Taxonomy and evolutionary history of *Alyssum montanum* (Brassicaceae) and related taxa in southwestern Europe and Morocco: Diversification driven by polyploidy, geographic and ecological isolation

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DOI http://dx.doi.org/10.12705/633.18

Abstract The *Alyssum montanum–A. repens* polyploid complex is a group of related perennial taxa with a diversity centre in the European (Sub)Mediterranean, controversial taxonomic treatments, and a poorly known evolutionary history. In the present study, morphological, ploidy level and genetic (AFLPs and chloroplast DNA sequences) data were collected to address the taxonomy and evolution of a sublineage of this complex distributed in southwestern Europe and Morocco. As a result, a new taxonomic treatment, differing substantially from recent concepts, is presented, including an identification key, synonymy and typifications. The recognition of several previously described but recently not accepted endemics is favoured (*A. flexicaule*, *A. orophilum*, *A. rhodanense*), whereas the existence of southern Iberian endemics is not supported. Most of the Iberian Peninsula is occupied by a single species for which the name *A. fastigiatum* is applicable. Populations from the summit areas of the Pyrenees represent a separate species, which is described here as *A. cacuminum*. Populations from coastal sand dunes in the Basque country (Bay of Biscay) and Galicia, recently recognised as two subspecies of *A. loiseleurii*, are elevated to species rank according to their genetic and morphological divergence. *Alyssum atlanticum* is resolved as a species confined to northern Africa and not reaching southern Spain. The distribution of *A. montanum* in a strict sense is much more restricted than previously reported, being delimited by the Pyrenees in the south, and the Alps in the northeast. The species complex studied here is composed of several polyploid stenoendemics confined to different mountain ranges or specific lowland habitats, and a few relatively widely distributed species. We infer that hybridisation and polyploidisation events, along with ecological and geographic isolation, have stimulated speciation in this complex. Hypotheses about the origin and evolutionary history of the species are discussed.

Keywords AFLPs; *Alyssum*; Iberian Peninsula; morphometrics; polyploidy; taxonomy

Supplementary Material Electronic Supplements 1 (Tables S1, S2, Fig. S1, Appendix S1) to 3 and alignment are available in the Supplementary Data section of the online version of this article at http://www.ingentaconnect.com/content/iapt/tax

■ INTRODUCTION

Southwestern Europe has been recognised as an important biodiversity centre. As part of the Mediterranean Basin, it harbours multiple areas of endemism and regional hotspots of plant diversity, many of which have been identified as refugia that have ensured long-term stability and persistence of species and genetic diversity (Médail & Quézel, 1997; Thompson, 2005; Médail & Diadema, 2009). Environmental heterogeneity in terms of climate, geology and topography, as well as the complex geological and biogeographic history of the area, explain such prominent accumulation of diversity. In contrast to central and northern Europe, species extinction during the Pleistocene climatic oscillations was limited in southern Europe. A lower impact of the ice ages and a high diversity of ecological niches available even at a fine scale probably induced only small-scale range shifts and often altitudinal rather than latitudinal migrations. These processes favoured not only long-term species persistence but also stimulated allopatric diversification and speciation, possibly leading to secondary contacts causing the genetic admixture of differentiated lineages and hybrid speciation (Nieto Feliner, 2011). Endemism and diversity hotspots largely coincide with mountain ranges (e.g., SW Alps, Pyrenees and Baetic mountain ranges), although low altitude and coastal regions have been identified as diversity hotspots as well (Lobo & al., 2001; García-Barros & al., 2002; Peñas & al., 2005; Casazza & al., 2008; Médail & Diadema, 2009). In addition, the flora of the southern Iberian region is enriched by northern African floristic elements (Baetic-Rifan hotspot; Médail & Quézel, 1997), with the Strait of Gibraltar

Received: 29 Nov 2013 | returned for first revision: 22 Jan 2014 | last revision received: 8 Feb 2014 | accepted: 12 Feb 2014 | not published online ahead of inclusion in print and online issues

intermittently acting as a land bridge that has affected species migration and diversification processes (Lavergne & al., 2013).

Detailed biogeographic and molecular systematic studies focusing on taxa in southwestern Europe have revealed diverse evolutionary and colonisation histories. Rapid diversification and speciation driven by isolation or ecological adaptation, reticulate evolution, as well as disjunct distributions resulting from both vicariance and long-distance dispersal have been reported (e.g., Gutiérrez Larena & al., 2002; Piñeiro & al., 2007; Balao & al., 2010; Salvo & al., 2010; Blanco-Pastor & al., 2012; Santos-Gally & al., 2012; Tison & al., 2013), contributing to the complexity of the observed genetic and taxonomic patterns. Many plant genera and species complexes with diversity centres in the western Mediterranean, however, have remained unexplored. The *Alyssum montanum–A. repens* group (Brassicaceae) is one of these cases, being a polyploid complex of perennial species distributed in Europe, western Asia and northern Africa, with the highest species diversity encountered in (Sub)Mediterranean areas (Dudley, 1965; Maire, 1967; Jalas & al., 1996; Hartvig, 2002; Španiel & al., 2011b, 2012b). The genus itself, with almost 200 species and a worldwide distribution, is polyphyletic and in need of taxonomic revision. The studied polyploid complex is a well-circumscribed monophyletic lineage in the *Alyssum* s.str. clade (Rešetnik & al., 2013). Our previous studies of this complex focused on species distributed in Central Europe and the Apennine Peninsula and unravelled variation patterns that contradicted traditional taxonomic treatments (Španiel & al., 2011a, b, 2012a, b). In contrast, little is known about populations and taxa from southwestern Europe. Our pilot studies encompassing the entire European area (see Results), however, have indicated that these species represent a monophyletic sublineage within this polyploid complex and are studied in detail here.

Numerous species and infraspecific taxa of the *Alyssum montanum–A. repens* complex were described and reported from southwestern Europe, inhabiting diverse habitats from sea level (coastal sand dunes) to high-mountain screes; however, taxonomic treatments have been fairly controversial (Ball & Dudley, 1993; Küpfer & Nieto Feliner, 1993; Jalas & al., 1996; Kerguélen, 1999). Eight species have been mostly accepted in more recent Floras and checklists: *A. atlanticum* Desf., *A. cuneifolium* Ten., *A. diffusum* Ten., *A. fastigiatum* Heywood, *A. gadorense* P.Küpfer, *A. loiseleurii* P.Fourn., *A. montanum* L., and *A. nevadense* Wilmott ex P.W.Ball & T.R.Dudley. *Alyssum montanum* (in a strict sense) was recorded as a highly polymorphic species (or subspecies) throughout the study area, in France, Spain and Morocco (Maire, 1967; Küpfer & Nieto Feliner, 1993; Jalas & al., 1996; Kerguélen, 1999). Four infraspecific taxa were reported from France (Kerguélen, 1999), and some related species were also described by Jordan & Fourreau (1868; e.g., *A. brigantiacum*, *A. orophilum* and *A. rhodanense*), but were later treated as synonyms of *A. montanum* (Kerguélen, 1993, 1999). Several infraspecific taxa of *A. montanum* were also described from Spain (e.g., Bolòs & Vigo, 1974); later, all of them were considered synonyms of *A. montanum* (Küpfer & Nieto Feliner, 1993, with updates at http://www.floraiberica.es/). A number of varieties have also been reported from northern Africa (Maire, 1967).

Alyssum diffusum, described from central to southern Italy and recently studied in the Apennines (Španiel & al., 2011b, 2012b), has also been reported from the Pyrenees and its foothills (Rouy & Foucaud, 1895; Guinochet & Vilmorin, 1982; Saule, 1991; Jalas & al., 1996), and from the Sierra Nevada (as var. *corymbosum* Pau, Pau, 1909, which, however, was more recently synonymised with *A. nevadense*, Küpfer & Nieto Feliner, 1993). Another species described from high altitudes of the Abruzzo Mts., *A. cuneifolium*, has also been reported from the screes of the uppermost parts of the mountain crests in the Pirin Mts. (Bulgaria; Stojanov, 1970), the Smolikas Mts. (Greece; Hartvig, 2002), Mt. Ventoux (France; Rouy & Foucaud, 1895; Coste, 1900) and the Pyrenees (Bolòs & Vigo, 1990; Saule, 1991; Küpfer & Nieto Feliner, 1993; Jalas & al., 1996). A separate subspecies, *A. cuneifolium* subsp. *losanum* P.Monts., was described from the Sierra de Guara (Pre-Pyrenees, Montserrat & Villar, 1974).

Alyssum loiseleurii was reported from coastal sand dunes in the Basque country, Bay of Biscay (Aquitaine, France; Guipúzcoa, Spain) and in Galicia, Spain (e.g., Küpfer & Nieto Feliner, 1993; Jalas & al., 1996). Populations from these two disjunct areas were later described as two subspecies, subsp. *loiseleurii* and subsp. *gallaecicum* S.Ortiz (Ortiz & Rodriguez Oubiña, 2005).

Alyssum atlanticum was originally described from the Tlemcen Mts. in Algeria (Desfontaines, 1798), and was also reported from Morocco (Maire, 1967, as *A. montanum* var. *atlanticum* (Desf.) Boiss.; Valdés & al., 2002, as *A. atlanticum*) and Sierra de Mijas in southern Spain (Küpfer & Nieto Feliner, 1993), although some authors also recorded its occurrence in several other southern Iberian mountain ranges of the Baetic System (Baumgartner, 1908; Molero Mesa & Pérez Raya, 1987; Morales Torres & al., 1988; Bolòs & Vigo, 1990). Three stenoendemics of the Baetic mountain ranges, *A. nevadense* (Sierra Nevada), *A. gadorense* (Sierra de Gádor), and *A. fastigiatum* (Sierra de Cazorla; more recently synonymised with *A. montanum*, Küpfer & Nieto Feliner, 1993) were also recognised (Ball & Dudley, 1993; Küpfer & Nieto Feliner, 1993; Jalas & al., 1996).

Most recently, we have rediscovered the high-alpine species *A. orophilum* Jord. & Fourr. from the southwestern Alps (Španiel & al., 2011b) that was not recognised as distinct from *A. montanum* in recent taxonomic accounts (e.g., Kerguélen, 1999).

Chromosome number records from the study area are fairly scarce; diploid chromosome numbers of $2n = 16$ were determined for *A. atlanticum* from Morocco and southern Spain (Quézel, 1957; Galland, 1988; Morales Torres & al., 1988), *A. nevadense* (as *A. diffusum* subsp. *corymbosum*; Morales Torres & al., 1988) and *A. montanum* from Spain and France (Küpfer, 1974), and a tetraploid number of $2n = 32$ for *A. loiseleurii* from France (as *A. arenarium*, Delay & Vivant, 1978). In other parts of Europe, the species complex includes diploids to hexaploids (Španiel & al., 2011a, b, 2012a).

To resolve the contradicting taxonomic treatments of the species listed above, and to outline their relationships and evolutionary history, we use ploidy-level screening, detailed morphometric analyses, and genetic approaches combining AFLPs (amplified fragment length polymorphisms) and chloroplast

DNA (cpDNA) sequence variation. The AFLP technique generates a high number of biparentally inherited markers capturing genome polymorphisms, and despite some limitations (e.g., potential fragment size homoplasy, dominant and anonymous nature of markers, their potential non-independence, low information content), it has proven informative in evolutionary and phylogenetic inferences among closely related congeners (Meudt & Clarke, 2007; García-Pereira & al., 2010). Its efficiency has also been demonstrated in several polyploid complexes (e.g., Balao & al., 2010; Bardy & al., 2010; Bendiksby & al., 2011; Rebernig & al., 2012; Greiner & al., 2013; Kuzmanović & al., 2013) that posed great taxonomic and evolutionary challenges. Highly variable intergenic spacers of maternally inherited, non-recombinant cpDNA have also been useful to determine relationships and evolution of closely related species (e.g., Borsch & Quandt, 2009; Consaul & al., 2010; Lakušić & al., 2013). Multivariate morphometrics allows for in-depth examination of morphological variation which, in comparison with genetic data, is essential to define and delimit taxa (Pessoa & al., 2012). More specifically, our aims were to (1) determine how many and which taxa can be recognised in the study area, (2) clarify their circumscription and geographic distribution, (3) unravel their relationships and evolutionary history, and (4) identify the main evolutionary processes that have driven speciation in this species complex.

■ MATERIALS AND METHODS

Study species, sampling design. — Populations were sampled throughout the entire European range of the studied species complex (Electr. Suppl. 1: Appendix S1). Our initial analyses indicated that the southwestern European and Moroccan populations form a monophyletic lineage of the complex. Representative populations of the taxa distributed in other parts of Europe (Central Europe, Apennine and Balkan Peninsulas; several of them recently studied and taxonomically revised, Španiel & al., 2011b, 2012a, b; Magauer & al., in prep.) were included in our molecular analyses, but the main focus was on populations and taxa from Spain, France and neighbouring regions in Italy, Switzerland and Germany, and in Morocco (Fig. 1; Table 1). *Alyssum montanum* subsp. *montanum* (here referred to as *A. montanum* s.str.) has recently been newly circumscribed (Španiel & al., 2012a) based on populations from Germany and Switzerland, with only two populations available from France; the present sampling extends the previous one by including samples from throughout France to determine and encompass the entire taxon range and variation. *Alyssum orophilum*, a recently rediscovered species from the high altitudes of the southwestern Alps in the Italian/French border region (Španiel & al., 2011b), was sampled and studied here in more detail.

Our sampling was based on detailed herbarium, database and literature surveys with the aim to obtain samples from the entire species ranges, encompassing their overall variation, and including type localities of as many described taxa as possible. The type localities or localities mentioned in the protologues (loci classici) are highlighted in Appendix S1 (Electr. Suppl. 1). Table 1 indicates our initial taxonomic assignment, traditional treatments (reflecting different taxonomic concepts applied over time by various authors), and the final treatment adopted as a result of the present study. The initial taxonomic assignments were based on the treatments in recent Floras and studies, but because we could not unambiguously recognise and delimit the traditional southern Iberian endemics (except for the populations from Sierra de Mijas, denoted as "*A. atlanticum*"), the mountain populations distributed throughout most of the Iberian Peninsula (i.e., apart from the coastal regions and the Pyrenees) and Morocco were treated as *A. montanum* s.l.

Multiple individuals per population were sampled and analysed; usually 10–30 individuals were used for morphometric analyses (preserved as herbarium specimens), and 10 leaf samples (taken from a subset of those 10–30 individuals) were dried in silica gel and the dehydrated tissue was subsequently used for DNA ploidy level determination (flow cytometry, all 10 samples) and DNA isolation (seven samples were used in AFLP analyses, and two of them for cpDNA sequence variation, based on our previous findings of no or little intrapopulation cpDNA variation in this species complex).

DNA ploidy level determination. — Flow cytometry using an AT-selective DAPI fluorochrome was employed to determine the DNA ploidy level of the studied populations. To relate the fluorescence intensity to DNA ploidy level, plants with known chromosome numbers (chromosomes counted here or taken from Španiel & al., 2011a, b, 2012a) were measured. The analyses were performed using a Partec Cyflow ML instrument equipped with an HBO-100 mercury arc lamp (Partec, Münster, Germany) following the protocol of Španiel & al. (2011a).

AFLP fingerprinting and data analyses. — Total genomic DNA was extracted using the DNeasy 96 Plant Kit (Qiagen, Hilden, Germany). The AFLP procedure followed the method of Vos & al. (1995) using the protocol as described in detail in Španiel & al. (2011a). Six primer pairs that yielded reproducible, polymorphic and well-scorable profiles were selected: *Eco*RI-ATC-(6-FAM)/*Mse*I-CAC, *Eco*RI-AGG-(VIC)/*Mse*I-CAC, *Eco*RI-AGG-(VIC)/*Mse*I-CAT, *Eco*RI-AGC-(NED)/*Mse*I-CAC, *Eco*RI-AGC-(PET)/*Mse*I-CAG, *Eco*RI-AGC-(PET)/*Mse*I-CTG. The amplification products were submitted for fragment analysis to the DNA Sequencing Laboratory, Faculty of Science, Charles University in Prague (3130 Genetic Analyzer, Life Technologies, Carlsbad, California, U.S.A.). PeakScanner v.1.0 software (Life Technologies) was used to read the AFLP electropherograms, to detect peaks and calculate their intensity and size. Light peak smoothing was applied to the electropherograms, the parameters of peak detection were kept at default values, and the filtering threshold of the minimal fluorescence was set to 50 relative fluorescent units (rfu). Binning and scoring were performed in RawGeno v.2.0 (R CRAN library; Arrigo & al., 2009) with the following settings: minimum bin width 1.5 bp, maximum bin width 2 bp, scoring range 80–500 bp, and discarding bins with low fluorescence peaks (<100 rfu). A binary data matrix was assembled. Usually one individual per population (12.5% of the final dataset) was replicated to estimate the reproducibility of the AFLP data generation and scoring (Bonin & al., 2004).

The genetic structure and relationships among the studied species and populations were estimated using multiple distance-based methods, Bayesian clustering, and analyses of molecular variance (AMOVA). Principal coordinate (PCoA; Krzanowski, 1990) and neighbour-joining analyses (NJ; Saitou & Nei, 1987) were both performed in FAMD v.1.30 (Schlüter & Harris, 2006) using Jaccard's coefficient for calculating pairwise genetic distances between individuals. For NJ, the group support was assessed by bootstrap analyses with 5000 replicates. Because all analysed populations appeared genetically coherent (clustering patterns strongly followed population assignment), NJ was also computed on a population level using pairwise ΦST values as an estimation of genetic distance (FAMD v.1.30; Schlüter & Harris, 2006).

To obtain deeper insights into the genetic structure of certain taxa or lineages, Bayesian clustering and AMOVA were performed. AMOVA was computed with Arlequin v.3.5 (Excoffier & al., 2005) using Euclidean pairwise distances and a significance test with 1000 permutations. Two Bayesian approaches were applied to estimate the optimal number of genetic clusters, identify the assignment of the analysed individuals, and reveal potential genetic admixture: the method

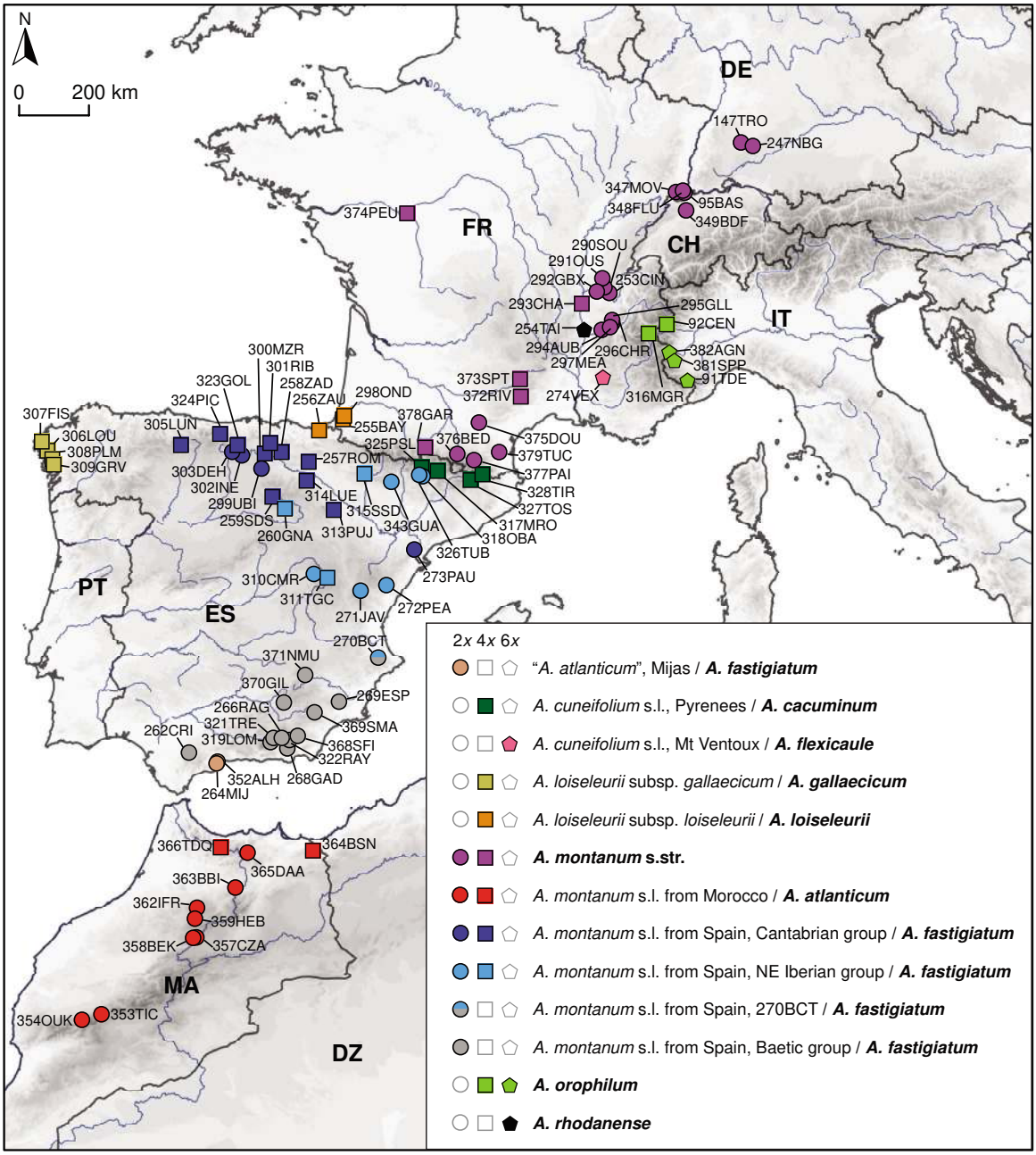

Fig. 1. Map of sampling sites. Symbol shapes indicate ploidy level, and colours depict species or group affiliation (if different, both the initial taxonomic assignment/final taxonomic treatments are given, separated by a slash, the latter in bold). Population codes follow Table 1.

Table 1. Overview of the studied *Alyssum* species and populations from southwestern Europe and Morocco and their characteristics. The initial taxonomic assignment or designation / traditional treatments / **final treatment as adopted here** are shown. Population code, country, location and altitude (Locality), DNA ploidy level (2*n*), and number of plants included in the flow cytometric, morphometric, AFLP and cpDNA analyses are indicated (N_{ind}). The AFLP columns also include the mean number of AFLP markers per individual ± standard deviation ($N_{loci/ind}$±SD), Nei's gene diversity (D_{Nei}), and the rarity index DW (rarity 2, AFLPdat, Ehrich, 2006) reported for populations and averaged for taxa; the cpDNA columns list the number of haplotypes (N_{hapl}, for populations) and haplotype (H) and nucleotide diversity (π_n) for taxa.

			Morph.	AFLP				cpDNA	
Pop. code	Locality	2*n* / N_{ind}	N_{ind}	N_{ind}	$N_{loci/ind}$±SD	D_{Nei}	DW	N_{ind}	N_{hapl}
"*A. atlanticum*" from Sierra de Mijas / *A. atlanticum* / ***A. fastigiatum***									
264MIJ	ES, Mijas, 641 m	2*x*/10	23	7	118±9	0.0500	16.19	2	1
352ALH	ES, Alhaurín el Grande, 620 m	2*x*/10	13	7	123±8	0.0562	13.90	2	1
		20	**36**	see *A. fastigiatum* – Baetic group for average species values					
A. cuneifolium s.l. from the Pyrenees / *A. cuneifolium* / ***A. cacuminum***									
317MRO	ES, Montorroio, 2730 m	4*x*/ 9 6*x*/ 1	29	7	147±9	0.0661	20.08	2	1
325PSL	ES, Tuca de Possolobino, 2603 m	4*x*/10	28	7	158±9	0.0731	22.16	2	1
327TOS	ES, La Tosa d'Alp, 2511 m	4*x*/10	12	7	163±9	0.0740	20.34	2	1
328TIR	ES, Coll de Tirapits, 2613 m	4*x*/10	24	7	147±9	0.0723	23.73	2	2
		40	**93**	**28**	**156**±7	**0.0714**±0.0036	**21.58**±1.71	***H* = 0.893,**	**π_n = 0.01458**
A. cuneifolium s.l. from Mt. Ventoux / ***A. flexicaule***									
274VEX	FR, Mt. Ventoux, 1686 m	6*x*/10	28	7	175±5	0.0729	27.68	2	1
A. loiseleurii subsp. *gallaecicum* / *A. loiseleurii* subsp. *gallaecicum* / ***A. gallaecicum***									
306LOU	ES, Praia de Louro, 7 m	4*x*/10	22	7	132±7	0.0580	14.33	2	1
307FIS	ES, Fisterra, 20 m	4*x**/7	–	6	129±11	0.0394	10.00	2	1
308PLM	ES, Palmeira, 8 m	4*x*/10	21	7	132±9	0.0469	13.47	2	1
309GRV	ES, O Grove, 7 m	4*x*/10	27	7	130±11	0.0472	10.31	2	1
		37	**70**	**27**	**130**±2	**0.0453**±0.0040	**12.03**±2.19	***H* = 0.429,**	**π_n = 0.00024**
A. loiseleurii subsp. *loiseleurii* / *A. loiseleurii* subsp. *loiseleurii* / ***A. loiseleurii***									
255BAY	FR, Bayonne, Anglet, 8 m	4*x*/12	–	7	131±10	0.0362	9.19	2	1
256ZAU	ES, Zarautz, 3 m	4*x*/14	19	6	132±7	0.0362	9.15	2	2
298OND	FR, Ondres, 11 m	4*x*/10	14	7	131±12	0.0444	9.67	2	1
		36	**33**	**20**	**131**±1	**0.0389**±0.0048	**9.34**±0.29	***H* = 0.333,**	**π_n = 0.00019**
A. montanum s.l. from Spain, Cantabrian group / *A. montanum*, *A. diffusum* / ***A. fastigiatum* – Cantabrian group**									
257ROM	ES, San Román de Campezo, 1033 m	4*x*/10	24	7	155±10	0.0616	22.99	2	1
258ZAD	ES, Jurisdicción de San Zadornil, 630 m	4*x*/10	27	7	139±9	0.0578	15.57	2	1
259SDS	ES, Santo Domingo de Silos, 1100 m	4*x*/10	25	7	143±8	0.0714	25.11	2	2
273PAU	ES, Paüls, 531 m	2*x*/10	–	6	145±13	0.0806	19.58	2	1
299UBI	ES, Cañón del río Ubierna, 905 m	2*x*/10	28	7	109±7	0.0343	10.93	2	1
300MZR	ES, Puerto de la Mazora, 990 m	4*x*/10	–	6	141±11	0.0690	19.59	2	1
301RIB	ES, El Ribero, 765 m	4*x*/10	22	7	137±19	0.0613	17.74	2	1
302INE	ES, Renedo de la Inera, 929 m	2*x*/10	20	6	123±18	0.0598	17.18	2	1
303DEH	ES, Dehesa de Montejo, 1120 m	2*x*/10	–	6	129±7	0.0496	13.74	2	1
305LUN	ES, Caldas de Luna, 1200 m	4*x*/10	19	7	155±10	0.0770	30.48	2	1
313PUJ	ES, Purujosa, 1525 m	4*x*/10	29	7	156±6	0.0777	27.56	2	1
314LUE	ES, Luezas, 1290 m	4*x*/10	30	7	149±4	0.0634	16.52	2	1
323GOL	ES, El Golobar, 1990 m	4*x*/10	18	6	143±11	0.0640	17.32	2	1
324PIC	ES, Picos de Europa, 2070 m	4*x*/ 9	–	6	150±8	0.0701	24.17	2	2
		139	**242**	**92**	**141**±13	**0.0641**±0.0121	**19.89**±5.50	***H* = 0.905,**	**π_n = 0.00504**

Table 1. Continued.

			Morph.	AFLP				cpDNA	
Pop. code	Locality	$2n/N_{ind}$	N_{ind}	N_{ind}	$N_{loci/ind}$±SD	D_{Nei}	DW	N_{ind}	N_{hapl}
A. montanum s.l. from Spain, NE Iberian group / *A. cuneifolium* subsp. *losanum*, *A. montanum* / ***A. fastigiatum* – NE Iberian group**									
260GNA	ES, Mirador de la Galiana, 1120 m	4*x*/10	24	7	122±6	0.0439	11.98	2	1
271JAV	ES, Sierra de Javalambre, 1853 m	2*x*/10	25	7	123±9	0.0554	22.83	2	1
272PEA	ES, Mt. Penyagolosa, 1695 m	2*x*/10	25	7	119±14	0.0556	17.41	2	1
310CMR	ES, Cañamares, 868 m	2*x*/10	20	6	121±5	0.0558	14.87	2	1
311TGC	ES, Tragacete, 1445 m	4*x*/10	27	7	147±10	0.0680	21.41	2	1
315SSD	ES, Sierra de Santo Domingo, 1236 m	4*x*/10	28	7	145±10	0.0531	15.08	2	2
318OBA	ES, Congosto de Obarra, 985 m	2*x*/10	–	7	108±9	0.0394	14.56	2	1
326TUB	ES, El Turbón, 1770 m	2*x*/10	–	7	141±7	0.0670	20.83	2	1
343GUA	ES, Tozal de Guara, 2077 m	2*x*/ 7	–	7	129±8	0.0476	12.62	2	1
		87	**149**	**62**	**128**±13	**0.0540**±0.0095	**16.84**±3.98	***H* = 0.935, π_n = 0.01327**	
A. montanum s.l. from Spain, Baetic group / *A. atlanticum*, *A. gadorense*, *A. fastigiatum*, *A. montanum*, *A. nevadense* / ***A. fastigiatum* – Baetic group**									
262CRI	ES, Cerro de San Cristobal, 1238 m	2*x*/10	24	5	125±12	0.0554	14.72	2	1
266RAG	ES, Puerto de la Ragua, 2120 m	2*x*/10	20	7	128±10	0.0552	15.78	2	1
268GAD	ES, Sierra de Gador, 1851 m	2*x*/10	25	7	133±11	0.0670	18.28	2	1
269ESP	ES, Sierra de Espuña, 1500 m	2*x*/10	10	7	128±20	0.0557	17.04	2	1
319LOM	ES, Loma del Mulhacén, 3021 m	2*x*/10	27	7	126±13	0.0615	17.96	2	1
321TRE	ES, Puerto del Trevélez, 2756 m	2*x*/10	26	7	127±5	0.0643	19.74	2	2
322RAY	ES, Cerro del Rayo, 2398 m	2*x*/10	4	7	131±10	0.0715	22.06	2	2
368SFI	ES, Sierra de los Filabres, 1912 m	2*x*/10	24	7	138±6	0.0694	26.29	2	2
369SMA	ES, Sierra de María, 1525 m	2*x*/10	24	7	137±11	0.0700	23.26	2	1
370GIL	ES, Puerto Gilillo, 1748 m	2*x*/10	22	6	126±6	0.0640	18.11	2	1
371NMU	ES, Nacimiento del río Mundo, 1050 m	2*x*/10	–	7	119±10	0.0555	18.24	2	1
		110	**206**	**74**	**127**±6[#]	**0.0609**±0.0068[#]	**18.69**±3.40[#]	***H* = 0.95, π_n = 0.00848[#]**	
A. montanum s.l. from Spain, pop. 270BCT / *A. montanum* / ***A. fastigiatum***									
270BCT	ES, Bocairent, 895 m	2*x*/10	20	7	119±9	0.0571	20.11	2	1
A. montanum s.l. from Morocco / *A. montanum*, *A. atlanticum* / ***A. atlanticum***									
353TIC	MA, Tizi-n-Tichka, 2100 m	2*x*/10	13	7	160±14	0.0742	31.51	2	1
354OUK	MA, Oukaïmeden, 2620 m	2*x*/10	25	7	149±12	0.0776	32.61	2	1
357CZA	MA, Col du Zad, 2210 m	2*x*/10	24	7	149±12	0.0737	32.00	2	2
358BEK	MA, Bekrite, 2075 m	2*x*/10	14	7	139±9	0.0745	31.13	2	2
359HEB	MA, Jbel Hebri, 1935 m	2*x*/10	18	6	140±7	0.0714	28.93	2	2
362IFR	MA, Ifrane, 1517 m	2*x*/10	23	7	132±11	0.0602	23.31	2	2
363BBI	MA, Bab Bou Idir, 1825 m	2*x*/ 2	–	–	–	–	–	–	–
364BSN	MA, Beni-Snassen Mts, 1504 m	4*x*/10	29	7	147±13	0.0631	22.96	2	1
365DAA	MA, Jbel Azrou Akchar, 1820 m	2*x*/10	28	5	147±11	0.0717	20.41	2	1
366TDQ	MA, Jbel Tidighine, 2280 m	4*x*/10	31	7	152±8	0.0601	21.34	2	1
		92	**205**	**60**	**146**±8	**0.0696**±0.0066	**27.13**±5.03	***H* = 0.961, π_n = 0.01127**	

Table 1. Continued.

Pop. code	Locality	$2n/N_{ind}$	Morph. N_{ind}	AFLP N_{ind}	AFLP $N_{loci/ind}$±SD	AFLP D_{Nei}	AFLP DW	cpDNA N_{ind}	cpDNA N_{hapl}
A. montanum subsp. *montanum* / *A. montanum* subsp. *montanum*, subsp. *collicolum* / ***A. montanum* s.str. – northern diploids**									
95BAS	CH, Basel, 390 m	2*x*[a]/10	22	6	121±8	0.0218	8.37	2	2
147TRO	DE, Trochtelfingen, 715 m	2*x*[a]/12	20	7	127±7	0.0426	13.52	2	2
247NBG	DE, Neuburg, 544 m	2*x*[c]/10	14	7	131±4	0.0267	8.73	2	1
253CIN	FR, Cerin, 700 m	2*x*[c]/10	25	6	126±4	0.0380	10.11	2	1
290SOU	FR, Souclin, 739 m	2*x*/ 10	4	7	121±7	0.0291	11.41	2	1
291OUS	FR, Oussiat, 245 m	2*x*/ 10	24	6	122±5	0.0376	10.26	2	1
292GBX	FR, Les Gaboureaux, 198 m	2*x*/ 10	10	7	108±7	0.0256	8.60	2	1
294AUB	FR, Auberives en Royans, 208 m	2*x*/ 10	22	7	119±8	0.0369	12.17	2	1
295GLL	FR, La Graille, 1251 m	2*x*/ 10	26	7	124±6	0.0362	9.17	2	1
296CHR	FR, Mt. Charande, 1699 m	2*x*/ 10	13	7	117±7	0.0277	7.97	2	1
297MEA	FR, Gorges du Méaudret, 1000 m	2*x*/ 10	22	7	126±5	0.0452	11.89	2	1
347MOV	CH, Movelier, 870 m	2*x*/ 7	–	7	136±9	0.0412	10.82	2	2
348FLU	CH, Hofstetten-Flüh, 530 m	2*x*/ 7	–	6	119±5	0.0239	7.40	2	1
349BDF	CH, Burgdorf, 543 m	2*x*/ 7	–	7	116±5	0.0259	9.03	2	1
		133	**202**	**94**	**122**±7	**0.0327**±**0.0077**	**9.96**±**1.80**	***H* = 0.698, π_n = 0.00853**	
A. montanum subsp. *montanum* / *A. montanum* subsp. *montanum*, subsp. *collicolum*, *A. diffusum* / ***A. montanum* s.str. – southern diploids**									
375DOU	FR, Dourgne, 337 m	2*x*/10	–	6	112±6	0.0284	7.81	2	1
376BED	FR, Bédeilhac-et-Aynat, 720 m	2*x*/10	20	6	122±6	0.0301	9.06	2	1
377PAI	FR, Col de Pailhères, 1979 m	2*x*/10	19	6	125±7	0.0398	9.84	2	1
379TUC	FR, Tuchan, 877 m	2*x*/10	–	7	125±5	0.0403	12.19	2	1
		40	**39**	**25**	**121**±6	**0.0347**±0.0063	**9.73**±1.84	***H* = 0.429, π_n = 0.00024**	
A. montanum subsp. *montanum* / *A. montanum* subsp. *montanum*, subsp. *collicolum*, *A. diffusum* / ***A. montanum* s.str. – tetraploids**									
293CHA	FR, Chasse sur Rhône, 176 m	4*x*/10	21	7	146±7	0.0537	15.78	2	1
372RIV	FR, Les Rives, 774 m	4*x*/10	25	7	146±15	0.0689	21.31	2	1
373SPT	FR, St Pierre des Tripiers, 976 m	4*x*/10	25	7	155±11	0.0766	24.49	2	1
374PEU	FR, Le Pérou, 73 m	4*x**/9	–	5	162±13	0.0751	17.78	2	1
378GAR	FR, Pic du Gar, 1654 m	4*x*/10	16	7	178±9	0.0703	26.47	2	1
		49	**87**	**33**	**158**±14	**0.0689**±0.0091	**21.17**±4.46	***H* = 0.800, π_n = 0.01235**	
A. orophilum / *A. montanum*, *A. orophilum*, *A. pedemontanum* / ***A. orophilum***									
91TDE	FR, Col de Tende, 1851 m	6*x*[b]/ 7	30	7	179±14	0.0774	35.91	4	1
92CEN	FR, Lac du Mont Cenis, 2086 m	4*x*[b]/ 7	30	7	164±12	0.0801	28.36	4	3
316MGR	FR, Montgenèvre, 1990 m	4*x*/10	28	7	150±14	0.0723	22.01	2	2
381SPP	IT, Stroppo, 1916 m	6*x*/10	20	7	157±11	0.0594	21.13	2	1
382AGN	FR, Col d'Agnel, 2430 m	6*x*/10	22	7	164±14	0.0594	23.47	2	1
		44	**130**	**35**	**163**±11	**0.0697**±0.0098	**26.43**±5.50	***H* = 0.659, π_n = 0.00809**	
A. rhodanense / *A. montanum*, *A. rhodanense* / ***A. rhodanense***									
254TAI	FR, Tain l'Hermitage, 168 m	6*x*/10	21	7	155±11	0.0590	19.84	2	1

DNA ploidy levels were determined here or taken from previous studies: [a] = Španiel & al. (2011a), [b] = Španiel & al. (2011b), [c] = Španiel & al. (2012a); asterisks (*) indicate that the tetraploid level (pop. 374PEU, 307FIS) was confirmed here as $2n = 32$ by chromosome counts. # = values including data of the two populations from Sierra de Mijas (264MIJ, 352ALH) listed separately. See Electr. Suppl. 1: Appendix S1 for more details on localities, collection data, type localities, and GenBank accession numbers.

based on stochastic optimisation as implemented in BAPS v.5.2 (Corander & al., 2006) and that based on the Markov chain Monte Carlo (MCMC) algorithm in STRUCTURE v.2.3.3 (Pritchard & al., 2000). For the BAPS analyses, the module clustering of individuals was used, setting the maximal number of clusters (*K*) to 10–20 (based on the dataset used), conducting both mixture and admixture analyses, and repeating 10 times to check the consistency of the clustering outputs. For the STRUCTURE computations, the approach that enables the handling of dominant data (Falush & al., 2007) was employed, using a model with admixture and assuming the independence of allele frequencies among populations. Ten replicates were run for each K = 1–10 (user-defined number of clusters) with a burn-in length of 100,000 generations and data collection for an additional 1,000,000 generations. The STRUCTURE analyses were performed on the Bioportal of the University of Oslo (www.bioportal.uio.no; Kumar & al., 2009). The STRUCTURE output data were parsed using the STRUCTURE HARVESTER program (Earl & von Holdt, 2012) and R-script Structure-sum-2009 (part of AFLPdat; Ehrich, 2006) to calculate similarity coefficients between the replicate runs and to determine the optimal *K* value following the method of Evanno & al. (2005). An alignment of cluster assignments across the replicate analyses was then conducted in CLUMPP v.1.1.2b (Jakobsson & Rosenberg, 2007) and visualised using DISTRUCT v.1.1 (Rosenberg, 2004).

Genetic diversity of the analysed populations and taxa was estimated by calculating the mean number of AFLP markers scored per individual and the average proportion of pairwise differences between individuals (Nei's gene diversity—D_{Nei}; Nei, 1987) using R-script AFLPdat (Ehrich, 2006). The statistical significance of the observed differences was explored using the *t*-test (pairwise comparisons) or Tukey-Kramer test (multiple comparisons) in SAS/STAT v.9.2 (SAS Institute, 2009). The AFLP marker specificity, rarity (the frequency down-weighed marker value, as implemented in AFLPdat), and sharing patterns were explored as well.

cpDNA amplification, sequencing and data analyses. — Two intergenic spacers of cpDNA that proved to be variable in our previous studies of this species complex (Španiel & al., 2011b, 2012a) were amplified and sequenced: *rpoB-trnC* (Shaw & al., 2005) and *rpl32-trnL*(UAG) (Shaw & al., 2007). The PCR amplification protocol followed Španiel & al. (2011b). PCR products were purified using FastAP thermosensitive alkaline phosphatase and exonuclease I following the manufacturer's protocol (Fermentas, Thermo Fisher Scientific, Waltham, Massachusetts, U.S.A.). The purified PCR products were submitted for sequencing to the GATC Biotech company (Konstanz, Germany). Both forward and reverse strands were sequenced. The sequences were proofread, trimmed of ambiguous ends and aligned in Geneious v.6.1.5 (Biomatters, Auckland, New Zealand) using the MAFFT v.6.814b (Katoh & al., 2002) plug-in. Phylogenetically informative indels were coded as binary characters following the simple indel coding approach by Simmons & Ochoterena (2000) using the GapCoder software (Young & Healy, 2003). Gaps in the microsatellite stretches of 1–3 bp-motifs that may be homoplasious were ignored.

Phylogenetic trees were reconstructed using Bayesian inference based on the MCMC algorithm (Huelsenbeck & Ronquist, 2001) in MrBayes v.3.2.1 (run at the Bioportal at the University of Oslo). The best-fit models of nucleotide substitutions were selected in jModelTest v.0.1.1 (Darriba & al., 2012) using Akaike (AIC) and Bayesian (BIC) information criteria and implemented in the Bayesian computations. The two cpDNA alignments were concatenated. Indel scoring, appended to the nucleotide datasets, was defined as a separate partition and treated under the restriction model as implemented in MrBayes. Four chains of MCMC were run for two million generations with a sampling frequency of every 100 generations. Convergence of all parameters was verified by inspecting the graphical output from the Bioportal website. The first 5000 trees (500,000 generations) were discarded as the burn-in period, and the consensus tree was generated from the remaining trees, computing also the Bayesian posterior probabilities for each node. The close relatives *A. turkestanicum* Regel & Schmalh., *A. minutum* DC. and *A. alyssoides* (L.) L. (GenBank acc. nos. F703784–88, JF703911–15) were used as outgroups.

Statistical parsimony was also used to construct a haplotype network (TCS v.1.18; Clement & al., 2000). TCS was run with a 95% connection limit, with gaps treated as missing data (but with gap scoring appended to the nucleotide dataset). Genetic diversity based on the cpDNA sequence data was assessed by calculating gene (haplotype) diversity (*H*) and nucleotide diversity (π_n, Nei, 1987) with the software Arlequin v.3.5 (Excoffier & al., 2005).

Multivariate morphometrics. — Discriminant analyses (canonical discriminant analysis, CDA, and classificatory discriminant analysis, classificatory DA; Klecka, 1980; Marhold, 2011) were employed to assess the morphological differentiation between the studied taxa and genetically defined entities. The morphological characters that were measured or scored included those reported as diagnostic in determination keys and Floras, those recognised as taxonomically relevant in our previous studies (Španiel & al., 2011a, b, 2012a), as well as characters that appeared variable based on our field observations (for the list of characters, see Table 2). Most characters were measured or scored directly on the herbarium specimens that were obtained from the field sampling. The floral characters were measured on scanned floral parts using the software CARNOY (Schols & al., 2002). Trichomes on stem leaves were observed and measured using a stereomicroscope (Olympus SZ61) and the software QuickPHOTO Industrial v.2.3. Two characters were semiquantitative (trichome coverage on upper and lower surface of stem leaves) and the others were quantitative. Two main matrices were assembled: (1) character values measured on individual plants (1542 plants × 20 characters) and (2) population means of the measured characters (72 populations × 20 characters). Partial matrices encompassing only a selection of individuals and populations were assembled as well. First, the Shapiro-Wilk statistic was used to test the normal distribution of the measured character values. Correlations between each character pair were then computed to reveal the correlation structure among the characters and to ensure that no very high correlations (>0.95–0.99) were present (potentially distorting

discriminant analyses). In CDA, the discriminant functions were derived to express the extent of morphological differentiation between predefined groups. The 95% isodensity circles, expected to contain 95% of the members of the group (Podani, 2000, 2001), were calculated for the first two canonical axes and drawn on the CDA diagrams. Nonparametric *k*-nearest neighbours classificatory DA using a cross-validation procedure were performed to estimate the percentage of plants that were correctly assigned to the predefined groups. Discriminant analyses generally require a multivariate normal distribution of the characters; nevertheless, they have been shown to be considerably robust against deviations in this respect (Thorpe, 1976; Klecka, 1980). Descriptive statistics of each measured character were also computed for the groups of populations/taxa delimited here. The analyses were performed using the SAS/STAT v.9.2 (SAS Institute, 2009) and SYN-TAX 2000 (Podani, 2001) software.

Table 2. List of characters and their acronyms used in morphometric analyses.

Vegetative characters
StemLength—length of longest stem on plant, measured from bottom (including its ascending part) to pedicel base of lowermost silicule/flower (mm)
NrLatBranches—number of lateral branches on main stem (excluding branches in basal, ascending part of stem)
Length8thLeaf—length of 8th stem leaf (counted downward from pedicel base of lowermost silicule/flower) (mm)
Width8thLeaf—width of 8th stem leaf (counted downward from pedicel base of lowermost silicule/flower) (mm)
Length15thLeaf—length of 15th stem leaf (counted downward from pedicel base of lowermost silicule/flower) (mm)
Width15thLeaf—width of 15th stem leaf (counted downward from pedicel base of lowermost silicule/flower) (mm)
Dist8–15thLeaf—distance between 8th and 15th stem leaf (counted downward from pedicel base of lowermost silicule/flower) (mm)
LengthTrichRay—length of longest ray of stellate trichomes on lower surface of middle stem leaf (mean value of three measurements) (mm)
NrRaysTrichLower—number of rays of stellate trichomes on lower surface of middle stem leaf (mean value of three counts)
TrichDensityLower—number of trichomes per 0.5 mm² (area with homogeneous indumentum is preferred, not including median vein) on the lower surface of middle stem leaf
TrichCoverageLower—coverage of trichomes on lower surface of middle stem leaf (0: 0%–33% coverage, 1: 33%–66% coverage, 2: 66%–95% coverage, 3: 95%–100% coverage)
NrRaysTrichUpper—number of rays of stellate trichomes on upper surface of middle stem leaf (mean value of three counts)
TrichDensityUpper—number of trichomes per 0.5 mm² (area with homogeneous indumentum is preferred, not including median vein) on upper surface of middle stem leaf
TrichCoverageUpper—coverage of trichomes on upper surface of middle stem leaf (0: 0%–33% coverage, 1: 33%–66% coverage, 2: 66%–95% coverage, 3: 95%–100% coverage)
Floral characters
PetalLength—maximum petal length in one of largest flowers (mm), emarginate apical part of petal with petal sinus not included
PetalSinus—depth of sinus on emarginate petal tip (mm)
PetalWidth—width of longest petal in one of the largest flowers (mm)
SepalLength—maximum sepal length in one of the largest flowers (mm)
FilamentLength—length of longest filament in one of the largest flowers (mm)
StyleLength—length of style in one of the largest flowers (mm)

■ RESULTS

DNA ploidy levels. — DNA ploidy level of the populations analysed here are given in Table 1. Diploid to hexaploid populations were recorded. Mixed-ploidy level populations were very rare (see population 317MRO in Table 1), but two different ploidy levels within a single species were commonly observed. *Alyssum montanum* s.str. included both diploids and tetraploids ($2n = 32$ counted in the 374PEU population); a hexaploid population from Rhône-Alpes, France (254TAI), initially assigned to this species is, based on its distinction (see below), further treated as *A. rhodanense*. *Alyssum orophilum* comprised tetraploids and hexaploids, and populations of *A. montanum* s.l. both from Spain and Morocco included diploids and tetraploids. *Alyssum cuneifolium* s.l. from the Pyrenees was tetraploid, whereas the population from Mt. Ventoux was hexaploid. Both subspecies of *A. loiseleurii* were tetraploid ($2n = 32$ counted in the 307FIS population).

AFLPs. — Replicate samples indicated a high degree of reproducibility of the generated AFLP data, with error rates in the datasets from individual primer pairs ranging from 0.53% to 1.23%. The complete AFLP dataset included 777 individuals from 118 populations and 1660 scored AFLP loci. To decrease the complexity of the data and to gain deeper insights into the genetic structure, three partial datasets based on the major clusters (presumably representing monophyletic groups) were also assembled: (1) dataset of the focus taxa, i.e., the southwestern European and Moroccan samples (586 individuals from 87 populations with 1563 loci; populations listed in Table 1); (2) dataset of *A. montanum* s.l. from Spain and *A. loiseleurii* subsp. *gallaecicum* (277 individuals from 41 populations with 1350 loci); and (3) dataset of *A. montanum* s.str. and *A. rhodanense* (159 individuals from 24 populations with 986 loci).

Complete dataset. — Both NJ (Fig. 2) and PCoA (not shown) placed the taxa from central Europe and the Apennine and Balkan Peninsulas into a single cluster (delimited by dashed lines in Fig. 2), which could be clearly differentiated from the here-studied southwestern European and Moroccan populations. Only the positions of the southwestern Alpine polyploid *A. orophilum* and *A. cuneifolium* s.l. from Mt. Ventoux (France) were uncertain to some extent; in the NJ analysis based on pairwise distances between individuals, these two species formed a separate cluster (Fig. 2), but in the NJ analysis based on population ΦST values (graph not shown),

they were placed close to some Balkan and Apennine species, and the same was observed for *A. orophilum* in the PCoA.

High-mountain scree populations of *A. cuneifolium* s.l. from geographically distant areas—Balkans (Pirin Mts., treated as *A. cuneifolium* subsp. *pirinicum*; Olympos Mts., as *A. handelii*), Apennines (*A. cuneifolium* s.str.), Mt. Ventoux, and Pyrenees—appeared in four clearly distinct positions across the NJ tree/PCoA plot, suggesting their polyphyletic origin. In general, the NJ tree displayed several distinct lineages (some supported by bootstrap support, BS > 50%), but the internal branch lengths were mostly short, resulting in poorly resolved relationships.

Dataset of southwestern European and Moroccan taxa. — The analyses focusing on the southwestern European and Moroccan populations showed several clusters (NJ based on ΦST in Fig. 3; NJ and PCoA based on Jaccard distances not shown) corresponding to the following species/lineages: *A. montanum* s.str. incl. *A. rhodanense* (BS = 64%), *A. orophilum* (BS = 100%), *A. cuneifolium* s.l. from Mt. Ventoux (BS = 100%), *A. cuneifolium* s.l. from the Pyrenees (BS = 52%), *A. loiseleurii* subsp. *loiseleurii* (BS = 100%), *A. montanum* s.l. from Morocco (BS = 100%), and *A. montanum* s.l. from Spain (in two clusters, no BS) including *A. loiseleurii* subsp. *gallaecicum*. The

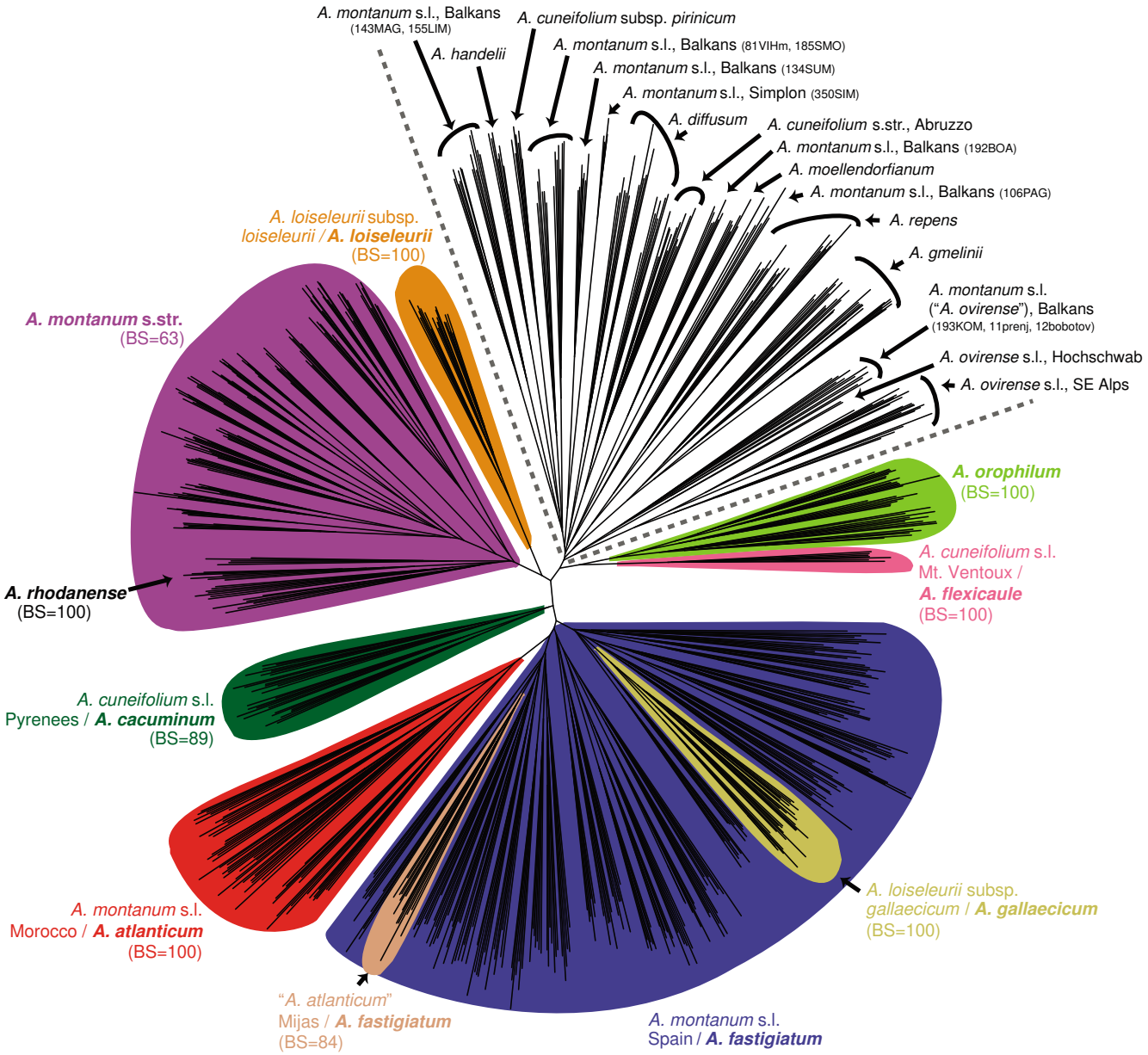

Fig. 2. Neighbour-joining tree based on the AFLP data of the complete dataset (777 individuals from 118 populations) inferred from Jaccard's pairwise genetic distances between individuals. The cluster delimited by dashed lines comprises taxa from Central Europe, the Apennines and the Balkans (see Appendix S1 for more details). Focus taxa from the area depicted in Fig. 1 are in colour. The initial taxonomic assignment/final taxonomic treatment (if different, separated by a slash) is given for the focus taxa. Bootstrap values (BS) >50% are indicated only for major branches.

clustering patterns indicate relatedness between the polyploids *A. orophilum* and *A. cuneifolium* s.l. from Mt. Ventoux (BS = 65%). The two subspecies of *A. loiseleurii* from the coastal sand dunes are clearly genetically distinct: subsp. *loiseleurii* (Aquitaine, France; Guipúzcoa, Spain) appears as a separate lineage, whereas subsp. *gallaecicum* (Galicia, Spain) is very close to the Cantabrian populations of *A. montanum* s.l. Several lineages showed geographically correlated substructure (Fig. 3): *A. montanum* s.str. (see also the detailed analyses below), *A. montanum* s.l. from Morocco (three clusters corresponding to the High Atlas, Middle Atlas and Rif Mts.), and *A. montanum* s.l. from Spain (Baetic, NE Iberian and Cantabrian regions, see the detailed analyses below).

Dataset of *A. montanum* s.l. from Spain and *A. loiseleurii* subsp. *gallaecicum*. — Both Bayesian clustering methods (BAPS, STRUCTURE; Fig. 4C) suggested optimal genetic partitioning into two clusters as follows: (1) northwestern Iberian populations, comprising mountain *A. montanum* s.l. mainly from the Cantabrian range and extending to the Iberian System (for simplicity denoted here as Cantabrian), and *A. loiseleurii* subsp. *gallaecicum* from coastal sand dunes in Galicia, and (2) the rest of the mountain populations of *A. montanum* s.l. that extend from the Baetic System in the south through the Iberian System to the Pre-Pyrenees in the north (Figs. 1, 4). A few populations showed slight admixture between the two main genetic clusters. Both the PCoA and NJ tree, however, also suggest a more detailed structure, and four population groupings can be delimited (Fig. 4A–B): (1) *A. montanum* s.l. from the Baetic System, (2) *A. montanum* s.l. from the Iberian System and Pre-Pyrenees (denoted as NE Iberian), (3) Cantabrian *A. montanum* s.l., and (4) *A. loiseleurii* subsp. *gallaecicum*. The population from the Sierra de Mariola (270BCT) falls both genetically and geographically between the first two groups. The Baetic group encompasses the type localities of *A. gadorense* (268GAD), *A. nevadense* (322RAY) and *A. fastigiatum* (370GIL), as well as the populations from Sierra de Mijas (264MIJ, 352ALH)

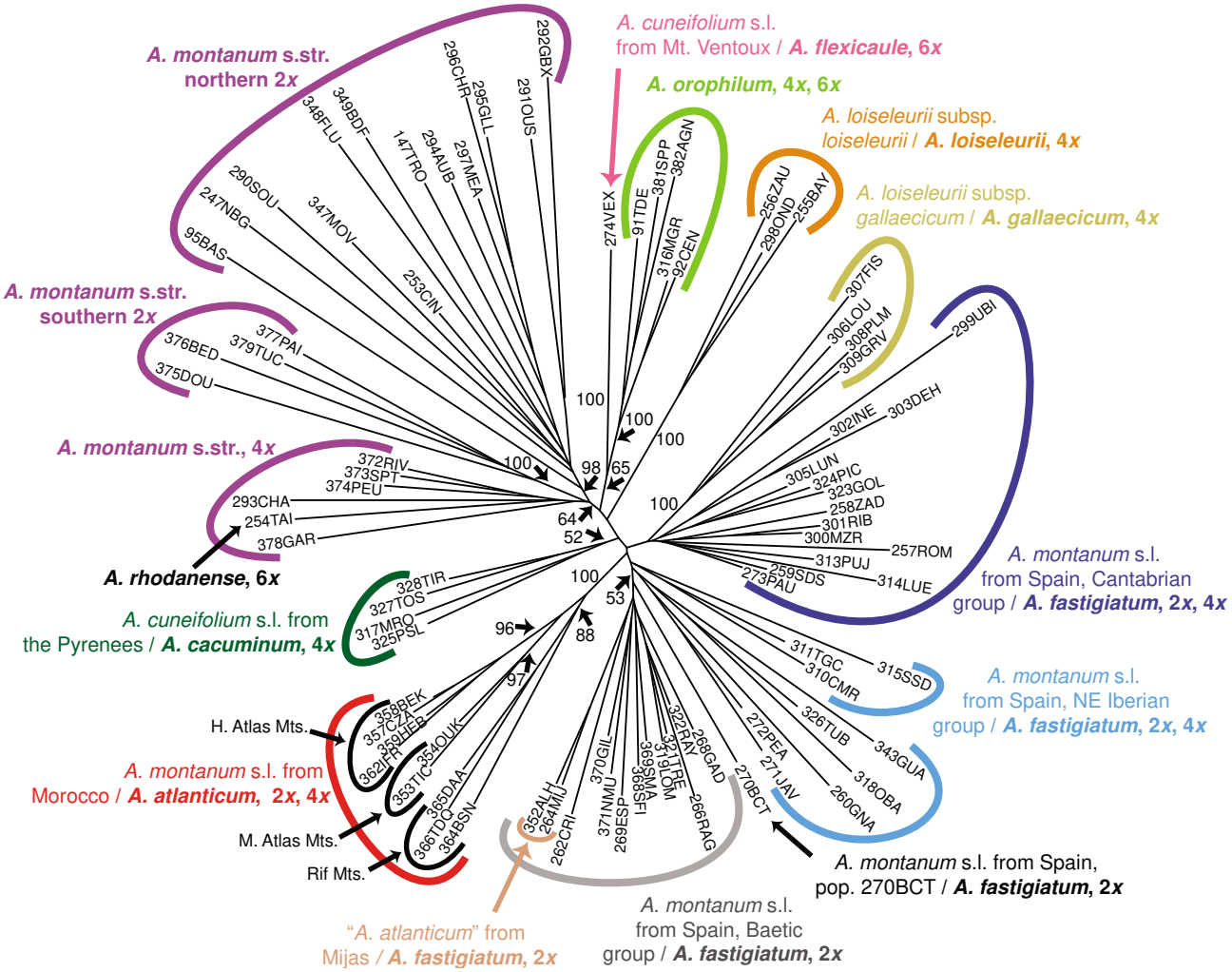

Fig. 3. Neighbour-joining tree based on the AFLP data of the southwestern European and Moroccan populations (i.e., focus taxa, 586 individuals from 87 populations) inferred from pairwise ΦST values between populations. The initial taxonomic assignment/final taxonomic treatment (if different, separated by a slash) is given. Bootstrap values >50% from the analysis based on Jaccard's pairwise genetic distances between individuals (not shown) are mapped along the corresponding branches. Population codes follow Table 1.

that are traditionally assigned to *A. atlanticum* (here referred to as "*A. atlanticum*").

The non-hierarchical AMOVA indicated that approximately two-thirds of the total genetic variation is present within populations (67.36%), whereas one-third is attributable to the among-population (32.64%) component. The hierarchical analyses with two (as suggested by the BAPS and STRUCTURE clustering) and four groups (based on PCoA and NJ, excluding the intermediate 270BCT population) apportioned 8.33% and 9.99% of the total variation, respectively, to the between-group component. Private markers (none of them fixed within the group, however) were observed for each of the four groups, ranging from 9 in *A. loiseleurii* subsp. *gallaecicum* to as many as 157 in the Cantabrian group (Table 3). The mean number of total AFLP markers recorded per individual was significantly ($P < 0.05$) higher in the tetraploids than in the diploids when considering the entire dataset. Similar differences, although not statistically significant, were observed also within the two geographic groups that include two cytotypes (Cantabrian, NE Iberian; see Table 3). Nei's gene diversity values, however, did not show significant differences between cytotypes. The Cantabrian and Baetic groups showed similarly high values of Nei's gene diversity, while *A. loiseleurii* subsp. *gallaecicum* was significantly less diverse (Tables 1, 3). Diversity values correlated positively with rarity values, both at the population (Spearman $r = 0.84$, $P < 0.0001$) and species/group levels (Table 1).

Dataset of *A. montanum* s.str. and *A. rhodanense*. — Three clusters related to geographic origin and ploidy level can be recognised in the PCoA (Fig. 5A) and NJ tree (not shown), which are also supported by the Bayesian BAPS clustering: (1) all four diploid populations sampled in the Midi-Pyrénées

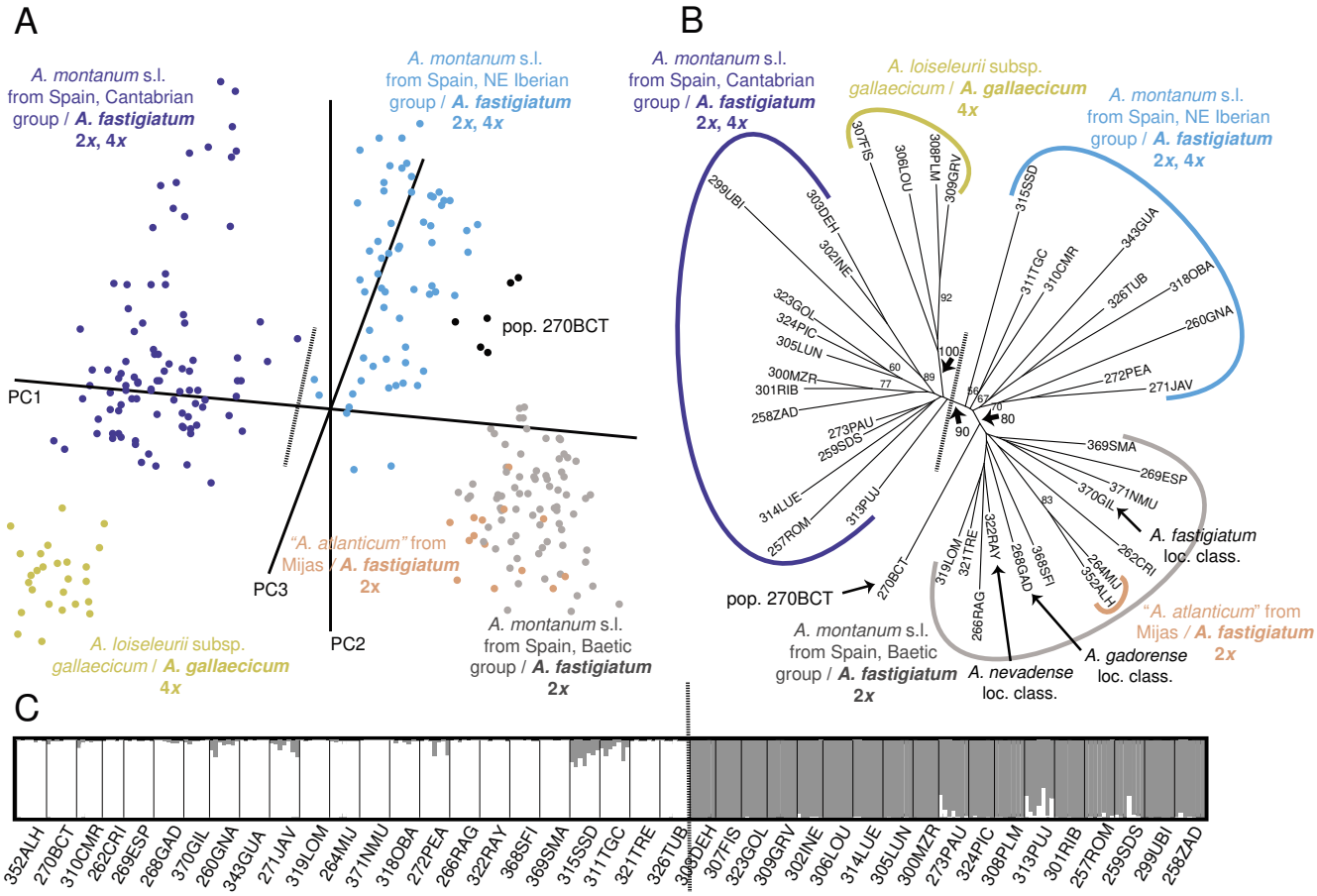

Fig. 4. Genetic structure of *A. montanum* s.l. from Spain (*A. fastigiatum* in the final treatment) and *A. loiseleurii* subsp. *gallaecicum* (*A. gallaecicum* in the final treatment) (277 individuals from 41 populations) based on the AFLP data. The colours indicate four groups: Baetic (grey), NE Iberian (light blue) and Cantabrian group (dark blue) of *A. fastigiatum*, and *A. gallaecicum* (yellow). Some populations are highlighted: the genetically and geographically intermediate population 270BCT, populations from the type localities, and two populations from Sierra de Mijas ("*A. atlanticum*"). Population codes follow Table 1. **A,** Principal coordinate analysis based on Jaccard's pairwise genetic distances between individuals. The first three axes explain 7.18, 3.57 and 3.30% of the variation. **B,** Neighbour-joining tree inferred from pairwise ΦST values between populations. Bootstrap values >50% from the analysis based on Jaccard's pairwise genetic distances between individuals (not shown) are mapped along the corresponding branches. **C,** Bayesian model-based clustering (STRUCTURE program) at $K = 2$. Vertical lines demarcate populations, with widths of corresponding boxes proportional to the number of individuals analysed in populations. Grey-white colouring indicates each individual's proportional cluster assignment: grey, Cantabrian group and *A. gallaecicum*; white, Baetic and NE Iberian groups. The dashed thick line in parts A and B indicates the main split following this STRUCTURE-based clustering.

and Languedoc-Roussillon regions of France (denoted here as southern diploids, BS = 100%), (2) the other diploid populations from several eastern to northeastern regions of France, from Switzerland and Germany (northern diploids, BS = 80%), and (3) all five tetraploid populations scattered across several regions in France, including the hexaploid *A. rhodanense* (BS = 50%). Within the polyploid cluster, the easternmost population (293CHA) appears in a somewhat outlying position (seen both in the NJ and PCoA, Fig. 5A). Bayesian STRUCTURE analyses (Fig. 5B) revealed the most optimal partitioning at $K = 2$ (having the highest ΔK and similarity coefficient values), with the northern diploids as one cluster, and the southern diploids together with the polyploids as the second cluster (this result coincides with the main division along the first axis of the PCoA). Genetic admixture was observed within the tetraploid 293CHA population (Fig. 5B).

The non-hierarchical AMOVA showed that approximately the same amount of variation is explained by the within- (51.58%) and among-population (48.42%) components. The hierarchical AMOVA analyses with two groups, either defined on the basis of ploidy level (diploid vs. polyploid) or as suggested by the STRUCTURE analyses (omitting the admixed 293CHA population) revealed 9.68% and 11.11% of total variation, respectively, attributable to the between-group component. The AMOVA analyses based on the three groups (PCoA, Fig. 5A) showed that 13.64% of the total variation can be explained by the among-group difference.

Patterns of AFLP marker specificity and sharing supported the existence of three groups. Private markers (none of them fixed within the group, however) were observed for each of these groups: 44 in the southern diploids, 177 in the northern diploids and as many as 252 in the polyploids. Disregarding the polyploids, the values increased to 103 private markers in the southern diploids and 370 in the northern diploids; 59 (57%) of the markers specific to the southern diploids (i.e., absent from the northern diploids) were shared with the polyploids, and 193 (52%) of the markers specific to the northern diploids (absent from the southern diploids) were shared with the polyploids. Nei's gene diversity values as well as the mean number of AFLP markers recorded per individual illustrate that the polyploids are significantly ($P < 0.05$) more diverse than the diploids. No differences were observed between the northern and southern diploids (Tables 1, 3). Nei's gene diversity correlated positively with the rarity values (Spearman $r = 0.92$, $P < 0.0001$).

cpDNA sequence variation. — The cpDNA sequences included 26 populations of taxa from Central Europe, the Apennines and the Balkans (mostly generated in our previous studies, Španiel & al., 2011b, 2012a, see Electr. Suppl. 1: Appendix S1 for more details) and 87 populations from this study, which focused on taxa from southwestern Europe and Morocco. The

Table 3. AFLP data statistics for *Alyssum montanum* s.str., *A. fastigiatum* (= *A. montanum* s.l. from Spain), and *A. gallaecicum* (= *A. loiseleurii* subsp. *gallaecicum*) considering their cytotypes and geographic groupings.

Taxon / Group	*N* pop/ind	Ploidy level	$N_{priv.}$	$N_{loci/ind}$ (±SD)	D_{Nei} (±SD)
***A. montanum* s. str.**					
southern diploids	4/25	2*x*	44(103)	120.875[a] (±6.025)	0.035[a] (±0.006)
northern diploids	14/94	2*x*	177(370)	122.369[a] (±6.896)	0.033[a] (±0.008)
tetra- and hexaploids*	5/33+1/7	4*x*+6*x*	252(–)	157.257[b] (±12.129)	0.067[b] (±0.009)
A. fastigiatum* + *A. gallaecicum					
A. fastigiatum – Baetic	14/95	2*x*	125	126.992[b] (±6.336)	0.061[a] (±0.007)
A. fastigiatum – NE Iberian	9/62	2*x*, 4*x*	82	128.343[b] (±13.185)	0.054[ab] (±0.010)
A. fastigiatum – Cantabrian	14/92	2*x*, 4*x*	157	140.947[a] (±13.472)	0.064[a] (±0.012)
A. gallaecicum	4/27	4*x*	9	130.446[ab] (±1.625)	0.045[b] (±0.004)
Pairwise comparisons within *A. fastigiatum* + *A. gallaecicum*: diploids vs. tetraploids					
A. fastigiatum – diploids	24/161	2*x*		125.156[a] (±8.178)	0.058[a] (±0.010)
A. fastigiatum+*A. gallaecicum* – tetraploids	17/115	4*x*		141.635[b] (±9.888)	0.060[a] (±0.012)
Pairwise comparisons within NE Iberian *A. fastigiatum*: diploids vs. tetraploids					
NE Iberian diploids	6/41	2*x*		123.467[a] (±10.878)	0.053[a] (±0.009)
NE Iberian tetraploids	3/21	4*x*		138.095[a] (±13.622)	0.055[a] (±0.012)
Pairwise comparisons within Cantabrian *A. fastigiatum*: diploids vs. tetraploids					
Cantabrian diploids	4/25	2*x*		119.968[a] (±10.316)	0.056[a] (±0.019)
Cantabrian tetraploids	10/67	4*x*		146.669[a] (±6.826)	0.067[a] (±0.007)

N pop/ind, number of analysed populations and individuals; $N_{priv.}$, number of private AFLP markers (values in brackets refer to numbers when excluding the polyploids); $N_{loci/ind}$ (±SD), mean number of AFLP markers per individual (average of population-level values) ± standard deviation; D_{Nei} (±SD), Nei's gene diversity (average of population-level values) ± standard deviation.

[a,b] Letters in superscript show the results of the Tukey-Kramer (multiple comparisons) or *t*-tests (pairwise comparisons): different letters indicate statistically significant differences at $P < 0.05$.

* The hexaploid population refers to *A. rhodanense*, but is treated here as part of *A. montanum* s.str.

GenBank accession numbers are listed in the Electr. Suppl. 1: Appendix 1. In total, the cpDNA alignment contained 236 ingroup individuals. The *rpoB-trnC* alignment was 933 bp long with 11 scored indels; the *rpl32-trnL*(UAG) alignment was 972 bp long with 14 scored indels. Of the 1905 bp of the concatenated dataset, 133 (7%) positions were polymorphic, and 87 ingroup haplotypes were resolved. The model TPM1uf+I+G was determined for both cpDNA regions as best fitting the data. The complete concatenated cpDNA alignment including the outgroup sequences is available as supplementary data.

The Bayesian majority-rule consensus tree (Electr. Suppl. 1: Fig. S1) showed low resolution at the backbone with many well-supported but rather small clades in a polytomy and few weakly supported higher-level clades. Three main haplotype groups can be delimited, comprising: clades resolved in the basal polytomy of the tree (denoted as paraphyletic group I); clades in the polytomy of a single large clade (denoted as paraphyletic group II); and a derived monophyletic clade III. The basal polytomy (group I) comprised all taxa from Central Europe and the Apennine and Balkan Peninsulas and some of the populations of *A. montanum* s.str., *A. orophilum*, and *A. montanum* s.l. from Morocco. The remaining two groups (II and III) comprised only southwestern European and Moroccan accessions.

The statistical parsimony analysis resolved a network in which these three groups of haplotypes can be outlined (Fig. 6). The network shows that a few haplotypes are rather frequent, either species-specific and shared by several conspecific populations, or shared by multiple taxa (two haplotypes found in the central positions of groups II and III). Most haplotypes, however, were rare and confined to individuals from a single population. The individuals sampled from the same population mostly showed identical or closely related haplotypes. All species and lineages delimited by the AFLP data harbour substantial cpDNA variation, with multiple, mostly non-monophyletic haplotypes. This was most pronounced in *A. montanum* s.l. from Morocco and Spain, *A. cuneifolium* from the Pyrenees, and tetraploid *A. montanum* s.str. (showing also the highest gene and nucleotide diversity values, Table 1), and least pronounced in the two subspecies of *A. loiseleurii*. In accordance with the AFLP data, *A. loiseleurii* subsp. *gallaecicum* possessed two related haplotypes that were closest to the Cantabrian accessions of *A. montanum* s.l. In contrast to the AFLP patterns, hexaploid *A. rhodanense* shared a haplotype with two Cantabrian diploid populations, being distant from those recorded in *A. montanum* s.str.

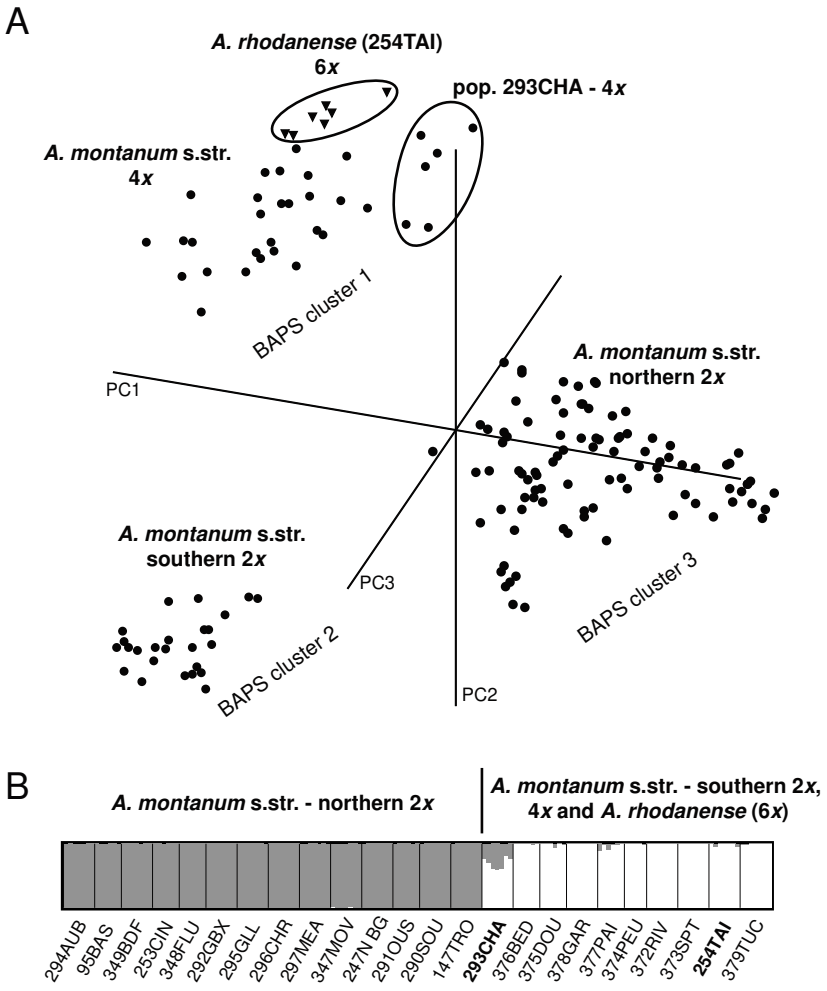

Fig. 5. Genetic structure of *A. montanum* s.str. and *A. rhodanense* (159 individuals from 24 populations) based on the AFLP data. **A,** Principal coordinate analysis based on Jaccard's pairwise genetic distances between individuals. The first three axes explain 10.40%, 6.94% and 5.80% of the variation. The three geographic/cytotype groupings corresponding to the three clusters as also revealed by the BAPS Bayesian clustering are highlighted: southern diploids, northern diploids, and polyploids (tetraploid *A. montanum* s.str. and hexaploid *A. rhodanense*). **B,** Bayesian model-based clustering (STRUCTURE program) at $K = 2$. For the figure description see legend of Fig. 4C. White, southern diploids and polyploids; grey, northern diploids. Population codes follow Table 1.

Multivariate morphometrics. — Because the distribution of most characters departed from normality, we used the non-parametric correlation coefficient (Spearman) and non-parametric classificatory discriminant analyses. The correlations did not exceed the thresholds of 0.95 or 0.99 for any character pair; therefore, all measured characters were retained for further analyses. The highest correlation (r = 0.90) was found between the number of trichome rays on the lower and upper leaf surfaces.

A series of canonical (CDA 1–CDA 7) and classificatory discriminant analyses (classificatory DA 1–DA 7) presented below aimed at exploring the morphological differentiation among the genetic entities as proposed and delimited by the AFLP data. The samples from Sierra de Mijas (264MIJ, 352ALH; referred to as "*A. atlanticum*"), however, were kept as a separate group in the morphometric analyses because of their conspicuous morphological distinction from the rest of

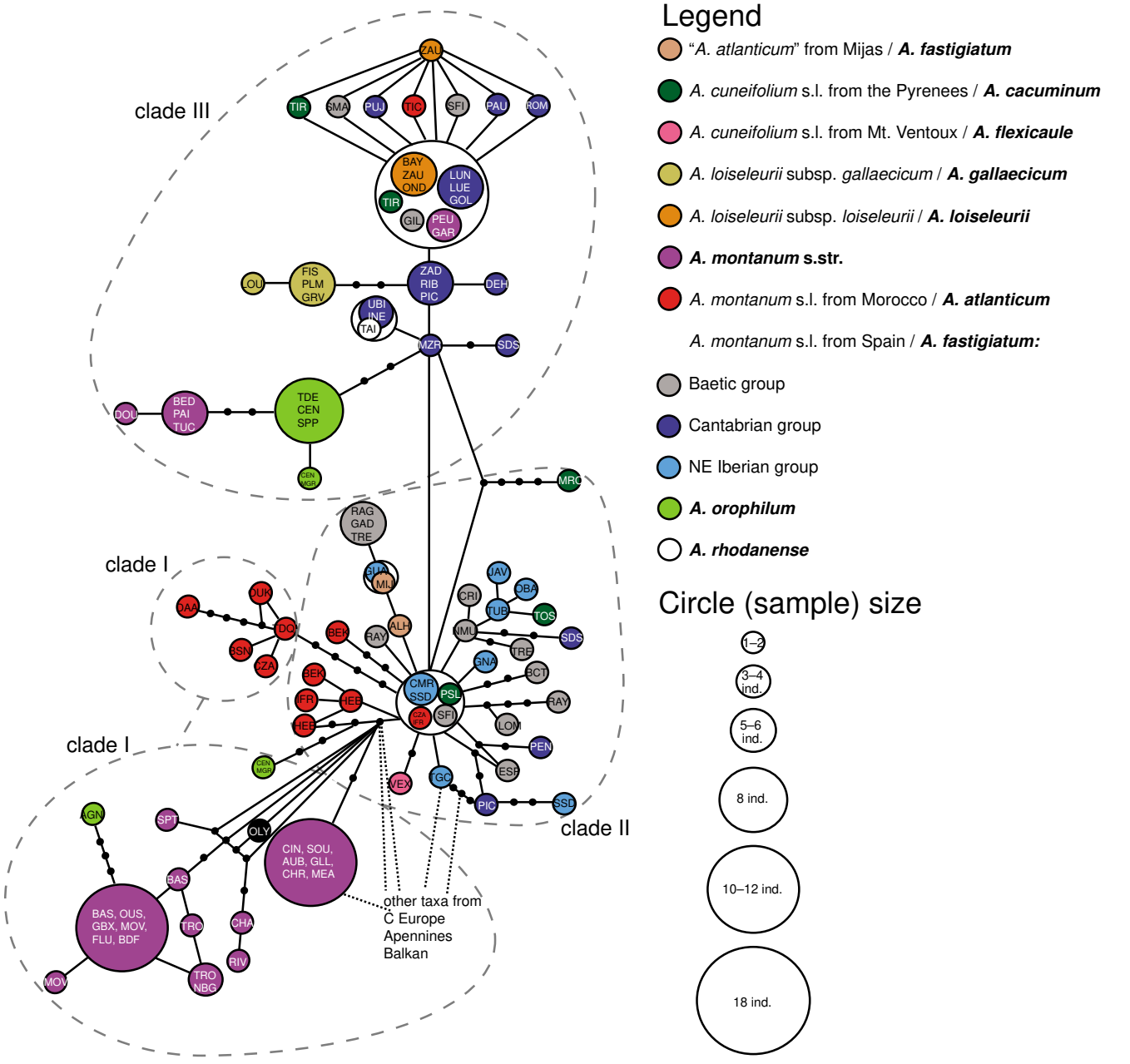

Fig. 6. Maximum parsimony network of the cpDNA (concatenated *rpoB-trnC* and *rpl32-trnL*(UAG) alignments) haplotypes of the studied south-western European and Moroccan *Alyssum* species. Haplotypes of the taxa from Central Europe and the Balkan and Apennine Peninsulas are not shown, but their positions within the network are indicated (see dotted lines). Circle sizes are proportional to haplotype frequencies (see scale), lines represent mutational steps, and black dots are not sampled haplotypes. Outlines shown in dashed lines encompass the three haplotype groups (I, II and III) as suggested by the Bayesian tree (Electr. Suppl. 1: Fig. S1). Colours indicate species or group affiliation, and both the initial taxonomic assignment and final taxonomic treatment (if different, separated by a slash) are given. Population codes follow Table 1.

the Baetic group of *A. montanum* s.l. Keeping these samples within this group would disrupt the interpretation of the results. In the analyses where *A. montanum* s.l. from Spain was divided into three geographic groups following AFLP-based structuring (Cantabrian, NE Iberian and Baetic groups in CDA 1 and CDA 2 below), the 270BCT population was omitted due to its ambiguous assignment (see above).

CDA 1 (based on population means, Fig. 7) and classificatory DA 1 (based on individual plants) were computed based on the data matrix of the southwestern European and Moroccan samples, and 12 predefined groups as revealed by the AFLP data (see Fig. 3). Most of them formed their own clouds in the ordination space, with some overlapping observed especially for the three groups of *A. montanum* s.l. from Spain and *A. rhodanense*, and less for *A. orophilum* and *A. montanum* s.l. from Morocco. The populations of *A. montanum* s.str. were resolved as the most differentiated grouping. For the characters most correlated with the canonical axes and thus contributing to the differentiation of particular groups, see Table 4, column CDA 1. In the classificatory DA 1 (k = 21), the percentage of correctly classified individuals exceeded 80% for most groupings, except for *A. montanum* s.l. from Spain and Morocco with a high proportion of misclassified plants (see Electr. Suppl. 1: Table S1 for details).

CDA 2 and 3 and the respective classificatory DA 2 and 3 were computed to obtain greater insight into the four morphologically most overlapping (but genetically distinct) groupings of CDA 1: three groups of *A. montanum* s.l. from Spain (Cantabrian, NE Iberian and Baetic groups) and *A. montanum* s.l. from Morocco. CDA 2, based on individual plants and these four groupings, revealed substantial overlap (Fig. 8A). The Moroccan plants were slightly differentiated from the rest along the first axis, whereas the remaining three groups overlapped much more, with only slight shifts observed along the second axis. The characters that were most highly correlated with the respective axes are highlighted in Table 4, column CDA 2. Classificatory DA 2, in congruence, resulted in a rather low percentage of correctly classified individuals (59.9%–88%, see Fig. 8A). In CDA 3, with the Spanish and Moroccan plants of *A. montanum* s.l. predefined as two groups, the overlap between them was still rather marked, although the percentages of correct assignments in the respective classificatory DA 3 were high (90% and 93.2%) (Fig. 8B). Petal and sepal length contributed most to this differentiation (the floral parts being larger in the Moroccan populations), followed by leaf width (slightly wider leaves in the Moroccan plants) and trichome coverage on the lower leaf surface (higher in the Moroccan plants; see Table 4, column CDA 3).

In CDA 4, we examined morphological differentiation within the dataset of *A. montanum* s.l. from Spain and *A. loiseleurii* subsp. *gallaecicum* (corresponding to the AFLP dataset presented in Fig. 4). *Alyssum montanum* s.l. from Spain was treated as a single group (based on the results of CDA 2 above), and the morphological differentiation of *A. loiseleurii* subsp. *gallaecicum* (genetically close to the Cantabrian group) and "*A. atlanticum*" from Sierra de Mijas (genetically indistinguishable from the Baetic group) was evaluated here (Fig. 9A). The three predefined groups were very well separated from each other, as also illustrated by high percentages of correctly classified plants in the respective classificatory DA 4, ranging from 96.4%–98.6% (see Fig. 9A). The characters that were most correlated with the first canonical axis, thus contributing to the separation of *A. montanum* s.l., were related to leaf trichomes (trichome density, coverage, and number of trichome rays on

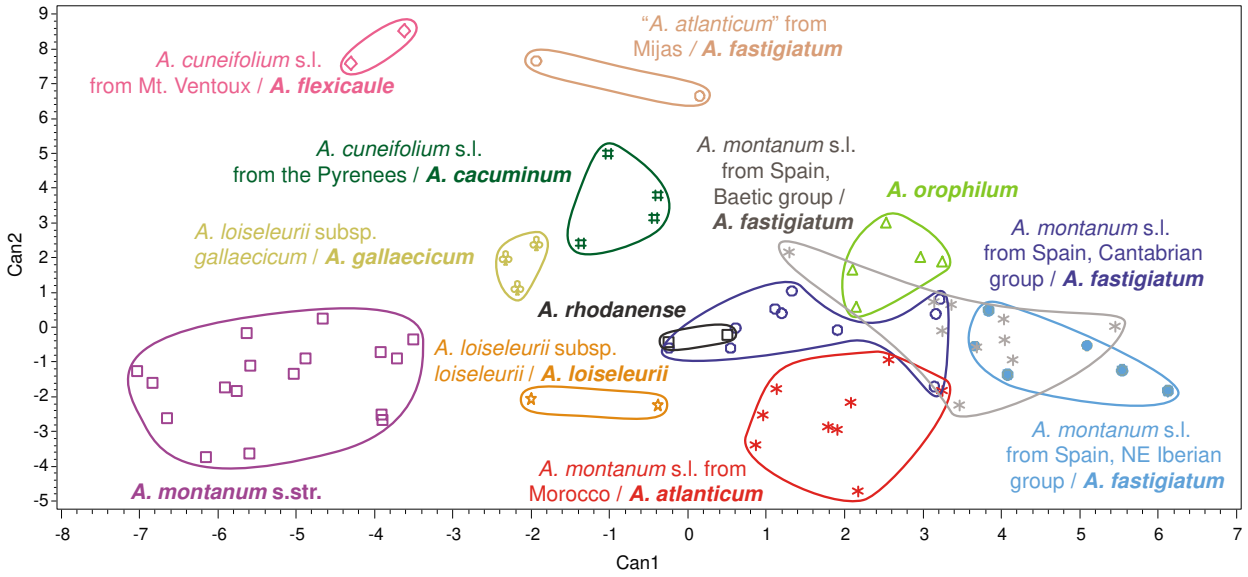

Fig. 7. Canonical discriminant analysis (CDA 1) of 12 predefined groups of the focus taxa from southwestern Europe and Morocco based on 20 morphological characters and population averages. The populations of *A. cuneifolium* s.l. from Mt. Ventoux and *A. rhodanense* were randomly split into two artificial subpopulations to satisfy the requirement of having at least two objects present in each of the predefined groups in the CDA. For total canonical structure, see Table 4. If different, the initial taxonomic assignment/final taxonomic treatment is given (separated by a slash).

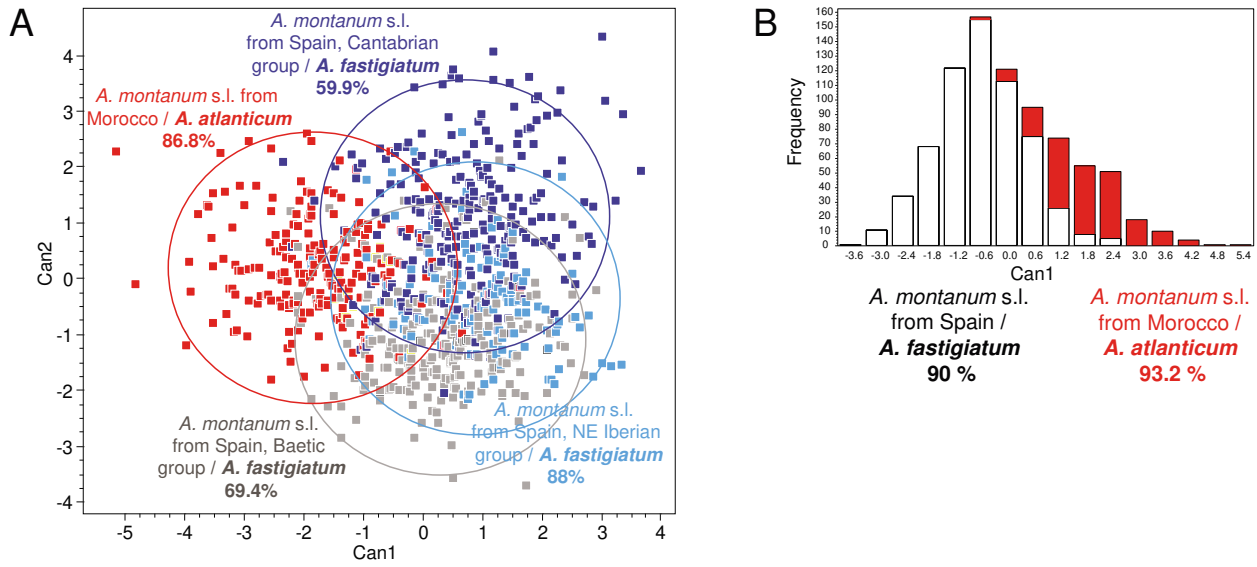

Fig. 8. Discriminant analyses performed on the Spanish and Moroccan populations of *A. montanum* s.l. (*A. fastigiatum* and *A. atlanticum* in the final treatment, respectively), based on individual plants and 20 morphological characters. The results of the canonical discriminant analyses (CDA) are depicted graphically, while the results of the classificatory discriminant analyses (classificatory DA) are presented as percentage values. The initial taxonomic assignment/final taxonomic treatment (separated by a slash) is given. For total canonical structure, see Table 4. **A,** CDA 2 and the respective classificatory DA 2 (k = 21) with four predefined groups: Cantabrian (242 plants), NE Iberian (150 plants) and Baetic group of *A. fastigiatum* (206 plants, excl. populations from Sierra de Mijas), and *A. atlanticum* from Morocco (205 plants). The 95% isodensity circles are depicted. **B,** CDA 3 and the respective classificatory DA 3 (k = 13) with two predefined groups: *A. fastigiatum* (618 plants) and *A. atlanticum* (205 plants).

Table 4. Results of canonical discriminant analyses (CDA 1–CDA 6) showing total canonical structure values that express correlations of morphological characters with canonical axes. High values are in bold. Character acronyms are explained in Table 2.

	CDA 1 (Fig. 7)		CDA 2 (Fig. 8A)		CDA 3 (Fig. 8B)	CDA 4 (Fig. 9A)		CDA 5 (Fig. 9B)	CDA 6 (Fig. 10)	
	Can 1	Can 2	Can 1	Can 2	Can 1	Can 1	Can 2	Can 1	Can 1	Can 2
PetalLength	0.194	0.393	**−0.402**	0.365	**0.475**	0.247	**0.474**	−0.253	−0.018	−0.284
PetalSinus	−0.056	−0.015	**0.414**	**0.512**	−0.328	−0.137	−0.090	0.357	−0.144	−0.228
PetalWidth	−0.337	0.341	−0.095	**0.691**	0.216	0.133	0.300	**−0.700**	**−0.580**	−0.131
SepalLength	0.331	**0.516**	**−0.459**	0.276	**0.514**	0.462	**0.432**	0.005	0.286	**−0.369**
FilamentLength	0.221	**0.527**	0.045	0.428	0.062	0.117	**0.479**	−0.075	0.055	−0.115
StyleLength	0.515	**0.536**	0.207	0.230	−0.145	0.144	0.371	0.540	0.367	−0.233
StemLength	−0.239	−0.136	0.310	**0.495**	−0.200	0.177	−0.218	0.581	−0.243	−0.126
Length15thLeaf	0.028	0.015	0.201	0.273	−0.141	−0.027	0.353	0.238	−0.218	−0.233
Width15thLeaf	−0.009	0.223	−0.252	0.336	0.343	0.252	0.132	−0.376	−0.027	−0.047
Length8Leaf	0.004	−0.049	0.270	0.300	−0.190	−0.173	**0.432**	−0.098	−0.298	−0.136
Width8Leaf	0.001	0.199	−0.195	0.428	0.304	0.180	0.147	−0.429	0.084	0.197
Dist8–15Leaf	−0.014	−0.323	0.155	0.370	−0.060	−0.290	0.048	−0.245	−0.245	0.100
LengthTrichRay	**0.840**	−0.003	−0.090	−0.402	0.063	−0.309	0.176	**−0.748**	**0.585**	−0.281
NrRaysTrichLower	**−0.876**	0.199	−0.114	0.277	0.092	**0.806**	0.039	**0.794**	−0.083	−0.171
TrichDensityLower	**−0.869**	−0.248	−0.182	0.459	0.184	0.624	−0.184	0.616	−0.276	**0.342**
TrichCoverageLower	**−0.789**	0.020	−0.358	0.170	0.318	**0.860**	−0.035	**0.792**	0.001	0.111
NrRaysTrichUpper	−0.685	0.419	−0.206	0.079	0.167	**0.812**	0.187	**0.786**	0.133	−0.197
TrichDensityUpper	−0.639	0.053	−0.215	0.207	0.185	**0.724**	−0.121	0.484	0.127	0.253
TrichCoverageUpper	−0.285	0.431	−0.253	−0.130	0.182	**0.780**	0.204	0.560	0.291	−0.019
NrLatBranches	−0.327	0.032	0.032	−0.047	−0.048	0.220	−0.060	0.169	0.031	**0.517**

both sides of the leaves, all being lower in this species, see Table 4, column CDA 4). Those correlated with the second axis, differentiating between *A. loiseleurii* subsp. *gallaecicum* and "*A. atlanticum*", were petal, sepal, filament, and leaf length (all longer in "*A. atlanticum*").

CDA 5 and classificatory DA 5 (Fig. 9B) revealed a morphological distinction between the two subspecies of *A. loiseleurii*, subsp. *loiseleurii* and subsp. *gallaecicum*, that appeared genetically divergent. Number and length of trichome rays (more rays and shorter rays in subsp. *gallaecicum*), trichome coverage (higher in subsp. *gallaecicum*), and petal width (narrower in subsp. *gallaecicum*) accounted for most of this differentiation (see Table 4, column CDA 5).

In CDA 6 and classificatory DA 6 (Fig. 10), we explored the morphological differentiation within *A. montanum* s.str. with three predefined groups based on their genetic distinction (Fig. 4): diploids from the northern populations, diploids from the southern populations, and tetraploids. CDA 6 showed certain morphological trends, but with considerable overlap among groups (Fig. 10), in congruence with rather low to moderate values of correct classification (69.2%–85.1%). For the characters that were most highly correlated with the canonical axes see Table 4, column CDA 6.

Finally, CDA 7 (figure not shown) and classificatory DA 7 (resulting in 100% correct classification) confirmed strong morphological differentiation of *A. rhodanense* from *A. montanum* s.str., in congruence with the above CDA 1, Fig. 7), which is mostly due to number and length of trichome rays (more

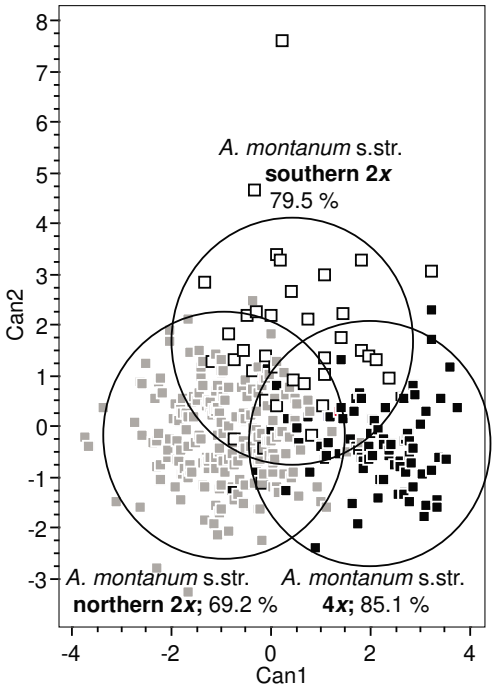

Fig. 10. Canonical discriminant analysis (CDA 6) of *A. montanum* s.str. based on 20 morphological characters and three predefined groupings: southern diploids (39 plants), northern diploids (202 plants), and tetraploids (87 plants). The 95% isodensity circles are depicted. For total canonical structure, see Table 4. Results of the respective classificatory DA 6 (k = 39) are shown in percentage values.

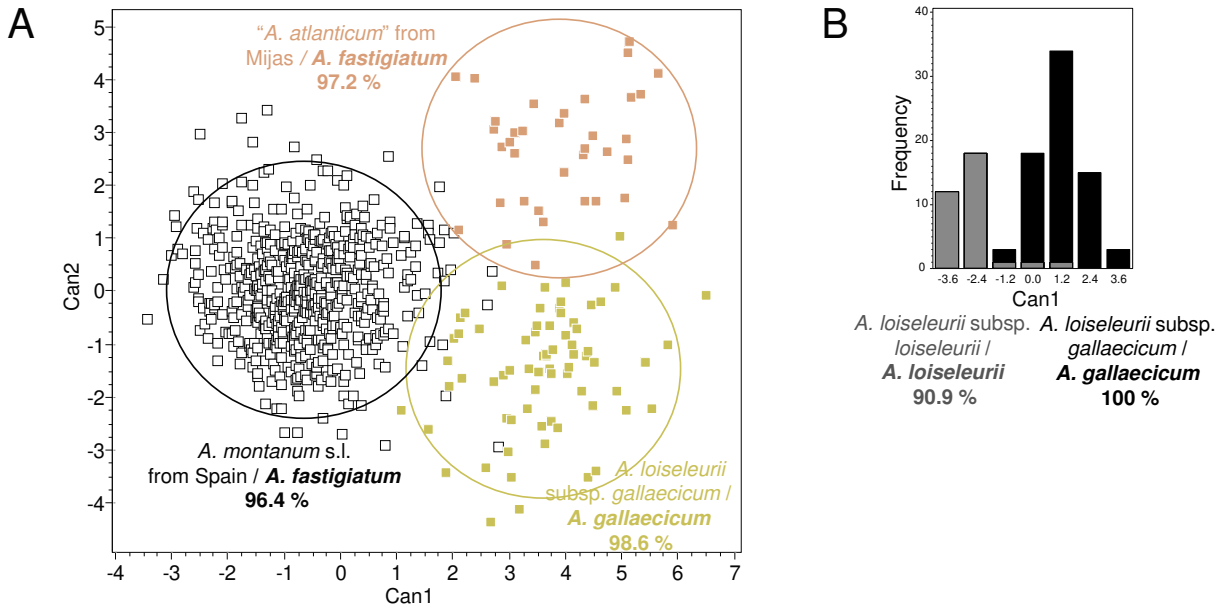

Fig. 9. Discriminant analyses performed on selected Iberian taxa or population groups, based on individual plants and 20 morphological characters. The results of the canonical discriminant analyses (CDA) are depicted graphically, while the results of the classificatory discriminant analyses (classificatory DA) are presented as percentage values. The initial taxonomic assignment/final taxonomic treatment (separated by a slash) is given. For total canonical structure, see Table 4. **A,** CDA 4 and the respective classificatory DA 4 (k = 5) based on three predefined groupings: *A. loiseleurii* subsp. *gallaecicum* (*A. gallaecicum* in the final treatment, 70 plants), *A. montanum* s.l. from Spain (*A. fastigiatum* in the final treatment, 618 plants), and "*A. atlanticum*" from Sierra de Mijas (36 plants). The 95% isodensity circles are depicted. **B,** CDA 5 and the respective classificatory DA 5 (k = 13) based on two groupings: *A. loiseleurii* (33 plants) and *A. gallaecicum* (70 plants).

rays and shorter rays present in *A. montanum* s.str.), trichome density and coverage (higher in *A. montanum* s.str.), and floral characters (shorter floral parts in *A. montanum* s.str.).

The morphological characteristics and differentiation of all entities and taxa recognised here are summarised by descriptive statistics presented in Electr. Suppl. 1: Table S2 and in the identification key given in the Taxonomic Treatment below.

■ DISCUSSION

Revised taxonomic treatment of the *Alyssum montanum–A. repens* complex in southwestern Europe and Morocco. — Full reliance on morphology in species recognition and delimitation may be problematic or even misleading in the polyploid complex of *Alyssum montanum–A. repens*. This fact, besides the lack of large-scale comparative studies spanning broader geographic areas, has most likely led to previous taxonomic contradictions (see Introduction). The present study shows that only a few characters are taxonomically relevant, which are, in addition, mostly quantitative and show overlap and can even reappear in certain combinations in unrelated species. The taxonomically most significant traits include the morphology of stellate trichomes on leaves (number and length of trichome rays), trichome density and coverage, and size and shape of floral parts and leaves, although floral size is reduced towards the end of the flowering stage, and leaf size may also be highly variable. In species complexes with such intricate variation pattern in morphology, multiple lines of evidence including several unlinked genetic markers are desirable to arrive at a sound taxonomic treatment (Padial & al., 2010; Pessoa & al., 2012). An AFLP-based structure was used here as a starting point to delimit genetic entities (potential taxa) and their taxonomic status was examined by detailed morphometric analyses. AFLPs, indeed, helped to revise the taxonomy in several other polyploid complexes (e.g., Bardy & al., 2010; Bendiksby & al., 2011; Meudt, 2011; Greiner & al., 2013; Kuzmanović & al., 2013), and have also been successfully used in the Central European and Apennine representatives of the *A. montanum–A. repens* complex (Španiel & al., 2011b, 2012a). Chloroplast DNA variation, however, did not aid in species delimitation, as it showed peculiar, partially species-independent patterns (see below), which is not rare in recently diverged species (e.g., Comes & Abbott, 2001; Albaladejo & al., 2005; Jakob & Blattner, 2006). The final taxonomic treatment adopted here, therefore, relies on the concordant AFLP- and morphology-based patterns, considering also the distributional and ploidy level data, in line with the integrative approach to taxonomy (see Padial & al., 2010). As a result, we here recognise nine species (see Taxonomic Treatment below). Species-level is preferred to subspecies-level treatment in several cases here. Even if morphological distinction is often not well pronounced, keeping the traditionally recognised subspecies (subspecies of *A. montanum* and *A. loiseleurii*) would result in polyphyletic species.

Alyssum montanum L. in a strict sense (reported also as subsp. *montanum*, Dudley, 1965; Jalas & al., 1996; Kerguélen, 1999; Španiel & al., 2012a) is supported here as a distinct species comprising diploid and tetraploid populations and growing in lowland to montane (rarely subalpine) dry grasslands, pastures and inland sandy or rocky (calcareous) sites. Although it was reported as widespread in Europe (Jalas & al., 1996), here we show that its distribution is much more restricted and that the species is confined to France, SW Germany, and W Switzerland. The species does not occur in Central and eastern Europe (those populations should be classified as *A. gmelinii* Jord. & Fourr.; see Španiel & al., 2012a), neither in the Apennines (Španiel & al., 2011b) nor in the Balkans (Španiel & al., in prep.). The present study revealed that *A. montanum* s.str. does not occur in Spain or northern Africa, contrary to previous reports (Maire, 1967; Küpfer & Nieto Feliner, 1993). The genetic variation patterns argue for a species- rather than subspecies-level treatment of *A. montanum*. Thus, the other two well-supported taxa, *A. montanum* subsp. *gmelinii* (Jord. & Fourr.) Em.Schmid (Španiel & al., 2012a; included also here, see Fig. 2) and subsp. *pluscanescens* (Jos.Baumgartner) Trpin (a hypothesised allohexaploid, Španiel & al., 2011a), should also be treated as separate species. *Alyssum montanum* s.str. displays geographically structured variation, both in ploidy level and genetic patterns, but this is not reflected in the morphology, and we do not recognise this variation taxonomically.

Populations distributed throughout most of the Iberian Peninsula, previously assigned mostly to *A. montanum* but also to *A. cuneifolium* subsp. *losanum* and *A. diffusum* in the north and *A. atlanticum*, *A. fastigiatum*, *A. gadorense* and *A. nevadense* in the south (Dudley, 1965; Küpfer & Nieto Feliner, 1993), are suggested here to represent a single polymorphic species. Following the nomenclatural priority, the name *A. fastigiatum* Heywood, based on plants from Sierra de Cazorla (Heywood, 1954), should be applied. This species encompasses diploid populations in the south (Baetic range) and both diploid and tetraploid populations in the north (Iberian, Cantabrian range), growing in the montane to alpine belts usually on calcareous bedrock. The populations are genetically differentiated into three para- to allopatric groupings, which, however, cannot be distinguished with morphometric data, suggesting that they reflect the species' phylogeographic history (see below) and do not merit taxonomic recognition. Herewith we also reject the occurrence of *A. diffusum* in the Iberian Peninsula and confirm that it is an exclusively Apennine species. The southern Iberian stenoendemics from Sierra de Cazorla (*A. fastigiatum*), Sierra Nevada (*A. nevadense*) and Sierra de Gádor (*A. gadorense*) are not supported and should be treated as part of the widespread *A. fastigiatum* in the present circumscription. Only the populations from Sierra de Mijas deserve special attention. These populations, dwelling on crystalline dolomitic sands, show a distinct morphology (silvery pubescence being the most prominent feature), but genetically, based on both AFLP and cpDNA data, they are not differentiated from the widespread *A. fastigiatum*. We tentatively keep them within *A. fastigiatum*, but future studies including cultivation experiments and examining the genetic basis of trichome development and density variation (Symonds & al., 2005) should be conducted to determine whether the observed morphological distinction mirrors phenotypic plasticity or is indicative of incipient ecological speciation

not captured by the neutral markers employed here. The Sierra de Mijas populations are usually treated as *A. atlanticum* (e.g., Küpfer & Nieto Feliner, 1993), but here we revealed that *A. atlanticum* is a different species restricted to northern Africa.

The Moroccan populations represent a distinct lineage that we assign to *A. atlanticum*. This species was described from the Tlemcen Mts. in Algeria (Desfontaines, 1798), and although we had no material from this area for detailed genetic and morphometric studies, both the morphology of the type specimen and the geographic proximity of the material sampled by us and the type locality (Rif Mts. being adjacent to the Tlemcen Mts.) strongly suggest that the Moroccan populations are conspecific with those from the Tlemcen Mts. Thus, this species is restricted to northern Africa and does not reach southern Spain as previously reported (Bolòs & Vigo, 1990; Küpfer & Nieto Feliner, 1993). The taxonomic status of the populations from the highest altitudes in the Atlas Mts. (above 3500 m a.s.l.) described as *A. embergeri* Quézel and *A. flahaultianum* Emb. ex Emb. (Emberger, 1935; Quézel, 1953; Greuter & Raus, 1985) remains unclear. They may be conspecific with *A. atlanticum* or represent close relatives (see the Taxonomic Treatment below). More detailed studies throughout the North African regions are needed in future.

A species described by Jordan & Fourreau (1868) but not accepted later, *A. rhodanense*, is resurrected here, based on its distinct morphology, ecology (granite bedrock), hexaploid level, and incongruent cpDNA and AFLP patterns suggesting its allopolyploid origin. It is a stenoendemic species; apart from the here studied population from Tain l'Hermitage in Rhône-Alpes, another population on granite rocks was documented from the geographically close Sarras in Ardèche (herbarium P, e.g., P04630419).

Furthermore, three polyploid, high-mountain endemic species are recognised. Populations from the summit areas of the Pyrenees and Mt. Ventoux, traditionally assigned to *A. cuneifolium*, are distinct from this species of the central Apennines as well as from each other, lending support to the recognition of two endemics: hexaploid *A. flexicaule* already described by Jordan (1846) from Mt. Ventoux, and tetraploid *A. cacuminum* described here from the Pyrenees as a new species. These species indeed show a similar morphology, such as a procumbent habit, flexuous stems, relatively wide petals, congested fruit racemes, and large, elliptic to broadly elliptic silicules, which we, however, attribute to parallel evolution in adaptation to high altitudes and unstable scree habitats. The third endemic, *A. orophilum*, was recently re-discovered (Španiel & al., 2011b) and is supported here, comprising tetraploids and hexaploids from the southwestern Alps.

Finally, the tetraploids from the coastal sand dunes in the Basque country (Bay of Biscay) and Galicia, recently recognised as two subspecies of *A. loiseleurii* (Ortiz & Rodriguez, 2005), are uncovered here as two separate lineages, favouring species-level treatment, as *A. gallaecicum* and *A. loiseleurii*. Apart from the previously reported differences in fruit and seed morphology (Ortiz & Rodriguez, 2005), the differentiation in vegetative (trichome morphology and density) and floral traits (petal width) discovered here provide further arguments for elevating them to species level.

Chloroplast genealogy: discordance with taxonomy, retention of ancestral variation and recent diversification. — Maternally inherited (Johannessen & al., 2005) cpDNA sequences did not yield patterns congruent with the predominantly nuclear-derived (Meudt & Clarke, 2007) AFLP markers. The plastid markers have limited value for species delimitation and phylogenetic inference in the studied species complex. Although high diversity and numerous haplotypes were observed, variation was poorly structured, resulting in a polytomy in the tree-based inferences and star-like patterns in the network-generating analyses. Haplotype sharing among genetically (AFLP) and morphologically well-distinct entities was also found. Such discordant patterns and little correspondence with taxonomy could be caused by hybridisation and/or retention and differential sorting of ancestral variation (incomplete lineage sorting; Wendel & Doyle, 1998). Distinguishing between these two processes is a difficult task, and both may act in concert (e.g., Albadalejo & al., 2005; Edwards & al., 2008).

The two haplotypes found to be shared by multiple species and recorded throughout most of the study area occupied internal positions within the respective subnetworks (Fig. 6). The derived haplotypes found at the tips of the network, in contrast, were mostly species- or population-specific and were found at low frequencies in small geographic ranges. Following the coalescence theory (Castelloe & Templeton, 1994; Pleines & al., 2009), these patterns suggest that haplotype sharing is due to retention of ancestral variation through speciation events and incomplete lineage sorting (e.g., Jakob & Blattner, 2006; Martín-Bravo & al., 2010) rather than recent hybridisation. Additionally, the species sharing haplotypes are currently allopatric and ecologically differentiated (e.g., *A. cacuminum* from high alpine screes vs. *A. loiseleurii* from coastal sand dunes) precluding current gene flow among them. Recent hybridisation is also not observed in the more rapidly evolving AFLP markers. The presence of multiple rare and derived haplotypes with a star-like structure especially in the subnetwork of group II indicate recent diversification and limited gene flow among conspecific populations, in accordance with the geographic structuring of AFLP variation within the species. Although we can exclude recent hybridisation, some patterns of intraspecific haplotype variation or haplotype sharing might indicate more ancient hybridisation (introgression during glacial and early postglacial range shifts, see Heuertz & al., 2006; Dixon & al., 2007) and shed light on the origin of polyploids. This refers to *A. montanum* that displays highly divergent haplotypes in the southern and northern diploids, and a similar divergence without geographic structure is observed in the polyploid *A. orophilum*, endemic to the southwestern Alps. The haplotype of the stenoendemic hexaploid *A. rhodanense* is shared with the geographically distant *A. fastigiatum*, which contrasts with the AFLP patterns, suggesting past hybridisation and an allopolyploid origin (see below).

The evolutionary inference based on cpDNA (Electr. Suppl. 1: Fig. S1) also implies that the ancestral species of the studied complex occur in the Balkan and Apennine Peninsulas, also harbouring the highest diversity, whereas the southwestern European species are of more recent origins.

Evolutionary and phylogeographic history of the study species in southwestern Europe and Morocco: the role of polyploidy and allopatric differentiation. — The Mediterranean Basin is recognised as a biodiversity centre and provides a challenge for the reconstruction of plant evolution in several species groups (Thompson, 2005). The here-studied *Alyssum montanum–A. repens* complex has its diversity centre in the (Sub)Mediterranean region, as can be concluded from the number of species and endemics reported here (Jalas & al., 1996) and as is supported by the results of our on-going studies (present study, Španiel & al., 2011b, and study in progress). There are no species with a circum-Mediterranean distribution, and several taxa occupy fairly restricted ranges. Such distribution patterns most likely result (1) from limited seed dispersal in these species (mainly epizoochorous cattle dispersal, see Gerth & al., 2011) that hampers the effective colonisation across geographic barriers or large distances and is supported here by geographically structured genetic variation in more widespread species, as well as (2) from the recent diversification of this polyploid complex. Rough age estimates of divergence events in the genus (Rešetnik & al., 2013) imply that the studied complex may have originated and began to diversify from the late Pliocene to the early Pleistocene, in between the onset of the Mediterranean climate (3.2 Ma) and the Pleistocene climatic oscillations (2.5 Ma). This is in accordance with recent speciation events documented in several other mainly Mediterranean species complexes (e.g., Alarcón & al., 2012; Blanco-Pastor & al., 2012; Santos-Gally & al., 2012). The extent to which the restricted distribution ranges reflect the species' narrow ecological niches remains to be explored.

We conclude that most of the Iberian Peninsula, apart from the Pyrenees and coastal areas, is occupied by a single species, *A. fastigiatum*, not supporting the existence of the previously recognised southern Iberian endemics. This species exhibits substantial geographically structured variation; nevertheless, the southern Baetic populations are not significantly more diverse than the more northern Cantabrian populations. This finding contrasts with other plant groups that show a high incidence of endemism and species diversity in the (Sub)Baetic region, supporting this region as a melting pot for biodiversity and as a speciation centre (Giménez & al., 2004; Peñas & al., 2005), as studied in detail in *Armeria* (Fuertes Aguilar & al., 2011 and references therein). *Alyssum fastigiatum* grows in different mountain systems of the Peninsula across a large altitudinal range, from 500 m to 2000 m in the Pre-Pyrenees and Picos de Europa, and to 3000 m in the Sierra Nevada. Altitudinal and small-scale latitudinal range shifts are expected to have occurred in such southern European mountain species during the glacial periods (Nieto Feliner, 2011). The geographical structuring of genetic variation (AFLPs) indicates the presence of three lineages that may have evolved in partial isolation, allowing for the accumulation of genetic differences. Gene flow has been insufficient to homogenise these gene pools, but genetic admixture is observed in contact zones (pop. 270BCT). Interestingly, the NE Iberian lineage is composed of populations from two mountain ranges (Iberian System and Pre-Pyrenees) separated by the Ebro valley that may be a prominent dispersal barrier for mountain species (Médail & Diadema, 2009), but suitable ecological niches may have been present at lower altitudes in glacial periods, allowing for migration between these mountain ranges (see, e.g., Kropf & al., 2008). Genetic diversity and distinctiveness usually help to identify refugial regions and colonisation histories (Hewitt, 1996). Intrapopulational genetic diversity values in *A. fastigiatum* largely coincided with the rarity values but did not reveal clear geographic patterns, supporting the absence of large range shifts in the past. Highly diverse populations were found to be scattered across the entire area and were observed mainly among the tetraploids in Cantabria but also in the Baetic diploids. Tetraploids were recorded within the Cantabrian and NE Iberian lineages for which the present data (AFLP, cpDNA, morphology) suggest autopolyploid origins.

A disjunct occurrence on both sides of the Strait of Gibraltar was assumed for *A. atlanticum* (Küpfer & Nieto Feliner, 1993); however, we can not verify its occurrence on the European continent. The Moroccan *A. atlanticum* and Iberian *A. fastigiatum* represent two distinct species. Although species relationships are poorly resolved with the present data, all available evidence, including AFLP patterns, cpDNA variation and weak morphological differentiation, indicate that they are close relatives and that their common ancestor may have been distributed on both sides of the Strait. Since the Strait opened around 5.3 Ma, it has acted both as a biogeographical bridge and barrier (Rodríguez-Sánchez & al., 2008; Lavergne & al., 2013). Migration events and gene flow across the strait, also enhanced by sea level lowering during the last glacial maximum, have been reported for several herbaceous species (e.g., Ortiz & al., 2007, 2008; Guzmán & Vargas, 2009; Arrigo & al., 2010), whereas gene exchange was not observed in other cases, promoting divergence and speciation (e.g., Escudero & al., 2008). The latter scenario is observed in the species studied here. The Iberian and Moroccan populations share an ancestral cpDNA haplotype that radiated into a series of derived haplotypes specific either to the Iberian or the Moroccan species. There are no indications of more recent migration or gene exchange events between *A. atlanticum* and *A. fastigiatum* either in the AFLP or the cpDNA data. Furthermore, the AFLP data show clustering patterns congruent with geographic regions in both species, reflecting their limited gene flow across larger distances. This pattern is especially pronounced in *A. atlanticum*, which harbours high, geographically structured diversity, apparently resulting from isolation at high altitudes of the Moroccan massifs. This species comprises both diploids and tetraploids; the latter are most likely autopolyploids that originated in situ.

The conspicuous south–north differentiation between the diploids of *A. montanum* is attributable to their long-term geographic separation, putatively reflecting different glacial refugia and inefficient colonisation and gene flow across the species range. Intrapopulational diversity and rarity values were highly correlated, and populations having the highest values were scattered across the entire range, suggesting multiple refugia and a glacial and postglacial history without major large-scale range shifts, as previously hypothesised in the eastern range of this species (Španiel & al., 2012a). The

intrapopulational genetic variation, however, was substantially lower when compared to the other species studied here, most likely reflecting a past reduction in population size and diversity, as well as a more recent decline and survival in small and isolated populations that are often threatened by overgrowing forests (see Rusterholz & al., 2012). The tetraploids, in contrast, were significantly more diverse, and the AFLP patterns suggest that they originated by hybridisation of the two diploid lineages. Nevertheless, the tetraploids also harboured a high proportion of specific fragments not found elsewhere, allowing for two alternative hypotheses. The tetraploid-specific markers can either result from genomic changes triggered by the polyploidisation events and subsequent independent evolution of the polyploids (Leitch & Leitch, 2008; Soltis & Soltis, 2009), or indicate the contribution of another (not identified here, possibly extinct) progenitor species (as documented, e.g., by Jakob & Blattner, 2010). An allopolyploid origin is furthermore hypothesised for the hexaploid stenoendemic *A. rhodanense*, assuming hybridisation between the tetraploid *A. montanum* (supported by AFLP data) and the diploid *A. fastigiatum* (cpDNA haplotype sharing and morphological similarity). This would imply past contact between these two currently allopatric species that are separated by the Pyrenees, which is intriguing as it indicates either past substantial range shifts/expansion of *A. fastigiatum* or an exceptional long-distance dispersal event. Formerly wider distribution areas accompanied by hybridisation events between currently allopatric species have also been inferred in other cases, such as in *Androsace* in the Pyrenees (Dixon & al., 2007). The AFLP data did not show admixture patterns in *A. rhodanense*, suggesting an old (presumably glacial) hybridisation event, but additional markers such as low-copy nuclear genes still need to be explored to disentangle the polyploid origins of both *A. montanum* and *A. rhodanense*.

The coastal species *A. gallaecicum* appears genetically very close to the Cantabrian mountain populations of *A. fastigiatum*, which might suggest past area fragmentation at the distribution edge of the more widespread *A. fastigiatum*, colonisation of sand dunes and ecological speciation in allopatry (Schluter, 2001). The presence of only few *A. gallaecicum*-specific AFLP markers and cpDNA haplotypes derived from those of *A. fastigiatum* indeed favour this scenario. Alternatively, the observed genetic affinity may also have been caused by past (glacial) secondary contact and introgression. The origin of *A. loiseleurii* from the coast of the Bay of Biscay remains uncertain; the AFLP data resolved it as a separate lineage, and its major cpDNA haplotype is shared with three other species (*A. fastigiatum*, *A. cacuminum*, *A. montanum*). The overall morphological resemblance with *A. gallaecicum* in some traits (procumbent habit, dense and greyish leaf indumentum) might result from parallel adaptation to the sand dune habitat and similar selective pressure (Wake & al., 2011). Both species are endemics distributed along short strips of coastal sand dunes (Ortiz & Rodriguez, 2005) deserving a high conservation priority. A significant decline in the number of populations due to habitat destruction and degradation has been documented for *A. loiseleurii* in the Bay of Biscay (Frey & al., 2012).

The polyploid origins of the high-mountain endemics *A. orophilum* (SW Alps), *A. flexicaule* (Mt. Ventoux) and *A. cacuminum* (Pyrenees) cannot be resolved with certainty here. The cpDNA patterns show a close relationship of *A. orophilum* to the geographically adjacent *A. montanum* as well as the Cantabrian populations of *A. fastigiatum*, suggesting these species as potential progenitors, but the AFLP data indicated a closer relationship to the group of Apennine and Balkan species. Thus, an allopolyploid origin involving progenitors distributed east and west of the Alps could be considered. The southwestern Alps have been recognised both as a glacial refugium and a secondary contact zone (Hewitt, 1999; Petit & al., 2003; Schönswetter & al., 2005). We hypothesise that distinct *Alyssum* lineages may have met there during glacial/interglacial range shifts, resulting in the origin of *A. orophilum*. The presence of two rare but highly distinct and derived cpDNA haplotypes indicates either multiple origins or later introgression accompanied by chloroplast capture. *Alyssum flexicaule*, so far confirmed only from Mt. Ventoux (possibly also Pic de Bure in the Dauphiné Alps, M. de la Harpe & al., pers. comm.), is resolved as a close relative of *A. orophilum*, which could be explained by their shared parentage and subsequent differentiation due to postglacial isolation or by introgression in glacial times. It is assumed that in the cold periods of the Pleistocene, high-mountain plants were distributed more widely and continuously at lower altitudes, allowing for inter-mountain contact and gene exchange, and that these ranges became fragmented during postglacial retreat (Kropf & al., 2008). The Pyrenean *A. cacuminum* forms a distinct lineage with high genetic diversity in both the AFLP and cpDNA data, indicative of multiple polyploid origins and/or recurrent introgression with relatives.

Conclusions. — The present study infers that both polyploidy and allopatric differentiation have driven the diversification in the *Alyssum montanum–A. repens* species complex. Polyploidisation has increased the diversity directly by polyploid speciation (*A. orophilum*, *A. cacuminum*, *A. flexicaule*, *A. rhodanense*), as well as indirectly by polyploidisation events enriching the intraspecific variation (*A. fastigiatum*, *A. atlanticum*). Hybridisation and polyploidisation may have facilitated the colonisation of new niches reinforcing speciation, as suggested for the coastal *A. gallaecicum* and *A. loiseleurii* or the alpine scree species *A. cacuminum*. Apart from polyploidy, divergence driven by geographic isolation and genetic drift has presumably played an evolutionary role as well. The strong geographical structuring of genetic variation in several species (*A. montanum*, *A. atlanticum*, *A. fastigiatum*), limited seed dispersal, and the presence of several narrow endemics imply highly restricted gene flow over large distances and low colonisation ability across barriers (river valleys, high mountain crests and marine straits) that stimulate speciation in allopatry.

■ TAXONOMIC TREATMENT

We present a revised taxonomic treatment of the *Alyssum montanum–A. repens* complex in southwestern Europe and Morocco. New circumscriptions are provided for *A. atlanticum*

and *A. fastigiatum*. We resurrect *A. rhodanense*, *A. flexicaule* and *A. orophilum*. Species-level treatment is advocated for *A. montanum*, *A. loiseleurii* and *A. gallaecicum*. The Pyrenean populations previously assigned to *A. cuneifolium* are described as a new species, *A. cacuminum*. The populations from Sierra de Mijas (southern Spain) are provisionally treated under *A. fastigiatum*, pending further studies.

Due to the character range overlaps, morphological identification may not be straightforward in every case, and therefore, several specimens per population should be examined. Ecological and geographical data also aid in correct species identification and are also specified in the key. The value ranges of the quantitative characters represent the 5th and 95th percentiles; asterisks indicate mean values of three random counts per leaf surface. Petal size should be measured on the largest flowers found on the plants and recorded during the early flowering period.

Apart from the standard identification key, an interactive identification key created in the Xper tool (Ung & al., 2010) as well as the corresponding file in SDD (Structured Descriptive Data) format (endorsed by the International Taxonomic Databases Working Group as a standard for interchange of descriptive data; Hagedorn & al., 2005) are provided as Electronic Supplements 2 and 3.

Key to the species of the *Alyssum montanum*–*A. repens* complex from southwestern Europe and Morocco

1. Leaves on both sides equally densely hairy and silvery-white; lower and upper leaves of almost equal size and shape, oblanceolate to narrowly oblanceolate; flowering stems ascending to erect …… ***A. fastigiatum*** (diploid plants; Sierra de Mijas, Spain; rocks and dolomitic sands)
1. Leaves sparsely hairy and green, or lower leaf surface densely hairy and greyish green to silvery-white and upper leaf surface less hairy and green to greyish green; lower leaves suborbicular or spathulate to widely oblanceolate, upper leaves oblanceolate to narrowly oblanceolate; flowering stems procumbent to ascending …… 2
2. Plants growing on coastal sand dunes …… 3
2. Plants growing on inland sand, on rock, dry grassland and in alpine habitats …… 4
3. Silicules 5.5–6.0 mm long; stellate trichomes on lower surface of middle stem leaf with 13–18* terminal rays, those on upper surface with 8–13* terminal rays ….. ***A. loiseleurii*** (tetraploid plants; Basque country: Guipúzcoa, Spain, Aquitaine, France; coastal sand dunes)
3. Silicules 2.9–4 mm long; stellate trichomes on lower surface of middle stem leaf with 17–25* terminal rays, those on upper surface with 13–20* terminal rays …… …… ***A. gallaecicum*** (tetraploid plants; Galicia, Spain; coastal sand dunes)
4. Silicules elliptic or broadly elliptic; fruiting racemes mostly congested, umbel-like; stems mostly flexuous, twisted in the basal procumbent part …… 5
4. Silicules suborbicular; fruiting racemes mostly not congested, elongated; stems mostly straight and ascending in the basal part (fruiting racemes sometimes congested and stems flexuous and twisted in plants from Tozal de Guara) …… 6
5. Stellate trichomes on lower surface of middle stem leaf with 19–29* terminal rays, those on upper surface with 14–24* terminal rays; lower surface of middle stem leaf mostly densely hairy, with 7–14 trichomes per 0.5 mm² area …… ***A. flexicaule*** (hexaploid plants; Mt. Ventoux, France; alpine calcareous screes)
5. Stellate trichomes on lower surface of middle stem leaf with 12–21* terminal rays, those on upper surface with 9–17* terminal rays; lower surface of middle stem leaf mostly sparsely hairy, with 4–11 trichomes per 0.5 mm² area …… ***A. cacuminum*** (tetraploid, exceptionally hexaploid plants, Pyrenees, France, Spain; alpine screes, rocks and dry grasslands)
6. Lower surface of middle stem leaf densely hairy, usually silvery-white and entirely covered by stellate trichomes, leaf epidermis often invisible underneath the layer of trichomes, 14–27 trichomes per 0.5 mm² area with 16–27* terminal rays 0.18–0.30 mm long … ***A. montanum*** s.str. (diploid and tetraploid plants; SW Germany, France, W Switzerland; rocks, dry grassland, inland sand in lowlands and mountains, rarely subalpine pastures)
6. Lower surface of middle stem leaf sparsely or densely hairy, green or greyish green, rarely entirely covered by trichomes, leaf epidermis usually at least partly visible underneath the layer of trichomes, 2–14 trichomes per 0.5 mm² area with 7–19* terminal rays 0.28–0.48 mm long …… 7
7. Petals 2.2–4.5 mm wide; lower surface of middle stem leaf with 2–7 stellate trichomes per 0.5 mm² area …… …… ***A. orophilum*** (tetraploid and hexaploid plants; southwestern Alps, France, Italy; alpine open sites and dry grasslands)
7. Petals 1.2–3.2 mm wide; lower surface of middle stem leaf with 3–14 stellate trichomes per 0.5 mm² area …… 8
8. Stem less densely leafy, with the distance between the 8th and 15th leaf base (counted downward) 2.5–8.3 cm; 15th stem leaf 7.7–25.7 mm long and 2.4–5.1 mm wide, 8th stem leaf 9.5–22.3 mm long and 2.0–4.6 mm wide; petals 2.2–3.5 mm wide, longer (inner) filaments 4.0–5.0 mm long, style 2.8–3.7 mm long …… ***A. rhodanense*** (hexaploid plants; Drôme, France; granite rocks, lowlands)
8. Stem more densely leafy, with the distance between the 8th and 15th leaf base 0.9–6.8 cm; 15th stem leaf 5.0–15.8 mm long and 1.3–3.7 mm wide, 8th stem leaf 6.2–16.9 mm long and 1.3–3.5 mm wide; petals 1.3–3.1 mm wide, longer (inner) filaments 3.0–4.9 mm long, style 1.8–3.8 mm long …… 9
9. Petals 4.5–7.4 mm long (emarginate apical part of the petal with petal sinus not included); depth of sinus on emarginate petal tip 0.10–0.53 mm; sepals 2.8–4.0 mm long; 15th stem leaf 1.6–4.7 mm wide, 8th stem leaf 1.5–4.0 mm wide …. …… ***A. atlanticum*** (diploid and tetraploid plants; Morocco; high-mountain dry grasslands, rocks and alpine pastures)

9. Petals 4.0–6.4 mm long (emarginate apical part of the petal with petal sinus not included); depth of sinus on emarginate petal tip 0.13–0.61 mm; sepals 2.5–3.6 mm long; 15th stem leaf 1.3–3.4 mm wide, 8th stem leaf 1.3–3.3 mm wide …. ……***A. fastigiatum*** (diploid and tetraploid plants; Spain; montane to alpine dry grasslands, rocks and pastures)

Alyssum atlanticum Desf., Fl. Atlant. 2: 71, t. 149. 1798 ≡ *A. montanum* var. *atlanticum* (Desf.) Boiss., Voy. Bot. Espagne 2: 44. 1839 ≡ *A. montanum* subsp. *atlanticum* (Desf.) Nyman, Consp. Fl. Eur.: 56. 1878 (O. Bolòs & Vigo in Butl. Inst. Catalana Hist. Nat. 38, Secc. Bot. 1: 78. 1974, isonym) – Ind. loc.: "Habitat in cacumine Atlantis prope Tlemsen" – **Lectotype (designated here):** [MOROCCO]. Habitat in cacumine Atlantis prope Tlemsen, s.d., [*Desfontaines*] *s.n.* (P-Desf. barcode P00680156!).

= *A. montanum* var. *subspeciosum* Maire & Sam. in Ark. Bot., n.s., 11: 15. 1939 – Ind. loc.: "Moyen Atlas: Bab Metik (in montibus ad merid. opp. Taza), in rupibus calcareis, circ. 1350 m s.m. (no. 7159)" – **Lectotype (designated here):** [MOROCCO]. Atlas Medius: in montibus ad austr. ab opp. Taza, Bab Metik, in rupibus calcareis, ca. 1350 m s.m., 27 Apr 1936, *G. Samuelsson 7159* (MPU barcode MPU006207!).

?= *A. embergeri* Quézel in Bull. Soc. Sci. Nat. Maroc 31 (1951): 254. 1953 – Ind. loc.: "Éboulis calcaires culminaux du massif de l'Ayachi (3.700 m. et plus)" – **Lectotype (designated here):** Haut Atlas: D. Ayachi, 3500 m [sic!], Aug 1951, *Quézel s.n.* (RAB no. 012475!).

?= *A. flahaultianum* Emb. ex Emb. in Willdenowia 15: 62. 1985 – Ind. loc.: "Maroc, Grand-Atlas central, massif de l'Erdouz-Igtet, sur le sommet de l'Igtet, 3615 m, Thibaudet" – **Lectotype (designated here):** Grand Atlas central: sur le sommet du Dj Igtet (Haut N'Fis), 3650 m [sic!], 8 Aug 1933, *Thibaudet s.n.* (RAB no. 012476!).

Alyssum cacuminum Španiel, Marhold & Lihová, **sp. nov.** – Holotype: SPAIN. Catalunya, Lleida, Pyrenees, N of Cabdella, SW of Montorroio (2861 m), rocks and screes, 42°29.79′ N, 01°01.36′ E, 2730 m, 24 Jun 2011, *Španiel, Zozomová-Lihová, Letz 317MRO2* [i.e., plant no. 2 from coll. *317MRO*] (SAV; isotype: *317MRO3* [plant no. 3 from coll. *317MRO*] MA).

Diagnosis. – Similar to *Alyssum flexicaule* Jord. and *A. cuneifolium* Ten., differing from both by fewer rays of stellate trichomes on the upper [(9–)10–17(–17) rays] and lower [(12–)14–19(–21) rays] surfaces of middle stem leaves and by a lower trichome density on the lower leaf surface [(3–)4–11(–11) stellate trichomes per 0.5 mm²] that, therefore, appears green or grey-green instead of greyish. It also differs from *A. cuneifolium* by slightly longer styles [(2–)2.3–3.6(–3.8) mm] and narrower petals [(1.9–)2.3–3.6(–3.8) mm].

Etymology. – The specific epithet is derived from the species' occurrence on mountain crests often reaching summit scree sites.

Chromosome number. – $2n = 4x = 32$ (rarely $2n = 6x = 48$).

Habitats. – Alpine screes, rocks and dry grasslands, alt. 1800–2800 m.

Distribution area. – Pyrenees (Spain, France).

Alyssum fastigiatum Heywood in Bull. Brit. Mus. (Nat. Hist.), Bot. 1: 92. 1954 ≡ *A. montanum* subsp. *fastigiatum* (Heywood) Malag., Sinopsis Fl. Iber. 29: 455. 1976 – Ind. loc.: "Prov. Jaén: Montes de Cazorla, Pico de la Garganta, near La Nava de San Pedro, rocky places, c. 1500 m., 25 June 1948, Heywood & Davis 153 (BM, holotype; E; K)" – Holotype: [SPAIN]. Montes de Cazorla (Jaén), Pico de Gargantas, rocky places, alt. ca. 1500 m s.m., 25 Jun 1948, *Heywood & Davis 153* (BM barcode BM000750131!; isotypes: A barcode 00018505!, E barcode E00438389!).

= *A. montanum* var. *hispanicum* Huter in Oesterr. Bot. Z. 54: 190. 1904 – Ind. loc.: "Exsc. H.P.R. 1879, Sierra Tejeda (Nr. 35b, sub nomine *A. diffusum*), P.R. 1891, Nr. 361, Sierra de Alcaraz (*A. diffusum*), P.R. 1895, Cerro de Cristobal (*A. montanum*)" – **Lectotype (designated here):** Regn. Murcicum, in pascuis rupestrib. Sierrae Alcaraz et Padron de Bienservida et Calar del Mundo, sol. calcar., 1000–2000 m. s.m., 25 Jun 1891, *Porta & Rigo*, Iter III hispanicum No. 361, (WU! [specimen annotated by Baumgartner in 1908 as "*Alyssum hispanicum* Hut."]).

Note. – Baumgartner (1908: 33–35) suggested to keep this name in the sense of the specimen from the Sierra de Alcaraz, and he considered the other material mentioned in the protologue to belong to other taxa. This was considered as lectotypification of this name by the herbarium sheet deposited in WU, collected by Porta & Rigo, as part of the Iter III. hispanicum, No. 361 and annotated by Baumgartner (W. Gutermann, 1981, in sched.). Nevertheless, there are plants collected on two different dates (25 Jun and 4 Jul 1891) on this herbarium sheet, and, therefore, lectotypification with a single gathering is done here.

= *A. diffusum* var. *corymbosum* Pau in Bol. Soc. Aragonesa Ci. Nat. 8: 112. 1909 ≡ *A. diffusum* subsp. *corymbosum* (Pau) Molero Mesa & Pérez Raya, Fl. Sierra Nevada: 101. 1987 – Ind. loc.: "Región mantana de Jérez subiendo al Puerto de Trevélez, entre matas leñosas" – Type: not located.

Note. – We have not been able to trace the original material of this name. The synonymisation is based on the locality indicated in the protologue (our collection no. 321TRE corresponds to the type locality).

= *A. montanum* var. *almijarrense* Pau in Mém. Mus. Ci. Nat. Barcelona, Ser. Bot. 1: 21. 1922 – Ind. loc.: "Hab. Cerro Lucero (1916)" – Type: not located.

Note. – We have not been able to trace the original material of this name. The synonymisation is based on the locality indicated in the protologue.

= *A. nevadense* Wilmott ex P.W.Ball & T.R.Dudley in J. Arnold Arbor. 45: 364. 1964 – Ind. loc.: "Holotype: Spain, Sierra Nevada, Almeria, main ridge west of Cerro del Rayo, 25 June 1926, Wilmott (BM)" – **Lectotype (designated here):** [SPAIN]. Sierra Nevada (Prov. Almeria), main ridge west

of Cerro del Rayo, 25 Jun 1926, *Wilmott & Lofthouse* [sic!] *s.n.* (BM barcode BM001118256!).

Note. – The holotype indicated by Ball & Dudley (Dudley, 1964: 364) consists of several specimens of the same gathering deposited in BM. Following Art. 40.2 Note 1 of the *Code*, such specimens are syntypes, and, therefore, a lectotype is designated here from among them.

= *A. cuneifolium* subsp. *losanum* P.Monts. in Doc. Phytosoc. 7–8: 14. 1974 – Ind. loc.: "P. Montserrat (no. 323la/67) l.d. 'Puntón de Guara' (Huesca) in glareosis meridionalibus, 2030 m altitudine, die 12 junio 1967 Hb. JACA legebat" – Holotype: [SPAIN. Huesca], Glera al SSW del Puntón, Guara, 2000–2050 m, 12 Jun 1967, *P. Montserrat s.n.* (JACA no. 323167!).

= *A. montanum* var. *aitanicum* O.Bolòs & Vigo in Butl. Inst. Catalana Hist. Nat. 38, Secc. Bot. 1: 78. 1974 – Ind. loc.: "Serra d'Aitana, in Xeracantho-Erinacione, 1500 m, leg. A. & O. de Bolòs, 9-VII-1958 (BC 149671)" – Type: not located.

Note. – We have not been able to trace the type specimen of this name. The synonymisation is based on the locality indicated in the protologue.

= *A. montanum* var. *alcalatenicum* O. Bolòs & Vigo in Butl. Inst. Catalana Hist. Nat. 38, Secc. Bot. 1: 78. 1974 – Ind. loc.: "Penyagolosa, 1700 m, ubi legerunt A. et O. de Bolòs, 17-VII-1957, BC 151015" – Holotype: [SPAIN]. Maestrat: Penyagolosa, 1700 m, 11 Jul 1957 [sic!], *A. de Bolòs & O. de Bolòs s.n.* (BC no. 151015!).

= *A. montanum* var. *guilleriense* O. Bolòs & Vigo in Butl. Inst. Catalana Hist. Nat. 38, Secc. Bot. 1: 78. 1974 – Ind. loc.: "Viladrau, sables granitiques vers St. Hilari Sacalm, 900 m, leg. Soulié, 4-VI-1913, BC 4567" – Holotype: [SPAIN]. Catalogne, Viladrau, sables granitiques vers San Hilari Sacalm, 900 m, 4 Jun 1913, *Soulie s.n.* (BC no. 4567!).

= *A. montanum* var. *pradesense* O. Bolòs & Vigo in Butl. Inst. Catalana Hist. Nat. 38, Secc. Bot. 1: 78. 1974 ('*pradense*') – Ind. loc.: "Prades, 1000 m, ubi legit P. Font i Quer, 29-VI-1918, BC 109222" – Holotype: [SPAIN]. Prades, 1000 m (Tanag.), 29 Jun 1918, *F*[*ont i*] *Q*[*uer*] *s.n.* (BC no. 109222! [only sheet with label by O. Bolòs, dated 14 Mar 1969]).

Note. – There are two herbarium sheets of this collection bearing the same number (BC no. 109222); the one with a handwritten revision label by O. Bolòs, dated 14 Mar 1969, should be considered the holotype of the name *A. montanum* var. *pradense*.

= *A. gadorense* P.Küpfer in Castroviejo & al., Fl. Iber. 4: 175. 1983 ("*A. montanum* subsp. *gadorensis*" Losa & Rivas Goday in Arch. Inst. Aclim. Cons. Super. Invest. Ci. 13: 184. 1974, nom. nud.) – Ind. loc.: "Almería: Sierra de Gádor, pr. Castala, WF18, 2050 m, zona cacuminal, suelo pedregoso calizo, 16. VI. 1986, Bayón, Galán & Nieto Feliner nr. 1388GN" – Holotype: [SPAIN]. Almería: Sierra de Gádor, pr. Castala, WF18, 2050 m, zona cacuminal, suelo pedregoso calizo, 16 Jun 1986, *Bayón, Galán & Nieto Feliner 1388GN* (MA no. 321725!).

Alyssum flexicaule Jord., Observ. Pl. Nouv. 1: 12, pl. 1. 1846 – Ind. loc.: "… parmi les rochers et dans les lieux secs et pierreux du mont Ventoux, près Avignon, où je l'ai récoltée en juillet 1841" – **Lectotype (designated here):** [FRANCE]. Mt. Ventoux près Avignon, 31 Jul 1841, [*Jordan*] *s.n.* (LY [herb. Jordan]!).

Note. – There is also one additional sheet with plants from Mt. Ventoux with the collection date 1841 in LY [herb. Jordan].

Alyssum gallaecicum (S.Ortiz) Španiel, Marhold & Lihová, **comb. & stat. nov.** ≡ *A. loiseleurii* subsp. *gallaecicum* S.Ortiz in Nova Acta Ci. Compostelana, Biol. 14: 54. 2005 – Ind. loc.: "O Grove, San Vicente do Mar, 29TNH0502, 14.VII.1983, en duna fixadas sobre forte aporte de CO_3Ca procedente de restos de cunchas, X.R. García s.n. (SANT 12463, holotype; MA 411056, isotype)" – Holotype: [SPAIN]. Pontevedra: O Grove, S. Vicente de Mar, 29TNH 0502, sobre dunas fijadas con fuerte aporte de CO_3Ca procedente de restos de conchas, 14 Jul 1983, *X.R. García Martínez s.n.* (SANT no. 12463; isotype: MA no. 411056!).

= *A. montanum* var. *parviflorum* Pau in Brotéria, Sér. Bot. 10: 130. 1912 – Ind. loc.: "… muy propaganda por los arenales del Grove (Melojo) S.ta Eugenia de Riveira, Olveira, Corrubedo …" – Type: not located.

Note. – We have not been able to trace the original material of this name. The synonymisation is based on the locality indicated in the protologue (our collection no. 309GRV is from O Grove).

Alyssum loiseleurii P.Fourn., Quatre Fl. France: 425. 1936 ≡ *A. arenarium* Loisel., Fl. Gall.: 401. 1807, non Kit. ex Spreng. 1801 ≡ *A. montanum* var. *arenarium* DC., Prodr. 1: 162. 1824 ≡ *A. montanum* subsp. *arenarium* (DC.) Rouy & Foucaud, Fl. France 2: 182. 1895 – Ind. loc.: "Habitat in arenosis maritimis circà Baionam" – **Lectotype (designated here):** [FRANCE]. Dans les dunes aux environs de Bayonne, 15[?] May 1803, *herb. Loiseleur s.n.* (P!).

Alyssum montanum L., Sp. Pl.: 650. 1753, nom. cons. – Ind. loc.: "Habitat in Helvetia" – Type proposed for conservation (Marhold & al. in Taxon 60: 237. 2011; recommended by the Committee for Vascular Plants, see Applequist in Taxon 61: 1110. 2012): SWITZERLAND. Baselland, S of Basel, Aesch, below the castle ruin Pfeffingen, 47°27.175′ N, 07°35.649′ E, 390 m, 13 Apr 2008, *T. Brodtbeck, K. Marhold & J. Zozomová-Lihová 95BAS/24* [i.e., plant no. 24 from coll. *95BAS*] (SAV!; isolectotype: *95BAS/13* [i.e., plant no. 13 from coll. *95BAS*] STU!).

= *A. montanum* var. *obovatum* Rchb., Fl. Germ. Excurs: 670. 1832 – Ind. loc.: "auf den Würtemb. Alpen auf Felsen und Ruinen: Hchst. [Höchstetter?]" – Type: not located.

Note. – We have not been able to trace the original material of this name. The synonymisation is based on the region indicated in the protologue.

= *A. beugesiacum* Jord. & Fourr., Brev. Pl. Nov. 2: 10. 1868 – Ind. loc.: "Hab. in rupibus calcareis Galliae orientalis, secus Rhodani ripas: Saint-Sorlin (Ain)" – **Lectotype (designated here):** St. Sorlin (Ain) 1866, mj. [mon jardin] 19 Apr 1867, [*Jordan*] *s.n.* (LY [herb. Jordan]!—flowering stem in the upper left corner of the sheet).

Note. – There are four flowering and two fruiting plants on the same sheet. The material was apparently gathered in 1866 at the original locality, cultivated and then collected for the herbarium specimens on 19 April 1867 (flowering plants, which belong to the original material) and in June 1870 (fruiting plants, which are not part of the original material).

= *A. brevifolium* Jord. & Fourr., Brev. Pl. Nov. 2: 11. 1868 ≡ *A. montanum* var. *brevifolium* (Jord. & Fourr.) Rouy & Foucaud, Fl. France 2: 180. 1895 – Ind. loc.: "Hab. in rupibus calcareis Galliae orientalis: Charette prope Crémieu (Isère), ex dom. E. Reverchon" – **Neotype (designated here):** de Charette (Isère), 1867, mj. [mon jardin] 28 May 1869, [*Jordan*] *s.n.* (MPU barcode MPU017006!; isoneotype: MPU barcode MPU017007!)

Note. – We have not been able to trace the original material related to this name either in P, LY or MPU, which is why a neotype is selected here. The material on the herbarium sheets was apparently gathered in 1867 at the original locality, cultivated and then collected for the herbarium specimens on 28 May 1869 (flowering plants, which constitute the neotype) and on 1 June 1870 (fruiting plants, which are not part of the neotype).

= *A. psammeum* Jord. & Fourr., Brev. Pl. Nov. 2: 9. 1868 – Ind. loc.: "Hab. in arenosis prope Lugdunum: Chasse (Isère)" – **Lectotype (designated here):** de Chasse (Isère), 1864, mj. [mon jardin] 30 Jun 1866, [*Jordan*] *s.n.* (MPU barcode MPU016995!).

Note. – The material on the herbarium sheet was apparently gathered in 1864 at the original locality, cultivated and then collected for the herbarium specimen on 30 June 1866.

= *A. xerophilum* Jord. & Fourr., Brev. Pl. Nov. 2: 10. 1868 – Ind. loc.: "Hab. in arenosis Galliae centalis: Montmorillon prope Fontainebleau (Seine-et-Marne)" – **Neotype (designated here):** Fontainebleau (S. et Marne), mj. [mon jardin] May 1870, [*Jordan*] *s.n.* (P barcode P05364288! [flowering plants only]).

Note. – We have not been able to trace the original material related to this name either in P, LY or MPU, which is why a neotype is selected here.

= *A. montanum* [unranked] *collicola* Rouy & Foucaud, Fl. France 2: 180. 1895 ('*collicolum*') ≡ *A. collinum* Jord. & Fourr., Brev. Pl. Nov. 2: 9. 1868 non Brot. 1827 ≡ *A. montanum* subsp. *collicola* (Rouy & Foucaud) P.Fourn., Quatre Fl. France: 425. 1936 ('*collicolum*') – Ind. loc.: "Hab. in collibus Galliae centralis: Chinon (Indre-et-Loire)" – **Lectotype (designated here):** [FRANCE]. Chinon (Indre-et-Loire), May 1867, [*Jordan*] *s.n.* [P barcode P05364829! [only plant in flower].

Note. – There is also one plant in fruit on the same sheet, collected in June 1867, which is not part of the lectotype.

= *A. montanum* var. *thiebautii* Liou in Arch. Bot. Mém. 3: 201. 1929 – Ind. loc.: "arènes dolomitiques de tous les grands Causses, surtout Larzac (type déposé dans les herbiers de la Faculté des Sciences de Clermont-Ferrand et de M.J. Thiébaut)" – Type: not located.

Note. – We have not been able to trace the original material of this name (despite a search in CLF; Gilles Thébaud, Clermont-Ferrand, pers. comm.). The synonymisation is based on the locality indicated in the protologue (our collection no. 372RIV is located close to Larzac, France).

Alyssum orophilum Jord. & Fourr., Brev. Pl. Nov. 2: 11. 1868 – Ind. loc.: "Hab. in rupibus Alpium Delphinatus: Briançon (Hautes-Alpes), etc." – **Lectotype (designated here):** Mt. Cenis, 4 Sep 1842, [*Jordan*] *s.n.* (LY [herb. Jordan]!).

= *A. brigantiacum* Jord. & Fourr., Brev. Pl. Nov. 2: 12. 1868 – Ind. loc.: "Hab. in rupibus Alpium Delphinatus; monte Gondran prope Briançon (Hautes-Alpes)" – **Lectotype (designated here):** Mt. Gondran près Briançon (Htes Alpes), 20 May 1867, [*Jordan*] *s.n.* (MPU barcode MPU017005!).

= *A. pedemontanum* Rupr. in Mém. Acad. Imp. Sci. Saint Pétersbourg, Sér. 7, 15(2) [= Fl. Caucasi]: 102. 1869 – Ind. loc.: "… e pascuis alpinis supra Tenda et m. Cenis …" – **Lectotype (designated here):** Mont Cenis, 1853, *Rchb.* [*Reichenbach*?] *s.n.* (LE!—specimen is annotated by Ruprecht as "*A. pedemontanum* m.").

Alyssum rhodanense Jord. & Fourr., Brev. Pl. Nov. 2: 10. 1868 – Ind. loc.: "Hab. in collibus graniticis, ad Rhodani ripas: Tain (Drôme)" – **Lectotype (designated here):** [FRANCE]. de Tain, m/j [mon jardin], May 1844, [*Jordan*] *s.n.* (LY [herb. Jordan]!).

■ ACKNOWLEDGEMENTS

We sincerely thank D.R. Letz, P. Mereďa (both Bratislava), and E. Záveská (Prague) for their company and assistance in the field; M. de la Harpe (Neuchâtel), N. Alvarez (Lausanne) for providing plant samples from several localities; S. Ortiz and his collaborators (Santiago de Compostela) and J.-M. Tison (L'Isle d'Abeau) for advice on some localities; B. Frajman, P. Schönswetter and M. Magauer (all Innsbruck) for sharing plant material and unpublished data; N. Lavapout-Sauter (Bagnères-de-Bigorre) for providing access to the database of the Conservatoire botanique national des Pyrénées et de Midi-Pyrénées; G. Nieto Feliner and J. Fuertes Aguilar (both Madrid) for discussion and useful comments on the results; D. Senko (Bratislava) for generating the map in Fig. 1; T. Burguiere (Paris) for the help with Xper² files; and the curators and employees of the herbaria (BC, BCN, BM, CLF, JACA, LE, LY, MA, MPU and P) for their assistance

during our herbarium studies. Thanks are due to Junta de Andalucia, Consejería de Medio Ambiente (*A. atlanticum* and *A. nevadense*) and Direction régionale de l'environnement, de l'aménagement et du logement d'Aquitaine, Bordeaux Cedex (*A. loiseleurii*, Demande cerfa no. 11634, issued to M. de la Harpe) for plant collection permissions. This study was financially supported by the Grant Agency VEGA, Bratislava, Slovak Republic (grant no. 2/0004/13 to J.Z.-L.), the Slovak Research and Development Agency (APVV; grant no. APVV-0139-12 to J.Z.-L.), and the Grant Agency of the Czech Republic (P506/12/0668 to K.M.). Studies of the herbarium specimens in MA were supported by the EU FP7 SYNTHESYS project (fellowships no. ES-TAF-3099 to K.M., ES-TAF-3213 to J.Z.-L. and ES-TAF-3216 to S.Š.). The field work was partly supported by the joint Slovak-Spanish research project supported by the Slovak Academy of Sciences, Bratislava and Consejo Superior de Investigaciones Científicas, Madrid. This publication was co-financed by the European Social Fund and the state budget of the Czech Republic (Project no. CZ.1.07/2.3.00/30.0022 to S.Š.).

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