

**GEOGRAPHIC VARIATION IN THE LEAF ESSENTIAL OILS
OF *HESPEROCYPARIS (CUPRESSUS) ABRAMSIANA*, *H.*
GOVENIANA AND *H. MACROCARPA*:
SYSTEMATIC IMPLICATIONS**

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ABSTRACT

The compositions of the volatile leaf essential oils of *Hesperocyparis abramsiana* and its putative subspecies (*Cupressus abramsiana* subsp. *locatellii*, *opleri*, *neolomondensis*, and *butanoensis*) are presented along with *H. goveniana*, *H. pygmaea*, and *H. macrocarpa*. Most of the putative subspecies of *H. abramsiana* oils contained large amounts of umbellulone (16-21.8%), while the putative *C. a.* subsp. *neolomondensis* (type 2 oil) and *H. pygmaea* contained the unusual terpene karahanaenone (18.4, 2.2%). With the possible exception of *C. abramsiana* subsp. *butanoensis*, none of the subspecies proposed by Silba (2003) was supported. *Phytologia* 91(2): 226-243 (August, 2009).

KEY WORDS: *Hesperocyparis abramsiana*; *Cupressus abramsiana* subsp. *butanoensis*, *locatellii*, *neolomondensis*, *opleri*; *Hesperocyparis goveniana*; *Cupressus goveniana* subsp. *gibsonensis*; *Hesperocyparis macrocarpa*; *Cupressus macrocarpa* subsp. *lobosensis*; *Callitropsis*, Cupressaceae; essential oil composition; taxonomy.

Silba (2003) recently described four new subspecies of *Cupressus abramsiana* Wolf: *C. a.* subsp. *locatellii* Silba, Eagle Rock, CA; *C. a.* subsp. *opleri* Silba, Bracken Brae, Santa Cruz, CA; *C. a.* subsp. *neolomondensis* Silba, Majors Creek, CA; and *C. a.* subsp.

butanoensis Silba, Butano Ridge, CA. In addition, Silba (2003) split *C. goveniana* Gordon and *C. macrocarpa* Hartw. into subspp. *gibsonensis* and *lobosensis*, respectively. Because these proposed new subspecies are morphologically rather indistinct, we collected samples of fresh foliage from five separate trees from all of the type localities from which we then extracted and analyzed the leaf volatile oils to gather additional genetic information.

Recent DNA sequencing of *Cupressus sensu lato* (Little et al., 2004, Little, 2006) demonstrated that the Western Hemisphere species form a well-supported clade quite separated from the Eastern Hemisphere cypresses. As a result, Little (2006) not only confined the genus *Cupressus* to the Eastern Hemisphere, he also used *Callitropsis nootkatensis* and its generic epithet for the Western Hemisphere cypresses and *Xanthocyparis vietnamensis*. Debreczy et al. (2009) later argued, on morphological grounds, that *Ca. nootkatensis* is a monotypic genus. Sequencing by Adams et al. (2009) of two additional nuclear genes and petN-psbM further supported the recognition of *Ca. nootkatensis* as a monotypic genus. Because *Callitropsis*, therefore, should not be applied to the Western Hemisphere cypresses, Bartel and Price in Adams et al. (2009) described a new genus, *Hesperocyparis*, for the Western Hemisphere cypresses (exclusive of *X. vietnamensis* and *Ca. nootkatensis*). However, when referring to Silba's subspecies, *Cupressus* is used throughout this paper to avoid creating any new name combinations.

The volatile leaf oil of *Cupressus macrocarpa* has been examined by several authors: Briggs and Sutherland (1942); Zavarin et al. (1971); Briggs and Kingsford (1974); Malizia et al. (2000); Floreani et al. (1982); Cool (2005); El-Ghorab et al. (2007); Manimaran et al. (2007). However, only Zavarin et al. (1971) and Cool (2005) examined oils from trees native to California. Zavarin et al. (1971) confined their analysis to the monoterpenes, and concluded that *Cupressus macrocarpa* was distinct in its leaf oil. Cool (2005) focused on the sesquiterpenes of *C. macrocarpa* and identified several new sesquiterpenes.

The volatile leaf oils of *Cupressus goveniana* appear to have only been analyzed by Zavarin et al. (1971) and that report was confined to the monoterpenes.

The monoterpenes of the volatile leaf oils of *Cupressus abramsiana* were reported by Zavarin et al. (1971). Jolad et al. (1984) reported the isolation of cupresol from *C. abramsiana*.

Cool et al. (1994) reported the occurrence of karahanaenone in trace or small amounts in *Cupressus abramsiana*, *C. forbesii*, *C. goveniana*, and *C. stephensonii*. However, they found individuals of *C. pygmaea* and *C. sargentii* whose oil contained over 20% concentrations of karahanaenone.

No analyses have been made of the volatile leaf oils of the new subspecies of *Cupressus* proposed by Silba (2003). Thus, we present below analyses of the leaf essential oils of *Hesperocyparis abramsiana* (C. B. Wolf) Bartel, *H. goveniana* (Gordon) Bartel, and *H. macrocarpa* (Hartw. ex Gordon) Bartel and compare these oils with the Silba's putative subspecies.

MATERIAL AND METHODS

Plant material - Specimens used in this study: *H. abramsiana*, Bonny Doon, Santa Cruz Co., CA, *Bartel 1598a-e*; *C. abramsiana* subsp. *butanoensis*, Pescadero Creek County Park, Butano Ridge, San Mateo Co., CA, *Bartel 1605a-e*; *C. abramsiana* subsp. *locatellii*, Eagle Rock, Santa Cruz Co., CA, *Bartel 1599a-e*; *C. abramsiana* subsp. *neolomondensis*, Wilder Ranch State Park, Santa Cruz Co., CA, *Bartel 1604a-e*; *C. a.* subsp. *opleri*, Bracken Brae, Santa Cruz Co., CA, *Bartel 1600a-e*; *H. goveniana*, SFB Morse Botanical Reserve, Monterey Co., CA, *Bartel 1596a-e*; *C. goveniana* subsp. *gibsonensis*, Point Lobos Ranch, Monterey Co., CA, *Bartel 1595a-e*; *H. pygmaea*, Albion Ridge, Mendocino Co., CA, *Bartel 1601a-e*; Little River Airport, *Bartel 1602a-e*; Casper Little Lake Rd., CA, *Bartel 1603a-e*; *C. macrocarpa* subsp. *lobosensis*, Point Lobos State Reserve, Allan Memorial Grove, Monterey Co., CA, *Bartel 1593a-e*, Point Lobos State Reserve, East Grove, *Bartel 1594a-e*; *H. macrocarpa*, Crocker Grove, Monterey Co.,

CA, Bartel 1597a-e. Voucher specimens currently are held in Bartel's personal herbarium in Carlsbad, California.

Isolation of Oils - Fresh leaves (200 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at -20°C until analyzed. The extracted leaves were oven dried (100°C, 48 h) for determination of oil yields.

Chemical Analyses - Oils from 5-10 trees of each of the taxa were analyzed and both average and individual values are reported. The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see 5 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2006), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

Data Analysis - Terpenoids (as per cent total oil) were coded and compared among the species by use of the Gower metric (1971). Principal coordinate analysis was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967).

RESULTS AND DISCUSSION

The leaf oils of *H. abramsiana* are dominated (table 1) by umbellone (16-21.4%), terpinen-4-ol (11.9 - 16.8%), and nezukol (6.1 - 12.1%) with moderate amounts of sabinene (7.5 - 9.6%), and β -phellandrene (7.3 - 9.4%). However, the *neolomondensis* population sample contained 3 individuals (neo1, table 1) with high amounts of karahanaenone and α -terpinyl acetate as found in *H. pygmaea*. In fact, the oils of the *neolomondensis* - neo1 plants share two unique compounds with *H. pygmaea* (pyg, table 1): (Z)-nuciferol and β -(Z)-curcumen-12-ol as well as similar quantities of sabinene, camphor,

karahanaenone, terpinen-4-ol, 3-thujanol acetate, 4-terpinyl acetate, α -terpinyl acetate, and nezukol.

The leaf oils of *H. goveniana* were dominated by sabinene (15.2 - 26.3%), terpinen-4-ol (9.5 - 15.7%) and nezukol (11.1-26.3%) with moderate amounts of γ -terpinene (3.1-7.5%). *Hesperocyparis pygmaea* has also been treated as a subspecies of *H. goveniana*, but for this discussion it is treated as a species. The oil of *H. pygmaea* was not typical of *H. goveniana* in having a very high amount of karahanaenone (14.6%, table 1), camphor (8.7%), α -terpineol (3.2%) and α -terpinyl acetate (4.2%).

Table 1 shows that both *H. macrocarpa* oils are high in sabinene (27.0, 23.3%), α -pinene (22.2, 19.8%), terpinen-4-ol (11.7, 14.7%) with moderate amounts of γ -terpinene (5.6, 5.1%), isophyllocladene (4.4, 4.9%), myrcene (3.6, 3.2%), β -pinene (2.6, 2.0%) and phyllocladene (2.3, 2.0%). Of the 71 compounds identified, these subspecies seemed differ in only nezukol (0, 2.2%), citronellal (0.6, 0.3%) and piperitone (0, 0.3%). Clearly, the oils are nearly identical in both composition and component amounts (table 1).

To examine the overall similarities of the oils, a Principal Coordinates Ordination (PCO) was performed on the mean oils of the eleven taxa. Figure 1 shows the ordination based on 23 terpenoids (each greater than 1.0% of the oil). *Hesperocyparis pygmaea* is quite separated from *H. goveniana* in this PCO (Fig. 1). As mentioned above, three individuals of *neolomondensis* had oils that were high in karahanaenone and α -terpinyl acetate as found in *H. pygmaea*. The mean values of compounds are designated as AN1 in table 1 and figure 1. The mean values of the other two individuals (low in karahanaenone) are designated as AN2 in table 1 and figure 1. The oil of *neolomondensis*, type 1 (AN1) is most similar to *H. pygmaea* (Fig. 1), whereas *neolomondensis*, type 2 oil (AN2) is most similar to other *H. abramsiana* populations. Though not entirely unique, the Butano Ridge population grows on a sandstone outcrop surrounded by a dense canopy redwood forest. The oil of *butanoensis* (AB, Fig. 1) appears to be a little different from other *H. abramsiana* oils.

Hesperocyparis abramsiana appeared to show some infra-specific variation (Fig. 1, Table 1). A PCO analysis of individuals of *C.*

abramsiana from all five putative subspecies was made and is shown in Figure 2. The three individuals from *neolomondensis*, high in karahanaenone, group with *H. pygmaea*, whereas the other two plants of *neolomondensis* are imbedded with other *abramsiana* plants (Fig. 2). The individuals of *butanoensis* form a group somewhat distinct from other *abramsiana* individuals. The individuals of *H. abramsiana*, and putative subspecies *locatellii* and *opleri* are interspersed (Fig. 2). The PCO offers no support for the recognition of *locatellii* or *opleri*

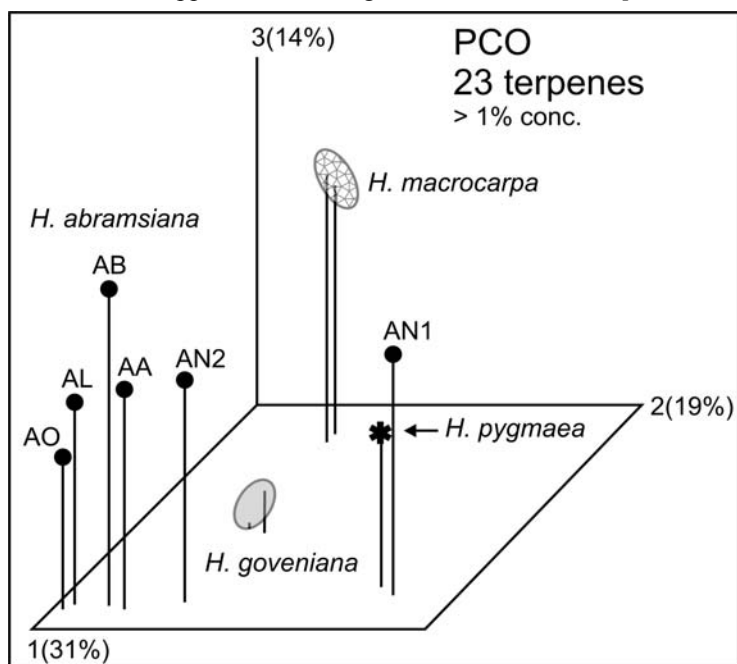


Figure 1. PCO of *Hesperocyparis* taxa using 23 terpenes that occurred in 1.0% or greater concentration.

- AA = *H. abramsiana*, Bonny Doon
- AB = *C. a.* subsp. *butanoensis*, Butano Ridge
- AL = *C. a.* subsp. *locatellii*, Eagle Rock
- AN1 = *C. a.* subsp. *neolomondensis*, high karahanaenone
- AN2 = *C. a.* subsp. *neolomondensis*, low karahanaenone
- AO = *C. a.* subsp. *opleri*, Bracken Brae

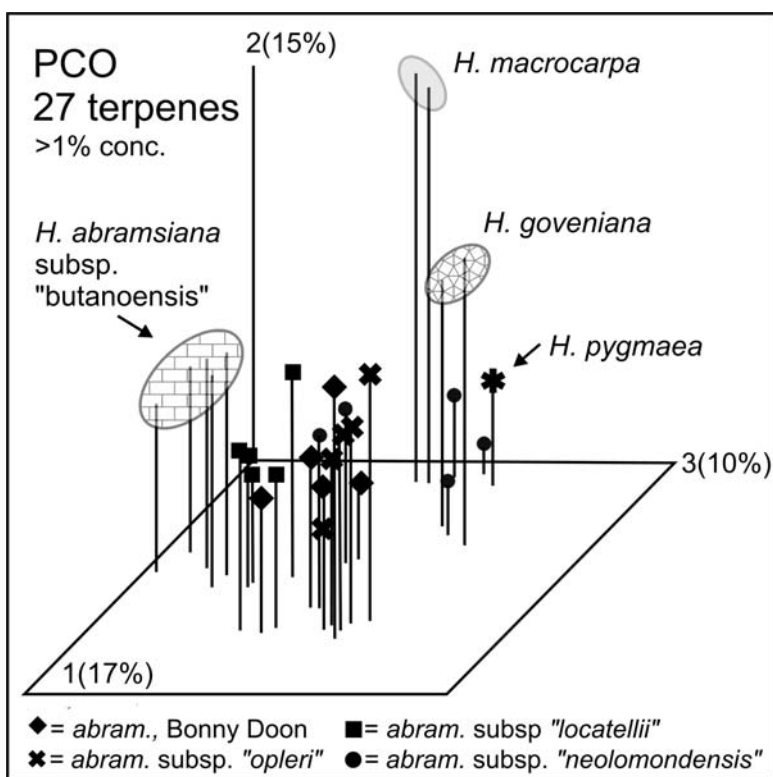


Figure 2. PCO of individuals of *H. abramsiana* based on 27 terpenes.

The initial analysis of *H. macrocarpa* subsp. *macrocarpa* and *C. m.* subsp. *lobosensis* average leaf oils (Fig. 1, Table 1) indicated that the oils were very similar with scarcely any differences (Table 1). PCO analysis of the individuals of *H. macrocarpa* confirm the overall trend. The individuals are interspersed (Fig. 3) implying that these two subspecies are behaving as one large population. We found no support in the leaf oil data to support the recognition of Silba's *C. abramsiana* subsp. *lobosensis*.

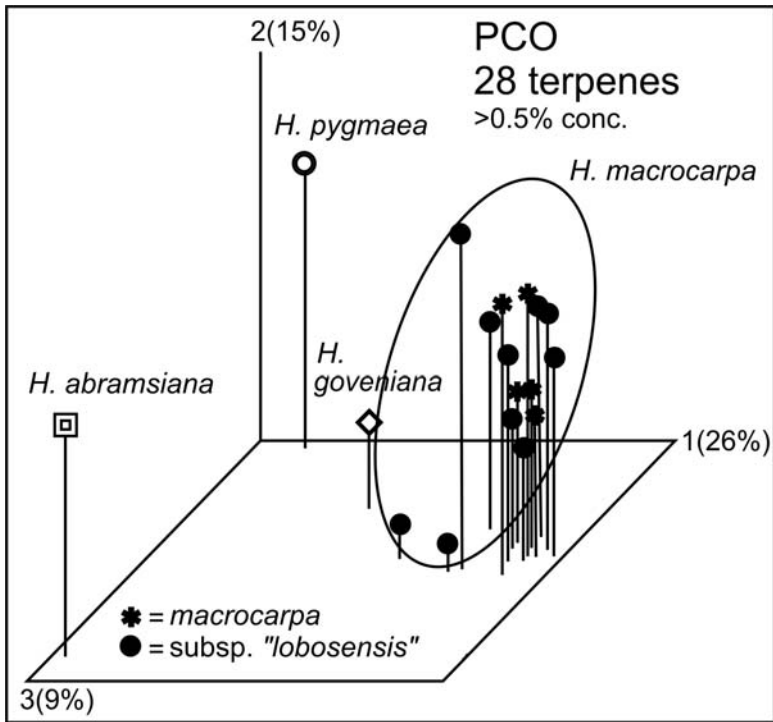


Figure 3. PCO of *H. macrocarpa* and Silba's putative *C. m.* subsp. *lobosensis* individuals along with mean values of *H. abramsiana*, *H. goveniana*, and *H. pygmaea*.

To examine differences among *H. goveniana*, putative *C. g.* subsp. *gibsonensis*, and *H. pygmaea*, a PCO analysis was made and is shown in figure 4. A slight separation exists between *H. pygmaea* and *H. goveniana* (Fig. 4), while there seems to be no difference between *H. goveniana* and Silba's putative *C. g.* subsp. *gibsonensis*, as these individuals are intermixed (Fig. 4). The three high karahanaenone individuals of *neolomondensis* were also included in the analysis and these seem close, but not conspecific with *H. pygmaea* (Fig. 4). Possibly these plants are the result of relictual or current hybridization

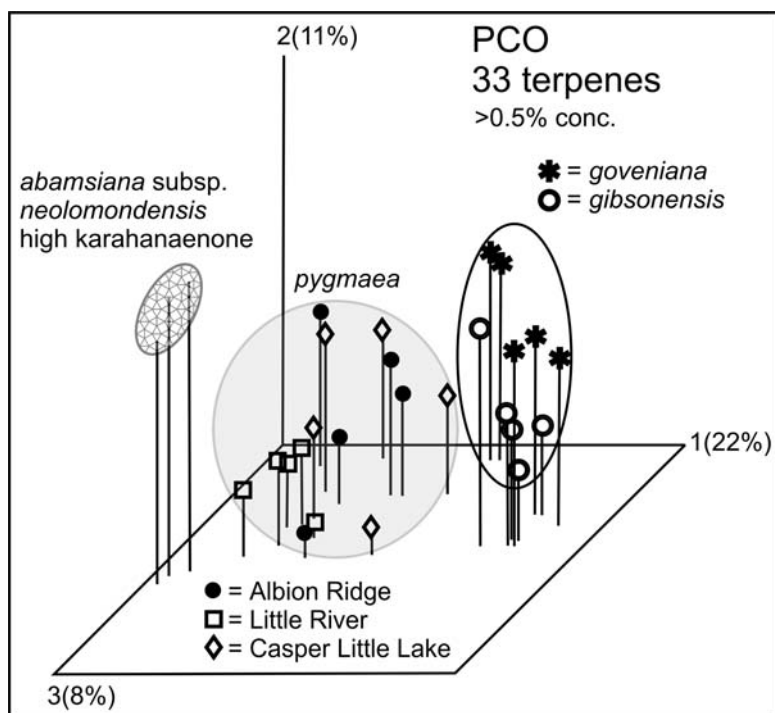


Figure 4. PCO of individuals of *H. goveniana*, putative *C. g.* subsp. *gibsonensis* and *H. pygmaea* plus three plants of putative *C. subsp. neolomondensis*, high karahanaenone type.

between *H. abramsiana* and *H. pygmaea*. Additional research using DNA markers is in progress to aid in resolving this situation. It should be noted that while each of the subspecies described by Silba (2003) are geographically isolated from one another, the individuals from the putative subspecies generally did not cluster geographically, but rather were randomly interspersed within each species as one would expect with an interbreeding population.

In summary, the leaf oils of putative *C. a.* subsp. *butanoensis*, Butano Ridge, showed some differentiation from *H. abramsiana*, Bonny Doon. The three individuals of putative *C. a.* subsp.

neolomondensis with high karahanaenone (and other components), seem intermediate to *H. pygmaea*. Additional research is needed to resolve this problem.

None of the new subspecies proposed by Silba (2003), (*C. abramsiana* subsp. *locatellii*, *C. a.* subsp. *opleri*, *C. goveniana* subsp. *gibsonensis*, *C. macrocarpa* subsp. *lobosensis*) is supported by differentiation of their volatile leaf oils, except possibly *C. a.* subsp. *butanoensis*.

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Al	compound	<i>H. abramsiana</i>					<i>H. pvg H. goveniana H. macrocarpa</i>					
		abr	loc	opl	but	neo2	neol	pyg	gov	gib	mac	lob
1025	β-phellandrene	7.8	9.4	7.3	4.0	4.9	5.2	1.5	1.8	1.5	1.1	1.0
1026	1,8-cineole	-	-	-	-	-	t	0.1	-	1.2	-	-
1044	(E)-β-ocimene	t	t	0.1	0.1	0.1	0.1	0.1	-	-	-	t
1054	γ-terpinene	3.6	4.2	5.2	4.2	3.7	2.4	3.1	7.5	6.2	5.6	5.0
1065	cis-sabinene hydrate	0.6	0.5	0.5	0.4	0.2	0.1	0.5	0.8	0.5	1.0	1.4
1073	2-methyl nonanone*	0.2	-	0.5	0.1	0.3	1.4	0.3	0.2	0.2	-	-
1086	terpinolene	2.2	3.2	2.6	2.7	1.7	1.0	1.3	2.9	2.0	2.1	1.8
1088	isobutyl tiglate	0.1	-	0.1	0.1	0.1	0.6	-	-	-	-	-
1090	6,7-epoxymenthyl acetate	-	-	-	-	-	-	-	-	-	0.2	0.1
1096	trans-sabinene hydrate	0.4	0.7	t	0.4	0.1	t	0.3	0.6	0.5	1.4	1.5
1096	linalool	1.0	t	3.1	0.1	0.1	0.9	0.7	0.6	0.1	-	-
1100	n-nonanal	t	-	t	0.1	0.1	-	0.1	0.1	0.2	t	t
1112	trans-thujone	-	-	-	0.1	0.1	0.1	0.1	0.1	0.1	-	-
1118	cis-p-menth-2-en-1-ol	0.9	0.8	1.1	0.6	0.5	0.4	0.5	0.9	0.7	0.7	0.8
1122	α-campholenal	-	-	-	-	-	-	-	-	-	0.1	0.2
1136	trans-p-menth-2-en-1-ol	0.4	0.6	0.8	0.5	0.4	0.2	0.3	0.9	0.5	0.6	0.7
1137	trans-verbenone	-	-	-	-	-	-	-	-	-	t	0.2
1141	camphor	12.4	0.1	1.5	0.6	6.5	13.5	8.7	0.2	1.8	0.3	0.1
1145	camphene hydrate	0.5	t	0.2	t	0.3	0.5	0.4	-	0.2	0.1	0.2
1154	sabina ketone	-	-	-	-	t	-	-	-	-	-	-

Al	compound	<i>H. abramsiana</i>					<i>H. pyg H. goveniana H. macrocarpa</i>					
		abr	loc	opl	but	neo2	neol	pyg	gov	gib	mac	lob
1148	citronellal	0.2	t	0.2	-	-	-	t	0.5	-	0.6	0.2
1152	(3Z)-nonen-1-ol	-	-	-	-	-	-	-	-	-	t	t
1154	karahanaenone	-	-	-	-	-	18.4	14.6	2.2	0.9	-	-
1165	borneol	-	-	-	-	-	-	t	-	0.8	0.1	0.3
1167	umbellulone	21.4	20.8	16.0	21.1	10.7	0.9	-	-	-	-	-
1174	terpinen-4-ol	12.8	11.9	16.8	9.5	9.7	6.6	9.5	15.7	13.9	11.7	14.5
1179	p-cymen-8-ol	-	-	-	0.2	0.3	t	t	0.3	t	t	0.2
1183	cryptone	0.2	0.4	0.9	0.1	-	-	-	-	-	-	-
1186	α -terpineol	1.2	0.7	1.9	0.8	0.7	1.6	3.2	0.9	0.6	0.8	0.8
1195	cis-piperitol	0.3	0.2	0.3	0.2	0.1	t	t	0.2	0.2	0.2	0.2
1198	shisofuran	0.2	0.1	0.1	0.2	0.1	-	-	-	-	-	-
1207	trans-piperitol	0.3	0.3	0.4	0.2	0.2	0.1	0.2	0.4	0.3	0.4	0.4
1223	citronellol	1.3	0.3	0.6	0.2	0.2	1.2	2.2	1.1	2.2	1.1	0.8
1232	thymol, methyl ether	0.1	0.2	0.1	0.1	0.1	-	-	-	-	-	-
1241	carvacrol, methyl ether	-	-	-	0.2	-	-	t	-	-	0.1	0.3
1249	piperitone	0.3	0.2	0.3	0.2	0.2	t	0.1	0.1	-	-	0.2
1287	bornyl acetate	0.3	-	-	0.1	0.3	0.9	-	-	0.2	-	-
1289	thymol	0.1	0.1	0.1	0.1	-	-	-	-	-	-	-
1293	2-undecanone	-	-	-	-	-	-	t	0.3	0.2	-	-
1295	3-thujanol acetate	-	-	-	0.2	0.2	1.0	0.1	-	-	-	-

Al	compound	<i>H. abramsiana</i>										<i>H. pyg H. goveniana H. macrocarpa</i>			
		abr	loc	opl	but	neo2	neol	pyg	gov	gib	mac	mac	lob		
1649	β -eudesmol	-	-	-	-	-	-	-	-	-	-	-	t	t	t
1652	α -eudesmol	-	-	-	-	-	-	-	-	-	-	-	t	t	0.1
1652	α -cadinol	-	-	-	-	-	-	-	-	-	-	-	t	t	0.1
1683	epi- α -bisabolol	0.2	t	0.5	t	t	0.3	t	0.1	0.1	-	-	-	-	-
1715	(2Z,6E)-farnesol	-	-	-	-	-	-	-	-	-	-	-	t	t	0.3
1724	(Z)-nuciferol	-	-	-	-	-	t	-	-	-	-	-	-	-	-
1754	β -(Z)-curcumen-12-ol	-	-	-	-	-	t	-	-	-	-	-	-	-	-
1896	rimuene	t	t	t	t	0.3	0.1	t	t	t	-	-	-	-	-
1900	pimara-9(11),15-diene*	0.1	0.2	0.2	0.2	0.9	0.3	1.1	0.2	0.5	0.1	0.1	0.1	0.1	0.1
1905	isopimara-9(11),15-diene	0.1	0.2	0.3	t	0.6	0.1	0.2	t	0.1	t	t	t	t	t
1925	diterpene, <u>4L</u> , 106, 257, 272	0.1	0.2	t	0.2	t	0.3	t	-	-	0.8	0.9	-	-	-
1948	pimaradiene	t	0.2	-	0.1	0.2	0.1	t	-	-	-	0.1	-	-	0.1
1960	iso-sandaracopimara-8(14),15-diene	0.2	0.7	0.3	t	t	t	t	t	0.2	0.4	-	-	-	-
1966	isophyllocladene	0.5	1.1	0.5	1.8	0.8	1.8	0.4	-	-	4.4	4.0	-	-	-
1978	manoyl oxide	0.7	0.3	1.3	0.1	2.3	1.2	0.5	0.8	2.2	t	-	-	-	-
2009	epi-13-manoyl oxide	0.1	0.1	0.2	-	0.4	0.2	0.1	t	-	-	-	-	-	-
2016	phyllocladene	0.1	0.1	0.1	0.2	-	0.2	0.1	t	0.5	0.6	0.6	0.6	0.6	0.6

AI	compound	<i>H. abramsiana</i>					<i>H. pyg</i>					<i>H. goveniana</i>			<i>H. macrocarpa</i>	
		abr	loc	opl	but	neo2	neol	pyg	gov	gib	mac	lob				
2055	abietatriene	t	0.1	t	0.2	0.2	0.1	0.1	0.1	t	0.2	0.1				
2091	iso-nezukul*	0.2	0.2	0.3	0.2	0.7	0.2	0.2	0.1	0.4	-	-				
2105	iso-abienol	-	-	-	-	-	t	t	0.1	t	-	-				
2132	nezukul	6.1	10.4	12.1	6.5	29.1	10.8	13.2	11.1	26.3	t	2.2				
2184	sandaracopimarinal	0.1	0.2	0.1	0.2	0.2	0.1	0.1	t	0.1	t	t				
2209	phylocladanol	-	-	-	-	-	-	-	-	-	2.3	1.9				
2269	sandaracopimarinal	t	0.2	t	-	-	-	-	t	-	-	t				
2282	sempervitrol	0.1	0.3	0.3	0.2	0.5	0.2	0.3	0.6	0.4	0.2	0.4				
2314	trans-totarol	t	0.3	0.1	0.4	0.4	0.3	t	0.4	0.3	0.2	0.3				
2331	trans-ferruginol	t	0.2	t	0.1	0.1	0.1	t	2.0	t	0.1	0.2				

AI = Arithmetic Index on DB-5 column. Values less than 0.05% are denoted as traces (t). Unidentified components less than 0.5% are not reported. Those compounds that appear to distinguish taxa are in boldface.