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How many taxa? Spatiotemporal evolution and taxonomy of *Amphoricarpos* (Asteraceae, Carduoideae) on the Balkan Peninsula

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Abstract *Amphoricarpos* Vis. is an early diverging genus within tribe Cardueae (Carduoideae, Asteraceae), which is disjunctly distributed in the Balkan Peninsula, Anatolia and the Caucasus; the Anatolian and Caucasian taxa are sometimes treated as separate genus *Alboviodoxa*. We focus on the monophyletic Balkan populations, which have been treated very inconsistently in previous taxonomic accounts (one polymorphic species with or without varying sets of intraspecific taxa vs. two species, one of them with two subspecies). In order to disentangle relationships among populations across the entire distribution area of *Amphoricarpos* on the Balkan Peninsula, we employed amplified fragment length polymorphisms (AFLPs) as well as nuclear and plastid DNA sequences (ITS and *rps16–trnK*) to a dense sampling of populations. ITS was also used to reconstruct the genus' spatiotemporal evolution. In addition, we contrasted the genetic results with morphological data to provide a sound taxonomic revision of *Amphoricarpos* on the Balkan Peninsula. The split between the Balkan populations and the Anatolian *A. exsul* took place in the late Miocene or early Pliocene, whereas diversification within the Balkan lineage is much younger and likely started in the Pleistocene. The deepest splits seen in AFLPs and/or ITS separate the geographically disjunct northern- and southern-most populations. Divergence within the continuous distribution area in the centre is shallower, but allowed recognition of three largely allopatric clusters. Morphometric data, however, were neither in line with previous multi-taxon treatments nor with patterns of genetic divergence. We therefore refrain from recognising any of the genetic groups as a distinct taxonomic entity and rather suggest treating all Balkan populations as a single, genetically, morphologically and ecologically variable species, *Amphoricarpos neumayerianus* (Vis.) Greuter, without intraspecific taxa.

Keywords *Amphoricarpos* · Balkan peninsula · Morphometrics · Over-splitting · Taxonomy

Introduction

The Balkan Peninsula is a hotspot of European plant diversity and an important centre of endemism (Hayek 1924–1933; Turrill 1929; Markgraf 1932; Horvat et al. 1974; Polunin 1987; Davis et al. 1994; Kryštufek and Reed 2004; Stevanović et al. 2007). Causes for this diversity may be sought in the geographic position at the transition of different floral provinces as well as in the region's topographic, climatic and geological complexity (Polunin 1987; Griffiths et al. 2004; Hewitt 2011; Nieto Feliner 2014). In addition, the mountains of the Balkan Peninsula were much less affected by Pleistocene glaciations than, for instance, the Alps and the Pyrenees, enabling the survival of Tertiary biota (e.g. Comes and Kadereit 1998; Hewitt 2000; Petit et al. 2003; Griffiths et al. 2004; Hewitt 2011) and fostering divergence in multiple Pleistocene microrefugia ('refugia-within-refugia hypothesis': Gómez and Lunt 2007; confirmed for the Balkans by, e.g.

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Surina et al. 2011, 2014; Kutnjak et al. 2014; see also Nieto Feliner 2014).

In spite of its extraordinary richness the Balkan Peninsula remains botanically poorly explored. Several new species have been described recently (e.g. Niketić and Stevanović 2007; Schönswetter and Schneeweiss 2009; Surina et al. 2009; Shuka et al. 2010, 2012; Meyer 2011; Lakušić et al. 2013; Bogdanović et al. 2014) and disjunct localities of rare species have been discovered (Biel and Tan 2010; Barina et al. 2013; Frajman et al. 2013, 2014). A few phylogenetic studies accompanied with dating analyses have shown that many Balkan endemics originated in the Tertiary (e.g. *Campanula comosiformis*; Frajman and Schneeweiss 2009; *Heliosperma macranthum*, Frajman et al. 2009a; *Viscaria asterias* and likely *Atocion lerchenfeldianum*; Frajman et al. 2009b); exceptions are, for instance, species from the *Heliosperma pusillum* group (Frajman et al. 2009a) and *Wulfenia* (Surina et al. 2014) that likely diversified in the Pleistocene.

One of the early diverging genera of the sunflower family is *Amphoricarpos* Vis. (Asteraceae, Carduoideae, Cardueae), which originated in the late Oligocene roughly 25 Ma (Barres et al. 2013). It is disjunctly distributed in the Balkan Peninsula, Anatolia (Turkey) and the Caucasus (Georgia; Euro + Med 2006–). *Amphoricarpos exsul* O. Schwarz and *A. praedictus* Ayasligil & Grierson are endemic to southwestern Anatolia (Schwarz 1970; Ayasligil 1984), whereas *A. elegans* Albov is distributed in the Caucasus (Georgia). These three species are sometimes treated as separate genus *Alboviodoxa* (Grossheim 1949). Molecular phylogenetic studies have shown that *A. exsul* is sister to the Balkan *A. neumayeri* (Vis.) Vis.; the split between the species was dated to the early Pliocene, ca. 5 Ma (Barres et al. 2013), which is significantly older than the diversification of the similarly distributed *Atocion compactum* (Frajman et al. 2009b) and *Wulfenia* (Surina et al. 2014), which likely took place in the Pleistocene.

The type species of *Amphoricarpos*, *A. neumayeri*, was described by Visiani (1842) based on a specimen collected by F. Neumayer in the Dinaric Mountains on Mt. Orjen at the border between Montenegro and Bosnia and Herzegovina. Initially included in *Jurinea* Cass.,Visiani (1847) later described a separate genus *Amphoricarpos*, based on amphora-shaped achenes. Further investigations on the Balkan Peninsula showed considerable variation in habit, leaf shape and floral characters across populations. Murbeck (1891) described a variety with broad leaves from Hercegovina as var. *velezenzis* Murbeck, which he suggested to occur across most of the genus' distribution on the Balkans (cf. Rohlena 1907; Maly 1928). However, Beck (1894) considered that the leaf shape is variable and not correlated with other characters; he therefore reduced the broad-leafed plants to f. *latifolia* G. Beck. Whereas Baldacci (1894) and Rohlena (1907) doubted the taxonomic value of leaf shape variability, Bošnjak (1936) described broad-leafed plants as subsp. *murbeckii* Bošnjak. Fukarek (1965), who extensively studied the distribution and ecological characteristics of *Amphoricarpos* on the Balkans, observed that the variability in leaf shape is high even within populations. Plants with long narrow acuminate leaves (var. *neumayeri*) tend to be more common close to the Adriatic Sea (mountain ranges Orjen, Bijela gora, Lovćen), whereas in other areas the broad-leafed var. *murbecki* and intermediate forms ("var. *intermedia*") prevail (Fukarek 1965). Fukarek, however, refrained from classifying populations into these three entities. Blečić and Mayer (1967) described broad-leafed populations as *A. autariatus* Blečić et Mayer and noted that besides the differences in leaf shape both taxa can be differentiated by the shape of achenes, the width of their wings and the shape of the involucral bracts. Within *A. autariatus* they separated two subspecies, subsp. *autariatus* and subsp. *bertisceus* Blečić et Mayer. The former has a northwestern distribution, being endemic to Bosnia and Herzegovina and Montenegro, whereas the latter has a southeastern range including Montenegro, Macedonia, Kosovo, Albania and northern Greece (Blečić and Mayer 1967; Niketić et al. 2014). In the Durmitor mountain range in Montenegro both subspecies were suggested to co-occur, the first in gorges (600–900 m a.s.l.), and the latter in higher altitudes (above 1, 600 m; Blečić and Mayer 1967). Blečić and Mayer (1967), however, supported their taxonomic treatment only descriptively without providing morphometric data for different populations. Accordingly, Schwarz (1970) recognised only a single species *A. neumayeri* with three subspecies, subsp. *neumayeri*, subsp. *bertisceus* and subsp. *murbeckii*, treating *A. autariatus* subsp. *autariatus* as a synonym of the latter. Also Webb (1976) in his treatment of *Amphoricarpos* in Flora Europea recognised only *A. neumayeri* with two subspecies, subsp. *neumayeri* and subsp. *murbeckii*, treating *A. autariatus* as a synonym of the latter. The former subspecies is distributed in coastal mountains of Montenegro (Orjen and Lovćen) and adjacent Hercegovina, whereas the latter occurs in all other parts of the species' distribution area (Webb 1976). This approach was followed also by Strid and Tan (1991). In contrast, Euro + Med (2006–) follows the treatment of Blečić and Mayer (1967). Farr et al. (1979) and Greuter et al. (1993) pointed out the illegitimacy of the name *A. neumayeri*, as it is based on *Jurinea neumayeriana* Vis., and Greuter (2003) proposed the new combination *A. neumayerianus* (Vis.) Greuter.

In view of the inconsistency of previous accounts, the first aim of our study is to disentangle relationships among populations across the entire distribution area of *Amphoricarpos* on the Balkan Peninsula using amplified fragment length polymorphisms (AFLPs) as well as nuclear and plastid DNA sequences. More specifically, we test (1) whether

Amphoricarpos on the Balkan Peninsula diversified in the Pleistocene or earlier, (2) whether phylogenetic patterns support the current taxonomy and (3) whether the genetically inferred groups have support in morphological diversification. Based on our results we provide a sound taxonomic revision of *Amphoricarpos* on the Balkan Peninsula.

Materials and methods

Plant material and DNA extraction

Leaf material for molecular analyses was collected in the field in 2013, dried and stored in silica gel. Twenty-nine populations of *Amphoricarpos* from five countries were sampled, covering its entire distribution area on the Balkan Peninsula. Additional fruiting material for morphometric analyses was collected in 2014. Flow-cytometric screening of genome size with DAPI-stained nuclei revealed that all populations share the same ploidy level, but the low quality of the peaks prevented full presentation of the data. The sampling localities of plants used in genetic analyses are shown in Fig. 1 and voucher details are provided in Table 1. Populations were assigned to taxa based on their distribution following Blečić and Mayer (1967). Total genomic DNA was extracted from ca. 10 mg silica-gel dried leaf material with the DNeasy 96 Plant Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions.

Amplification and sequencing of plastid and nuclear DNA markers

Three plastid DNA regions totalling ca. 6500 bp, that is *ndhJ–trnT*, *trnL*$^{(UAG)}$*–ndhF* and *trnQ*$^{(UUG)}$ *–5′rps16–5′trnK*$^{(UUU)}$ (Shaw et al. 2005, 2007) were inspected for variability. Only the *5′rps16–5′trnK*$^{(UUU)}$region (referred to as *rps16–trnK* from here on) was variable, and was amplified for one individual per population using the primers trnQ$^{(UUG)}$ and trnK$^{(UUU)}$ (Shaw et al. 2007), as well as for the outgroup taxa *A. exsul* (E00077734, leg. Duman and Duran 1996; GenBank number KR704922), *Dipterocome pusilla* (W 1960–0003931, leg. Rechinger 1957; KR704920) and *Xeranthemum longepapposum*

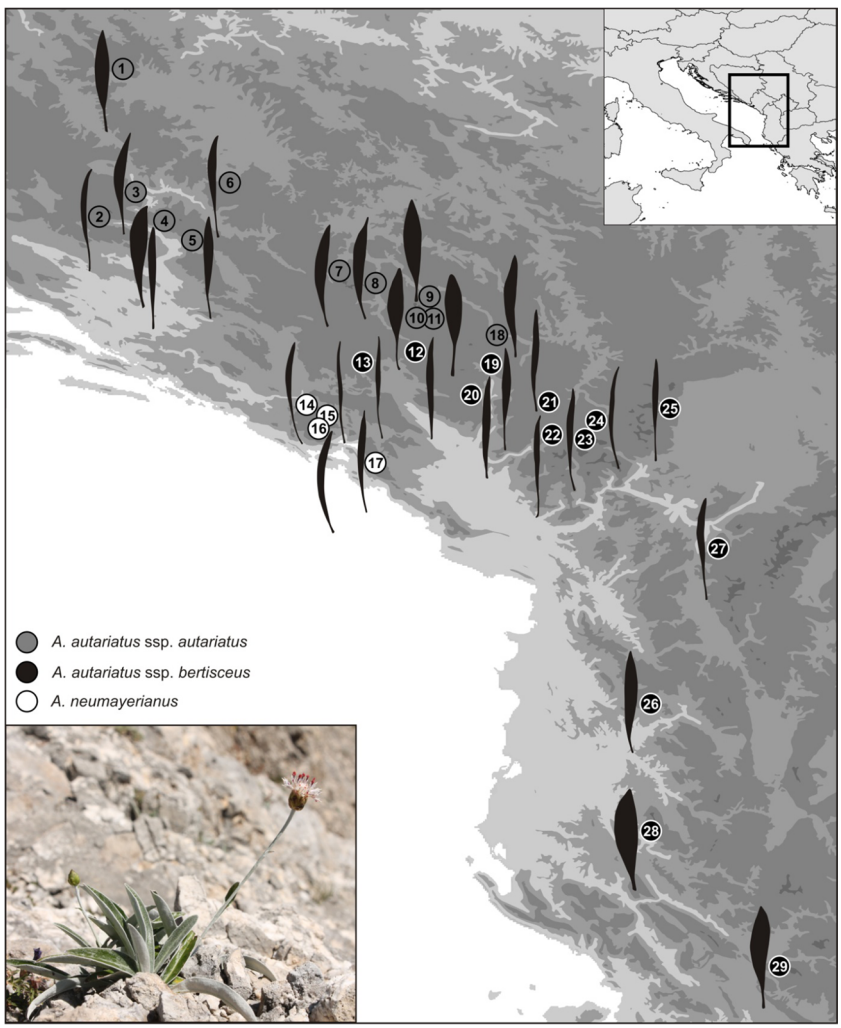

Fig. 1 Sampled populations of *Amphoricarpos* on the Balkan Peninsula. The *inserts* show the position of the sampled area in southeastern Europe and a plant from population 26. The taxonomic assignment follows Blečić and Mayer (1967)

Table 1 List of studied populations of *Amphoricarpus* on the Balkan Peninsula

Pop. ID	Taxon (identification based on distribution)	Taxon1	Taxon2	Taxon3	Country	Sampling locality	Altitude (m)	Lon.E/Lat.N	N_{AFLP}	Nei	Voucher number	Genbank (ITS; *rps16–trnK*)
1	*A. autariatus* subsp. *autariatus*	Ab	Ab	A	BH	Vlašić: Paklarske stijene	1756	17.62583/44.27611	3	0.050	BF & PS 13768 (WU)	KR704954; KR704926
2	*A. autariatus* subsp. *autariatus*	Aa	Aa	A	BH	between Čvrsnica and Čabulja: Bare	1460	17.51472/43.57027	3	0.060	FB 13960 (WU)	KR704965; KR704937
3	*A. autariatus* subsp. *autariatus*	Aa	Ab	A	BH	Čvrsnica: Doljanka valley	410	17.68833/43.68944	4	0.093	FB 13959 (WU)	KR704968; KR704940
4	*A. autariatus* subsp. *autariatus*	Aa	Ab	A	BH	Prenj: Tisovica valley	1740	17.82555/43.55111	4	0.079	FB 13897 (WU)	KR704966; KR704938
5	*A. autariatus* subsp. *autariatus*	Aa	Aa	A	BH	Prenj: Saddle Rujište	970	17.95916/43.45888	2	–	FB 13896 (WU)	KR704953; KR704925
6	*A. autariatus* subsp. *autariatus*	Aa	Ab	A	BH	Bjelašnica: Mala Hranisava	1770	18.13805/43.73555	4	0.076	FB 13898 (WU)	KR704967; KR704939
7	*A. autariatus* subsp. *autariatus*	Aa	Aa	A	BH	Sutjeska valley	673	18.66333/43.3150	5	0.106	BF & PS 13775 (WU)	KR704955; KR704927
8	*A. autariatus* subsp. *autariatus*	Ab	Aa	N	MNE	Piva Canyon: Mratinje	660	18.83638/43.25833	5	0.107	DC & DS 13974 (WU)	KR704958; KR704930
9	*A. autariatus* subsp. *autariatus*	Aa	Aa	N	MNE	Durmitor: Ćurevac, slopes directed to Tara Canyon	1560	19.09444/43.19416	5	0.105	DC & DS 13975 (WU)	KR704971; KR704943
10	*A. autariatus* subsp. *autariatus*	Ab	Aa	N	MNE	Durmitor: Lojanik, slopes directed towards the spring of river Komarnica	1620	19.03166/43.09194	5	0.068	DC & DS 13976 (WU)	KR704972; KR704944
11	*A. autariatus* subsp. *autariatus*	Aa	Aa	A	MNE	Komarnica Canyon: Klještina	1450	19.03555/43.09166	5	0.076	DC & DS 13973 (WU)	KR704969; KR704941
12	*A. autariatus* subsp. *bertisceus*	Ab	N	N	MNE	Vojnik: Mramorni peak	1780	19.02694/42.92944	4	0.098	DC & DS 13968 (WU)	KR704970; KR704942
13	*A. autariatus* subsp. *bertisceus*	A	Ab	A	MNE	Njegoš: between Veliki Kijac and Mali Kijac	1460	18.77527/42.87916	5	0.121	DC & DS 13977 (WU)	KR704979; KR704950
14	*A. neumayerianus*	Ab	N	N	BH	Bijela gora: W of Skočigrm	854	18.50777/42.67444	5	0.085	BF & PS 13779 (WU)	KR704962; KR704934
15	*A. neumayerianus*	N	Ab	N	MNE	Bijela gora: Lisac	1520	18.60638/42.62305	4	0.124	DC & DS 13967 (WU)	KR704977; KR704948
16	*A. neumayerianus*	A	N	A	MNE	Orjen: between Crkvice and Sedlo	1440	18.5650/42.56166	5	0.071	DC & DS 13966 (WU)	KR704978; KR704949
17	*A. neumayerianus*	N	Aa	A	MNE	Lovćen: Jezerski vrh	1659	18.83750/42.39972	5	0.118	BF & PS 13833 (WU)	KR704963; KR704935
18	*A. autariatus* subsp. *autariatus*	Aa	Aa	A	MNE	Tara Canyon: Crna Poda	830	19.41638/43.01111	5	0.125	DC & DS 13970 (WU)	KR704975; KR704946
19	*A. autariatus* subsp. *bertisceus*	A	Ab	A	MNE	Sinjajevina: Katunine	1440	19.38944/42.87333	5	0.112	DC & DS 13969 (WU)	KR704974; KR704945
20	*A. autariatus* subsp. *bertisceus*	Aa	Ab	A	MNE	Maganik: Medjedji peak	1680	19.29416/42.72444	4	0.116	DS 13971 (WU)	KR704976; KR704947
21	*A. autariatus* subsp. *bertisceus*	Ab	N	A	MNE	Komovi: NW slopes of Kom Vasojevićki	1900	19.6650/42.69277	4	0.132	PS 13835 (WU)	KR704957; KR704929
22	*A. autariatus* subsp. *bertisceus*	Aa	Ab	A	AL	Alpet Shqiptare (Prokletije): west of Gropat e Selces	1122	19.68166/42.53555	5	0.171	BF & PS 13790 (WU)	KR704960; KR704932
23	*A. autariatus* subsp. *bertisceus*	Ab	Ab	A	MNE	Prokletije: Ropojana valley	1024	19.83777/42.51444	4	0.098	BF & PS 13782 (WU)	KR704961; KR704933
24	*A. autariatus* subsp. *bertisceus*	N/Ab	Ab	N	MNE	Prokletije: SE slopes of Visitor	1988	19.89083/42.60305	4	0.092	BF & PS 13785 (WU)	KR704959; KR704931
25	*A. autariatus* subsp. *bertisceus*	Aa	Ab	N	KS	Rugovska klisura west of Peć	580	20.24333/42.66138	3	0.106	BF & PS 13795 (WU)	KR704964; KR704936
26	*A. autariatus* subsp. *bertisceus*	Ab	Aa	N	AL	Mali i Dejës: above village Macukull	1720	20.15611/41.25083	1	–	Z. Barina, D. Pifkó 15909 (BP)	KR704973; –
27	*A. autariatus* subsp. *bertisceus*	A	Ab	N	AL	Kukës: surroundings of Bicaj	900	20.43222/41.98416	4	0.154	BF & PS 13818 (WU)	KR704951; KR704923
28	*A. autariatus* subsp. *bertisceus*	Aa	Ab	A	AL	Skrapar: Mali i Tomorit	2233	20.15888/40.64416	4	0.143	BF & PS 13804 (WU)	KR704952; KR704924
29	*A. autariatus* subsp. *bertisceus*	Aa	Ab	A	GR	Ioanninon/Dodonis: Timfi	1293	20.7650/39.97583	5	0.089	BF & PS 13814 (WU)	KR704956; KR704928

Taxon1, identification based on the rosette leaf width; *Taxon2*, identification based on the ratio rosette leaf length/stem length; *Taxon3*, identification based on the width/length ratio of involucral bracts

A, *A. autariatus*; *Aa*, *A. autariatus* subsp. *autariatus*; *Ab*, *A. autariatus* subsp. *bertisceus*; *N*, *A. neumayerianus*. *BH* Bosnia and Herzegovina, *MNE* Montenegro, *AL* Albania, *KS* Kosovo, *GR* Greece. N_{AFLP}, number of individuals investigated with amplified fragment length polymorphism. *Nei*, Nei's (1987) gene diversity. Collectors: *BF* B. Frajman, *FB* F. Bogunić, *DC* D. Caković, *DS* D. Stešević, *PS* P. Schönswetter

(WU 080558, leg. Ehrendorfer et al. 1979; KR704921). The reaction mix (total volume 16 μl) contained 1.56 μl of 10× TaKaRa Buffer, 1.23 μl of TaKaRa dNTP Mixture (Takara Bio Inc.), 0.63 μl of both primers (10 μM) and 1 μl of template DNA. Cycling conditions were 5 min at 85 °C, 35 cycles of 30 s at 94 °C, 30 s at 52 °C and 4 min at 72 °C, followed by 10 min at 72 °C. The nuclear ribosomal internal transcribed spacer (ITS; Sun et al. 1994) was amplified and sequenced for one individual per population. The PCR was performed in a total volume of 16.5 μl, comprising 6 μl of RedTaq PCR Reaction Mix (Sigma-Aldrich), 0.7 μl of BSA (1 mg/ml; Promega), 0.4 μl of both primers 17SE and 26SE (10 μM; Sun et al. 1994) and 1 μl template DNA. PCR conditions were 5 min at 94 °C, 35 cycles of 30 s at 94 °C, 30 s at 56 °C and 1 min at 72 °C, followed by 10 min at 72 °C. All reactions were carried out in a MasterCycler Gradient thermocycler (Eppendorf).

The quality of the PCR products was checked on 1 % TBE-agarose gels. Subsequently, the amplification products were purified enzymatically using Exonuclease I and Shrimp Alkaline Phosphatase (SAP; Fermentas) according to the manufacturer's instructions. Cycle sequencing reactions were performed separately for each primer using BigDye Terminator chemistry (Applied Biosystems) according to the manufacturer's protocol. The same primers were used as for amplification, with addition of a newly designed primer Amph_rps16 (CAGGAAGGACGCTAAATATAA) for *rps16–trnK*. Electrophoresis was performed on an ABI 3130xl Genetic Analyzer capillary sequencer (Applied Biosystems). Geneious 5.5.6 (Biomatters) was used to assemble and edit the contigs and to align the sequences.

Analyses of sequence data

The alignment of the plastid region was analysed using statistical parsimony as implemented in TCS 1.21 (Clement et al. 2000) with the connection limit set to 95 %; gaps were treated as fifth character state. For this analysis, an indel longer than 1 bp was reduced to a single base pair column allowing this structural mutation to be counted as single base pair mutation only. Maximum parsimony (MP) analyses were performed for both, ITS and plastid markers using PAUP 4.0b10 (Swofford 2002). The most parsimonious trees were searched for heuristically with 1,000 replicates of random sequence addition, multrees on, TBR branch swapping, and treating characters as unordered and equally weighted. Sequences of close relatives were added to the alignment for rooting purposes, i.e., *Centaurea diffusa* Lam. (KJ690264), *Guizotia abyssinica* Cass. (EU549769) and *Lactuca sativa* L. (DQ383816) from GenBank and newly sequenced *A. exsul*, *D. pusilla* and *X. longepapposum* in the case of the plastid dataset and *A. exsul* O.Schwarz (AY826228), *Atractylodes lancea* DC. (DQ159944), *Chardinia orientalis* (L.) Kuntze (AY826260), *D. pusilla* Fisch. & C.A.Mey. (FJ813487) and *X. longepapposum* Fisch. & C.A.Mey. (AY826348) from GenBank in the case of ITS. Clade support was assessed via bootstrapping with 1,000 replicates using five random sequence addition replicates, TBR branch swapping, and MulTrees off. Bayesian analyses were performed employing MrBayes 3.2.1 (Ronquist et al. 2012) applying the GTR + Γ substitution model proposed by the Akaike information criterion implemented in MrAIC.pl 1.4 (Nylander 2004). Values for all parameters, such as the shape of the gamma distribution, were estimated during the analyses. The settings for the Metropolis-coupled Markov chain Monte Carlo (MC^3) process included four runs with four chains each (three heated ones using the default heating scheme), run simultaneously for 10,000,000 generations each, sampling trees every 1,000th generation using default priors. Posterior probabilities (PPs) were determined from the combined set of trees, discarding the first 1,001 trees of each run as burn-in.

Dating of the ITS data set was performed using BEAST ver. 1.6.2 (Drummond and Rambaut 2007). The trees were rooted with *D. pusilla*, forcing the ingroup to become a monophyletic sister group, based on previous analyses. The prior age of the root was set to 34.31 Ma, with a normally distributed standard deviation of 4, which corresponds to the age and the 95 % confidence intervals obtained by Barres et al. (2013). Analyses were performed with a Yule tree prior, GTR + Γ substitution model and a strict clock. Two independent MCMC chains were run for 30,000,000 generations with tree and parameter values saved every 3,000th generation. Tracer ver. 1.5 (Rambaut and Drummond 2009) was used to determine the degree of mixing, the shape of the probability density distributions, and 95 % credibility intervals for estimated divergence dates. Both the effective sample sizes and mixing were appropriate. FigTree 1.4 (Rambaut 2006) was used to display the maximum clade credibility tree after combining the tree files using LogCombiner and summarising the information using TreeAnnotator (both programs available in BEAST package).

AFLP fingerprinting

AFLP fingerprinting was performed for all 29 populations with usually five individuals per population (Table 1). AFLP profiles were generated following established protocols (Vos et al. 1995) with modifications described in Schönswetter et al. (2009). Two blanks (DNA replaced by water) were included to test for contamination, and 14 samples were used as replicates between PCR batches to test the reproducibility of AFLP fingerprinting. Based on an initial primer trial the following three selective primer combinations were chosen for selective PCR (fluorescent dye in brackets): *Eco*RI (6-FAM)ACA / *Mse*I-CAG, *Eco*RI (VIC)AGG / *Mse*I-CAT, and *Eco*RI (NED)AGC / *Mse*I-CAG (6-FAM-labelled primers: Sigma-Aldrich; NED- and VIC-labelled primers:

Applied Biosystems). The selective PCR mix for the VIC and 6-FAM labelled primers contained 1 μl 10×RedTaq PCR reaction buffer (Sigma-Aldrich), 0.25 RedTaq (Sigma-Aldrich), 0.22 μl dNTPs (10 mM; Applied Biosystems), 0.54 μl of each selective primer (*Mse*I-primer, 5 μM; *Eco*RI-primer, 1 μM, Sigma-Aldrich) and 2 μl of the diluted preselective amplification product. The reaction mix for the NED-labelled primer contained 0.4 U RedTaq. The selective PCR product was purified using Sephadex G-50 Fine (GE Healthcare Bio-Sciences, Uppsala, Sweden) applied to a MultiScreen-HV plate (Millipore, Molsheim, France) in three steps of 200 μl each and packed at 600 g for 1, 1 and 5 min, respectively. Then 1.2 μl of the elution product was mixed with 10 μl formamide (Applied Biosystems) and 0.1 μl GeneScan 500 ROX (Applied Biosystems) and run on an ABI 3130 automated capillary sequencer.

Analyses of AFLP data

Electropherograms were analysed with Peak Scanner version 1.0 (Applied Biosystems) using default peak detection parameters except employing light peak smoothing. The minimum fluorescent threshold was set to 50 relative fluorescence units (RFUs). Automated binning and scoring of the AFLP fragments were performed using RawGeno 2.0-1 (Arrigo et al. 2009) for R 2.15.0 (R Development Core Team 2011) with the following settings: scoring range=50–500 bp, minimum intensity=50 RFUs, minimum bin width=1 bp, and maximum bin width=1.5 bp. Fragments with a reproducibility lower than 80 % based on sample-replicate comparisons were eliminated. The error rate (Bonin et al. 2004) was calculated as the ratio of mismatches (scoring 1 versus 0) over phenotypic comparisons in AFLP profiles of replicated individuals. Fragments present/absent in only one individual were excluded.

Nei's (1987) gene diversity index was calculated for each population with at least three investigated individuals using the R script AFLPdat (Ehrich 2006). A Neighbor-Joining (NJ) analysis based on Nei-Li genetic distances (Nei and Li 1979) was conducted and bootstrapped (1000 pseudo-replicates) with TREECON v.1.3b (van de Peer and De Wachter 1997). SplitsTree4 12.6 (Huson and Bryant 2006) was used to produce a Neighbor-Net diagram based on uncorrected P-distances. A principal coordinate analysis (PCoA) based on a matrix of Jaccard distances among individuals was calculated using FAMD (Schluter and Harris 2006).

We identified genetically homogeneous groups employing the Bayesian multilocus assignment method implemented in BAPS 5.2 (Bayesian Analysis of Population Structure; Corander et al. 2003) using the "Clustering with linked loci" option, which is particularly suited for dominant markers such as AFLPs (Corander and Tang 2007) for the mixture analysis and the default settings for admixture analysis. BAPS uses stochastic optimization (Corander et al. 2006), treats K (the appropriate number of groups) as a variable to estimate and gives a list of the best partitioning and their likelihood scores. Individuals were used as clustering units, and values of K (the maximum number of clusters) in the range 2–29 (i.e., the number of investigated populations; BAPS does not allow exploration of $K=1$; Corander and Tang 2007) were explored using ten replicates for each value of K.

Morphometric analyses

Material for morphometric analyses included vouchers of molecularly investigated populations (except for population 28; Table 1), supplemented with herbarium vouchers stored in SA (43236–43238, 43240–43242, 43245–43249, 43252–43254, 43256–43266, 48542) and IB (Schönswetter and Frajman 13996, 13998), totalling 90 individuals. Thirty characters were selected, most of which were used previously for taxon recognition—17 metric, 7 qualitative and 6 ratios (Table 2). Leaf characters were measured on one leaf from the middle of the rosette and on the lowest stem leaf. Involucral bracts were taken from the third row. Since the outer achenes differ from the inner ones, fruit characters were measured on both. In some plants, achenes were missing; missing character values were replaced with mean values. Characters of involucral bracts and fruits were measured on magnified images taken with an Olympus UC 30 wide zoom camera on an Olympus SZX9 binocular.

All qualitative characters (2, 5, 9, 16, 28, 29 and 30 in Table 2) were invariable and were excluded from further analysis. Correlation among metric characters was tested employing Pearson or Spearman correlation coefficients dependent on character distribution. After standardization to zero mean and one unit variance multivariate statistics were performed. Morphological separation of three and five predefined groups based on current taxonomy ("autariatus", "bertisceus", "neumayerianus"; Blečić and Mayer 1967) and the results of genetic admixture analysis with BAPS, respectively, was tested using canonical discriminant analysis (CDA) in order to clarify the relative importance of characters as discriminators between groups and the relative positions of the individuals of those groups (Manly 1986). Herbarium material not included in the molecular analyses was assigned to the BAPS groups based on the geographic proximity of the samples analysed genetically. All samples from the herbarium SA were from the western parts of Bosnia and Herzegovina and were thus included in the northernmost BAPS group in order to explore whether the deepest genetic split is also reflected in morphology. Two specimens from the Albanian Alps stored in the herbarium IB were included in the BAPS group constituted by populations 21–27 from the same area. Principal component analysis was applied to display the overall variation pattern along the first three components, based on

Table 2 Morphological characters studied in *Amphoricarpos* from the Balkan Peninsula

	Character	Abbreviation
1	Inner achene length, mm	IAL
2	Inner achene indumentum: densely hairy (1); sparsely hairy (2)	IAI
3	Inner achene pappus, mm	IAP
4	Outer achene length, mm	OAL
5	Outer achene indumentum: densely hairy (1); sparsely hairy (2)	OAI
6	Outer achene pappus, mm	OAP
7	Outer achene wing width, mm	OAW
8	Outer achene auricles length, mm	OAA
9	Outer achene ribs: no ribs (0); with ribs (1)	OAR
10	Involucral bract width, mm	ILW
11	Involucral bract length, mm	ILL
12	Ratio of width and length of involucral bract	ILW/ILL
13	Ratio of distance from the basis to widest part of involucral bract and its length	ILMW/ILL
14	Involucral bract width of the scarious margin, mm	ILSM
15	Ratio of width of the scarious margin and total width of involucral bract	ILSM/ILW
16	Involucral bract apex: obtuse (0); acute (1)	ILA
17	Involucral bract mucro length, mm	ILML
18	Distance of insertion of mucro from tip of involucral bract, mm	MP
19	Rosette leaf width, mm	RLW
20	Rosette leaf length, mm	RLL
21	Ratio of width and length of rosette leaf	RLW/RLL
22	Ratio of distance from the basis to widest part of rosette leaf and its length	RLMW/RLL
23	Width of revolute margin of rosette leaf, mm	RMRL
24	Rosette leaf indumentum on upper surface: glabrous to sparse (0); sparse (1)	RLIA
25	Stem leaf width, mm	SLW
26	Stem leaf length, mm	SLL
27	Ratio of width and length of stem leaf	SLW/SLL
28	Stem leaf apex: acute (0); acuminate (1)	SLA
29	Stem leaf indumentum on upper surface: glabrous to sparse (0); sparse (1)	SLIA
30	Plant height (mm)	PH

the same dataset. Tukey's HSD post hoc test was performed for all variable characters to evaluate differences among the three and five groups described above. Statistical analyses were performed with Statistica (version 5.1.; StatSoft 1996).

Results

ITS and plastid DNA sequences

The number of terminals, included characters, parsimony informative characters, percentage of parsimony informative characters, number and lengths of MP trees, consistency and retention indices for both DNA regions, as well as the model of evolution proposed by MrAIC and used in MrBayes and BEAST analyses are presented in Table 3.

The ITS sequences of Balkan *Amphoricarpos* were 793–794 bp long (GenBank accession numbers KR704951–KR704979) and included seven variable sites, of which four were parsimony informative. Relationships inferred by maximum parsimony and Bayesian analyses were congruent (Fig. 2). All Balkan populations formed a monophyletic group (BS 100 %, PP 1), sister to *A. exsul* (BS 99 %, PP 0.99). The relationships among the Balkan populations followed geography rather than taxonomy: six westernmost populations (1–6) formed a clade (BS 87 %, PP 1) sister to all other accessions (BS 63 %, PP 0.94); within the latter, the southernmost populations 28 from Albania and 29 from Greece were divergent from all other populations, which formed a clade with low support (BS 63 %, PP 0.83). According to the dating analyses (Fig. 2) the Balkan populations diverged from the Anatolian *A. exsul* about 4.9 Ma (2.7–7.6 Ma, 95 % highest posterior densities, HPDs), whereas the deep split between the Bosnian populations 1–6 and all other populations occurred about 1.7 Ma (0.8–2.7 Ma, 95 % HPDs) and the southernmost populations diverged about 1.2 Ma (0.5–1.8 Ma, 95 % HPDs).

Table 3 Matrix and phylogenetic analyses statistics for the two DNA regions analysed as well as substitution models proposed by MrAIC and used in the Bayesian analyses

	ITS	*rps16–trnK*
Number of terminals	34	34
Number of included characters	798	1989
Number / percentage of parsimony informative characters (within the ingroup)	74 (4) / 9.3 % (0.9 %)	54 (5) / 2.7 % (0.25 %)
Length / number of MP trees	275 / 9366	278 / 1
Consistency index (CI; excluding uninformative characters)	0.88 (0.73)	0.94 (0.77)
Retention index (RI)	0.77	0.78
Substitution model	GTR + Γ	GTR + Γ

The *rps16–trnK* sequences of Balkan *Amphoricarpos* were 1,814–1,835 bp long (GenBank accession numbers KR704923–KR704950) and included eight variable sites, of which five were parsimony informative. We failed to amplify this region for population 26 most likely due to a mutation in the priming site. Relationships inferred by maximum parsimony and Bayesian analyses were congruent (Fig. 3). All Balkan populations formed a monophyletic group (BS 63 %, PP 0.91), sister to *A. exsul* (BS 98 %, PP 1). Sixteen individuals possessed the same central haplotype, from which haplotypes from the three southern populations 27–29 were separated by one or two mutational steps. Nine populations formed a clade with low support (BS 65 %, PP 0.96), based on one substitution; two individuals of *A. neumayerianus* from Bijela gora (populations 14 and 16) were separated by four mutational steps and formed a highly supported subclade (BS 96 %, PP 1).

AFLP data

We scored 492 fragments in 122 individuals; 60 bands were found in only one individual and one fragment was monomorphic. These latter fragments were excluded from further analyses. The error rate before the exclusion of non-reproducible fragments was 3.5 %.

Nei's gene diversity index varied from 0.049 in population 1 to 0.171 in population 22 (Table 1). Low-altitude populations sampled at <1,000 m a.s.l. were not characterised by lower gene diversity than populations from higher altitudes (*t* test, $P=0.339$). The NJ analysis (not shown) identified several clusters with high bootstrap support, which were also supported by strongly weighted splits identified by the NeighborNet analysis (Fig. 4). The most divergent cluster with maximum bootstrap support contained all populations of *A. autariatus* subsp. *autariatus* from western Bosnia and Herzegovina (populations 1–6). Another cluster (BS 97 %) comprised the southernmost populations of *A. autariatus* subsp. *bertisceus* from Albania (28) and Greece (29), which were both genetically divergent (each supported by BS 100 %). The largest cluster (BS 86 %) included *A. autariatus* subsp. *autariatus* (7–11, 18) from south-eastern Bosnia and Herzegovina and Montenegro, and one population of *A. autariatus* subsp. *bertisceus* (12) from Montenegro. The relationships among other populations were not resolved. Populations of *A. neumayerianus* (14–17) shared several unique splits in the NeighborNet analysis, but received no bootstrap support.

Model-based admixture clustering with BAPS resulted in an optimal partition (marginal likelihood=−15,913) with five clusters, which closely matched the results of the distance-based analyses (Fig. 5a). Most populations were assigned to a single cluster, only population 22 was strongly admixed.

The principal coordinate analysis (Fig. 5b–e) revealed good congruence with the five groups identified by BAPS, while there were major discrepancies with the taxonomic assignment following Blečić and Mayer (1967).

Morphometry

As correlation coefficients did not exceed 0.9 for any character pair, all characters were retained for further analyses. The PCA (first two axes cumulatively explaining 28.2 % of the total variation) did neither reflect current taxonomy (Fig. 6a) nor the five groups identified by BAPS analysis of the AFLP data (Fig. 6c). The characters with highest loading are the leaf characters RLW/RLL, RMRL, RLW, SLL (first axis) and the pappus and achene characters IAP, OAP, IAL and OAL (second axis). The CDA (Fig. 6b, d) showed strong overlap of the above-mentioned three or five groups, the sole exception being the northernmost populations 1–6, which were somewhat separated (middle grey dots in Fig. 6d). In the CDA for three groups (Fig. 6b), the involucrum and leaf characters ILSM, ILMW/ILW, SLL and RLW contributed most to the discrimination along the first axis, and the involucrum, pappus and achene characters ILL, ILW/ILL, ILW and OAL contributed most to the second axis. In the CDA for five groups (Fig. 6d) these characters were RLW, ILW, SLL, OAP for the first axis and ILSM, ILMW/ILW, ILW and RLW for the second axis. Boxplots of characters with high discriminatory power among the groups (RLW, RMRL, SLL, RLW/RLL, ILMW/ILL,

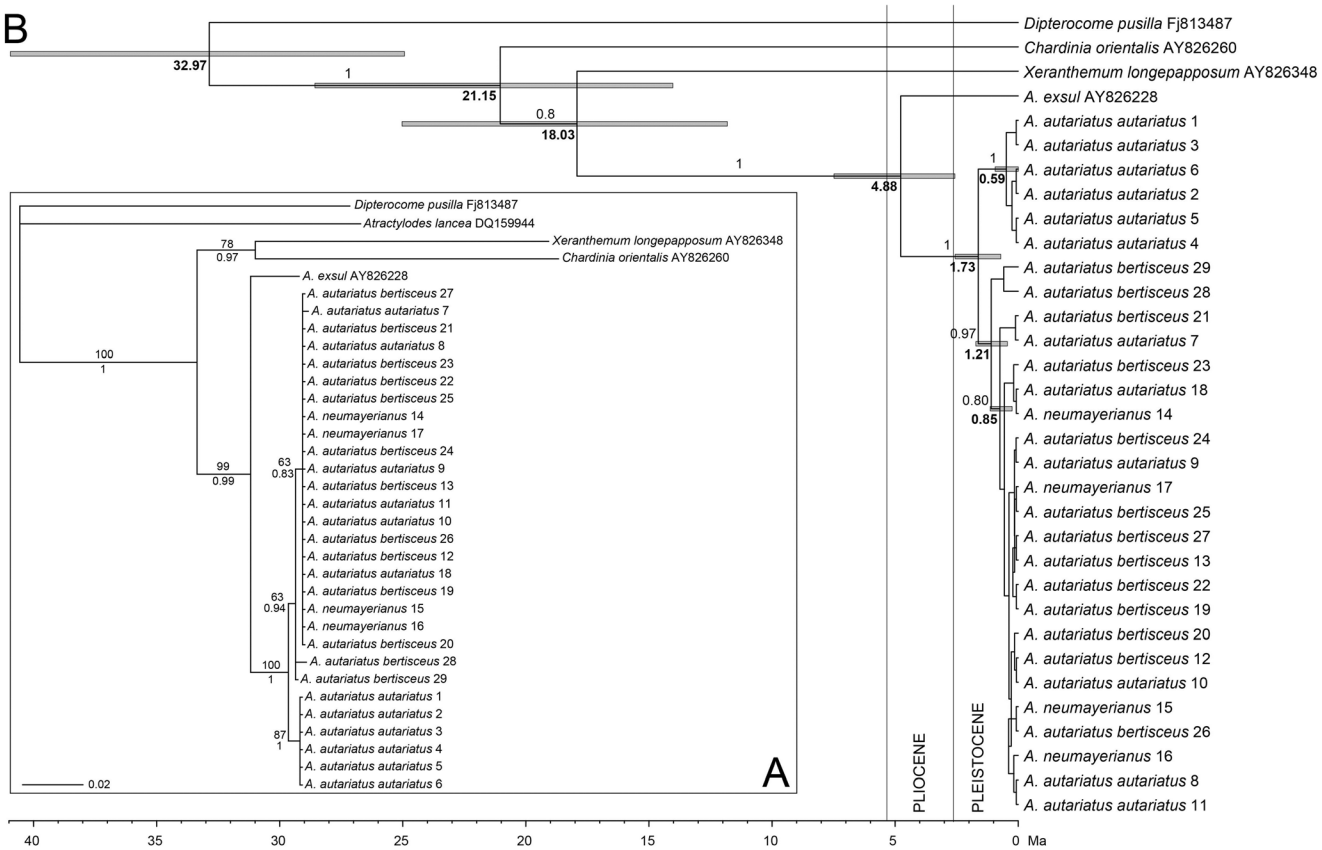

Fig. 2 Relationships of *Amphoricarpos* from the Balkan Peninsula inferred from phylogenetic analyses of Internal Transcribed Spacer (*ITS*) sequences. **a** Bayesian consensus phylogram; numbers above branches are bootstrap values >50 %, those below branches *PP* values >0.50. **b** Bayesian consensus chronogram (obtained with BEAST); numbers above branches are *PP* values >0.50, *numbers associated with nodes* indicate the mean crown group age in millions of years of the clade diversifying at that node and the *bars* correspond to the 95 % highest posterior densities of the age estimates

SLW) revealed by Tukey's HSD post hoc test ($p<0.001$) are shown in the Supplementary Fig. 1.

Discussion

Spatiotemporal diversification of Balkan *Amphoricarpos*

The split between the Balkan populations of *Amphoricarpos* and the Anatolian *A. exsul* took place about 4.88 Ma, which is largely congruent with the estimate of Barres et al. (2013). In both studies broad HPDs were inferred, but it is likely that the split happened in the late Miocene or early Pliocene, which coincides with deepening and extension of the South Aegean Basin between the Balkan Peninsula and Asia Minor after the end of the Messinian salinity crisis 5.34 Ma (Meulenkamp and Sissingh 2003). We are aware that the inclusion of the Anatolian *A. praedictus* and the Caucasian *A. elegans* might change our age estimates and thus suggest viewing them with appropriate caution. Tertiary origin of Balkan *Amphoricarpos* contrasts with diversification within Balkan *Atocion compactum* and *Wulfenia*, whose divergence from Anatolian relatives was dated to the Pleistocene (Frajman et al. 2009b; Surina et al. 2014). Acknowledging the contribution of the Asian flora to the species richness of the Balkan Peninsula (Turrill 1929; Stevanović 1996; Nieto Feliner 2014), additional studies are needed to explore temporal diversification patterns in other plants with similar distribution. Several species are common to the southern Balkans and Asia Minor, of which some, such as *Anemone blanda*, *Atocion compactum*, *Euphorbia myrsinites*, *Juniperus excelsa*, *J. foetidissima* and *Ranunculus marginatus*, extend their distribution to the western Balkans on the one, and the Caucasus on the other hand (Turrill 1929; Stevanović 1996), thus exhibiting the same distribution pattern as *Amphoricarpos*.

Diversification within the Balkan lineage is much younger and likely started in the Pleistocene, following the climatic changes which led to migrations of biota, formation of local and regional (environmental) barriers and local extinctions. The deep genetic split within Balkan *Amphoricarpos* suggested by ITS (Fig. 2) and AFLPs (Figs. 4 and 5) is dated to 1.7 Ma and geographically coincides with a distribution gap roughly situated in the area between the rivers Neretva and Sutjeska in Bosnia and Herzegovina (Niketić et al. 2014; situated roughly between populations 6 and 7 in Fig. 1).

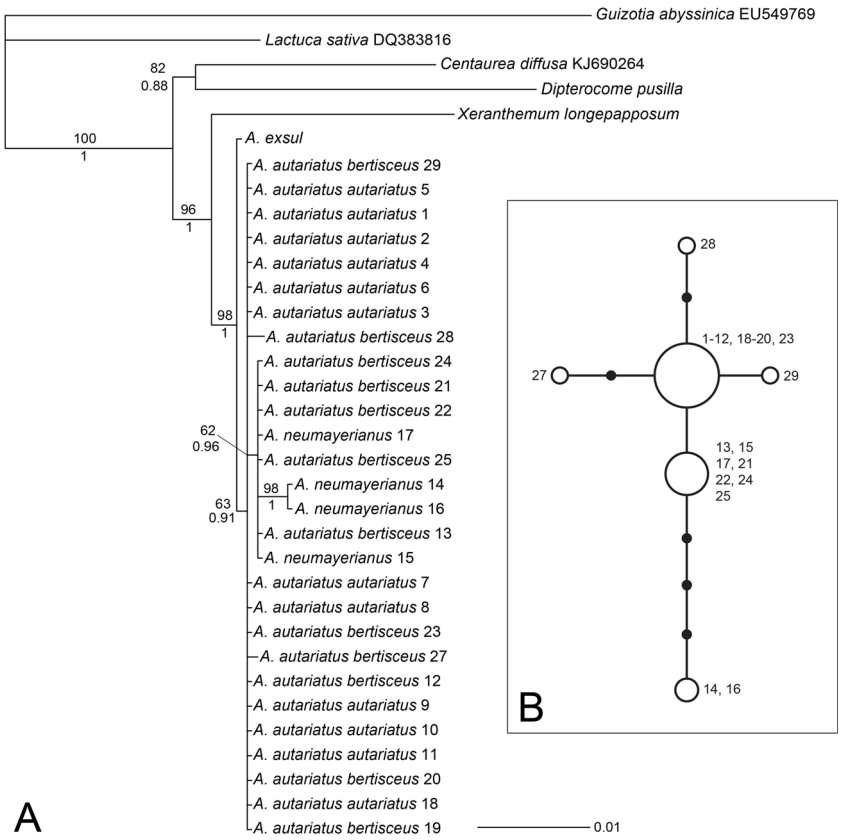

Fig. 3 Relationships of *Amphoricarpos* from the Balkan Peninsula inferred from phylogenetic analyses of plastid *rps16–trnK* sequences. **a**, Bayesian consensus phylogram; numbers above branches are bootstrap values >50 %, those below branches *PP* values >0.50. **b**, Statistical parsimony network. *Small black dots* represent unsampled haplotypes, numbers are population identifiers as in Fig. 1 and Table 1

Interestingly, similar genetic breaks coinciding with the Neretva river valley have been observed in several plant and animal taxa (see Kutnjak et al. 2014 for the latest review), whereas in the *H. pusillum* group the genetic split in plastid data dated to the Pliocene matches the Sutjeska and Drina rivers in easternmost Bosnia and Herzegovina (Frajman and Oxelman 2007). Even if the time of divergence was not identified in most previous studies, it is likely that its onset was in the Pleistocene, when the northern shore of the Adriatic Sea oscillated in north–south direction and significantly influenced the environmental conditions and thus the distribution of species (Lakušić et al. 2013; Kutnjak et al. 2014).

Similarly as in *Edraianthus graminifolius* (Surina et al. 2014) populations in the south of the distribution area of Balkan *Amphoricarpos* are highly disjunct (Niketić et al. 2014). The genetic divergence of the southernmost populations 28 and 29 of *Amphoricarpos* from Albania and northern Greece is likely caused by both, geographic isolation preventing contemporary gene flow, and long-term in situ persistence (Surina et al. 2014).

Genetic structure within Balkan *Amphoricarpos* does not reflect taxonomy

Results of our genetic analyses do not corroborate any of the previous taxonomic treatments—two species, one of them with two subspecies (Blečić and Mayer 1967) or, alternatively, one species with two or three subspecies (e.g. Schwarz 1970; Webb 1976; Strid and Tan 1991)—but rather suggests a more complex structure (Figs. 2, 3, 4, 5 and 6). In addition to the separation of the northwestern populations 1–6 and the southernmost populations 28–29, BAPS analysis of AFLP data (Figs. 4 and 5) revealed three geographically correlated genetic clusters in southeastern Bosnia, Montenegro and northern Albania (populations 7–27; Fig. 1). This pattern, which is not contradicted by the unresolved ITS relationships, is likely a result of relatively recent divergence, probably dating to the Pleistocene (Fig. 2). The area is topographically highly complex, with mountain ranges separated by deep valleys, which could have served as refugia for *Amphoricarpos* during Pleistocene glacials; several gorges still harbour low-altitude populations of *Amphoricarpos* on cliffs with low competitive pressure (personal observation).

The northern of the three BAPS clusters included six populations of *A. autariatus* subsp. *autariatus* (7–11, 18) and one population of *A. autariatus* subsp. *bertisceus* (12); it was identical to a clade with 86 % BS in the NJ tree, visible also in the NeighborNet (Fig. 4). Geographically, this cluster covers the catchment area of Drina, which ultimately drains into the Black Sea. Populations of *A. neumayerianus* (14–17) shared several unique splits in the NeighborNet analysis (Fig. 4) but received no bootstrap support and in the BAPS analysis they

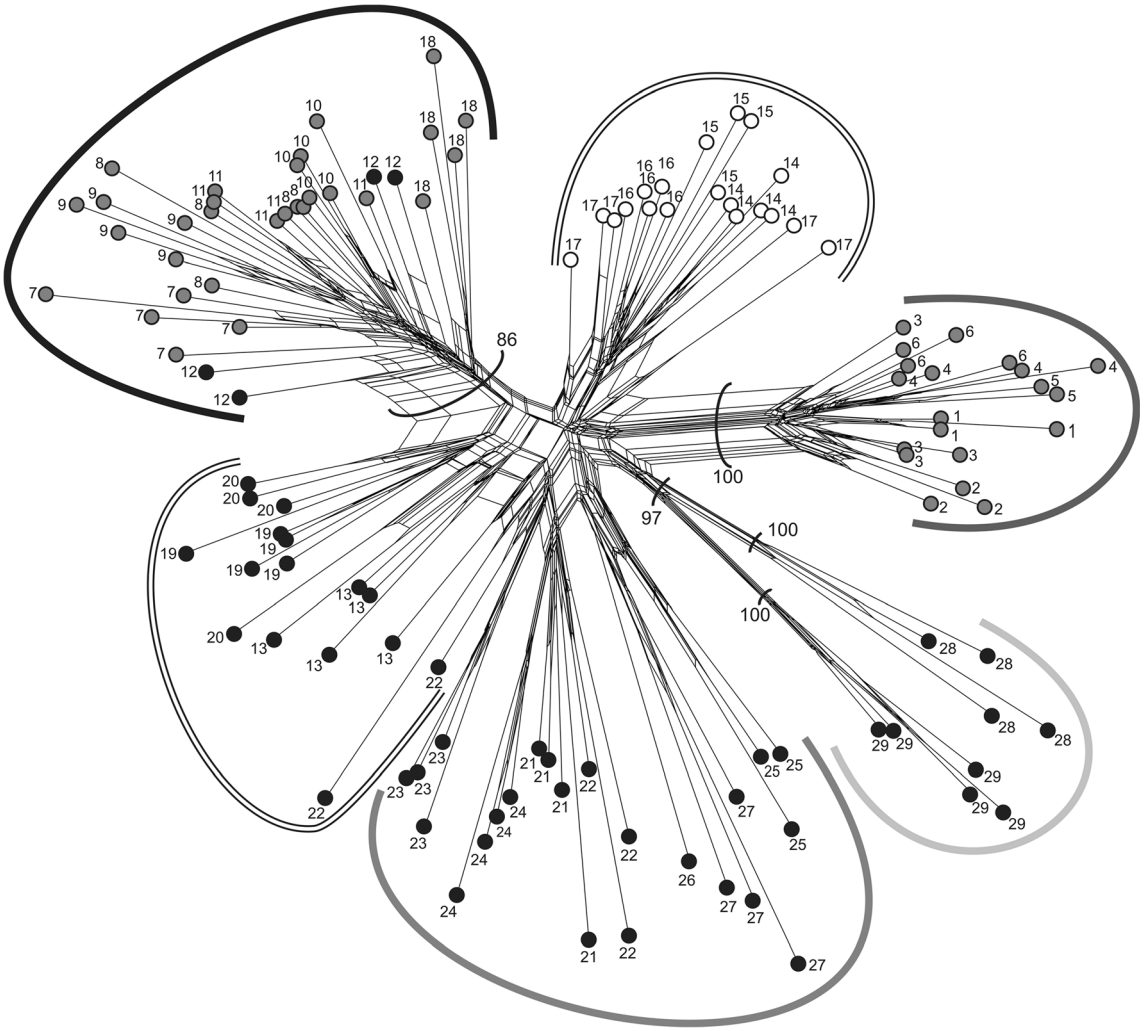

Fig. 4 NeighborNet diagram based on uncorrected P distances derived from AFLP data of *Amphoricarpos* from the Balkan Peninsula. *Numbers* positioned along the splits are bootstrap values derived from Neighbour-joining analysis (1,000 replicates). Populations are coded as in Fig. 1 and Table 1. Results of the BAPS analysis from Fig. 5a are included (*black*, *dark grey*, *middle grey*, *faint grey* or *white lines*) to allow comparison of distance-based and model-based analyses

clustered with three populations (13, 19, 20) of *A. autariatus* subsp. *bertisceus*. This group is geographically more complex but covers only areas draining ultimately into the Adriatic Sea. All other populations of *A. autariatus* subsp. *bertisceus* from Montenegro and Albania (21–27) formed a third cluster and shared several splits with other populations of *A. autariatus* subsp. *bertisceus* (Fig. 4). These populations inhabit the Albanian Alps (Prokletije / Alpet Shqiptare), Maja e Gjalices and Mali i Dejës. The valleys surrounding the Albanian Alps still host *Amphoricarpos* populations at low altitudes; the interpretation of such populations as remnants of glacial refugia is, however, not supported by genetic diversity estimates that showed no elevated values as compared to populations from higher altitudes. The present study thus reveals divergence in multiple Pleistocene microrefugia also in Balkan *Amphoricarpos* and thus provides additional support to the 'refugia-within-refugia hypothesis' (Gómez and Lunt 2007; for the Balkans: Surina et al. 2011; Kutnjak et al. 2014, Nieto Feliner 2014).

Contrary to the largely congruent structure revealed by ITS and AFLPs, plastid DNA variation was not clearly geographically correlated. Sequence variability was low and several regions (>5,000 bp) tested for five individuals spanning the entire geographic area were invariable. Sequences of the only variable region, *rps16–trnK*, were identical for most populations. Only some populations from the central part of the distribution pertaining to *A. neumayerianus* and *A. autariatus* subsp. *bertisceus* were divergent and formed a clade supported by a single substitution (Fig. 3). In addition, the three disjunct southernmost populations 27–29 exhibited divergent haplotypes separated by one or two mutations from the central haplotype. Small isolated populations are more susceptible to stochastic events (faster spread and fixation, but also faster elimination of new mutations) as compared to larger populations

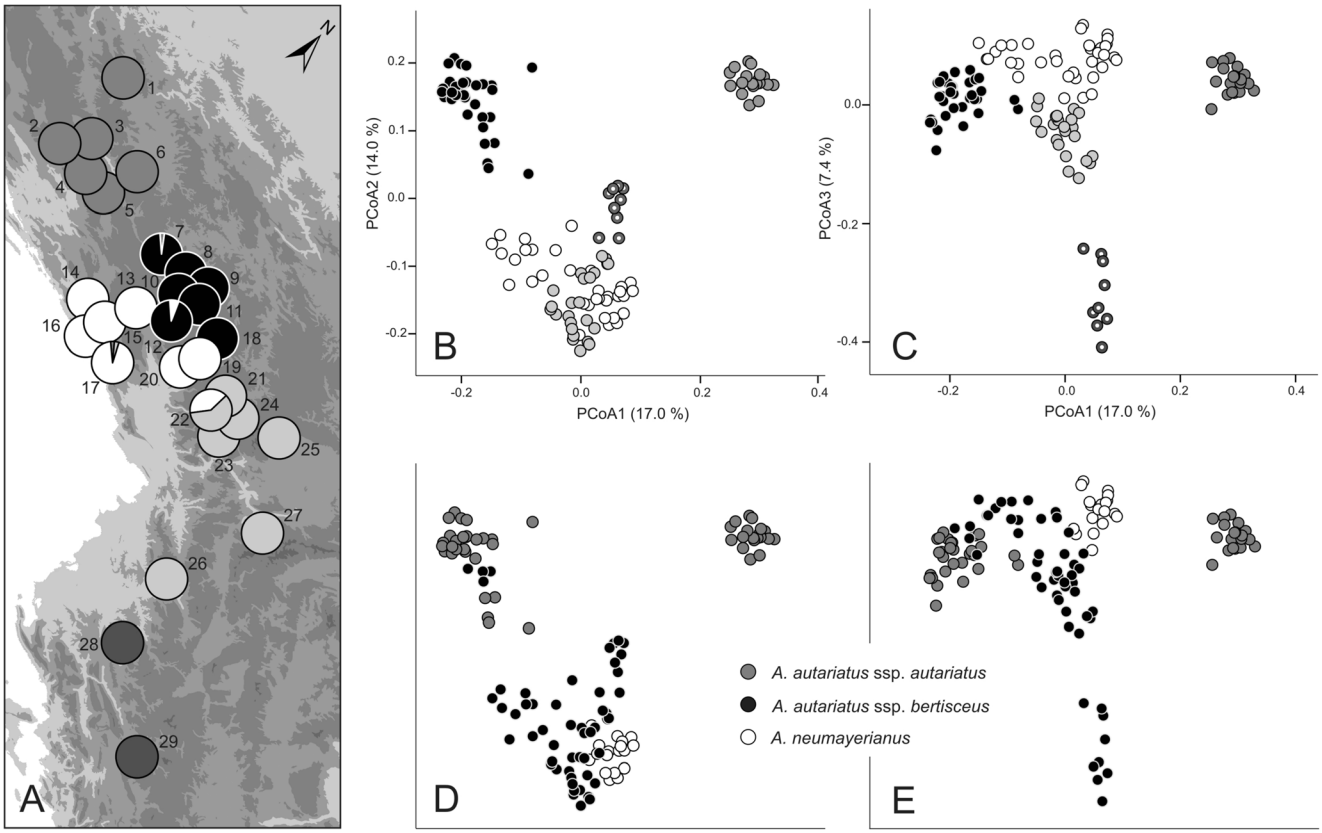

Fig. 5 AFLP variation in *Amphoricarpos* on the Balkan Peninsula. **a**, Bayesian clustering with the software BAPS resulted in five clusters, whose distributions are shown averaged for populations. **b**–**e**, scatterplots of the first three factors extracted by a principal coordinate analysis based on Jaccard distances. **b**–**c**, coding follows the BAPS analysis shown in **a**. The only exception is the southern-most cluster (populations 28–29), which is illustrated with white-centred *dark grey dots* to increase legibility. Admixed individuals were assigned to the predominant cluster. **d**–**e**, ordination as in **b** and **c**, respectively, but coding follows Blečić and Mayer (1967)

interconnected by gene flow (Freeland et al. 2011; Masel 2011). This might, in combination with the uniparental mode of plastid inheritance and the thus smaller effective population size of the plastid genome, explain the observed pattern. The divergence of populations 14 and 16 from the centre of the distribution area is more difficult to explain, as the species is relatively common there, but incongruences between nuclear and plastid phylogenies are frequent in shallow phylogenies (e.g. Frajman et al. 2009a; Kučera et al. 2010; Kutnjak et al. 2014).

How many taxa of *Amphoricarpos* are there on the Balkan Peninsula?

Incongruence between our phylogenetic data and previous taxonomic treatments of Balkan *Amphoricarpos* is evident. Important diagnostic characters in the three-taxon concept of Blečić and Mayer (1967), and the two-taxon concept of Webb (1976) were the width of the basal leaves, shape of the leaf apex, leaf margin (revolute or not), shape of involucral bracts, length of their mucro, width of their wings, shape of auricles and indumentum of outer achenes. From these, shape of the leaf apex and the length of the mucro on involucral bracts, along with other five qualitative characters were invariable. Principal component analysis (Fig. 6a, c) showed that neither the three taxa of Blečić and Mayer (1967) nor the five BAPS clusters are morphologically distinct. Moreover, the shape of the leaves, which was considered the most important diagnostic character, varied considerably across taxa, but also within populations (Fig. 1: population 4). The application of the most important diagnostic characters (width of the rosette leaves, length of the rosette leaves compared to the plant height, width/length ratio of the involucral bracts) listed by Blečić and Mayer (1967) results in inconsistent determinations, which also differ from the authors' geography-correlated classification (Table 1).

In canonical discriminant analyses with the same three or five predefined groupings as above the groups still showed large overlaps (Fig. 6b, d). In both groupings, a similar set of characters, which were drawn from involucral bracts, rosette and stem leaves, achenes and pappus, contributed to the separation. However, post hoc tests did not reveal significant differences among the three or five groups for most of these characters. Only in five morphological characters one of the

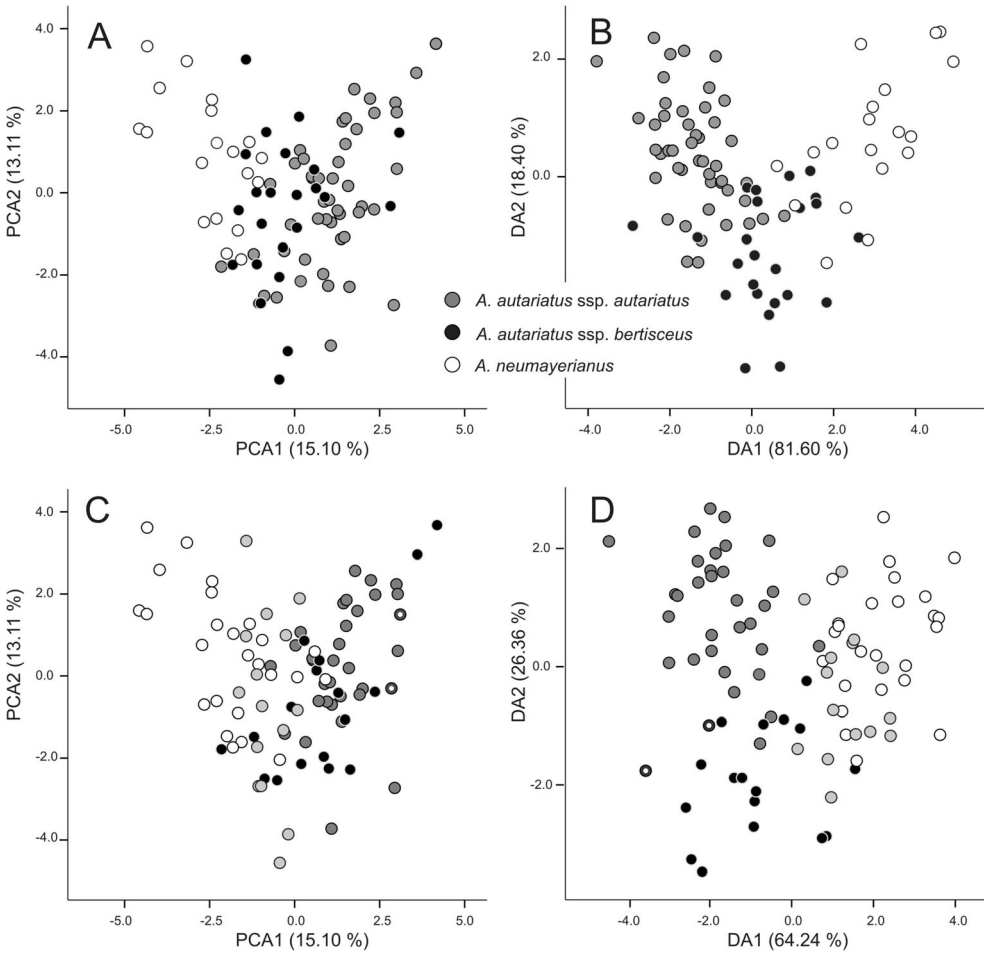

Fig. 6 Morphological variation in *Amphoricarpos* on the Balkan Peninsula based on 17 metric characters and six ratios. **a**, **c** Principal component analysis. **b**, **d** Canonical discriminant analyses. Labelling and grouping in **a** and **b** follow Blečić and Mayer (1967), in **c** and **d** they reflect the five BAPS clusters shown in Fig. 5b–e

three taxa was significantly different from at least one other taxon, but overlaps were strong in all cases (Supplementary Fig. 1). *Amphoricarpos neumayerianus* significantly differed from *A. autariatus* only in the width of the revolute margin of rosette leaves, but almost 50 % of the values overlapped. *A. autariatus* subsp. *autariatus* also had significantly broader rosette leaves than both other taxa, but again the overlap was considerable.

Altogether, both our genetic data as well as the results of the morphological analyses clearly show that the current taxonomy is highly artificial. The genetic structure allows for various groupings (two groups, five groups), which, however, cannot be morphologically discriminated. The northernmost populations, which were phylogenetically most distinct, differed from two BAPS groups in three characters whereas they were indistinguishable from two other BAPS groups. Also in this case, the overlap of the characters among the groups was high and we therefore refrain from recognising any of the genetic groups as a distinct taxonomic entity. Based on our data, we rather suggest treating all Balkan populations as a single, genetically, morphologically and ecologically variable species, *A. neumayerianus* without intraspecific taxa.

Taxonomic treatment

A. neumayerianus (Vis.) Greuter in Willdenowia 33: 51. 2003≡*J. neumayeriana* Vis., Fl. Dalmat.: t. 10, f. 2. 1842≡*Amphoricarpos neumayeri* (Vis.) Vis., nom. illeg., in Giorn. Bot. Ital. 1: 196. 1844.—Type: "Ex Monte Orien / Dalmazia" / Comm. Visiani, ex Herb. J. Ball, F. R. S., August, 1890 (K00768965!), neotype designated here.

Note: We traced two herbarium specimens collected on Mt. Orjen in Montenegro with Visiani's handwriting; one is cited above, and the other is deposited at W (293684!). It is not clear when they were collected and if they were part of the original material. However, as they are not labeled as *J. neumayeriana*, we assume that they were collected later. We thus designate the former, which is better preserved, as a neotype. Another option would be to designate the illustration of *J. neumayeriana* in Flora Dalmatica (Visiani 1842) as a lectotype,but also in this case it is not clear if it relates to the original material (see Ross 2002).

= *A. neumayeri* var. *velezensis* Murbeck in Lunds Univ. Årsskr. 27: 100. 1892.—Type: „Bosnia and Hercegovina, Hercegovina, in abruptis montis Velez, ca. 1,800 m, leg.

Murbeck. S. (LD1081780!; http://plants.jstor.org/specimen/ld1081780?s=t), lectotype designated here. Syntypes deposited at LD, S and W.
= *A. neumayeri* f. *latifolius* Beck, Jahreskat.Wiener bot.Tauschverein: 20. 1894.—Type: "Flora Bosniaca, Travnik, loc. Smahidins Kok [Smajin Kuk], leg. E. Brandis, Spt. 1893". (PRC!; lectotype, designated here).
= *A. neumayeri* subsp. *murbeckii* Bošnjak in Glasn. Hrvatsk. Prir. Društva 41–48: 62–63. 1936≡ *A. neumayeri* var. *murbeckii* (Bošnjak) Fukarek in Glasn. Zem. Muz. Saraj. 3–4: 161. 1965. Type: not designated (specimen not in ZA!).
= *A. neumayeri* var. *intermedia* Fukarek, nom. nud., in Glasn. Zem. Muz. Saraj. 3–4: 161. 1965.
= *A. autariatus* Blečić & E.Mayer subsp. *autariatus* in Phyton (Horn) 12: 155. 1967.—Type: "Jugoslavia: Montenegro (Crna Gora): Durmitor, canjon Pive prope Mratinje, in rupium fissuris, solo calcareo ca. 850 m s.m., leg. V. Blečić et Mayer, 15.8.1962". (LJU 52970!; holotype)
= *A. autariatus* subsp. *bertisceus* Blečić & E. Mayer in Phyton (Horn) 12: 156. 1967≡*A. neumayeri* subsp. *bertisceus* (Blečić & E. Mayer) O. Schwarz in Phyton (Horn) 14: 132. 1970.—Type: "Jugoslavia: SW Serbia (Metohia): Prokletije (Bertiscus): Rugovska klisura inter Peć et Čakor- in rupium fissuris, solo calcareo, ca. 800 m s.m., leg. V. Blečić, 20.8.1965" (LJU 52952!; holotype)

Description (13–)20–50(–65)-cm high perennial with short woody stock. Stems erect to overhanging (plants growing in cliffs), with whitish indumentum. Rosette leaves alternate, (0.15–) 0.25–0.50(–0.65) as long as the stem, on the upper surface green, glabrous to sparsely hairy, on the lower surface white-tomentose, linear, lanceolate to ovate-, elliptic- or obovate-oblong, narrowed gradually into a short petiole or subsessile, (3–) 5–25(–40)× long as wide, (5–) 7–20(–23) cm long, (5–) 6–22(–32) mm wide, entire, with flat to (up to 1.3 mm) revolute margin, acute. Stem leaves (3–) 4–7(–10), alternate, linear, lanceolate, rarely lanceolate-obovate, entire, smaller, the lowest (1.5–)6–32(–40)×long as wide, (1.4–)4–17(–22) cm long, (2–)4–14(–18) mm wide, entire, with flat to revolute margin, acute; indumentum similar as on the basal leaves. Capitula terminal solitary or rarely two to four, 1.5–2(–2.5) cm in diameter. Involucral bracts mostly oblong-ovate to ovate-orbicular, in the third row (1–)1.2–2 (–2.5)×long as wide, (4–)4.5–8(–9.5) mm long and (2.6–)3–4.5(–5) mm wide, usually mucronate, with (0.2–)0.35–0.8(–0.95)-mm-wide scarious margin. Receptacle convex, with entire or lacerate scales. Florets pink to whitish. Achenes (3.5–)4.5–7.5(–8.5) mm long, sparsely to densely hairy, ribbed, the outer compressed, the inner cylindrical. Outer achenes with (0.1–)0.2–0.6(–0.8) wide wings and (0.1–)0.2–1.1(–1.4) long auricles. Pappus (4.5–)6–10(–11) mm long. $2n=24$.

Distribution Western and southern Balkan Peninsula from Mt. Vlašić (Bosnia and Herzegovina) in the north to Mt. Timphi (Greece) in the south, spanning Albania, Bosnia and Herzegovina, Greece, Kosovo, Macedonia, Montenegro and Serbia.

Habitat Rock crevices, cliffs, stabilised screes on calcareous substrate from the submontane (river gorges) to the alpine belt.

Conservation *A. neumayerianus* is not threatened.

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Conflict of Interest The authors declare that they have no conflict of interest.

References

Arrigo, N., Tuszynski, J. W., Ehrich, D., Gerdes, T., & Alvarez, N. (2009). Evaluating the impact of scoring parameters on the structure of intra-specific genetic variation using RawGeno, an R package for automating AFLP scoring. *BMC Bioinformatics, 10*, 33. doi:10.1186/1471-2105-10-33.

Ayasligil, Y. (1984). New taxa and records from southwestern Turkey. *Notes from the Royal Botanic Garden Edinburgh, 42*, 69–76.

Baldacci, A. (1894). Revista della collezione botanica fatta nel 1892 nell'Albania. *Malpighia, 8*, 69–87.

Barina, Z., Rakaj, M., & Pifkó, D. (2013). Contributions to the flora of Albania, 4. *Willdenowia, 43*, 165–184. doi:10.3372/wi.43.43119.

Barres, L., Sanmartín, I., Anderson, C. L, Susanna, A., Buerki, S., Galbany-Casals, M., & Vilatersana, R. (2013). Reconstructing the

evolution and biogeographic history of tribe Cardueae (Compositae). *American Journal of Botany, 100*(5), 867–882. doi:10.3732/ajb.1200058, doi:10.3372/wi.43.43119.

Beck, G. (1894). V. Gruppe. In I. Dörfler (Ed.), *Jahreskatalog des Wiener botanischen Tauschvereines* (p. 20). Wien.

Biel, B., & Tan, K. (2010). Reports 3–23. In: V. Vladimirov, F. Dante, K. Tan (Eds.), *New floristic records in the Balkans: 13* (pp. 144–148). *Phytologia Balcanica, 16(1),* 143–165.

Blečić, V., & Mayer, E. (1967). Die europäischen Sippen der Gattung *Amphoricarpos* Visiani. *Phyton, 12*, 150–158.

Bogdanović, S., Brullo, S., Rešetnik, I., Šatović, Z., & Liber, Z. (2014). *Campanula teutina*, a new isophyllous *Campanula* (Campanulaceae) from the Adriatic region. *Phytotaxa, 162*(1), 1–17. doi:10.11646/phytotaxa.162.1.1.

Bonin, A., Bellemain, E., Bronken Eidesen, P., Pompanon, F., Brochmann, C., & Taberlet, P. (2004). How to track and assess genotyping errors in population genetics studies. *Molecular Ecology, 13*(11), 3261–3273. doi:10.1111/j.1365-294X.2004.02346.x.

Bošnjak, K. (1936). Iz hercegovačke flore. *Glasnik Hrvatskog Prirodoslovnoga Društva, 41–48*, 62–63.

Clement, M., Posada, D., & Crandall, K. A. (2000). TCS: a computer program to estimate gene genealogies. *Molecular Ecology, 9*(10), 1657–1659. doi:10.1046/j.1365-294x.2000.01020.x.

Comes, P. H., & Kadereit, J. W. (1998). The effects of quaternary climatic changes on plant distribution and evolution. *Trends in Plant Sciences, 3*, 432–438. doi:10.1016/S1360-1385(98)01327-2.

Corander, J., & Tang, J. (2007). Bayesian analysis of population structure based on linked molecular information. *Mathematical Bioscience, 205*(1), 19–31. doi:10.1016/j.mbs.2006.09.015.

Corander, J., Waldmann, P., Marttinen, P., & Sillanpää, M. J. (2003). BAPS 2: enhanced possibilities for the analysis of genetic population structure. *Bioinformatics, 20*, 2363–2369. doi:10.1093/bioinformatics/bth250.

Corander, J., Marttinen, P., & Mäntyniemi, S. (2006). Bayesian identification of stock mixtures from molecular marker data. *Fishery Bulletin, 104*, 550–558.

Davis, S. D., Heywood, V. H., & Hamilton, A. C. (1994). *Centres of plant diversity, vol. 1, Europe, Africa, southwest Asia and the Middle East*. Gland: International Union for the Conservation of Nature and Natural Resources.

Drummond, A. J., & Rambaut, A. (2007). BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology, 7*, 214. doi:10.1186/1471-2148-7-214.

Ehrich, D. (2006). AFLPdat: a collection of R functions for convenient handling of AFLP data. *Molecular Ecology Notes, 6*, 603–604. doi:10.1111/j.1471-8286.2006.01380.x.

Euro + Med (2006–). Euro + Med PlantBase—the information resource for Euro-Mediterranean plant diversity. http://ww2.bgbm.org/EuroPlusMed. Accessed 15 Oct 2014.

Farr, E. R., Leussink, J. A., & Stafleu, F. A. (1979). Index nominum genericorum (plantarum), 1 (Aa-Epochnium). *Regnum Vegetabile, 100*, 74.

Frajman, B., & Oxelman, B. (2007). Reticulate phylogenetics and phytogeographical structure of *Heliosperma* (Sileneae, Caryophyllaceae) inferred from chloroplast and nuclear DNA sequences. *Molecular Phylogenetics and Evolution, 43*(1), 140–155. doi:10.1016/j.ympev.2006.11.003.

Frajman, B., & Schneeweiss, G. M. (2009). A Campanulaceous fate: the Albanian stenoendemic *Asyneuma comosiforme* in fact belongs to isophyllous *Campanula*. *Systematic Botany, 34*(3), 595–601. doi:10.1600/036364409789271173.

Frajman, B., Eggens, F., & Oxelman, B. (2009a). Hybrid origins and homoploid reticulate evolution within *Heliosperma* (Sileneae, Caryophyllaceae)—a multigene phylogenetic approach with relative dating. *Systematic Biology, 58*(3), 328–345. doi:10.1093/sysbio/syp030.

Frajman, B., Heidari, N., & Oxelman, B. (2009b). Phylogenetic relationships of *Atocion* and *Viscaria* (Sileneae, Caryophyllaceae) inferred from chloroplast, nuclear ribosomal, and low-copy gene DNA sequences. *Taxon, 58*(3), 811–824.

Frajman, B., Carlón, L., Kosachev, P., Sánchez Pedraja, Ó., Schneeweiss, G. M., & Schönswetter, P. (2013). Phylogenetic position and taxonomy of the enigmatic *Orobanche krylowii* (Orobanchaceae), a predominatly Asian species newly found in Albania (SE Europe). *Phytotaxa, 137*(1), 1–14. doi:10.11646/phytotaxa.137.1.1.

Frajman, B., Pachschwöll, C., & Schönswetter, P. (2014). Contributions to the knowledge of the flora of the Dinarides (Balkan Peninsula). *Phyton, 54*(1), 27–46.

Freeland, J. R., Kirk, H., & Petersen, S. D. (2011). *Molecular ecology* (2nd ed.). Chichester: Wiley-Blackwell.

Fukarek, P. (1965). Rasprostranjenost i ekološke karakteristike krčagovine *Amphoricarpus neumayeri* Vis. *Glasnik Zemaljskog Muzeja Sarajevo, 3–4*, 159–180.

Gómez, A., & Lunt, D. H. (2007). Refugia within refugia: patterns of phylogeographic concordance in the Iberian Peninsula. In S. Weiss & N. Ferrand (Eds.), *Phylogeography of southern European refugia* (pp. 155–188). Berlin: Springer.

Greuter, W. (2003). The Euro + Med treatment of *Cardueae* (*Compositae*)—generic concepts and required new names. *Willdenowia, 33*, 49–61.

Greuter, W., Brummitt, R. K., Farr, E., Kilian, N., Kirk, P. M., & Silva, P. C. (1993). Names in current use for extant plant genera. *Regnum Vegetabile, 129*, 48.

Griffiths, H. I., Kryštufek, B., & Reed, J. M. (Eds.). (2004). *Balkan biodiversity: pattern and process in the European hotspot*. Dordrecht: Kluwer.

Grossheim, A. A. (1949). *Opredelitel' rastenii kavkaza*. Moskva.

Hayek, A. (1924–1933). Prodromus Florae peninsulae Balcanicae. *Repertorium Specierum Novarum Regni Vegetabilis, 30,* 1–1193, 1–1152, 1–472.

Hewitt, G. M. (2000). The genetic legacy of the quaternary ice ages. *Nature, 405*, 907–913.

Hewitt, G. M. (2011). Mediterranean peninsulas: the evolution of hotspots. In F. E. Zachos & J. C. Habel (Eds.), *Biodiversity hotspots* (pp. 123–147). Berlin: Springer.

Horvat, I., Glavač, V., & Ellenberg, H. (1974). *Vegetation Südosteuropas*. Stuttgart: Gustav Fischer.

Huson, D. H., & Bryant, D. (2006). Application of phylogenetic networks in evolutionary studies. *Molecular Biology and Evolution, 23*(2), 254–267. doi:10.1093/molbev/msj030.

Kryštufek, B., & Reed, J. M. (2004). Pattern and processes in Balkan biodiversity—an overview. In H. I. Griffits, B. Kryštufek, & J. M. Reed (Eds.), *Balkan biodiversity, pattern and process in the European hotspot* (pp. 203–217). Dordrecht: Kluwer.

Kučera, J., Marhold, K., & Lihová, J. (2010). *Cardamine maritima* group (Brassicaceae) in the amphi-Adriatic area: a hotspot of species diversity revealed by DNA sequences and morphological variation. *Taxon, 59*(1), 148–164.

Kutnjak, D., Schönswetter, P., Dullinger, S., Kuttner, M., Niketić, M., & Frajman, B. (2014). Escaping to the summits: phylogeography and predicted range dynamics of *Cerastium dinaricum*, an endangered high mountain plant endemic to the western Balkan Peninsula. *Molecular Phylogenetics and Evolution, 78*(1), 365–374. doi:10.1016/j.ympev.2014.05.015.

Lakušić, D., Liber, Z., Nikolić, T., Surina, B., Kovačić, S., Bogdanović, S., & Stefanović, S. (2013). Molecular phylogeny of the *Campanula pyramidalis* species complex (Campanulaceae) inferred from chloroplast and nuclear non-coding sequences and its taxonomic implications. *Taxon, 62*(3), 505–524.

Maly, K. (1928). Prilozi za floru Bosne i Hercegovine. *Glasnik Zemaljskog Muzeja Bosne i Hercegovine, 40*, 107–166.

Manly, F. J. B. (1986). *Multivariate statistical methods—a primer*. New York: Chapman and Hall.

Markgraf, F. (1932). Pflanzengeographie von Albanien. Ihre Bedeutung für Vegetation und Flora der Mittelmeerländer. *Bibliotheca Botanica, 26*(105), 100–132.

Masel, J. (2011). Genetic drift. *Current Biology, 21*(20), R837–R838. doi:10.1016/j.cub.2011.08.007.

Meulenkamp, J. E., & Sissingh, W. (2003). Tertiary palaeogeography and tectonostratigraphic evolution of the Northern and Southern Peri-Tethys platforms and the intermediate domains of the African–Eurasian convergent plate boundary zone. *Palaeogeography, Palaeoclimatology, Palaeoecology, 196*(1–2), 209–228. doi:10.1016/S0031-0182(03)00319-5.

Meyer, F. K. (2011). Beiträge zur Flora von Albanien. *Haussknechtia Beihefte, 15*, 1–220.

Murbeck, S. (1891). Beiträge zur Kenntnis der Flora von Südbosnien und der Hercegovina. *Acta Universitatis Lundensis, 27*, 1–182.

Nei, M. (1987). *Molecular evolutionary genetics*. New York: Columbia University Press.

Nei, M., & Li, W. H. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences of the United States of America, 76*(10), 5269–5273. doi:10.1073/pnas.76.10.5269.

Nieto Feliner, G. (2014). Patterns and processes in plant phylogeography in the Mediterranean Basin. A review. *Perspectives in Plant Ecology, Evolution and Systematics, 16*(5), 265–278. doi:10.1016/j.ppees.2014.07.002.

Niketić, M., & Stevanović, V. (2007). A new species of *Heliosperma* (Caryophyllaceae) from Serbia and Montenegro. *Botanical Journal of the Linnean Society, 154*(1), 55–63. doi:10.1111/j.1095-8339.2007.00643.x.

Niketić, M., Tomović, G., Melovski, L., Stevanović, V., & Matevski, V. (2014). New species for the vascular flora of Republic of Macedonia and their distribution in the Balkan Peninsula. *Botanica Serbica, 38*(1), 57–67.

Nylander, J. A. A. (2004). *MrAIC pl. Program distributed by the author*. Evolutionary Biology Centre, Uppsala University.

Petit, R. J., Aguinagalde, I., de Beaulieu, J. L., Bittkau, C., Brewer, S., Cheddadi, R., Ennos, R., Fineschi, S., Grivet, D., Lascoux, M., Mohanty, A., Müller-Starck, G., Demesure-Musch, B., Palme, A., Martin, J. P., Rendell, S., & Vendramin, G. G. (2003). Glacial refugia: hotspots but not melting pots of genetic diversity. *Science, 300*(5625), 1563–1565. doi:10.1126/science.1083264.

Polunin, O. (1987). *Flowers of Greece and the Balkans—a field guide*. Oxford: Oxford University Press.

R Development Core Team. (2011). *R: A language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing.

Rambaut, A. (2006). FigTree v1.0, http://tree.bio.ed.ac.uk/software/figtree/.

Rambaut, A., & Drummond, A. (2009). *Tracer, version 1.5*. http://tree.bio.ed.ac.uk/software/tracer/.

Rohlena, J. (1907). Beitrag zur Flora von Montenegro. *Magyar Botanikai Lapok, 6*, 158.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. A., & Huelsenbeck, J. P. (2012). MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology, 61*(3), 539–542. doi:10.1093/sysbio/sys029.

Ross, R. (2002). Which illustrations are original material? *Taxon, 51*(4), 523–524. doi:10.2307/1554867.

Schluter, P. M., & Harris, S. A. (2006). Analysis of multilocus fingerprinting data sets containing missing data. *Molecular Ecology Notes, 6*(2), 569–572. doi:10.1111/j.1471-8286.2006.01225.x.

Schönswetter, P., & Schneeweiss, G. M. (2009). *Androsace komovensis* sp. nov., a long mistaken local endemic from the southern Balkan Peninsula with biogeographic links to the Eastern Alps. *Taxon, 58*(2), 544–549.

Schönswetter, P., Solstad, H., Garcia, P. E., & Elven, R. (2009). A combined molecular and morphological approach to the taxonomically intricate European mountain plant *Papaver alpinum s.l.* (Papaveraceae)—taxa or informal phylogeographical groups? *Taxon, 58*(4), 1326–1343.

Schwarz, O. (1970). Eine bemerkenswerte neue *Amphoricarpos*-Art aus Südwest-Anatolien. *Phyton, 14*, 125–133.

Shaw, J., Lickey, E. B., Beck, J. T., Farmer, S. B., Liu, W., Miller, J., Siripun, K. C., Winder, C. T., Schilling, E. E., & Small, R. L. (2005). The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *American Journal of Botany, 92*(1), 142–166. doi:10.3732/ajb.92.1.142.

Shaw, J., Lickey, E. B., Schilling, E. E., & Small, R. L. (2007). Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. *American Journal of Botany, 94*(3), 275–288. doi:10.3732/ajb.94.3.275.

Shuka, L., Tan, K., & Siljak-Yakovlev, S. (2010). *Tulipa albanica* (Liliaceae), a new species from northeastern Albania. *Phytotaxa, 10*, 17–25. doi:10.11646/phytotaxa.10.1.2.

Shuka, L., Tan, K., & Krasniqi, R. (2012). *Tulipa kosovarica* (Liliaceae), a new species of tulip from Kosovo. *Phytotaxa, 62*, 1–9.

StatSoft. (1996). *STATISTICA (data analysis software system), version 5.1*. Tulsa: StatSoft Inc. www.statsoft.com.

Stevanović, V. (1996). Analysis of the Central European and Mediterranean orophytic element on the mountains of the W. and Central Balkan Peninsula, with special reference to endemics. *Bocconea, 5*, 77–97.

Stevanović, V., Kit, T., & Petrova, A. (2007). Mapping the endemic flora of the Balkans—a progress report. *Bocconea, 21*, 131–137.

Strid, A., & Tan, K. (1991). *Mountain flora of Greece, vol. 2*. Edinburgh: Edinburgh University Press.

Sun, Y., Skinner, D., Liang, G., & Hulbert, S. (1994). Phylogenetic analysis of *Sorghum* and related taxa using internal transcribed spacers of nuclear ribosomal DNA. *Theoretical and Applied Genetics, 89*, 26–32. doi:10.1007/BF00226978.

Surina, B., Rakić, T., Stefanović, S., Stevanović, V., & Lakušić, D. (2009). One new species of the genus *Edraianthus*, and a change in taxonomic status for *Edraianthus serpyllifolius* f. *pilosulus* (Campanulaceae) from the Balkan Peninsula. *Systematic Botany, 34*(3), 602–608. doi:10.1600/036364409789271236.

Surina, B., Schönswetter, P., & Schneeweiss, G. M. (2011). Quaternary range dynamics of ecologically divergent species (*Edraianthus serpyllifolius* and *E. tenuifolius*, Campanulaceae) within the Balkan refugium. *Journal of Biogeography, 38*, 1381–1393. doi:10.1111/j.1365-2699.2011.02493.x.

Surina, B., Pfanzelt, S., Einzmann, H. J. R., & Albach, D. C. (2014). Bridging the Alps and the Middle East: evolution, phylogeny and systematics of the genus *Wulfenia* (Plantaginaceae). *Taxon, 63*(4), 843–858. doi:10.12705/634.18.

Swofford, D. L. (2002). *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods), ver. 4.0 beta 10*. Sunderland: Sinauer Associates.

Turrill, W. B. (1929). *The plant life of the Balkan peninsula: a phytogeographical study*. Oxford: Clarendon.

van de Peer, Y., & de Wachter, R. (1997). Construction of evolutionary distance trees with TREECON for Windows: accounting for

variation in nucleotide substitution rate among sites. *Computer Applications in the Biosciences, 13*(3), 227–230. doi:10.1093/bioinformatics/13.3.227.

Visiani, R. (1842). *Flora Dalmatica. Vol. I*. Lipsiae.

Visiani, R. (1847). *Flora Dalmatica. Vol. II*. Lipsiae.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., & Zabeau, M. (1995). AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research, 23*(21), 4407–4414. doi:10.1093/nar/23.21.4407.

Webb, D. A. (1976). *Amphoricarpos* Vis. Pp. 243. In T. G. Tutin, V. H. Heywood, N. A. Burges, D. M. Moore, D. H. Valentine, S. M. Walters, & D. A. Webb (Eds.), *Flora Europaea, vol. 4, Plantaginaceae to Compositae (and Rubiaceae)*. Cambridge: Cambridge University Press.