

**DETERMINATION OF THE MOLECULAR SIGNATURE OF FOSSIL CONIFERS
BY EXPERIMENTAL PALAEOCHEMOTAXONOMY : CONTRIBUTION TO
PALAEOFLORESTIC AND PALAEOCLIMATIC RECONSTRUCTIONS**

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Terrestrial vascular plants, and more especially conifers, synthesize a great variety of terpenoids which are major components of their essential oils and resins. The composition of these bioterpenoids differs significantly from a taxon to another and it is thus possible to distinguish the different conifer families by their molecular signature (Otto and Wilde, 2001). These bioterpenoids are then transported from palaeophytocenoses to sedimentary basins in which they are partially preserved within sediments. During diagenesis, bioterpenoids are transformed into geoterpenoids which can keep, partially or totally, their initial chemotaxonomic value. Therefore, plant biomarkers are excellent palaeofloristic, and thus palaeoclimatic, proxies (Otto and Simoneit, 2001; Hautevelle et al. 2006a).

Classically, palaeobotany and palynology are the most improved approaches for palaeofloristic and palaeoclimatic reconstructions but present some disadvantages. Indeed, recognizable plant macrofossils are very scarce in the sedimentary record and often concentrate within layers characterized by exceptional preservation. Spores and pollens, as for them, are common but not easily reliable to plant taxa, especially for pre-Cenozoic times. Conversely, plant biomarkers are very usual in the sedimentary record and can be directly linked to plant taxa. This makes botanical palaeochemotaxonomy a complementary, valuable and innovative approach for palaeofloristic and palaeoclimatic reconstructions. However, for now, available palaeochemotaxonomic data associating the biomarkers to their source plants are not sufficient to interpret biomarker assemblages in terms of palaeofloristic composition systematically. Currently, palaeochemotaxonomic data are derived from the extrapolation of chemotaxonomic data (Otto and Wilde, 2001) and the analysis of well identified fossils (Otto and Simoneit, 2001).

In order to fill this gap, we developed recently an original approach in order to gain access to new paleochemotaxonomic data based on artificial maturation of extant plants using confined pyrolysis (Hautevelle et al., 2006b). This method allows to determine the molecular signature of the fossil counterpart of each pyrolysed plant.

In this study, we investigated the palaeochemotaxonomy of the Coniferales order (i.e. conifers) which are present in almost all types of vegetation and are major components of Meso- and Cenozoic floras. We pyrolysed nearly 70 conifer species which represent very well the diversity of the coniferales order. We studied 11 Araucariaceae representatives (8 *Araucaria*, 2 *Agathis*, 1 *Wollemia*), 17 Cupressaceae (1 *Calocedrus*, 4 *Chamaecyparis*, 2 *Cupressus*, 5 *Juniperus*, 1 *Microbiota*, 3 *Thuja*, 1 *Thujopsis*), 21 Pinaceae (4 *Abies*, 3 *Cedrus*, 4 *Larix*, 4 *Picea*, 4 *Pinus*, 1 *Pseudotsuga*, 1 *Tsuga*), 4 Podocarpaceae (*Podocarpus*), the unique Sciadopitaceae species, 5 Taxaceae (2 *Cephalotaxus*, 2 *Taxus*, 1 *Torreya*) and 8 Taxodiaceae (1 *Cryptomeria*, 2 *Cunninghamia*, 1 *Metasequoia*, 1 *Sequoia*, 1 *Sequoiadendron*, 2 *Taxodium*).

The data obtained highlights the molecular specificities and terpenoid association of each families as well as intra-family differences and inter-family resemblances. Our palaeochemotaxonomical data confirms that the different families can be distinguished from their respective molecular signatures. For instance, Araucariaceae are characterized by very high amount of tetracyclic diterpanes compared to tricyclic diterpanes and by the occurrence of not well identified bicyclic diterpanes (other than labdanes which are found in all conifer species). However, the nature of the tetracyclic diterpanes varies from a species to another (beyerane, phyllocladanes, *ent*-kauranes). Cupressaceae and Taxodiaceae show a very high diversity of molecular signatures which allows the distinction of many of their genus by means of their terpenoids compositions. Pinaceae are characterized by the abundance of hydrocarbon and acid abietanes coupled with the lack of tetracyclic diterpanes.

These data allow to fill the gap in our knowledge of botanical palaeochemotaxonomy and will improve the use of organic geochemistry as a competitive approach to palaeobotany and palynology in the field of palaeofloristic and palaeoclimatic reconstruction.

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PLANT-WAX LIPIDS AS (PALAEO-) CLIMATE RECORDERS: HOW FRESH ARE THEIR SIGNALS?

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Plant-wax lipids and their isotope signatures are favoured for (palaeo-) climate reconstructions due to their relative stability against degradation. Their refractory nature, however, potentially leads to extensive storage in intermediate reservoirs, such as soils, which might cause a significant delay between incorporation of environmental signals and ultimate deposition in sedimentary archives.

In order to resolve fresh and pre-aged fractions within distinct lipid biomarkers and shed light on their potential to carry contemporary climate signals, extensive compound-specific radiocarbon dating was undertaken on a riverbed sample from the Congo River estuary, central Africa. Organic matter of this surface sediment sample, which was collected in 1976, should have recorded elevated radiocarbon contents resulting from the atmospheric ¹⁴C “bomb spike” due to above-ground nuclear weapons testing in prior decades, if it contains recently photosynthetically fixed carbon. Among the investigated fractions are total organic carbon (TOC) and series of *n*-alkanes, *n*-alkanoic acids, and *n*-alkanols. Stable carbon isotope compositions of TOC and long-chain *n*-alkyl lipids indicate predominant C₃ plant sources with comparable hydrogen isotope compositions reflecting a consistent continental hydrological signal. High carbon-preference indices of the *n*-alkyl lipids suggest relative freshness of the plant-wax lipids, whereas a high BIT index also indicates significant soil-derived carbon. The presence of degraded vascular plant-derived organic matter is also suggested by lignin degradation parameters. Compound-specific radiocarbon concentrations of *n*-alkyl lipids range from about +460 permil, higher than the atmospheric value at the time of sampling, to around -220 permil, equivalent to about 2000 years age. Within *n*-alkyl lipid classes distinct patterns of radiocarbon contents with chain-length and relative abundance emerge, indicating common sources and selective degradation processes. Mass-balance calculations allow distinguishing relative fresh vs. pre-aged fractions within single lipids. The significance of these results will be discussed in the context of production and preservation mechanisms and with regard to the applicability of plant lipids for (palaeo-) climate reconstructions.

C₄₀ BIS-DITERPENOIDS, NOVEL CHEMOTAXONOMIC BIOMARKERS OF PODOCARPACEAE IN SEDIMENTS?

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In the course of a detailed palaeoenvironmental study based on biomarker distributions in sediments from the Lower Oligocene (Rupelian, Lower Oligocene) of the Rhine rift valley, an outcrop sample rich in higher plant fossil remains was collected in a quarry located near the city of Burnhaupt-le-Haut (South of Alsace, France). In agreement with the macrofossil assemblage, most of the biomarkers were related to terrigenous inputs, as illustrated by the gas chromatogram of the aromatic hydrocarbons shown in Fig. 1a. Indeed, the aromatic hydrocarbons are strongly dominated by diterpenoid- and triterpenoid-related biomarkers attesting of contributions from gymnosperm and angiosperm precursors. However, a rather complex series of unknown high molecular weight compounds eluted at the end of the aromatic hydrocarbon fraction was also present (filled and empty circles in Fig. 1a), showing molecular ions at M^+ 570 and 552. Given the rather poor information obtained from their mass spectra (Figs. 1b), three homologues (**1-3**; Fig. 1a) were isolated by liquid chromatography (LC, HPLC) in order to characterize their structures by NMR. The first isolated compound (**1**) with a molecular mass of 570 Da. was shown to have a dimeric structure made of two phenolic totarol sub-units linked by an ether bond, whereas the two other compounds with a molecular weight of 552 Da. (**2-3**) correspond to dimers made of totarol (**2**) and mixed totarol-sempervirol sub-units (**3**) bearing a central dibenzofuran moiety. Although C₄₀ bis-diterpenoids are not reported yet to occur in the geological record, there are few reports on the occurrence of bis-diterpenoids made of totarol sub-units linked by a carbon-carbon bond in the Plant kingdom. Such is the case of podototarol, a diphenolic diterpenoid which has been shown to be present among the heartwood of various species of Podocarpaceae and which is thought to be formed by the enzymatic oxidative coupling between the two phenolic diterpenoid sub-units. Hence, it can be envisaged that the various bis-diterpenoids present in the sediment sample investigated represent diagenetic transformation products of bis-diterpenoids from Podocarpaceae. However, since “mixed” bis-diterpenoids such as **3** and bis-diterpenoids with a structure related to **1** have never been

characterized among biolipids, an alternative hypothesis regarding the origin and mode of formation of the sedimentary bis-diterpenoids has to be considered. We propose that these novel biomarkers may be formed by purely diagenetic processes, with a first step involving an oxidative coupling between the phenolic sub-units (i.e., totarol and semperviol) following a free radical mechanism and leading to the formation of an ether bond as in the case for **1**, or a carbon-carbon bond (**2-3**), followed by a second, acido-catalysed cyclization forming the central dibenzofuran moiety as in the case for compounds **2** and **3**.

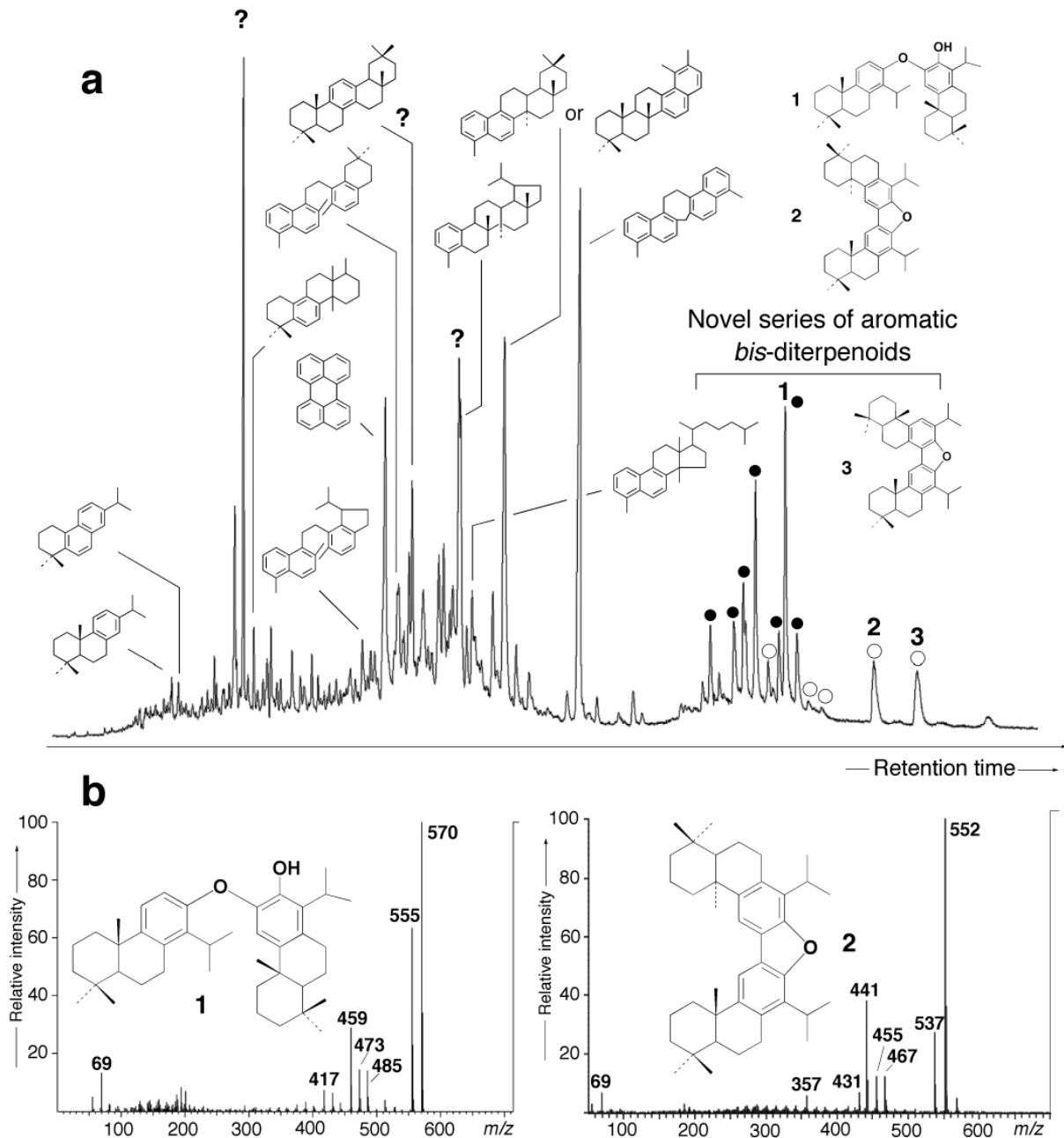


Figure 1: (a) Gas chromatogram (RIC) of the aromatic hydrocarbon fraction from a sample rich in plant fossils collected in an outcrop of the Meletta bed formation (Lower Oligocene) located near Burnhaupt-le-Haut (France). Filled circles: compounds with a M_w of 570 Da.; Empty circles: compounds with a M_w of 552 Da.; (b) Mass spectra of compounds **1-2**.

TERPENES FROM BOGWOOD OF CRIPTOMERIA JAPONICA D. DON

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The essential oils from a bogwood stump of sugi (*Cryptomeria japonica* D. Don) from Fukui, Japan, (F-bogwood) preserved for c. 3,000-3,500 years in sediment of a muddy field were analyzed using gas chromatography-mass spectrometry (GC/MS).

Twenty-six sesquiterpenes and 6 diterpenes were identified. The main components were *cis*-calamenene (23.1%), cadalene (18.1%), δ -cadinene (7.9%) and simonellite (4.5%). These results were compared with previous results for oils from a stump of sugi fresh wood and two bogwood samples from Yamaguchi (Y-bogwood, muddy field, c. 3,000 years) (Narita *et al.*, 2006a) and Shimane (S-bogwood, volcanic ash, c. 3,500-3,800 years) (Narita and Yatagai, 2006b).

The yields of the oils from 100 g oven-dried wood powder were 1.6 ml for F-bogwood, 2.3 ml for Y-bogwood, 0.19 g for S-bogwood and 1.6 ml for fresh wood; the sesquiterpene contents were 86.8%, 97.0%, 42.4% and 98.5%, respectively. Thus the essential oil in F-bogwood is preserved in a fairly high level. Sesquiterpene alcohols, abundant in fresh wood, seems to have been dehydrated to hydrocarbons in the early stage of diagenesis; therefore the alcohol/ketone fraction was lost in the bogwoods in the order of S- > F- > Y-. This order agrees with that of maturity (Narita and Yatagai, 2006b).

Sesquiterpene hydrocarbons can be classified by *m/z* value (Fig. 1). Fresh wood is rich in *m/z* 204 compounds. The *m/z* 202 and 198 compounds, *i.e.* the dehydrogenation products from *m/z* 204, are abundant in F-bogwood, whereas *m/z* 208 compounds, hydrogenation products from *m/z* 204, are scarce. Thus, dehydrogenation seems to have been dominant in F-bogwood; however, it also contains small amount of *m/z* 206 (partially hydrogenated). On the other hand, the *m/z* 202 component of F-bogwood contains small amount of tetrahydrocadalene, which is found in no other samples. It is known that the early degradation stage of cadinane-type sesquiterpenes is dehydrogenation to form calamenenes *m/z* 202, which are different from tetrahydrocadalene in dehydrogenation position. Based on these results we propose a new possible diagenetic process of cadinane-type hydrocarbons in F-bogwood as follows: Firstly, both hydrogenation and dehydrogenation take place simultaneously; secondly, the *m/z* 208 species are dehydrogenated to cadalene (*m/z* 198) via compounds *m/z* 202. In the case of F-bogwood, this dehydrogenation may proceed via

multiple pathways at the m/z 202 stage containing tetrahydrocadalene, which has not been found in other bogwoods and fresh wood.

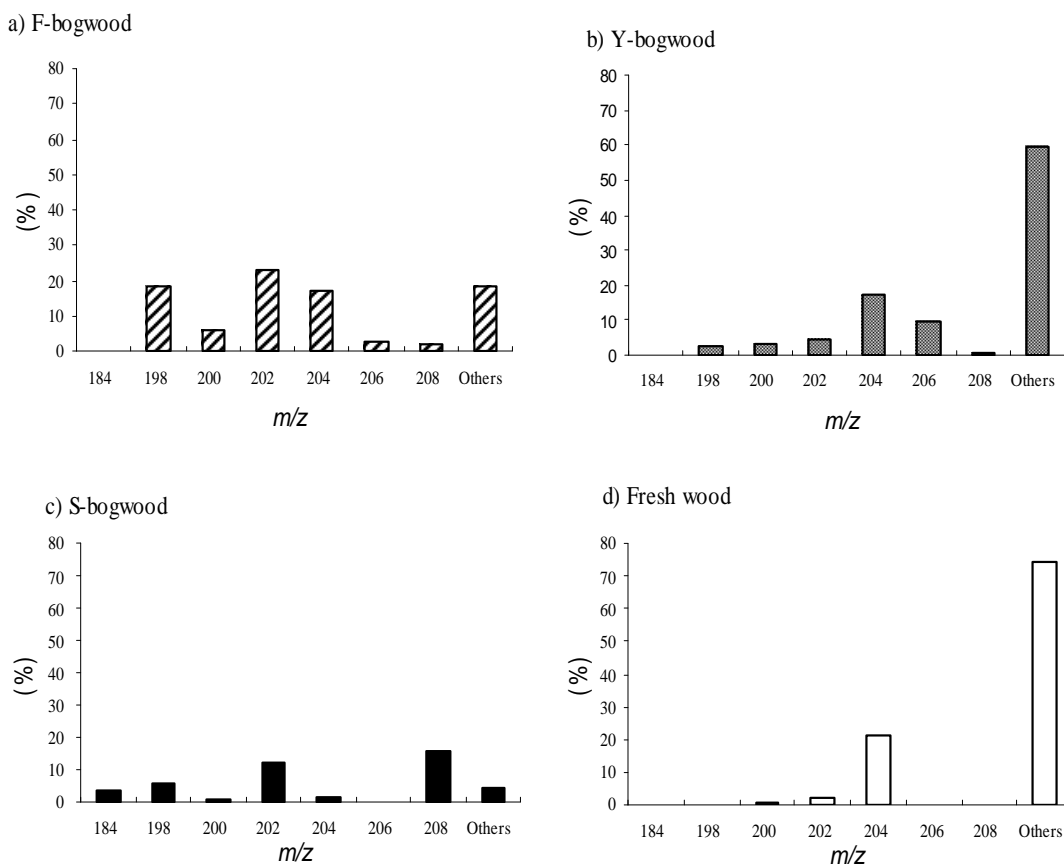


Figure 1. Compositions of sesquiterpenes in essential oils from *Cryptomeria japonica* D. Don: a) F-bogwood, b) Y-bogwood, c) S-bogwood and d) Fresh wood. "Others" represents alcohols and ketones.

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BIOMARKERS FROM LATEX DRYING PLANTS IN SEDIMENTS OF THE SIAK RIVER, E SUMATRA, INDONESIA

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In Pekanbaru, the capital of Riau Province, E Sumatra, Indonesia, several latex drying facilities discharge drying liquor into the Siak River. Effluents from rubber and latex processing factories include wash water, small amounts of uncoagulated latex and also contain small quantities of dissolved organic compounds such as protein, carbohydrates and lipids. Since large amounts of acid are used in processing, the effluent is usually acidic and contains high proportions of suspended solids and nitrogen. This discharge hence represents a source of oxygen-consuming and eutrophication compounds to the river system.

Bark from *Hevea brasiliensis*, the tree used for natural latex production in Sumatra, showed a characteristic composition of terpenoid alcohols and ketones including friedelin (friedelan-3-one) and two as yet unidentified tetraterpene alcohols (TTA) with m/z 500 and 510 for their molecular ion (TMS). Particulate matter, sampled directly at the wastewater discharge point of a latex processing factory, showed the same compounds with a comparable relative composition to be present. In the Siak River sediment sample, taken approximately 100 m downstream from the discharge point, the TTA with m/z 500 was missing while the compound with m/z 510 had its molecular mass increased by two units suggesting a loss of a double bond by oxidation.

The uncharacterised TTA's could not be found in any other of 40 plant sources from the Siak bank vegetation and hence appear to be unique to *Hevea brasiliensis* thus providing a biomarker for inputs from latex processing. This approach provides together with the results of the joint projects [1], comprehensive information for political and administrative bodies to assess the environmental situation in this area.

REFERENCE

[1] <http://www.zmt-bremen.de>

MOLECULAR CHARACTERIZATION OF FRESH AND ARCHAEOLOGICAL DIPTEROCARPACEAE TRITERPENIC RESINS

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Vegetal triterpenic resins, available throughout the world, have been used since ancient times, due to their particular properties, for a wide range of applications: painting, varnishing, sealing, waterproofing and caulking (Lampert, 2003). Therefore, significant trade networks were widely established and then, such resins were often found in organic remains on archaeological items.

The “Junk of Brunei” shipwreck (XVth – XVIth centuries), excavated in 1997, constitutes one of the most significant archaeological discovery in the China Sea: it enclosed particularly a great number of storage jars, still full of resin-like substances, which may be of *Dipterocarpaceae* origin (Dammar resins), according to palynological data. This discovery remains until today the only known evidence of the importance of resin trade routes in the China Sea, during the Hongzhi Period (1488-1506).

A molecular and isotopic study of a diversified sampling of archaeological and ethnological Dammar resins (cargo of the Brunei shipwreck, lumps unearthed in Thai excavations, ship-hull caulking mixture coating a Vietnamese Thuyen Thung bamboo boat), based on chromatographic techniques and mass spectrometry (GC-MS-MS, LC-MS and GC-irm-MS), was carried out. This investigation was completed by the study of fresh *Dipterocarpaceae* resins from different genus, in order to determine a diagnostic molecular fingerprint, even in very altered samples, which allow to differentiate Dammars from other triterpenic resins, like Mastics (*Anacardiaceae*). Indeed the later which originate from the Mediterranean Basin, have also been used for the same purposes (Scalarone *et al.*, 2003). The determination of the Dammar specific sesquiterpenic and mostly triterpenic distributions, characterised by compounds from the oleanane, ursane and dammarane series, enables this discrimination. After the comparison of the Brunei resins with fresh Dammars from the genus *Shorea* and *Dipterocarpus*, we were able to conclude that the archaeological aged resins belong to the *Dipterocarpus* genus: indeed they exhibit the characteristic composition of *Dipterocarpus* and enclose some compounds which were never found in *Shorea* resins. A resin from the same genus was also identified in the caulking agent of the Thung boat.

Natural ageing and human handling transform resin composition significantly: some triterpenic compounds disappear (dammaradienone), whereas altered species and new compounds are likely to be generated due to oxidation, polymerisation, biodegradation... Hence, the characterization of a resin has, in certain cases, to be done on the basis of degradation byproducts only.

In that respect, a set of compounds has been detected in the aromatic hydrocarbons fraction of the Brunei resins which are not present in fresh resins, but result from the progressive aromatization under microbial influence (Fig. 1) of pentacyclic triterpenic precursors bearing an oxygen functionality (Lohmann *et al.*, 1990). Precise molecular identification of other compounds, only detected in archaeological samples from Brunei shipwreck, is in progress.

Oxidation appears to be a very important process in ageing of resins: the relative abundance of varying oxidised species (20,24-epoxy-25-hydroxy-dammaran-3-one, Fig. 1) could enable to determine if a resin is well preserved or was highly oxidized during ageing.

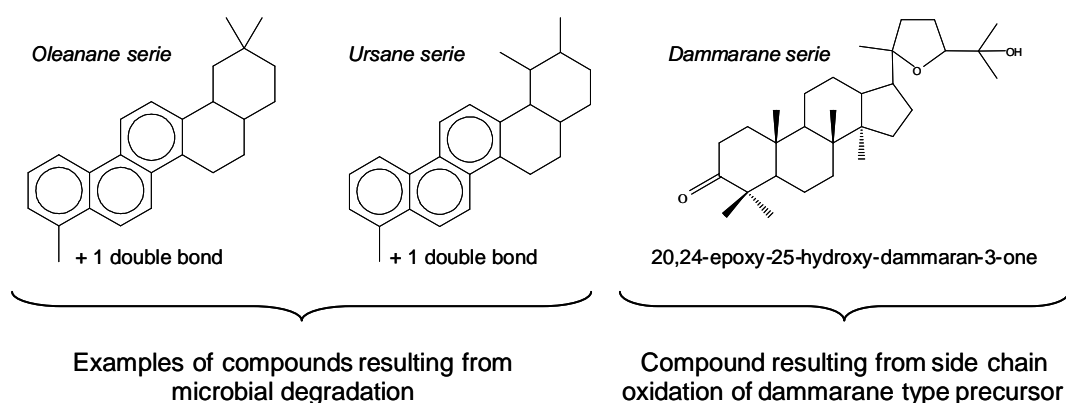


Figure 1. Triterpenoids occurring in aged Dammar resins.

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CHEMOTAXONOMIC STUDY ON REFRACTORY MACROMOLECULES IN FOSSILS OF PLANT ACHENES AND CONES FROM THE NEOGENE TOKAI GROUP OF CENTRAL JAPAN

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Recently, higher plant-derived biomarkers such as terpenoids have suggested being applicable as molecular palaeontological indicators such as chemotaxonomic and biogeochemical markers (*e. g.* van Aarssen et al., 2000). Alternatively, (bio)macromolecule constituting plant 'organic body' is refractory and well preserved even in ancient fossil, and therefore, can be also powerful tool for reconstructing plant chemotaxonomy and paleo-vegetation. However, there few studies for chemotaxonomy in fossil plant macromolecule. We have analyzed higher plant biomarkers such as triterpenoids and diterpenoids bound in macromolecule of plant macrofossils collected from sediments of Neogene Tokai Group (Tokiguchi Porcelain Clay Formation and Toki Sand and Gravel Formation), which were deposited in fluvial system and contained excellent-preserved plant macrofossils, in Tounou area of central Japan (Arai et al., 2003). In this study, we focus on alkyl compounds obtained as hydrolytic products from refractory (geo)macromolecules in the Neogene plant macrofossils from Tokai Group in order to examine their potentials as chemotaxonomic markers.

A pinecone fossil of *Pinus fujiii* from Miocene Tokiguchi Porcelain Clay Formation (ca. 11Ma), a pinecone fossil of *Pinus trifolia* from Toki Sand and Gravel Formation KS-2 (ca. 10-9Ma), and an achene fossil of *Liquidambar formosana* and an endocarp fossil of *Nyssa* sp. from Toki Sand and Gravel Formation NN06 (ca. 4Ma) were used. Also, extant plant achenes of *L. formosana* and *Liquidambar styraciflua*, and pinecones of *Pinus ridida* were used. Plant samples were treated by hydrolysis (saponification) with KOH/MeOH. The hydrolytic products were fractionated by silicagel column chromatography, and analyzed by gas chromatography (GC) and gas chromatography / mass spectrometer (GC/MS).

Lignin phenol, n-alkanol, α , ω -diol, fatty acid, α , ω -dicarboxylic acid, and ω -hydroxylic acid were mainly detected as hydrolytic (saponified) products from the plant macrofossils. Fatty acids characterized by higher even carbon number predominance (CPI>4.3), showing low maturity level. Detection of lignin phenol by saponification indicated that structure of lignin polymers, which were commonly polymerized with ether

bond, altered to that constructing with ester bond in these macrofossils. Syringic compounds, angiospermous lignin phenol, were detected in *L. formosana* and *Nyssa* sp. hydrolyzate. Vanilic compounds generally detected in hydrolyzates of both angiosperm and gymnosperm were identified in all the samples. In addition, oleanoidal triterpenoids (angiospermous biomarker) and pimarane- and abietane-type diterpenoids (coniferous biomarker) have been identified in *L. formosana*, as well as *P. trifolia* and *P. fujiii*, respectively (Arai et al., 2003). Hence, it was confirmed that the lignin phenols and the terpenoid biomarkers in fossil plant can be useful as chemotaxonomic markers at the broad level of taxa.

Compared fossil with extant plant samples, carbon number distributions of alkyl compounds such as carboxylic and hydroxyl acids as well as diol in hydrolyzates were quite different between these samples. This fact indicates that the structure of alkyl chains within macromolecules significantly altered by cleavage accompanied with oxidation and/or defunctionalization during diagenesis. Nevertheless, it was found that carbon number distributions of only n-alkanols in hydrolyzates were almost similar between fossil and extant plant samples. The n-alkanol is known to be one of the monomer composed of suberin polymer in periderm tissue. Thus, the n-alkanol moiety bound in suberin polymer with ester bond is presumably well preserved in plant fossil. Furthermore, distribution patterns of n-alkanols in hydrolyzates (maximal peak of C₂₄ homologue) of fossil and extant *L. formosana* as well as extant *L. styraciflua* were similar to that of Fagaceae in (sub)class Hamamelidae, which includes liquidamber (Holloway, 1983). Also, maximal peak of n-C₂₀ alkanol in hydrolyzates was observed in fossil *Nyssa* sp., which agreed with the results of Aceraceae, (sub)class Rosidae (including *Nyssa*) (Holloway, 1983). From these results, it is suggested that the carbon number distribution of n-alkanols constituting suberin polymer within plant fossil can be applicable and reliable as chemotaxonomic marker at the level of (sub)class, although examination is necessary.

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**MOLECULAR ISOTOPIC COMPOSITION AND DISTRIBUTION PATTERNS OF
LONG-CHAIN *N*-ALKANES AND *N*-ALKANOLS FROM C₃ PLANTS GROWING IN
DIFFERENT TROPICAL HABITATS**

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Contents, distribution patterns and molecular stable isotope compositions of long-chain *n*-alkanes and *n*-alkan-1-ols have been used as indicators for the contribution of terrestrial organic matter to dusts, soils and sediments (e.g. Schefuß et al., 2003; Rommerskirchen et al., 2003, 2006a). These parameters vary according to the proportions of land plants with different metabolic pathways (C₃ versus C₄). Most grasses of the arid grasslands are C₄ plants, and thus a higher contribution of C₄ plants is related to a larger proportion of organic material from arid environments. Tropical vegetation may change from arid grasslands over shrubby to woody savannas and finally to rain forests. Trees, shrubs, lianas and most herbs of these habitats are C₃ species. There are many suggestions regarding the lipid composition of these plants but no study has evaluated all the necessary parameters. The present study closes this gap and provides significant background information relevant to the use of long-chain aliphatic biomarkers in studies of palaeovegetation changes.

We analysed leaf waxes of 50 savanna plants (28 trees, 11 shrubs, 11 herbs) and 22 rain forest species (8 trees, 8 shrubs, 6 lianas) collected in several African countries. Averaged *n*-alkane distribution patterns for trees, shrubs and herbs of the savanna show significant similarities. The same is evident for the patterns of trees, shrubs and lianas of the rain forest. Hence it is possible to average the distribution patterns of all species collected in the same habitat. A comparison of the averaged *n*-alkane distribution patterns from C₃ rain forest and C₃ savanna species with the distribution pattern of C₄ grasses (Rommerskirchen et al., 2006b) shows a shift to longer chain length of the *n*-alkanes. This can be numerically displayed, e.g., by an increase of the mean average carbon chain length of the odd carbon numbered *n*-C₂₇ to *n*-C₃₃ alkanes (ACL₂₇₋₃₃, Fig. 1). The same shift to longer chain lengths is also evident in the *n*-alkanol distribution patterns. The molecular stable isotope compositions of C₄ and C₃ plants differ due to their metabolic pathways. Among the C₃ plants variations originate from different isotopic compositions of the source CO₂ caused by recycling effects in the closed canopy of the rain forest. The molecular $\delta^{13}\text{C}$ values decrease from C₄ grasses over C₃ savanna vegetation to C₃ rain forest plants.

The results of the present study verify distribution patterns and molecular stable isotope composition of long-chain *n*-alkanes and *n*-alkan-1-ols to be suitable indicators to assess the changing contribution of land plants with different metabolic pathways. Also for biomass input from plants of the same metabolic type information can be obtained about the different climates the plant material came from. Thus, these parameters are useful indicators for palaeoenvironmental climate studies.

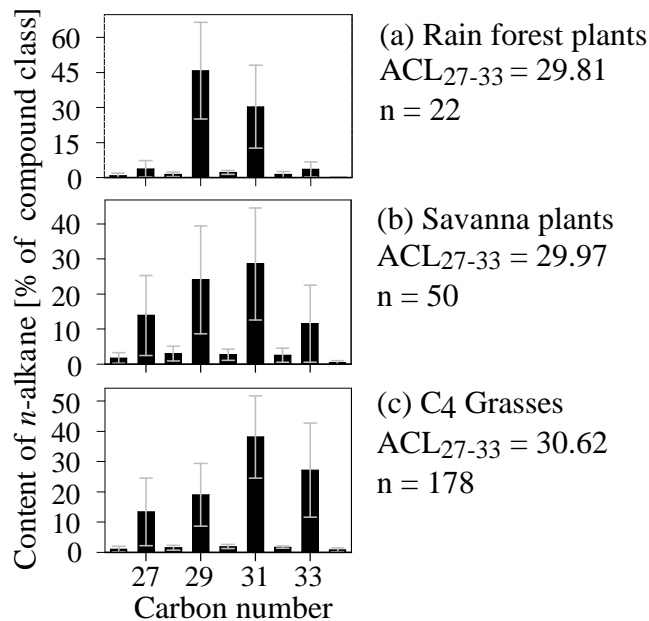


Figure 1. Averaged histogram representation for (a) rain forest plants, (b) savanna plants and (c) C₄ grasses of contents of *n*-C₂₆ to *n*-C₃₄ alkanes. C₄ grass data are from Rommerskirchen et al. (2006b). The diagrams are individually normalised to the most abundant homologue. ACL: mean average carbon chain length of the odd-carbon-numbered *n*-C₂₇ to *n*-C₃₃ alkanes. n: number of species used for the averaging.

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PEAT-FORMING PLANT SPECIFIC BIOMARKERS AS INDICATORS OF PALAEOENVIRONMENTAL CHANGES IN *SPHAGNUM*-DOMINATED PEATLANDS

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The high preservation of organic material in peatlands that results from low pH, anoxia and rapid burial make the peat archives particularly useful for reconstructing natural or anthropogenic environmental changes. Nevertheless, up to now biochemical composition of peat OM has rarely been used as indicator for past environmental conditions, particularly in ombrotrophic peatlands. Within RECIPE, a European Union Framework 5 initiative, we aimed to identify combinations of site physico-chemical conditions, vegetation composition and below-ground microbiological characteristics that are beneficial to the long-term regeneration of cut-over peatland biodiversity and restoration of the carbon sink function (Chapman *et al.*, 2003).

To infer plant inputs and identify biopolymer degradation in response to these anthropogenic changes, bulk and molecular indicators of modern peat-forming plants were determined and compared to those recovered from ca. 50 cm peat deposited in La Chau d'Abel peatland (Jura Mountains, Switzerland). Particular emphasis was devoted to carbohydrate and lipid analysis given (i) their important role in the constitution and metabolism of the peat-forming plants and (ii) the slight degradation they undergo in the peat, particularly carbohydrates. In fact, contrary to commonly perceived ideas which stipulate a highly biodegradability, we recently demonstrated that sugar compounds are well preserved in peat allowing their use both as indicators of humification and as tracers of plant sources (Comont *et al.*, 2006).

13 species of living plants: 10 mosses (9 *Sphagnum* species & *Polytrichum strictum*) and 3 sedges (*Eriophorum vaginatum*, *E. angustifolium* and *Carex rostrata*), as well as the peat core sections have been examined for C/N, light microscopy and distributions of their monosaccharide and lipid biomarkers. Among *Sphagnum* mosses, characteristic hummock-forming species (*Sphagnum fuscum*, *S. magenalicum*, *S. acufolium*) and hollow species (*Sphagnum cuspidatum*, *S. phallax*) were collected to test whether they differ in composition or not. Fatty acids were determined from plant derived neutral, glyco and phospholipids and

are designated as NLFA, GLFA and PLFA, respectively. Neutral sugars were recovered after two hydrolyses releasing the total and the hemicellulosic sugars, respectively. The cellulosic sugars were determined by difference.

Neutral fatty acids (NLFA) showed the highest concentration in the investigated plants followed by PLFA and GLFA. The median fatty acid concentration was $0.16 \mu\text{mol g}^{-1}$ plant dry matter. In all three lipid fractions saturated fatty acids (SATFA) were the most dominating (60.2-99.0% of total lipids), whereas mono- and polyunsaturated (MUFA and PUFA) occurred at similar percentages at far lower levels. Lipid concentrations (absolute value) did not vary significantly between the different plant genera in contrast, to the percentages (relative values) of SATFA, MUFA, PUFA.

Overall, no significant differences in total sugar contents were detected between the *Sphagnum* mosses (305 to 400 mg.g^{-1}) and the sedges (289 to 440 mg.g^{-1}). However, in contrast to the *Sphagnum* mosses, the sedges are much richer in hemicelluloses than in cellulose (ca. 223 vs. 157 mg.g^{-1}) as previously reported for Cyperaceae plants collected in a tropical peaty marsh (Bourdon *et al.*, 2000). Among the *Sphagnum* mosses that exhibit comparable TOC values (41.2 to 44.4 %), the typical hollow species, i.e. *S. cuspidatum*, are richer in total sugars (ca. 400 mg.g^{-1}) than the hummock ones, i.e. *S. fuscum* (ca. 300 mg.g^{-1}). The distribution of the hemicellulose monosaccharide content (wt%) shows contrasted signatures between the plants. The dominant hemicellulose monosaccharides are (i) galactose (13 to 19 wt%) and rhamnose (6 to 10 wt%) in the nine *Sphagnum* species, (ii) mannose (25 to 29 wt%) in *Polytrichum strictum*, and (iii) xylose (25 to 36 wt%) and arabinose (9 to 16 wt%) in the sedges. The richness of sedges in xylose and arabinose has already been reported in several studies, while to our knowledge only very few studies mentioned the occurrence of high proportions of galactose, rhamnose and mannose in the hemicellulose of the primary cell walls of mosses; these latter compounds being usually attributed to microbial syntheses.

These results show the source biomarker potential for sugars and fatty acids, which are currently under-utilised as proxies for plant inputs in peatland ecosystems. Another outcome of this study is the applicability of various PLFA biomarkers for microbial investigations in peatland ecology.

RECONSTRUCTING PALEOALTIMETRY WITH D/H MEASUREMENTS OF LIPID BIOMARKERS

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Paleoelevation is a crucial variable in tectonics and climate research. Observation-based estimates provide a real-world benchmark to compare with models of mountain formation and help constrain the complex interactions driving uplift, denudation and climate. A striking example of their value is provided by the Cenozoic history of the Himalaya. Different models of the Himalayan orogen result in markedly different predictions of area and elevation histories for the Tibetan plateau. For example, thickening and northward expansion of the Tibetan plateau as a simple function of India-Asia convergence predicts a time-transgressive history of uplift, with regions near the Himalayan front experiencing uplift earlier than regions further to the north. In contrast, models that invoke convective removal of the mantle lithosphere predict rapid uplift of large regions at discrete time intervals. Estimates of past elevations are one of the few means to test different models of the uplift history of the Tibetan plateau.

Rowley et al. (2001) established the theoretical basis for using the stable isotopes of oxygen and hydrogen in precipitation for paleoaltimetry. Subsequently, this technique and closely related approaches have been applied to carbonate-based $\delta^{18}\text{O}$ records from different localities. However, no studies have investigated the hydrogen isotope composition of precipitation for quantitative paleoaltimetry reconstructions, primarily because there are few substrates that preserve the D/H ratio of precipitation, surface or groundwater. Here we use the δD composition of *n*-alkanes from epicuticular plant waxes to reconstruct the δD of precipitation in Cenozoic basins that have been elevated as part of the Tibetan plateau. These precipitation δD ratios are converted to estimates of paleoelevation using the isotope-altitude relationship derived from a simple thermodynamic model that calculates the isotopic composition of water vapor and precipitation as an air parcel is lifted in the atmosphere (Rowley *et al.*, 2001).

Samples of Cenozoic lacustrine limestones from the Lunpola and Hoh-Xil basins on the Tibetan Plateau were analyzed for plant-wax δD paleoaltimetry. *n*-Alkane distributions exhibit a short-chain maximum with no odd/even preference (OEP) and a long chain

maximum (C₂₉ or C₃₁) with a strong OEP. The long- and short-chain maxima and OEP are typical of modern lake sediments that receive organic material from both terrestrial plants (C₂₉₋₃₃, high OEP) and aquatic sources (C₁₆₋₁₈, low OEP). Low thermal maturity of the samples is indicated by sterane and hopane maturation indices while preservation of the D/H signal is documented by the D/H offset between *n*-alkyl and isoprenoid biomarkers (Pedentchouk *et al.*, 2006).

Simultaneous molecular δD and carbonate $\delta^{18}\text{O}$ determinations (Rowley & Currie, 2006) of meteoric water composition from the Lunpola basin show excellent agreement despite their distinct source waters (precipitation vs. lake water), materials (calcite vs. organic molecules), modes of incorporation (mineral precipitation vs. biosynthesis) and diagenetic pathways. The similarity of the paleoaltimetry results from both the carbonate $\delta^{18}\text{O}$ and biomarker δD analyses provides strong support for the presence of an unaltered precipitation signal in both archives. We have also analyzed molecular D/H ratios on Miocene samples from the Hoh-Xil basin for which no other paleoaltimetry data are available. Our results for these samples indicate elevations >3.5 km, demonstrating the basin was near its present elevation by the late-Miocene. By comparison, Cyr *et al.* (2005) reconstructed late-Eocene elevations for the Hoh-Xil basin of < 2 km based upon the $\delta^{18}\text{O}$ of lacustrine limestones. This difference in elevation is consistent with uplift of the basin ~25 Ma, as predicted by scaling plateau width to India-Asia convergence (Rowley & Currie, 2006). Our results show that lipid biomarker D/H ratios can be used to reconstruct paleoelevation. In addition, our findings support a time-transgress model for plateau growth that scales the width of the plateau to the convergence of the Indian and Eurasian lithospheric plates.

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NOVEL PROXIES FOR CONTINENTAL PALAEO TEMPERATURE AND SOIL PH BASED ON TETRAETHER MEMBRANE LIPIDS OF SOIL BACTERIA

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Several proxies based on organic compounds exist of which the U^{K}_{37} and the TEX_{86} are now well established quantitative temperature proxies applied in the marine realm. For the terrestrial realm biomarker proxies exist based on for example $\delta^{13}C$ and δD values of plant wax *n*-alkanes, however, these are mainly qualitative. Here we introduce two new proxies based on terrestrial derived branched glycerol dialkyl glycerol tetraether (GDGT) membrane lipids to quantitatively estimate continental palaeo temperatures and palaeo soil pH.

Branched GDGTs, which are ubiquitous in soils and peat bogs (Weijers et al., 2006a), possess variable amounts of methyl groups and cyclopentyl moieties in their carbon chains and their distribution in soils is strongly related to soil pH and annual mean air temperature (MAT) (Weijers et al., 2006b). As these branched GDGTs are fluvially transported to the oceans and become part of the marine sedimentary archive, it might be possible to use these relations for reconstructing past continental temperatures and soil pH by analysing marine sediment cores in front of large river outflows. As an advantage over current terrestrial temperature proxies, this approach yields continuous long-term high resolution records of river basin integrated continental temperatures and soil pH. In this study, for the first time, we applied these potential proxies in several sediment cores. Application in a sediment core (GeoB 6518) in front of the Congo River outflow, spanning the last deglaciation, shows that the reconstructed annual MAT gradually increased by 4°C, from 21 to 25°C starting around 17 ka BP, in equatorial central Africa. The record of palaeo soil pH co-varies with humidity records, which is explained by stronger and weaker soil leaching processes with higher and lower precipitation intensity, respectively. Additionally, this approach also allows for direct comparison of palaeo land and seawater temperatures. The record of land-sea temperature differences over the last deglaciation for central Africa shows variability which co-varies with humidity records (Schefuß et al., 2005), indicating that this difference exerts strong control on central African hydrology.

Another application of this new temperature proxy involves a record for the Palaeocene-Eocene thermal maximum in the Arctic. The reconstructed annual MAT increased by ~8°C yielding subtropical temperatures of ca. 25°C for the Arctic continent during this time interval. These temperatures are comparable with Arctic Ocean sea surface temperatures

reconstructed previously with the TEX_{86} ' proxy (Sluijs et al., 2006) (Fig. 1). Moreover, we have detected branched GDGTs in all soils analysed so far and also in an Eemian palaeo soil, in an immature lignite of Late-Palaeocene age and in marine sedimentary records up to 95 Ma ago. This shows that these new biomarker based proxies can be applied in both marine and ancient terrestrial sequences up to the mid-Cretaceous, making these proxies an excellent and very promising tool for continental palaeoclimate research.

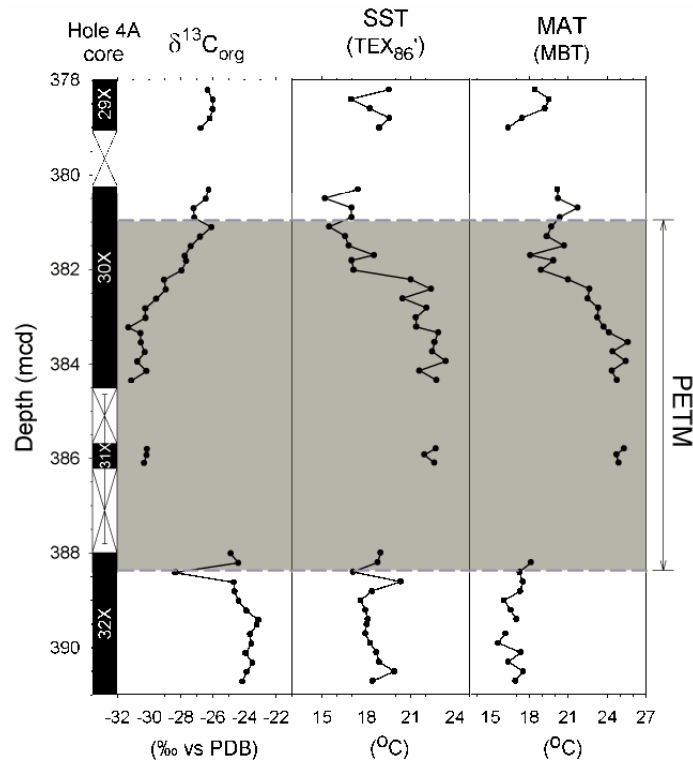


Figure 1. The record of terrestrial annual MAT for the Palaeocene-Eocene thermal maximum in the Arctic region compares well with the reconstructed Arctic Ocean SST

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