

Interactive comment on “Determination of the molecular signature of fossil conifers by experimental palaeochemotaxonomy – Part 1: The Araucariaceae family” by Y. Lu et al.

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We wish to thank the editor of Biogeoscience and the two reviewers for their appreciation to our work and their numerous relevant comments to this paper.

Mr. Sawada pointed out "Table 1; P10522, L3-L5: The authors should give a detailed explanation for plant parts (leaf or twig) of individual Araucariaceae samples using the experiments. The terpenoid biomarker trends are known to vary in parts of a single plant species. Also, for example, conifer cone fossil is thought to be easier for morphological identification of its fossil species and comparison with the other cone fossils. Do the authors have the terpenoid data for the cone of Araucariaceae?" In this study,

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mixtures of leaves and twigs were used. This kind of details was not given in Table 1, but in the text (P10522, lines 9-10). Moreover, table 1 was designed to give general information about each Araucariaceae representative, such as phylogenetic relationship, native geographic distribution as well as the provenance of the samples. The reasons we used leaves and twigs as plant materials in this study are listed below. - Leaves and twigs constitute the material that is mainly produced by conifer trees during their entire life; it is mostly these materials rather than cones, trunks, limbs or roots is exported from the tree communities to the sedimentary basins. - Plant biomarkers investigated in this paper derive from resin bioterpenoids. They constitute the major components of essential oils and resins. The resin circulates in all parts of the plant through vessels without significant change in its molecular composition, even if these different parts can present different molecular compositions (lignin, cellulose, cutine) from one to another. For instance, regarding the cones pointed by Mr. Sawada, they are probably richer in lignin than the leaves of the same tree but there is no reason that the molecular composition of the resin in the cone significantly differs from that contemporaneously present in the leaves. - Moreover, available chemotaxonomic data was mainly acquired from the analysis of leaves and twigs (Hegnauer, 1967; Langenheim, 1994; Mongrand, et al., 2001; Otto and Wilde, 2001, etc.). This is why we believe that acquisition of palaeochemotaxonomic data based on the pyrolysis of leaves and twigs is more logical and relevant than via the pyrolysis of other parts of the plants. - In addition, we do not have more information concerning a hypothetic different composition between the terpenoids present in the cone of Araucariaceae and those present in the leaves. Furthermore, according to Otto et al., (2007), fossil seed cones of some conifer species show similar molecular patterns to the resins of their extant counterparts. For all these reasons, we chose to investigate the leaves and twigs of Araucariaceae representatives. These details are added in the new version.

Mr. Sawada pointed out "Table 2: As mentioned above, the quantitative data for concentrations (or yields) of sesqui- and diterpenoids in Araucariaceae after the experimental simulation should be added. In addition, the class distributions of these com-

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pounds based on the concentration (or yield) data should be shown in a table and figure(s). It can be expected that the class distributions vary within genus *Agathis* and *Araucaria*, despite of similarity of kinds of compounds detected, and can be more valuable data for paleochemotaxonomy." As well as that was mentioned by the anonymous reviewer "h/. In Table 2, you have used crosses to show the relative concentrations of particular compounds. Maybe better will be presentation based on abundance of particular compound in relation to major peak = 100, as it was presented in: Marynowski et al., 2007. *Appl. Geochem.* 22, 2456-2485 and Marynowski et al., 2008. *N. Jb. Geol. Paläont. Abd.* 247, 177-189." Both reviewers criticized the use of a semi-quantitative and subjective way to represent the relative proportion of each class of compounds. The latter was presented in the 'Table 2' in the first version of paper by a set of crosses: from '++++' for major compounds to '+'/tr.' for minor or trace compounds. The reasons we did not use a more quantitative way to present our results are listed below. - For each species, their molecular signature of their fossil counterpart was obtained from combination of data acquired by two separate pyrolysis experiments: a first one with a reducing agent (LiAlH₄) to preferentially generate saturated terpanes and a second one without the reducing agent to obtain aromatic & polar terpenoids. Obviously, the two pyrolysates were analysed separately after fractionation, and it is very difficult, if not impossible to make quantitative comparisons between compounds found in these different fractions. - In nature, the molecular composition of fossil plants varies depending on numerous diagenetic parameters. This means that a same species of fossil plant can provide different molecular signatures according to the diagenetic parameters. For instance, if we consider abietanes, polar compounds like abietic acid and ferruginol represent their biological precursors. 1) In the case of a low-degree of diagenesis, biological precursors are less degraded and still functionalised compounds like dehydrabietic acid as well as some partially aromatised compounds will mainly represent abietanes. Inversely, in the case of a high-degree of diagenesis, polar compounds will be completely defunctionalised and abietanes will be mainly represented by more transformed hydrocarbons like retene. 2) Moreover, in the case of reducing conditions,

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saturated abietanes will be preferentially formed whereas in more oxidizing conditions, aromatic abietanes will predominate over saturates. This example illustrates very well the fact that, for each species, the precise proportion of each biomarker is not really relevant. However, it is important to note that, despite this diagenetic effect, each plant or group of plants is characterized by palaeochemotaxonomic trends, which allows to distinguish them from the others. We still believe that these palaeochemotaxonomic trends, due to their own diversity, are actually better represented by crosses than by the relative abundances of compounds found in the pyrolysates. As indicated by Hautevelle et al. (2006) and in this paper, the pyrolysis parameters were determined in order to generate the broadest range of molecular plant biomarkers that will theoretically be present in the fossil counterpart of extant plants, from the less to the more diagenetised. However, the obtained signature will not necessarily correspond exactly to those of fossils that found in sedimentary deposits due to the possible multiple diagenetic pathways. To answer to the comments of the two reviewers, two tables are added: 'Table 2' (with reducing agent) and 'Table 3' (without reducing agent) in the new version of paper. And the Table 2 in the first version became Table 4. Related modifications are done in the text as well.

Mr. Sawada also pointed out "P10529, L1-L2: If possible, data for polar functionalized terpenoids such as phyllocladanol and kauran-16-ol detected in extant fresh plant samples should be shown. There were few detailed descriptions of polar bioterpenoid in living plant, precursor of geoterpenoid, in biomarker geochemistry. I think that the pathways of diagenetic alterations from tetra-cyclic bioditerpenoids to phyllocladane-type geoterpenoids are still unclear, although those from abietanoic acid to abietane-type geoterpenoids are relatively well known." Compositions of biomolecules are largely detailed in numerous papers concerning chemotaxonomy of *Araucariaceae*, like in Brophy et al., (2000), Otto et Wilde, (2001), Staniek et al., (2010), and numerous paper of Caputo et al., (1974, 1976) as well as Caputo and Mangoni, (1974). As be pointed out by Mr. Sawada, diagenetic pathway of tetracyclic diterpenoids including beyerane, phyllocladanes as well as kauranes, are still unclear. Our results show that tetracyclic

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bioterpenoids progressively lost the oxygenated functions and double bonds. Due to the presence of quaternary carbons in their carbon skeletons, it is possible that the A-ring may be aromatized, and it is not the case for the other rings (B, C, D). We checked the presence of monoaromatic tetracyclic diterpenes in pyrolysates, and no compound presents a mass spectrum that could be attributed to such chemical structure. This is why we believe that aromatisation of tetracyclic diterpenoids is unlikely. Furthermore, it is possible that degradation of tetracyclic compounds leads to formation of some tricyclic compounds that could be mistaken with, for example, diagenetic products of abietane- and pimarane-type compounds (Ellis et al., 1996).

Moreover, Mr. Sawada proposed to delete the sentence P10514, L7-L9: 'Such knowledge is . . . and environmental studies.' In the abstract. We believe that this sentence is important in the abstract since it point out that, even if botanical palaeochemotaxonomy mainly aims to provide data for palaeobotanical investigations, it can be alternatively used for other purposes (environment, archaeology,...). We prefer to keep this sentence in the abstract.

To answer the other remarks of two reviewers, we accepted the following propositions and corrections were directly done in the text. From the anonymous reviewer: a/. Page 105016, Line 9. Change "diagenesis" to "diagenetic". b/. In my opinion, Fig 2 should be separated into two Figures (Fig 2 & 3), showing separately enlarged aliphatic and aromatic fraction. This important figure should be as large and informative as possible. The same according to Fig. 5 and Fig. 8. We agree with the reviewer about the layout of figures 2, 3 and 8. In order to give a better quality, illustrations about the distributions of sesqui- and diterpenoids in each genus should be separated. This has been improved in the new version. c/. Page 105018, Line 18 to 21. Add references to confirm this information. Additional references were added. "Its maximum worldwide distribution was achieved in both hemispheres during the Cretaceous (Krassilov, 1978; Kunzmann, 2007b)." d/. Page 105018, Line 8. Change "K-T boundary" to "K-Pg boundary". According to new decisions Tertiary should be changed to Paleogene. e/.

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Page 105023, Line 7. Add more information about silylation procedure (time, temperature ect.) More information about the silylation method was added "... Polar fractions were silylated using N,O-bis-(trimethylsilyl)trifluoroacetamide (BSTFA) at 60°C during 15 min before analysis." f/. Page 105028, Line 19. Change "a same" to "the same" g/. Spectrum from Fig. 6c is for sure not methylretene. However it is also not trimethylphenanthrene (as You mention on the 10528 page, Line 20 and on the Fig. 6c). It is probable one of the penthamethyl-phenanthrene isomers (in trimethyl-phenanthrenes molecular and base peak is $M^+ = 220$). We agree with you that this peak should be proposed as a penthamethyl-phenanthrene and not trimethyl. The "trimethyl" was replaced in text and figure.

As well as those are proposed by Mr. Sawada: 1) 'diagenetized' -> 'diagenetic'. 2) 'infra-generic' -> 'intra-generic'. 3) 'palaeofloristic' -> 'palaeofloral'. 4) Figure caption etc.: 'pyrolysis products' -> 'pyrolysates'.

1) P10514, L3: 'invested' -> 'investigated' 3) P10514, L10-L11: 'using gas chromatography-mass spectrometry' should be omitted. 4) P10514, L12: 'tetracyclic diterpenoids.' -> 'tetracyclic diterpenoids including phyllocladane and ent-kaurane.' 5) P10514, L14: 'compounds of the cadalane-type compounds' -> 'the cadalane-type compounds' 6) P10514, L16: 'the labdane-type, isopimarane, abietane-type' -> 'the labdane, isopimarane and abietane-types'. 7) P10515, L15: 'palaeoflora'-> 'palaeofloral'. 8) P10515, L25: 'anthropic' -> 'anthropogenic'. 9) P10516, L1: 'diet habits' -> 'dietary habits'. 10) P10516, L4: 'some part' -> 'some parts' 11) P10516, L24-L25: 'on palaeoflora, palaeoclimatic reconstruction, archaeology, environmental research.' -> 'on palaeofloral and palaeoclimatic reconstruction, archaeology, as well as environmental research.' 12) P10533, L18, L25: 'by the sesquiterpenoids' -> 'by the high abundances of the sesquiterpenoids'

In addition, we made some minor changes in the text, which do not alter the meaning of the previous version. These changes are listed as follows : P10514, L1-7: at the beginning of the abstract "Several extant species of the Araucariaceae family (one of the

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families of conifers) were investigated for the experimental artificial maturation by confined pyrolysis, in order to realize the transformation of biomolecules to geomolecules in laboratory conditions. The experimental study of diagenetic molecular signatures of the Araucariaceae species (common, inter- and infra-generic characteristics) allow to complete our knowledge in botanical palaeochemotaxonomy. Such knowledge is relevant to the reconstitution of palaeoflora and palaeoclimatic reconstruction, archaeology and environmental studies." was improved by "Twelve species of the conifer family Araucariaceae family, including Araucaria (6 species), Agathis (3 species) and Wollemia (1 species) genera, were submitted to artificial maturation by confined pyrolysis. The aim of these experiments is to transform the biomolecules synthesized by these species into their homologous geomolecules in laboratory conditions. Determination of the diagenetic molecular signatures of Araucariaceae through experimentation on extant representatives allows to complete our knowledge in botanical palaeochemotaxonomy." P10517, L7: "living environment"-> " living environments" P10518, L12: "record"-> removed P10519, L18: "Araucariaceae interests"-> "Usefulness of Araucariaceae from the past to present days" P10520, L10: ". . . diterpenoids (C20) are here summarised"-> More details were given in the new version ". . . diterpenoids (C20) of many Araucariaceae representatives are here summarised" P10533, L12-14: "Major structures of sesqui- and diterpenoids allow to trace the transformation of biomolecules into geomolecules (biomarkers)." -> removed P10533, L14: "by a remarkable predominance"-> "by the remarkable predominance" P10533, L15: "tetracyclic diterpenoids"-> More details were given in the new version "tetracyclic diterpenoids including beyerane, phyllocladanes as well as kauranes" P10533, L16; "However, a high abundance of sesquiterpenoids and abietanes derived could also be pointed out." -> More details were given in the new version "This family is also characterised by a relatively high abundance of sesquiterpenoids (like cadalanes, bisabolane, eudesmane as well as chamazulene) and diterpenoids like abietanes."

New version of this paper is given in 'Supplement Document'

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Please also note the supplement to this comment:

<http://www.biogeosciences-discuss.net/9/C6846/2012/bgd-9-C6846-2012-supplement.zip>

Interactive comment on Biogeosciences Discuss., 9, 10513, 2012.

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