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Chemotaxonomic significance of leaf wax *n*-alkanes in the Pinales (Coniferales)

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The chemotaxonomic significance of leaf wax *n*-alkanes was studied in 112 species and cultivars belonging to the Pinaceae, Cupressaceae, Podocarpaceae, Araucariaceae, Cephalotaxaceae, Sciadopityaceae and Taxaceae (Pinales). In general, *n*-alkanes ranged from 18 to 34 carbon numbers. In the Pinales, C_{31} was the most abundant *n*-alkane (20.17%±1.68), followed by C_{27} (2.84%±0.41), C_{29} (2.59%±0.49) and C_{25} (2.41%±0.22). In the Araucariaceae, *n*-alkane composition was characterized by low relative percentages of C_{31} (5.23%±1.58), whereas the Cephalotaxaceae were characterized by high percentages of C_{29} (31.95%±2.05) and C_{27} (28.00%±1.00). The Cupressaceae had a mean composition of *n*-alkanes characterized by moderate percentages of C_{31} (18.31%±2.32) and C_{33} (5.36%±1.07), whereas in the Pinaceae, C_{31} was the main *n*-alkane (25.40%±2.56). The Podocarpaceae were characterized by moderate percentages of C_{29} (12.69%±9.16), C_{31} (10.77%±2.70), C_{27} (7.37%±5.83) and C_{33} (6.59%±5.71), whereas the Taxaceae had high percentages of C_{31} (34.94%±7.85). *Sciadopitys verticillata* showed low percentages of all *n*-alkanes. Discriminant Analysis (DA) of the Araucariaceae, Cupressaceae and Pinaceae showed a good discrimination among subfamilies. Cluster Analysis (CA) and Principal Component Analysis (PCA) performed on species of the Pinales, showed a good separation among the families. The direct comparison of the present data with those obtained on species belonging to eleven angiosperm families provided further evidence of the chemotaxonomic significance of leaf wax *n*-alkanes.

Key words: Pinales, Chemotaxonomy, Leaf wax *n*-alkanes, Principal Component Analysis, Discriminant Analysis, Cluster Analysis.

INTRODUCTION

At the beginning of the sixties, Eglinton and co-workers (1962) have published a taxonomic survey on the hydrocarbon constituents of leaf wax coatings, which form a hydrophobic layer, the cuticle. This has a fundamental importance in photosynthesis, transpiration (Baker, 1970; Tuomisto & Neuvonen, 1993) and attacking by pathogenic fungi and bacteria (Taiz & Zeiger, 2002). In their work, Eglinton and co-workers have first noted the advantages of the use of cuticular secondary compounds as a criterion for systematic classification. This was mainly due to the universality of occurrence of waxy coatings, the species variation in composition, the simplicity of sampling, and the availability of rapid analytical tools (Eglinton *et al.*, 1962).

A few years later, Herbin & Robins (1969) have reviewed the taxonomic usefulness of leaf wax alkanes, which represent a minor portion of the overall wax composition, by narrowing their field of application to limited groupings of plants. At the end of the seventies, Tulloch & Hoffman (1976), by studying the wax composition of *Agropyron intermedium*, have stressed the commercial applications of these compounds, while in the eighties, Baker (1982) has updated the state of the art of the chemical wax composition.

Later on, alkanes have been used in estimating the species composition of herbage mixtures (Dove, 1992), in pasture sampling for the estimation of herbal intake (Vulich *et al.*, 1993), in leaf feeding patterns (Bergman *et al.*, 1991; Bodnaryk, 1992; Adati & Matsuda, 1993), in chilling injury (Rosen-

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qvist & Laakso, 1991; Maffei *et al.*, 1993; McDonald *et al.*, 1993), in edible oil characterization (Bianchi *et al.*, 1992; McGill *et al.*, 1993), and in bioindicating of the general degree of air pollution (Young & Wang, 2002) in plants (Hellqvist *et al.*, 1992; Salter & Hewitt, 1992; Tuomisto & Neuvonen, 1993 and references cited therein; Bryselbout *et al.*, 1998; Bytnerowicz *et al.*, 1998) and lichens (Zygaldo *et al.*, 1993; Piervittori *et al.*, 1996).

The chemotaxonomic significance of wax alkanes has been demonstrated in studies on Solanaceae (Zygadlo *et al.*, 1994), Crassulaceae (Stevens *et al.*, 1994), Cactaceae (Maffei *et al.*, 1997), Labiatae (and four related plant families) (Maffei, 1994), Gramineae (Maffei, 1996a), Compositae (Maffei, 1996b), Umbelliferae, Cruciferae and Leguminosae (Maffei, 1996c).

Schnable *et al.* (1994), Gülz (1994) and Reynhardt & Riederer (1994) have reviewed the genetics of cuticular wax biosynthesis, the epicuticular leaf waxes in the evolution of the plant kingdom and the molecular dynamics of plant waxes, respectively. Recently, the molecular genetics of epicuticular wax biosynthesis (Lemieux, 1996), the biosynthesis of lipid components of epicuticular wax (Kroumova & Wagner, 1999) and the biosynthesis and secretion of plant cuticular wax (Kunst & Samuels, 2003) have been reviewed.

In continuation of our studies on the chemotaxonomic significance of plant surface wax alkanes (Maffei, 1994, 1996a, 1996b, 1996c; Maffei *et al.*, 1997), we examined the usefulness of these wax constituents as chemotaxonomic markers in the order Pinales (Coniferales). Recently, Chaw *et al.* (1997) by analyzing 18S rRNA sequences have demonstrated that this order is monophyletic, whereas Mongrand *et al.* (2001) have performed taxonomic studies through multivariate analyses by using the leaf fatty acid composition of Gymnospermae, including the Pinales.

The results of our study showed that leaf wax alkanes extracted from Pinales needles are good chemotaxonomic markers, able to allow separation at the familial and subfamilial level. Furthermore, by comparing the present data with those obtained from species belonging to eleven plant families of the angiosperms, we show that the order Pinales is quite well separated from angiosperm dicotyledons and monocotyledons.

MATERIALS AND METHODS

Plant material

Mature leaves of plants belonging to the Pinales (Pinaceae, Cupressaceae, Podocarpaceae, Araucariaceae, Cephalotaxaceae, Sciadopityaceae and Taxaceae) were collected during the summer from the Botanical Garden of the University of Turin, Italy (*Araucaria cunninghamii*, *Abies balsamea*, *Abies numidica, Abies pinsapo, Cedrus atlantica*, *Cedrus deodara, Cedrus libani, Cephalotaxus fortunei, Cephalotaxus harringtonia*, *Chamaecyparis lawsoniana*, *Cryptomeria japonica, Cunninghamia lanceolata, Cupressus sempervirens*, *Juniperus chinensis*, *Juniperus communis, Larix gmelinii, Metasequoia glyptostroboides, Picea abies*, *Picea engelmannii, Picea glauca, Picea orientalis, Pinus pinea, Pinus radiata*, *Pinus sylvestris, Pinus wallichiana, Sciadopitys verticillata*, *Sequoia sempervirens, Taxodium distichum*, *Taxus baccata*, *Tsuga canadensis*) and from some nurseries present in the surroundings of the Turin area: Vivaio Regione Piemonte-Piossasco: *Abies alba, Abies nordmanniana*, *Calocedrus decurrens, Cedrus deodara* var. *pectinata*, *Larix decidua*, *Picea abies* var. *alpestris, Picea albertiana, Picea excelsa, Picea pungens, Pinus cembra* var. *glauca, Pinus excelsa, Pinus mugo* var. *pumilio*, *Pinus nigra*, *Pinus strobus*, *Pinus sylvestris* var. *iberica*, *Thuja occidentalis, Thuja orientalis*. Vivaio Sartorelli-Chieri: *Abies koreana, Abies kosteriana, Araucaria araucana, Calocedrus decurrens*, *Chamaecyparis obtusa* var. *nana*, *Picea asperata*, *Picea kosteriana, Picea kosteriana* var. *nana, Pinus cembra*, *Pinus nana, Platycladus orientalis, Pseudotsuga menziesii*, *Sequoiadendron giganteum*. Vivaio Coppo, Grugliasco: *Calocedrus macrolepis*, *Chamaecyparis lawsoniana*, *Chamaecyparis lawsoniana* var. *aurea*, *Chamaecyparis pisifera*, *Juniperus sabina, Picea pungens*, *Pinus mugo, Pinus pumila*, *Thuja occidentalis* var. *pyramidalis*. Vivaio Tomaino, Ciriè: *Abies nidiformis, Cryptomeria elegans, Juniperus chinensis* var. *plumosa aurea*, *Juniperus communis* var. *nana*, *Juniperus recurva, Juniperus sabina* var. *tamariscifolia*, *Juniperus squamata* var. *meyeri, Picea omorika, Pinus heldreichii* var. *leucodermis, Pinus parviflora, Pinus parviflora* var. *pentaphylla, Taxus iberica, Taxus media*. Comune di Torino, Parco del Valentino: *Abies concolor, Picea breweriana, Podocarpus chinensis*, *Pseudotsuga menziesii* var. *glauca, Tetraclinis articulata, Thuja globosa*. Exotic plants were obtained from the Botanical Garden of the University of Wageningen, The Netherlands (*Juniperus alpina, Ju-*

niperus horizontalis, Juniperus procumbens, Juniperus procumbens var. *nana, Larix kaempferi, Larix laricina, Picea likiangensis, Picea schrenkiana, Picea spinulosa, Pinus aristata, Pinus bungeana, Pinus contorta, Pinus thunbergii, Podocarpus nivalis, Taxus cuspidata, Thujopsis dolobrata, Torreya californica, Torreya grandis*) and from the Mount Tomah Botanic Gardens, Australia (*Agathis moorei, Agathis robusta*, *Araucaria bidwillii*, *Araucaria columnaris, Araucaria cunninghamii*, *Araucaria heterophylla, Calocedrus macrolepis, Keteleeria fortunei, Prumnopitys ladei, Tetraclinis articulata*). Samples of *Juniperus indica* and *Juniperus recurva* were collected from the areas North of Namche Bazar, Nepal. A voucher specimen of all taxa is deposited at the Herbarium Generale (TO), Department of Plant Biology of the University of Turin, Italy.

Leaf wax n-alkane analysis

Leaves at the same growth stage were randomly collected from each species and 1 g of fresh material was immediately extracted with 10 ml hexane for 60 s; 30 µg of *n*-tricosane were added as internal standard. A total of three extractions and injections per species were made. The extract was then concentrated by a gentle stream of N_2, passed through a column of anhydrous $MgSO_4$ and analyzed by gas chromatography. The extract (1 µl) was injected into an on-column injector of an Agilent Technologies 6890 gas chromatograph, equipped with a Flame Ionization Detector (FID). Separation was accomplished with a 25 m × 0.2 mm × 0.33 µm (film thickness) HP-5 capillary column with the following program: 100 °C for 5 min, then an increasing rate of 10 °C min^{-1} up to 280 °C held for 50 min (detector 300 °C, injector 280 °C, carrier gas He at 0.28 m s^{-1}). Peak areas and concentrations were calculated using an electronic integrator. Peak identification was based on R_t compared with pure standards and GC-MS. Compounds were identified using gas chromatography-mass spectrometry equipped with a 50-meter HP-1 capillary column. The helium carrier gas flow rate was 1.5 ml min^{-1}. Mass spectroscopy was performed at 70 eV. The injector temperature was 230 °C and the ion source temperature 250 °C. At least one sample per species was run on GC-MS for peak identification. Compounds, even- and odd-numbered *n*-alkanes, were identified by direct comparison with pure standard mass spectra. Compounds typical of gymnosperms, such as 10-nonacosanol, docosanol, eicosanol and corresponding diols, were clearly separated and distinguished from *n*-alkanes.

The following abbreviations and diagnostic ions (*m/z*) of the identified alkanes were used: C_{18} *n*-octadecane (254), C_{19} *n*-nonadecane (268), C_{20} *n*-eicosane (282), C_{21} *n*-eneicosane (296), C_{22} *n*-docosane (310), C_{23} *n*-tricosane (324), C_{24} *n*-tetracosane (338), C_{25} *n*-pentacosane (352), C_{26} *n*-hexacosane (366), C_{27} *n*-heptacosane (380), C_{28} *n*-octacosane (394), C_{29} *n*-nonacosane (408), C_{30} *n*-triacontane (422), C_{31} *n*-hentriacontane (436), C_{32} *n*-dotriacontane (450), C_{33} *n*-tritriacontane (464), C_{34} *n*-tetratriacontane (478).

Statistical analysis

All data were statistically processed using a Systat 5.2 software for Macintosh. Analyses included: a) cluster analysis (CA) calculated from GC analyses using the Euclidean or 1-Pearson distances with single, Ward and complete linkage method; b) principal component analysis (PCA), using the Factor option with a varimax rotation; and c) discriminant analysis (DA) using the Fully Factorial, Anova, Manova option defining as dependent variable all the identified compounds and, as Factor variable, the families or the subfamilies. A test of effects was estimated using the latter as between subjects to obtain original group membership (GROUP) and membership predicted by the model (PREDICT) to be associated to the related taxa. The option Table was used to tabulate (percentage) the actual group membership against that predicted.

RESULTS

n-Alkane content and composition of the Pinales

Epicuticular waxes of Pinales needles are represented by *n*-alkanes ranging from 18 to 34 carbon numbers. Table 1 shows the chemical composition of wax alkanes from 112 species belonging to seven families of the order Pinales. Species are grouped to subfamilies and families, and for each group mean values and SEM are indicated. From the total mean values of all species it follows that, C_{31} is the most abundant *n*-alkane (20.17%±1.68), followed by C_{27} (2.84%±0.41), C_{29} (2.59%±0.49) and C_{25} (2.41%± 0.22). Considering the total *n*-alkane content, the species analyzed gave a total mean value of 6.18 (±0.38) µg g^{-1} d. wt.

In the Araucariaceae, species belonging to the Agatheae had relatively high percentages of C_{31}, C_{33}

TABLE 1. Chemical composition of leaf wax alkanes in families belonging to the order Pinales (Coniferales). Values are expressed as percentage. Content is expressed as µg g-1 d.wt

Families (subfamilies) and Species	**Content**	C_{18}	C_{19}	C_{20}	C_{21}	C_{22}	C_{23}	C_{24}	C_{25}	C_{26}	C_{27}	C_{28}	C_{29}	C_{30}	C_{31}	C_{32}	C_{33}	C_{34}
ARAUCARIACEAE Henkel et W. Hoscht.																		
Agatheae																		
Agathis moorei	12.61	0.56	0.00	0.73	1.93	0.00	0.00	1.86	1.43	1.07	0.00	0.75	0.00	1.31	13.20	1.57	12.00	0.63
Agathis robusta	11.10	0.57	0.30	0.67	1.39	1.62	0.00	0.99	2.02	0.58	0.87	0.68	4.97	2.34	2.67	2.78	0.49	0.12
Mean values (SEM)	11.86	0.60	0.15	0.70	1.65	0.80	0.00	1.45	1.70	0.85	0.45	0.75	2.50	1.80	7.85	2.20	6.25	0.35
	(0.76)	(0.00)	(0.15)	(0.00)	(0.25)	(0.80)		(0.45)	(0.30)	(0.25)	(0.45)	(0.05)	(2.50)	(0.50)	(5.15)	(0.60)	(5.75)	(0.25)
Araucarieae																		
Araucaria araucana	3.94	0.66	0.65	0.85	0.51	1.75	0.00	6.95	7.22	10.6	8.14	8.48	6.70	4.78	7.26	1.87	1.47	0.57
Araucaria bidwillii	3.52	1.11	0.00	0.53	1.19	1.65	0.00	0.92	0.00	0.4	0.48	0.00	5.27	2.08	1.15	0.00	0.00	0.00
Araucaria columnaris	4.16	0.00	0.00	2.46	3.82	0.00	4.92	3.49	2.77	2.14	1.59	1.09	0.72	0.47	1.47	0.11	0.12	0.00
Araucaria cunninghamii	3.93	0.88	0.00	2.24	3.31	0.00	0.00	2.33	1.50	1.63	0.79	0.72	0.52	0.46	6.71	0.00	1.05	0.00
Araucaria heterophylla	9.06	0.00	0.00	0.00	2.43	3.42	0.00	2.00	1.62	1.36	1.36	0.94	1.03	0.38	4.15	0.00	0.00	0.00
Mean values (SEM)	4.92	0.54	0.14	1.22	2.24	1.38	0.98	3.14	2.62	3.30	2.48	2.24	2.84	1.66	4.18	0.40	0.54	0.12
	(1.04)	(0.23)	(0.14)	(0.49)	(0.62)	(0.64)	(0.98)	(1.05)	(1.23)	(1.94)	(1.42)	(1.58)	(1.31)	(0.85)	(1.27)	(0.38)	(0.32)	(0.12)
Family mean values (SEM)	6.90	0.56	0.14	1.07	2.07	1.21	0.70	2.66	2.36	2.60	1.90	1.81	2.74	1.70	5.23	0.91	2.17	0.19
	(1.48)	(0.16)	(0.10)	(0.35)	(0.44)	(0.49)	(0.70)	(0.79)	(0.87)	(1.42)	(1.05)	(1.12)	(1.74)	(0.59)	(1.58)	(0.44)	(1.65)	(0.11)
CEPHALOTAXACEAE Neger																		
Cephalotaxus fortunei	18.13	0.00	0.00	0.92	0.34	0.81	0.00	1.41	4.36	1.93	27.0	2.74	33.9	1.41	9.43	0.25	1.13	0.00
Cephalotaxus harringtonia	21.82	0.00	0.00	0.00	1.61	0.00	3.11	3.36	5.99	2.84	28.9	0.00	29.9	0.95	6.29	0.16	0.99	0.00
Family mean values (SEM)	19.98	0.00	0.00	0.45	0.95	0.40	1.55	2.40	5.20	2.35	28.00	1.35	31.95	1.03	7.85	0.23	1.05	0.00
	(1.85)			(0.45)	(0.65)	(0.40)	(1.55)	(1.00)	(0.80)	(0.45)	(1.00)	(1.35)	(2.05)	(0.38)	(1.56)	(0.07)	(0.06)	
CUPRESSACEAE Rich. Ex Bartling																		
Thujoideae																		
Calocedrus decurrens	12.69	0.69	0.43	0.68	2.38	2.69	4.17	3.98	3.38	2.61	2.10	1.00	1.95	0.75	28.50	0.35	11.20	0.71
Calocedrus macrolepis	9.50	0.91	0.00	1.37	1.95	2.51	0.00	1.31	0.87	0.60	0.77	0.59	3.21	0.59	15.50	0.00	0.00	0.00
Platycladus orientalis	3.61	1.10	0.00	0.00	3.94	0.00	0.00	3.71	2.99	2.46	1.58	0.78	0.98	0.14	0.00	0.25	0.00	0.79
Thuja globosa	3.22	0.00	1.70	0.00	0.00	1.36	0.00	0.00	0.00	0.29	0.86	4.90	0.68	0.00	10.60	0.00	11.90	1.11
Thuja occidentalis	1.35	0.30	0.00	0.00	0.00	1.17	1.73	1.85	1.60	1.10	0.90	0.39	1.84	0.00	0.00	0.00	2.46	0.47
Thuja occidentalis var. *pyramidalis*	2.85	0.00	2.35	0.00	0.00	0.00	1.38	0.00	0.00	0.00	0.00	0.00	1.18	0.00	20.5	0.56	10.2	0.91
Thuja orientalis	4.26	0.91	2.29	0.00	0.00	0.00	1.37	1.13	1.33	1.20	1.55	0.74	1.45	0.32	21.1	0.55	9.24	0.95
Thujopsis dolobrata	2.52	0.00	0.00	0.00	0.00	0.53	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.34	0.00	0.00
Mean values (SEM)	5.00	0.49	0.85	0.26	1.04	1.22	0.91	1.50	1.27	1.04	0.99	1.20	1.27	2.85	9.70	1.46	4.45	0.51
	(1.40)	(0.16)	(0.39)	(0.18)	(0.54)	(0.36)	(0.53)	(0.57)	(0.47)	(0.37)	(0.27)	(0.54)	(0.38)	(2.60)	(4.02)	(1.22)	(1.88)	(0.16)

Families (subfamilies) and Species	Content	C_{18}	C_{19}	C_{20}	C_{21}	C_{22}	C_{23}	C_{24}	C_{25}	C_{26}	C_{27}	C_{28}	C_{29}	C_{30}	C_{31}	C_{32}	C_{33}	C_{34}
Cupressoideae																		
Chamaecyparis bouleward	17.07	0.00	0.00	0.52	0.00	0.00	0.91	0.00	9.91	0.38	2.97	0.25	1.54	1.35	19.60	0.09	2.55	0.19
Chamaecyparis lawsoniana	11.87	0.11	2.03	1.08	0.31	0.68	0.61	1.50	0.49	0.50	1.28	0.25	0.89	1.99	15.20	0.33	6.97	0.26
Chamaecyparis lawsoniana var. *aurea*	3.51	0.00	0.00	0.00	0.00	0.00	0.00	0.28	0.35	0.33	0.41	0.19	0.63	2.39	43.9	0.74	6.22	0.37
Chamaecyparis obtusa var. *nana*	5.85	1.24	0.00	0.00	1.64	1.94	3.01	3.32	3.86	2.50	12.70	0.95	1.61	0.00	13.00	0.00	0.92	0.00
Chamaecyparis pisifera	19.89	0.00	0.77	0.00	0.00	0.00	0.46	0.42	1.55	0.82	5.18	0.60	2.34	0.00	47.3	0.18	3.74	0.45
Cupressus sempervirens	4.62	0.16	0.97	0.00	15.1	0.00	2.38	0.69	0.69	0.47	0.58	0.00	1.90	0.17	19.7	0.45	9.57	0.64
Mean values (SEM)	10.47	0.25	0.63	0.27	2.82	0.43	1.23	1.03	2.83	0.83	3.92	0.40	1.47	1.00	26.50	0.30	5.00	0.33
	(2.82)	(0.19)	(0.33)	(0.19)	(2.45)	(0.31)	(0.48)	(0.50)	(1.51)	(0.34)	(1.96)	(0.14)	(0.26)	(0.44)	(6.13)	(0.11)	(1.30)	(0.09)
Cryptomerieae																		
Cryptomeria elegans	10.78	1.03	1.33	1.97	1.59	0.82	0.00	2.09	1.60	2.02	1.54	1.39	1.23	0.53	4.61	0.23	1.00	0.22
Cryptomeria japonica	3.25	0.00	0.00	0.78	0.94	0.84	0.89	0.91	0.94	0.66	2.36	0.00	0.00	0.31	7.44	0.00	1.95	0.00
Mean values (SEM)	7.02	0.50	0.65	1.40	1.25	0.80	0.45	1.50	1.25	1.35	1.95	0.70	0.60	0.40	6.00	0.10	1.50	0.10
	(3.77)	(0.50)	(0.65)	(0.60)	(0.35)	(0.00)	(0.45)	(0.60)	(0.35)	(0.65)	(0.45)	(0.70)	(0.60)	(0.10)	(1.40)	(0.10)	(0.50)	(0.10)
Cunnimghameae																		
Cunninghamia lanceolata	4.62	0.00	0.00	0.00	3.16	0.00	6.16	7.68	6.62	4.83	3.14	1.36	0.89	0.17	10.3	0.00	0.00	0.00
Juniperoideae																		
Juniperus alpina	6.04	0.07	4.64	0.00	0.08	0.00	0.00	0.10	0.10	0.00	0.47	0.10	1.02	0.34	57.99	0.57	20.29	0.64
Juniperus chinensis	4.53	0.00	0.00	0.30	0.53	0.60	0.71	0.71	0.68	0.46	0.57	0.33	0.91	0.58	16.6	0.56	2.94	0.56
Juniperus chinensis var. *plumosa aurea*	8.28	0.19	0.21	0.00	0.00	0.00	1.42	0.00	0.74	0.72	0.87	0.47	0.85	1.00	31.8	0.00	5.81	0.38
Juniperus communis	1.34	3.42	3.63	0.24	0.00	0.00	0.88	1.24	0.89	0.41	1.08	0.22	3.76	0.31	0.00	0.27	6.57	0.41
Juniperus communis var. *aurea*	3.02	0.51	0.38	0.43	0.00	0.00	0.55	0.81	0.56	0.74	0.75	0.55	0.99	0.48	18.8	0.71	11.4	0.55
Juniperus horizontalis	7.54	0.03	0.09	0.00	0.10	4.22	0.00	0.10	0.00	0.03	0.00	0.03	1.43	0.14	20.00	0.59	0.00	1.05
Juniperus indica	6.42	3.57	1.10	1.09	5.43	0.00	1.08	0.00	0.62	0.00	2.58	0.00	11.01	2.18	25.57	0.92	3.26	0.00
Juniperus procumbens	5.56	0.11	0.00	0.00	0.17	0.09	0.00	1.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.81	0.20	1.59
Juniperus procumbens var. *nana*	2.09	0.12	0.00	0.00	0.00	0.00	0.88	0.00	0.32	0.17	0.25	0.00	0.76	0.38	43.85	1.04	26.14	1.50
Juniperus recurva	12.35	1.00	1.19	1.86	1.61	0.00	0.00	2.51	1.90	2.21	1.38	1.42	1.06	0.63	17.1	0.34	2.77	0.00
Juniperus sabina	4.39	0.29	0.63	0.00	1.02	0.00	0.00	0.00	0.69	1.00	0.63	0.65	0.00	0.00	22.2	0.73	15.70	0.81
Juniperus sabina var. *tamariscifolia*	6.97	0.00	0.00	0.00	0.00	0.00	0.00	1.85	0.64	0.56	0.67	0.00	0.00	0.00	30.8	0.54	12.50	0.48
Juniperus squamata var. *meyeri*	3.29	0.63	0.77	1.06	1.43	0.00	2.55	0.88	1.85	0.88	3.47	0.66	4.32	2.45	22.00	0.00	8.60	0.43
Mean values (SEM)	5.52	0.76	0.97	0.38	0.79	0.38	0.63	0.72	0.70	0.55	0.99	0.35	2.02	0.66	23.65	0.54	8.89	0.65
	(0.82)	(0.34)	(0.41)	(0.17)	(0.42)	(0.32)	(0.21)	(0.22)	(0.17)	(0.17)	(0.28)	(0.12)	(0.83)	(0.22)	(4.36)	(0.09)	(2.22)	(0.14)
Metasequoieae																		
Metasequoia glyptostroboides	1.16	0.00	0.00	0.00	0.00	1.34	0.00	1.53	0.00	1.09	1.21	0.86	4.10	0.00	12.20	0.00	0.00	0.00

Families (subfamilies) and Species	Content	C_{18}	C_{19}	C_{20}	C_{21}	C_{22}	C_{23}	C_{24}	C_{25}	C_{26}	C_{27}	C_{28}	C_{29}	C_{30}	C_{31}	C_{32}	C_{33}	C_{34}
Sequoieae																		
Sequoia sempervirens	8.70	0.00	0.13	0.39	0.90	0.00	2.76	2.85	4.45	1.94	1.08	0.43	0.35	0.00	5.60	0.00	0.00	0.00
Sequoiadendron giganteum	2.80	2.31	1.42	0.00	0.00	0.64	0.94	1.24	1.30	1.11	1.31	0.65	2.01	0.00	37.00	0.00	0.00	0.00
Mean values (SEM)	5.75	1.15	0.75	0.20	0.45	0.30	1.85	2.05	2.90	1.50	1.20	0.55	1.20	0.00	21.30	0.00	0.00	0.00
	(2.95)	(1.15)	(0.65)	(0.20)	(0.45)	(0.30)	(0.95)	(0.85)	(1.60)	(0.40)	(0.10)	(0.15)	(0.80)		(15.70)			
Taxodieae																		
Taxodium distichum	2.76	2.93	1.60	1.10	0.00	0.62	0.00	0.00	0.80	0.39	0.69	0.17	0.45	0.19	17.90	0.00	3.28	0.00
Callitroideae																		
Tetraclinis articulata	2.01	0.00	0.90	0.00	0.00	0.00	2.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.08	0.00	0.00	0.95
Family mean values (SEM)	5.89	0.61	0.82	0.37	1.20	0.61	1.02	1.25	1.48	0.93	1.59	0.62	1.55	1.10	18.31	0.59	5.36	0.45
	(0.59)	(0.17)	(0.12)	(0.10)	(0.46)	(0.16)	(0.24)	(0.27)	(0.35)	(0.17)	(0.39)	(0.15)	(0.34)	(0.60)	(2.32)	(0.28)	(1.07)	(0.08)
PINACEAE Lindley																		
Abietoideae																		
Abies alba	2.01	0.00	0.00	0.00	0.00	0.00	0.00	0.72	2.74	0.00	0.00	2.51	2.37	1.79	5.20	0.90	0.53	0.46
Abies balsamea	7.49	0.88	0.00	0.00	3.68	5.20	7.06	7.71	6.42	4.64	2.89	1.22	0.71	0.61	6.51	0.00	0.00	0.00
Abies concolor	4.37	0.00	0.00	0.00	0.00	1.41	3.75	1.52	3.03	1.37	3.58	1.27	5.47	0.00	51.7	0.00	0.00	0.00
Abies koreana	8.95	0.44	0.53	0.73	3.81	0.00	0.00	2.47	0.00	1.39	1.14	0.83	0.61	1.04	4.02	0.17	0.33	0.10
Abies kosteriana	1.79	2.64	0.00	2.26	0.00	0.00	1.00	0.91	0.00	0.87	2.12	0.63	1.16	1.87	15.70	0.00	0.00	0.71
Abies nidiformis	6.62	0.63	0.63	0.93	0.57	1.89	0.00	5.21	5.55	6.43	5.08	4.30	3.67	2.10	30.30	0.75	0.40	0.00
Abies nordmanniana	10.56	0.00	0.00	0.38	2.46	0.00	3.90	3.73	3.90	2.93	3.07	1.49	2.27	0.00	39.70	0.23	0.17	0.00
Abies numidica	3.55	0.00	0.00	1.61	0.51	6.03	6.55	5.68	5.27	4.31	3.33	2.25	1.83	0.93	8.39	0.34	0.00	0.00
Abies pinsapo	3.84	1.34	0.00	0.30	0.25	0.68	0.00	0.00	0.00	0.00	9.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cedrus atlantica	4.36	1.83	0.83	0.30	0.32	0.48	1.07	0.91	1.49	0.87	1.55	0.56	1.76	3.08	67.9	0.00	0.00	0.00
Cedrus deodara	4.33	0.47	0.40	0.58	1.34	1.75	2.36	2.68	2.68	2.58	1.90	1.85	0.99	51.4	0.00	0.00	0.00	0.00
Cedrus deodara var. *pectinata*	4.09	1.56	0.94	0.66	0.84	1.46	2.70	4.03	5.98	4.46	4.63	2.07	3.34	1.02	41.50	0.00	0.00	0.00
Cedrus libani	3.83	1.68	0.85	0.51	0.00	1.19	1.59	1.77	2.10	1.40	1.83	0.91	1.78	3.38	39.2	0.00	0.00	0.66
Keteleeria fortunei	9.21	0.59	0.00	0.00	3.04	0.00	3.58	2.42	1.79	1.49	2.01	1.17	2.17	0.54	1.14	0.32	1.76	0.00
Tsuga canadensis	7.48	2.63	1.04	0.61	4.19	1.37	2.37	2.02	2.35	1.60	1.66	0.79	0.94	0.00	28.3	0.00	0.00	0.00
Mean values (SEM)	5.50	0.98	0.34	0.59	1.40	1.44	2.41	2.78	2.89	2.29	2.92	1.47	1.95	4.49	22.61	0.18	0.21	0.13
	(0.70)	(0.24)	(0.10)	(0.16)	(0.41)	(0.47)	(0.59)	(0.55)	(0.56)	(0.49)	(0.55)	(0.27)	(0.36)	(3.33)	(5.59)	(0.08)	(0.12)	(0.07)
Laricoideae																		
Larix decidua	4.14	0.00	0.00	0.00	0.00	0.00	0.00	3.76	2.30	0.37	0.37	0.86	1.68	3.56	12.40	0.00	0.00	0.00
Larix gmelinii	6.26	5.58	2.18	1.04	0.67	0.49	1.00	0.71	0.91	0.26	0.66	0.09	1.36	1.76	32.50	0.00	0.00	0.00
Larix Kaempferi	5.76	0.10	0.00	0.00	0.00	0.00	0.15	0.19	0.16	0.00	0.26	0.13	0.14	6.86	0.00	0.00	0.00	0.00

Families (subfamilies) and Species	Content	C_{18}	C_{19}	C_{20}	C_{21}	C_{22}	C_{23}	C_{24}	C_{25}	C_{26}	C_{27}	C_{28}	C_{29}	C_{30}	C_{31}	C_{32}	C_{33}	C_{34}
Larix laricina	2.64	1.85	0.00	0.00	0.00	0.00	0.34	0.18	0.28	0.00	0.54	0.00	4.55	2.43	60.92	0.00	0.00	0.00
Pseudotsuga menziesii	8.00	3.15	1.79	1.57	2.94	3.14	5.01	4.86	4.44	2.71	1.84	0.86	1.29	2.09	18.70	0.00	0.00	0.00
Pseudotsuga menziesii var. *glauca*	7.30	0.55	0.00	0.00	3.72	3.56	0.30	4.48	4.53	2.76	2.24	1.13	1.49	1.11	21.80	0.00	0.00	0.00
Mean values (SEM)	5.68	1.89	0.67	0.43	1.22	1.20	1.13	2.38	2.09	1.03	0.98	0.52	1.76	2.98	24.49	0.00	0.00	0.00
	(0.82)	(0.89)	(0.42)	(0.28)	(0.68)	(0.69)	(0.79)	(0.92)	(0.81)	(0.55)	(0.33)	(0.20)	(0.60)	(0.85)	(8.55)			
Piceoideae																		
Picea abies	4.87	0.00	0.00	0.00	3.18	0.00	7.68	9.07	8.34	6.19	4.18	1.97	1.20	1.43	20.10	0.00	0.00	0.00
Picea abies var. *alpestris*	4.86	0.39	0.00	0.00	2.31	1.42	1.58	1.41	0.99	0.56	0.59	0.32	0.57	0.92	15.40	0.00	0.56	0.00
Picea albertiana	3.42	0.00	6.12	0.00	0.00	1.38	0.00	0.00	5.40	0.00	7.69	0.76	1.70	0.00	33.20	0.00	0.45	0.00
Picea asperata	2.89	0.00	0.00	0.00	1.44	2.59	0.00	2.02	1.98	1.66	1.38	1.64	2.62	1.58	29.50	0.37	0.61	0.00
Picea breweriana	4.62	0.00	4.85	0.00	0.00	1.85	2.81	3.00	3.09	2.24	2.01	0.00	1.23	3.49	48.40	0.00	0.00	0.00
Picea engelmannii	4.27	0.46	0.00	0.00	0.00	0.79	0.94	0.90	1.04	0.85	1.06	0.00	0.00	2.00	59.70	0.00	0.00	0.00
Picea excelsa	3.99	0.00	0.00	0.00	0.00	1.62	3.88	2.16	4.93	2.50	4.05	2.14	8.64	2.97	35.70	0.61	0.99	0.00
Picea glauca	5.78	0.00	0.00	0.00	2.88	3.70	8.10	8.94	6.30	4.90	3.36	1.39	1.12	1.32	25.10	0.00	0.20	0.00
Picea kosteriana	6.91	1.39	0.84	2.00	4.48	3.54	3.40	2.24	2.56	1.62	1.95	1.12	1.97	0.44	24.3	0.19	0.00	0.00
Picea kosteriana var. *nana*	6.80	1.39	1.46	1.69	1.42	0.00	0.00	2.21	1.78	1.93	1.77	1.32	1.77	1.15	38.6	0.00	0.00	0.00
Picea likiangensis	4.78	0.07	0.00	0.00	0.00	0.11	0.42	0.46	0.95	0.90	1.17	8.38	1.90	11.00	53.85	0.86	0.54	0.26
Picea omorika	8.24	1.02	1.26	2.11	1.77	3.24	0.00	4.17	2.95	3.32	2.03	2.08	1.91	0.90	15.70	0.30	0.15	0.00
Picea orientalis	3.98	1.27	1.22	2.00	1.28	2.28	0.00	4.75	4.05	4.78	3.21	2.80	2.28	1.22	23.4	0.39	0.00	0.00
Picea pungens	10.02	0.00	2.10	0.00	0.30	0.40	0.75	0.66	8.86	0.00	13.3	0.39	2.07	1.08	45.9	0.00	0.00	0.00
Picea schrenkiana	14.34	0.30	0.00	0.00	0.29	0.00	0.33	0.36	0.45	0.00	0.41	1.02	1.03	2.03	72.56	0.00	0.77	0.00
Picea spinulosa	4.31	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.62	0.17	3.42	0.00	0.35	1.19	0.51	0.00	0.00
Mean values (SEM)	5.88	0.41	0.99	0.50	1.27	1.58	2.09	2.93	3.12	2.29	2.42	1.93	1.87	1.99	32.10	0.24	0.51	0.03
	(0.73)	(0.14)	(0.46)	(0.22)	(0.35)	(0.33)	(0.68)	(0.72)	(0.59)	(0.48)	(0.48)	(0.49)	(0.49)	(0.64)	(4.39)	(0.07)	(0.23)	(0.02)
Pineoideae																		
Pinus aristata	8.29	0.33	0.00	0.00	0.62	0.00	0.00	0.40	0.00	0.00	0.00	0.00	1.02	1.71	57.04	0.00	0.00	0.00
Pinus bungeana	4.58	0.86	0.00	0.00	0.00	0.38	0.59	0.78	0.40	0.59	0.74	0.00	2.39	0.00	66.26	0.00	1.84	0.00
Pinus cembra	6.18	1.16	1.13	1.51	1.20	2.40	0.00	5.04	5.14	5.26	6.64	3.04	5.17	1.31	20.00	0.42	0.33	0.00
Pinus cembra var. glauca	2.27	0.00	0.00	0.00	2.16	2.20	3.38	4.00	4.23	2.99	1.87	0.90	0.44	0.29	4.51	0.00	0.00	0.00
Pinus contorta	5.31	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.29	0.00	2.42	0.00	25.78	0.00	0.00	0.00
Pinus excelsa	5.57	0.71	0.00	0.00	0.00	0.00	0.00	0.00	4.83	0.00	9.52	0.11	1.00	1.33	33.20	0.00	0.34	0.00
Pinus heldreichii var. *leucodermis*	3.22	0.83	0.71	0.86	1.13	2.66	3.01	3.66	3.57	3.53	3.14	2.28	2.30	1.03	28.60	0.00	0.00	0.00
Pinus mugo	15.22	1.35	1.31	2.15	1.67	1.50	0.00	2.67	1.87	2.26	1.61	1.87	1.39	1.05	6.22	0.00	0.26	0.22
Pinus mugo var. *pumilio*	1.91	0.11	0.06	0.00	0.64	0.89	0.00	1.21	0.00	0.45	0.92	0.00	0.00	0.00	22.4	0.00	0.62	0.00
Pinus nana	6.79	0.00	1.43	0.88	1.93	1.80	3.04	4.12	5.30	5.53	5.16	4.08	3.48	2.27	18.00	1.00	0.65	0.31

Families (subfamilies) and Species	Content	C_{18}	C_{19}	C_{20}	C_{21}	C_{22}	C_{23}	C_{24}	C_{25}	C_{26}	C_{27}	C_{28}	C_{29}	C_{30}	C_{31}	C_{32}	C_{33}	C_{34}
Pinus nigra	3.29	0.00	0.00	0.00	2.49	4.94	9.87	5.95	6.40	3.71	3.40	1.45	1.41	0.00	23.7	0.00	0.39	0.00
Pinus parviflora	10.84	0.00	0.00	0.00	2.04	0.69	1.31	1.42	1.71	1.26	1.25	0.00	1.36	0.00	50.3	0.00	0.00	0.00
Pinus parviflora var. *pentaphylla*	5.19	1.47	1.84	2.92	2.29	0.00	1.81	1.79	1.13	1.18	0.55	0.47	0.39	0.17	3.08	0.00	0.00	0.00
Pinus pinea	11.78	1.27	0.00	0.00	4.67	4.37	7.02	8.31	7.27	5.75	3.90	1.92	1.13	0.39	6.84	0.09	0.00	0.00
Pinus pumila	4.94	0.00	0.00	0.00	0.00	1.56	1.25	1.92	0.9	0.68	0.77	0.40	0.53	0.11	19.00	0.00	0.00	0.00
Pinus radiata	3.11	0.00	0.00	0.00	0.00	0.00	2.99	3.62	3.39	2.42	1.74	0.83	0.90	0.00	18.3	0.00	0.51	0.00
Pinus strobus	4.90	1.12	0.87	0.97	0.80	1.75	2.35	2.80	2.78	2.76	2.23	1.71	1.63	1.13	34.3	0.00	0.00	0.00
Pinus sylvestris	8.93	0.81	0.00	0.00	3.77	4.02	7.32	9.03	8.11	6.20	4.10	1.96	1.44	0.35	24.8	0.00	0.20	0.00
Pinus sylvestris var. *iberica*	1.50	1.95	3.61	0.00	0.00	0.00	4.79	0.17	0.00	0.00	0.81	0.62	0.89	0.30	0.00	0.00	0.81	0.00
Pinus thunbergii	0.32	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.59	0.00	5.18	0.00	0.00	0.00	0.00	0.00
Pinus wallichiana	2.05	3.55	1.74	0.81	0.54	0.56	0.95	0.87	1.67	1.02	1.73	0.86	1.57	0.59	11.80	0.00	0.46	0.00
Mean values (SEM)	5.53	0.75	0.60	0.49	1.23	1.42	2.37	2.75	2.80	2.18	2.71	1.08	1.71	0.58	22.56	0.07	0.31	0.02
	(0.82)	(0.20)	(0.21)	(0.18)	(0.29)	(0.34)	(0.61)	(0.57)	(0.57)	(0.46)	(0.52)	(0.25)	(0.31)	(0.15)	(3.95)	(0.05)	(0.10)	(0.02)
Family mean values (SEM)	5.64	0.83	0.65	0.51	1.28	1.45	2.18	2.77	2.84	2.12	2.51	1.35	1.82	2.23	25.40	0.14	0.31	0.05
	(0.40)	(0.14)	(0.16)	(0.10)	(0.19)	(0.20)	(0.33)	(0.33)	(0.31)	(0.25)	(0.28)	(0.18)	(0.20)	(0.89)	(2.56)	(0.03)	(0.08)	(0.02)
SCIADOPITYACEAE Luerss.																		
Sciadopitys verticillata	3.17	0.49	1.02	1.28	1.08	2.23	0.00	4.43	4.13	6.48	5.06	5.49	3.08	3.05	4.65	1.25	0.80	0.55
PODOCARPACEAE Endl.																		
Podocarpus chinensis	6.75	0.00	0.00	0.00	0.00	0.63	1.02	1.14	3.11	2.24	19.40	3.26	30.80	2.30	12.6	0.47	1.04	0.33
Podocarpus nivalis	3.04	0.00	0.00	0.00	0.66	0.94	3.25	0.56	0.01	11.60	0.90	0.00	2.97	0.00	13.90	0.59	0.76	0.00
Prumnopitys ladei	7.77	1.45	0.00	0.70	0.00	0.00	0.79	1.00	1.64	1.91	2.19	1.89	4.13	2.32	5.44	3.36	17.5	0.73
Family mean values (SEM)	5.85	0.50	0.00	0.23	0.22	0.51	1.68	0.89	1.57	5.23	7.37	1.73	12.69	1.53	10.77	1.50	6.59	0.33
	(1.44)	(0.50)		(0.23)	(0.22)	(0.27)	(0.79)	(0.17)	(0.89)	(3.18)	(5.83)	(0.96)	(9.16)	(0.77)	(2.70)	(0.95)	(5.71)	(0.20)
TAXACEAE Gray																		
Taxus baccata	7.19	0.99	0.00	0.77	3.10	0.00	7.42	8.45	8.09	6.86	6.15	3.33	2.71	1.14	15.00	0.43	0.31	0.00
Taxus cuspidata	2.18	0.18	0.00	0.00	0.00	0.14	0.24	0.32	0.50	0.51	0.76	0.50	0.69	1.76	61.60	0.00	0.33	0.00
Taxus iberica	6.16	0.92	0.81	0.95	1.03	2.31	0.00	4.27	4.87	4.97	6.53	2.52	3.26	0.68	36.30	0.23	1.58	0.00
Taxus media	8.68	0.83	0.90	1.28	0.86	0.00	0.00	4.45	4.32	5.55	5.03	3.48	2.71	1.45	12.30	0.50	0.27	0.15
Torreya californica	12.36	0.08	0.12	1.05	0.17	0.05	0.00	0.37	0.25	0.00	1.39	0.20	0.00	0.00	49.59	2.31	1.43	0.00
Torreya grandis	8.52	0.00	0.75	0.00	0.25	0.87	1.92	0.85	0.32	0.48	0.76	0.64	0.01	0.00	35.46	0.00	0.00	0.00
Family mean values (SEM)	7.52	0.49	0.43	0.69	0.90	0.56	1.59	3.14	3.06	3.08	3.44	1.77	1.57	0.84	34.94	0.57	0.66	0.03
	(1.37)	(0.19)	(0.18)	(0.23)	(0.47)	(0.37)	(1.20)	(1.33)	(1.32)	(1.26)	(1.13)	(0.61)	(0.61)	(0.30)	(7.85)	(0.36)	(0.28)	(0.03)
Order mean values over	6.18	0.70	0.62	0.50	1.24	1.09	1.60	2.25	2.41	1.85	2.84	1.21	2.59	1.72	20.17	0.38	2.18	0.20
112 species (SEM)	(0.38)	(0.09)	(0.10)	(0.07)	(0.18)	(0.13)	(0.21)	(0.22)	(0.22)	(0.19)	(0.41)	(0.14)	(0.49)	(0.50)	(1.68)	(0.10)	(0.43)	(0.03)

and C_{29}, whereas those belonging to the Araucarieae had lower percentages of C_{31} and higher percentages of *n*-alkanees ranging from C_{24} to C_{29}. These results lead to a general *n*-alkane composition characterized by low relative percentages of C_{31} (5.23%±1.58). The total *n*-alkane content of the Agatheae was significantly higher than that of the Araucarieae (Table 1).

Species belonging to the Cephalotaxaceae were characterized by high percentages of C_{29} (31.95% ±2.05) and C_{27} (28.00%±1.00), followed by good percentages of C_{31} (7.85%±1.56) and C_{25} (5.20% ±0.80). In these species, the total *n*-alkane content was quite high (19.98±1.85 μg g^{-1} d. wt.).

Species belonging to the family Cupressaceae are grouped in nine subfamilies. In the Thujoideae, the main *n*-alkanes were C_{31} (9.70%±4.02) and C_{33} (4.45%±1.88). The high standard error of C_{31} shows the consistent variability inside this subfamily for this *n*-alkane. In fact, *Thuja globosa*, *Thuja orientalis*, *Thuja occidentalis* var. *pyramidalis* and both *Calocedrus* species had high percentages of C_{31}, whereas this compound was absent in *Platycladus orientalis*, *Thujopsis dolobrata* and *Thuja occidentalis*. *Calocedrus macrolepis* was completely devoid of C_{33}, which however was present in a good percentage in *Calocedrus decurrens*. In the Cupressoideae, the main *n*-alkane was C_{31} (26.50%±6.13), followed by C_{33} (5.00%±1.30), C_{27} (3.92%±1.92) and C_{21} (2.82% ±2.45). The latter compound, however, had a high SEM value, due to the very high percentages found only in *Cupressus sempervirens*. In the Cryptomerieae, *n*-alkanes were present in low percentages, with C_{31} (6.00%±1.40) being the most abundant compound. The only species of the Cunninghameae had high percentages of C_{31} and *n*-alkanes ranging from C_{23} to C_{27}. The Juniperoideae were mostly characterized by C_{31} (23.65%±4.36) and C_{33} (8.89%±2.22). The highest percentages of C_{31} were assessed in *Juniperus alpina* (57.99), *Juniperus procumbens* var. *nana* (43.85%) and *Juniperus chinensis* var. *plumosa aurea* (31.80%), while high percentages of C_{33} were also found in *J. procumbens* var. *nana* (26.14%) and *J. sabina* (15.70%). *Metasequoia glyptostroboides* (Metasequoieae) had moderate percentages of C_{31} (12.20%). The raised SEM of C_{31} (21.30%±15.70) in the Sequoieae depended on the high percentage of this compound in *Sequoiadendron giganteum* (37.00%) and the low percentage in *Sequoia sempervirens* (5.60%). *Taxodium disticum* (Taxodieae) had a relatively high percentage of C_{31} (17.90%), whereas *Tetraclinis articulata* (Callitroideae) had a very low percentage of all *n*-alkanes. These results gave the Crupressaceae a mean composition of *n*-alkanes characterized by moderate percentages of C_{31} (18.31%±2.32) and C_{33} (5.36% ±1.07). The highest total *n*-alkane content was found in the Cupressoideae (10.47±2.82 μg g^{-1} d. wt.), while no statistical difference was found among the other subfamilies, with the exception of the Metasequoieae, Taxodieae and Callitroideae, which had lower values.

Species belonging to the family Pinaceae are grouped in four subfamilies. The chemical pattern of *n*-alkane distribution in these subfamilies is quite similar. In general, C_{31} was the main *n*-alkane (25.40%±2.56) followed by *n*-alkanes ranging from C_{21} to C_{30} [percentages from 1.28 (±0.19) to 2.84 (±0.31)]. The highest percentage of C_{31} was encountered in the Piceoideae (32.10%±4.39), with high values in *Picea schrenkiana* (72.56%), *Picea engelmannii* (59.70%) and *Picea likiangensis* (53.85%). The latter had also the highest percentage of C_{30} (11.00%). The Abietoideae, Laricoideae and Pineoideae had almost the same percentage of C_{31} (22.61%±5.59; 24.49%±8.55; 22.56%±3.95, respectively), with *Larix laricina* (60.92%) and *Pinus bungeana* (66.26) having the highest values. No statistical differences were found among the total *n*-alkane contents of the four subfamilies. *Sciadopitys verticillata* showed low percentages of all *n*-alkanes, with moderate values of C_{26} (6.48).

The Podocarpaceae were characterized by moderate percentages of the *n*-alkanes C_{29} (12.69% ±9.16), C_{31} (10.77%±2.70), C_{27} (7.37%±5.83) and C_{33} (6.59%±5.71), with *Prumnopitys ladei* having the highest total *n*-alkane content (7.77 μg g^{-1} d. wt) and percentage of C_{33} (17.5%). *Podocarpus chinensis* had the highest percentage of C_{29} (30.80%).

The Taxaceae had high percentages of C_{31} (34.94%±7.85), but low percentages of all other *n*-alkanes. The highest percentages of C_{31} were observed in *Taxus cuspidata* (61.60%) and *Torreya californica* (49.59%). The latter had also the highest total *n*-alkane content (12.36 μg g^{-1} d. wt).

Discriminant analysis of the Araucariaceae, Cupressaceae and Pinaceae

Discriminant analysis (DA) of the Araucariaceae, Cupressaceae and Pinaceae was performed using the data matrix of Table 1. DA of the Araucariaceae showed a complete separation of the two subfamilies

TABLE 2. Group (rows) by predict (columns) frequencies and in brackets row percents from the discriminant analysis. Discrimination was done considering the subfamilies of the Araucariaceae

	Agatheae	Araucarieae	Total
Agatheae	2 (100)	0	2 (100)
Araucarieae	0	5 (100)	5 (100)
Total	2 (28.57)	5 (71.43)	7 (100)

(Table 2), while in the Cupressaceae, a complete separation was obtained only for the Callitroideae, the Cryptomerieae, the Cunninghameae, the Metasequoieae, the Sequoieae and the Taxodieae (Table 3). In the Cupressoideae, *Chamaecyparis pisifera* was assigned to the Cryptomerieae, while in the Juniperoideae, *Juniperus horizontalis* to the Cryptomerieae, *J. procumbens* var. *nana* to the Cupressoideae, *Juniperus squamata* var. *meyeri* to the Sequoieae and *Juniperus sabina* var. *tamariscifolia*, *Juniperus chinensis* var. *plumosa aurea* and *Juniperus procumbens* to the Thujoideae. In the Thujoideae, *Thuja occidentalis* was assigned to the Cryptomerieae and *T.occidentalis* var. *pyramidalis* to the Juniperoideae (Table 3).

DA of the Pinaceae showed a good discrimination of the Laricoideae, followed by the Pineoideae and the Piceoideae, whereas the Abietoideae were not sufficiently discriminated (Table 4). In the Abietoideae, *Abies alba*, was assigned to the Laricoideae, *Abies nidiformis* and *Keteleeria fortunei* to the Piceoideae and *Abies concolor*, *Cedrus deodara* var. *pectinata* and *Cedrus deodara* to the Pineoideae. In the Laricoideae, *Larix kaempferi* was assigned to the Pineoideae, while in the Piceoideae, *Picea abies*, *Picea kosteriana* and *Picea kosteriana* var. *nana* were assigned to the Abietoideae. *Picea breweriana*, *Picea excelsa* and *Picea spinulosa* were assigned to the Pineoideae. Finally, in the Pineoideae, *Pinus excelsa*, *Pinus nana* and *Pinus pinea* were assigned to the Abietoideae, *Pinus wallichiana* to the Laricoideae and *Pinus bungeana* and *Pinus aristata* to the Piceoideae (Table 4).

Chemotaxonomic significance of n-alkanes in the Pinales

The data matrix of Table 1 was used to calculate the Cluster Analysis (CA) of the families belonging to the Pinales. The CA was calculated by using the Euclidean distance metric with the Ward minimum variance method, showing two clusters (Fig. 1). The first cluster

TABLE 3. Group (rows) by predict (columns) frequencies and in brackets row percents from the discriminant analysis. Discrimination was done considering the subfamilies of the Cupressaceae

	Callitroideae	Cryptomerieae	Cunninghameae	Cupressoideae	Juniperoideae	Metasequoieae	Sequoieae	Taxodieae	Thujoideae	Total
Callitroideae	1 (100)	0	0	0	0	0	0	0	0	1 (100)
Cryptomerieae	0	3 (100)	0	0	0	0	0	0	0	3 (100)
Cunninghameae	0	0	1 (100)	0	0	0	0	0	0	1 (100)
Cupressoideae	0	1 (14.29)	0	6 (85.71)	0	0	0	0	0	7 (100)
Juniperoideae	0	1 (6.67)	0	1 (6.67)	9 (60)	0	1 (6.67)	0	3 (20.00)	15 (100)
Metasequoieae	0	0	0	0	0	1 (100)	0	0	0	1 (100)
Sequoieae	0	0	0	0	0	0	2 (100)	0	0	2 (100)
Taxodieae	0	0	0	0	0	0	0	1 (100)	0	1 (100)
Thujoideae	0	2 (22.22)	0	0	1 (11.11)	0	0	0	6 (66.67)	9 (100)
Total	1 (2.50)	7 (17.50)	1 (2.50)	7 (17.50)	10 (25.00)	1 (2.50)	3 (7.30)	1 (2.50)	9 (22.50)	40 (100)

TABLE 4. Group (rows) by predict (columns) frequencies and in brackets row percents from the discriminant analysis. Discrimination was done considering the subfamilies of the Pinaceae

	Abietoideae	Laricoideae	Piceoideae	Pineoideae	Total
Abietoideae	9 (56.25)	1 (6.25)	2 (12.50)	4 (25.00)	16 (100)
Laricoideae	0	5(83.33)	0	1 (16.67)	6 (100)
Piceoideae	3 (17.65)	0	11 (64.71)	3 (17.65)	17 (100)
Pineoideae	3 (14.29)	1 (4.76)	2 (9.52)	15 (71.43)	21 (100)
Total	15 (25.00)	7 (11.67)	15 (25.00)	23 (38.23)	60 (100)

was made by the Cephalotaxaceae and by a subcluster gathering the Podocarpaceae, the Araucariaceae and the Sciadopityaceae. In this cluster, the Cephalotaxaceae were separated from the other families because of their high percentages of C_{27}, C_{28} and C_{29}. The second cluster was made by the Cupressaceae, the Pinaceae and the Taxaceae and was separated from the first cluster owing to its high percentage of C_{31} and low percentage of C_{27} and C_{29}. In this cluster, the Taxaceae were separated from the other two families owing to their higher percentage of C_{31} (Fig. 1).

Principal Component Analysis (PCA) calculated on the data matrix of Table 1, explained 85.79% of total variance for the three main PCs (PC_1, 40.08%; PC_2, 25.03%; PC_3, 20.68%). The plot of factor scores on the three main axes showed a clear separation among the families (Fig. 2). The Cupressaceae, Pinaceae and Taxaceae, were separated by positive PC_2 factor scores, dominated by the value of C_{31} component loadings, whereas the Sciadopityaceae, Cephalotaxaceae and Araucariaceae were separated by positive PC_1 factor scores, dominated by the value of C_{24} C_{26} and C_{28} component loadings. Finally, the Podocarpaceae were separated by positive PC_3 factor scores, dominated by the value of C_{29} and C_{33} component loadings (Fig. 2). The size of circles is proportional to the number of sampled species.

DA calculated on the data matrix of Table 1 showed a complete discrimination of the Cephalotaxaceae, Sciadopityaceae and Taxaceae (Table 5). A good discrimination was found for the Araucariaceae, even though *Agathis moorei* was assigned to the Cupressaceae and *Araucaria araucana* to the Sciadopityaceae. In the Cupressaceae, *Cryptomeria elegans*, *Juniperus recurva*, *Calocedrus macrolepis* and *Thuja occidentalis* var. *pyramidalis* were assigned to the Araucariaceae, *Sequoiadendron giganteum* and *Taxodium disticum* to the Pinaceae, *Juniperus indica* to the Podocarpaceae and *Cryptomeria japonica*, *Metasequoia glyptostroboides*, *Sequoia sempervirens*, *Chamaecyparis obtusa* var. *nana*, *Thujopsis dolobrata* and *Cunninghamia lanceolata* to the Taxaceae. In the Pinaceae, *Abies koreana*, *Picea omorika*, *Picea orientalis*, *Pinus cembra*, *Pinus mugo* and *Pinus parviflora* var. *pentaphylla* were assigned to the Araucariaceae, *Abies alba* and *Abies kosteriana* to the Cupressaceae, *Pinus nana* to the Sciadopityaceae and *Abies nidiformis*, *Abies pinsapo*, *Keteleeria fortunei*, *Larix kaempferi*, *Picea abies*, *Picea spinulosa*, *Pinus radiata*, *Pinus cembra* var. *glauca*, *Pinus thunbergii* and *Pinus contorta* to the Taxaceae. Finally, in the Podocarpaceae, *Podocarpus chinensis* was assigned to the Cephalotaxaceae, while *Podocarpus nivalis* to the Taxaceae. The plot of the canonical scores on the three main axes of the DA evidenced the separation among species belonging to the seven families (Fig. 3). In particular, the close chemical relation between *A. araucana* and *S. verticillata*, the presence of *A. alba* and *A. moorei* inside the discriminant space of the Cupressaceae, the position of *M. glyp-*

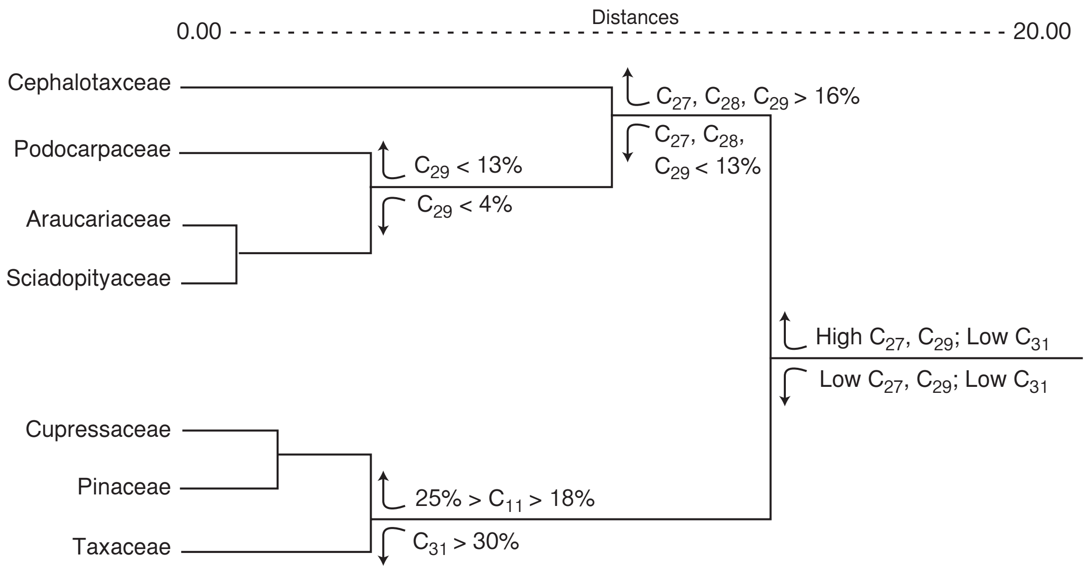

FIG. 1. Cluster analysis performed on the data matrix of Table 1 and calculated using the Euclidean distance with Ward minimum variance. Two main clusters are evident, the first made by families with a high percentage of C_{31} and the second composed by two subclusters one represented by the Cephalotaxaceae and the other by the remaining families.

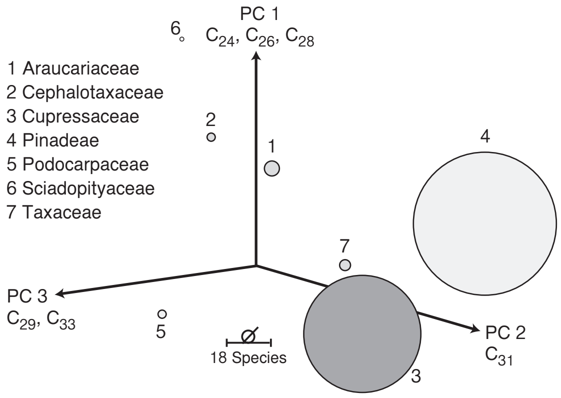

FIG. 2. Scatter plot of the factor scores of the Principal Component Analysis indicating a clear separation between the families. The total variance explained by the three principal components was greater than 85%. The area of circles is proportional to the number of sampled species (indicated by the metric bar).

tostroboides inside the Taxaceae and of *P. chinensis* inside the discriminant space of the Cephalotaxaceae, are evident (Fig. 3).

Chemotaxonomic significance of n-alkanes in the Pinales and some families belonging to the angiosperms

In order to better asses the chemotaxonomic significance of wax alkanes extracted from the Pinales, the data matrix of Table 1 was integrated into data matrices obtained from the analysis of leaf wax alkanes of species belonging to eleven angiosperm families [Labiatae, Verbenaceae, Scrophulariaceae, Solanaceae (Maffei, 1994), Gramineae (Maffei, 1996a), Compositae (Maffei, 1996b), Umbelliferae, Cruciferae, Leguminosae (Maffei, 1996c) and Cactaceae (Maffei *et al.*, 1997)] for a total of about 700 species.

The CA which was calculated on this combined data matrix using the 1-Pearson distance with single linkage method, showed the presence of four main clusters (Fig. 4). The first cluster was represented by the Gramineae and the second by the Pinales, showing a clear separation from the other families. In the third cluster, there was a close statistical linkage between the Labiatae and the Verbenaceae, which were linked to the Cactaceae. The last cluster was made by four subclusters. The first subcluster was made by the Umbelliferae, the second by the Leguminosae, the third by the Compositae and the Cruciferae (showing a very close statistical linkage), which in turn were linked to the Boraginaceae. In the fourth subcluster, a close statistical linkage linked the Scrophulariaceae with the Solanaceae (Fig. 4).

The PCA which was calculated with the varimax option on the Pinales and on the eleven angiosperm families, explained 71.93% of total variance on the main three PCs (PC_1, 30.04%; PC_2, 18.21%; PC_3, 23.68%). The plot of factor score coefficients on the three main axes of the PCA showed an evident separation of the Pinales from the angiosperms (Fig. 5). The Compositae, Labiatae, Verbenaceae, Cruciferae, Solanaceae and Scrophulariaceae were clearly separated by PC_2 and PC_3, while the Cactaceae and Gramineae by PC_1 and PC_3. Positive scores of PC_1 separated the Umbelliferae, whereas positive PC_2 scores separated the Pinales. PC_1 and PC_2 separated the Leguminosae and Boraginaceae (Fig. 5). The size of circles is proportional to the number of species considered.

DISCUSSION

Epicuticular waxes coat the surfaces of fleshy plant organs and serve to protect the plant from desiccation pest attacks, as well as to control leaf temperature, frost hardiness and signaling between pollen and stigma, etc. (Herbin & Robins, 1968; Lemieux, 1996; Kroumova & Wagner, 1999; Taiz & Zeiger,

TABLE 5. Group (rows) by predict (columns) frequencies and in brackets row percents from the discriminant analysis. Discrimination was done considering the families of the Pinales

	Araucariaceae	Cephalotaxaceae	Cupressaceae	Pinaceae	Podocarpaceae	Sciadopityaceae	Taxaceae	Total
Araucariaceae	5 (71.43)	0	1(14.29)	0	0	1 (14.29)	0	7 (100)
Cephalotaxaceae	0	2 (100)	0	0	0	0	0	2 (100)
Cupressaceae	4 (11.43)	0	22 (62.86)	2 (5.71)	1 (2.86)	0	6 (17.14)	35 (100)
Pinaceae	6 (10.34)	0	2 (3.45)	39 (67.24)	0	1(1.72)	10 (17.24)	58 (100)
Podocarpaceae	0	1 (33.33)	0	0	1 (33.33)	0	1 (33.33)	3 (100)
Sciadopityaceae	0	0	0	0	0	1 (100)	0	1 (100)
Taxaceae	0	0	0	0	0	0	6 (100)	6 (100)
Total	15 (13.39)	3 (2.68)	25 (22.32)	41 (36.61)	2 (1.79)	3 (2.68)	23 (20.54)	112 (100)

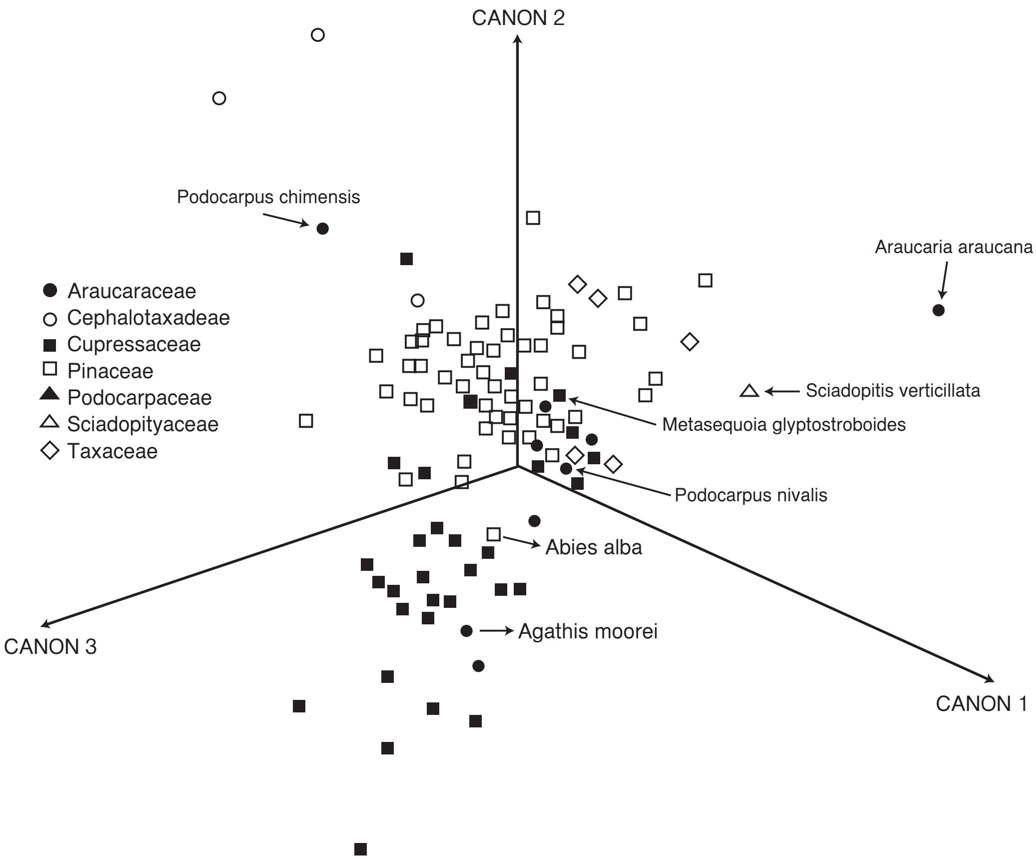

FIG. 3. Scatter plot of the species listed in Table 1 on the three main axes of the discriminant analysis (factor scores). A clear separation is evident between a group of Cupressaceae and a group of Pinaceae. The Cephalotaxaceae are present in the upper left part of the scatter plot.

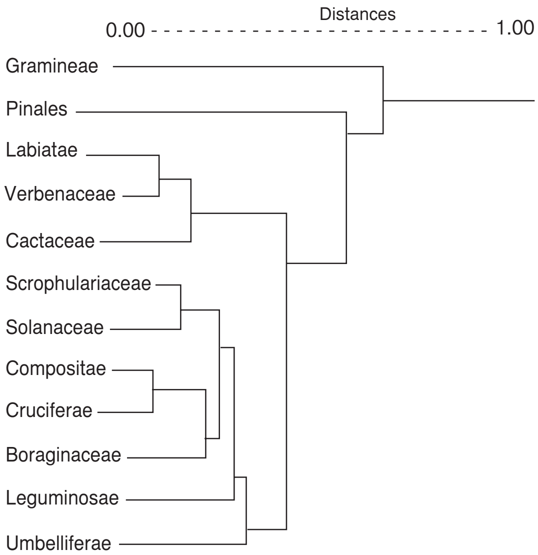

FIG. 4. Cluster Analysis calculated considering the data matrix of Table 1 and the data matrices of Tables reported in the literature (Maffei, 1994, 1996a,b,c; Maffei *et al.* 1997) related to eleven angiosperm families for a total of almost 700 species. CA calculated on this combined data matrix using the 1-Pearson distance with single linkage method showed the presence of four main clusters. See text for comments.

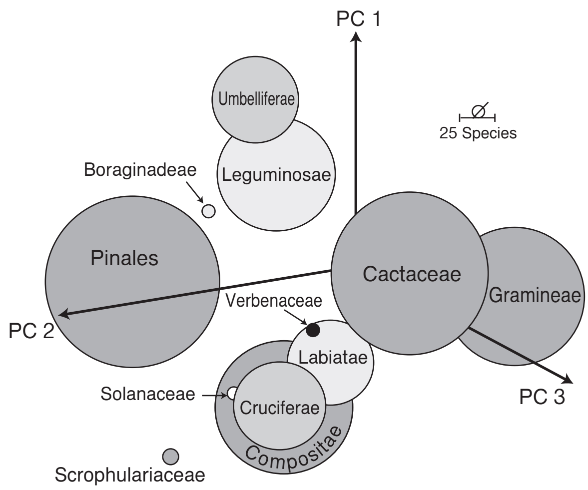

FIG. 5. Scatter plot of the 11 angiosperm families and the Pinales on the three main axes of the Principal Component Analysis. The three main PCs explained almost 72% of total variance. An evident separation of the Pinales from the angiosperms is clearly shown. The area of circles is proportional to the number of sampled species (indicated by the metric bar).

2002). Epicuticular waxes refer to surface lipids forming crystalloids or a smooth film exterior to the cuticle. A consistent part of epicuticular waxes is made of alkanes with predominant chain lengths from 18 to 34 carbon atoms (Kunst & Samuels, 2003). Beside biochemical, physiological and molecular considerations, wax alkanes have been also considered for their chemotaxonomic value.

In the Pinales, leaf wax *n*-alkane composition showed a chemical profile indicating that qualitatively the composition does not markedly differ from that of other plant families (Zygadlo *et al.*, 1994; Stevens *et al.*, 1994; Maffei, 1994; 1996a,b,c; Maffei *et al.*, 1997). Even though the most discriminant *n*-alkanes are represented by odd-numbered molecules, even-numbered alkanes were also present, as confirmed by direct comparison with pure MS standards. However, a direct comparison with angiosperm families indicates that the content of *n*-alkanes in the Pinales is quite different. In the Pinales, C_{31} is the main compound with percentages similar to those found in the Compositae (Maffei, 1996b), Labiatae (Maffei, 1994) and Cruciferae (Maffei, 1996c). However, in these angiosperm families, the content of C_{29} is always superior to that of the Pinales. The latter also possess a lower content of C_{27} and C_{33} when compared to most of the angiosperms studied so far (Zygadlo *et al.*, 1994; Stevens *et al.*, 1994; Maffei, 1994, 1996a, 1996b, 1996c; Maffei *et al.*, 1997). This different pattern of *n*-alkane quantitative distribution allows the chemotaxonomic separation of the Pinales from some angiosperm families as demonstrated by CA (Fig. 4) and PCA (Fig. 5) performed on almost 700 species belonging to a total of eighteen families. Our results are in agreement with studies performed on gymnosperms and some angiosperm species using storage protein (legumin) cDNA (Häger & Dank, 1996), plastid *rbc*L, nuclear 18S rDNA, mitochondrial *cox1* and *atp*A gene sequences (Bowe *et al.*, 2000 and reference cited therein), mitochondrial small subunit rRNA sequences and chloroplast *rbc*L gene (Chaw *et al.*, 2000) and nuclear 18S rRNA (Chaw *et al.*, 1997).

Within the Pinales, the Pinaceae, Cupressaceae and Taxaceae, share a high percentage of C_{31} (Fig. 1), which separates these families from the other Pinales (Fig. 2). Considering the Pinaceae, the clear separation of the family shown by PCA analysis (Fig. 2) agrees with the results obtained by Mongrand *et al.* (2001) who have performed leaf fatty acid analysis. The Pinaceae have been found to exhibit the greatest variation in nuclear ribosomal DNA ITS region length when compared to the Cupressaceae and Taxaceae (Liston *et al.*, 1996). Moreover, phylogenetic analyses using 18S rDNA sequences have shown that the Pinaceae are monophyletic and basal (Chaw *et al.*, 1997). Discriminant analysis of the Pinaceae (Table 4, Fig. 3) showed an almost perfect discrimination of the Laricoideae. The assignment of *Larix kaempferi* to the Pineoideae is in agreement with fatty acid analysis data of Mongrand *et al.* (2001), reflecting the close relationship between phenotypic expression of the same gene pools when *n*-alkanes and fatty acids are considered. A good separation was assessed for the Pineoideae even though, as found for fatty acid distribution (Mongrand *et al.*, 2001), some species showed close relationships with the Piceoideae. From the chemical point of view, the high percentage of the *n*-alkane C_{31} in *Pinus strobus* confirms previous works conducted by Herbin & Robins (1968). However, the relative percentage of this compound in our sample does not correspond to the one reported by these authors, possibly owing to the different growth conditions and/or developmental stage of the samples. A completely different alkane percentage was found in *Pinus sylvestris* when data were compared to those of Streibl *et al.* (1978). In the latter work, plants were sampled in Central Bohemia, where temperature and growth conditions are different from ours. As stated above, environmental conditions may exert a consistent pressure on gene activation and this is the main reason why plant chemotaxonomy will always be a comparative method to help plant classification, not the standard method.

Within the Araucariaceae, DA showed a complete separation between the Agatheae and the Araucarieae (Tab. 2). Our data are in agreement with those obtained using the *rbcL* gene of cpDNA from 29 species of the Araucariaceae, where the phylogenetic trees determined by the parsimony method indicated that the Araucariaceae are well defined by *rbcL* sequences and also that the monophyly of *Agathis* or *Araucaria* is well supported by high bootstrap values (Hiroaki *et al.*, 1998). A close statistical linkage was assessed between the Araucariaceae and the Sciadopityaceae (Fig. 1). Liston *et al.* (1996) found that the observed ITS region lengths in these two families are larger than those of other Pinales. However, the Sciadopityaceae are present in a different clade than that of the Araucariaceae and appear to form an outgroup of the Taxaceae, Cephalotaxaceae and Cupressaceae when 18S rRNA sequences are used to study evolutionary relationships (Chaw *et al.*, 1997).

The morphologically and ecologically diverse family of the Podocarpaceae showed a statistical linkage with the Araucariaceae and the Sciadopityaceae (Fig. 1). However, the PCA plot showed a clear separation of this family from the other Pinales, as described by Mongrand *et al.* (2001). These data would confirm the monophyly of the Podocarpaceae as demonstrated by Kelch (1998), but DA scatters the Podocarpaceae among the Taxaceae and the Cephalotaxaceae (Tab. 5), indicating that the familial relationship between the Podocarpaceae and the other families of the Pinales cannot be resolved by *n*-alkane data.

The Cephalotaxaceae are completely separated from the Taxaceae by using *n*-alkane profiles. Even though there is a current debate whether the Cephalotaxaceae should continue to be recognized as a separate family from the Taxaceae (Earle, 2003), our data clearly indicate a chemical differentiation between these two families when *n*-alkanes are considered.

Finally, the Cupressaceae have been formerly divided into Cupressaceae *sensu stricto* and Taxodiaceae. A recent study on plastid (*rbc*L) DNA sequences has confirmed the close relationship between the Cupressaceae *s.str.* and the genera formerly assigned to the Taxodiaceae (Gadek *et al.*, 2000; Farjon *et al.*, 2002). In the Cupressaceae, DA data indicated a perfect discrimination of six over nine subfamilies (Tab. 3). This is in accordance with genetic analyses using *matK* chloroplast gene locus (Gadek *et al.*, 2000) and fatty acid analyses (Mongrand *et al.*, 2001). Previous works on *Cupressus sempervirens* have reported a high percentage of C_{33} (Piovetti *et al.*, 1981). Our data confirm the high relative percentages of this *n*-alkane, though at lower values. Even in this case, different environmental conditions and developmental stage of sampling may be responsible for the difference in area percentage values.

In conclusion, the results of this work confirm the chemotaxonomic usefulness of surface wax *n*-alkanes, particularly at the familial level. The presence of even-numbered alkanes has to be taken with due caution, owing to the possible contamination from exogenous sources, as discussed by Reddy *et al.* (2000). The direct comparison of the present data with those obtained on angiosperms provides further evidence for the utility of *n*-alkane chemical analysis as a quick, reliable and inexpensive method to assess preliminary chemotaxonomic relationships. However, since *n*-alkanes are epicuticular depositions, these molecules should be used with caution and always in combination with other chemical and molecular data for phylogenetic or systematic studies. In fact, phenotypic plasticity may overcome genetic variability, particularly when plant developmental stages are considered along with abiotic and biotic stress conditions.

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