

Original Article

Disentangling relationships in *Sorbus* subgen. *Tormaria* (Rosaceae) from the western Balkan Peninsula (south-east Europe)

Alma Hajrudinović-Bogunić^{1,✉}, Božo Frajman^{2,*,✉}, Peter Schönswetter^{2,✉}, Sonja Siljak-Yakovlev^{3,✉}, Ante Begić⁴, Faruk Bogunić^{1,*,✉}

¹Faculty of Forestry, University of Sarajevo, 71000 Sarajevo, Bosnia and Herzegovina

²Department of Botany, University of Innsbruck, 6020 Innsbruck, Austria

³Université Paris-Saclay, CNRS, AgroParisTech, Ecologie Systématique Evolution, 91190 Gif-sur-Yvette, France

⁴Šumsko-gospodarsko društvo Županije Zapadnohercegovačke d.o.o, 88240 Posušje, Bosnia and Herzegovina

*Corresponding authors. Faruk Bogunić, Faculty of Forestry, University of Sarajevo, Zagrebačka 20, 71000 Sarajevo, Bosnia and Herzegovina. E-mail: fbogunic@sfsa.unsa.ba and Božo Frajman, Department of Botany, University of Innsbruck, Sternwartestrasse 15, 6020 Innsbruck, Austria. E-mail: bozo.frajman@uibk.ac.at

ABSTRACT

Hybridization and polyploidization are the main drivers of diversification in *Sorbus*, and have generated numerous evolutionary lineages across Europe. Newly derived polyploid lineages usually reproduce via apomixis and represent novel genetic and morphological entities, usually circumscribed as distinct species. Whereas *Sorbus* has been thoroughly studied in Central and Western Europe, its diversity in the Balkans remains less explored. This is especially the case for *Sorbus* subgen. *Tormaria* that includes derivatives of crosses between *S.* subgen. *Aria* and *S. torminalis*. Here, we report the discovery of a triploid population of *S.* subgen. *Tormaria* in south-western Bosnia and Herzegovina. We performed amplified fragment length polymorphism fingerprinting, evaluation of nuclear microsatellites, plastid DNA sequencing, flow cytometric ploidy level estimation, reproduction mode screening, and morphological characterization to disentangle its relationship to the previously reported diploid populations from Bosnia and Herzegovina and congeners from Central Europe, as well as to both parents. The data revealed that the aforementioned population includes mostly triploid facultative apomicts that are genetically divergent from other taxa. We therefore describe it as a new species, *Sorbus hercegovinae*, and provide its description, distribution data, and conservation status. Our study highlights the Balkan Peninsula as one of the hotspots of whitebeam diversity.

Keywords: apomixis; hybridization; new species; polyploidy; *Sorbus*

INTRODUCTION

The Balkan Peninsula represents a hotspot of European plant diversity due to heterogeneous geological, topographic, and climatic conditions, leading to high variability of habitats, as well as due to milder influence of Pleistocene glaciations enabling survival of biota in different refugia (Kryštufek and Reed 2004, Nieto Feliner 2011, Rešetnik and Španiel 2022). Hybridization and polyploidization are among the most important drivers contributing to this remarkable diversity, resulting in a high number of endemic species (Lakušić *et al.* 2009, Bjedov *et al.* 2015, Hajrudinović *et al.* 2015a, Hajrudinović-Bogunić *et al.* 2023, Lazarević *et al.* 2015, 2022, Olšovská *et al.* 2016, Španiel *et al.* 2017, Niketić

et al. 2022, Rešetnik *et al.* 2023, Kuzmanović *et al.* 2024). Allopolyploidy represents a strong force that affects genome variation through recombination, epigenetic expression, eco-physiological and phenotypic adaptation, as well as changes in reproduction mode (Leitch and Leitch 2008, Renny-Byfield *et al.* 2013, Weiss-Schneeweiss *et al.* 2013, Shimizu-Inatsugi *et al.* 2017).

Sorbus L. (Rosaceae, Malinae) is a woody plant genus, whose diversification was predominately shaped by recurrent homoploid hybridization as well as by polyploidization, generating numerous distinct lineages (Rich *et al.* 2010, Robertson *et al.* 2010, Kurtto *et al.* 2018). The newly derived polyploid lineages are often maintained by apomixis (asexual seed formation), which enables their

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propagation and long-term persistence (Robertson *et al.* 2010, Ludwig *et al.* 2013). Whereas diploid *Sorbus* species are sexual outcrossers, polyploids are mostly pseudogamous apomicts; triploids show nearly obligate apomixis and tetraploids retain some sexuality (facultative apomixis; Hajrudinović *et al.* 2015b, Lepšić *et al.* 2019). Although apomictic reproduction generates clonal offspring, recurrent pollen flow from polyploid apomicts to diploid outcrossers continuously generates new polyploid diversity with different mating systems (Robertson *et al.* 2010, Hajrudinović *et al.* 2015b). The taxonomy of these polyploid-apomictic complexes is challenging, therefore proper conservation efforts should rather focus on the evolutionary processes that drive production of the novel biodiversity (Ennos *et al.* 2005, 2012).

The Balkan Peninsula hosts ~15 *Sorbus* taxa, including four sexual and 10 apomictic species (Kurto *et al.* 2018, Sennikov and Kurto 2017). Recent studies of Balkan *Sorbus* revealed complex patterns of karyological, genetic, and morphological diversity and uncovered numerous cryptic lineages, especially in *Sorbus* subgen. *Soraria* Májovský et Bernátová, which includes hybrid derivatives of crosses between *S.* subgen. *Aria* and *S. aucuparia* L. (Hajrudinović *et al.* 2015a, 2015b, Hajrudinović-Bogunić *et al.* 2023). On the other hand, *Sorbus* subgen. *Tormaria* Májovský et Bernátová that includes a series of di-, tri-, and tetraploid taxa generated by hybridization between *S.* subgen. *Aria* and *S. torminalis* L. (Meyer *et al.* 2005, Lepšić *et al.* 2008, 2009, Rich *et al.* 2010) was neglected in previous studies of Balkan plants. The highest species diversity within this subgenus, which is at least partly related to focused systematic research, is in parts of Central Europe (Meyer *et al.* 2005, Németh 2007, 2012, 2015, Lepšić *et al.* 2008, 2009, Sennikov and Kurto 2017) and Great Britain (Rich *et al.* 2010), where numerous apomictic species have been described recently. Elsewhere, hybrids between *S.* subgen. *Aria* and *S. torminalis* have been mostly referred to as *S. latifolia* (Lam) Pers. s.l. (e.g. in Austria: Fischer *et al.* 2008). This is also the case for the Balkan Peninsula, where this taxon was reported from scattered localities in Slovenia (Daksobler *et al.* 2014), Bosnia and Herzegovina (Hajrudinović *et al.* 2012), Montenegro and Serbia (Tomović *et al.* 2020), North Macedonia (Teofilovski *et al.* 2015, Teofilovski 2017), and Bulgaria (Zieliński and Vladimirov 2013). With the exception of two populations from the western Balkan Peninsula (Bosnia and Herzegovina) that were revealed to be diploid and thus likely a primary F1 hybrid (Hajrudinović *et al.* 2012), nothing is known about the ploidy levels and reproduction modes of other Balkan populations.

Here, we report the discovery of a polyploid population of *S.* subgen. *Tormaria* composed of ~50 fructiferous plants from Posušje area (sites Crne Lokve and Gradac) in south-western Bosnia and Herzegovina. We performed amplified fragment length polymorphism (AFLP) fingerprinting, evaluation of nuclear microsatellites, plastid DNA sequencing, flow cytometric genome size and ploidy level estimation, reproduction mode screening, and morphological characterization to evaluate the status of this population and to disentangle its relationship to the previously reported diploid populations from Bosnia and Herzegovina as well as to both parents. In addition, we included several recently described Central European species belonging to this subgenus (Lepšić *et al.* 2008, 2009), which have never been studied phylogenetically, as a reference. As we inferred that the population from

Posušje area is predominantly triploid, and represents an independent evolutionary lineage, we describe this triploid lineage as a new species, *Sorbus hercegovinae*, and provide a morphological characterization. For simplicity, we apply the name *S. hercegovinae* hereafter.

MATERIALS AND METHODS

Plant material

Leaf material from 26 localities belonging to *S.* subgen. *Aria* Pers. (diploids, triploids, and tetraploids of *S. aria* aggregate, from 14 localities), *S.* subgen. *Tormaria* (di-, tri-, and tetraploids of *S. aria* × *torminalis*, from 15 localities), and *S.* subgen. *Torminaria* (diploids of *S. torminalis*, from six localities) was collected and silica-dried for molecular analyses and herbarized for morphological analyses (Fig. 1A; Supporting Information, Data S1); material from localities 18–26 was used only for molecular analyses. The taxa were identified using *Flora Europaea* (Warburg and Kárpáti 1968), Euro+Med Plantbase (Kurto 2009+), and national floras (Jovanović 1972, Fischer *et al.* 2008, Rich *et al.* 2010, Nikolić 2020). Voucher specimens are kept at the National Museum of Bosnia and Herzegovina (SARA).

Genome size estimation

We estimated genome size using flow cytometry as described by Hajrudinović *et al.* (2015b). Briefly, fresh leaves of 49 individuals from eight populations (Supporting Information, Data S1) were co-chopped with fresh leaves of internal standard *Medicago truncatula* Gaertn. cv. R108-1 (0.98 pg; Marie and Brown 1993) with a razor blade in 600 mL of cold Gif nuclear buffer (Bourge *et al.* 2018). The suspension was filtered through a 50- μ m nylon mesh (CellTrics, Partec) and RNase (Roche) was added to 25 U mL⁻¹. The nuclei were stained with propidium iodide (Sigma-Aldrich) in a final concentration of 50 mg mL⁻¹ and incubated on ice for 5–10 min before analysis. The fluorescence of ~3000 nuclei was recorded for each sample using a Partec CyFlow SL3 (Partec, Münster, Germany) 532 nm laser cytometer or CyFlow Ploidy Analyser (Sysmex Europe SE) 532 nm laser. The 2C DNA values were obtained and DNA-ploidy levels (Suda *et al.* 2006), were inferred by comparison with 2C DNA values of individuals of known chromosome counts (Siljak-Yakovlev *et al.* 2010).

For the purpose of reproduction mode identification, we conducted flow cytometric seed screening (FCSS) on 46 seeds (Supporting Information, Data S1), following Hajrudinović *et al.* (2015a). Seeds were collected from previously cytotyped mother individuals (Supporting Information, Data S1). Well-formed seeds were cleaned, shortly dried at room temperature and kept in paper bags at 4 °C prior to analysis. Each seed was analysed separately. Endosperm (*end*) ploidy was calculated using the inferred monoploid genome size of the embryo (*emb*). DNA ploidies of embryo and endosperm were compared to distinguish between sexual and apomictic origin of each seed following Hajrudinović *et al.* (2015a).

Amplified fragment length polymorphism (AFLP)

One to 19 individuals per locality, totalling 110 individuals from 26 localities, were included in the AFLP analysis (Supporting Information, Data S1). A modified CTAB-procedure (Tel-Zur

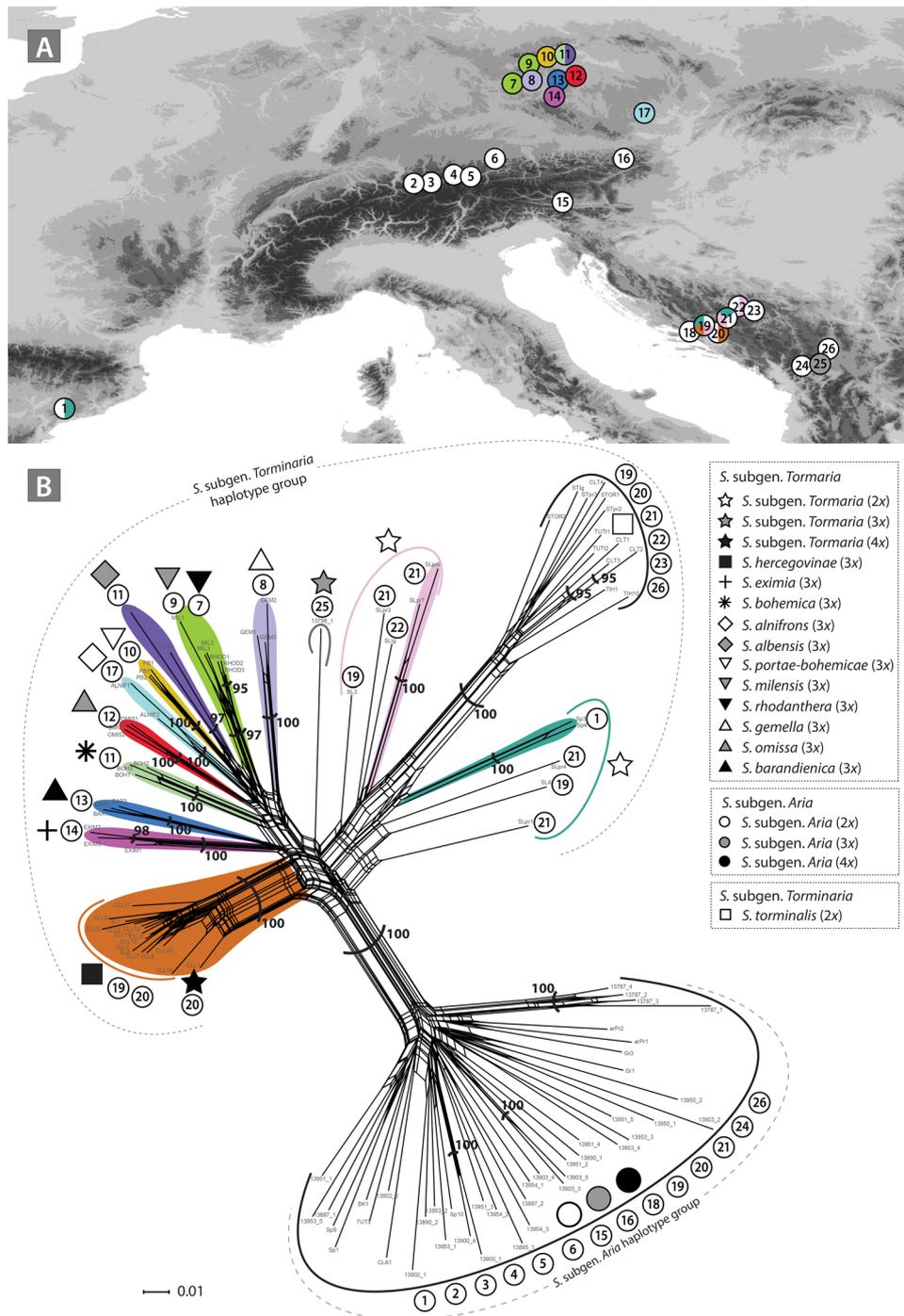


Figure 1. A, Geographic origin and B, NeighborNet based on AFLP within *Sorbus* supplemented with bootstrap values $\geq 95\%$ derived from a neighbour-joining analysis (Supporting Information, Fig. S1). Locality numbers correspond to Supporting Information, Data S1. Dashed lines in (B) denote the plastid *trnT-trnF* haplotype affiliation according to Figure 3. Note that *trnT-trnF* was only sequenced for a subset of individuals.

et al. 1999) was used for extraction of total genomic DNA from ~20 mg of silica-dried leaf material. The AFLP protocol followed Vos *et al.* (1995) with the modifications described in Hajrudinović *et al.* (2015b). We used the following primer combinations for the selective PCR (fluorescent dye in brackets): EcoRI (6-FAM)-ACA/MseI-CAC, EcoRI (VIC)-AAG/MseI-CTG, and EcoRI (NED)-ACC/MseI-CAG (MseI and EcoRI primers: Sigma-Aldrich). Reproducibility was evaluated using 15 replicated

samples. Electropherograms were analysed using Peak Scanner v.1.0 (Applied Biosystems) with default peak detection parameters. The minimum fluorescent threshold was set to 50 relative fluorescence units (RFU). RawGeno v.2.0 (Arrigo *et al.* 2009), a package for R (R Core Team 2022) was used for automated data scoring with the following settings: 75–500 bp scoring range, 50 RFU minimum intensity, and bin width 1.0–1.5. Fragments with reproducibility lower than 80% based on sample-replicate

comparisons were excluded. A neighbour-joining analysis based on Nei–Li genetic distances (Nei and Li 1979) was conducted and bootstrapped (2000 pseudo-replicates) with TREECON v.1.3b (Van de Peer and De Wachter 1997). A NeighborNet was produced from a matrix of uncorrected P distances using Split-Tree v.4.12 (Huson 1998). A principal coordinate analysis (PCoA) based on Jaccard distances was conducted using PAST v.2.15 (Hammer *et al.* 2001).

Analysis of nuclear microsatellites

Amplification of six nuclear microsatellite-specific loci (CH01F02, MSS5, MSS13, MSS16, D11, and H10) was successfully performed for 107 individuals from 26 sampled localities (Supporting Information, Data S1), following Robertson *et al.* (2004, 2010). An ABI PRISM 310 Genetic Analyzer (Applied Biosystems) was used for electrophoretic separation of the PCR products. Alleles were sized relative to the internal size standard TAMRA 500 (Applied Biosystems). Electropherograms were analysed using GeneMapper (Applied Biosystems). To study the genetic diversity, we determined the multilocus genotype (MG) for each individual on the basis of microsatellite alleles for each of the six loci using the software GenoType v.1.2 (Meirmans and Van Tienderen 2004). Assignment of individuals to a particular clone was done using the algorithm of Meirmans and Van Tienderen (2004) based on the calculation of a genetic distance matrix and a threshold value (set to 3 after testing different thresholds as recommended) under the stepwise mutation model option. Relationships among MGs were visualized by PCoA based on Jaccard distances using PAST v.2.15 (Hammer *et al.* 2001). If the ploidy level could not be identified with flow cytometry, we used the maximum number of alleles per locus to infer it.

Plastid *trnT*–*trnF* sequencing and phylogenetic analyses

We sequenced the plastid *trnT*–*trnF* region for totally 12 individuals from 10 localities, belonging to *S.* subgen. *Tormaria* (eight individuals from seven localities), *S.* subgen. *Torminaria* (three individuals from three localities) as well as one individual from *S.* subgen. *Aria* (Supporting Information, Data S1), following the procedure described by Hajrudinović *et al.* (2015a) and using the primers TabA, TabC, and TabF (Taberlet *et al.* 1991). In addition, we included 54 sequences belonging to different outgroup *Sorbus* taxa from Hajrudinović-Bogunić *et al.* (2023) as well as *Pyrus pyrifolia* (Burm.f.) Nakai from GenBank (AP012207) for rooting. Sequences were edited and aligned with Geneious Pro v.5.5.9 (Kearse *et al.* 2012). We coded indels as binary characters applying simple gap coding (Simmons and Ochoterena 2000) with SeqState v.1.25 (Müller 2005).

Maximum parsimony (MP) and MP bootstrap (MPB) analyses were performed using PAUP v.4.0b10 (Swofford 2002). The most parsimonious trees were searched for heuristically with 100 replicates of random sequence addition, TBR swapping, and MulTrees on. All characters were equally weighted and unordered. The data set was bootstrapped using full heuristics, 1000 replicates, TBR branch swapping, MulTrees option off, and random addition sequence with five replicates. Bayesian analyses were performed using MrBayes v.3.2.1 (Ronquist *et al.* 2012) applying the HKY85 substitution model proposed by the Akaike information criterion implemented in MrAIC.pl v.1.4 (Nylander 2004). The alignment

was partitioned into nucleotide and indel data sets, and the latter was treated as morphological data according to the model of Lewis (2001). 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 process included four runs with four chains each (three heated ones using the default heating scheme), run simultaneously for 1000000 generations each, sampling trees every 1000th generation using default priors. The posterior probabilities (PP) of the phylogeny and its branches were determined from the combined set of trees, discarding the first 1001 trees of each run as burn-in.

Morphological characterization

Morphological measurements of 29 quantitative leaf, flower, and fruit characters (Supporting Information, Data S2), previously shown to be informative (Lepší *et al.* 2008, 2009, Rich *et al.* 2010), were conducted using a digital calliper on 15 triploid *S. hercegovinae* individuals (representing ~30% of the population) from two adjacent localities, Crne Lokve and Gradac in Bosnia and Herzegovina (Supporting Information, Data S1). Arithmetic means of three to five measurements per leaf character were calculated for each individual, based on broad mid-leaves from different short sterile shots, following the recommendation of Rich *et al.* (2010). In the species' morphological description, value ranges correspond to the 10th and 90th percentiles, supplemented by minimum and maximum values in parentheses.

In addition, principal component analysis (PCA) based on the correlation matrix was performed on a sample of 24 individuals (Supporting Information, Fig. S4) to assess the overall morphological variation among triploid *S. hercegovinae* and diploid and tetraploid *S.* subgen. *Tormaria* individuals from Bosnia and Herzegovina (Supporting Information, Data S1). Individuals are labelled with the number of the corresponding MG, as in Table 2.

A comparative assessment of morphological character variation in *S. hercegovinae* and morphologically similar taxa from Central Europe (primarily those included in this study along with additional taxa from Hungary) is provided in Supporting Information, Data S4 (morphological data were obtained from published sources). The genetically analysed species from the Czech Republic, along with additional taxa from Hungary, served as reference material as they represent the geographically closest (albeit located at distances exceeding 450 km) members of the *Tormaria* group in relation to *S. hercegovinae*.

RESULTS

Genome size (GS), ploidy level and reproductive mode

Estimations of nuclear DNA content yielded stable histograms and high-resolution peaks, providing an accurate estimation with low values of coefficients of variation (mean CV = 2.98%). Nuclear DNA content averages span from 1.29 pg in diploid *S.* subgen. *Tormaria* to 2.73 pg in tetraploid *S.* subgen. *Tormaria* (Supporting Information, Data S1) and correspond to di-, tri-, and tetraploids. The values of diploid *S. aria* range from 1.38 to 1.51 pg, whereas one tetraploid accession of *S. aria* had GS 2.71 pg. In *S.* subgen. *Tormaria* the GS values range from 1.29 to 1.66 pg in diploids and from 2.06 to 2.34 pg in triploid accessions of *S. hercegovinae*; the only tetraploid individual of *S.* subgen. *Tormaria* has GS 2.73 pg.

Sorbus torminalis is exclusively diploid with GS values ranging from 1.38 to 1.53 pg.

The diploid hybrids yielded exclusively seeds of sexual origin having the same ($2x$) or an increased ploidy level of the embryo ($3x$) compared with the mother plant (Table 1, Fig. 2A, B). The seeds from triploid mother plants have different apomictic and sexual cytometric profiles (Table 1, Fig. 2C–E, I–J). The most common apomictic profile is $3x\ emb: 8x\ end$ (Fig. 2I, five seeds), whereas a single seed has a profile $3x\ emb: 9x\ end$ (Fig. 2J). Three sexual profiles are documented in triploids (i.e. *S. hercegovinae*), i.e. $4x\ emb: 7x\ end$ (Fig. 2C), $4x\ emb: 8x\ end$ (Fig. 2D), and $5x\ emb: 8x\ end$ (Fig. 2E), exhibiting increased ploidy level of the embryo compared with the mother tree. Three different modes of sexual seed formation are observed in tetraploid individuals, namely the sexual profiles $4x\ emb: 6x\ end$ (Fig. 2H), $4x\ emb: 5x\ end$ (Fig. 2G), and $3x\ emb: 5x\ end$ (Fig. 2F); the single apomictic one is $4x\ emb: 12x\ end$ (Fig. 2K).

AFLP fingerprinting

We obtained 520 high-quality and reproducible AFLP fragments from 110 individuals. The initial average error rate is 3.4%. The neighbour-joining analysis (Supporting Information, Fig. S1) infers two main clusters with 100% bootstrap support (BS), corresponding to *S. subgen. Aria* and *S. subgen. Tormaria*, with *S. torminalis* (*S. subgen. Torminaria*; BS 100%) nested within the latter cluster. Within *S. subgen. Tormaria* several clusters with BS >95% are resolved, mostly corresponding to different species. One cluster (BS 100%) includes triploid *S. hercegovinae* and a tetraploid individual belonging to *S. subgen. Tormaria*. Diploid *Tormaria* samples form a grade of branches closely related to *S. torminalis*. This structure is reflected in the NeighborNet (Fig. 1B), where the two most divergent clusters contain the parental species *S. torminalis* and *S. subgen. Aria*. All other clusters corresponding to *S. subgen. Tormaria* are intermediate between the parental taxa, with the diploid samples of *Tormaria* sharing several common splits with *S. torminalis*. All Central European taxa of *S. subgen. Tormaria* with the exception of *S. milensis* M.Lepší, K.Boublík, P.Lepší et P.Vít, and *S. rhodanthera* Kovanda form divergent clusters (BS >95%). Likewise, all triploid individuals, also including the tetraploid *S. subgen. Tormaria* individual, from the adjacent populations 19 and 20 (Crne lokve and Gradac, Bosnia and Herzegovina), which are 3 km apart, form separate cluster pertaining to *S. hercegovinae*. Relationships between hybrid populations/taxa and parental species show a similar pattern in the PCoA scatterplot, where the two most divergent clusters along the first PCoA axis correspond to *S. torminalis* and *S. subgen. Aria* (Supporting Information, Fig. S2A). The diploid and triploid accessions of *S. subgen. Tormaria* are positioned between the parental taxa and clearly separated along the second PCoA axis. Ordination of the accessions belonging to *S. subgen. Tormaria* shows a clear separation of *S. hercegovinae* from the Central European taxa along the first PCoA axis (Supporting Information, Fig. S2B). A single triploid individual from Kosovo is grouped with *S. eximia* Kovanda and *S. barrandienica* Vít, M.Lepší & P.Lepší, which are divergent from other Central European taxa. Diploid cytotypes of *S. subgen. Tormaria* are clearly divergent along the second axis.

Nuclear microsatellites

A total of 65 MGs are found within 107 accessions (Supporting Information, Data S3). Diploid cytotypes of *S. subgen. Aria*, *S. torminalis* and *S. subgen. Tormaria* have unique MGs, whereas most polyploid accessions contain at least one MG shared by a different number of individuals within a locality; from localities 18 and 25, we only sampled one individual each (Supporting Information, Data S3). Within polyploid *S. subgen. Tormaria*, Central European species (with >2 sampled individuals per population) are completely clonal, and *S. milensis* and *S. rhodanthera* share the same MG (Table 2). On the other hand, individuals of *S. hercegovinae* exhibit four MGs, with the dominance of MG8 shared by 10 individuals (Table 2).

The relationships among MGs (Supporting Information, Fig. S3) are consistent with AFLP data (Supporting Information, Fig. S2). Namely, PCoA shows a strong divergence of parental *S. subgen. Aria* and *S. torminalis* MGs along PCo1, whereas all diploid hybrids have intermediate positions (Supporting Information, Fig. S3A). The MGs belonging to the Central European taxa show strong divergence along PCo2. The MGs of *S. hercegovinae* are closer to *S. torminalis* and divergent from the Central European taxa.

Plastid *trnT-trnF* phylogenetic relationships

The *trnT-trnF* alignment of concatenated *trnT-trnL* intergenic spacer and *trnL-trnF* partial sequences is 1965 bp long, whereas the sequence of *S. hercegovinae* is 1821 bp long. Thirty-six characters are parsimony-informative and Bayesian and parsimony analyses resulted in congruent phylogenies (Fig. 3). Two main clades are resolved, one (posterior probability, pp, 1; parsimony bootstrap, BS, 100%) includes *S. aucuparia* as well as its hybrid species with *S. subgen. Aria* pertaining to *S. subgen. Soraria* [e.g. *S. austriaca* (Beck) Prain] with unresolved relationships. The second main clade (PP 1, BS 99%) is divided into a clade (PP 1, BS 86%) including several accessions of *S. subgen. Aria*, one accession of *S. subgen. Aria* × *S. austriaca*, and one of *S. pauca* M.Lepší & P.Lepší, and a clade (PP 1, BS 97%) including all other taxa. This latter clade is genetically most diverse and includes species of *S. subgen. Tormaria* and *Torminaria*; one diploid individual of *S. subgen. Tormaria* is sister to a clade (PP 0.98, BS 86%) that is divided into three subclades. One (PP 0.98, BS 65%) includes Central European *S. barrandienica* and *S. milensis* along with another diploid accession from the Balkan Peninsula. The second (PP 0.96, BS 56%) includes two accessions of *S. torminalis* and diploids of *S. subgen. Tormaria* from the western Balkan Peninsula, whereas the third (PP 0.98, BS 62%) is composed of two accessions of *S. hercegovinae*, one of *S. torminalis* (both western Balkan Peninsula), as well as one accession of *S. eximia* from Central Europe.

Morphological variation

PCA yielded seven significant principal components. The first two components accounted for 47.6% of the total variance (PC1 = 29.2%, PC2 = 18.4%) and exhibited moderate correlations with most of the associated morphological traits. The PCA ordination diagram (Supporting Information, Fig. S4) revealed a clear pattern, whereby triploid *S. hercegovinae* individuals (representing four multilocus genotypes MG8, MG9, MG10, and MG11;

Table 1. Ploidy and corresponding genome sizes as a result of FCSS followed by the proposed pathways of seed formation and seed origin of analysed *Sorbus* taxa/cytotypes.

| Taxon | Mother ID | Locality No. | Mother ploidy | FCSS | | | | Hypothesized pathways of seed formation | | | | | | Seed origin | |
|---|-----------|--------------|---------------|------------------|---------------------|---------------|------------------|---|-----------------|------------------------------|-------------------------|---|---|-------------|--|
| | | | | Embryo (mean pg) | Endosperm (mean pg) | Embryo ploidy | Endosperm ploidy | Number of seeds | Egg cell ploidy | Number of polar cells nuclei | Ploidy of spermal cells | Ploidy of spermal cells of polar cells nuclei | Number of spermal cells fecundating endosperm | | Maternal: paternal genome in endosperm |
| S. subgen. <i>Tormaria</i> <i>S. hercegovinae</i> | SL1 | 21 | 2x | 1.41 | 2.11 | 2x | 3x | 8 | 1x | 1x/2 | 1x | 1 | 2:1 | 1.5 | Sexual |
| | | | 2x | 2.07 | | 3x | - | 1 | 1x | | 2x | | | | Sexual |
| | | | 3x | 2.09 | 5.69 | 3x | 8x | 5 | 3x | 3x/2 | 1x or 2x | 2 or 1 | 3:1 | 2.7 | Apomictic |
| | | | 3x | 2.12 | 6.67 | 3x | 9x | 1 | 3x | 3x/2 | 3x | 1 | 2:1 | 3.1 | Apomictic |
| | | | 3x | 2.72 | 5.02 | 4x | 7x | 3 | 3x | 3x/2 | 1x | 1 | 6:1 | 1.8 | Sexual |
| S. subgen. <i>Tormaria</i> | | | 3x | 2.85 | 4.84 | 4x | 8x | 2 | 3x | 3x/2 | 1x, 2x | 1 | 3:1 | 1.7 | Sexual |
| | | | 3x | 3.27 | 5.52 | 5x | 8x | 1 | 3x | 3x/2 | 2x | 1 | 3:1 | 1.7 | Sexual |
| | | | 4x | 2.11 | 3.61 | 3x | 5x | 1 | 2x | 2x/2 | 1x | 1 | 4:1 | 1.7 | Sexual |
| | | | 4x | 2.68 | 3.51 | 4x | 5x | 1 | 2x | 2x/2 | 1x, 2x | 1 | 4:1 | 1.3 | Sexual |
| | | | 4x | 2.73 | 4.16 | 4x | 6x | 6 | 2x | 2x/2 | 2x | 1 | 2:1 | 1.5 | Sexual |
| S. subgen. <i>Torminaria</i> | | | 4x | 2.71 | 8.42 | 4x | 12x | 1 | 4x | 4x/2 | 2x | 2 | 2:1 | 3.1 | Apomictic |
| | | | 2x | 1.47 | 2.22 | 2x | 3x | 10 | 1x | 1x/2 | 1x | 1 | 2:1 | 1.5 | Sexual |
| | | | 20, 23, 26 | | | | | | | | | | | | |
| S. subgen. <i>Aria</i> | | | 2x | 1.40 | 2.10 | 2x | 3x | 5 | 1x | 1x/2 | 1x | 1 | 2:1 | 1.5 | Sexual |
| | | | 19, 20, 21 | | | | | | | | | | | | |
| | | | 26 | 2.77 | 8.15 | 4x | 12x | 1 | 4x | 4x/2 | 2x | 2 | 2:1 | 2.9 | Apomictic |
| | | | | | | TOTAL | 46 | | | | | | | | |

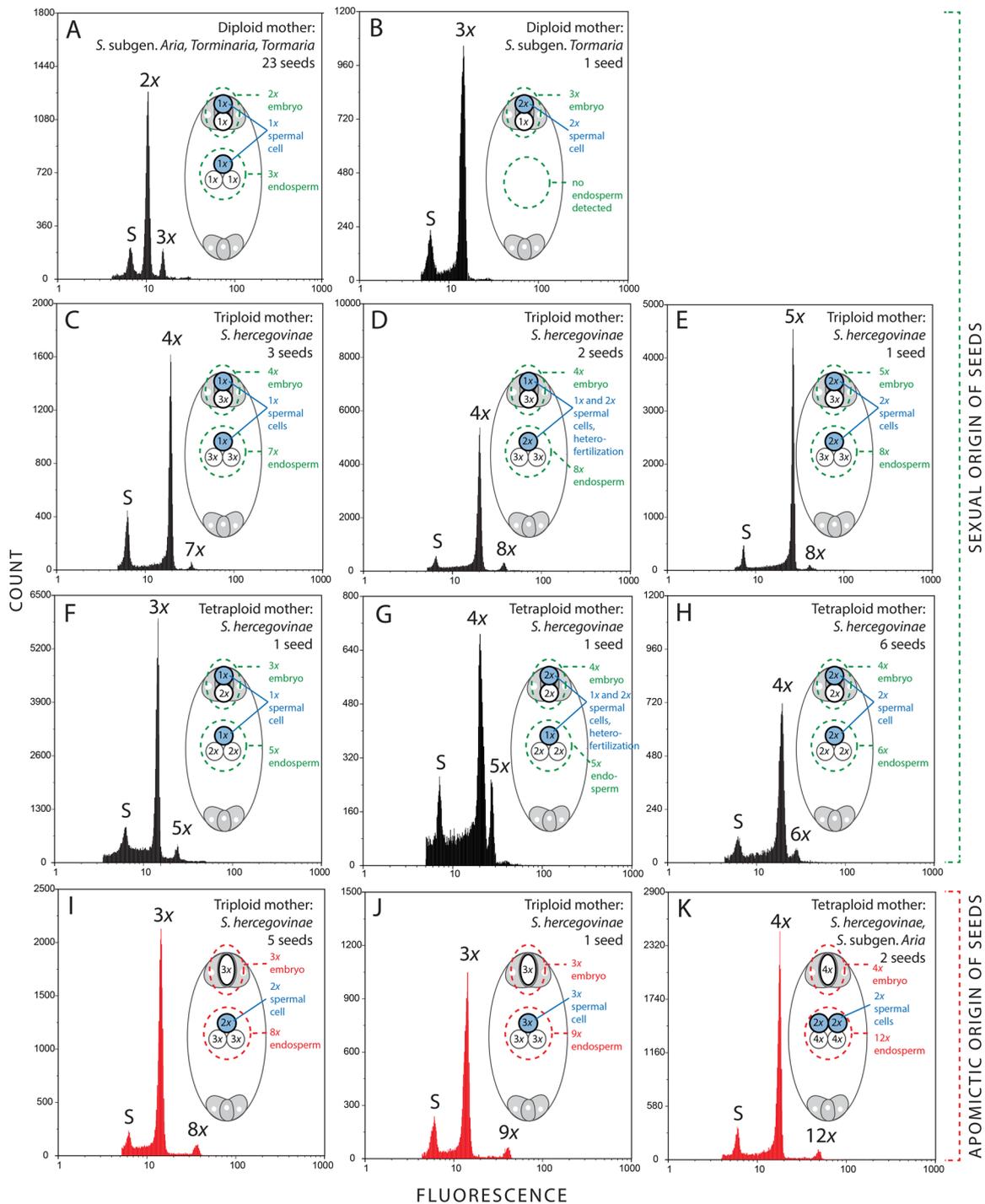


Figure 2. Flow cytometric seed screen histograms (log abscissa) of *Sorbus* seeds originated sexually (A–H) and apomictically (I–K). The first fluorescence peak in each panel corresponds to the internal standard (S, *Oryza sativa* L. ssp. *japonica* ‘Nipponbare’), the second to the embryo and the third to the endosperm. The histograms are complemented with illustrations of the proposed pathways of seed formation.

see Table 2) formed a homogenous cluster, with the exception of a single individual (CLL13, MG8). The tetraploid *S. subgen. Tormaria* individual was interspersed within the *S. hercegovinae* cluster, while diploid individuals were more randomly distributed.

The traits contributing to the first principal component included the length of the third nerve (3NERV), lamina width (WLEAV), and the distance from the leaf base to the point of maximum leaf width (MXWLEAV). Traits contributing to the

second principal component were the length of the third tooth (1SEINL) and the ratio of ‘lamina width’ to ‘distance from the leaf base to maximum leaf width’ (WLEAV/MXLEAV).

A comparative review of trait variation in the studied species (Supporting Information, Data S4) revealed notable similarities between *S. hercegovinae* and related taxa from the Czech Republic and Hungary. Among these, *S. barrandienica* and *S. bohémica* share the highest number of traits in common with *S. hercegovinae*

Table 2. MGs and allele composition of the studied accessions of *Sorbus* subgen. *Tormaria*.

| MG | Taxon | Ploidy | ID | N per MG | CH01F02 | MSS5 | Loci | | | | H10 | Locality (Locality No.) |
|----|----------------------------|-----------------|------------------|-----------|---------------|---------------|---------------|---------------|---------------|--------|--|-------------------------|
| | | | | | | | MSS13 | MSS16 | D11 | MSS16 | | |
| 1 | S. subgen. <i>Tormaria</i> | 2x ^a | SLlg | 1 | 188, 194 | 110, 126 | 189, 201 | 158, 168 | 147 | 78, 82 | Gřrkarica, Mt. Igman, B&H (22) | |
| 2 | | 2x ^a | SLpr1 | 1 | 186, 196 | 120, 130 | 191, 201 | 158, 184 | 153, 155 | 78 | Pratača, Mt. Igman, B&H (21) | |
| 3 | | 2x ^a | SLpr3 | 1 | 186, 196 | 120, 122 | 191, 195 | 158, 184 | 171 | 78 | Pratača, Mt. Igman, B&H (21) | |
| 4 | | 2x ^a | SLpr4 | 1 | 186, 200 | 118, 130 | 191, 201 | 158, 184 | 171 | 78 | Pratača, Mt. Igman, B&H (21) | |
| 5 | | 2x ^a | SLpr6, 7 | 2 | 186, 196 | 124, 130 | 187, 195 | 160, 180 | 171 | 78 | Pratača, Mt. Igman, B&H (21) | |
| 6 | | 2x ^a | SL3 | 1 | 186, 198 | 120 | 189, 195 | 156, 162 | 169, 181 | 78 | Gradac, B&H (19) | |
| 7 | | 2x ^a | SL4 | 1 | 186, 196 | 110, 134 | 193 | 166, 184 | 137, 189 | 78 | Gradac, B&H (19) | |
| 8 | <i>S. hercegovinae</i> | 3x ^a | CLL3, 6–8, 10–15 | 10 | 186, 200 | 122, 124 | 189, 193 | 158, 202 | 149, 151, 193 | 78 | Crne Lokve, B&H (20) | |
| 9 | | 3x ^a | CLL5, 9 | 2 | 186, 200 | 122, 124 | 189, 193 | 154, 158 | 149, 151, 193 | 78 | Crne Lokve, B&H (20) | |
| 10 | | 3x ^a | CLL2 | 1 | 186, 200 | 122, 124 | 189, 193 | 158 | 151 | 78 | Crne Lokve, B&H (20) | |
| 11 | | 3x ^a | SL1, 2 | 2 | 186, 200 | 122, 124 | 189, 193 | 156 | 151, 189 | 78 | Gradac, B&H (20) | |
| 12 | S. subgen. <i>Tormaria</i> | 3x ^b | 13798_1 | 1 | 186, 196 | 110, 122 | 187, 195 | 154, 158 | 137, 149, 151 | – | Rugovska klisura, Kosovo (25) | |
| 13 | S. subgen. <i>Tormaria</i> | 4x ^a | CLL1 | 1 | 186, 196, 200 | 118, 122, 124 | 189, 193 | 158, 162 | 149, 151, 195 | 78 | Crne Lokve, B&H (20) | |
| 14 | <i>S. milensis</i> | 3x ^c | MIL1–3 | 6 | 188, 196, 200 | 124, 126, 136 | 193, 201 | 158, 160, 182 | 151, 157 | 78 | Milá; Chlumská hora; Czech Republic (7, 9) | |
| 15 | <i>S. rhodantha</i> | 3x ^c | RHOD1–3 | 2 | 186, 190, 196 | 112, 124, 126 | 191, 193 | 156, 160, 178 | 151 | 78 | Kamýk, Czech Republic (11) | |
| 16 | <i>S. albensis</i> | 3x ^c | ALB1, 3 | 2 | 186, 190, 200 | 112, 118, 124 | 193, 201 | 156, 158, 198 | 151 | 78 | Jamolice, Czech Republic (17) | |
| 17 | <i>S. alnifrons</i> | 3x ^c | ALNIF1, 2 | 2 | 186, 190, 200 | 112, 124, 130 | 193, 201 | 156, 160, 196 | 151, 183 | 78 | Kamýk, Czech Republic (11) | |
| 18 | <i>S. bohemica</i> | 3x ^c | BOH1–3 | 3 | 186, 196, 200 | 112, 124, 132 | 193, 201 | 156, 182 | 151 | 78 | Markvarec, Czech Republic (8) | |
| 19 | <i>S. gemella</i> | 3x ^c | GEMI–3 | 3 | 186, 190, 200 | 112, 124 | 195, 201 | 160, 166 | 151, 205 | 78 | Koda, Czech Republic (14) | |
| 20 | <i>S. extimia</i> | 3x ^c | EXIMI–3 | 3 | 186, 196, 200 | 120, 124, 126 | 193, 201 | 156, 158, 182 | 151, 193 | 78 | Úholičky, Czech Republic (12) | |
| 21 | <i>S. omissa</i> | 3x ^c | OMIS1–3 | 3 | 186, 196, 200 | 112, 126, 136 | 191, 193, 201 | 158, 160, 184 | 151 | 78 | Litochovice nad Labem, Czech Republic (10) | |
| 22 | <i>S. portae-bohemicae</i> | 3x ^c | PB1–3 | 3 | 186, 190, 196 | 112, 124 | 191, 193, 201 | 162, 192 | 151, 197 | 78 | Paní hora, Czech Republic (13) | |
| | <i>S. barrandienica</i> | 3x ^c | BARI–3 | 3 | 186, 196 | 112, 124 | 191, 193, 201 | 162, 192 | 151, 197 | 78 | | |
| | Total | | | 53 | | | | | | | | |

^aPloidy level from own flow cytometric measurements.^bPloidy level estimated from nuclear microsatellites.^cPloidy level from Lepšić *et al.* 2019.

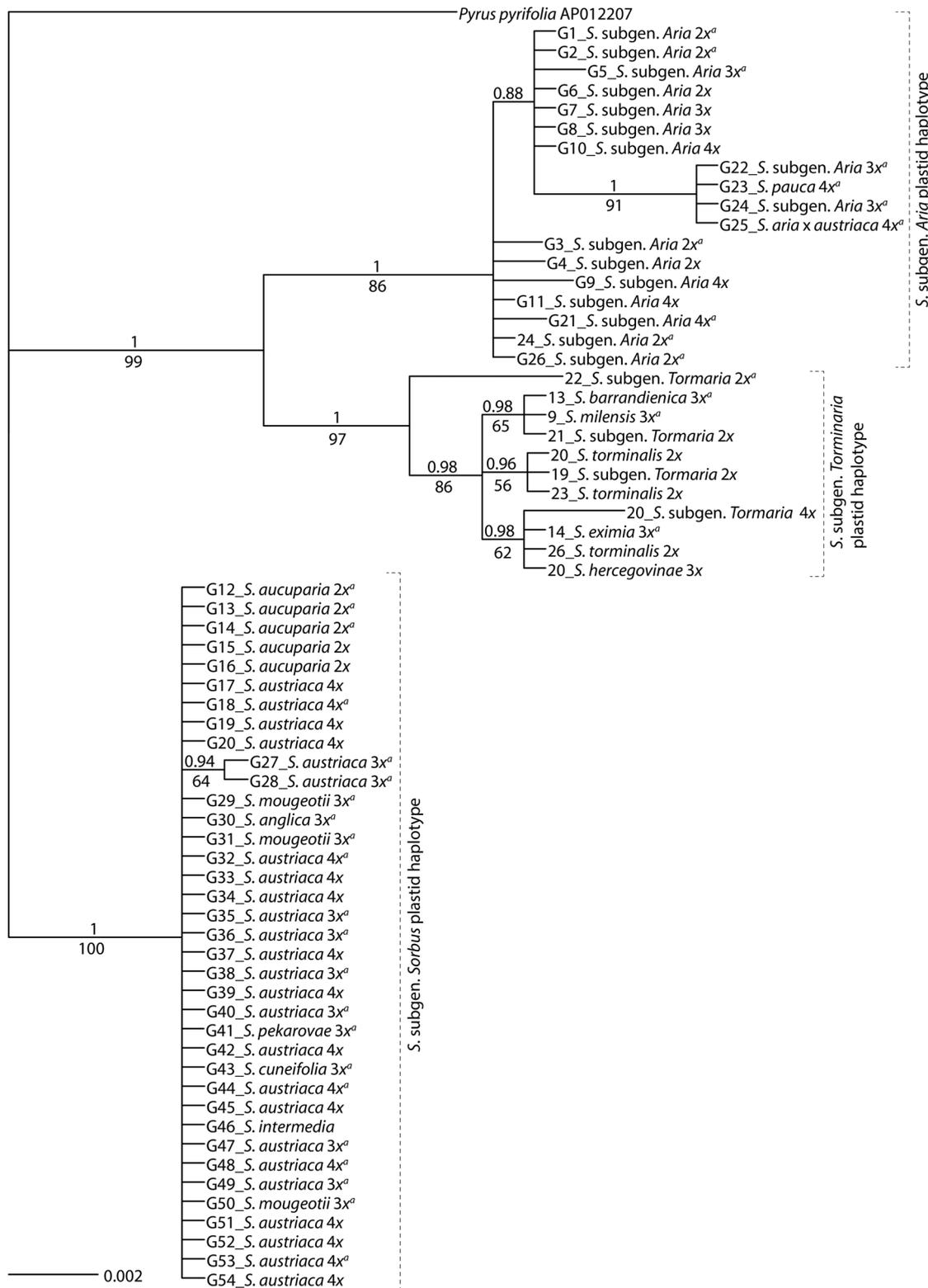


Figure 3. Bayesian consensus phylogram inferred from phylogenetic analyses of plastid *trnT-trnF* sequences. Numbers above branches are PP >0.50 and those below branches MP bootstrap values > 50%. Locality numbers and outgroup haplotypes marked with G1 to G54 correspond to Supporting Information, Data S1. Ploidy level is indicated if available (*ploidy estimated from nuclear microsatellites).

(Supporting Information, Data S4). These similarities are primarily reflected in leaf shape, colour of indumentum, as well as fruit and floral traits. The most pronounced similarities between *S. hercegovinae* and *S. barrandienica* are observed in lamina shape, shape of the lamina apex, the number of lateral leaf veins, fruit

colour, and fruit size. The greatest similarities with *S. bohemica* are in lamina shape, the number of lateral leaf veins, and fruit colour. However, *S. hercegovinae* also possesses distinguishing characteristics that set it apart from both of these species. These include differences in the shape of the lamina apex (*S. hercegovinae* vs.

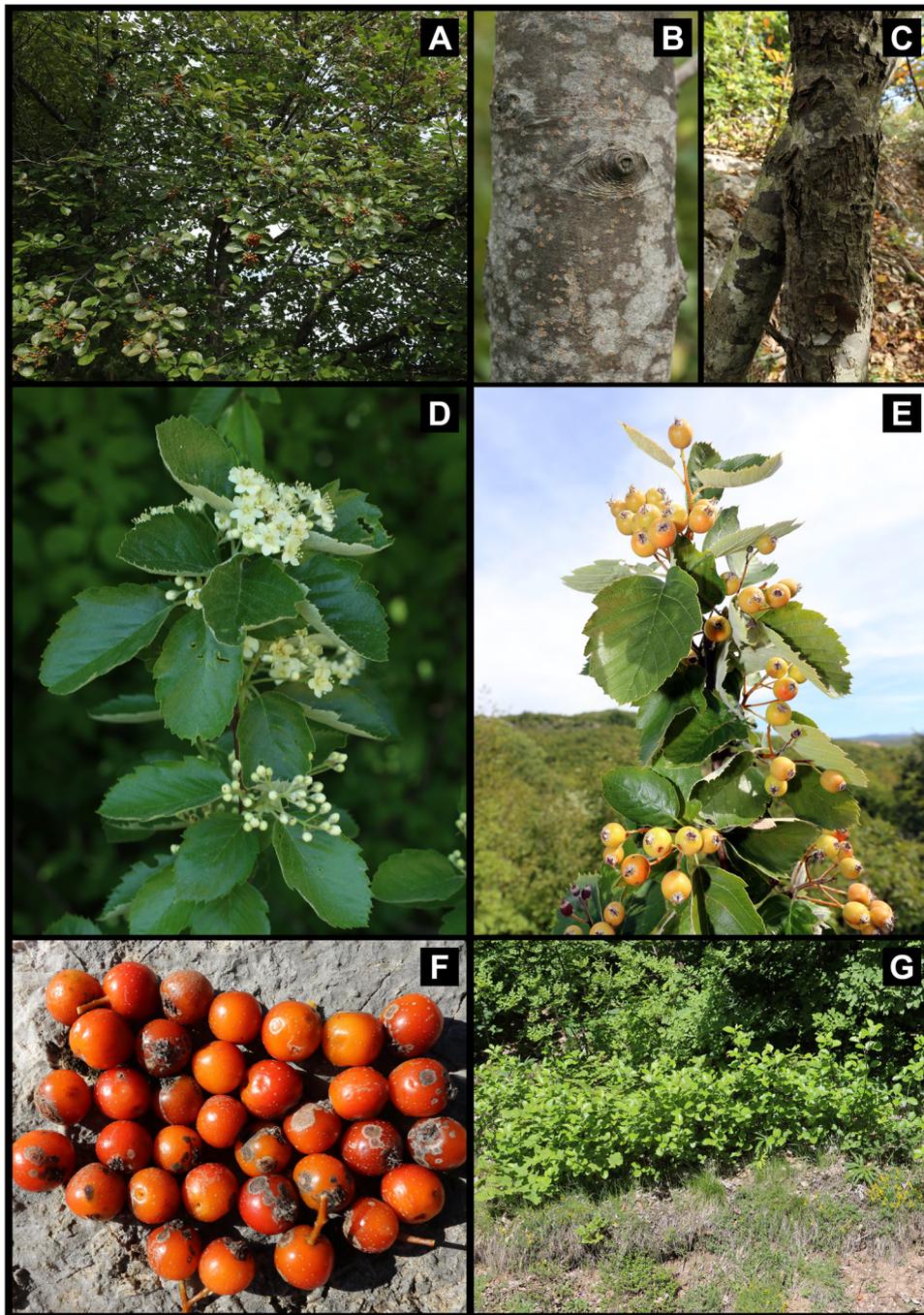


Figure 4. *Sorbus hercegovinae*: A, canopy; B, young tree bark; C, mature tree bark; D, flowering twig; E, twig with unripe fruits; F, ripe fruits; and G, juvenile trees.

S. bohemica) and shape of the lamina base (*S. hercegovinae* vs. *S. barrandienica*). The key traits that distinguish *S. hercegovinae* from the two aforementioned species—as well as from all other compared taxa—are the significantly lower number of lenticels and the point of style fusion in the pistil.

DISCUSSION

Our study based on AFLP fingerprinting, plastid DNA sequences, nuclear microsatellites, and flow cytometric GS estimations revealed a distinct evolutionary lineage within *S.* subgen. *Tormaria*

from the western Balkan Peninsula (Bosnia and Herzegovina), which we describe as a new species, *S. hercegovinae*. This apomictic triploid species is probably one of several lineages within *S.* subgen. *Tormaria*, for which scattered floristic records exist from the Balkan Peninsula (Slovenia, Daksobler *et al.* 2014; Montenegro and Serbia, Tomović *et al.* 2020; North Macedonia, Teofilovski *et al.* 2015, Teofilovski 2017; Bulgaria, Zieliński and Vladimirov 2013). The newly described taxon includes triploids (Fig. 1B; Supporting Information, Data S1); to date, it is the southernmost known polyploid member of *S.* subgen. *Tormaria*. Our data do not only reveal an independent evolutionary origin for *S. hercegovinae*,

but also show clear genetic differentiation among narrow-endemic triploid species of *S.* subgen. *Tormaria* in Central Europe (Czech Republic, Fig. 1B). The exceptions are the species *S. milensis* and *S. rhodanthera* from the Czech Republic (both from the *loci classici*), which are closely clustered in the AFLP NeighborNet (Fig. 1B) and share the same MG (Table 2). *Sorbus milensis* and *S. rhodanthera* represent the most closely related taxa from the *S.* subgen. *Tormaria* group in Bohemia, showing minor morphological and genetic differentiation (Lepší *et al.* 2008). The differences between the present and aforementioned results may be due to the different nuclear microsatellite primers used. Also, the weak differentiation of the two taxa may be due to the same or very similar parental combinations. On the other hand, the diploid members of *S.* subgen. *Tormaria* from the western Balkan Peninsula are genetically closer to parental *S. torminalis* and are likely F1 hybrids between members of *S.* subgen. *Aria* and *S. torminalis*.

Our results are in line with a general pattern of diversification within *Sorbus* in Europe, where polyploid hybridization and apomixis drive diversification at different geographical scales (Lepší *et al.* 2008, 2009, Robertson *et al.* 2010, Hamston *et al.* 2018, Levin *et al.* 2018, Hajrudinović-Bogunić *et al.* 2023). The lack of reproductive barriers among the parental taxa fosters continuous hybridization when they occur in sympatry, producing a series of morphologically similar and genetically related apomictic polyploid species (Robertson *et al.* 2004, 2010, Levin *et al.* 2018). On the other side, genetic uniqueness and stability of polyploid hybrid derivatives are maintained via apomixis over long periods (Lepší *et al.* 2008, 2009, Velebil *et al.* 2022), which led to description of numerous narrow-range endemics of conservation concern (Rivers *et al.* 2019) predominately in Central Europe and Great Britain (Rich *et al.* 2010, Németh 2007, 2012, 2015, Somlyay and Sennikov 2014, Velebil *et al.* 2012, 2022).

Sorbus hercegovinae originated after hybridization between

S. subgen. *Aria* and *S. torminalis* and represents an allotriploid apomictic lineage, similar to most species belonging to *S.* subgen. *Tormaria* in Europe (Lepší *et al.* 2008, 2009, Rich *et al.* 2010, Feulner *et al.* 2013, 2023). Plastid DNA sequences positioned *S. hercegovinae* in a clade with *S. torminalis* (Fig. 3), which acted as maternal parent, similarly as in most other hybridogenous taxa of *S.* subgen. *Tormaria* (Chester *et al.* 2007, Robertson *et al.* 2010, Hamston *et al.* 2018, Feulner *et al.* 2023). Whereas both AFLP data and nuclear microsatellites confirmed the genetic segregation of *S. hercegovinae* from other taxa (BS 100%; Fig. 1B; Supporting Information, Fig. S3), its exact origin remains unclear. The principal mechanism of polyploid formation in *Sorbus* involves hybridization between a sexual diploid and an apomictic polyploid, resulting in heteroploid offspring (Robertson *et al.* 2010, Ludwig *et al.* 2013, Hajrudinović *et al.* 2015a, Lepší *et al.* 2019). In most hybridizations across Europe, the polyploid male parent is a member of *S.* subgen. *Aria* [for example, *S. baldacci* (C.K.Schneid.) Zinserl., *S. collina* M.Lepší, P.Lepší & N.Mey., *S. danubialis* (Jáv.) Prodan, *S. rupicola* (Syme) Hedl.], pollinating sexual diploid *S. torminalis* (Velebil *et al.* 2022, Feulner *et al.* 2023). However, we have not observed any polyploid member of *S.* subgen. *Aria* in the studied area (see also Hajrudinović *et al.* 2015b). This suggests that *S. hercegovinae* may have arisen after hybridization between the two diploid parents present in the Posušje area, thus involving

polyploidization via unreduced gametes of either parent (Ramsey and Schemske 1998). Alternatively, polyploids of *S.* subgen. *Aria* got extinct in the study area or have not been discovered; the ploidy was screened in only 28 individuals from this area (Supporting Information, Data S1; Hajrudinović *et al.* 2015a). Finally, as the nearest known population of *S.* subgen. *Aria* containing di-, tri-, and tetraploids is at Bosiljna (Hajrudinović *et al.* 2015a), which is only 4 km from Crne Lokve, we cannot exclude long-distance transfer of pollen from this locality.

Genetic variability within *S. hercegovinae*, evident both from AFLP (Fig. 1B) as well as microsatellite data (Table 2), is probably a result of the complex breeding system (Hamston *et al.* 2018). Low values of genetic variability, namely the existence of several MGs shared by different individuals (Table 2) confirm predominantly apomictic reproduction. While *Sorbus* polyploids are characterized by pseudogamous apomixis and rarely retain sexual reproduction (Robertson *et al.* 2010, Hamston *et al.* 2018), our FCSS confirmed that sexual reproduction is present in *S. hercegovinae* (Table 1, Fig. 2). Sexual reproduction of *S. hercegovinae* involves three different embryo and endosperm formation pathways with contribution of heteroploid gametes (Table 1, Fig. 2). As a consequence of interploid crossings, the sexually originated seeds of triploid *S. hercegovinae* include tetraploid embryos (and one pentaploid), indicating a dynamic nature of the breeding system through ongoing gene exchange and hybridization. However, it remains questionable whether sexual seeds from triploids are viable due to unbalanced genome sizes of embryo and endosperm (Lepší *et al.* 2019). Half of the analysed seeds of triploid *S. hercegovinae* are apomictic, while the remaining seeds, which are of sexual origin, consistently exhibit higher embryo ploidy levels (Table 2). It is evident that the new triploid generation is maintained clonally and will likely predominate as mature individuals in natural populations. The detection of only a single tetraploid *S.* subgen. *Tormaria* individual in the *S. hercegovinae* population indicates a low rate of survival and persistence of such individuals. Furthermore, the existence of different MGs within *S. hercegovinae* (Table 2) may suggest independent hybridization events of the same parental combination or reflects ‘clone mates’ (Paun *et al.* 2006) that have accumulated somatic mutations over time.

The morphological differentiation among *S. hercegovinae* and the other related taxa is not strongly pronounced, but rather manifested as mosaic and gappy variation in morphological character states (Supporting Information, Data S4). *Sorbus hercegovinae* is morphologically differentiated to a similar extent from the other taxa, as they are among each-other. This type of overlapping variation probably results from recurrent hybridization involving the same or similar parental combinations—*S. torminalis* and the same or different members of *S.* subgen. *Aria*—the exact parental origins of which often remain unidentified. However, certain traits, such as the low number of fruit lenticels and the long length of the fusion of two styles in the pistil, are found only in *S. hercegovinae*. Altogether, several lines of evidence, including morphological divergence combined with allotriploidy, a clonal mode of reproduction, and a restricted geographic distribution significantly distant from the nearest known populations of other polyploid *Tormaria* species, justify a distinct position within *S.* subgen. *Tormaria*, which includes apomictic taxa across Europe. This finding,



Figure 5. Holotype of *Sorbus hercegovinae* (SARA 385).

along with previous studies (Hajrudinović *et al.* 2015a, 2015b, Hajrudinović-Bogunić *et al.* 2023), highlights the Balkan Peninsula as one of the hotspots of *Sorbus* diversity. The sites harbouring mixtures of species, cytotypes, and the interacting sexual and asexual lineages represent potential reservoirs of novel biodiversity, which are valuable from a biodiversity conservation perspective (Ennos *et al.* 2005, 2012).

Taxonomic treatment

Sorbus hercegovinae Bogunić, Hajrud., Frajman, Schönsw., Siljak-Yak. & Begić, *sp. nov.* (Figs 4, 5). Type: Bosna and Herzegovina, western Herzegovina: Crne Lokve, 2.5 km east of Posušje,

700 m, 43.440833° N, 17.464167° E. Leg. F. Bogunić, A. Hajrudinović-Bogunić & A. Begić, 19 May 2015. Holotype: SARA 385. Isotype: W 0356951 (<https://w.jacq.org/W0356951>).

An individual (CLL3) belonging to the most common multi-locus genotype (MG8) within the population was selected as the type specimen of *S. hercegovinae*.

Description: Small, mostly monocormous tree up to 8 m, with ovate to rounded crown. Trunks up to 35 cm in diameter. Bark grey to dark grey, smooth when young, vertical fissures present at maturity (particularly at the trunk base), bark lenticels 6–8(–11) mm long and (3–)5–7(–12) mm wide. Twigs greyish-brown to brown and glabrous, young shoots pale brown, with numerous small

lenticels and sparsely tomentose. Buds 6–14 mm long, 3–6 mm wide, ovoid; scales yellow-greenish to pale brown with narrow brown tomentose margins. Leaves (of sterile short shoots) simple, upper surface dark green and glabrous, lower surface greyish-green tomentose; lamina (65–)74–83(–84) mm long, (44–)55–64(–68) mm wide, 1.2–1.4 times as long as wide, ovate elliptical to broadly ovate elliptical; apex acute to subacute, base broadly cuneate to broadly rounded, lobed with acute or acuminate lobes, first lobe (1.8–)1.9–3.8(–4.2) mm long, second lobe (2–)3.8–5.9(–7.2) mm long, third lobe (3.6–)3.9–6.3(–6.4) mm long; margins mostly double to triple serrate with acuminate teeth; secondary veins 14–16(–17), first vein (from the lamina base) at an angle (50–)52–58(–62)°, the second at (40–)41–48(–51)°, and the third at (31–)33–39(–42)°. Petioles (9.6–)9.8–14(–17) mm long, sparsely tomentose. Inflorescence up to 10 cm in diameter, moderately dense or lax, branchlets densely tomentose, with (21–)22–35(–43) flowers. Sepals triangular, acuminate to acute, densely tomentose on both sides, (1.7–)2–2.9(–3.1) mm long, (1.5–)1.6–2.6(–2.7) mm wide. Petals elliptic to ovate, white, pilose at base of upper surface, (5.2–)5.3–7.4(–7.8) mm long, 4.1–5.2(–5.3) mm wide. Stamens ~20, anthers creamy yellow. Styles two, mostly connate up to near the tip, rarely free, tomentose at base, (2.3–)2.5–4.5(–4.6) mm long. Fruits (10.9–)11.1–12.8(–13.1) mm long and (10.8–)11–12.4(–13) mm wide, fruit length/width ratio 0.9–1.1, globose to subglobose, with (5–)5.2–9.7(–11) medium sized lenticels per 0.5 cm², orange-red to reddish when mature. DNA-ploidy triploid, reproduction facultatively apomictic. Flowers in May and fruits ripe in September to October.

Distribution and ecology: Surroundings of Gradac and Crne Lokve villages, ~2.5 km east of the town Posušje (south-western Bosnia and Herzegovina). The species covers an area of ~30 km² with an altitudinal range from 630 to 900 m a.s.l., with south-east and south-west exposure. The population includes ~50 adult fructiferous individuals and many juveniles, which are scattered across its range. Plants thrive in a mosaic of mixed and degraded submediterranean forests of *Quercus pubescens* Willd., *Q. cerris* L., *Carpinus orientalis* L., and *Fraxinus ornus* L., which are in various stages of degradation (coppices and scrublands), on calcareous dolomitic substrate and shallow stony soils (calcic cambisols). The most frequent accompanying species are *Acer obtusatum* Waldst. et Kit., *Acer hyrcanum* Fisch. & C.A.Mey., *Astragalus monspessulanus* subsp. *illyricus* (Bernh.) Chater, *Centaurea montana* L., *C. rupestris* L., *Genista sylvestris* subsp. *dalmatica* (Bartl.) H. Lindb., *Juniperus oxycedrus* L., *Petteria ramentacea* (Sieber) C. Presl., *Sorbus aria* (L.) Crantz, *S. torminalis* (L.) Crantz., and *Spiraea cana* Waldst. et Kit.

Etymology: The epithet *hercegovinae* refers to Hercegovina (as a geographical part of Bosnia and Herzegovina), where the new species has its *locus classicus*.

Conservation status: Based on the current knowledge of the species' distribution with extent of occurrence and the area of occupancy of only 30 km² and including ~50 individuals, *S. hercegovinae* should be classified as critically endangered (CRD; IUCN, 2012).

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AUTHOR CONTRIBUTIONS

All authors made a substantial contribution to the concept and design of the study, to data collection, analysis, and interpretation, writing and revising the paper, and adding intellectual content.

SUPPLEMENTARY DATA

Supplementary data is available at *Botanical Journal of the Linnean Society* online.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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DATA AVAILABILITY STATEMENT

The data underlying this article are available in the article, its online [supplementary material](#), deposited in GenBank (DNA sequences) or will be shared on request by the corresponding authors (AFLP data).

REFERENCES

- Arrigo N, Tuszynski JW, Ehrich D *et al.* 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* 2009; **10**:33. <https://doi.org/10.1186/1471-2105-10-33>
- Bjedov I, Obratov-Petković D, Mišić D *et al.* Genetic patterns in range-edge populations of *Vaccinium* species from the central Balkans: implications on conservation prospects and sustainable usage. *Silva Fennica* 2015; **49**:1283. <https://doi.org/10.14214/sf.1283>
- Bourge M, Brown SC, Siljak-Yakovlev S. Flow cytometry as tool in plant sciences, with emphasis on genome size and ploidy level assessment. *Genetics & Applications* 2018; **2**:1–12. <https://doi.org/10.31383/ga.vol2iss2pp1-12>
- Chester M, Cowan SC, Fay M *et al.* Parentage of endemic *Sorbus* L. (Rosaceae) species in the British Isles: evidence from plastid DNA. *Botanical Journal of the Linnean Society* 2007; **154**:291–304. <https://doi.org/10.1111/j.1095-8339.2007.00669.x>
- Dakskobler I, Kutnar L, Zupančič M. *Toploljubni listnati gozdovi v Sloveniji: toploljubni gozdovi kraškega gabra, puhastega hrasta, gradna, črnega gabra in malega jesena v submediteranskem fitogeografskem območju in ponekod v notranjosti države*. Ljubljana: Silva Slovenica, Gozdarski inštitut Slovenije, 2014.

- Ennos RA, French GC, Hollingsworth PM. Conserving taxonomic complexity. *Trends in Ecology & Evolution* 2005;**20**:164–8. <https://doi.org/10.1016/j.tree.2005.01.012>
- Ennos RA, Whitlock R, Fay MF *et al.* Process-based species action plans: an approach to conserve contemporary evolutionary processes that sustain diversity in taxonomically complex groups. *Botanical Journal of the Linnean Society* 2012;**168**:194–203. <https://doi.org/10.1111/j.1095-8339.2011.01206.x>
- Feulner M, Aas G, Urbon T *et al.* Low rates of apomixis and polyploidy in progeny of Thuringian *Sorbus* subgenus *Tormaria*. *Plant Systematics and Evolution* 2023;**309**: <https://doi.org/10.1007/s00606-023-01850-6>.
- Feulner M, Liede-Schumann S, Meve U *et al.* Genetic structure of *Sorbus latifolia* (Lam.) Pers. taxa endemic to Northern Bavaria. *Plant Systematics and Evolution* 2013;**299**:1065–74. <https://doi.org/10.1007/s00606-013-0778-x>
- Fischer MA, Oswald K, Adler W. *Exkursionsflora für Österreich, Liechtenstein und Südtirol*. 3rd edn. Linz: Biologiezentrum der Oberösterreichischen Landesmuseen, 2008.
- Hajrudinović A, Bašić N, Bogunić F. *Sorbus latifolia* (Rosaceae): a new species in the flora of Bosnia and Herzegovina. *Academy of Sciences and Arts of Bosnia and Herzegovina, Special Editions CXLVIII, Proceedings* 2012;**22**:175–86.
- Hajrudinović A, Frajman B, Schönschwetter P *et al.* Towards a better understanding of polyploid *Sorbus* (Rosaceae) from Bosnia and Herzegovina (Balkan Peninsula), including description of a novel, tetraploid apomictic species. *Botanical Journal of the Linnean Society* 2015a;**178**:670–85. <https://doi.org/10.1111/boj.12289>
- Hajrudinović A, Siljak-Yakovlev S, Brown SC *et al.* When sexual meets apomict: genome size, ploidy level and reproductive mode variation of *Sorbus aria* s.l. and *S. austriaca* (Rosaceae) in Bosnia and Herzegovina. *Annals of Botany* 2015b;**116**:301–12. <https://doi.org/10.1093/aob/mcv093>
- Hajrudinović-Bogunić A, Frajman B, Schönschwetter P *et al.* Apomictic mountain whitebeam (*Sorbus austriaca*, Rosaceae) comprises several genetically and morphologically divergent lineages. *Biology* 2023;**12**:380. <https://doi.org/10.3390/biology12030380>
- Hammer Ø, Harper DAT, Ryan PD. PAST: paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* 2001;**4**:1–9. http://palaeo-electronica.org/2001_1/past/issue1_01.htm
- Hamston TJ, de Vere N, King AR *et al.* Apomixis and hybridization drives reticulate evolution and phyletic differentiation in *Sorbus* L.: implications for conservation. *Frontiers in Plant Science* 2018;**9**:1796. <https://doi.org/10.3389/fpls.2018.01796>
- Huson DH. SplitsTree: a program for analyzing and visualizing evolutionary data. *Bioinformatics* 1998;**14**:68–73. <https://doi.org/10.1093/bioinformatics/14.1.68>
- Jovanović B. Rod *Sorbus* L. In: Josifović M. (ed.), *Flora of SR Serbia Vol. 4*. Beograd: Srpska akademija Nauka i Umetnosti, Odeljenje Prirodno-Matematičkih Nauka, 1972, 150–60.
- Kearse M, Moir R, Wilson A *et al.* Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics (Oxford, England)* 2012;**28**:1647–9. <https://doi.org/10.1093/bioinformatics/bts199>
- Kryštufek B, Reed JM. Pattern and process in Balkan biodiversity—an overview. In: Griffiths HI, Kryštufek B, Reed JM (eds), *Balkan Biodiversity: Pattern and Process in the European Hotspot*. Dordrecht: Kluwer, 2004, 217. https://doi.org/10.1007/978-1-4020-2854-0_1
- Kurtto A, Sennikov AN, Lampinen R (eds), *Atlas Florae Europaeae. Distribution of Vascular Plants in Europe, 17. Rosaceae (Sorbus s. lato)*. Helsinki: The Committee for Mapping the Flora of Europe & Societas Biologica Fennica Vanamo, 2018.
- Kurtto A Rosaceae (pro parte majore). In: *Euro+Med Plantbase—the information resource for Euro-Mediterranean plant diversity*, 2009. Available at: <http://ww2.bgbm.org/EuroPlusMed/> (15 August 2024, date last accessed).
- Kuzmanović N, Lakušić D, Stevanoski I *et al.* *Carpinus austrobalcanica*—a new highly polyploid species from the Balkan Peninsula closely related to European hornbeam. *Perspectives in Plant Ecology, Evolution and Systematics* 2024;**64**:125812. <https://doi.org/10.1016/j.ppees.2024.125812>
- Lakušić D, Rakić T, Stefanović S *et al.* *Edraianthus × lakusicii* (Campanulaceae) a new intersectional natural hybrid: Morphological and molecular evidence. *Plant Systematics and Evolution* 2009;**280**:77–88. <https://doi.org/10.1007/s00606-009-0168-6>
- Lazarević M, Kuzmanović N, Lakušić D *et al.* Patterns of cytotype distribution and genome size variation in the genus *Sesleria* Scop. (Poaceae). *Botanical Journal of the Linnean Society* 2015;**179**:126–43. <https://doi.org/10.1111/boj.12306>
- Lazarević M, Siljak-Yakovlev S, Sanino A *et al.* Genetic variability in Balkan paleoendemic resurrection plants *Ramonda serbica* and *R. nathaliae* across their range and in the zone of sympatry. *Frontiers in Plant Science* 2022;**13**:873471. <https://doi.org/10.3389/fpls.2022.873471>
- Leitch AR, Leitch IJ. Genomic plasticity and the diversity of polyploid plants. *Science* 2008;**320**:481–3. <https://doi.org/10.1126/science.1153585>
- Lepšić M, Koutecký P, Nosková J *et al.* Versatility of reproductive modes and ploidy level interactions in *Sorbus* s.l. (Malinae, Rosaceae). *Botanical Journal of the Linnean Society* 2019;**191**:502–22. <https://doi.org/10.1093/botlinnean/boz054>
- Lepšić M, Vit P, Lepšić P *et al.* *Sorbus milensis*, a new hybridogenous species from northwestern Bohemia. *Preslia* 2008;**80**:229–44.
- Lepšić M, Vit P, Lepšić P *et al.* *Sorbus portae-bohemicae* and *Sorbus albensis*, two new apomictic endemic species recognized based on revision of *Sorbus bohemica*. *Preslia* 2009;**81**:63–89.
- Levin J, Fay MF, Pellicer J *et al.* Multiple independent origins of intermediate species between *Sorbus aucuparia* and *S. hybrida* (Rosaceae) in the Baltic region. *Nordic Journal of Botany* 2018;**36**: <https://doi.org/10.1111/njb.02035>
- Lewis PO. A likelihood approach to estimating phylogeny from discrete morphological character data. *Systematic Biology* 2001;**50**:913–25. <https://doi.org/10.1080/106351501753462876>
- Ludwig S, Robertson A, Rich TC *et al.* Breeding systems, hybridization and continuing evolution in Avon Gorge *Sorbus*. *Annals of Botany* 2013;**111**:563–75. <https://doi.org/10.1093/aob/mct013>
- Marie D, Brown SC. A cytometric exercise in plant DNA histograms, with 2C values for 70 species. *Biology of the Cell* 1993;**78**:41–51. [https://doi.org/10.1016/0248-4900\(93\)90113-s](https://doi.org/10.1016/0248-4900(93)90113-s)
- Meirmans PG, Van Tienderen PH. GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms. *Molecular Ecology Notes* 2004;**4**:792–4. <https://doi.org/10.1111/j.1471-8286.2004.00770.x>
- Meyer N, Meierott L, Schuwerk H *et al.* Beiträge zur Gattung *Sorbus* in Bayern. *Berichte der Bayerischen Botanischen Gesellschaft, Sonderband* 2005;**75**:5–216.
- Müller K. SeqState: primer design and sequence statistics for phylogenetic DNA datasets. *Applied Bioinformatics* 2005;**4**:65–9. <https://doi.org/10.2165/00822942-200504010-00008>
- Nei M, Li WH. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences of the United States of America* 1979;**76**:5269–73. <https://doi.org/10.1073/pnas.76.10.5269>
- Németh C. *Sorbus tobani* NÉMETH, a new hybridogeneous species of the genus *Sorbus* L. emend. CR. from the Bakony Mountains, Hungary (in Hungarian). *Flora Pannonica Journal of Phytogeography & Taxonomy* 2007;**5**:173–84.
- Németh C. Two new *Sorbus* (Rosaceae) species from the Bakony Mts, Hungary. *Acta Botanica Hungarica* 2012;**54**:131–44. <https://doi.org/10.1556/ABot.54.2012.1-2.15>
- Németh C. Taxonomical revision of *Sorbus pseudosemiincisa* (Rosaceae), a stenoendemic whitebeam from the Vértes Mts (Hungary), with the description of a new species, *Sorbus pyricarpa*. *Studia Botanica Hungarica* 2015;**46**:157–74. <https://doi.org/10.17110/StudBot.2015.46.2.157>
- Nieto Feliner G. Southern European glacial refugia: a tale of tales. *Taxon* 2011;**60**:365–72. <https://doi.org/10.1002/tax.602007>
- Niketić M, Đurović SZ, Tomović G *et al.* Diversification within ploidy-variable Balkan endemic *Cerastium decalvans* (Caryophyllaceae) reconstructed based on genetic, morphological and ecological evidence.

- Botanical Journal of the Linnean Society* 2022; **199**:578–608. <https://doi.org/10.1093/botlinnean/boab037>
- Nikolić T. *Flora Croatica 3—vaskularna flora Republike Hrvatske*. Zagreb: ALFA d.d., 2020.
- Nylander JAA. *MrAIC.pl. Program Distributed by the Author*. Uppsala: Evolutionary Biology Centre, Uppsala University, 2004.
- Olšavská K, Slovák M, Marhold K *et al.* On the origins of Balkan endemics: the studies on complex evolutionary history of the *Cyanus napulifer* group (Asteraceae). *Annals of Botany* 2016; **118**:1071–88. <https://doi.org/10.1093/aob/mcw142>
- Paun O, Greilhuber J, Temsch E *et al.* Patterns, sources and ecological implications of clonal diversity in apomictic *Ranunculus carpaticola* (*Ranunculus auricomus* complex, Ranunculaceae). *Molecular Ecology* 2006; **15**:897–910. <https://doi.org/10.1111/j.1365-294X.2006.02800.x>
- R Core Team. R: a language and environment for statistical computing. R Foundation for Statistical Computing: Vienna, Austria, 2022. <http://www.r-project.org/>
- Ramsey J, Schemske DW. Pathways, mechanisms, and rates of polyploid formation in flowering plants. *Annual Review of Ecology and Systematics* 1998; **29**:467–501. <https://doi.org/10.1146/annurev.ecolsys.29.1.467>
- Renny-Byfield S, Kovarik A, Kelly LJ *et al.* Diploidization and genome size change in allopolyploids is associated with differential dynamics of low- and high-copy sequences. *The Plant Journal: For Cell and Molecular Biology* 2013; **74**:829–39. <https://doi.org/10.1111/tpj.12168>
- Rešetnik I, Schönswetter P, Temunović M *et al.* Diploid chastity vs. polyploid promiscuity—Extensive gene flow among polyploid cytotypes blurs genetic, morphological and taxonomic boundaries among Dinaric taxa of *Knautia*. *Perspectives in Plant Ecology, Evolution and Systematics* 2023; **59**:125730. <https://doi.org/10.1016/j.ppees.2023.125730>
- Rešetnik I, Španiel S. Plants of the Balkan Peninsula in space and time. *Plant Systematics and Evolution* 2022; **308**:39. <https://doi.org/10.1007/s00606-022-01830-2>
- Rich TCG, Houston L, Robertson A *et al.* *Whitebeams, Rowans and Service Trees of Britain and Ireland. A Monograph of British and Irish Sorbus L.* BSBI Handbook No. 14, 1st edn. London: Botanical Society of the British Isles, 2010.
- Rivers MC, Beech E, Bazos I *et al.* *European Red List of Trees*. Cambridge, UK and Brussels, Belgium: IUCN. 2019. <https://doi.org/10.2305/IUCN.CH.2019.ERL.1.en>
- Robertson A, Newton AC, Ennos RA. Multiple hybrid origins, genetic diversity and population genetic structure of two endemic *Sorbus* taxa on the Isle of Arran, Scotland. *Molecular Ecology* 2004; **13**:123–34. <https://doi.org/10.1046/j.1365-294X.2003.02025.x>
- Robertson A, Rich TCG, Allen AM *et al.* Hybridization and polyploidy as drivers of continuing evolution and speciation in *Sorbus*. *Molecular Ecology* 2010; **19**:1675–90. <https://doi.org/10.1111/j.1365-294X.2010.04585.x>
- Ronquist F, Teslenko M, van der Mark P *et al.* MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 2012; **61**:539–42. <https://doi.org/10.1093/sysbio/sys029>
- Sennikov AN, Kurtto A. A phylogenetic checklist of *Sorbus s.l.* (Rosaceae) in Europe. *Memoranda Societatis Pro Fauna et Flora Fennica* 2017; **93**:1–78. <http://hdl.handle.net/10138/230914>
- Shimizu-Inatsugi R, Terada A, Hirose K *et al.* Plant adaptive radiation mediated by polyploid plasticity in transcriptomes. *Molecular Ecology* 2017; **26**:193–207. <https://doi.org/10.1111/mec.13738>
- Siljak-Yakovlev S, Pustahija F, Šolić ME *et al.* Towards a genome size and chromosome number database of Balkan flora: C-values in 343 taxa with novel values for 242. *Advanced Science Letters* 2010; **3**:190–213. <https://doi.org/10.1166/asl.2010.1115>
- Simmons MP, Ochoterena H. Gaps as characters in sequence-based phylogenetic analyses. *Systematic Biology* 2000; **49**:369–81. <https://doi.org/10.1093/sysbio/49.2.369>
- Somlyay L, Sennikov A. Atlas Florae Europaeae notes 23. The typification and revised taxonomic circumscription of *Sorbus bakonyensis* (Rosaceae), with a description of *Sorbus udvardyana*, a new apomictic species endemic to Hungary. *Phytotaxa* 2014; **164**:265–75. <http://dx.doi.org/10.11646/phytotaxa.164.4.5>
- Suda J, Krahulcová A, Trávníček P *et al.* Ploidy level versus DNA ploidy level: an appeal for consistent terminology. *Taxon* 2006; **55**:447–50. <https://doi.org/10.2307/25065591>
- Swofford D. PAUP. Phylogenetic Analysis Using Parsimony (and Other Methods), Version 4.0b10. Sinauer Associates: Sunderland, USA, 2002. <https://doi.org/10.1111/j.0014-3820.2002.tb00191.x>
- Španiel S, Marhold K, Zozomová-Lihová J. The polyploid *Alyssum montanum-A. repens* complex in the Balkans: a hotspot of species and genetic diversity. *Plant Systematics and Evolution* 2017; **303**:1443–65. <https://doi.org/10.1007/s00606-017-1470-3>
- Taberlet P, Gielly L, Pautou G *et al.* Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 1991; **17**:1105–9. <https://doi.org/10.1007/BF00037152>
- Tel-Zur N, Abbo S, Myslabodski D *et al.* Modified CTAB procedure for DNA isolation from epiphytic cacti of genera *Hylocereus* and *Selenicereus* (Cactaceae). *Plant Molecular Biology Reporter* 1999; **17**:249–54. <https://doi.org/10.1023/A:1007656315275>
- Teofilovski A. Contribution to knowledge of the flora of the Republic Macedonia. *Botanica Serbica* 2017; **41**:99–103. [10.5281/zenodo.456499](https://doi.org/10.5281/zenodo.456499)
- Teofilovski A, Zieliński J, Vladimirov V. Report 106. In: Vladimirov V, Dane F, Matevski V, Tan K (eds), *New Floristic Records in the Balkans, Vol. 27*. Phytologia Balcanica 2015; 21:212.
- Tomović G, Sabovljević MS, Djokić I *et al.* New records and noteworthy data of plants, algae and fungi in SE Europe and adjacent regions, 2. *Botanica Serbica* 2020; **44**:251–9. <https://doi.org/10.2298/BOTSERB2002251T>
- Van de Peer Y, De Wachter R. Construction of evolutionary distance trees with TREECON for Windows: accounting for variation in nucleotide substitution rate among sites. *Computer Applications in the Biosciences: CABIOS* 1997; **13**:227–30. <https://doi.org/10.1093/bioinformatics/13.3.227>
- Velebil J. *Sorbus omissa*, a new endemic hybridogenous species from the lower Vltava river valley. *Preslia* 2012; **84**:375–90.
- Velebil J, Lepší M, Nosková J *et al.* Taxonomic assessment of *Sorbus* subgenus *Aria* in the Malé Karpaty Mountains. *Preslia* 2022; **94**:305–34. <https://doi.org/10.23855/preslia.2022.305>
- Vos P, Hogers R, Bleeker M *et al.* AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research* 1995; **23**:4407–14. <https://doi.org/10.1093/nar/23.21.4407>
- Warburg EF, Kárpáti ZE. *Sorbus L.* In: Tutin TG, Heywood VH, Burgess NA, Moore DM, Valentine DH, Walters SM, Webb DA (eds), *Flora Europaea*, 2nd edn. Cambridge: Cambridge University Press, 1968, 67–71.
- Weiss-Schneeweiss H, Emadzade K, Jang TS *et al.* Evolutionary consequences, constraints and potential of polyploidy in plants. *Cytogenetic and Genome Research* 2013; **140**:137–50. <https://doi.org/10.1159/000351727>
- Zieliński J, Vladimirov V. *Sorbus × latifolia* (Rosaceae) in the Balkan peninsula and SW Asia. *Phytologia Balcanica* 2013; **19**:39–46.