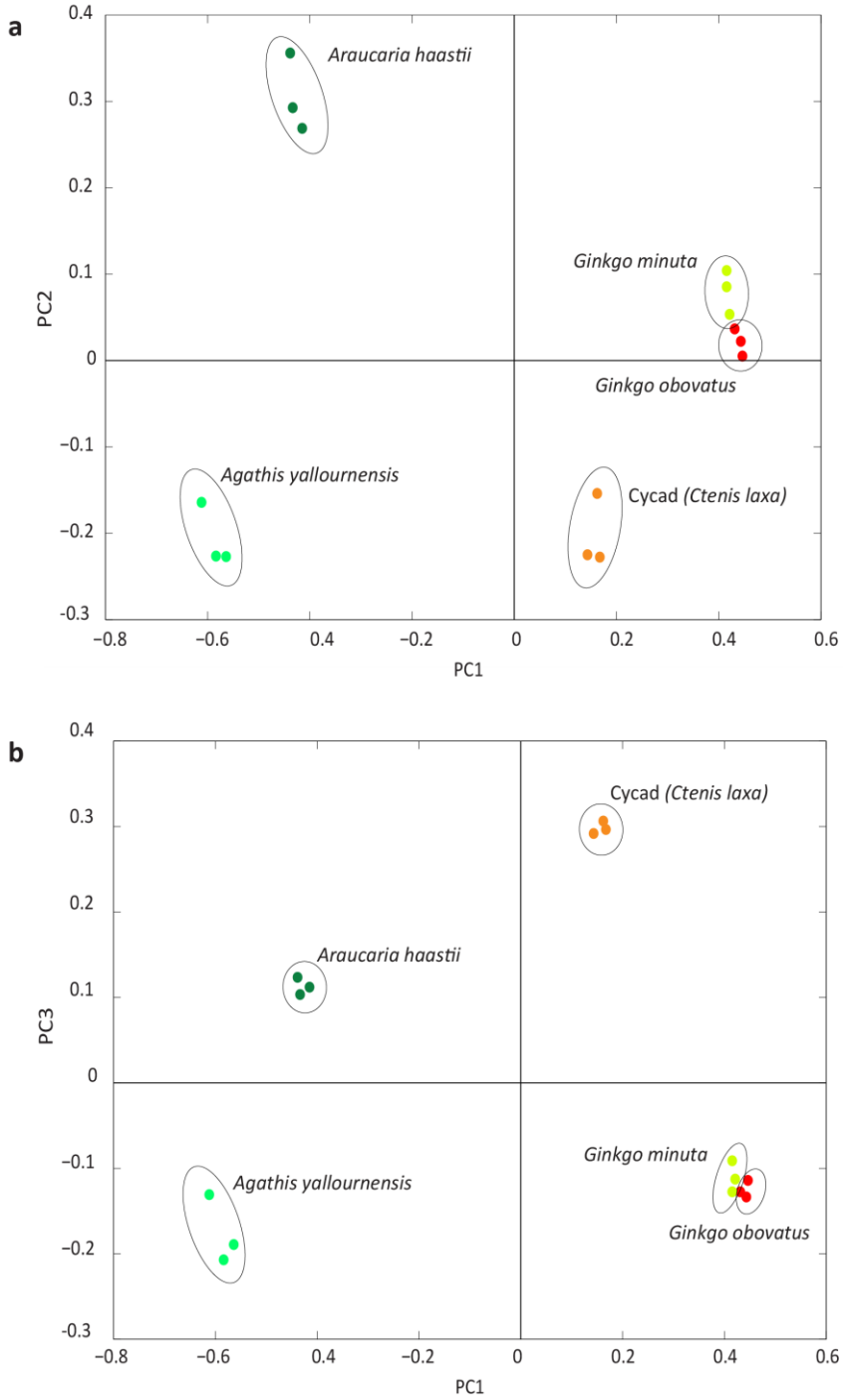
Principal Component Analysis (PCA) was used as an additional tool to identify relations between the data (Supplementary Fig. 2–4). PCA generates new variables—principal components (PCs) —which are linear combinations of the original dataset. Each PC represents variance of the data and, therefore, can be used for highlighting differences between the spectra. PCA was performed using the Matlab function *princomp*, which employs a covariance matrix to calculate the PCs. PC score plots were compiled from the first three components (accounting for approximately 95% of variance depending on the sample set) in order to observe the relationships between the sample groups.

The PCA analysis yields similar results to the HCA and supports the conclusions of this work. It should be noted, however, that the PCA does not group the data, but rather shows the relationship between the data points. Nevertheless, PCA score plots (PC1 vs. PC2 and PC1 vs. PC3) reveal that spectra of each plant – modern or fossil – form discrete clusters.



Supplementary Figure 2. PCA score plots based on IR absorption spectra of the cuticles of <u>extant</u> gymnosperms.

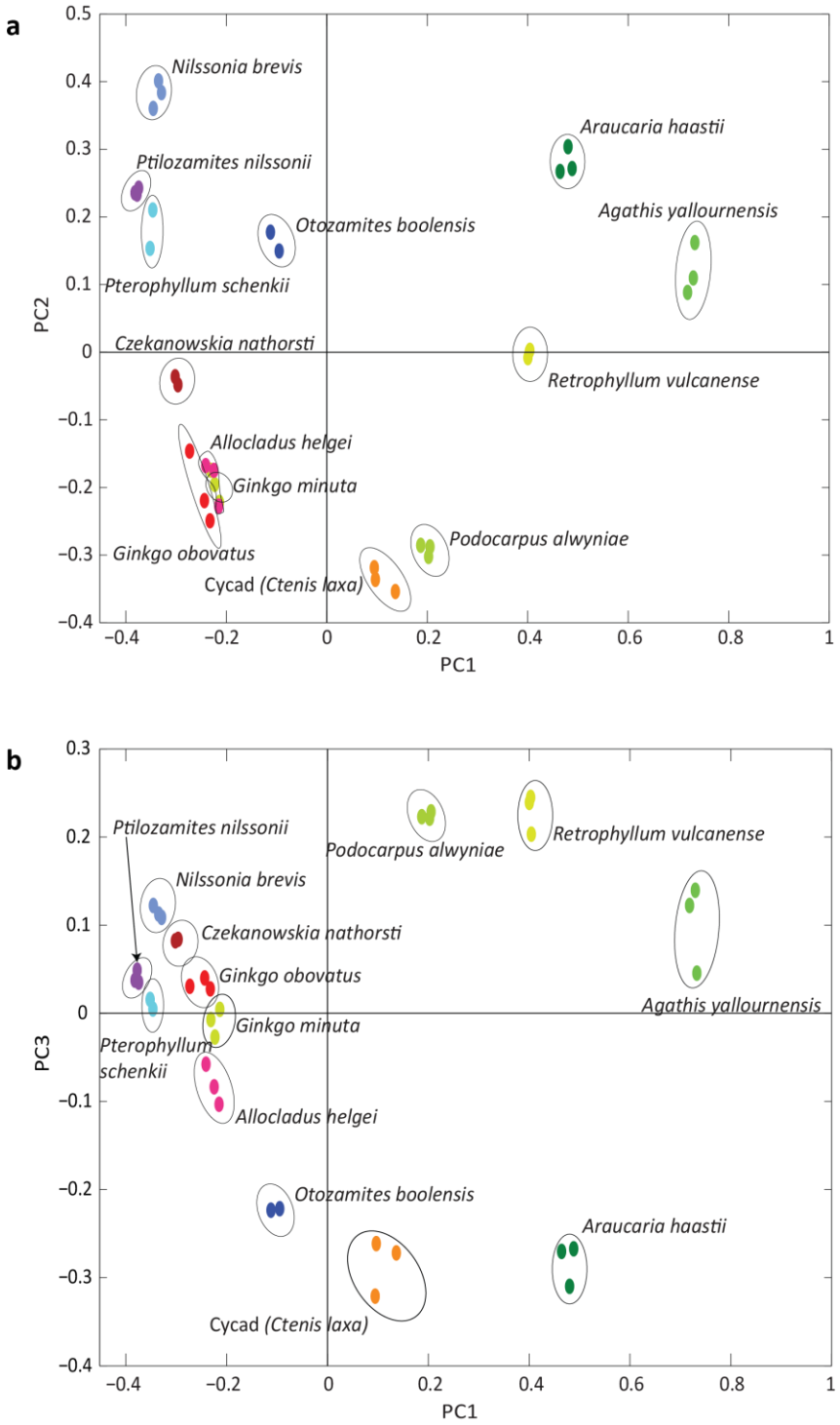
Two major groups of data points can be separated: (I) cycads and ginkgos and (II) Araucariaceae. Although the cycads and ginkgoaleans plot close to each other in one dimension (**b**), they are relatively remote in the other (**a**) indicating substantial divergence between these subgroups. Araucariaceae are always clustered together.



Supplementary Figure 3.

PCA score plots based on IR absorption spectra of the cuticles of <u>fossil</u> gymnosperms related to the extant taxa analysed in Supplementary Figure 2.

As for the modern plants, the same two major groups of data points can be identified amongst the fossils: (I) cycads and ginkgos and (II) Araucariaceae. Although the cycads and ginkgoaleans plot close to each other in one dimension (**a**), they are, just as in the modern counterparts, relatively remote in the other (**b**) indicating substantial divergence between these subgroups.



Supplementary Figure 4.

PCA score plots based on IR absorption spectra of the cuticles of 13 fossil gymnosperms. Despite more taxon sampling, the conifers (Araucariaceae and Podocarpaceae) group in a discrete sector of the ordination space (**a, b**). The extinct Nilssoniales and *Ptilozamites* are more closely allied with Bennettitales rather than with Cycadales. A similar close relationship is evident between Ginkgoales and the extinct Leptostrobales.

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