

A new proposal concerning the botanical origin of Baltic amber

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Baltic amber constitutes the largest known deposit of fossil plant resin and the richest repository of fossil insects of any age. Despite a remarkable legacy of archaeological, geochemical and palaeobiological investigation, the botanical origin of this exceptional resource remains controversial. Here, we use taxonomically explicit applications of solid-state Fourier-transform infrared (FTIR) microspectroscopy, coupled with multivariate clustering and palaeobotanical observations, to propose that conifers of the family Sciadopityaceae, closely allied to the sole extant representative, *Sciadopitys verticillata*, were involved in the genesis of Baltic amber. The fidelity of FTIR-based chemotaxonomic inferences is upheld by modern–fossil comparisons of resins from additional conifer families and genera (Cupressaceae: *Metasequoia*; Pinaceae: *Pinus* and *Pseudolarix*). Our conclusions challenge hypotheses advocating members of either of the families Araucariaceae or Pinaceae as the primary amber-producing trees and correlate favourably with the progressive demise of subtropical forest biomes from northern Europe as palaeotemperatures cooled following the Eocene climate optimum.

Keywords: Baltic amber; conifer resin; Fourier-transform infrared microspectroscopy; Sciadopityaceae; *Sciadopitys verticillata*; Eocene

1. INTRODUCTION

It has been estimated that over 10⁵ tons of Baltic amber were produced by Palaeogene forests in the pan-Tethyan region of northern Europe, making this the largest single known deposit of fossilized plant resin (Poinar 1992; Grimaldi 1996; Weitschat & Wichard 2002). Baltic amber contains the most diverse assemblage of fossil insects of any age, which are typically preserved in exquisite anatomical detail (Grimaldi & Engel 2005). The cultural and economic significance of Baltic amber should not be understated: it has been recognized as a luxury item in archaeological contexts as old as Palaeolithic, and subsequently became the basis for important trade routes in Roman and Medieval times (Spekke 1957). Baltic amber is still mined and traded actively, with large intact blocks and specimens containing entombed insects commanding the highest prices. However, Baltic amber is never found in its original stratigraphic position, having been redeposited in Eocene glauconitic marine sediments as well as Neogene fluvial and glacial deposits. Although Baltic amber has been linked to resin production in forests dominated either by the conifer family Araucariaceae (Langenheim 1969; Gough & Mills 1972; Mills *et al.* 1984) or Pinaceae (Conwentz 1890; Schubert 1961; Weitschat & Wichard 2002), neither group fully satisfies

the range of geochemical and phytogeographical criteria necessary for a conclusive linkage, resulting in a botanical conundrum that has endured for well over a century. A detailed review of the ‘Tertiary Baltic amber mystery’ is provided by Langenheim (2003).

Amber is polymerized from a broad range of isoprenoid compounds originally produced by plant secondary metabolism. These compounds include primarily terpenoids, carboxylic (resin) acids and associated alcohols. Terpenoids constitute the most diverse group of plant natural products, with approximately 25 000 known compounds (Croteau *et al.* 2000). These are produced by both the mevalonic acid pathway in the cytosol and the deoxyxylulose phosphate pathway in the plastid (McGarvey & Croteau 1995). Terpenoids perform a range of ecological functions, including biochemical defence and semiochemical signalling, in addition to key roles in defensive resinosis upon attack or injury of the tree, primarily by insects (Langenheim 1994; Trapp & Croteau 2001). Because terpenoid synthases are genetically programmed (Bohlmann *et al.* 1998), terpenoid chemical profiles retain some degree of taxonomic specificity.

Owing to the great diversity of organic compounds present in modern and fossil resins, the infrared spectra of these materials are of considerable use in establishing relationships between amber and source trees, as first illustrated by the pioneering works of Beck and Langenheim (Beck *et al.* 1964, 1965; Langenheim & Beck 1965; Langenheim 1969). Subsequent developments in Fourier-transform infrared (FTIR) instrumentation,

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coupled with a strategic array of modern and fossil samples, have reopened the investigation of the source of Baltic amber. Current solid-state micro-FTIR technology enables routine analysis of untreated small samples (1 mg or less) directly on NaCl stages, thereby precluding the need for embedding in KBr pellets prior to spectroscopic analysis. This has facilitated the analysis of fossil resins in direct association with identifiable plant macrofossils, such as leaves and cones. Consequently, it has been demonstrated that FTIR microspectroscopy of botanical fossils outperforms traditional FTIR microscopy of samples diluted in KBr pellets with respect to resolving discrete bands and their associated functionalities (Gupta *et al.* 2007). In the present study, we exploit these technological advances in order to build upon prior studies, and in doing so reveal a hitherto unsuspected candidate for the production of Baltic amber: conifers of the family Sciadopityaceae.

2. MATERIAL AND METHODS

(a) *Samples*

(i) *Modern conifer resins*

A database of 79 FTIR spectra from modern conifer exudates was compiled from collections of wild populations as well as cultivars. Resins were obtained directly from wood surfaces, or in some cases from ovulate cone scales. Hardened resins were preferentially sampled. The database includes all conifer families capable of producing geologically relevant quantities of resin. The most intensely sampled family is Pinaceae (41 specimens representing 26 species from eight genera), followed by Cupressaceae *sensu lato* (26 specimens representing 17 species from 11 genera). These were augmented by nine specimens of Araucariaceae representing two species of *Agathis* and three of *Araucaria*, and three exemplars of *Sciadopitys verticillata*, the sole extant representative of Sciadopityaceae. Wild specimens of several species indigenous to western North America were sampled from discrete populations across broad edaphic and climatic gradients (e.g. Pinaceae: *Pinus contorta*, *Pseudotsuga menziesii*; Cupressaceae: *Thuja plicata*), in order to verify that local ecological factors exert relatively little influence on exudate composition. A full taxonomic list including sampling localities is provided in appendix 1 in the electronic supplementary material.

(ii) *Fossil samples*

Comparative analyses of paired modern–fossil resins produced by three conifer genera were undertaken to assess critically the performance of FTIR as a chemotaxonomic tool. The fossil materials are of comparable age to Baltic amber (i.e. Middle Eocene) and include deposits in northern Canada with exceptional preservation: the Buchanan Lake Formation on Axel Heiberg Island (79°55' N, 89°02' W; Basinger 1991) and the Giraffe kimberlite locality (64°44' N, 109°45' W; Wolfe *et al.* 2006). Resin nodules were obtained directly from mummified ovulate cones of *Pinus* sp. (pine) and *Pseudolarix* sp. (golden larch) from Axel Heiberg Island and from *Metasequoia* (dawn redwood) litter in the Giraffe drill core. The modern and fossil materials described above were compared with Baltic amber specimens originating from Germany, Latvia, Poland, Russia and southern Sweden. These samples encompass the range of appearances that are typical of Baltic amber, with respect to colour (honey-yellow to dark brown), bubble content (clear to milky) and detrital content (mainly plant fragments, occasionally arthropod remains). The Bitterfeld amber variant

from Germany, which is probably younger and compositionally distinct from Baltic amber, was deliberately excluded from analysis.

(b) *FTIR microspectroscopy*

For the entire array of modern and fossil conifer resins, FTIR microspectroscopy was conducted with a Thermo-Nicolet Nexus 470 bench spectrometer fitted with a dual-aperture Continuum infrared microscope with motorized stage. Samples were first examined with a binocular microscope and crushed to fragments of less than 500 µm prior to mounting directly on NaCl stages. The infrared microscope was then used to search for even surfaces free of impurities prior to spectroscopic analysis. Beam size was set at either 50 or 100 µm, and the spectral resolution was 4 cm⁻¹. Each reported spectrum is the average of 200 complete scans, resulting in 869 absorption values over the 4000–650 cm⁻¹ interval, which is equivalent to wavelengths of 2.5–15 µm. A selection of the raw FTIR microspectroscopic results is presented in figure 1.

(c) *Data reduction and hierarchical cluster analysis*

Raw resin spectroscopic data were collated into *a priori* groupings according to tree family for each of Araucariaceae, Pinaceae and Sciadopityaceae. Resins from Cupressaceae *sensu stricto* were subdivided into cupressoid (*Chamaecyparis*, *Cupressus*, *Juniperus*, *Neocallitropsis*, *Tetraclinis*, *Thuja* and *Widdringtonia*) and taxodioid (*Cunninghamia*, *Metasequoia*, *Sequoia* and *Sequoiadendron*) genera. For each of these groupings, as well as the five specimens of Baltic amber, a normalized average FTIR spectrum was derived.

Hierarchical cluster analysis, an established technique for exploring FTIR spectral databases (Naumann *et al.* 1991), was then used to explore relationships between the six groupings of conifer resins (i.e. Araucariaceae, Baltic amber, cupressoid, Pinaceae, Sciadopityaceae and taxodioid). Two clustering strategies were used. First, six spectroscopic ratios that can be assigned to discrete functionalities within the modern and fossil resins were computed (table 1). The use of ratios obviates the influence of variable baseline absorbance, which can result from differences in sample thickness, purity and beam incidence angle. The selected ratios describe the principal areas of variable spectroscopic behaviour between modern resins from different conifer families. Furthermore, when comparing modern and fossil counterparts, the chosen ratios appear relatively insensitive to diagenetic overprinting associated with amber polymerization. This is in sharp contrast to additional ratios that are clearly modulated by sample oxidation history (figure 2). Second, FTIR absorbance values from the range 650–1300 cm⁻¹, the so-called fingerprint region (e.g. Galletti & Mazzeo 1993; McKellar *et al.* 2008), were compiled for the six resin categories and normalized to unity. This range is primarily associated with functional groups residing in resin terpenoids, alcohols and associated esters, with minimal influence from the hydration or oxidation state of the material. In both analyses, clustering was applied to dissimilarity matrices based on squared Euclidean distances, using Ward's (minimum variance) strategy with randomized sample input order. The fundamental difference between the two strategies lies in the number of variables used to characterize each resin category: 6 for the spectroscopic ratios and 169 for the fingerprint region.

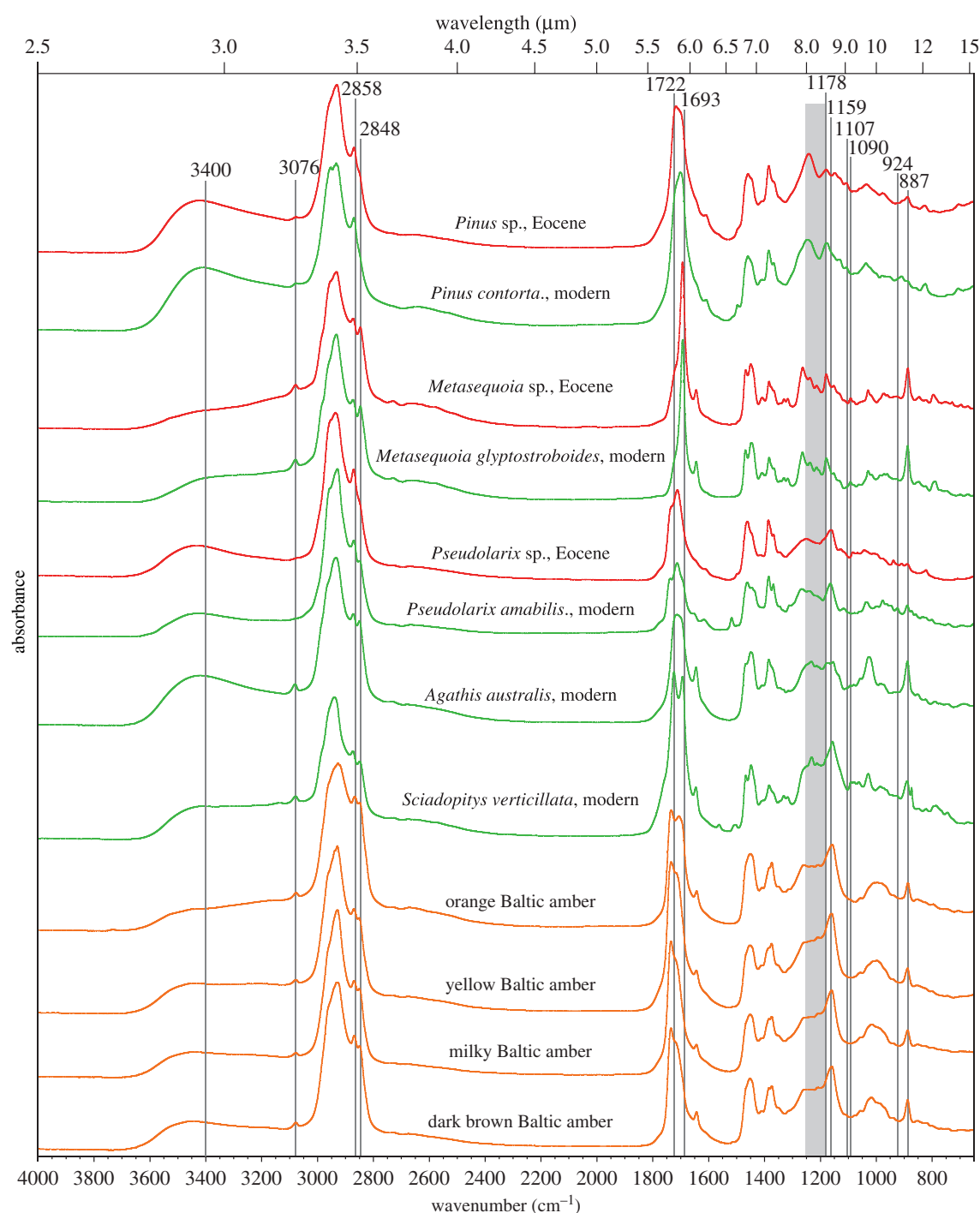


Figure 1. FTIR spectra of resins from selected modern and Eocene conifers and of Baltic amber. Vertical lines indicate spectral bands used to define the ratios subjected to cluster analysis (table 1). Shaded area is the Baltic shoulder, which is fully expressed in Baltic amber and *S. verticillata* and partially in *Pseudolarix*. Red, fossil resin; green, modern resin; orange, Baltic amber.

(d) Molecular characterization

Molecular profiling of Baltic amber and selected modern conifer exudates was undertaken using gas chromatography–mass spectrometry (GC–MS), in an attempt to evaluate the chemotaxonomic results obtained from FTIR microspectroscopy. Baltic amber was compared with modern resin and needle extracts from *Pinus ponderosa* and *S. verticillata*, given the possibility of linking explicitly two biomarkers: succinic acid from Baltic amber (Gough & Mills 1972) and verticillol from *S. verticillata* (Otto & Wilde 2001). A published diethyl ether/methanol extraction procedure was initially used (Mills *et al.* 1984) and subsequently refined by the addition of

basic and acid methanolytic extraction steps. In the case of Baltic amber, extraction was followed by treatment with diazomethane to yield dimethyl succinate. This facilitates GC–MS analysis because dimethyl succinate is more volatile than the parent diacid. Methanolysis reactions of plant resin and needle extracts under basic and acidic conditions were performed to release any succinic acid potentially present as diterpenoid esters in the plant material, as is known for *P. ponderosa* (Gough & Mills 1972). The basic methanolysis reactions were followed by diazomethane treatment to yield dimethyl succinate. The acidic methanolysis reaction yields dimethyl succinate directly.

Table 1. Summary of the FTIR spectroscopic ratios used in hierarchical cluster analysis.

wavenumber ratio	determinant functionalities and interpretation	Baltic amber (<i>n</i> = 5)	<i>S. verticillata</i> (<i>n</i> = 3)	Pinaceae (<i>n</i> = 41)	Araucariaceae (<i>n</i> = 9)	Cupressaceae <i>sensu stricto</i>	
						Cupressoid (<i>n</i> = 20)	Taxodioid (<i>n</i> = 6)
3400 cm ⁻¹ /3076 cm ⁻¹	OH stretching/asymmetric CH stretching of terminal alkene	0.94 ± 0.10	0.73 ± 0.06	0.93 ± 0.37	0.78 ± 0.21	0.81 ± 0.28	0.93 ± 0.21
2858 cm ⁻¹ /2848 cm ⁻¹	CH methyl/CH methylene	0.99 ± 0.01	0.93 ± 0.03	1.11 ± 0.05	0.96 ± 0.05	0.94 ± 0.04	0.98 ± 0.05
1722 cm ⁻¹ /1693 cm ⁻¹	C=O carboxylic esters/C=O carboxylic acids	1.32 ± 0.17	0.74 ± 0.24	0.71 ± 0.38	0.67 ± 0.27	0.51 ± 0.22	0.68 ± 0.24
1159 cm ⁻¹ /1178 cm ⁻¹	Baltic shoulder: patterns of C–O stretching in aliphatic esters	1.11 ± 0.01	1.30 ± 0.12	0.93 ± 0.10	0.88 ± 0.11	0.78 ± 0.13	0.87 ± 0.11
1107 cm ⁻¹ /1090 cm ⁻¹	β-unsaturated and aromatic esters/C–O stretching secondary alcohols	1.02 ± 0.02	1.04 ± 0.02	1.18 ± 0.16	0.91 ± 0.10	0.91 ± 0.10	0.95 ± 0.09
924 cm ⁻¹ /887 cm ⁻¹	C–C stretching/out-of-plane terminal methylene	0.79 ± 0.03	0.55 ± 0.20	0.97 ± 0.09	0.66 ± 0.20	0.66 ± 0.20	0.72 ± 0.15

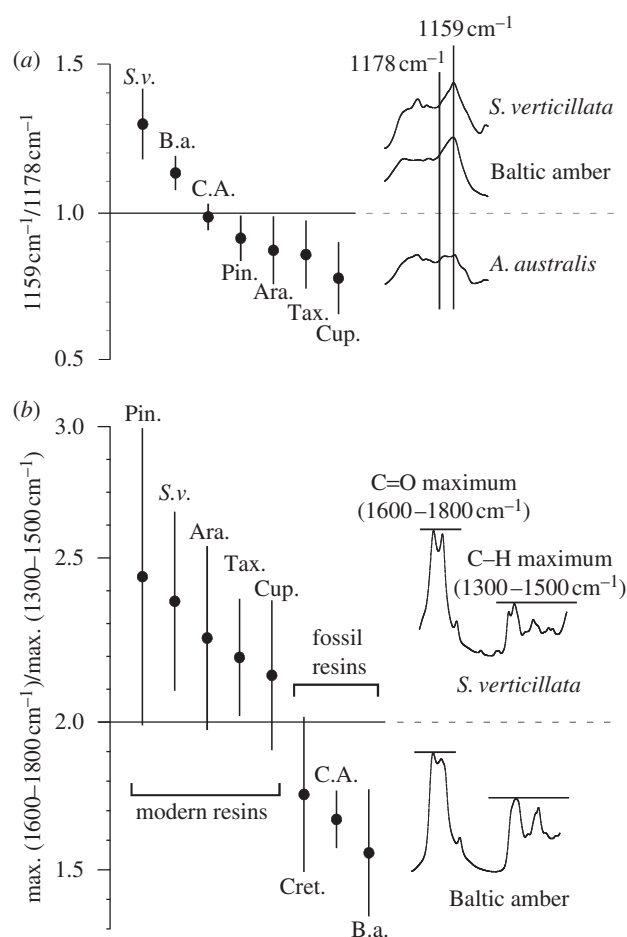


Figure 2. Examples of wavenumber ratios calculated from the FTIR spectral database. (a) The 1159 cm⁻¹/1178 cm⁻¹ ratio reflects the amplitude of the Baltic shoulder and is positive only for modern *S. verticillata* (*S.v.*) resin and Baltic amber (B.a.). This ratio is not overprinted strongly by diagenesis. (b) The ratio of maximum C=O absorbance to that of secondary C–H, in contrast, is controlled by oxidation history and retains no chemotaxonomic significance. Additional abbreviations are as follows: C.A., Eocene Canadian Arctic (all Giraffe and Buchanan Lake samples); Pin., Pinaceae; Ara., Araucariaceae; Tax., taxodioid Cupressaceae; Cup., cupressoid Cupressaceae; Cret., Canadian Cretaceous ambers from McKellar *et al.* (2008).

(e) *Palaeobotany*

Chemotaxonomic inferences were augmented by a range of palaeobotanical observations from Baltic amber, as well as detailed considerations of modern *S. verticillata*. A small wood sample (300 mg) was obtained beneath cambium and bark appressed to the surface of a large block of Baltic amber housed in Copenhagen (Ravhuset, Nyhavn), part of the private collection of Søren Fehrn. Because the grain of the wood is parallel to flow lines in the amber, they are believed to have originated from the same tree, making this specimen exceptionally valuable. The untreated wood was mounted to Al stubs for scanning electron microscopy (SEM) using Ag paint, then sputter-coated with Au prior to examination with a JEOL-6301F field emission instrument. Thin sections of Baltic amber were ground and examined for pollen in light microscopy (LM). Modern wood and pollen of *S. verticillata* were also examined using LM and SEM.

3. RESULTS AND DISCUSSION

(a) *Microspectroscopy of modern and fossil resins*

For each of *Pinus*, *Pseudolarix* and *Metasequoia*, FTIR spectra from paired modern–Eocene resins are visually indistinguishable (figure 1), despite age differences of 40 million years (Myr) or more. Although conversion of some biomolecules to their diagenetic geomolecular counterparts has undoubtedly occurred in the fossil resins (Otto & Wilde 2001), the range and frequency of functional groups that modulate FTIR spectra are highly conserved for all three genera on this timescale. While there is an over-arching similarity between the FTIR spectra of all conifer resins (figure 1), taxonomically diagnostic differences are also evident between these genera, based on features present in both modern and fossil samples. For example, *Pseudolarix* lacks the olefinic C–H absorption band at 3076 cm^{-1} present in other taxa, whereas *Metasequoia* is distinguished by a remarkably steep and narrow carbonyl peak centred at 1700 cm^{-1} . Members of Pinaceae (*Pinus* and *Pseudolarix*) lack the well-defined doublet observed in other spectra at $2850\text{--}2900\text{ cm}^{-1}$, implying distinct arrangements of methyl and methylene groups in resins from this family. Over the full FTIR spectrum (i.e. 869 data points per sample), correlation coefficients (r^2) between modern and Eocene *Pinus*, *Pseudolarix* and *Metasequoia* are 0.99, 0.90 and 0.89, respectively. Such high degrees of congeneric similarity imply minimal evolutionary change in resin composition since the Middle Eocene. Furthermore, FTIR spectra obtained from modern conifer resins sampled across broad ecological gradients in western Canada reveal that variation among species is far greater than that within species, confirming that resin composition is ultimately determined by taxonomic identity and not environmental factors (fig. S1 in the electronic supplementary material).

These observations support the contention that a modern resin FTIR spectrum matching that of the Baltic amber will reveal a botanical affinity, although to date such a match has proven elusive. The FTIR spectrum of Baltic amber itself has been considered exhaustively (Langenheim 1969; Larsson 1978), exhibiting a characteristic feature referred to as the Baltic shoulder ($1175\text{--}1250\text{ cm}^{-1}$) flanked by an absorbance peak at 1160 cm^{-1} (figure 2). Given the remarkable consistency of this spectroscopic feature across the geographical range of Baltic amber (eastern UK, Denmark, southern Sweden, Germany, Poland, Russia, Ukraine and the Baltic republics), it appears that extensive forests of closely related amber-producing conifers were the source, and it is possible that a single species was responsible. Indeed, our analyses (figure 1) reveal that the Baltic shoulder is consistently expressed in Baltic amber specimens of different colour, bubble content and detrital content, as originally observed by Beck *et al.* (1964, 1965). However, the Baltic shoulder has not been reported previously from any extant conifer resin. Of the modern representatives of resin-producing conifers, only *S. verticillata* (Japanese umbrella pine, Koyamaki) consistently produces FTIR spectra closely matching those of Baltic amber (figure 1). Pairwise correlations between Baltic amber and *S. verticillata* FTIR spectra range from 0.77 to 0.99 (mean: 0.88, $n = 15$ pairs), which are

comparable to the correlations obtained between paired Eocene–modern *Pinus*, *Pseudolarix* and *Metasequoia* resins.

These elements of similarity are corroborated by the cluster analysis results (figure 3). Whether spectroscopic ratios or bands from the fingerprint region are used to generate the dissimilarity matrix, the resulting dendrograms produce essentially the same result. Two major clusters are defined, one containing Baltic amber and *S. verticillata* and the other containing Pinaceae, Araucariaceae and the two subgroups of Cupressaceae. Within the latter cluster, Pinaceae is the most dissimilar, whereas resins from Araucariaceae, cupressoid and taxodioid are very similar to each other (figure 3). The degree of dissimilarity between *Sciadopitys* and all other extant conifer groups is not surprising, given the relict status of this genus conferred from morphology (Peirce 1935; Farjon 2005), biogeography (Page 1990), palaeobotany (Ohsawa 1994) and molecular phylogeny (Brunsfeld *et al.* 1994). From considerations of both the raw FTIR spectra (figure 1) and their subsequent multivariate treatment (figure 3), the hypothesis emerges that Baltic amber was derived from Palaeogene sciadopityaceous conifers.

(b) *Palaeobiology of sciadopityaceous conifers*

Although *S. verticillata* is the sole surviving representative of the family Sciadopityaceae and native only to Japan, the fossil record establishes that these conifers were more taxonomically diverse and geographically widespread in the past (Florin 1922; Farjon 2005). Sciadopityaceous palynomorphs (e.g. *Sciadopityspollenites serratus*) are sufficiently common in Palaeocene to early Eocene sediments from Baffin and Ellesmere islands, west Greenland, Iceland and Spitsbergen to imply the common presence of these trees in high northern latitude forests (Manum 1962). In younger Palaeogene sediments from central Europe, *Sciadopitys*-type pollen grains occur in mass accumulations reaching 60 per cent of assemblages (Kruttsch 1971; Gothan & Weyland 1973), and indicating that the geographical range of these trees extended well into the subtropical mid-latitudes. Such abundances imply that these conifers were at times dominant elements within forest communities, and therefore contributing organic matter to the Baltic catchment of the Tethyan Seaway. Forests dominated by *Sciadopitys* occurred as localized enclaves well into the Miocene, as evidenced by the fossil record of Rhineland brown coal (Mosbrugger *et al.* 1994). Pollen data suggest that *Sciadopitys* persisted in Europe as vestigial populations until the Pliocene (van der Hammen *et al.* 1971). *Sciadopitys* and several other conifer genera (including *Metasequoia* and *Pseudolarix*) were relegated successively to Asia during the Miocene and Pliocene, eventually defining their modern distributions. These taxa exemplify the progressive extirpation of subtropical forest taxa from northern Europe, which availed new ecological space for Neogene expansions of boreal forest.

Sciadopitys has been recognized in Baltic amber since the nineteenth century (Goeppert & Menge 1883), primarily as distinctive needles that comprise two longitudinally fused lobes with furrows on both abaxial and adaxial surfaces (figure 4). Sciadopityaceous pollen grains are also present in Baltic amber. However, a direct linkage to the amber itself has not previously been proposed, owing to longstanding preconceptions that source trees must

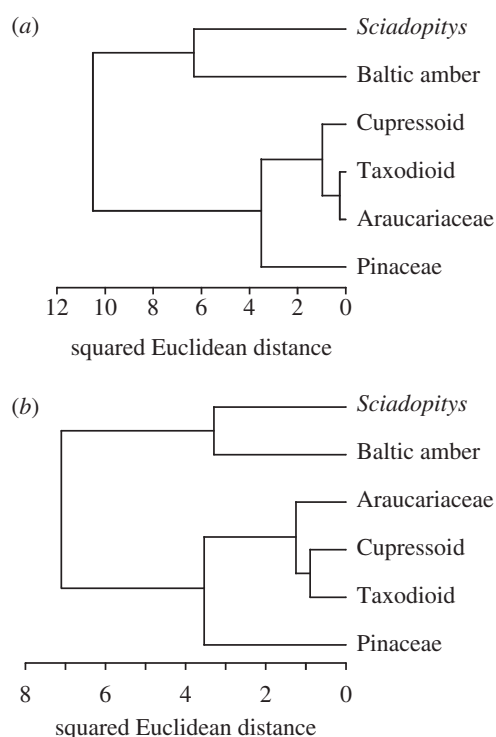


Figure 3. Hierarchical cluster analysis based on averaged FTIR spectra for five groupings of modern conifer resins and Baltic amber. (a) Dendrogram based on the six FTIR spectral ratios described in table 1. (b) Dendrogram based on the 169 bands that define the fingerprint region between 650 and 1300 cm⁻¹.

belong to either Pinaceae or Araucariaceae. Although Langenheim (2003) mentioned that resin production by *Sciadopitys* is unknown, it is evident that this tree has evolved the capacity to produce copiously, typically yielding a cloudy, water-insoluble labdanoid exudate that oxidizes rapidly upon exposure (figure 4). Examination of wood preserved in association with successive resin flows (*Schlaube*) in Baltic amber reveals a simple wood anatomy that is entirely consistent with that of *Sciadopitys* (Peirce 1935; Farjon 2005). The cupressoid–taxodioid bark of the Ravhuset specimen is furthermore consistent with these elements of similarity and contrasts with the bark of most pinaceous and araucarian conifers.

In light of our spectroscopic and palaeobotanical observations, we revisit arguments (Langenheim 2003) favouring alternately Araucariaceae or Pinaceae as the putative source trees for Baltic amber. Araucarians have been advanced by simple analogy to extant taxa that are copious resin producers, principally *Agathis australis* (kauri). Furthermore, the suite of monoterpenes present in *Agathis* resin reflects a certain degree of geochemical affinity with Baltic amber (Mills *et al.* 1984). However, the pollen and macrofossil record suggest that Araucariaceae were already restricted to the southern hemisphere by the Eocene, while *Agathis* lacks any northern hemisphere fossil record whatsoever (Stockey 1982, 1994). Accordingly, Araucariaceae is not represented in the Baltic amber flora. Biogeographically, Pinaceae are therefore a much more parsimonious candidate, given widespread occurrence within northern European Eocene forests (Utescher & Mosbrugger 2007), and good representation in Baltic amber. Anatomical

investigations of wood inclusions in Baltic amber have supported a pinaceous origin (Schubert 1961), although this diagnosis is not unequivocal (figure 4). Few extant pines appear capable of producing the quantities of resin demanded by the recognized volume of Baltic amber, and, accordingly, pines have not been conclusively linked to any other major amber deposit globally (Poinar 1992; Grimaldi 1996). Poor preservation potential of pinaceous resins is consistent with higher proportions of volatile mono- and sesquiterpenoids relative to other conifer families (McGarvey & Croteau 1995) and a lack of structural features conducive to the polymerization of resin into amber (Langenheim 2003). Finally, it is worth noting that no modern araucarian or pinaceous resin is known that produces the diagnostic Baltic shoulder in FTIR spectra. For example, the FTIR spectrum of modern *A. australis* lacks the associated peak at 1160 cm⁻¹ (figure 2), suggesting that any similarities between *Agathis* resin and Baltic amber are compromised by pronounced skeletal differences, independent of apparent biogeographic constraints. Other than *S. verticillata*, *Pseudolarix amabilis* is the only modern conifer in our extensive survey that expresses the 1160 cm⁻¹ peak in association with the Baltic shoulder, albeit as a subdued and somewhat distorted expression (figure 1). *Pseudolarix* has been evaluated critically as a potential source for Baltic amber, but rejected because the labdanes in its resin have enantiomeric and not regular configurations as in Baltic amber (Anderson & LePage 1995). Thus, similarities between Baltic amber and modern resins from both Araucariaceae and Pinaceae are insufficient to establish a conclusive association or even to advance either family as a more likely candidate for involvement in amber production.

(c) Resin composition and diagenetic history

Baltic amber is a (co)polymer of labdanoid diterpenes, principally communic acid and communol, that furthermore incorporates succinic acid (C₄H₆O₄; HOOC-CH₂-CH₂-COOH), a non-terpenoid dicarboxylic acid (Mills *et al.* 1984; Anderson 1995). Indeed, elevated concentrations of total succinates in Baltic amber (1–8% by mass) are a defining chemical characteristic, and the reason it is commonly referred to as succinite. However, free crystalline succinic acid comprises only 0.005 to 0.04 per cent of typical Baltic amber by mass (i.e. 50–400 ppm; Tonidandel *et al.* 2009). This implies that it is the range of more common succinates in Baltic amber that act as cross-linking agents within the polymer framework. Some of these compounds (e.g. dimonoterpanyl succinate) are not known from exudates of living conifers, so it remains uncertain whether the high succinate content of Baltic amber reflects source plant metabolism or diagenetic alteration (Yamamoto *et al.* 2006). No extant conifer produces succinic acid and related compounds so copiously as is found in Baltic amber (Otto & Wilde 2001; Otto *et al.* 2007).

Our new methanolytic GC–MS results (table 2) revealed the presence of succinic acid and esterified derivatives in Baltic amber and needle extracts of *P. ponderosa*, but its absence in modern resins from either *P. ponderosa* or *S. verticillata*. Verticillol, an alcohol of the cembrane-type macrocyclic diterpenoid verticillane

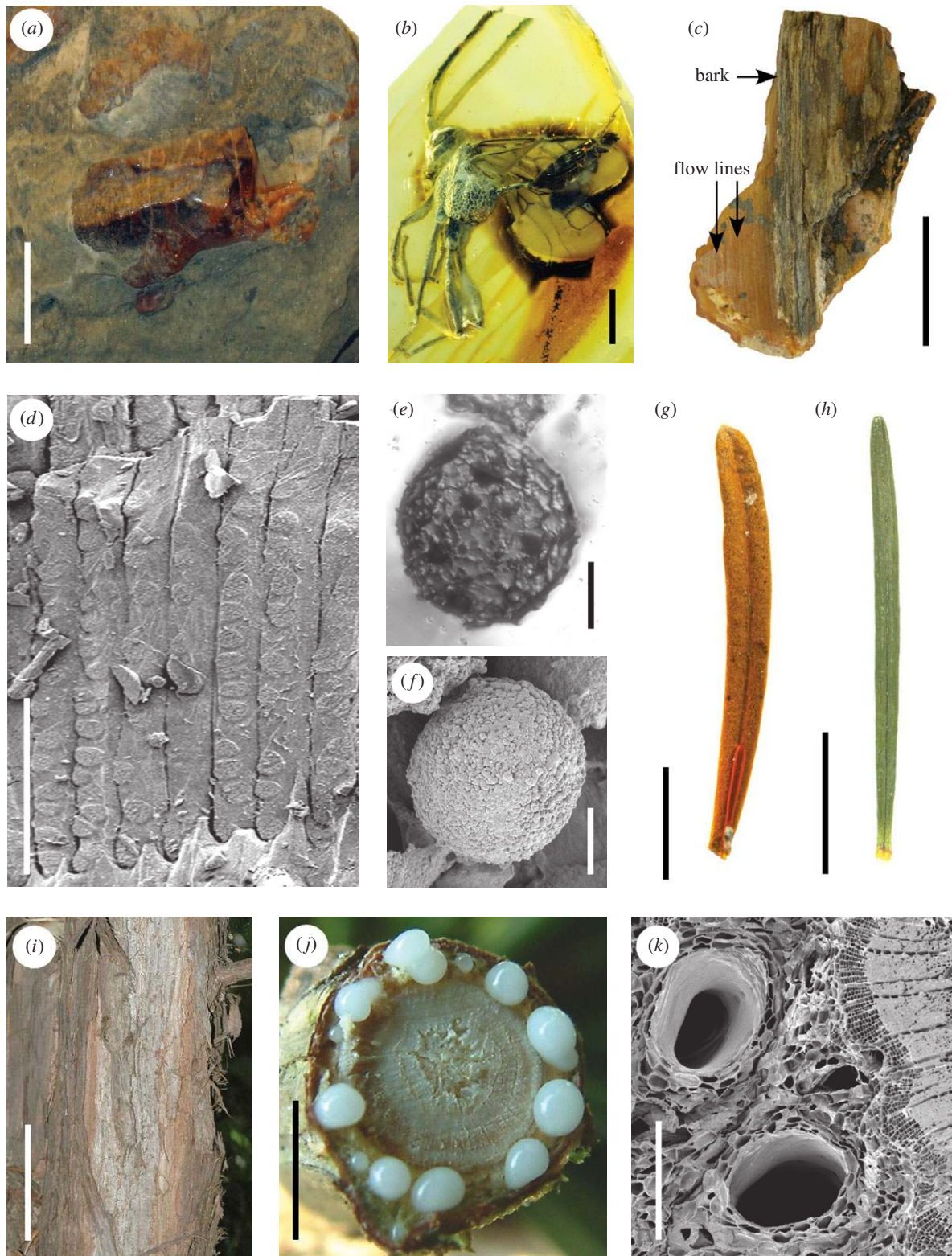


Figure 4. Baltic amber and *S. verticillata*. (a) Fragments of Baltic amber in Eocene glauconitic sediments from Kaliningrad Oblast, Russia. (b) *Parevania producta*, an evaniid (or ensign) wasp preserved in Polish Baltic amber, demonstrating the quality of arthropod preservation witnessed in the deposit. (c) Large Baltic amber block with bark and wood occluded to the specimen by successive resin flows (*Schlaube*). The bark possesses a cupressoid–taxodioid morphology similar to modern *S. verticillata* (i), but distinct from either *Pinus* or *Agathis*. (d) Wood anatomy from the specimen illustrated in (c) is simple, lacking parenchyma and intercellular canals. Radial walls of xylem tracheids have uniseriate ovoid to circular bordered pits. Vertical tracheids frequently terminate at perpendicular ray parenchymal cells arranged radially in vertical rows of one to eight cells. In each of these aspects, wood anatomy compares favourably with modern *Sciadopitys*, but bears no resemblance to *Agathis*, for which bordered pits are multiseriate. (e) *Sciadopityspollenites* palynomorph from Swedish Baltic amber, compared with modern *S. verticillata* pollen (f) which is comparable in terms of both size and exine morphology. (g) Conifer needle from Baltic amber showing morphological similarity to modern *S. verticillata* (h). (j) Resin bled from a severed twig of *S. verticillata*. (k) Dense resin canals in *S. verticillata* phloem are well developed for defensive resinosis. Scale bars, (a) 2 cm, (b) 1 mm, (c, i) 10 cm, (d) 250 μm , (e, f) 10 μm , (g, h) 1 cm, (j) 0.5 cm and (k) 100 μm .

previously considered specific to *S. verticillata* (Otto & Wilde 2001), was detected in resin from *P. ponderosa* and needle extracts of *S. verticillata*, yet was absent from all other fractions, including Baltic amber and *S. verticillata* resin. These equivocal results indicate that, if sciadopityaceous conifers were indeed the source of Baltic amber as our FTIR and palaeobotanical analyses imply, then diagenetic reactions must be invoked for molecular transformations including succinylation and volatile loss of compounds such as verticillol. There is compelling evidence that this is exactly the case.

Liquid inclusions in Baltic amber contain succinate and acetate anions, while terpenoids are conspicuously absent (Buchberger *et al.* 1997). This indicates a decoupling between succinate production and plant natural product chemistry. Furthermore, air bubbles in Baltic amber have elevated CO₂ concentrations that are directly attributable to respiration (Berner & Landis 1988). Succinate, acetate and CO₂ are the characteristic by-products of a wide range of bacterial and fungal fermentation processes, which are relatively unselective with regard to the specific carbohydrate used as substrate (van der Werf *et al.* 1997; McKinlay *et al.* 2005). We therefore consider the possibility that succinic acid in Baltic amber is crystallized from succinates originally produced by the fermentation of associated materials in the sedimentary environment, including, but not limited to, plant cellulose and resin polysaccharides. For example, in modern environments, sediment anoxia coupled with enhanced organic matter supply (as pulp-mill effluent) has been linked directly to elevated sediment succinate concentrations (Miller *et al.* 1979). The depositional history of Baltic amber involved fluvial transport from source forests to nearshore marine environments. This was followed by long-term burial in fine-grained marine sediments where the iron silicate glauconite formed authigenically, a defining characteristic of the *Blau Erde* (blue earth) in which Baltic amber is principally located (Grimaldi 1996; Weitschat & Wichard 2002). Because glauconite formation involves the redox cycle of iron with the participation of sedimentary organic matter as a reducing agent (Ireland *et al.* 1983), its very presence ensures that Baltic amber was entombed in anoxic conditions during early diagenesis.

Following strategies taken in coal geology (Goodarzi & McFarlane 1991), we developed an index for the oxidation history of modern resins, Baltic amber and other fossil resins using FTIR spectroscopic data. Peak values of C=O absorbance were normalized to those of adjacent C–H bands (figure 2b). This approach reveals that Baltic amber and fossil resins are consistently less oxidized than their modern resin counterparts, which is consistent with our hypothesis that succinic acid in Baltic amber is largely a diagenetic product, and therefore of no chemotaxonomic significance. These inferences are supported by the scattered reports of succinic acid in botanical fossils other than Baltic amber. Succinic acid has been detected in wood of the extinct conifer *Frenelopsis* (Cheirolepidiaceae) from the Cretaceous of France (Nguyen Tu *et al.* 2000), as well as in Eocene resinites of *Taxodium* (swamp cypress) from Germany (Otto & Simoneit 2001) and *Pseudolarix* from Axel Heiberg Island (Anderson & LePage 1995). In each of these cases, associated depositional environments appear to have

Table 2. Summary of GC–MS results after extraction and methanolysis experiments. Abbreviations are as follows: A, extraction and methylation of Baltic amber; B, general procedure for extraction of plant resins; C, general procedure for extraction of plant needles; D, basic methanolysis; E, acidic methanolysis. Dashes indicate the absence of either compound in the corresponding sample.

sample	experimental	GC-MS result
Baltic amber	A	dimethyl succinate
<i>S. verticillata</i> resin	B	—
	B, D	—
	B, E	—
<i>P. ponderosa</i> resin	B	—
	B, D	—
	B, E	verticillol
<i>S. verticillata</i> needles	C	verticillol
	C, E	verticillol
<i>P. ponderosa</i> needles	C	—
	C, E	dimethyl succinate

been conducive to succinate production by fermentation of organic quantities such as lignin degradation products, with the attendant participation of microbial communities in active biotransformations. Strikingly, neither *Frenelopsis* wood specimens lacking morphological evidence of fungal infestation nor *Taxodium* from younger Oligocene (Germany) and Miocene (USA) sediments contains succinic acid or related compounds (Nguyen Tu *et al.* 2000; Otto *et al.* 2002).

(d) *Palaeoclimatic context of Baltic amber formation*

The most precise radiometric ages for marine strata containing Baltic amber are 44.1 ± 1.1 and 47.0 ± 1.5 Ma, based on ⁴⁰Ar/⁴⁰K analyses of glauconite (Ritzkowski 1997). As these represent minimum ages for amber-producing forests, initial formation of Baltic amber followed closely the early Eocene climate optimum and accompanied subsequent cooling during the Middle Eocene. Global deep-sea temperatures declined monotonically by approximately 0.7°C Myr^{−1} in the period 50–44 Ma (Zachos *et al.* 2001). Although most Baltic amber lacks stratigraphic context, the record of fossil inclusions suggests that amber production persisted during this protracted interval of secular global cooling. This is because plant and insect assemblages from Baltic amber include taxa with both subtropical and boreal ecological affinities (Goepfert & Menge 1883; Larsson 1978; Poinar 1992; Kobbert 2005).

The envisaged sciadopityaceous amber forest is likely to have been of the subtropical broadleaf evergreen variety (Utescher & Mosbrugger 2007) in association with oak, which is represented in Baltic amber by stellate trichomes, the most common botanical fossil (Weitschat & Wichard 2002). Because resin production persisted as boreal plants and insects replaced warm stenotherms displaced by cold, the conifers responsible for Baltic amber required sufficient thermal tolerances to endure secular cooling. Accordingly, modern *S. verticillata* thrives under a broad range of mean annual temperatures: 7.4–16.6°C (Utescher & Mosbrugger 2006). Eocene palaeotemperatures across northern Europe probably remained in this

range for millions of years, allowing sciadopityaceous conifers to prosper despite considerable local ecological turnover. This is an important consideration because overall tree health is necessary to maintain elevated resin production (McGarvey & Croteau, 1995), irrespective of the inducing vector (e.g. insect infestations, forest pathogens, physical injury). A sudden demise of the amber-producing forest is inconsistent with either the physiology of resin production or the ecological diversity encapsulated by Baltic amber, and, furthermore, is unlikely to account for the sheer volume of Baltic amber preserved in the geological record.

4. CONCLUSIONS

FTIR microspectroscopy, coupled with Palaeogene forest biogeography and climate history, is consistent with the hypothesis that Baltic amber derived from conifers of the family Sciadopityaceae. Although this hypothesis is supported by similarities between *S. verticillata* and a range of Baltic amber botanical fossils (figure 4), it requires that the ubiquity of succinic acid and related compounds in Baltic amber be associated with post-depositional processes and not the metabolism of source trees. This new interpretation contrasts sharply with views favouring either an araucarian or a pinaceous origin for this outstanding palaeontological resource and offers new directions for future research. For example, it remains possible that the unique immunological (Price & Lowenstein 1989) and allelopathic (Yates et al. 2006) properties of *S. verticillata* contributed directly to the exceptional preservation of Baltic amber fossils. These unexplored factors may equally pertain to broader questions concerning the very survival of such remarkable volumes of conifer resin in the geological record.

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