

## A combined molecular and morphological approach to the taxonomically intricate European mountain plant *Papaver alpinum* s.l. (Papaveraceae)—taxa or informal phylogeographical groups?

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*Papaver alpinum* s.l. is an extraordinarily polymorphic taxon distributed throughout southern and central European mountain ranges. We tested previous hypotheses about relationships and taxonomical status of the numerous described taxa within this species or species group by applying different molecular approaches. In addition we re-evaluated morphological characters used in previous taxonomic treatments in the light of the molecular results. The ancestral sequence haplotypes were widespread and dominant throughout the Alps, whereas peripheral populations in other mountain ranges were often characterised by haplotypes directly derived from the central haplotypes involving one or two mutational steps. The AFLP data corroborated the pattern of a ‘centrifugal radiation’ and additionally showed that most populations were genetically distinct, presumably due to the effect of genetic drift in small and isolated populations. The morphological data did not reveal clear patterns of variation; only the Pyrenean and Sierra Nevada populations differed in two non-overlapping and presumably independent characters. Altogether, our study implies that most previous taxonomic concepts of *P. alpinum* s.l. were highly artificial, and that either nearly all populations have to be raised to some taxonomic rank or that, preferably, no infraspecific taxa should be recognised at all. The only segregate possibly deserving taxonomic rank, based on both morphology and genetics, is the Iberian *P. alpinum* subsp. *lapeyrousianum*.

**KEYWORDS:** AFLPs, central and southern European mountain ranges, low-copy nuclear region *RPA2*, morphology, *Papaver alpinum* s.l., phylogeography, plastid region *psbE-petL*, taxonomy

### INTRODUCTION

The Alpine poppy, *Papaver alpinum* L., belongs to the monophyletic *P. sect. Meconella* Spach, which is sister to the Himalayan representatives of *Meconopsis* Vig. (Carolan & al., 2006). *Papaver sect. Meconella* has a circumpolar arctic distribution, but also occurs in the alpine and subalpine belts of several temperate mountain ranges, as well as in steppes and river valleys of Central and northern Asia (e.g., Rändel, 1974; Hultén & Fries, 1986; Peschkova, 1994). The section is absent from the western Asian mountains and the Caucasus. Whereas the arctic distribution is nearly continuous, the temperate taxa often occupy disjunct ranges in mountain areas, as is the case for the central and southern European *P. alpinum*.

*Papaver alpinum* is diploid (summarised in Jalas & Suominen, 1991), a ploidy level documented in other species from Central Asia, Siberia, Beringia and the central Rocky Mountains (e.g., Hanelt, 1969; Rändel, 1974; Peschkova, 1994; Kiger & Murray, 1997; Petrovsky, 1999). The geographically closest relatives of *P. alpinum* are found in

Scandinavia and the western Eurasian Arctic (*P. dahlianum* Nordh., *P. lapponicum* (Tolm.) Nordh., *P. radicans* Rottb.), all being high polyploids. There is a large gap to the temperate relatives in the alpine belt of Asian Pamir and Tian-Shan (mainly *P. croceum* Ledeb. s.l. and *P. nudicaule* L. s.l.) and further east. The morphologically most similar species has been suggested to be the northeastern Asian *P. nivale* Tolm. (Rändel, 1974).

Analyses of DNA sequences (plastid, nuclear ribosomal, low-copy nuclear; Solstad & al., unpub.) show that *P. alpinum* is monophyletic. Establishment of sister-group relationships is more complicated as even analyses including only the diploid species of *P. sect. Meconella* revealed incongruent phylogenies for the different DNA regions. They were, however, congruent in indicating that the closest relatives are Asian low-ploid taxa. AFLP data (Solstad & al., unpub.) indicate that *P. alpinum* s.l. is distantly related to the northern European polyploid species and rather connects to low-ploid northeastern Asian and amphi-Pacific/Beringian species (*P. nivale* was not included).

*Papaver alpinum* is distributed throughout most of the major mountain areas of southern and Central Europe (Markgraf, 1958a; Jalas & Suominen, 1991; Bittkau & Kadereit, 2003; Aeschimann & al., 2004). The distribution is relatively continuous in the Alps (France, Switzerland, Germany, Italy, Austria, Slovenia); most populations and population groups are, however, more or less isolated due to preference for sparsely vegetated, mostly calcareous scree of varying degree of mobility. Geographically isolated population groups are found in the Sierra Nevada (southern Spain), the Pyrenees (Spain, France), the Apennines (the Gran Sasso area in Abruzzo, central Italy), the Tatras in the Western Carpathians (Poland, Slovakia), the Southern and Eastern Carpathians (Romania), the Pirin (Bulgaria), and the southern Dinaric Mountains (Bosnia and Hercegovina, Montenegro).

*Papaver alpinum* is extraordinarily polymorphic with respect to vegetative, floral and fruit characters. Variation can be encountered in, e.g., firmness of the basal tunica; leaf glaucescence and dissection; shape and symmetry of terminal leaf segments; presence and density of leaf indumentum; orientation and density of scape indumentum; colour and length of hairs on buds; petal colour, shape and degree of overlap; length of stamens relative to gynoeceum; shape of capsule and stigmatic disc; and number of stigmatic rays as well as degree of their decurrence. As easily observed morphological differences show some geographical pattern, they were early assigned taxonomic importance. Examples of characters used as diagnostic in previous treatments (e.g., Markgraf, 1958a; Mowat & Walters, 1964) are given in Table 1. In the last 250 years, numerous names at specific or subspecific levels have been coined for the different populations (summary in Markgraf, 1958a). Thirteen of these represent combined morphological and geographical entities that have been assigned specific or subspecific rank in recent accounts (Appendix 1\*; for keys, description and more information, see especially Markgraf 1958a). Here we apply the names without ranks in the geographical meanings given in Appendix 2.

Studies of *P. alpinum* in the last 50 years have reached strikingly different conclusions as regards subdivision of the complex and taxonomic ranks of the entities. Six species and several subspecies were accepted by Mowat & Walters (1964) in the first edition of *Flora Europaea*, and they informally commented on some additional subspecies. Two species were accepted by Markgraf (1958a), an Iberian species as *P. suaveolens* Lapeyr., and *P. alpinum* with eight subspecies, six of them in the Alps (Markgraf, 1958a). This approach was followed by Jalas & Suominen (1991) in *Atlas Florae Europaeae* with only small modifications (e.g., tentative indication of *P. occidentale*

**Table 1. Morphological characters studied in *Papaver alpinum* s.l.**

Char. No.	Character
1	Leaf sheath tunica: lax (1); medium (2); firm (3)
2	Blade colour: green (1); slightly glaucous (2); strongly glaucous (3)
3	Blade indumentum: glabrous or subglabrous (1); sparse (2); moderate or dense (3)
4	Blade dissection: number of times divided (1; 2; 3)
5	Main segment attachment: opposite (1); subopposite (2); alternate (3)
6	Terminal segment shape: linear or strap-shaped (1); (ob)lanceolate (2); (ob)ovate (3)
7	Terminal segment symmetry: symmetrical (1); slightly asymmetrical, broader on proximal side of midvein (2); distinctly asymmetrical, much broader on proximal side of midvein (3)
8	Terminal segment direction: divergent from main axis of main segment (1); straight (2); convergent towards main axis of main segment (3)
9	Scape hair density: sparse (1); moderate, hairs slightly overlapping (2); dense, hairs strongly overlapping (3)
10	Scape hair direction: subappressed (1); ascending to subpatent (2); patent (3)
11	Bud shape: globular or subglobular (1); ovoid (2); ellipsoid (3)
12	Bud indumentum colour: pale, not contrasting with scape (1); medium, some contrast with scape (2); dark, strong contrast with scape (3)
13	Petal length (broadest pair), mm
14	Petal width (broadest pair), mm
15	Petal overlap: none (1); contiguous or overlapping (2)
16	Petal colour: white (1); yellow (2)
17	Stamens compared with ovary: shorter (1); equal (2); longer (3)
18	Capsule widest part: at middle (1); between middle and upper third (2); within uppermost third (3)
19	Capsule length (below stigmatic disc): broad towards top (1); slightly constricted (2); strongly constricted (3)
20	Capsule (without stigmatic disc), mm
21	Capsule maximum width, mm
22	Capsule setae density: sparse, <10 per valve (1); moderate, 10–20 per valve (2); dense, >20 per valve (3)
23	Stigmatic disc shape: broad and convex (1); broad and slightly peaked (2); narrow and strongly peaked (3)
24	Decurrence of stigmatic rays on capsule; decurrence: none or slight, <5% of fruit length (1); 5%–20% of capsule length (2); >20% of capsule length (3)
25	Number of stigmatic rays: 4 (1); 5 (2); 6(–7) (3)

\*For all Appendices see the online version of this article.

(Markgr.) H.E. Hess & Landolt and *P. victoris* Škornik & Wraber as additional taxa). Kadereit (1990) accepted only one species with eight subspecies, among them the Iberian subsp. *lapeyrousianum* (Greuter & Burdet) Kerguélen. In the second edition of *Flora Europaea*, Kadereit (1993) accepted *P. lapeyrousianum* and *P. alpinum* as two species but did not accept subspecies of *P. alpinum*. Aeschimann & al. (2004) accepted seven species for the Alps alone.

The taxonomic treatment of some entities has been controversial (see also Appendix 1). Kadereit (1990) merged the *P. occidentale* populations in the northwestern Alps and the *P. alpinum* subsp. *tatricum* A. Nyár. s.str. populations in the Tatra in one taxon, whereas Markgraf (1958a) and Jalas & Suominen (1991) suggested that *P. tatricum* and *P. occidentale* should be treated at least as different varieties. Markgraf (1958a) and Jalas & Suominen (1991) reported two sympatric taxa in Abruzzo, central Italy: *P. ernesti-mayeri* (Markgr.) Wraber (otherwise in the southeastern Alps) and *P. alpinum* subsp. *degenii* (Urum. & Jár.) Markgr. (typified from Pirin in Bulgaria), whereas Kadereit (1990) accepted only *P. ernesti-mayeri* in the Abruzzo area. Plants in the southwestern Alps were originally described as *P. aurantiacum* Loisel. but were included by most later authors in subsp. *rhaeticum* (Leresche) Markgr. (Markgraf, 1958a, b; Kadereit, 1990; Jalas & Suominen, 1991). The name *P. aurantiacum* has priority if *P. aurantiacum* and *P. rhaeticum* are merged at species level (Aeschimann & al., 2004).

An attempt to resolve the taxonomy and biogeography of *P. alpinum* s.l. by studying RAPD variation throughout its range was judged by Bittkau & Kadereit (2003) as preliminary. Nevertheless, they concluded that most of the taxa were not monophyletic but instead belonged to five geographic groups, none of which had any bootstrap support: (1) the Iberian regions and the southwestern Alps (*lapeyrousianum* and *aurantiacum*); (2) the Gran Sasso area in Abruzzo, central Italy (plants assigned to *degenii* and/or *ernesti-mayeri*); (3) the western and northwestern Alps (*occidentale* and western parts of *sendtneri*); (4) the northeastern Alps and the Tatra (eastern parts of *sendtneri*, *alpinum*, and *tatricum* s.str.); and (5) the southeastern Alps, and the Carpathians (*rhaeticum* s.str., *kernerii*, *ernesti-mayeri* s.str., *corona-sancti-stephani*). Neither plants belonging to *victoris*, nor any populations from the Dinaric Mountains or the Pirin (*kernerii*, *degenii*) were included. Kropf & al. (2006) investigated phylogeographic relationships among populations of *P. alpinum* from the Spanish Sierra Nevada, the Pyrenees and the south-western Alps using AFLP markers. Plants in the different mountain ranges were resolved as monophyletic, except for one population from the western Pyrenees, which clustered with the Sierra Nevada population.

The advent of highly polymorphic and reproducible molecular markers such as Amplified Fragment Length

Polymorphism (AFLP; Vos & al., 1995), together with the development of sensitive Bayesian clustering techniques (Pritchard & al., 2000; Falush & al., 2007), form a solid basis for re-investigating systematics and biogeography of *P. alpinum*. By applying DNA sequencing (one plastid region and one low-copy nuclear region) and AFLP fingerprinting, we aim: (1) to test the results of Bittkau & Kadereit (2003) with an extended taxonomic sampling including a higher number of individuals per population; (2) to correlate the molecular data with morphological characters scored on DNA vouchers and supplementary specimens; (3) to discuss our data with respect to the growing body of literature exploring the phylogeography of southern European mountain ranges; and (4) to draw taxonomic conclusions.

## MATERIAL AND METHODS

**Sampling and DNA extraction.** — Leaf material from five plants (exceptions are populations L2, U4 and K27 with three, four and one collected individual, respectively) from 30 populations of *P. alpinum* s.l. (Fig. 1; Table 2) was sampled and dried in silica gel. All taxa proposed at species or subspecies level, except for *P. alpinum* subsp. *fatraemagnae* Bernát., are present with at least one population. The Dinaric populations are coded as *kernerii*, but it was difficult to separate morphologically population K27 from *rhaeticum* from the Alps. Voucher specimens are deposited at the Institute of Botany, University of Vienna, Austria (WU), the Botanical Museum, University of Oslo, Norway (O), and the Natural History Museum, Rijeka, Croatia. Total genomic DNA was extracted from dried tissue (ca. 10 mg) following a CTAB-protocol (Doyle & Doyle, 1987) with a few modifications: after precipitation with isopropanol and subsequent centrifugation, the DNA pellet was washed in 70% ethanol, dried at 37°C and re-suspended in TE-buffer. Extracts of population K29 were strongly coloured and were therefore re-extracted with the modified CTAB-protocol of Tel-Zur & al. (1999) including three washing steps with sorbitol buffer. Five samples from other populations were extracted with both protocols to detect possible influence of the extraction protocol on the AFLP banding pattern. The quality of the extracted DNA was checked on 1% TAE-agarose gels.

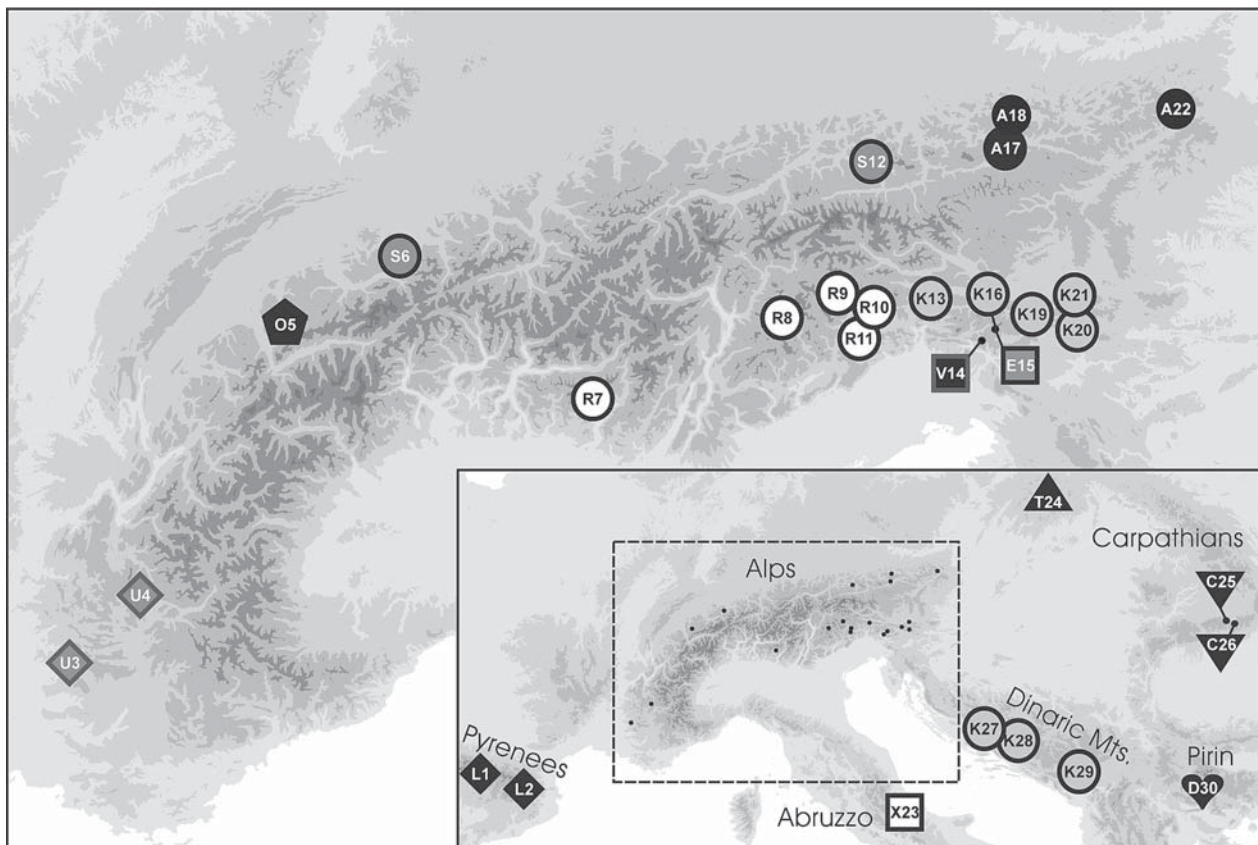
**AFLPs.** — The AFLP procedure followed Vos & al. (1995) with modifications. Total DNA was digested with *EcoRI* and *MseI* and ligated to double-stranded *EcoRI* and *MseI* adapters in one step at 37°C for 3 hrs, followed by 17°C overnight. The reaction mix (final volume 12 µl) contained 1.2 µl T4 DNA ligase buffer (Promega, Madison, Wisconsin, U.S.A.), 0.6 µl BSA (1 mg/ml; New England Biolabs, Beverly, Massachusetts, U.S.A.), 1.2 µl

0.5 M NaCl, 1 U *Mse*I (New England Biolabs), 5 U *Eco*RI (Promega), 0.6 U T4 DNA ligase (Promega), 1  $\mu$ l 50  $\mu$ M *Mse*I-adapters (genXpress, Wiener Neudorf, Austria) and 1  $\mu$ l 5  $\mu$ M *Eco*RI adapters (genXpress) and 6  $\mu$ l template DNA. Ligated DNA fragments were diluted 10-fold.

Both preselective amplification and selective amplification were carried out in a volume of 10  $\mu$ l in a thermocycler (GeneAmp PCR System 9700, Applied Biosystems, Foster City, California, U.S.A.). Cycling protocols followed Vos & al. (1995). The reaction mix for the preselective amplification contained 1.14  $\mu$ l 10 $\times$  RedTaq PCR Reaction buffer (Sigma, St. Louis, Missouri, U.S.A.), 0.2 U RedTaq, 0.22  $\mu$ l dNTPs (10 mM; Applied Biosystems), 0.58  $\mu$ l preselective primers (5  $\mu$ M; genXpress) and 2  $\mu$ l diluted product from the restriction/ligation step. The PCR product was diluted 10-fold. Initially selective primers were screened using twelve selective primer combinations. The five final primer combinations for the selective PCR (fluorescent dye in brackets) were *Eco*RI (6-Fam)-ACA/*Mse*I-CAC, *Eco*RI (VIC)AGG/*Mse*I CTG, *Eco*RI (NED)

ACC/*Mse*I-CAG, *Eco*RI (6-Fam)-ATC/*Mse*I-CTG, and *Eco*RI (NED)AAC/*Mse*I CAT. The reaction mixture for the selective amplification contained 1  $\mu$ l 10 $\times$  RedTaq PCR Reaction buffer (Sigma), 0.2 U RedTaq, 0.22  $\mu$ l dNTPs (10 mM; Applied Biosystems), 0.54  $\mu$ l of each selective primer (*Mse*I-primer: 5  $\mu$ M, genXpress; *Eco*RI-primer: 1  $\mu$ M, Applied Biosystems) and 2  $\mu$ l diluted product of the preselective amplification. 5  $\mu$ l of each selective PCR product were purified using Sephadex G-50 Superfine (GE Healthcare Bio-Sciences, Uppsala, Sweden) applied to a Multi Screen-HV plate (Millipore, Molsheim, France). 1.2  $\mu$ l of the elution product was mixed with 10  $\mu$ l formamide (Applied Biosystems) and 0.1  $\mu$ l GeneScan 500 ROX (Applied Biosystems) and run on an ABI 3130x automated capillary sequencer. Eleven individuals were replicated to calculate the error rate according to Bonin & al. (2004) and to exclude non-reproducible fragments from the analysis.

**AFLP analyses.** — Raw AFLP data were aligned with the internal size standard using ABI Prism GeneScan 3.7.1 (Applied Biosystems), and imported into



**Fig. 1.** Sampled populations of *Papaver alpinum* s.l. Symbols and letters indicate taxonomy: *alpinum* (A), black dots; *aurantiacum* (U), grey diamonds; *corona-sancti-stephani* (C), down-pointing black triangles; *degenii* (D), black heart; *ernestimayeri* (E), grey square; *kernerii* (K), light grey dots; *lapeyrousianum* (L), black diamonds; *occidentale* (O), black pentagon; *rhaeticum* (R), white dots; *sendtneri* (S), dark grey dots; *taticum* (T), black triangle; *victoris* (V), black square. The population from Abruzzo (X), central Italy, with uncertain taxonomic affiliation (*degenii* or *ernestimayeri*, see text) is indicated with a white square. See Table 2 for information about the sampled populations.

Table 2. Population codes and sampling localities for the investigated populations of *Papaver alpinum* s.l.

Code	Country	Sampling locality	Long./Lat.	Coll. No.	N	% <sub>poly</sub>	D	DW	H <sub>cp</sub>	H <sub>RPA2</sub>	GenBank accession No.
L1	E	Pyrenees: Pirineo Aragonés; Picos de Ballibierna	0.66/42.59	S&T 8843	5	1.9	0.010	5.70	I	II	FJ767970, FJ767940
L2	E	Pyrenees: Pirineo Catalán; Puigmal d'Err	2.13/42.38	S&T 8820	3	0.2	0.001	3.33	I	III	FJ767971, FJ767941
U3	F	Alps: Vacluse; Mont Ventoux	5.31/44.16	S&T 8913	5	11.3	0.057	3.27	I	II	FJ767972, FJ767942
U4	F	Alps: Dévoluy; Pic de Bure	5.93/44.63	S&T 9366	4	14.0	0.077	4.46	II	II	FJ767973, FJ767943
O5	CH	Alps: Waadter Alpen; Gummfluh	7.17/46.44	S&T 10910	5	11.1	0.055	2.84	II	II	FJ767974, FJ767944
S6	CH	Alps: Luzerner Alpen; Pilatus	8.24/46.89	S&T 9384	5	15.4	0.075	3.40	II	II	FJ767975, FJ767945
R7	I	Alps: Alpi Bergamasche; Monte Ferranto	10.03/45.97	S&T 8946	5	12.1	0.058	3.90	II	II	FJ767976, FJ767946
R8	I	Alps: Dolomiten; Passo Pordoi	11.82/46.48	S&T 9420	5	17.2	0.082	3.16	II	II	FJ767977, FJ767947
R9	I	Alps: Dolomiten; Büttelejoch	12.32/46.63	No voucher	5	18.3	0.091	3.38	II	IV	FJ767978, FJ767948
R10	I	Alps: Alpi Carniche; Monte Pramaggiore	12.56/46.37	S&T 9416	5	19.3	0.097	4.14	II	II	FJ767979, FJ767949
R11	I	Alps: Prealpi Carniche; Monte Tiarfin	12.59/46.46	S&T 8948	5	19.5	0.093	3.14	II	II	FJ767980, FJ767950
S12	A	Alps: Leoganger Steinberge; Griessener Rotschartl	12.69/47.48	S&T 9245	5	19.5	0.097	4.62	II	II	FJ767981, FJ767951
K13	A	Alps: Karnische Alpen; Trogkofel	13.22/46.57	S&T 9466	5	16.0	0.080	3.73	II	V	FJ767982, FJ767952
V14	SI	Alps: Julijiske Alpe; Krnsko jezero	13.68/46.28	S&T 11065	5	18.5	0.092	3.07	II	II	FJ767983, FJ767953
E15	SI	Alps: Julijiske Alpe; Hribarice	13.81/46.35	S&T 11061	5	21.6	0.109	2.45	II	II	FJ767984, FJ767954
K16	SI	Alps: Julijiske Alpe; Hribarice	13.81/46.35	S&T 11060	5	21.2	0.106	3.48	II	II	FJ767985, FJ767955
A17	A	Alps: Nordöstliche Kalkalpen; Grimming	14.01/47.52	S&T 11395	5	13.8	0.068	3.02	II	II	FJ767986, FJ767956
A18	A	Alps: Totes Gebirge; Spitzmauer	14.06/47.70	No voucher	5	18.1	0.089	3.10	II	VI	FJ767987, FJ767957
K19	A	Alps: Karawanken/Karavanke; Koschuta/Košuta	14.31/46.43	S&T 9145	5	16.4	0.083	3.53	II	II	FJ767988, FJ767958
K20	SI	Alps: Karnische Alpe; Skuta	14.56/46.36	S&T 9673	5	18.9	0.093	2.54	II	I	FJ767989, FJ767959
K21	A	Alps: Karawanken/Karavanke; Samtaler Sattel/Savinjsko sedlo	14.57/46.54	S&T 11049	5	16.0	0.080	2.81	II	I	FJ767990, FJ767960
A22	A	Alps: Nordöstliche Kalkalpen; Rax	15.67/47.69	S&T 8799	5	17.2	0.083	3.89	II	I	FJ767991, FJ767961
X23	I	Abruzzo: Majella; Monte Amaro	14.06/42.10	S&T 8933	5	16.0	0.077	4.82	II	IX	FJ767992, FJ767962
T24	PL	Carpathians: Vysoke Tatry; uppermost Litworowa dolina	19.91/49.23	S&T 10588	5	15.0	0.071	2.78	II	I	FJ767993, FJ767963
C25	RO	Carpathians: Piatra Craiului; Refugio Spiirla	25.18/45.56	S&T 10521	5	15.4	0.075	3.22	III	VII	FJ767994, FJ767964
C26	RO	Carpathians: Muntii Bucegi; Omu	25.45/45.44	S&T 10515	5	11.7	0.055	3.50	III	VII	FJ767995, FJ767965
K27	BiH	Dinaric Mountains: Dinara; Veliki Troglav	16.59/43.95	NHMR475/ PMR08876	1	—	—	—	—	II	FJ767996, FJ767966
K28	BiH	Dinaric Mountains: Čvrsnica; Veliki Vilinac	17.63/43.63	NHMR177/ PMR08595	5	13.6	0.067	4.07	II	II	FJ767997, FJ767967
K29	MNE	Dinaric Mountains: Komovi; Kom Vasojevički	19.68/42.69	NHMR 878/ PMR09275	5	10.3	0.051	5.06	II	II	FJ767998, FJ767968
D30	BU	Pirin; Vihren	23.41/41.76	S&T 11338	5	15.6	0.083	3.99	II	VIII	FJ767999, FJ767969

Coll. No., collection number (S&T; herbarium Schönswetter & Tribsch stored in WU; NHMR: Natural History Museum Rijeka, Croatia); N, number of individuals investigated with AFLP; %<sub>poly</sub>, percentage of polymorphic AFLP fragments; D, genetic diversity (Kosman, 2003); DW, frequency-down-weighted marker values according to Schönswetter & Tribsch (2005) with the modification of Ehrich & al. (2007); H<sub>cp</sub>, cpDNA haplotype derived from *psbE-petL* sequences; H<sub>RPA2</sub>, haplotype derived from *RPA2* sequences. In the column 'GenBank accession No.', the first number refers to the *psbE-petL* sequence and the second to the *RPA2* sequence.

Genographer v.1.6.0 (available at <http://hordeum.oscs.montana.edu/genographer>) for scoring. The error rate (Bonin & al., 2004) was calculated as the ratio of mismatches (scoring of 0 vs. 1) over matches (1 vs. 1) in AFLP profiles of replicated individuals.

The percentage of AFLP markers exhibiting intrapopulation variation was calculated, as well as genetic diversity estimated as the average number of pairwise differences among genotypes (Kosman, 2003). In order to quantify the number of rare markers without setting an arbitrary threshold, frequency-down-weighted marker values (DW) were calculated according to Schönswetter & Tribsch (2005). We used the average of individual values obtained in a population in order to diminish the effect of differences in sample size following Ehrich & al. (2007). Calculations were carried out using the R-script (R Development Core Team, 2004) AFLPdat (Ehrich, 2006). Analyses of molecular variance (AMOVAs) were conducted with Arlequin 3.11 (Excoffier & al., 2005).

Using the program SplitsTree4 ver. 4.6 (Huson & Bryant, 2006), a NeighbourNet (NNet) was calculated based on a matrix of uncorrected P-distances. A Neighbour-joining (NJ) analysis based on a matrix of Nei-Li distances (Nei & Li, 1979) including 2,000 pseudo-replicates was conducted in TreeCon 1.3b (van de Peer & de Wachter, 1997). Principal Coordinate Analyses (PCoAs) based on a matrix of Jaccard distances among individuals were calculated using NTSYS-pc 2.0 (Rohlf, 1998).

The software STRUCTURE 2.2 with a Bayesian clustering approach developed for dominant markers (Pritchard & al., 2000; Falush & al., 2007) was used with an admixture model with uncorrelated allele frequencies and recessive alleles. Ten replicate runs for each K (number of groups) ranging from 1 to 10 were carried out at the Biportal of the University of Oslo (<http://www.biportal.uio.no/>), using a burn-in of 100,000 iterations followed by 1,000,000 additional MCMC iterations. For comparison, we also ran a no-admixture model with the same MCMC parameters. Similarity among results of different runs for the same K was calculated according to Nordborg & al. (2005) using AFLPsum (Ehrich, 2006). We identified the optimal number of main groups as the value of K when the increase in likelihood started to flatten out; the results of replicate runs were identical, and gave no empty groups. The replicate runs of the best K were merged with CLUMPP 1.1.1.1 (Jakobsson & Rosenberg, 2007) using the full-search algorithm. The relative 'cluster membership coefficients' of all individuals were then averaged for each population. The genetic structure is often hierarchical in complex datasets, and several numbers of groups can be appropriate (Rosenberg & al., 2002). STRUCTURE was therefore run with an admixture model with uncorrelated allele frequencies and with recessive alleles to explore the substructure of the two major clusters.

**Sequencing of plastid DNA.** — One region of the plastid genome, the *psbE-petL* spacer (which has also been used for a phylogenetic study of *Papaver* sect. *Meconella*; Solstad & al., unpub.) was sequenced from one individual per population (five and four individuals, respectively, from populations U3 and U4) using the primer pair *psbE-RF* and *petL-R* (Popp & al., 2005). PCR conditions were 5 min at 95°C followed by 35 cycles of 45 s at 95°C, 45 s at 50°C and 90 s at 72°C, followed by 5 min at 72°C. Reaction volumes were 25 µl, comprising 9 µl REDTaq ReadyMix PCR Reaction Mix (Sigma-Aldrich, Vienna, Austria), 1 µl of 1 : 10 diluted template DNA of unknown concentration, 1 µl of each primer (10 µM; genXpress), and 1 µl BSA (1 mg/ml; New England Biolabs). The PCR products were cleaned with Exonuclease I and Calf Intestine Alkaline Phosphatase (Fermentas, St. Leon-Rot, Germany) according to the manufacturer's instructions. All reactions were carried out on a GeneAmp 9700 thermocycler (Applied Biosystems). BigDye Terminator chemistry (Applied Biosystems) was used according to the manufacturer's instructions for cycle sequencing following electrophoresis with an ABI 3130x capillary sequencer (Applied Biosystems).

Indels larger than 1 bp were coded as single characters. No phylogenetic analyses were performed as only three haplotypes were found.

**Sequencing of nuclear DNA.** — The low-copy nuclear *RPA2* intron region of the RNA polymerase gene family (which has been used for a phylogenetic study of *Papaver* sect. *Meconella*; Solstad & al., unpub.) was amplified for diploid species of *Papaver* sect. *Meconella* using the degenerated subunit specific primer pairs A2F and A2Ra (Popp & Oxelman, 2004). The sequences obtained were used to design *Papaver* specific primers pairs: PAP-A2-F3: CAT GCC TTC CCT TCT AGA ATG and PAP-A2-R: GAC CTC CAA CCC ATG ATA CTG. One individual per population was sequenced under the following PCR conditions: 1 min at 98°C followed by 35 cycles of 15 s at 98°C, 30 s at 58°C and 45 s at 72°C, followed by 5 min at 72°C. Reaction volumes were 20 µl, including 0.5 U Phusion Hot Start High-Fidelity DNA Polymerase (Finnzymes, Espoo, Finland), 2.5 µl 10× buffer as supplied with the enzyme, 2 µl BSA (1 mg/ml; New England Biolabs), 2 µl dNTPs (2 µM; Applied Biosystems), 1.5 µl 25 mM MgCl<sub>2</sub>, 1 µl of each primer (10 µM, genXpress), and 1 µl of 1 : 10 diluted template DNA of unknown concentration. PCR products were purified and sequenced as described above.

Indels larger than 1 bp were coded as single characters. No phylogenetic analyses were carried out as the relationships among the haplotypes were straightforward.

**Morphology.** — Material for morphological investigations included the DNA vouchers (Table 2) supplemented with 52 herbarium vouchers (WU and O; Appendix 2) to

increase the number of measurements to at least three for each character from each taxon and major geographic region except for *victoris* and *degenii* for which only one and two vouchers were available, respectively. Often not all necessary phenological stages (budding, flowering and fruiting stages) were available in each plant (indicated as missing data in Appendix 4). In addition, measurements of *Papaver* fruits on herbarium material are often difficult due to deformation of the capsules during drying and pressing. The shape also changes during maturation, and it is difficult to find material at the same stage for comparison.

Twenty-five characters, most of which have been determined as diagnostic in previous taxonomic and floristic treatments (Mowat & Walters, 1964; Hess & al., 1970; Binz & Heitz, 1986; Markgraf, 1958a; Kadereit, 1993), were selected (Table 1). The majority were qualitative as previous investigations applying more exact morphometrics on *P. sect. Meconella* (Solstad, unpub.) have largely failed, probably because of pronounced phenotypic plasticity of most characters. Only four characters are quantitative: petal length and width and fruit length and width.

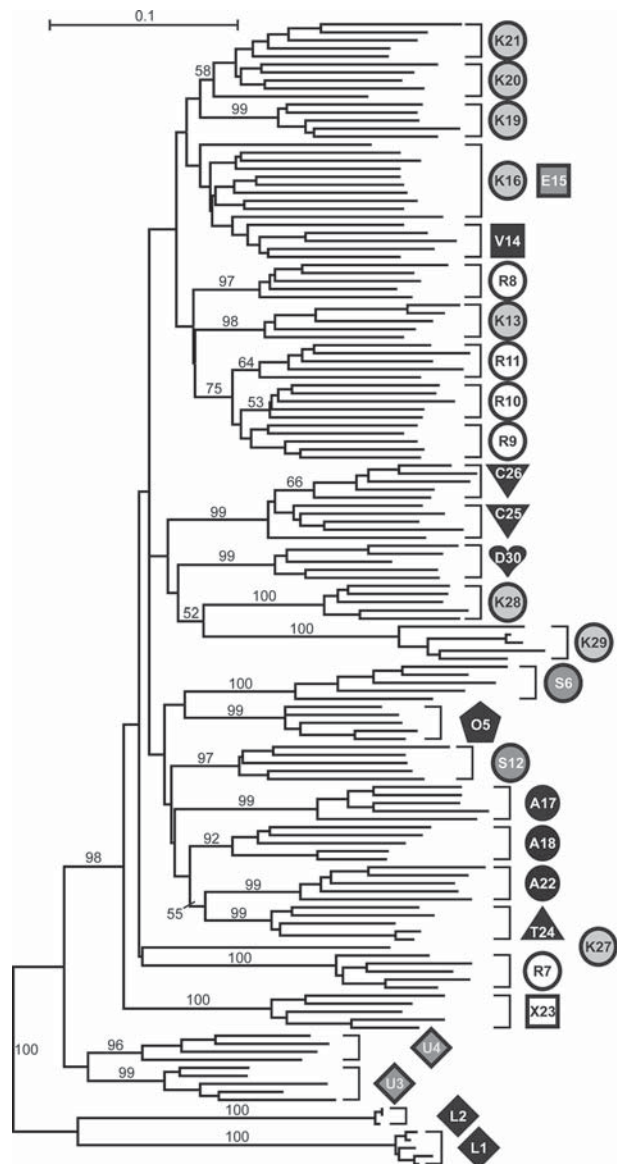
Since our molecular results indicated that numerous named entities belonging to *P. alpinum* s.l. are highly artificial, we grouped the vouchers both by taxa and according to geographic origin. The geographic regions circumscribed for this purpose include the following taxa: the Pyrenees in France and Spain and the Sierra Nevada in Spain (*lapeyrousianum*), yellow-flowered and broad-lobed plants from the southwestern Alps in Italy and France (*aurantiacum*), white-flowered and narrow-lobed plants from the western and northwestern Alps in Switzerland and France (*occidentale*), the Northern Limestone Alps in Switzerland, Austria and Germany (*alpinum*, *sendtneri*), the southern Alps in Italy (*rhaeticum*), the southeastern Alps as two subregions, one western centred in the Italian Dolomiti (*rhaeticum*) and one eastern from the Alpi Giulie/Julijske Alpe to Karawanken/Karavanke in Italy, Slovenia and Austria (*ernesti-mayeri*, *keneri*, *victoris*), the Tatras in Slovakia and Poland (*tatricum*), the southern Carpathians in Romania (*corona-sancti-stephani*), the Pirin in Bulgaria (*degenii*), the Dinaric Mountains in Bosnia-Herzegovina and Montenegro (*keneri*), and the Abruzzo in Italy (*ernesti-mayeri* and/or *degenii*).

## RESULTS

**AFLPs.** — We scored 513 AFLP fragments ranging from 51 to 505 base pairs, of which 118 (23.0%) were monomorphic. The error rate was low (0.37%). The extraction protocol did not influence the AFLP banding pattern. The percentage of AFLP markers with intrapopulation variation varied from 0.2% in population L2 from the

Pyrenees to 21.6% in population E15 from the southeastern Alps (average 15.0%, standard deviation 4.89%, see Table 2). Genetic diversity ranged from 0.001 in population L2 to 0.109 in population E15 ( $0.074 \pm 0.024$ ). DW showed a contrasting pattern and ranged from 2.45 in population E15 to 5.70 in population L1 ( $3.60 \pm 0.77$ ). For more details, see Table 2.

The neighbour-joining analysis (Fig. 2) identified two reciprocally strongly divergent populations L1 and L2 from the Pyrenees as different from all other populations



**Fig. 2.** Neighbour-joining analysis of AFLP phenotypes of *Papaver alpinum* s.l. based on Nei & Li's (1979) distances. Numbers above branches are bootstrap values higher than 50% (2,000 replicates; not shown for nodes within populations). Population and taxonomic codes as in Table 2 and Fig. 1.

with maximum bootstrap support (BS). Populations U3 and U4 from the southwestern Alps (BS < 50) are the consecutive ‘sister’ to all remaining populations (BS 98) that exhibited no supported internal structure at deeper nodes. Nearly all populations formed monophyletic groups, and most of them received high bootstrap support. Exceptions are populations R9, C25, K20 and K21, as well as V14, E15 and K16. The two last-mentioned populations were sampled on the same scree slope but include white- and yellow-flowered plants, respectively, which are currently classified as two different taxa. Of all taxa represented with more than one population, only the Pyrenean *lapeyrousianum* (populations L1, L2) and the

Southern Carpathian *corona-sancti-stephani* (C25, C26) formed monophyletic groups. The NeighbourNet (Fig. 3) was largely congruent with the NJ tree, and illustrates the distinctness of most populations. It did not reveal strongly weighted incompatible splits, indicative of reticulations between genetically differentiated lineages.

In the PCoA of the entire dataset (Fig. 4A), Pyrenean *lapeyrousianum* (L1, L2) was separated from all other populations along the first factor (explaining only 6.6% of the variation), whilst populations U3 and U4 of *aurantiacum* from the southwestern Alps were in intermediate positions. The second factor (5.1%) showed no clear grouping according to current taxonomy. In the PCoA excluding

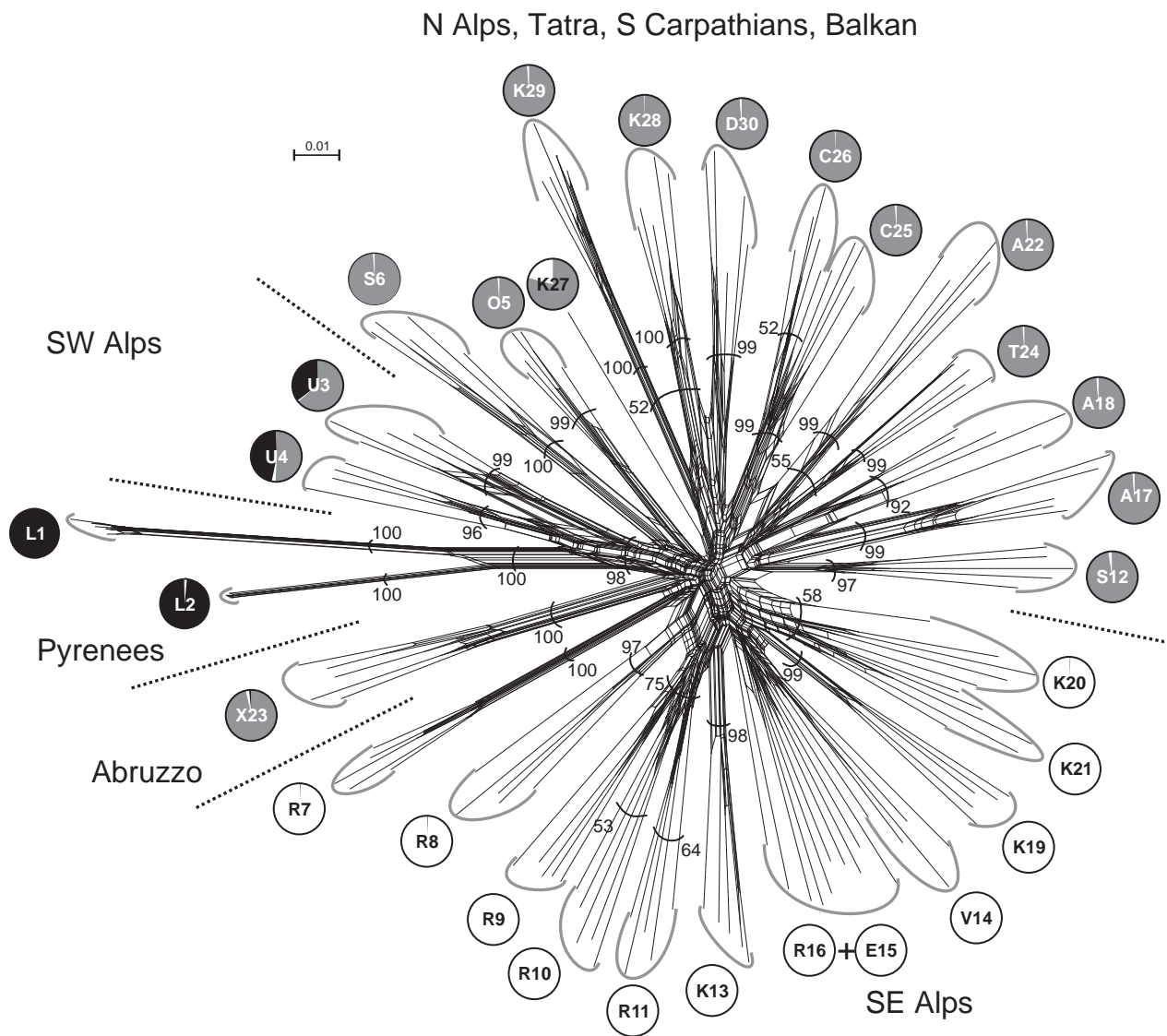


Fig. 3. NeighbourNet derived from AFLP data of *Papaver alpinum* s.l. Numbers are bootstrap values derived from Neighbour-joining analysis (2,000 replicates). Populations are coded as in Fig. 1 and Table 2. Results of the STRUCTURE analysis from Fig. 5C are included as piecharts (black, Pyrenean Cluster; grey, Northern Alps-Carpathian-Balkan-Abruzzo Cluster; white, Southeastern Alpine Cluster) to allow comparison of distance-based and model-based analyses.



populations L1, L2, U3, and U4 (Fig. 4B), grouping followed geography rather than taxonomy. The populations from the southern Alps were separated from all others along the first factor (6.0%), and the second factor (4.5%) separated the populations from the northern Alps, Tatras and Abruzzo from those from the Balkans and the Southern Carpathians.

Analysis of molecular variance (AMOVA) attributed 53.0% of the overall variation to the among-population component (*d.f.* 29;  $P < 0.01$ ).

The STRUCTURE analyses were largely in agreement with the results of PCoA, NNet and NJ analyses. For the entire dataset we obtained identical results for admixture and no-admixture models. Both search strategies gave  $K = 3$  as the appropriate number of groups. Higher values of  $K$  had higher likelihoods, but the clustering results differed strongly among runs and were consequently not considered (Appendix 3). Populational membership coefficients for the three identified clusters are presented on a geographical basis in Fig. 5C. The three clusters, the Pyrenean Cluster, the Northern Alps-Carpathian-Balkan-Abruzzo Cluster (NACBA-Cluster) and the Southeastern Alpine Cluster, do not correspond to previous taxonomic concepts but instead reflect geography. Most populations are assigned to a single cluster, but populations U3 and U4 from the southwestern Alps are admixed between the Pyrenean and the NACBA Clusters, and the single individual of population K27 from the Dinaric Mountains is admixed between the NACBA and Southeastern Alpine Clusters.

The Southeastern Alpine Cluster was subdivided into three sub-clusters in a separate STRUCTURE analysis (Fig. 5D; details in Appendix 3). One contained population R7 from Alpi Bergamasche, the second contained populations R8, R9, R10, and R11 from Dolomiti and western Alpi Carniche, and the third included all populations from the southeastern Alps from the eastern Alpi Carniche eastwards. The border between the central and eastern sub-clusters is congruent with the distribution of *rhaeticum* and *kernerii*. However, the eastern sub-cluster also included *victoris* and *ernesti-mayeri*. For the NACBA Cluster we did not obtain an unequivocally best clustering solution inasmuch as for  $K \leq 3$  the similarity among replicate runs was low and for  $K \geq 4$  empty groups were present in all runs (not shown). The exclusion of population X23 from Abruzzo improved the clustering significantly and  $K = 3$  was obtained as the best solution (Fig. 5E; details in Appendix 3). Populations from the northern Alps clustered with population T24 from the Tatras, whereas the Southern Carpathian (C25, C26) and most Balkan populations (K27, K28, D30) formed another cluster, and finally population K29 from the southern Dinaric Mountains formed a cluster on its own. Only population K27 was strongly admixed (Fig. 5E).

**Plastid DNA sequences.** — The *psbE-petL* sequences were 1,239–1,245 bp long. Alignment was trivial and gave a matrix of 1,245 bp. Three haplotypes were found (Table 2; Fig. 5A). The internal haplotype II was most frequent and occurred from the southwestern Alps to the southern Dinaric Mountains and Pirin. Two

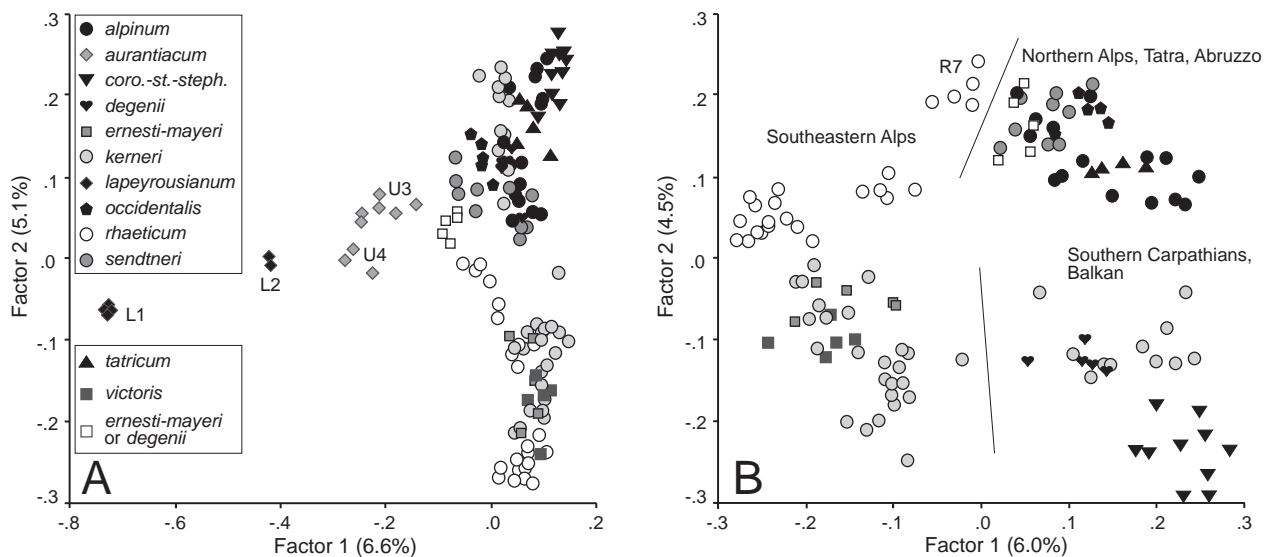


Fig. 4. Principal Coordinate Analysis (PCoA; first two factors) of AFLP data of *Papaver alpinum* s.l. The taxonomic coding of individuals is according to Fig. 1. A, ordination of the entire dataset; B, ordination with reduced dataset (populations L1, L2, U3 and U4 were excluded). Geographic provenance of groups is given. Populations of particular interest are labelled with their population codes.

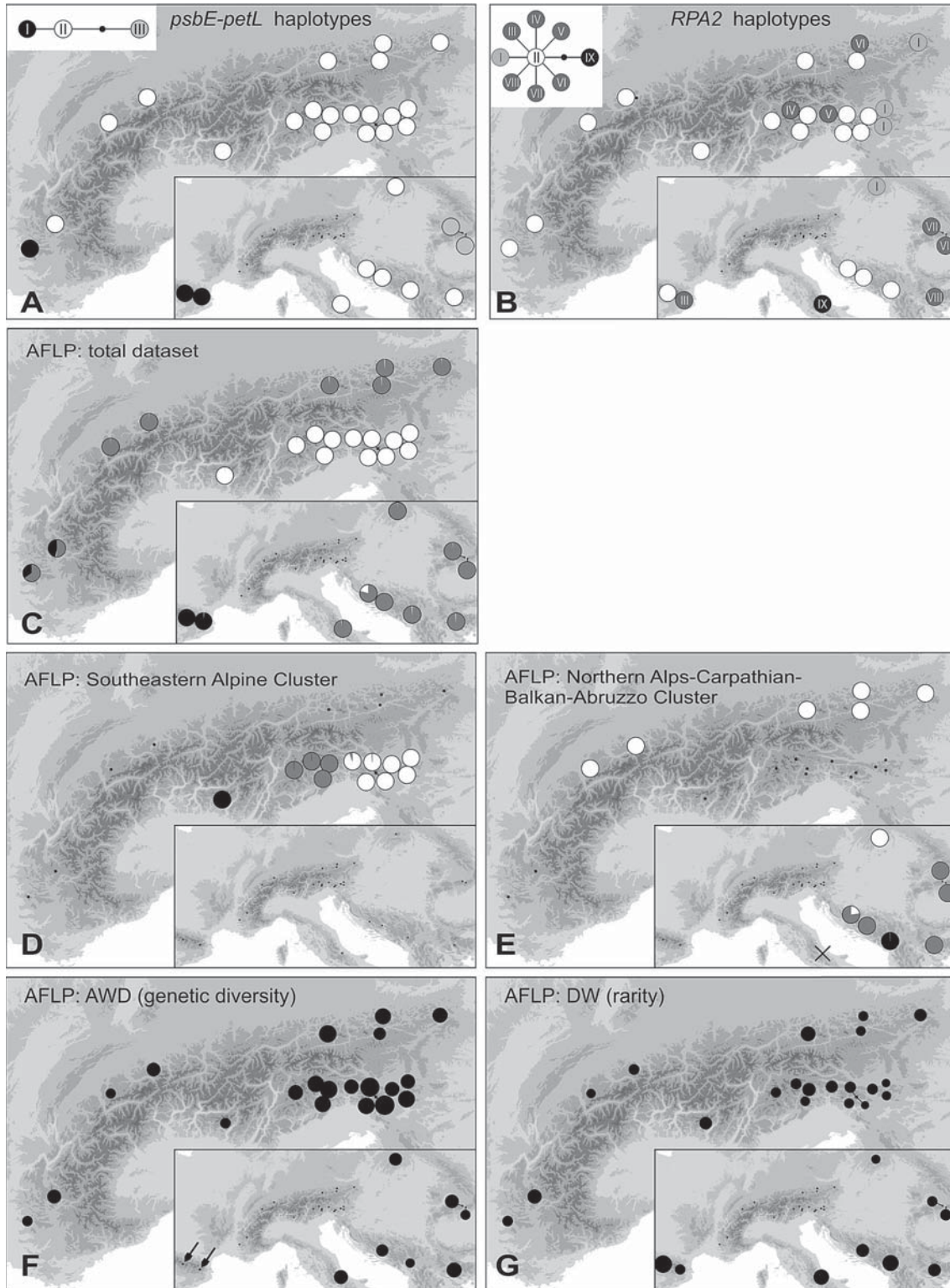


Fig. 5. Geographic patterns of genetic variation in *Papaver alpinum* s.l. A, plastid *psbE-petL* spacer haplotypes. B, nuclear low-copy region *RPA2* haplotypes. C–G, analyses of AFLP data; C–E, results of Bayesian clustering of AFLP data using the software STRUCTURE. C, total dataset. D, Southeastern Alpine Cluster. E, Northern Alps-Carpathian-Balkan-Abruzzo Cluster; population X23 from Abruzzo was excluded from the analysis and is marked with a cross. F, genetic diversity per population. G, frequency–down-weighted marker values per population. See text for further explanations. In A and B, haplotype II is more internal as compared to the other haplotypes in a rooted tree (Solstad et al., unpub.).

haplotypes, derived from it by one or two mutational steps, occurred at the western and eastern margin of the distribution area. Haplotype I, which differed in duplication of a TACTTT motif, was restricted to populations L1, L2 and U3 from the Pyrenees and the southwestern Alps. In population U3, haplotype I was detected in all five sequenced individuals, whereas all four investigated individuals of U4 had haplotype II. Haplotype III, characterised by one single-base insertion and one substitution, was found in populations C25 and C26 from the Southern Carpathians. Comparisons with sequences of other species of *P.* sect. *Meconella* (Solstad & al., unpub.) revealed the *P. alpinum* haplotypes as unique. The most common haplotype II is most similar to the rest of the section and thus likely to be the most ancestral one.

**Nuclear DNA sequences.** — The *RPA2* sequences were 920–1,001 bp long. Alignment was trivial and resulted in a matrix of 1,009 bp. Nine haplotypes were found (Table 2; Fig. 5B). The internal haplotype II was most frequent and occurred from the Pyrenees to the southern Dinaric Mountains. Comparisons with sequences of other species of *P.* sect. *Meconella* (Solstad & al., unpub.) revealed the *P. alpinum* haplotypes as unique. The most common haplotype II is most similar to the rest of the section and thus likely to be the most ancestral one. Haplotypes I and III–IX are derived by two steps at the most. Haplotype I, characterised by duplication of a 61-bp motif, is distributed along the eastern margin of the Alps and in the Tatras. Some peripheral populations from the Pyrenees (L2), Abruzzo (X23), Pirin (D30), and the Southern Carpathians (C25, C26), but also populations R9, K13 and R18 from the Alps, are characterised by derived haplotypes.

**Morphology.** — Measurements and scores of characters in Table 1 are presented in Appendix 4; arranged by geographic regions (for definition and extent of regions, see Fig. 6A) and previously recognised taxa. A selection of characters is presented in Figs. 6B–L and 7.

Only two of the investigated characters are discontinuous: petal length/width ratio [13]/[14] (Fig. 7B) and length of stamens relative to ovary at full anthesis [17] (Fig. 6H). The Iberian populations (*lapeyrousianum*) have narrow and non-overlapping petals ca. 1.5 times as long as wide, and stamens shorter than or (rarely) as long as the ovary, with no overlap in the material seen. These two characters were also pointed out as diagnostic by Markgraf (1958a) and Kadereit (1993), i.e., the petals of

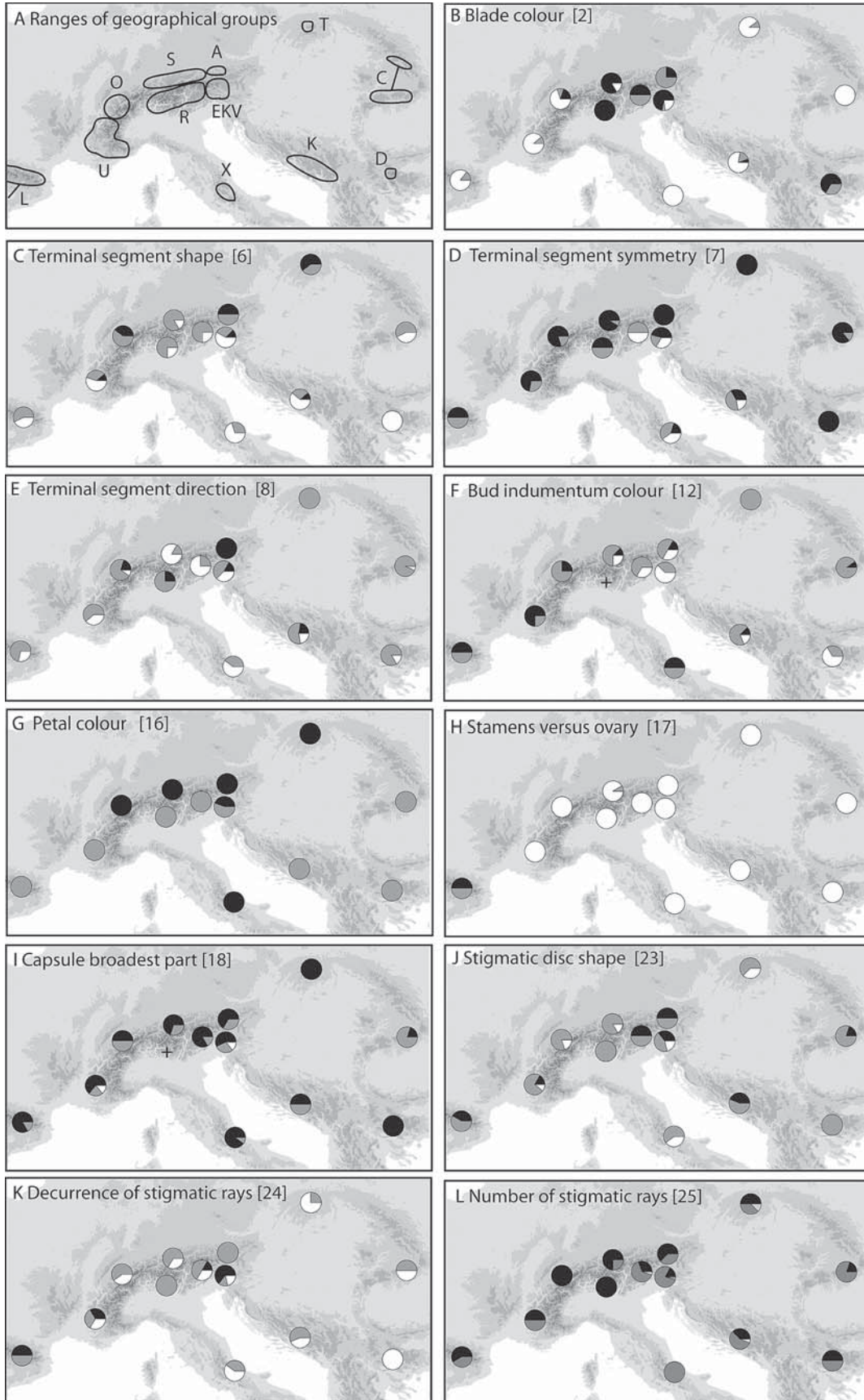
*P. lapeyrousianum* as ‘not overlapping’ versus ‘overlapping’ in all other constituents of *P. alpinum* s.l.

Most other characters vary strongly. Several characters reported to differentiate among races or species are difficult to score, e.g., firmness of leaf sheath tunica [1] (suggested by Mowat & Walters, 1964; Markgraf, 1958a); and shape of stigmatic disc [23] (Fig. 6J). These characters may be more easily observed in field conditions or on fresh material. Some characters vary with developmental stage, e.g., degree of dissection of blade [04]; bud shape [11], often subglobose in young buds, ovoid in larger buds, and ellipsoid just before anthesis; and degree of decurrence of stigmatic rays [24] (Fig. 6K), often much stronger in immature fruits than in mature fruits. Some characters vary in a random fashion without correspondence to regions or proposed taxa, e.g., density [9] and direction [10] of scape hairs; colour of bud indumentum [12]; and density of setae on fruit [22].

The leaf blades vary from rarely simply divided with broad, (ob)ovate segments to most often twice or a few times nearly thrice divided with nearly linear to (ob) lanceolate or (ob)ovate segments. Narrow segments are characteristic of most groups in the northern Alps and Tatras (*occidentale*, *sendtneri*, *alpinum*, *tatricum*), whereas the other groups mostly have broader segments [6] (Fig. 6C). The segment symmetry [7] (Fig. 6D), emphasised by Markgraf (1958a) as a diagnostic character, is not always easy to observe on herbarium specimens. Asymmetrical segments seem to be more frequent in the southeastern Alps (*rhaeticum* and the *ernesti-mayeri-kernerii-victoris* complex), in the Abruzzo and in the Dinaric Mountains than elsewhere. We did not find a close correlation between asymmetrical [7] and convergent [8] segments as proposed by Markgraf (1958a; Fig. 6D, E). Mowat & Walters (1964) proposed that some entities have alternate attachment of main blade segments, others opposite to subopposite attachments. We found subopposite attachment of the lower (proximal) main segments in nearly all plants investigated [5]. The colour of the blade varies from pure green to strongly glaucous [2] (Fig. 6B). Plants with pure green blades are nearly confined to the eastern Alps (predominant in *rhaeticum*, *sendtneri*, and the *ernesti-mayeri-kernerii-victoris* complex, partly in *alpinum*) and the Bulgarian Pirin (*degenii*). All other regions have a predominance of more or less glaucous blades.

The pattern in petal colour [16] (Fig. 6G) among proposed taxa is clear, as is to be expected since colour

**Fig. 6. Geographic patterns of morphological variation in *Papaver alpinum* s.l. A, ranges of geographical groups; letters as in Fig. 1. B–L, variation in eleven qualitative morphological characters. Numbers in brackets refer to the characters in Table 1. Pie charts show the proportional assignment of herbarium specimens and vouchers to character states 1 (black), 2 (grey) and 3 (white) as defined in Table 1. Intermediate scores were split up equally, i.e., score 1.5 was treated as 0.5 to 1 and 2, respectively.**



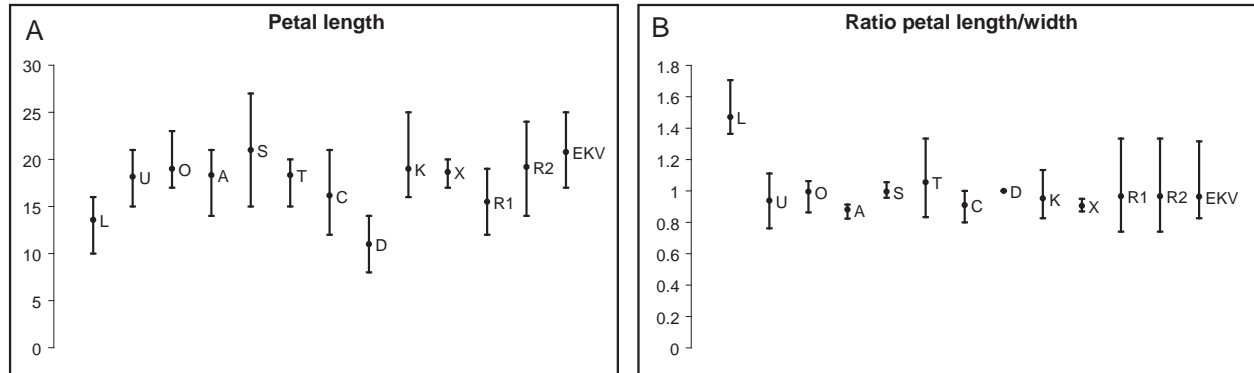


Fig. 7. Variation in two quantitative morphological characters scored in *Papaver alpinum* s.l. given as maximum and minimum values with mean values indicated. See Fig. 1 for explanation of abbreviations of taxa. A, petal length in mm; B, ratio of petal length and width.

has been one of the main diagnostic characters applied in each and every proposed regional subspecies or species. All groups of the northern Alps (*occidentale*, *sendtneri*, *alpinum* s.str.), the Tatra (*tatricum*) and the Abruzzo are white-flowered, as is the southeastern Alpine *ernesti-mayeri*; all others are yellow-flowered. However, white flowers are also reported from the Pyrenees (Markgraf, 1958a), and yellow flowers from Abruzzo (Markgraf, 1958a). Petal length [13] (Fig. 7A) also varies from very short petals in populations in the Iberian (*lapeyrouseanum*) and Bulgarian mountains (*degenii*) to especially long ones in the northern and eastern Alps (*sendtneri*, eastern *rhaeticum* and the *ernesti-mayeri*–*kernerii*–*victoriae* complex).

The shape of the capsule is highly variable [18–21, 23], and it has been assigned diagnostic value by most authors. The fruits have been described as urn-shaped (broadest near the middle and broad towards the top), obovoid (broadest above the middle and narrowed towards the top), and clavate (broadest near the top), and rarely subglobose. Our measurements and scores do not show any clear geographical patterns (Appendix 4; Fig. 6I–J). The number of stigmatic rays [25] has been treated as diagnostic (Mowat & Walters, 1964; Markgraf, 1958a). We cannot confirm this (Fig. 6L). Four rays predominate in population groups of the western and northern Alps (*occidentale*, *sendtneri*, *alpinum*, and also southern Alpine *rhaeticum*); nearly equal frequencies of four and five rays in the Pyrenees (*lapeyrouseanum*), the southwestern Alps (*aurantiacum*), the Tatra (*tatricum*), and the Pirin (*degenii*); whereas the other groups had a predominance of five rays. More than five rays were found in a few populations in the southeastern Alps (*ernesti-mayeri*, *rhaeticum*). The number of rays often varied within populations and even within individuals, i.e., smaller fruits having fewer rays than larger fruits from the same plant. It is unlikely that this is a good diagnostic character for any part of *P. alpinum* s.l.

## DISCUSSION

**Depth of genetic structure.** — Low-copy nuclear and plastid DNA sequences show a shallow overall genetic structure. The haplotypes in the population groups at the western, eastern and southeastern margin of the distribution area are differentiated from the widespread, presumably ancestral (Solstad, unpub.) haplotypes which are centred in the Alps (Fig. 5A, B). Interestingly, *RPA2* haplotype I implies a connection between the Northern and Southern Limestone Alps, a pattern deviating from that of the AFLP fingerprinting data (see below). Both Southern Carpathian populations (C25, C26) are characterised by shared, unique haplotypes in both sequenced regions, indicating relatively long-term isolation of the populations at the easternmost margin of the distribution area of *P. alpinum*. The closest diploid relatives of *P. alpinum* occur from Central Asia northeastwards (Rändel, 1974) and consequently the immigration of the ancestor of *P. alpinum* probably occurred from the east. The derived haplotypes of the easternmost populations (*corona-sancti-stephani*), however, preclude the—geographically plausible—hypothesis that the Southern Carpathians were colonised first.

**Distinctness of populations.** — In congruence with the results of Kropf & al. (2006) obtained for populations from the western part of the distribution area (Sierra Nevada, Pyrenees, southwestern Alps), our AFLP data revealed the distinctness of many populations. As we typically sampled five individuals per population (Table 2), our sampling design allowed recognition of an important factor that could not be detected by the individual-based sampling scheme of Bittkau & Kadereit (2003), i.e., that most populations form genetically divergent entities. This can be most straightforwardly observed in the NNet (Fig. 3) as well as in branch lengths and bootstrap

support values of the NJ tree (Fig. 2), but also in the non-hierarchical AMOVA where more than one half of the entire genetic variation was explained by the among-population component. There may be several, mutually non-exclusive explanations: (1) Most populations outside the northeastern and southeastern Alps often have low numbers of plants (Schönswetter, pers. obs.). This factor combined with a strongly fragmented distribution area due to specific habitat requirements certainly reinforce genetic drift (Fabergé, 1943). (2) *Papaver alpinum* reaches the reproductive state rapidly as compared to most other alpine perennials. Plants in culture can flower as early as three months after germination (Solstad, pers. obs.), in the field they do so most probably in the second growing season as is the case for *P. radicum* (Nordal & al., 1997). The short generation cycle promotes mutations, which are likely to become fixed relatively rapidly due to the small population size (Ellstrand & Elam, 1993). (3) *Papaver alpinum* is diploid. This is in contrast to the many presumably allopolyploid arctic representatives of *P. sect. Meconella*, where fixed heterozygosity likely reduces the effect of genetic drift. (4) The reproductive system may vary. Fabergé (1943) reported *P. alpinum* as a self-compatible species, whereas Knaben (1959) found no spontaneous self-pollination in isolated flowers. The latter result is in accordance with Hanelt (1969). Kadereit (1990) conducted crossing experiments within and between taxa and confirms these contradictory reports as he found both self-compatible and self-incompatible populations. Altogether, if selfing is predominant, this could enhance genetic drift in populations even without pronounced geographic isolation. (5) Local bottlenecks have likely shaped the genetic constitution of *P. alpinum*. Observations of individual plants or even large populations of *P. alpinum* s.l. in gravel river beds and road sides at low altitudes from the northeastern and southeastern Alps (Schönswetter, pers. obs.) suggest that *P. alpinum* was probably frequent in the vast alluvial terraces surrounding the southern European mountain ranges during cold and dry stages of the Pleistocene (Penck & Brückner, 1909). Under such a scenario, the species is in ‘refugial situations’ today, i.e., bottlenecks are more probably interglacial than glacial.

**Stepwise range expansion from the southwestern Alps to the Central Pyrenees.** — Our AFLP data, which are in large parts congruent with the RAPD data of Bittkau & Kadereit (2003), support the dynamic ‘Out of the Alps’ scenario implied by plastid and nuclear sequences. Although the Pyrenean populations L1 and L2 form a highly divergent group in the NJ analysis (Fig. 2) and are separated by splits in the NNet (Fig. 3), several lines of evidence show they are genetically strongly depauperate (Fig. 5F; Table 2) derivatives of populations from the southwestern Alps which were strongly affected

by genetic drift. (1) The STRUCTURE analysis of the entire dataset (Fig. 5C) separated both populations L1 and L2 as one cluster, but also showed that populations U3 and U4 from the southwestern Alps are strongly admixed between the Pyrenean Cluster and the NACBA Cluster. (2) In the PCoA of the entire AFLP dataset (Fig. 4), the sequence of populations followed an East-West pattern along factor 1, i.e., population L2 from the eastern Pyrenees was intermediate between population L1 from the western Pyrenees and the southwestern-Alpine populations U3 and U4, which themselves bridged populations from the Pyrenees and the northern and eastern Alps. Importantly, individuals of the eastern Pyrenean population L2 were resolved as more similar to populations U3 and U4 than to the western Pyrenean L1. (3) Whereas populations U3 and U4 are polymorphic with respect to their plastid DNA haplotype (haplotypes I and II; Fig. 5A), the Pyrenean populations are uniformly characterised by haplotype I. Altogether, our data show that the Pyrenees have been colonised from the southwestern Alps via stepwise leading edge migration. The high bootstrap support for the branch connecting populations L1 and L2 suggests one migration event, which was probably followed by independent bottlenecks in each population. Under this scenario, the most strongly divergent (e.g., Fig. 4A) population L1 is likely to be the end-product of a series of bottlenecks induced by stepwise leading-edge migration. Strong divergence between eastern and central Pyrenean populations of *P. alpinum* was also found by Kropf & al. (2006). We can only speculate about the mode of migration from the Alps to the Pyrenees. Palaeovegetation data (Frenzel & al., 1992) do not support a continuous distribution of *P. alpinum* between the Alps and Pyrenees during cold stages of the Pleistocene, as southern France was covered with steppe vegetation dominated by *Artemisia*, *Ephedra*, *Juniperus*, and *Asteraceae* rather than with equivalents of alpine vegetation. Although patchy occurrence of suitable habitats in a matrix of steppe vegetation cannot be ruled out, the low genetic variability of the Pyrenean population suggests long-distance dispersal, maybe involving a stepping-stone in the Massif Central, to be more likely. Gene flow from the Alps to the Pyrenees was previously inferred in, e.g., *Phyteuma globulariifolium* Sternb. & Hoppe (Schönswetter & al., 2002) and *Carex curvula* All. (Puşcaş & al., 2008). The alternative hypothesis, i.e., that the populations from the southwestern Alps represent a meeting zone between long isolated Pyrenean and Alpine gene pools, cannot be rejected, but appears unlikely as it requires an old dispersal event from the Alps to the Pyrenees and a more recent dispersal back towards the Alps, followed by a relatively homogeneous amalgamation of Pyrenean and southwestern Alpine gene pools (see Fig. 5C) in the southwestern Alps.

### North–South differentiation in the Alps. —

Within the middle and eastern Alps, the AFLP data suggest a main split into populations from the Northern and Southern Limestone Alps. This is seen in the STRUCTURE analysis of the entire dataset (Fig. 5C), the separation along the first factor of the PCoA of the reduced dataset (Fig. 4), as well as in the NNet (Fig. 3) and the NJ analysis (Fig. 2). The split, however, was neither strongly weighted in the NNet, nor did it receive bootstrap support in the NJ analysis.

A genetic break following the siliceous main divide of the Alps can be anticipated in predominantly calciphilous taxa such as *P. alpinum*. Having its main distribution area along the limestone ranges at the northern and southern periphery of the Alps, the mainly siliceous Central Alps present an obvious obstacle to gene flow. Similar results to ours have been found in the few available range-wide phylogeographic studies of widespread calciphilous species of the Alps (e.g., Ehrich & al., 2007; Paun & al., in press; Winkler & al., unpub.). The long-term importance of the Central Alps as an obstacle to North–South migration is also supported by the strong differentiation of the flora of the Northern and Southern Limestone Alps, as illustrated by patterns of endemism (Pawłowski, 1970; Tribsch, 2004).

The genetic structure is different between the Northern and the Southern Limestone Alps, as revealed by separate STRUCTURE analyses. Whereas no substructure was detected within the northern Alps (Fig. 5E), three subgroups could be identified in the southern and southeastern Alps, i.e., Alpi Bergamasche (R7); Dolomiti/Dolomiten (R9–R11); and Alpi Carniche to Karavanke/Karawanken (from K13 eastwards to K21; Fig. 1). This pattern is congruent with well-defined centres of endemism (Tribsch, 2004). Populations from the Dolomiti eastwards are on the one hand characterised by weak reciprocal differentiation, as illustrated by low values for the rarity index DW (Fig. 5G; Table 2); and on the other hand, they exhibit high values for the diversity index AWD (Fig. 5F; Table 2) and are among the most diverse with respect to morphological characters what resulted in the description of many taxa from that area. The weak differentiation may be partly because there is a higher density of sampled populations in the southeastern Alps than in the Northern Limestone Alps (Fig. 1; see below). We believe the observed high genetic variation and morphological plasticity are the result of a relatively incomplete Pleistocene glaciation of the southeastern Alps (van Husen, 1987), leaving ample space for large, interconnected populations within the two areas defined by the STRUCTURE analysis of southeastern Alpine populations (Dolomiti; Alpi Giulie/Julijske Alpe, Karavanke/Karawanken, Kamniške Alpe).

The distribution of the RPA2 haplotypes in the Northern and Southern Limestone Alps (Fig. 5B) contrasts with

the AFLP data (Fig. 5C). While haplotype IV, characterised by one point mutation, was found uniquely in population K13 from Alpi Carniche, haplotype I, characterised by a duplication of a 61-bp motif, was detected in the three easternmost sampled populations of *P. alpinum* from the Southern (K20, K21) as well as the Northern (A22) Limestone Alps. Disregarding the possibility of a homoplasious origin, which appears improbable given its non-random geographic distribution, haplotype I may indicate relatively old gene flow between the eastern margins of the Northern and Southern Limestone Alps that has been swamped by more recent gene exchange in the AFLP pattern.

**Ambiguous placement of the Abruzzo population. —** Population X23 from Abruzzo possesses the most strongly differentiated haplotype in the RPA2 dataset and groups with most Alpine and all Carpathian and Balkan populations in the NJ analysis (Fig. 2). In the NNet (Fig. 3), it was placed between the Pyrenean and southeastern Alps plants, whereas in the STRUCTURE analysis of the entire AFLP dataset (Fig. 5C) it was unambiguously assigned to the NACBA Cluster. The PCoA of the reduced dataset (Fig. 4) showed strong similarity to populations S6, S12 and A17, all from the Northern Limestone Alps. Why STRUCTURE failed to determine the placement of population X23 in the substructure of the NACBA Cluster remains elusive. Stable results among replicate runs were only obtained after its exclusion (Appendix 3).

**Carpathian and Balkan populations connect to the Northern Limestone Alps. —** The populations sampled to the east and southeast of the Alps are related to the northern Alps rather than to the southeastern Alps (Figs. 3, 4, 5C). From a geographical point of view, this connection is unexpected as there are connecting limestone mountains almost all the way from the southeastern Alps to the Dinaric Mountains, to Pirin, and also to the Carpathians. Even if interrupted in many places today, the connections between northern Alps, Carpathians and Balkan must have been extensive during glacial epochs when the treeline was situated at lower altitudes. The connection among populations from the northeastern Alps (A17, A22) and the Tatras (T24) (Figs. 4B, 5E) is expected from previous phylogeographic studies (e.g., *Pritzelago alpina* Kuntze: Kropf & al. 2003; *Ranunculus alpestris* L.: Paun & al., 2008). However, an entirely different pattern is shown by *Dryas octopetala* L.: populations from the Tatras connect to northeastern Europe and Siberia, whereas the Alpine plants connect to northwestern Europe (Skrede & al., 2006).

Within the NACBA-cluster, population T24 from the Tatras clusters with the Northern Alpine populations, not with the Southern Carpathian and Balkan populations (C25, C26, K28, K29, D30). The latter form their own—unsupported—branch in the NJ analysis (Fig. 2),

are separated along the second factor of the PCoA of the reduced dataset (Fig. 4B) and, finally, are assigned to two own clusters (C25, C26, K28, D30; K29) in the separate STRUCTURE analysis of the NACBA Cluster (Fig. 5E). We refrain from discussing the admixed state of the single analysed individual of population K27 in the STRUCTURE analyses (Fig. 5C, E) as the ambiguous results are likely an artefact caused by the sample size.

Whereas AFLP data may suggest migration from the northern Alps along the Carpathian arc to the mountains of the Balkan Peninsula, the presence of derived plastid (Fig. 5A) as well as *RPA2* (Fig. 5B) haplotypes in the Southern Carpathians does not support this hypothesis. Similarly as outlined above for migration from the southwestern Alps to the Pyrenees, although it appears unlikely that populations of *P. alpinum* thrived in the steppes and scrublands covering the intervening space between the present-day populations (Frenzel & al., 1992), occurrence in pockets of suitable alpine vegetation cannot be ruled out. Altogether, we do not know along which pathways *P. alpinum* reached the mountains of the Balkans from a presumed source population in the Alps, but it is rather unlikely that the Southern Carpathian populations represent relics of this migration.

**Taxonomical considerations.** — The intricate taxonomic structure proposed within *Papaver alpinum* s.l. (e.g., Markgraf, 1958a, b; Mowat & Walters, 1964; Jalas & Suominen, 1991; Aeschmann & al., 2004) is not supported by molecular investigations (Bittkau & Kadereit, 2003; present data). The main feature of the AFLP data (Fig. 3) is the distinctness of each population or small population group rather than a deeper hierarchy. The shallow molecular structure mostly runs across the proposed taxa, demonstrating that the morphological criteria applied for definition of the taxa do not correspond with genetic groups. Neither the STRUCTURE analysis of the entire AFLP data (Fig. 5C) nor the analyses of subsets (Fig. 5D, E) resulted in groups corresponding to proposed taxa but rather split entities (*kernerii*, *rhaeticum* s.l.) or merged them fully (southeastern Alpine *ernesti-mayeri*, *kernerii* and *victoris*). The exception is the Pyrenean *lapeyrousianum*, to some degree separate in all molecular dataset, but with intermediates in the southwestern Alps (Fig. 5A, C). Deviating plastid *psbE-petL* haplotypes occurred in *lapeyrousianum* and one of two populations of *aurantiacum* (*rhaeticum* of other authors), as well as in *corona-sancti-stephani* (Fig. 5A), but this pattern is shared neither by the nuclear *RPA2* haplotypes nor by AFLP markers. The pattern in the *RPA2* haplotypes (Fig. 5B) runs across all previously proposed taxonomic solutions, especially in the eastern Alps and on the Balkan Peninsula.

The morphological data (Fig. 6; Appendix 4) support the contention that *P. alpinum* s.l. is a morphologically hypervariable taxon, but does not give much support to the

proposed species or subspecies, again with *lapeyrousianum* as an exception. The suggested differential characters in, e.g., petal colour, fruit and stigmatic disc shape, and leaf architecture appear more or less scattered throughout the STRUCTURE groups in the AFLP analysis, except for the combined group of the southwestern Alps and the Pyrenees being uniformly yellow-flowered and broad-lobed. At least some of the hair characters as well as the petal colour may have a simple genetic basis (Fabergé, 1943, 1944).

Accordingly, there is not much support for recognition of taxa within *P. alpinum* s.l. in the Alps, the Carpathians, the Balkan mountains, or the Apennines. The only segregate possibly deserving taxonomic recognition is the Iberian *P. alpinum* subsp. *lapeyrousianum*, as proposed by Kadereit (1993, but as species). Morphological evidence (petal shape/size and relative length of stamens and gynoecium) seems to separate this group from all other constituents of *P. alpinum* s.l. We have studied only a small sample of *lapeyrousianum*, and plants with petals approximately as long as broad are known from Sierra Nevada (specimen accession number 344112 at MA). Non-overlapping petals, however, are reported to be diagnostic for all Iberian plants (Díaz González, 1986). From a genetic perspective, the Iberian plants are genetically depauperate derivatives of source populations in the southwestern Alps. Similar genetically slightly divergent (micro-) lineages are represented by the populations R7 (*rhaeticum* in the southern Alps, AFLPs), X23 (the Abruzzo plants, AFLPs), and C25 and C26 (*corona-sancti-stephani* in the South Carpathians, plastid haplotype), but without a parallel morphological discontinuity. From a phylogenetic point of view it can be argued that it is difficult to keep the deviating peripheral populations and population groups as separate taxa while keeping the core populations in the Alps, the Tatras and the Balkan mountains as one taxon (*P. alpinum* s.str. or *P. alpinum* subsp. *alpinum*). We thus refrain from accepting named specific or subspecific taxa within *P. alpinum* s.l., for the reasons outlined above. The data indicate that the Pleistocene fluctuations in ranges, leading to cycles of range contraction and expansion with secondary contact, have been too extensive and recent to result in a resolvable taxonomic structure.

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Appendix 1. Previously recognised taxa in *P. alpinum* s.l., current nomenclature and ranges.

*Papaver alpinum* L., Sp. Pl.: 507. 1753.

- (a) ***Papaver alpinum* subsp. *alpinum*** (*P. burseri* Crantz, Stirp. Austr. Fasc. 2: 129. 1763; *P. alpinum* subsp. *burseri* (Crantz) Fedde in Engler, Pflanzenreich IV.104(40): 373. 1909). – Linnaeus (1753) described the species with “Habitat in Helvetia, Sneeburg Austriae”. Kerner (1868) identified Mount Schneeberg in Niederösterreich as type locality. The lectotype is preserved in Herb. Burser IX:58 (UPS), designated by Markgraf (1965: 145). This white-flowered entity is restricted to the northeastern Austrian Alps (Markgraf, 1958a; Jalas & Suominen, 1991; Aeschmann & al., 2004).
- (b) ***Papaver alpinum* (subsp. *rhaeticum*) var. *aurantiacum*** (Loisel.) Markgr. in Phytion (Horn) 7: 306. 1958 (*P. aurantiacum* Loisel. in J. Bot. (Desvaux) 2: 340. 1807). – The yellow-flowered *P. aurantiacum* was described from a morphologically deviating and geographically isolated population on Mont Ventoux, southeastern France (Loiseleur-Deslongchamps, 1807). Markgraf (1958b) treated Loiseleur’s *P. aurantiacum* as a variety within subsp. *rhaeticum* and as sympatric with the type variety throughout nearly the entire range of the latter. This procedure was followed by, e.g., Markgraf (1958a), Kadereit (1990), Jalas & Suominen (1991), and also by Aeschmann & al. (2004), but by the last-mentioned authors as a species under the priority name *P. aurantiacum*. Markgraf (1958b) classified the Mont Ventoux population as a subvar. *aurantiacum* besides a subvar. *lancifolium*. We apply the name *aurantiacum* for the populations from the geographically separated western part of the distribution area of subsp. *rhaeticum* as mapped by Markgraf (1958a) and Jalas & Suominen (1991), or of *P. aurantiacum* as mapped by Aeschmann & al. (2004), i.e., in the western and southwestern Alps in France and Italy. As the presence of this taxon in the eastern Pyrenees is strongly doubted by, e.g., Diaz González (1986), it is indicated as questionable in Jalas & Suominen (1991).
- (c) ***Papaver alpinum* subsp. *corona-sancti-stephani*** (Zapał.) Markgr. in Phytion (Horn) 7: 306. 1958 (*P. corona-sancti-stephani* Zapał., Bull. Int. Acad. Sci. Cracovie, Sci. Nat., Sér. B, 1911: 620. 1911; *P. pyrenaicum* subsp. *corona-sancti-stephani* (Zapał.) Borza in Bul. Grad. Bot. Univ. Cluj 8: 114. 1928). – *Papaver corona-sancti-stephani* was described from Mount Ineu and is restricted to the Romanian Carpathians (Jalas & Suominen, 1991). It is accepted as a yellow-flowered subspecies or species by all authors that accept a subdivision of *P. alpinum*.
- (d) ***Papaver alpinum* subsp. *degenii*** (Urum. & Jáv.) Markgr. in Phytion (Horn) 7: 312. 1958 (*P. pyrenaicum* subsp. *degenii* Urum. & Jáv. in Magyar Bot. Lapok 19: 33. 1920). – This taxon was described from the Pirin, and in the strict sense, this yellow-flowered entity is restricted to that mountain range in Bulgaria (Jalas & Suominen, 1991). However, Markgraf (1958a) assigned the plants with reddish yellow flowers in the Gran Sasso area, Abruzzo, central Italy, to subsp. *degenii* as subvar. *rubicundum* (Bornm.) Markgr. Markgraf (1958b), followed by Jalas & Suominen (1991), reported subsp. *degenii* and subsp. *ernesti-mayeri* (white-flowered) as two taxa sympatric in Abruzzo. Kadereit (1990) synonymised the populations of subsp. *degenii* from the Pirin with subsp. *kernerii*, whereas he considered the Abruzzo populations as subsp. *ernesti-mayeri*.
- (e) ***Papaver alpinum* subsp. *ernesti-mayeri*** Markgr. in Phytion (Horn) 7: 312. 1958 (*P. ernesti-mayeri* (Markgr.) Wraber in Proteus (Ljubljana) 44: 238. 1982; *P. julicum* E. Mayer & Merxm. in Lazar, Ad Annuum Horti Bot. Labacensis Solemnem CL (Ljubljana): 28, 44. 1960 [nom. inval.]; *P. alpinum* var. *julicum* (E. Mayer & Merxm.) Á. Löve & D. Löve in Preslia 46: 129. 1974 [nom. inval.]). – Markgraf (1958b) described subsp. *ernesti-mayeri* based on a type from Slovenia: Julische Alpen, Triglav, Staničeva koča, 1956. He reported it as a white-flowered parallel to the largely sympatric yellow-flowered subsp. *kernerii* and stated its range to include the Alpi Giulie/Julijske Alpe in northeastern Italy and northwestern Slovenia, and also the Gran Sasso area, Abruzzo, central Italy (Zodda, 1964). This approach was followed by Markgraf (1958a), Jalas & Suominen (1991), and Aeschmann & al. (2004).
- (f) ***Papaver alpinum* subsp. *kernerii*** (Hayek) Fedde in Engler, Pflanzenreich IV.104(40): 375. 1909 (*P. kernerii* Hayek in Österr. Bot. Z. 53: 170. 1903). – The yellow-flowered subsp. *kernerii* was described from Karawanken/Karavanke and Kamniške Alpe/Steiner Alpen, i.e., northern Slovenia and southeastern Austria, with lectotype from Slovenia: Steiner Alpen, Korošica-Hütte, leg. Hayek (GB; Markgraf, 1958b). The range of subsp. *kernerii* var. *widderi* Markgr. overlaps slightly with the also yellow-flowered subsp. *rhaeticum* var. *angustius* Markgr. in northeastern Italy and northwestern Slovenia. In addition, Hayek (1927), followed by Markgraf (1958a) and Jalas & Suominen (1991), assigned several isolated populations in the Dinaric Mountains in Hercegovina and Montenegro to subsp. *kernerii*.
- (g) ***Papaver alpinum* subsp. *lapeyrousianum*** (Greuter & Burdet) Kerguelen, Index Synonym. Fl. France (Coll. Patrim. Nat.; 8): xv. 1993 (*P. lapeyrousianum* Gutermaun in Österr. Bot. Z. 122: 268. 1973 [nom. inval.]; *P. lapeyrousianum* Greuter & Burdet in Willdenowia 11: 43. 1981; *P. alpinum* subsp. *lapeyrousianum* (Gutermaun) Kadereit in Bot. Jahrb. Syst. 112: 84. 1990 [comb. inval.]; *P. suaveolens* Lapeyr., Hist. Abr. Pl. Pyr. Suppl.: 72. 1818 [nom. illegit.] pro parte [excl. typ.]; *P. alpinum* subsp. *suaveolens* Rändel in Feddes Repert. 84: 713. 1974 [comb. illegit.]). – This predominantly yellow- or red-flowered entity from the Iberian Peninsula (the Sierra Nevada in Spain, the Pyrenees in France) was first described as *P. suaveolens*. Greuter (1981) furnished the taxon with a valid name and designated a specimen collected by Lapeyrouse (as ‘*Argemone Pyrenaica*’, stored in B) as type. Kadereit (1993) considered this entity to include all plants of the Sierra Nevada and the Pyrenees. Mowat & Walters (1964) recognized two subspecies within their *P. suaveolens*: subsp. *suaveolens* in both the Pyrenees and Sierra Nevada, and subsp. *endressii* (Asch.) Mowat & Walters [comb. inval.] in the eastern Pyrenees. Greuter (1981) did the same but under the names *P. lapeyrousianum* subsp. *lapeyrousianum* and subsp. *endressii* (Asch.) Greuter & Burdet. Mowat & Walters (1964) stated that the Iberian plants uniformly have yellow or red flowers. However, we can confirm Markgraf’s (1958a) report of white-flowering populations from the Pyrenees (*P. Schönswetter*, pers. obs.).
- (h) ***Papaver alpinum* (subsp. *tatricum*) var. *occidentale*** Markgr. in Phytion (Horn) 7: 313. 1958 (*P. occidentale* (Markgr.) H.E. Hess & Landolt, Fl. Schweiz 3: 778. 1973). – Markgraf (1958a) reported this white-flowered taxon from several localities in the northwestern and western Alps in Switzerland and France, as a variety of *P. alpinum* subsp. *tatricum*. He specified a type from France: Haute-Savoie, Mont Vergy, leg. J. Thimothée (Dörfner, Herb. norm. 5209). Aeschmann & al. (2004) mapped *P. occidentale* as replacing *P. sendtneri* westwards in the northern and western Alps but as overlapping the range of *P. aurantiacum* south of Lac Léman. Kadereit (1990) synonymized var. *occidentale* with subsp. *tatricum*. Jalas & Suominen (1991: 45) informally mapped *P. alpinum* subsp. *tatricum* and *P. “occidentale”* as separate and stated that “*P. occidentale* ... needs further study”.
- (i) ***Papaver alpinum* subsp. *rhaeticum*** (Leresche) Nyman, Consp. Fl. Eur. Suppl. 2: 16. 1889 (*P. rhaeticum* Leresche in Gremli, Excursionsfl. Schweiz 66. 1874). – Leresche in Gremli (1874) only referred to plants from Engadin, southeastern Switzerland, and consequently the name *rhaeticum* in a strict sense (i.e., excluding *P. aurantiacum*, see above) must be connected to yellow-flowered plants in the southern-central and southeastern Alps (easternmost Switzerland, northern and northeastern Italy, southern Austria, and northwestern Slovenia, see Jalas & Suominen, 1981; Aeschmann & al., 2004). Within subsp. *rhaeticum*, Markgraf (1958b) observed a clinal variation from a western broad leafed var. *rhaeticum* centred in the Engadin in Switzerland over a widespread var. *lancifolium* A. Nyár. 1942 (type from France, Isère, Grand Veymont) to an eastern var. *angustius* Markgr. 1958 (type from Slovenia, Julijske Alpe, Hribarice) in the southeastern Alps, possibly connecting to subsp. *kernerii*. Hayek (1927), followed by Markgraf (1958a) and Jalas & Suominen (1991), also assigned some plants from the Dinaric Mountains in Hercegovina and Montenegro to this subspecies.
- (j) ***Papaver alpinum* subsp. *sendtneri*** (Kern. ex Hayek) Schinz & Keller, Fl. Schweiz, ed. 3, 1: 223. 1909 (*P. sendtneri* Kern. ex Hayek in Österr. Bot. Z. 53: 406. 1903). – According to Markgraf (1958a), Kerner designated a plant from Berchtesgaden collected by Sendtner as type. It is the most widespread entity in the northern and northeastern Alps extending eastwards from the Vierwaldstätter See area (Mt. Pilatus) in Switzerland to Austria (Markgraf, 1958a; Jalas & Suominen, 1991; Aeschmann & al., 2004). The entity is accepted in this circumscription and with this range by all authors recognizing several taxa within *P. alpinum* s.l.
- (k) ***Papaver alpinum* subsp. *tatricum*** A. Nyár. in Acta Geobot. Hung. 5: 19. 1942. – In the strict sense, the white-flowered subsp. *tatricum* is restricted to the Tatra in the Western Carpathians, in Poland and Slovakia (Markgraf, 1958a; Jalas & Suominen, 1991). Markgraf (1958b) refers to a type from “Karpaten, Hohe Tatra”, leg. Nyárády. In the wide sense (see above) subspecies *tatricum* is highly disjunct between the Tatra and the western Alps, with two other taxa (subsp. *sendtneri* and subsp. *alpinum*) present in the gap. For the proposed inclusion of *P. occidentale*, see above.

## Appendix 1. Continued.

- (l) *Papaver alpinum* subsp. *victoris* (Škornik & Wraber) Wraber in Hladnikia 10: 42. 1998 (*P. victoris* Škornik & Wraber in Biol. Vestn. 36(3): 82. 1988). – *Papaver victoris* was described as a locally distributed, yellow-flowered species from the southwestern Julijske Alpe in northwestern Slovenia. The diagnostic characters separating it from subsp. *kernerii* are few and quantitative. Due to its late description, it was not considered by Markgraf (1958a) and was overlooked by Kadereit (1990). However, it was accepted as a geographically restricted species by Aeschmann & al. (2004).
- (m) *Papaver alpinum* subsp. *fatraemagnae* Bernát., Fl. Slovenska 5(4): 765. 2002. – This white flowering stenoendemic taxon was only recently described from Veľká Fatra (Slovak Republic). It differs from subsp. *tatricum* in its broader leaf segments, smaller flowers and irregularly toothed petals.

## Appendix 2. Label information from the additional herbarium vouchers used for morphological investigations from WU, O and herbarium Gutermann stored at the Department of Biogeography, University of Vienna. Names based on identifications by the collectors and, if available, later revisions in brackets. Geographical grouping according to Fig. 6A.

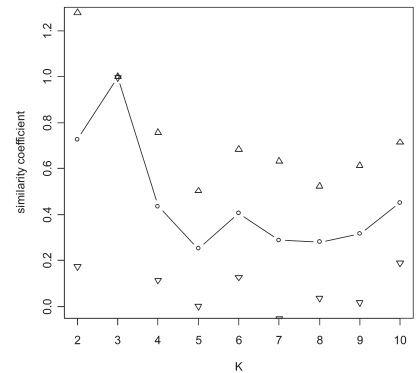
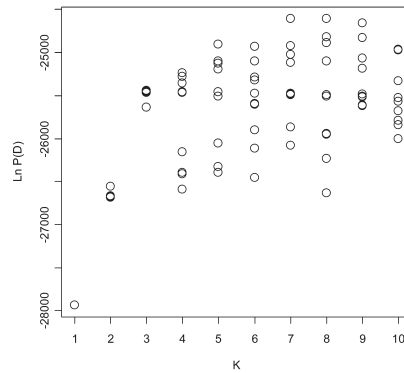
- Sierra Nevada in Spain, and Pyrenees in France & Spain ‘lapeyrousianum’:** L1WU, France, Dép. Pyrénées-Orientales, Ost-Pyrénées, Cambre d’Azeu, 2,450–2,600 m, 04.08.1970, D. Ernet & W. Gutermann 9022 (Herb. Gutermann) [*P. lapeyrousianum*]; L2O, France, Pyrenees, Cambres d’Aize, s.d., M.N. Blytt s.n. (O) [*P. alpinum*]; L3O, France, Pyrénées orientales, Vallée d’Eyne, 12.07.1892, G. Vidal, s.d., M.N. Blytt s.n. (O) [*P. pyrenaicum* & *P. alpinum*]; L4O, France, Pyrénées orientales, Ostpyrenäerne, Val Vennes, s.d., M.N. Blytt s.n. (O) [*P. pyrenaicum*]; L5WU Spain, W Sierra Nevada, Mulhacén, 3,481 m, 07.08.1924, A. Ginzberger s.n. (WU).
- SW Alps in France and Italy ‘aurantiacum’:** U1WU, France, Vaucluse, Mt. Ventoux, 1,200–1,912 m, 06.08.1955, Merxmüller & Wiedmann 1093 (Herb. Gutermann) [*P. aurantiacum*]; U2WU, France, Dép. Hautes-Alpes, Dévoluy, Montagne d’Arouse, Plateau de Bure–Pic de Bure, 2,500–2,550 m, 21.07.1983, L. Schrott & W. Gutermann 18557 (Herb. Gutermann) [*P. alpinum* subsp. *aurantiacum*]; U3WU, France, Dép. Hautes-Alpes, Mt. Arouse, Tal des Sigouste von Les Sauvas bis zum Plateau de Bure, 1,400–2,500 m, 08.08.1955, Merxmüller & Wiedmann 1237 (Herb. Gutermann) [*P. rhaeticum*]; U5WU, France, Savoie, Isère, massif de l’Obiou, 1,800–2,000 m, 02.08.1889, Abbé L. Guiguet s.n. (WU) [*P. alpinum* subsp. *occidentale* → *rhaeticum*]; U6WU, France, Savoie, Isère, Disans [Oisans?], 2,800 m, 13.08.1869, Faure s.n. (WU) [*P. alpinum* subsp. *rhaeticum*]; U4WU, Italy, Piemont, Limone, s.d., Huguemin [?] s.n. (WU) [*P. alpinum* subsp. *rhaeticum*].
- NW Alps in Switzerland and France ‘occidentale’:** O1O, France, Dép. Haute-Savoie, Vergy prope “Brizon”, 1,900 m, 07.1894, J. Timothée s.n. (O) [*P. burseri*]; O2O, France, Savoie, chaîne du Brezon près de Bonneville, s.d., Seringe s.n. (O) [*P. alpinum*]; O3WU, France, Savoie, Mont Trelod [Crelod?], s.d., Huguemin [?] s.n. (WU) [*P. alpinum* subsp. *occidentale* → *tatricum*]; O4WU, Schweiz, Wallis, Alpen in Untervallis, s.d., Em. Thomas s.n. (O) [*P. alpinum*, *occidentale*?]; O5WU, France, Haute-Savoie, Mont Vergy, 2,000 m, 28.07.1887, P. Gave s.n. (WU) [*P. alpinum* subsp. *occidentale* → *tatricum*].
- NW Alps in Switzerland & NE Alps in Austria ‘sendtneri’:** S1WU, Austria, Tirol, Speckkarspitze im Karwendelgebirge, 24.07.1901, Vollmann s.n. (Herb. Gutermann) [*P. rhaeticum* f. *albiflorum*]; S2WU, Austria, Tirol, Allgäuer Alpen, Hochvogel, 2,285–2,580 m, 18.08.1955, W. Gutermann 1752 (Herb. Gutermann) [*P. alpinum* subsp. *sendtneri*]; S3WU, Austria, Tirol, Stempeljoch bei Innsbruck, 1887, Zimeter s.n. (WU) [*P. alpinum* subsp. *sendtneri*]; S4O, Schweiz, Unterwalden, Pilatusberget, 24.07.1923, J. Holmboe s.n. (O) [*P. pyrenaicum* subsp. *sendtneri*].
- NE Alps in Austria ‘alpinum’:** A1WU, Austria, Steiermark, Johnsbach-Tal, Ödstein, 800–900 m, 17.07.1957, W. Gutermann 3034 (Herb. Gutermann) [*P. alpinum* subsp. *alpinum*]; A2WU, Austria, Steiermark, Johnsbach-Tal, Kaderalpschüttgraben, 650–700 m, 13.06.1969, W. Gutermann 7916 (Herb. Gutermann) [*P. alpinum*].
- Tatras in Poland & Slovakia ‘tatricum’:** T1O, Slovakia, Montes Magas Tatra, lacum “Késmárki Zöld”, 1,650 m, 13.07.1915, F. Filarszky & G. Timkó s.n. (O) [*P. burseri*]; T2O, Poland, Tatra, Czerwone Wierchy, 25.07.1928, J. Holmboe s.n. (O) [*P. burseri*]; T3O, Poland, Czerwone Wierchy, 1,800 m, 07.1923, T. Wisniewski s.n. (O) [*P. burseri*]; T4WU, Poland/Slovakia?, Koscielisko, in glareosis torrentis Dunajec, 22.07.1967, Heidenreich s.n. (WU) [*P. alpinum* subsp. *tatricum*].
- E Carpathians in Romania ‘corona-sancti-stephani’:** C1O, Romania, Bucegi-Gebirge, Omul, 2,510 m, 07.1928, C.C. Georgescu s.n. (O) [*P. pyrenaicum* subsp. *corona-sancti-stephani*]; C2O, Romania, Muntenia, distr. Prahova, Omul, 2,400–2,500 m, 12.08.1927, A. Borza s.n. (O) [*P. pyrenaicum* subsp. *corona-sancti-stephani*]; C3O, Romania, In reg. oppid. Brassó, in alp. Buceacs, 2,508 m, 22.07.1905, M. Futó s.n. (O) [*P. pyrenaicum*]; C4WU, Romania, Siebenbürgen, Königstein com Salzer, s.d., Kerner s.n. (WU) [*P. alpinum* subsp. *corona-sancti-stephani*]; C5WU, Romania, Transsilvania, in monte Ineu pr. Rodna, 12.08.1899, J. Dörfler s.n. (WU) [*P. alpinum* subsp. *corona-sancti-stephani*].
- Pirin in Bulgaria ‘degenii’:** D1WU, Bulgaria, Mt Jel-Tepe Perin dag, 2,500 m, 06.1909, Dimonie s.n. (WU) [*P. alpinum* subsp. *degenii* → *kernerii*].
- Dinaric Mountains in Bosnia and Herzegovina, Montenegro ‘kernerii’ or ‘rhaeticum’:** KR1WU, Bosnia-Hercegovina, Hercegovina centralis, montis Lupoglav (Prenj Planina), ca. 1,900 m, 08.1893, D.K. Vandaz s.n. (WU) [*P. alpinum* subsp. *kernerii*]; KR2WU, Bosnia-Hercegovina, Hercegovina, in monte Maglic Planina, 2,000 m, 07.1888, Adamovic 24.08.1889, S. Murbeck s.n. (WU) [*P. alpinum* subsp. *kernerii*]; KR3WU, Montenegro, Mt Kom Vasojevički, 21.08.1890, A. Baldacci s.n. (WU) [*P. alpinum* subsp. *rhaeticum* → *kernerii*]; KR4WU, Bosnia-Hercegovina, Čvrnsnica Planina, Gipfel des Veliki Vilinac, ca. 2,116 m, 12.07.1909, Handel-Mazetti s.n. (WU) [*P. alpinum* subsp. *rhaeticum* → *kernerii*]; KR5WU, Bosnia-Hercegovina, Čvrnsnigebiet, Gipfelregion der Čvrnsnica, 2,200 m, 29.07.1907, J. Stadlmann, F. Faltis & E. Wibiral s.n. (WU) [*P. alpinum* subsp. *rhaeticum* → *kernerii*]; KR6WU, Bosnia-Hercegovina, Bosnia SW, Dinara Planina, Troglav, Veliki Troglav, 1,880–1,930 m, 08.07.1973, F. Ehrendorfer & W. Gutermann s.n. (Herb. Gutermann) [*P. alpinum* subsp. *rhaeticum*].
- Abruzzo (Apennines), Italy:** X1WU, Italy, Abruzzo, in Mte Majellae, 6–7000’, 08.08.1874, Porta & Bigo s.n. (WU) [*P. alpinum* subsp. *ernesti-mayeri*]; X2WU, Italy, Abruzzo, Gran Sasso d’Italia, 08.1912, A. Ginzberger s.n. (WU) [*P. julicum*]; X3WU, Italy, Abruzzo, La Majella, M. Amaro, 2,800 m, 04.08.1899, G. Rigo s.n. (WU) [*P. alpinum* subsp. *ernesti-mayeri*]; X4WU, Italy, Abruzzo, La Majella, Campo di Grove zur Malga, ca. 2,000 m, 25.07.1924, Hayek s.n. (WU) [*P. alpinum* subsp. *ernesti-mayeri*].
- SE Alps in N-NE Italy ‘rhaeticum’:** R1WU, Italy, Como, Monte Braulio, s.d., s.c. (WU) [*P. alpinum* subsp. *rhaeticum*]; R2WU, Italy, Ortler-Gruppe, Stilfserjoch, Tibethütte zum Signalkogel, 2,760–2,780 m, 21.07.1982, L. Schrott & W. Gutermann 16812 (Herb. Gutermann) [*P. alpinum* subsp. *rhaeticum*]; R3WU, Italy, Prov. Sondrio, Orobische Alpen, Pizzo dei Tre Signori, 2,000–2,554 m, 31.07.1956, W. Gutermann 2531 (Herb. Gutermann) [*P. rhaeticum*]; R4WU, Italy, Prov. Bozen, Nordöstl. Dolomiten, Sella-Gruppe, Murfreitspitzen, 2,000–2,050 m, 13.07.1971, W. Gutermann 9937 (Herb. Gutermann) [*P. rhaeticum*]; R5WU, Italy, Prov. Belluno, Dolomiten, Sextener Dolomiten, vom Rif. Selva Piana zum Nordfuss der Cima Bagni, 1,700–1,950 m, 09.08.1982, L. Schrott & W. Gutermann 17261 (Herb. Gutermann) [*P. alpinum* subsp. *rhaeticum*].
- SE Alps in NE Italy, S Austria and NW Slovenia ‘kernerii’, ‘ernesti-mayeri’, ‘victoris’:** E1WU, Slovenia, Julische Alpen, Mangart-Massiv, Travnik, 2,100–2,200 m, 07.09.2006, W. Gutermann 38224 (Herb. Gutermann) [*P. alpinum* subsp. *ernesti-mayeri*]; E2WU, Italy, Udine, Julische Alpen, Lago del Predil, 965–980 m, 15.08.1956, W. Gutermann 2817 (Herb. Gutermann) [*P. alpinum* subsp. *ernesti-mayeri*]; E3WU, Italy, Udine, Jöf di Montasio, 1,700–1,980 m, 16.09.1979, W. Gutermann 13378 (Herb. Gutermann) [*P. alpinum* subsp. *ernesti-mayeri*]; K4WU, Austria, Steiermark, Süd-Steiermark, Logartal, 04.06.1904, Hayek & Kraskovits s.n. (WU) [*P. kernerii*]; E5WU, Italy, Raibler See, 25.07.1909, E. Galvagni s.n. (WU) [*P. alpinum* subsp. *ernesti-mayeri*].

**Appendix 3. Summary of analyses of the AFLP dataset of *Papaver alpinum* s.l. with the program STRUCTURE 2.2.**

- A) Entire dataset
- B) Northern Alps-Carpathian-Balkan-Abruzzo Cluster (NACBA Cluster)
- C) Southeastern Alpine Cluster

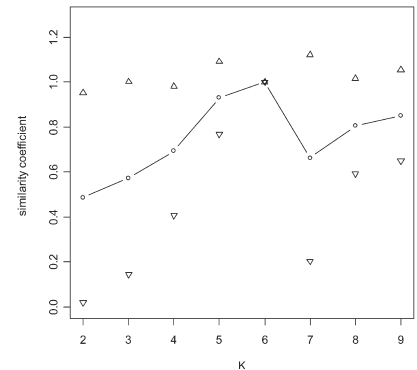
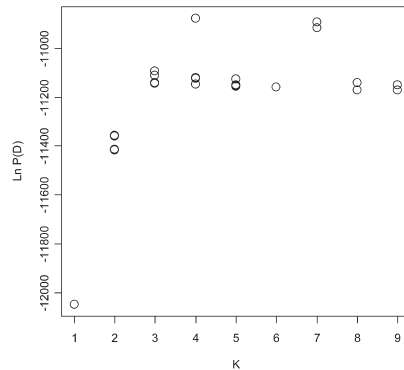
**A) Entire dataset**

In the graph to the left; likelihood of each number of groups (K) for each of 10 runs is plotted against K values. In the graph to the right; average similarity among runs is shown for each K value. Circles represent the mean of all pairwise comparisons among the 10 runs; whereas triangles indicate the standard deviation. Similarity among runs was calculated according to Rosenberg & al. (2002) with the R-script STRUCTURE-sum available from [www.nhm.uio.no/nhb](http://www.nhm.uio.no/nhb). According to Rosenberg & al. (2002); a similarity value above 0.85 corresponds to a generally similar population structure.



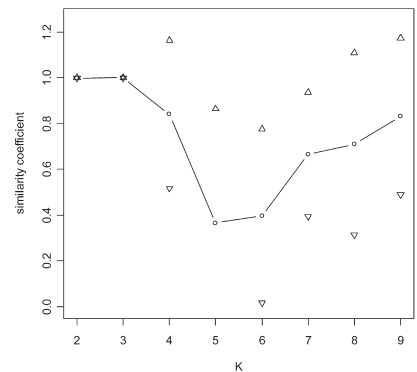
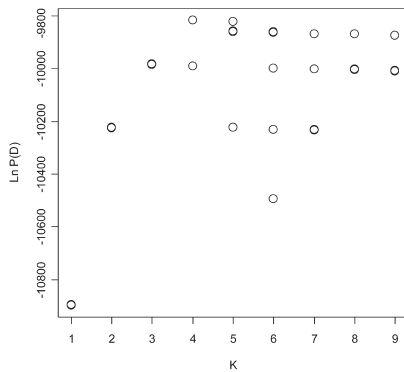
**B) Northern Alps-Carpathian-Balkan-Abruzzo Cluster**

Clustering with all populations of this group gave highly unstable results: Seven runs with the most deviating likelihoods are excluded in the figure below (2 runs for K = 2 and 5 runs for K = 5, both out of 29 runs)



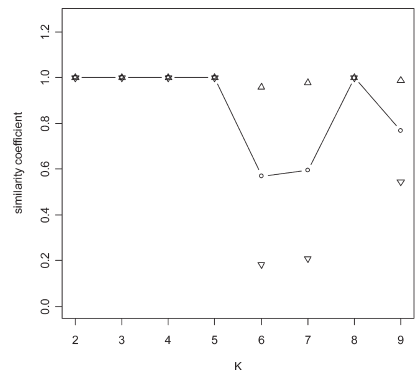
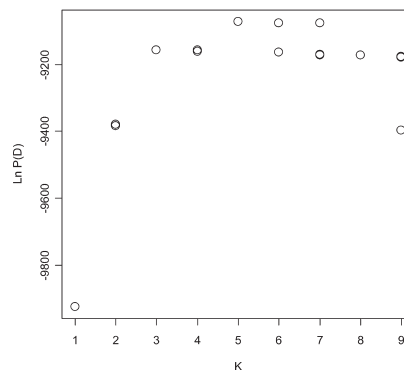
**Northern Alps-Carpathian-Balkan Cluster (without Abruzzo)**

The results improved significantly after population X23 from Abruzzo was excluded. Graph to the left; likelihood of each number of groups (K) for each of 10 runs plotted against the K values. Graph to the right; average similarity among runs for each K value.



**C) Southeastern Alpine Cluster**

Graph to the left; likelihood of each number of groups (K) for each of 10 runs plotted against the K values. Graph to the right; average similarity among runs for each K value.



**Appendix 4. Morphological measurements and scores (characters 1–25; see Table 1) arranged by regions and taxa. Numbers (No.) refer to herbarium and DNA vouchers from WU and O listed in Table 2 and Appendix 2. For the qualitative characters decimal numbers are intermediates. mv; missing value.**

Tax	Ctry	Reg	No	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	
lapey	France	PyrE	L20	2	3	2	2	2	2.5	2	2	2	1	2.5	1.5	15	11	1	2	1.5	1	3	8	5	2	1	1	4	
lapey	France	PyrE	L30	1.5	3	2	2	2	2	1.5	2	2	1	2	1.5	15	10	1	2	1.5	1	3	10	6	2	2	2	4	
lapey	France	PyrE	L1WU	1	3	1	2	2	2.5	1	3	2	1	2	2	11	8	1	2	1.5	mv	mv	mv	mv	mv	mv	mv	mv	
lapey	France	PyrE	L40	2	3	2	2	2	2	1.5	2	2	1	2	1	16	11	1	2	mv	1	3	9	6	2	2	2	4	
lapey	Spain	PyrE	L2	1.5	3	2	2	2	3	1.5	2	2	1	3	2	14.5	8.5	1	2	1.5	1	3	9	6.5	2	2	2	5	
lapey	Spain	PyrE	L1	mv	2	2	2	2	2.5	1	2	2	1	2	1	10	7	1	2	1.5	1	3	mv	mv	2	1	1	5	
lapey	Spain	SNEv	L5WU	2.5	3	2	1.5	2	2.5	2	3	2	3	mv	mv	mv	mv	mv	mv	mv	2	2	10.5	5.5	2.5	1.5	1	4.5	
auran	France	HAIp	U2WU	3	3	2	2	1.5	3	1	2	2.5	1	2	1	16	21	3	2	3	mv	mv	mv	mv	2	1	3	4.5	
auran	France	HAIp	U3WU	2	3	2	2	2	2	2	3	2.5	1	mv	mv	20	18	3	2	3	1	3	13	6	3	2	1	4	
auran	France	Savoy	U5WU	2.5	3	2	2	2	2	1	2	2.5	1	2	1	18	18	3	2	3	1	3	10	5	2	2	1.5	4	
auran	France	Savoy	R4	mv	2	3	2	2	2.5	2	2	3	1	mv	mv	21	22	3	2	3	1.5	3	8	4	3	2	2	5	
auran	France	Savoy	U6WU	2.5	3	3	2	2	3	1.5	3	3	1.5	mv	mv	15	mv	3	2	mv	1	2	9	5	2	2	1	4.5	
auran	France	Vaucl	U1WU	2.5	3	3	2	2	3	1	3	3	1/3	1	1	19	22	3	2	3	2	3	9	5	3	2	2	4.5	
auran	France	Vaucl	R3	mv	3	3	1.5	2	3	1	2.5	3	3	mv	mv	mv	mv	mv	2	3	mv	mv	mv	mv	mv	3	3	5	
auran	Italy	Piemo	U4WU	2	3	1	2	2	2.5	1	2	1	1	mv	mv	20	21	3	2	3	3	1.5	9	6	2	1.5	1.5	4	
occid	Switz	Wallis	O40	2	3	1	2	2	1.5	1	2	1	1	2	2	19	22	3	1	3	mv	mv	mv	mv	2	2	2	mv	
occid	Switz	Waadt	O5	mv	1	1	2.5	2	2	1	1	2	1	mv	mv	18	17	3	1	3	2	2	8	4	2	3	3	4	
occid	France	Savoy	O20	1	3	1	2	2	2	1.5	2	2	1	2	1.5	23	22	3	1	3	1.5	2	8	5.5	2	2	2	4	
occid	France	Savoy	O3WU	2.5	3	1	2	2	1	1	2	2	1	mv	mv	17	16	3	1	3	1	2.5	9	7	2	2	2	4	
occid	France	Savoy	O10	1.5	2.5	1	2	2	1.5	1.5	2.5	2	1	mv	mv	18	19	3	1	3	1.5	2	13	5	2	2	3	4	
occid	France	Savoy	O5WU	2	3	1	2	2	1	1	2	1	1	2	2	15	18	3	1	3	1	3	12.5	6	1	2	3	5	
alpin	Austr	Steie	A17	mv	2	1.5	2	2	2	1	1	2	1	2	1.5	mv	mv	mv	1	3	mv	mv	mv	mv	2	1.5	2	4.5	
alpin	Austr	Steie	A22	2	1	1.5	2	2	2	1	1	2	1	2	mv	14	17	3	1	3	2	2	9	5	2	2	2	4	
alpin	Austr	Steie	A1WU	2	2	1	2.5	1.5	1	1	1	1	1	2	2.5	20	22	3	1	3	1	2	8.5	6	1	1.5	2	4	
alpin	Austr	Steie	A2WU	2	2	1	2.5	1.5	1	1	1	1	1	2	2.5	21	23	3	1	3	1	2	10.5	7	1	1	2	5	
sendt	Austr	Tirol	S12	3	1	2	2	2	2	1	2.5	3	1	mv	mv	19	18	3	1	3	2	2	9	5	3	2	2	4	
sendt	Austr	Tirol	S1WU	3	1	3	2	1.5	3	1	2	3	1.5	1.5	2.5	22	23	3	1	3	mv	mv	mv	mv	2	2	3	4.5	
sendt	Austr	Tirol	S3WU	2	1	2	2	2	2.5	1	2	2	1	2	2.5	27	28	3	1	3	1	3	11	6	2	3	2	5	
sendt	Austr	Tirol	S2WU	3	1	3	2	2	2.5	1	2	3	2	2	2	23	23	3	1	3	1.5	2	mv	mv	2	2	3	4	
sendt	Switz	Untw	S6	mv	1	3	2	2	2	1	2	2	1	mv	mv	15	15	3	1	3	1	3	8	4	2	2	2	4	
sendt	Switz	Untw	S40	2	3	2	2	2	2	1.5	2.5	2	1	3	1.5	20	20	3	1	2.5	1	2	9	5.5	2	2	2	4	
tatri	Slova	Tatry	T10	2	3	1	2	2	1	1	2	2	1	1.5	2	20	15	3	1	3	mv	mv	mv	mv	2	3	3	5.5	
tatri	Polan	Tatry	T4WU	2	3	1	2	2	1	1	2	2	1.5	mv	mv	mv	mv	mv	mv	mv	1	2	10	6	2	2	3	4	
tatri	Polan	Tatry	T20	2	3	1	2	2	1.5	1	2	2	1	2	2	20	24	3	1	3	mv	mv	mv	mv	2	2	2	5	
tatri	Polan	Tatry	T3WU	2	3	1	2	2	2	1	2	2	1.5	mv	mv	15	15	3	1	3	1	3	mv	mv	2	2.5	3	4	
tatri	Polan	Tatry	T24	3	2.5	1	2	2	1.5	1	2	2	1	1	2	mv	mv	mv	mv	mv	mv	mv	mv	mv	mv	mv	mv	mv	mv
coron	Roman	CarpS	C10	2	3	1	2	2	3	1	2	2.5	1	mv	mv	15	16	3	2	3	mv	mv	mv	mv	mv	mv	mv	mv	
coron	Roman	CarpS	C30	3	3	1	2	2	2.5	1	2	2	1	2	1.5	15	15	3	2	3	2	2	9	5	2	2	3	4	
coron	Roman	CarpS	C20	2	3	1	2	2	2	1	2	2.5	1	mv	mv	12	mv	3	2	mv	2	2	9	6	2	2	3	5	
coron	Roman	CarpS	C26	3	3	1	2	2	3	1	2	2	1	mv	2	mv	mv	mv	mv	mv	mv	mv	mv	mv	mv	mv	mv	mv	
coron	Roman	CarpS	C4WU	3	3	1	2	2	2	1	2	1	1	2	2	16	20	3	2	3	2	2	9	5	2	2	2	5	
coron	Roman	CarpS	C5WU	2	3	1	2	2	2	2	2.5	2.5	1.5	2	2	18	20	3	2	3	2	2	7	5	2	2	2.5	5	
coron	Roman	CarpS	C25	3	3	1	2	2	2.5	1	2	2	1	2	2	21	23	3	2	3	1	1.5	mv	mv	2	1	2	5	

Appendix 4. Continued.

Tax	Ctry	Reg	No	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	
degen	Bulga	Pirin	D30B	3	1	1	2	2	3	1	2	2	1	1	3	8	8	3	2	3	mv	mv	mv	mv	mv	mv	mv	mv	mv
degen	Bulga	Pirin	D1WU	3	2	1.5	2	2	3	1	2.5	2	1	2	2	14	mv	3	2	3	1	1	6	4	2	2	3	4.5	
degen	Bulga	Pirin	D30A	3	1	2	2	2	3	1	2	2	1	1	3	mv	mv	mv	mv	mv	mv	mv	mv	mv	mv	mv	mv	mv	mv
rhaet?	Bosni	Dinar	K27	2	3	1	2	2	3	3	3	1.5	1	1.5	2	25	26	3	2	3	1.5	2	mv	mv	2	1	3	5	
kern?	Bosni	Dinar	K28	3	3	2	2	2	2.5	1	2	2	1	1.5	1.5	17	20	3	2	3	1	3	9	5	2.5	2	2	4	
kern?	Mnegr	Dinar	K29	3	3	2	2	2	2.5	2	2	2	1	1	1.5	16	16	3	2	3	2	2	9	4	2	2	2	4	
kern?	Bosni	Dinar	KR5WU	3	2	1	1.5	2	3	2	2	2	1	2	2	mv	mv	mv	2	3	mv	mv	mv	mv	2	1	3	5	
kern?	Bosni	Dinar	KR1WU	2	3	1	2	2	1.5	1	1	1.5	1	2	2.5	17	15	3	2	3	2	2	mv	mv	2	1.5	2	4	
kern?	Bosni	Dinar	KR2WU	2	3	1	2	2	1.5	1	1	1.5	1	2	2	19	20	3	2	3	2	2	10	6	1	2	2	5	
kern?	Bosni	Dinar	KR4WU	2	3	2	2	2	2.5	2	2	2	1	2	3	19	23	3	2	3	mv	mv	mv	mv	mv	mv	mv	mv	mv
kern?	Mnegr	Dinar	KR3WU	3	3	1	2	2	3	2	2	2	1	mv	mv	17	18	3	2	3	1	3	9	5	2	2	2.5	5.5	
rhaet?	Bosni	Dinar	KR6WU	3	1.5	1	2	2	3	3	3	2	1	2	2	22	23	3	2	3	1	2	mv	mv	2	1	3	5	
emay?	Italy	Abruz	X2WU	3	3	1	2	2	3	3	3	2	1	mv	mv	mv	mv	mv	mv	3	1	2	mv	mv	3	2	2	5	
emay?	Italy	Abruz	E23	2	3	2	2	2	2.5	2	2	2	1	mv	mv	mv	mv	mv	mv	mv	1	2	9	5	2	2	2	5	
emay?	Italy	Abruz	X1WU	2	3	2	2	2	2	1	2	2	1	2	2	17	19	3	1	3	1.5	2	mv	mv	2	3	3	5	
emay?	Italy	Abruz	X3WU	2	3	2	2	2	3	3	3	2	1	2	1.5	20	23	3	1	3	1	3	9	5	2	2	3	5	
emay?	Italy	Abruz	X4WU	3	3	2	2	2	3	2	3	2	1	2	1	19	20	3	1	3	1	3	12	7	3	3	3	5	
rhaet	Italy	Berga	R7	1	1	2	1.5	2	2	2	1.5	1	1	mv	mv	19	17	3	2	3	mv	mv	mv	mv	2	2	2	4	
rhaet	Italy	Berga	R1WU	mv	mv	2	2	2	2.5	1	2	2	1	mv	mv	12	12	3	2	3	mv	mv	mv	mv	mv	mv	mv	mv	
rhaet	Italy	Carni	R11	1.5	1	3	2	2	2.5	2	3	2	1	mv	mv	24	18	3	2	3	1	1	8	4.5	2	1	1	5	
rhaet	Italy	Dolom	R10	mv	1	3	2	2	3	2.5	3	mv	mv	mv	mv	mv	mv	mv	mv	mv	mv	mv	mv	mv	mv	mv	mv	mv	
rhaet	Italy	Dolom	R4WU	3	2	2	2	2	3	3	3	2.5	1	mv	mv	20	27	3	2	3	1	3	9	7	2	1	2	4.5	
rhaet	Italy	Dolom	R5WU	2	2	2	2	2	2.5	2	2	2	1	2	2	23	26	3	2	3	1	3	11	6.5	2	2	2	46	
rhaet	Italy	Dolom	R8	mv	1	1.5	2	1.5	3	2.5	3	2	1	mv	mv	mv	mv	mv	mv	mv	2	1	8	4	2	1	2	5	
rhaet	Italy	Trent	R2WU	2	2	2	1	2	3	3	3	3	1	2	3	14	15	3	2	3	1	3	9	7	2.5	2	3	4.5	
rhaet	Italy	Berga	R3WU	2	2	2	1.5	2	2.5	2	2	2	1	2	2	15	16	3	2	3	1	3	mv	mv	2.5	2	3	4.5	
kern	Italy	Carni	K13	mv	1	1	2	2	2.5	3	3	mv	mv	mv	mv	mv	mv	mv	mv	mv	mv	mv	mv	mv	mv	mv	mv	mv	
emay	Slove	Julij	E15	mv	1	3	2	2	3	1.5	2	2	1	mv	mv	20	21	3	1	3	mv	mv	mv	mv	mv	mv	mv	5	
emay	Slove	Julij	E1WU	2	1	2	1.5	2	2	3	3	2	1.5	mv	mv	19	23	3	1	3	1	2	9	6	3	1.5	1	4.5	
emay	Italy	Julij	E3WU	2	2.5	2	2	2	2.5	3	2	2	1	2	3	mv	mv	mv	mv	mv	2	2	10	7	2	2	1	4–7	
emay	Italy	Julij	E2WU	2	3	1	2	2	3	3	3	1.5	1.5	2	3	23	27	3	1	3	1	2	9	6.5	2	1	1	5	
emay	Austr	Kärnt	E5WU	2	mv	2	2	2	2.5	1	2	2	1.5	2	2.5	23	25	3	1	3	3	1	13	5.5	2.5	3	1.5	5	
kern	Slove	Julij	K16A	mv	1	1	2	2	3	1.5	2.5	2	1	mv	mv	17	19	3	2	3	mv	mv	mv	mv	mv	mv	mv	mv	
kern	Slove	Julij	K16B	mv	1	3	2	2	3	2	2.5	3	3	mv	mv	17	17	3	2	3	mv	mv	mv	mv	mv	mv	mv	5	
kern	Austr	Kärnt	K21	2	1	1	2.5	2	3	1	1	2	1	mv	mv	mv	mv	mv	mv	2	1	7	4	2	1	1	5		
kern	Austr	Kärnt	K19	2.5	1	1	2.5	2	1	1	2	1	1	mv	mv	21	23	3	2	3	mv	mv	mv	mv	mv	mv	mv	5	
kern	Austr	Steie	K4WU	2	3	1	2	2	1.5	1	1	2	1	2.5	2	22	22	3	2	3	1	2	mv	mv	2	2	3	4	
kern	Slove	Kamni	K20	2.5	1	3	2	2	2	1.5	2.5	2	1	mv	mv	mv	mv	mv	mv	mv	mv	mv	mv	mv	mv	mv	mv	mv	
victo	Slove	Julij	V14	mv	1	2	2	2	3	3	3	1	1	mv	mv	25	19	3	2	3	1	1	8	5	2	2.5	2.5	5	