

SUPPLEMENTARY MATERIAL

Morphological and chemical analysis of male scent organs in the butterfly genus *Pyrgus* (Lepidoptera: HesperIIDae)

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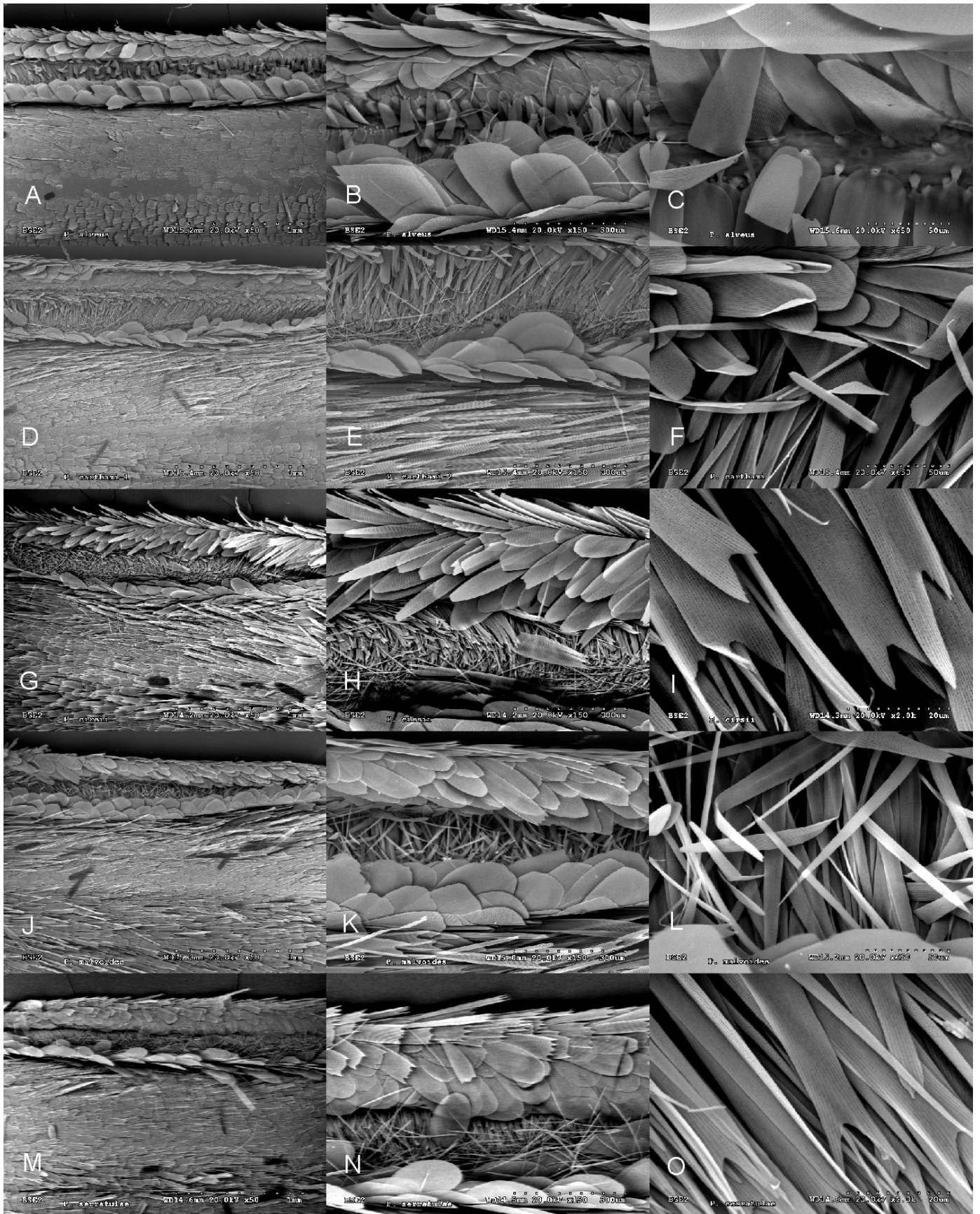


Figure S1 SEM photographs of the costal fold scent organs on the forewings of *Pyrgus* males. Dorsal view of the costal fold at lower (left) and higher magnification (center), and detail of the internal scent scales (androconia) (right) of *P. alveus* (A - C), *P. carthami* (D - F), *P. cirsii* (G - I), *P. malvoidea* (J - L) and *P. serratulae* (M - O).

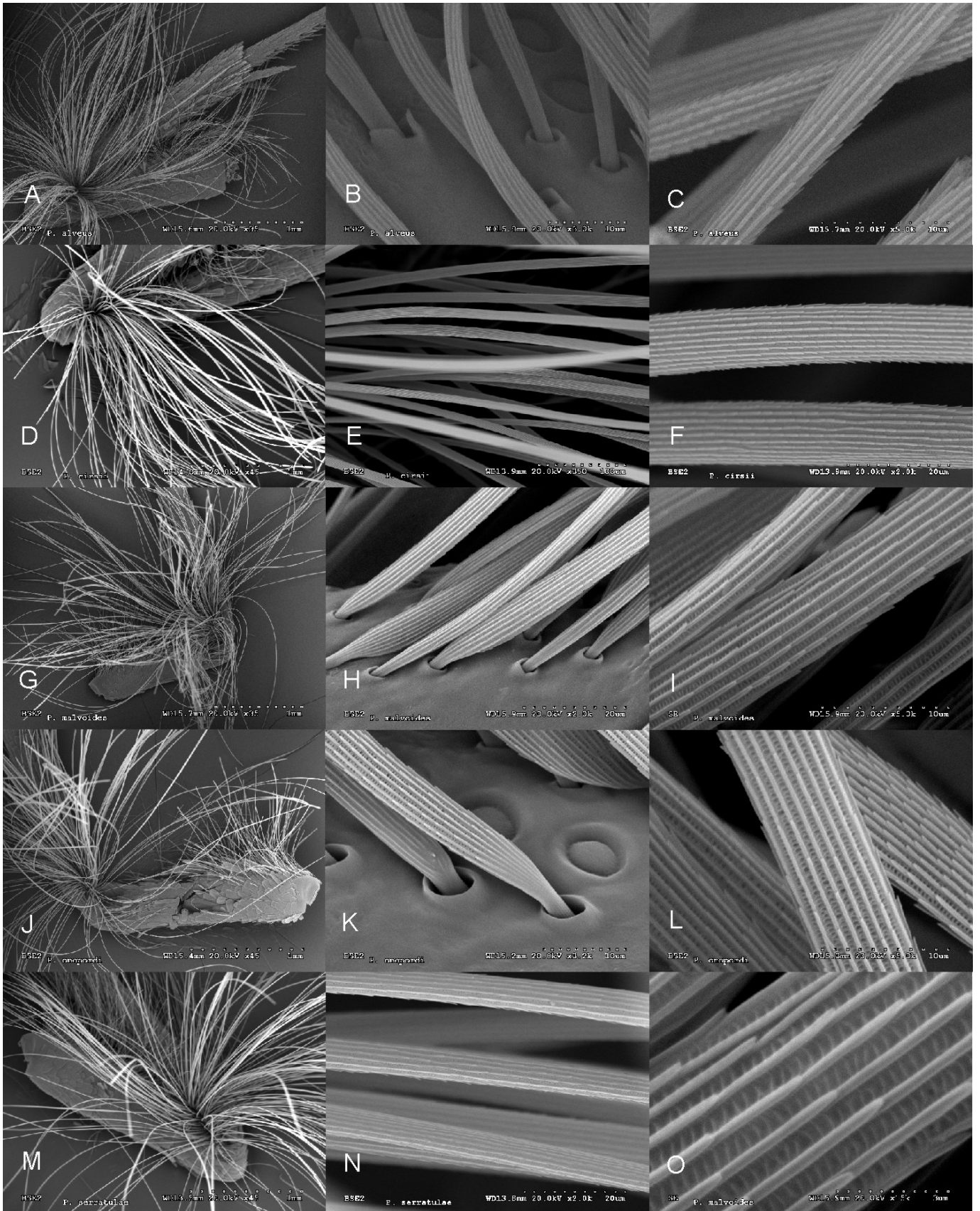


Figure S2 SEM photographs of the tibial tufts scent organs on the hind legs of *Pyrgus* males. General view of a hind leg showing the tibial tufts (left) and detailed view of the setae (center, right) of *P. alveus* (A - C), *P. cirsi* (D - F), *P. malvoides* (G - I, O), *P. onopordi* (J - L) and *P. serratulae* (M, N).

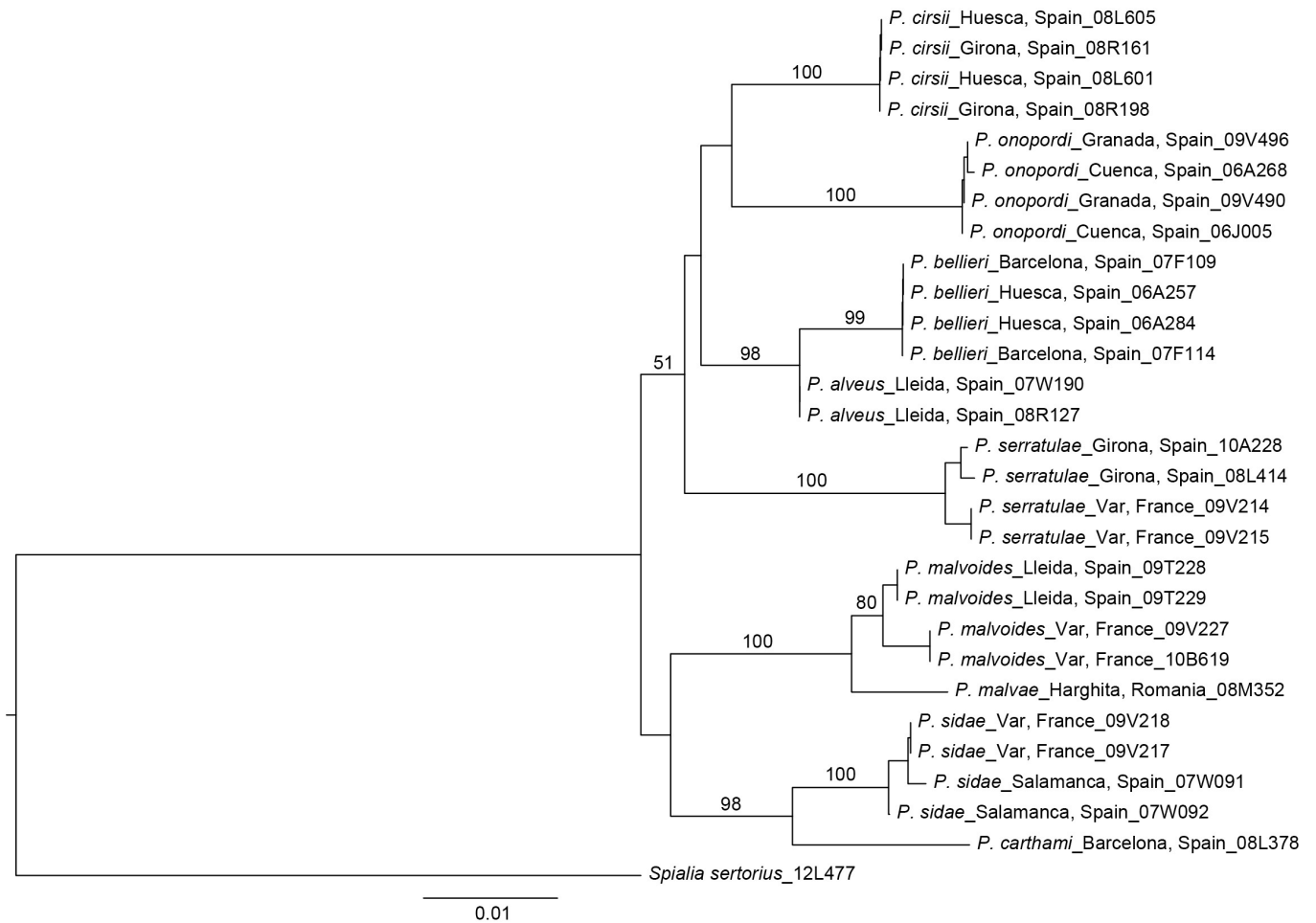


Figure S3 Inferred phylogenetic tree for the *Pyrgus* populations studied based on COI. Neighbour-joining tree with bootstrap supports (>50%) for species monophyly and deeper nodes are shown on the branches.

Table S1 List of *Pyrgus* populations studied by GC-MS. N = number of male specimens pooled in each case

Species	N	Date collected	Locality data	Province, Region	Country	Elevation (m)
<i>P. malvoides</i>	7	9.V.2009	Fonts del Cardener, La Coma, Solsonès	Lleida, Catalunya	Spain	1100
<i>P. malvoides</i>	6	19.V.2009	Domaine de la chasse de Puits de Rians, Rians	Var, Provence	France	450
<i>P. malvae</i>	7	2.VI.2009	Valea Belchia, Gheorgheni	Harghita	Romania	1000
<i>P. serratulae</i>	8	19.V.2009	Domaine de la chasse de Puits de Rians, Rians	Var, Provence	France	450
<i>P. serratulae</i>	9	11.VI.2009	Meranges, La Cerdanya	Girona, Catalunya	Spain	1700
<i>P. alveus</i>	5	2.VIII.2008	Port de la Bonaigua, Pallars Sobirà	Lleida, Catalunya	Spain	2100
<i>P. bellieri</i>	8	5.VIII.2008	El Brull, Osona	Barcelona, Catalunya	Spain	845
<i>P. bellieri</i>	9	8.VIII.2008	Río Alcanadre, Laguarda	Huesca, Aragón	Spain	1215
<i>P. cirsii</i>	9	8.VIII.2009	Río Alcanadre, Laguarda	Huesca, Aragón	Spain	1215
<i>P. cirsii</i>	8	7.VIII.2008	Llanars, Ripollès	Girona, Catalunya	Spain	1120
<i>P. onopordi</i>	9	17.VII.2009	Sierra de la Sagra	Granada, Andalucía	Spain	1670-1820
<i>P. onopordi</i>	9	24.VII.2009	Serranía de Cuenca, Tragacete	Cuenca, Castilla-La Mancha	Spain	1250
<i>P. sidae</i>	6	19.V.2009	Domaine de la chasse de Puits de Rians, Rians	Var, Provence	France	450
<i>P. sidae</i>	8	30.V.2009	Sierra de Candelario, Candelario	Salamanca, Castilla y León	Spain	1350
<i>P. carthami</i>	8	28.VI.2009	Collformic, El Brull	Barcelona, Catalunya	Spain	1170

Table S2 Spacing between transverse ribs of scent and normal scales in *Pyrgus* males. n= number of measurements.

Species	Spacing between transverse ribs (μm)	
	Scent scales	Normal scales
<i>P. alveus</i>	0.86 \pm 0.06 (n=5)	1.36 \pm 0.07 (n=3)
<i>P. carthami</i>	0.77 \pm 0.06 (n=3)	1.65 \pm 0.18 (n=7)
<i>P. cirsii</i>	0.65 \pm 0.02 (n=3)	1.49 \pm 0.05 (n=4)
<i>P. malvoides</i>	0.68 \pm 0.04 (n=3)	1.69 \pm 0.10 (n=4)
<i>P. onopordi</i>	0.67 \pm 0.06 (n=3)	1.36 \pm 0.15 (n=4)
<i>P. serratulae</i>	0.67 \pm 0.03 (n=3)	1.46 \pm 0.00 (n=3)

Table S3 Chemical compounds detected. Compounds are identified by a compound number. The reference library main match is provided only as additional information (it should not be taken as an identification). When available, the Chemical Abstracts Service (CAS) number corresponding to the library main match is indicated to avoid ambiguity. The “base peak” is the most intense peak detected, and the “experimental molecular peak” corresponds to the highest MW peak. If a chemical has been detected in more than one sample, the minimum and maximum match percentage obtained with respect to the reference library and the lowest and highest molecular weight (MW) corresponding to the experimental base and molecular peaks are indicated. Peak intensities on each total ion chromatogram (TIC) were normalised vs. the area of the most intense peak of the same TIC: 0, non-detected compounds; 1, compounds showing 1-33 % abundance compared to the reference peak; 2, compounds with 33-66 % abundance; 3, compounds with abundances above 66%.

Compound number	Detected component	Synonym	CAS No.	Empirical formula	match (%)	experimental base peak/s (MW, Da)	experimental molecular peak/s (MW, Da)	Costal fold <i>P. malvoides</i> (Solsonès, Lleida)	Tibial tufts <i>P. malvoides</i> (Solsonès, Lleida)	Costal fold <i>P. malvoides</i> (Puits de Rians, Provence)	Tibial tufts <i>P. malvoides</i> (Puits de Rians, Provence)	Costal fold <i>P. malvae</i> (Valea Belchia, Romania)	Tibial tufts <i>P. malvae</i> (Valea Belchia, Romania)	Costal fold <i>P. serratae</i> (Puits de Rians, Provence)	Tibial tufts <i>P. serratae</i> (Puits de Rians, Provence)	Costal fold <i>P. serratae</i> (Meranges, Girona)	Tibial tufts <i>P. serratae</i> (Meranges, Girona)	Costal fold <i>P. alveus</i> (Pallars Sobirà, Lleida)	Tibial tufts <i>P. alveus</i> (Pallars Sobirà, Lleida)	Costal fold <i>P. bellieri</i> (El Brull, Barcelona)	Tibial tufts <i>P. bellieri</i> (El Brull, Barcelona)	Costal fold <i>P. bellieri</i> (Laguarta, Huesca)	Tibial tufts <i>P. bellieri</i> (Laguarta, Huesca)	Costal fold <i>P. cirsii</i> (Laguarta, Huesca)	Tibial tufts <i>P. cirsii</i> (Laguarta, Huesca)	Costal fold <i>P. cirsii</i> (Llanars, Girona)	Tibial tufts <i>P. cirsii</i> (Llanars, Girona)	Costal fold <i>P. onopordi</i> (La Sagra, Granada)	Tibial tufts <i>P. onopordi</i> (La Sagra, Granada)	Costal fold <i>P. onopordi</i> (Tragacete, Cuenca)	Tibial tufts <i>P. onopordi</i> (Tragacete, Cuenca)	Costal fold <i>P. sidae</i> (Puits de Rians, Provence)	Tibial tufts <i>P. sidae</i> (Puits de Rians, Provence)	Costal fold <i>P. sidae</i> (Candelario, Salamanca)	Tibial tufts <i>P. sidae</i> (Candelario, Salamanca)	Costal fold <i>P. carthami</i> (El Brull, Barcelona)	Tibial tufts <i>P. carthami</i> (El Brull, Barcelona)						
1	2,3-butanediol		513-85-9	C4H10O2	78-82	45 77		0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0		
2	2,3-dimethyl-2,3-butanediol	pinacol	76-09-5	C6H14O2	77	59 85		0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0
3	(Z)-alpha-5-(2,3-dimethyltricyclo(2.2.1.0(2,6))hept-3-yl)-2-methyl-2-penten-1-ol	alpha santalol	115-71-9	C15H24O	50-79	93, 107 202, 220		0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 3	0 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0
4	1-docosanol	behenyl alcohol	661-19-8	C22H46O	83	41 326		0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0
5	1-pentadecanol		629-76-5	C15H32O	71	83 157		0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0
6	4,9,13,17-tetramethyl-4,8,12,16-octadecatetraen-1-ol		56882-10-1	C22H38O	71-86	95 149		0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 1	0 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 3	0 3	0 0	0 0	0 0	0 0	0 0	0 0	0 0
7	1-octanol		111-87-5	C8H18O	87-90	32, 55 84, 97		0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 1	0 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0

8	3-pentyl-2,4-pentadien-1-ol	3-ethenyl-2-octen-1-ol	666841-70-9	C10H18O	78	69	125	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0
9	benzyl alcohol	alpha toluenol	100-51-6	C7H8O	92	79	107	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	(E,E,E)-3,7,11,15-tetramethyl-2,6,10,14-hexadecatetraen-1-ol	geranylgeraniol	7614-21-3	C20H34O	83-85	41, 69	189, 205	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	3,7-dimethyl-6-octen-1-ol	citronellol or dihydrogeraniol	106-22-9	C10H20O	83	59	123	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	5,9,13-trimethyl-4,8,12-tetradecatrien-1-ol			C17H30O	83-87	81	163	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
13	3,7,11-trimethyl-2,6,10-dodecatrien-1-ol	farnesol	4602-84-0	C15H26O	75-88	69, 93	161, 205	0	3	0	3	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0
14	(2R)-6-methyl-2-[(1R)-4-methyl-1-cyclohex-3-enyl]hept-5-en-2-ol	alpha bisabolol	515-69-5	C15H26O	71-87	109, 119	138, 204	0	1	0	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
15	3,6,6-trimethylbicyclo[3.1.1]heptan-2-ol	3,6,6-trimethyl-2-norpinanol	29548-09-2	C10H18O	92	85	129	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
16	4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-ol	verbenol	473-67-6	C10H16O	86	81	137	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	2-methyl-5-(1-methylethenyl)-cyclohexanol	dihydrocarveol	619-01-2	C10H18O	79-82	83, 95	154	0	0	0	0	0	0	2	0	2	0	0	0	0	0	0	0	0	1	0
18	(3β)-lanosta-8,24-dien-3-ol	lanosterol	79-63-0	C30H50O	77	69	272	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0
19	(4aS,7S)-2,3,4,4a,5,6,7,8-octahydro-1,1,4a,7-tetramethyl-1H-benzocyclohepten-7-ol	widdrol	6892-80-4	C15H26O	71	96	222	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0
20	tricyclo[4.3.1.1.3,8]undecan-3-ol		14504-80-4	C11H18O	82	96	166	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
21	(6E)-3,7,11-trimethyl-1,6,10-dodecatrien-3-ol	(E)-nerolidol	40716-66-3	C15H26O	83-91	69, 93	161, 204	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0
22	2,3-dimethyl-1-undecen-3-ol		959050-83-0	C13H26O	92-93	85	85	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	2	0
23	3,7-dimethyl-1,6-octadien-3-ol	linalool	78-70-6	C10H18O	78	93	132	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24	(1R,3E,7E,11R,12R)-bicyclo[9.3.1]-4,8,12,15,15-pentamethylpentadeca-3,7-dien-12-ol	verticiol	70000-19-0	C20H34O	75-76	81	257	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	3	0
25	3-methylbutanal	isopentanal or isovaleral	590-86-3	C5H10O	80-82	41	86	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26	2-methylbutanal	2-methylbutyraldehyde	96-17-3	C5H10O	82	41	86	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

