High genetic and morphological diversification of the *Euphorbia verrucosa* alliance (Euphorbiaceae) in the Balkan and Iberian peninsulas

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**Abstract** We explored the diversification of the southern European *Euphorbia verrucosa* alliance applying molecular (amplified fragment length polymorphism fingerprinting [AFLP], sequencing of the nuclear ribosomal internal transcribed spacer), karyological (relative genome size estimations, chromosome counts) and morphometric methods. The AFLP data inferred four main phylogenetic lineages corresponding to western-southern Balkan *E. montenegrina*, central Balkan *E. serpentina*, northern Balkan–central European–north Italian *E. verrucosa* and Iberian–southern French *E. flavicoma*. Genetic diversification is strongest within the Iberian and the Balkan peninsulas, suggesting Pleistocene persistence of the species in different micro-refugia. In contrast, weak genetic structure in *E. verrucosa* suggests Holocene (after last glacial maximum) expansion to central and western Europe, likely from a northern Balkan refugium. Karyological data provide evidence for tetraploidisation events in *E. flavicoma* and *E. montenegrina*, but not in *E. verrucosa* and *E. serpentina*. By integrating phylogenetic data with multivariate morphometric analyses, we propose a new taxonomic treatment for this group, mainly by recognising the Balkan endemics *E. montenegrina* and *E. serpentina* as independent species and by redefining the distributions of *E. flavicoma* and *E. verrucosa*. Our study underlines the importance of the Balkan and Iberian peninsulas as major Pleistocene refugia.

**Keywords** AFLP; Mediterranean; morphometrics; phylogeography; polyploid evolution; relative genome size; taxonomy

**Supporting Information** may be found online in the Supporting Information section at the end of the article.

## INTRODUCTION

The Iberian, the Apennine and the Balkan peninsulas have been important glacial refugia, where distinct genetic lineages persisted through Pleistocene and Quaternary climatic fluctuations (Bilton et al., 1998; Hewitt, 1999, 2011; Petit et al., 2003; Schmitt, 2007; Nieto Feliner, 2014). They are recognised as important hotspots of genetic diversity (Petit et al., 2003) and areas of high endemism (Bilton et al., 1998; Thompson et al., 2005). Especially the Balkan and the Iberian peninsulas served as source areas for the postglacial recolonization of central and northern Europe (Taberlet et al., 1998; Hewitt, 1999; Schmitt, 2007). A number of temperate tree species such as buckthorn (*Frangula alnus* Miller; Hampe et al., 2003), common ash (*Fraxinus excelsior* L.; Heuertz & al., 2004), beech (*Fagus sylvatica* L.; Magri et al., 2006; Willner et al., 2009), hornbeam (*Carpinus betulus* L.; Grivet & Petit, 2003) and oaks (*Quercus* spp.; Petit et al., 2002), along with forest understory species (e.g., Willner et al., 2009; Bardy & al., 2010; Slovák & al., 2012; Rešetnik & al., 2016), colonised large parts of central and northern Europe from the Balkan Peninsula. In contrast, plant species that persisted in the Iberian Peninsula during the glaciations mostly colonised only north-easterly adjacent areas in France (Bucci et al., 2007; Magri et al., 2007), exceptions being white oaks (Petit et al., 2002) and ecologically specialised coastal plants (Kadereit & al., 2005; Berjano et al., 2015). Whereas widespread European forest species have received considerable attention in phylogeographic studies, little is known about the reaction of widespread temperate grassland species below the timberline to climate changes and how they expanded their range across Europe.

One of the widespread temperate grassland species in Europe is *Euphorbia verrucosa* L. (Euphorbiaceae), distributed from the southern Balkan Peninsula in Greece through the western Balkan Peninsula and the northern Apennine...
Penninsula as well as central Europe east, north and west of the Alps, to the northern Iberian Peninsula (Pyrenees) in Spain (Hegi, 1966; Radcliffe-Smith & Tutin, 1968; Meusel & al., 1978; Simon & Vicens, 1999; Govaerts & al., 2000; Cresti & al., 2019). A species considered closely related to or conspecific with *E. verrucosa* is *E. flavicoma* DC., which is bound to Mediterranean vegetation types and was suggested to be distributed from the Iberian Peninsula through southern France to northwestern Italy (Hegi, 1966; Radcliffe-Smith & Tutin, 1968; Meusel & al., 1978; Simon & Vicens, 1999, Govaerts & al., 2000; Pignatti, 2017; Bartolucci & al., 2018). Recently, Cresti & al. (2019) have shown that *E. flavicoma* is phylogenetically and morphologically distinct from *E. verrucosa* and also has a divergent genome size, but it remains unclear where the geographic boundary between these two taxa is situated, especially if the former reaches northwestern Italy. In addition, taxonomic value and relationships of the different subspecies of *E. flavicoma* (Simon & Vicens, 1999; Govaerts & al., 2000) remain to be established.

*Euphorbia montenegrina* (Bald.) K.Malý from mountainous areas in Montenegro in the southwestern Balkan Peninsula (Govaerts & al., 2000) was considered closely related to *E. epithymoides* L. (Radcliffe-Smith & Tutin, 1968), but phylogenetic studies (Frajman & Schönswetter, 2011; Riina & al., 2013; Cresti & al., 2019) have shown that it belongs to the *E. verrucosa* alliance, along with *E. flavicoma* and the Italian endemic *E. gasparrinii* Boiss. The study of Cresti & al. (2019) was focused on origin and diversification of *E. gasparrinii* and its relations to *E. flavicoma* and *E. verrucosa*. Cresti & al. (2019) also suggested that *E. montenegrina* might be conspecific with *E. verrucosa*, following Baldacci (1900) and Beck Mannagetta (1920), but the poor sampling prevented them from proposing taxonomic changes (even if, based on the results of this study, several populations studied by Cresti & al., 2019, actually belong to *E. montenegrina*). Niketić & al. (2014) suggested that *E. montenegrina* is widespread in the western Balkan Peninsula from Bosnia and Herzegovina in the north to Albania and North Macedonia in the south. Another Balkan endemic species that was neglected by Radcliffe-Smith & Tutin (1968) but considered related to *E. montenegrina* by Greuter & al. (1986) and Govaerts & al. (2000), is *E. serpentini* Novák, which is distributed over serpentine areas in western Serbia (Novák, 1924, 1927; Nikolić, 1977). Whereas *E. verrucosa* is diploid with $2n = 14$ and for *E. flavicoma* and *E. gasparrinii* both di- and tetraploids have been reported ($2n = 14$, 28 and $2n = 16$, 32, respectively), the chromosome number is unknown for *E. montenegrina* and *E. serpentini* (Cesca, 1966; Simon & al., 1997; Rice & al., 2015; Peruzzi & al., 2018; Cresti & al., 2019).

In order to elucidate the unclear taxonomic status of *E. montenegrina* and *E. serpentini* and their phylogenetic and morphological differentiation from related taxa, we here use an integrative approach. Specifically, we aim to (1) infer the phylogenetic positions of *E. montenegrina* and *E. serpentini* and their relationships with *E. flavicoma* and *E. verrucosa* using nuclear ribosomal internal transcribed spacer (ITS) sequences and amplified fragment length polymorphism (AFLP) fingerprinting, including samples from across the distribution areas of all investigated species; (2) explore phylogeographic patterns within the species using AFLPs and in particular investigate from where *E. verrucosa* has colonised central Europe after the Pleistocene. In addition, we aim to (3) explore the morphological (using multivariate morphometrics) and karyological (using flow cytometry to estimate relative genome size [RGS]) variation among and within these species, and establish the chromosome number for *E. montenegrina*. Based on the integrative approach, we (4) propose a revised taxonomic treatment for the *E. verrucosa* alliance, including species descriptions and an identification key. Finally, we (5) explore the elevational distribution of di- and tetraploids, in line with the hypothesis that higher-ploidy cytotypes are more successful at higher elevations compared to diploids (Löve & Löve, 1943).

## MATERIALS AND METHODS

**Plant material.**— Plant material (samples from 114 populations of the *E. verrucosa* group, encompassing *E. flavicoma*, *E. montenegrina*, *E. serpentini* and *E. verrucosa*) for RGS estimation, molecular and morphometric analyses was collected in the field between 1998 and 2019. For simplicity, the Balkan samples, which were originally named *E. verrucosa* s.l. according to their geographic origin but were grouped in the same genetic clusters with the type populations of *E. montenegrina* and *E. serpentini* by AFLP fingerprinting, respectively (see Results), are hereafter (including Appendix 1, the suppl. Table S1 and all the figures) referred to as *E. montenegrina* or *E. serpentini*. For the same reason, we refer to the collections 53 and 54 from the serpentine mountain Puy du Wolf in France as *E. verrucosa*, despite the fact that this is the focus classicus and the only locality of *E. flavicoma* subsp. costeana (Rouy) Greuter & Burdet (Simon & Vicens, 1999). In supplementary Table S1, we, however, additionally provide the original field identifications, which were mostly based on the treatments in national Floras (e.g., Hegi, 1966; Aldén, 1986; Benedi & al., 1997; Pignatti, 1982, 2017).

Molecular and RGS analyses were based on silica-gel dried leaf material, whereas morphometric measurements were performed on herbarium specimens. In addition to the specimens collected by us, 32 herbarium specimens from four herbaria (APP, BCN, G, MA) were used in the morphometric analyses. In total, 146 populations of *E. flavicoma*, *E. montenegrina*, *E. serpentini* and *E. verrucosa* were studied: 45 were included in ITS phylogenetic analyses, for 103 we provide RGS data, 67 were included in AFLP and 102 in morphometric analyses (see suppl. Table S1, Fig. 1 and suppl. Fig. S1 including population IDs, and the sections below for specific datasets).

**DNA extraction, ITS sequencing and analyses of sequence data.**— Extraction of total genomic DNA and ITS sequencing for 24 samples were performed as described by Frajman & Schönswetter (2011), with the exception that sequencing was carried out at Eurofins Genomics (Ebersberg, Germany). Contigs were assembled, edited and sequences
aligned (suppl. Appendix S1) using Geneious Pro v.5.5.9 (Kearse & al., 2012). Base polymorphisms were coded using NC-IUPAC ambiguity codes. In addition, we used 21 ITS sequences from previous studies deposited in GenBank, 3 from Frajman & Schönswetter (2011) and 18 from Cresti & al. (2019). It should be noted that several accessions from the southern Balkan Peninsula referred to as *E. verrucosa* by Cresti & al. (2019) belong to *E. montenegrina* or *E. serpentini* as circumscribed in this study (see Taxonomic treatment). GenBank accession numbers of all ingroup sequences are given in Appendix 1 and supplementary Table S1, and all previously published sequences of the outgroup taxa are given in the supplementary Fig. S2 and Appendix 2. Since Bayesian and maximum parsimony tree-inferring methods resulted in unresolved phylogenetic trees in the study of Cresti & al. (2019), and also a preliminary Bayesian analysis of our data (conducted as described in Cresti & al., 2019) produced a polytomy within the *E. verrucosa* alliance (suppl. Fig. S2), we only present a NeighborNet constructed using SplitsTree4 v.12.3 (Huson & Bryant, 2006).

**AFLP fingerprinting and analyses of AFLP data.** — The AFLP procedure followed Vos & al. (1995) with modifications described by Cresti & al. (2019), using the same primer combination and sequencing procedure, with the exception that 0.5 μl of the elution product was mixed with 10 μl formamide and 0.1 μl GeneScan 500 ROX (ThermoFisher Scientific, Waltham, Massachusetts, U.S.A.). Two blanks (DNA replaced by water) were included to test for contamination, and 21 samples were used as replicates between the two PCR batches to test the reproducibility of the technique. Electropherograms were analysed with Peak Scanner v.1.0 (Applied Biosystems) using default peak detection parameters except for 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 v.2.0-1 (Arrigo & al., 2009) for R v.2.15.2 (R Development Core Team, 2012) with the following settings: scoring range 75–500 bp, minimum intensity 100 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. Eleven individuals did not produce interpretable fingerprints and were excluded. The error rate was calculated from the replicated individuals. A matrix of 255 individuals (excluding the 21 replicates), including 738 presence/absence fragments, was finally produced and analysed as described below.

A neighbour-joining (NJ) tree was constructed and bootstrapped (1000 pseudo-replicates) with PAUP v.4.0b10 (Swofford, 2002) and plotted with FigTree v.1.4.4. (Rambaut, 2018). For 36 populations and 137 individuals of *E. verrucosa*, non-hierarchical K-means clustering (Hartigan & Wong, 1979) was performed using the script of Arrigo & al. (2010) in RStudio v.1.0.143 (RStudio Team, 2016, R-3.3.1). A total of 50,000 independent runs (i.e., starting from random points) were performed for each value for K (number of groups) ranging from 2 to 10, and for each population, the proportions of individuals assigned to K-means groups were displayed on a map.

**Chromosome counting and relative genome size measurements.** — Seeds of *E. montenegrina* populations 130 and 131 collected in the field were germinated, root tips...
treated and chromosomes counted as described by Cresti & al. (2019).

RGS was measured for 69 populations with a CyFlow space flow cytometer (Partec, Münster, Germany) using 4,6-diamidino-2-phenylindole (DAPI) and the reference standard Bellis perennis L. (2C = 3.38 pg; Schönswetter & al., 2007) following Suda & Trávníček (2006) and modifications described by Cresti & al. (2019). RGS was calculated as the ratio between the values of the mean relative fluorescence of the sample and the standard. In addition, RGS values of 34 populations estimated by Cresti & al. (2019) were included in the statistical analyses, totalling in 103 populations for which the RGS data were analysed. The statistical analyses were performed using RStudio v.1.0.143 (RStudio Team, 2016, R-3.3.1 version) with the visualisation package “ggplot2”. Box plots of holoploid and monoploid RGS were produced for all samples and species across both ploidy levels. The RGS values were tested for normality and homogeneity of variance; differences were tested using one-way ANOVA, Tukey's post hoc test and Kruskal-Wallis H test.

**Morphometric analyses.** — Individuals from 102 populations (including 44 populations from Cresti & al., 2019) that proportionally represent the species' distribution ranges were analysed morphometrically: 31 of E. flavicoma, 19 of E. montenegrina, 7 of E. serpentina and 45 of E. verrucosa (see suppl. Table S1 for details). Since not all of these populations were analysed genetically, we used several criteria to assign the remaining ones to the four taxa. Geographic proximity to genetically analysed samples was crucial in the case of E. montenegrina and E. serpentina. We also used the identification key of Cresti & al. (2019) and differences in populations’ RGS to distinguish between E. flavicoma and E. verrucosa.

Forty-two metric characters were measured or scored; additionally, 16 ratios were calculated (Table 1). Leaf characters, with the exception of teeth and trichome characters, were analysed on scanned images using ImageJ v.1.5.3 (Abrámoff & al., 2004). Stem leaves were from the middle part of the stem and raylet leaves from the basal bifurcations of rays. Plant height, stem length and width, and number, length and number of branchings of terminal rays were measured or scored manually. All other characters (cyathium, fruit and seed characters, leaf teeth and trichome characters) were measured on magnified images taken with a stereomicroscope Olympus SZX9 using the Olympus image analysis software analySIS pro v.3.2. Since fruits were developed only in a limited number of specimens, we scored these characters on two or three fruits per voucher, when available, to increase the sample size. Along the same line, as seeds were present only in a few specimens, we did not analyse their measurements statistically but used them for taxon descriptions only. Data missing in a few specimens were replaced in the final data matrix with mean values for the taxon. Based on the unequal representation of individuals across different sets of characters, two different datasets were produced, one for fruit characters and one for all other characters.

Statistical analyses were performed using Statistica v.5.1. (StatSoft; www.statsoft.de). Correlation among metric characters was tested employing Pearson and Spearman correlation coefficients, and one character from each character-pair yielding a correlation coefficient >0.95 was excluded from further analyses. Box plot diagrams were produced for all characters in order to visualise the variation among the four species. After standardization to zero mean and one unit variance, a principal component analysis (PCA) was performed. Subsequently, discriminant analyses (DA), including an a priori classificatory cross-checked DA, were performed to explore which characters are differentiating best among E. flavicoma, E. montenegrina, E. serpentina and E. verrucosa. Six characters (stem width, number of terminal rays, number of branchings of terminal rays, number of teeth along 5 mm leaf margin below the leaf tip, number of trichomes in 1 mm² upper leaf surface, distance from the base to the widest part of a wart) and four ratios (distance from the base to the widest part of a ray leaf/length of a ray leaf, length/width of a raylet leaf, length/width of cyathial gland, distance from the base to the widest part of the fruit/fruit length), for which the Tukey HSD post hoc test showed no discriminatory importance, were excluded from the DA. In addition, we performed the same PCA and DA analyses by including only the three similar species E. montenegrina, E. serpentina and E. verrucosa, which all occur in the Balkan Peninsula. Also in this case, the characters for which the Tukey HSD post hoc test showed no discriminatory importance, were excluded from the DA: 15 metric characters (number of teeth along 5 mm leaf margin below the leaf tip, number of trichomes in 1 mm² upper leaf surface, length of a middle stem leaf, distance from the base to the widest part of a middle stem leaf, area of a middle stem leaf, angle of leaf, length of a ray leaf, distance from the base to the widest part of a ray leaf, area of a ray leaf square, length of a raylet leaf, width of a raylet leaf, number of terminal rays, number of branchings of terminal rays, width of a wart on the fruit, distance from the base to the widest part of a wart) and 6 ratios (distance from the base to the widest part of a middle stem leaf/length of a middle stem leaf, distance from the base to the widest part of a ray leaf/length of a ray leaf, distance from the base to the widest part of a raylet leaf/length of a raylet leaf, length of cyathial gland/width of cyathial gland, distance from the base to the widest part of the fruit/fruit length, distance from the base to the widest part of a wart/length of a wart on the fruit).

Based on the morphometric data, we produced species descriptions and an identification key. Metric values presented there correspond to the 10 and 90 percentiles, supplemented by extreme values in parentheses.

### Results

**ITS NeighborNet.** — In the ITS NeighborNet (Fig. 2A), samples of E. flavicoma, E. montenegrina, E. serpentina and E. verrucosa were positioned along four diverging splits, which were mostly geographically (Fig. 2B) and partly taxonomically...
### Table 1. Characters studied in the morphometric analyses of *Euphorbia flavicoma*, *E. montenegrina*, *E. serpentini* and *E. verrucosa*.

| No. | Character                                      |
|-----|------------------------------------------------|
|     | **Total plant**                                |
| 1   | Plant height, cm                               |
|     | **Stem**                                       |
| 2   | Stem length, cm                                |
| 3   | Stem width, mm                                 |
|     | **Middle stem leaves**                         |
| 4   | Length of a middle stem leaf, mm               |
| 5   | Width of a middle stem leaf, mm                |
| 6   | Ratio length of a middle stem leaf/width of a middle stem leaf |
| 7   | Ratio of distance from the base to the widest part of a middle stem leaf/length of a middle stem leaf |
| 8   | Angle of leaf tip, °                           |
| 9   | Length of teeth below the leaf tip, mm         |
| 10  | Width of teeth below the leaf tip, mm          |
| 11  | Number of teeth along 5 mm leaf margin below the leaf tip |
| 12  | Number of trichomes in 1 mm² upper leaf surface |
| 13  | Distance from the base to the widest part of a middle stem leaf, mm |
| 14  | Area of a middle stem leaf, mm²                |
|     | **Terminal rays**                              |
| 15  | Number of terminal rays                        |
| 16  | Length the terminal rays, cm                   |
| 17  | Number of branchings of terminal rays          |
|     | **Ray leaves**                                 |
| 18  | Length of a ray leaf, mm                       |
| 19  | Width of a ray leaf, mm                        |
| 20  | Ratio length/width of a ray leaf               |
| 21  | Ratio of distance from the base to the widest part of a ray leaf/length of a ray leaf |
| 22  | Angle of tip of a ray leaf, °                  |
| 23  | Distance from the base to the widest part of a ray leaf, mm |
| 24  | Area of a ray leaf square, mm²                 |
|     | **Raylet leaves**                              |
| 25  | Length of a raylet leaf, mm                    |
| 26  | Width of a raylet leaf, mm                     |
| 27  | Ratio length/width of a raylet leaf            |
| 28  | Ratio of distance from the base to the widest part of a raylet leaf/length of a raylet leaf |
|     | **Cyathium**                                   |
| 29  | Angle of tip of a raylet leaf, °               |
| 30  | Distance from the base to the widest part of a raylet leaf, mm |
| 31  | Area of a raylet leaf square, mm²              |
|     | **Fruits**                                     |
| 32  | Length of cyathial involucre, mm               |
| 33  | Width of cyathial involucre, mm                |
| 34  | Ratio length of cyathial involucre/width of cyathial involucre |
| 35  | Length of cyathial gland, mm                   |
| 36  | Width of cyathial gland, mm                    |
| 37  | Ratio length of cyathial gland/width of cyathial gland |
|     | **Seeds**                                      |
| 38  | Fruit length, mm                               |
| 39  | Fruit width, mm                                |
| 40  | Ratio fruit length/fruit width                 |
| 41  | Ratio of distance from the base to the widest part of the fruit/fruit length |
| 42  | Length of a wart on the fruit, mm              |
| 43  | Width of a wart on the fruit, mm               |
| 44  | Ratio length/width of a wart on the fruit      |
| 45  | Ratio of distance from the base to the widest part of a wart/length of a wart on the fruit |
| 46  | Style length, mm                               |
| 47  | Distance from the base to the widest part of the fruit, mm |
| 48  | Distance from the base to the widest part of a wart, mm |
|     | **Caruncle**                                   |
| 49  | Seed length, mm                                |
| 50  | Seed width, mm                                 |
| 51  | Ratio seed length/seed width                   |
| 52  | Ratio of distance from the base to the widest part of a seed/seed length |
| 53  | Caruncle length, mm                            |
| 54  | Caruncle width, mm                             |
| 55  | Ratio caruncle length/caruncle width           |
| 56  | Ratio of distance from the base to the widest part of caruncle/caruncle length |
| 57  | Distance from the base to the widest part of a seed, mm |
| 58  | Distance from the base to the widest part of caruncle, mm |

(Continues)
correlated. In the central area (black), which connected all four divergent splits, most populations of *E. montenegrina*, two of *E. serpentini* and two Balkan and two central European populations from east and north of the Alps of *E. verrucosa* were positioned. One split led to all diploid Iberian populations of *E. flavicoma* (pink), the second included three northern populations of *E. montenegrina* (green), the third comprised northern Balkan and northern Apennine populations of *E. verrucosa* (yellow), and the fourth included most populations of *E. verrucosa* distributed from the Pyrenees to the central Balkan Peninsula (light blue), one population of *E. serpentini* (dark blue) and two strongly divergent tetraploid populations of *E. flavicoma* from France (red).

**AFLP data.** — A total of 738 fragments were scored in 276 individuals, including 21 replicates; 55 fragments were excluded because they were present or absent in a single individual only. The error rate (Bonin & al., 2004), calculated before the exclusion of non-reproducible fragments, was 2.6%. The NJ tree (suppl. Fig. S3, simplified version with major clusters shown in Fig. 3) revealed two main clusters; one included *E. flavicoma* (BS 100%) and the other, named the *E. verrucosa* lineage hereafter, was composed of *E. verrucosa* (BS 75%), and a cluster (BS 57%) including *E. montenegrina* (BS 84%) and *E. serpentini* (BS 90%). The *E. verrucosa* cluster also included the type population of *E. flavicoma* subsp. *costeana* from France. Relationships within *E. verrucosa* remained largely unresolved as most of the groups had low bootstrap support or formed a polytomy of populations. In contrast, within *E. montenegrina*, *E. serpentini* and *E. flavicoma*, several groups with high BS were inferred. Within *E. montenegrina*, several tetraploid populations were intermingled with diploid populations (suppl. Fig. S1). Within *E. flavicoma*, two main clusters were further subdivided into two subclusters each. The first cluster (BS 55%) included a subcluster of northwest Iberian populations of *E. flavicoma* subsp. *occidentalis* (BS 84%) and a subcluster of south Iberian (Andalusian) *E. flavicoma* subsp. *flavicoma* (BS 100%). The second cluster (BS 67%) included a subcluster of diploid east Iberian populations (BS 79%) and a subcluster of tetraploid French populations (BS 95%) of *E. flavicoma* subsp. *flavicoma*.

![AFLP data](image.png)

**Fig. 2.** ITS variation in *Euphorbia flavicoma*, *E. montenegrina*, *E. serpentini* and *E. verrucosa*. NeighborNet of ITS sequences (A) and geographic position of the ITS ribotype groups revealed by the NeighborNet (B). The asterisk in (A) denotes the population 116 of *E. serpentini*, which had the same ribotype as several populations of *E. montenegrina*; for better visibility, the symbols for both species are thus slightly displaced in the NeighborNet.
Non-hierarchical K-means clustering of *E. verrucosa* with increasing *K* revealed that genetic clusters from *K* = 2 to *K* = 8 are geographically correlated and that the main genetic diversification with increasing *K* is observed in the eastern part of the distribution, i.e., in the Balkan Peninsula and the areas southeast of the Alps (Fig. 4). At *K* = 2, the populations to the west and northwest of the Alps (the Western Cluster) were divergent from all other populations, which formed the Eastern Cluster. At *K* = 3 the Eastern Cluster got split into the populations north, east and south of the Alps (orange; the Northern Cluster), and the populations in the Balkan and Apennine peninsulas (green; the Southern Cluster). With *K* increasing to *K* = 6, only the Southern Cluster was further subdivided, and the easternmost population of the Northern Cluster was separated; furthermore, there was a certain degree of admixture between some clusters. Only at *K* = 7, the Western Cluster was also subdivided into populations east and west of the Massif Central.

**Chromosome counts and relative genome size.** — We recorded a chromosome number 2n = 28 for populations 130 and 131 of *Euphorbia montenegrina* (Fig. 5). Two clearly different DNA-ploidy levels (Suda & Trávníček, 2006), calibrated with the new chromosome counts of *E. montenegrina* and those of *E. verrucosa* published by Cresti & al. (2019), were revealed by the RGS data. Most populations were diploid, and 16 were tetraploid, 4 of *E. flavicoma* and 12 of *E. montenegrina* (Fig. 6). The holoploid RGS of diploids ranged from 0.439 (population 135 of *E. montenegrina*) to 0.608 (population 13 of *E. flavicoma*), and that of tetraploids from 0.843 (population 127 of *E. montenegrina*) to 1.164 (population 37 of *E. flavicoma*), in both cases being discretely distributed, i.e., exhibiting a gap in RGS values, between *E. flavicoma* and the other species. The RGS of *E. flavicoma* ranged from 0.527 to 0.608 in diploids and from 1.101 to 1.164 in tetraploids, that of *E. montenegrina* from 0.439 to 0.458 in diploids and from 0.843 to 0.907 in tetraploids, that of *E. serpentini* from 0.441 to 0.484, and that of *E. verrucosa* from 0.442 to 0.517 (suppl. Table S1, Fig. 6A). The monoploid RGS of *E. flavicoma* ranged from 0.264 to 0.304 in diploids and from 0.275 to 0.291 in tetraploids, that of *E. montenegrina* from 0.219 to 0.229 in diploids and from 0.211 to 0.227 in tetraploids, that of *E. serpentini* from 0.220 to 0.242, and that of *E. verrucosa* from 0.221 to 0.258 (Fig. 6B).

The variance among the diploid populations of the four species was homogenous (Levene’s test; *P* = 0.0196).
Differences in RGS of diploids were significant among the four species (Kruskal-Wallis H test; $P < 0.0001$). RGS differed significantly between all four species also in pairwise comparisons (ANOVA with Tukey’s test; $P < 0.05$). The variance of monoploid RGS between diploid and tetraploid populations of *E. flavicoma* and *E. montenegrina* was not homogenous (Kruskal-Wallis H test; $P = 0.4663$ and 0.1487, respectively), and their monoploid RGS did not differ.

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**Fig. 4.** Geographic distribution of the groups inferred by nonhierarchical K-means clustering of AFLP data of *Euphorbia verrucosa* at $K$ (the number of groups) ranging from 2 to 7 ($K = 8$ not shown). Groups are colour-coded; admixed populations are coded by two colours.

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**Fig. 5.** Metaphase plates of *Euphorbia montenegrina* from populations no. 130 (A) and 131 (B) showing $2n = 28$ chromosomes. — Scale bars: 1 μm. Population numbers correspond to supplementary Table S1.
significantly (ANOVA with Tukey’s test; $P = 0.2889$ and $P = 0.0997$).

Within *E. flavicoma*, diploids range from 60 to 900 m a.s.l. and tetraploids from 195 to 1050 m (Fig. 7). *Euphorbia verrucosa*, which is diploid, is distributed from 15 to 1700 m and *E. serpentina* from 290 to 1300 m, and within *E. montenegrina*, diploids range from 1210 to 1970 m and tetraploids from 1180 to 2210 m.

**Morphometry.** — The morphological character states are presented in supplementary Table S2 for the fruit characters and supplementary Table S3 for all other characters. Box plot diagrams of the most important differential characters (Tukey HSD post-hoc test, $P < 0.001$) are shown in supplementary Fig. S4. The correlation coefficients exceeded $r = 0.95$ in three pairs of characters both in the four-species and the three-species (*E. montenegrina*, *E. serpentina*, *E. verrucosa*) datasets (area of ray leaves/width of ray leaves; area of raylet leaves/width of raylet leaves; plant height/stem length) and the first-mentioned characters were thus excluded from the PCA and DA analyses.

The PCA scatter plot (first three axes explaining 28.66%, 10.38% and 9.51% of the total variation; supplementary Fig. S5A) based on vegetative and cyathium characters showed a pronounced overlap among the four species. The characters with the highest loading along the first axis, which indicated a weak separation of *E. flavicoma* from the other three species, were leaf (area, length and width of a middle stem leaf) and ray

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**Fig. 6.** Holoploid (A) and monoploid (B) relative genome size (RGS) variation in diploid (2x) and tetraploid (4x) populations of *Euphorbia flavicoma*, *E. montenegrina*, *E. serpentina* and *E. verrucosa*. 
leaf characters (area, length and width of a ray leaf, distance from the base to the widest part of a ray leaf). Along the second axis, *E. montenegrina* and *E. verrucosa* were weakly separated, whereas *E. serpentina* was scattered between them. The characters with the highest loading along the second axis were cyathial characters (length of cyathial gland, length of cyathial involucre, ratio length/width of cyathial involucre) as well as angle of the tip of a ray leaf.

In the classificatory cross-checked DA, 94.1% of individuals were correctly classified to the four predefined groups, and the DA scatter plot (Fig. 8A; suppl. Fig. S5B) showed a separation of *E. flavicoma*, *E. montenegrina* and *E. verrucosa*, with only a slight overlap among them. *Euphorbia serpentina* was scattered between the latter two species. Along the first factor, mostly *E. flavicoma* was separated from *E. montenegrina* and *E. serpentina* (Wilks’ Lambda = 0.04, Chi-square = 272.495, df = 84, \( P < 0.0001 \)), and along the second factor, *E. verrucosa* was separated from *E. flavicoma* and *E. montenegrina* with a slight overlap (Wilks’ Lambda = 0.15, Chi-square = 156.931, df = 54, \( P < 0.0001 \)). The characters contributing most to the first separation were width and length of a ray leaf, width of a raylet leaf, length of cyathial involucre and the ratio length/width of cyathial involucre. In the second factor, these characters were length of a middle stem leaf, the ratio length/width of a middle stem leaf, length of a raylet leaf, distance from the base to the widest part of a raylet leaf.

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**Fig. 7.** Elevationa distribution of diploid (2x) and tetraploid (4x) populations of *Euphorbia flavicoma*, *E. verrucosa*, *E. serpentina* (*E. serp.*), and *E. montenegrina*, following their distribution from west to east and with increasing elevation within each group. Elevational data were recorded in the field and are given in supplementary Table S1.

**Fig. 8.** Morphological differentiation revealed by discriminant analysis (DA) of 39 metric vegetative and cyathium characters and 16 ratios among: A, *Euphorbia flavicoma* (squares), *E. montenegrina* (triangles), *E. serpentina* (stars) and *E. verrucosa* (circles); B, *E. montenegrina* (triangles), *E. serpentina* (stars) and *E. verrucosa* (circles).
For fruit characters, the PCA (first three axes explaining 31.77%, 17.37% and 15.64% of the total variation; suppl. Fig. S5C) showed a strong overlap among the species. In the classificatory cross-checked DA, 70.5% of individuals were correctly classified to the four predefined groups, and in the DA scatter plot (Wilks’ Lambda = 0.22, Chi-square = 211.489, df=27, P < 0.0001 for the first factor and Wilks’ Lambda = 0.54, Chi-square = 86.896, df = 16, P < 0.0001 for the second factor, suppl. Fig. S5D), the separation was slightly more pronounced compared to the PCA. Fruit length, fruit width and length of a wart on the fruit contributed most to the separation along the first factor, along which *E. verrucosa* showed an intermediate position between *E. flavicoma*, *E. montenegrina* and *E. serpentina*. Fruit width, fruit length and ratio fruit length/width contributed most to the separation along the second factor, along which *E. verrucosa* was weakly separated from the other three species.

The PCA scatter plot based on vegetative and cyathium characters of *E. montenegrina*, *E. serpentina* and *E. verrucosa* (first three axes explaining 24.09%, 13.90% and 9.46%; suppl. Fig. S6A) showed a strong overlap among the taxa. The characters with the highest loading along the first axis were leaf characters (length of a middle stem leaf, area of a middle stem leaf) and ray leaf characters (length of a ray leaf, width of a ray leaf, area of a ray leaf), those with the highest loading along the second axis were length of cyathial involucre, length of cyathial gland, length of the terminal rays and angle of tip of a raylet leaf.

In the classificatory cross-checked DA of the vegetative and cyathium data, 94.2% of the individuals were correctly classified to the three predefined groups, and the DA scatter plot (Fig. 8B, suppl. Fig. S6B) showed a separation of *E. montenegrina* from the other two species along the first factor (Wilks’ Lambda = 0.18, Chi-square = 98.78, df = 32, P < 0.0001) and a separation of *E. serpentina* from the other two species along the second factor (Wilks’ Lambda = 0.54, Chi-square = 35.800, df = 15, P = 0.002). Cyathium characters (length of cyathial involucre, width of cyathial involucre, ratio length/width of cyathial involucre) contributed most to the separation along the first factor. The same cyathium characters, as well as stem length and width of a middle stem leaf, were most important for the separation along the second factor.

For fruit characters, the PCA scatterplot (first three axes explaining 34.23%, 17.65% and 13.41% of the total variation; not shown) showed a strong overlap among the species. In the classificatory cross-checked DA, 76.2% of individuals were correctly classified to the three predefined groups, and in the DA scatter plot (Wilks’ Lambda = 0.32, Chi-square = 110.107, df=18, P < 0.0001 for the first factor and Wilks’ Lambda = 0.65, Chi-square = 42.364, df = 18, P < 0.0001 for the second factor, suppl. Fig. S6C) the overlap was slightly less pronounced compared to the PCA. Fruit length, fruit width and length of a wart on the fruit contributed most to the separation along the first factor, with a weak separation between *E. serpentina* and *E. verrucosa*. The same characters were also most important for the weak separation of *E. montenegrina* from the other two species along the second factor.

**DISCUSSION**

In a previous paper, Cresti & al. (2019) suggested that the Iberian, Apennine and Balkan peninsulas have acted as glacial refugia for the *Euphorbia verrucosa* alliance during the Pleistocene, ultimately leading to allopatric speciation and evolution of *E. flavicoma*, *E. gasparrinii* and *E. verrucosa*. In Cresti & al. (2019), we focused on Italian endemic *E. gasparrinii* and explored its relationships with *E. flavicoma* and *E. verrucosa*. Here, by substantially extending the geographic and taxonomic sampling, we show that the Iberian and the Balkan peninsulas were indeed centres of diversification of the *E. verrucosa* alliance, both in terms of genetic as well as morphological and taxonomic diversity, whereas the populations in central Europe are genetically homogenous. Based on our data, we propose to recognise *E. montenegrina* and *E. serpentina*, two species endemic to the Balkan Peninsula. For these two species, we provide a new circumscription of their distributions and morphologies. On this basis, we redefine *E. verrucosa*, also in relation to the western Mediterranean *E. flavicoma*.

**The Balkan Peninsula: a cradle for diversification of the *Euphorbia verrucosa* lineage.** — The Balkan Peninsula, which was the main source of lineages for the postglacial recolonization of central and northern Europe (e.g., Taberlet & al., 1998; Hewitt, 1999; Hampe & al., 2003; Schmitt, 2007; Willner & al., 2009; Rešetnik & al., 2016), was also a cradle for the diversification of the *E. verrucosa* lineage. This widespread lineage is distributed from the Iberian Peninsula through central Europe to the Balkan Peninsula, where it exhibits the highest genetic, karyological and morphological diversity. In line with the results of Cresti & al. (2019), it is clearly divergent from the Western Mediterranean *E. flavicoma* in the AFLP tree (Fig. 3). Its divergence from the latter is also supported by ITS (Fig. 2) and RGS (Fig. 6) data. By including population 129 of *E. montenegrina* from the locus classicus in Montenegro, we corroborated previous studies (Frajman & Schönswetter, 2011; Riina & al., 2013; Cresti & al., 2019), which indicated a close relationship between *E. verrucosa* and *E. montenegrina* based on ITS sequences (Fig. 2). In addition, *E. montenegrina* — along with all southern Balkan populations southeast of central Bosnia and Herzegovina, which were in the past mostly treated as *E. verrucosa*, some populations occasionally as *E. serpentina* — forms a cluster in the AFLP tree (Fig. 3). This cluster is sister to the *E. verrucosa* populations distributed in northerly adjacent areas ranging from the northern Balkan Peninsula to the northern Iberian Peninsula. Interestingly, this main phylogenetic split in the Balkan Peninsula in our study group coincides with the Neretva and Bosna river valleys in central Bosnia and Herzegovina. This is the most prominent phylogeographic break in the western Balkan Peninsula inferred for many ecologically different plants from various elevational zones (e.g., Frajman & Oxelman, 2007; Lukić & al., 2013; Kutnjak & al., 2014; Caković & al., 2015; Falch & al., 2019).

Following the main genetic boundary inferred by AFLPs in the Balkan Peninsula (Fig. 3), we assign the southern Balkan subcluster to *E. montenegrina* and circumscribe this species
much more broadly than proposed by Radcliffe-Smith & Tutin (1968) and Govaerts & al. (2000), thus largely confirming the circumscription of Niketić & al. (2014). The latter study, however, also included the Bosnian populations of *E. serpentini* in *E. montenegrina*. Compared to Niketić & al. (2014), our circumscription of *E. montenegrina* also includes the Albanian and Greek populations that were treated as *E. verrucosa* by Barina (2017) and as *E. flavidoma* (but “resembling subsp. verrucosa (Fiori) Pignatti”) by Aldén (1986). These results are in agreement with our field experience in the southern Balkan Peninsula, where it was often difficult to unambiguously classify the plants as either *E. montenegrina* or *E. verrucosa*. This was also due to a lack of diagnostic characters in the literature, as the only key including both species was in *Flora Europaea* (Radcliffe-Smith & Tutin, 1968), and the key point differentiating between them was “Stems stout; ray-leaves orbicular”, vs. “Stems very slender; ray-leaves ovate to obovate”. Additional diagnostic characters of *E. montenegrina* provided by Baldacci (1900) and Rohlena (1942) were the shorter terminal rays, only slightly exceeding the ray leaves, and denser, longer, cylindrical purple tubercules on the capsules.

Our morphometric analyses (Fig. 8, suppl. Figs. S3–S5; Taxonomic treatment) showed that the above-mentioned diagnostic characters are of limited value and that the morphological differentiation between *E. montenegrina* and *E. verrucosa* is not always clear-cut. However, with a combination of different characters (see Identification key) it is in most cases possible to identify both species; furthermore, they are strictly allopatric. Contrary to the purely diploid *E. verrucosa*, a grassland species usually restricted to low altitudes, *E. montenegrina* includes both diploids and tetraploids and is restricted to the mountains of the western and southern Balkan Peninsula, often above timberline in subalpine or low-alpine vegetation. In that, as well as in the preference of calcareous substrate, it resembles the closely related Italian endemic *E. gasparrinii*, with which it also shares the prolonged, vermicular fruit tubercles (Cresti & al., 2019).

In addition to the clear genetic (Fig. 3) and RGS (Fig. 6) divergence between *E. montenegrina* and *E. verrucosa*, AFLP data (Fig. 3) also inferred a central Balkan cluster sister to *E. montenegrina*, which included the population from the *locus classicus* of *E. serpentini* in western Serbia, plus all populations from central and eastern Bosnia and Herzegovina growing on serpentine below timberline. All these populations were diploid and differed in RGS (Fig. 6) from both *E. montenegrina* and *E. verrucosa*, from which they were also morphologically differentiated (Fig. 8). Following the clear AFLP divergence, which is in line with morphological and ecological differentiation, we here propose to recognise *E. serpentini*, which was neglected by Radcliffe-Smith & Tutin (1968), and confirm the suggestion of Govaerts & al. (2000), who deemed it closely related to *E. montenegrina*. Stevanović & al. (2003) treated these two species as ecological vicariants, the former inhabiting calcareous and the latter serpentine bedrock.

Plants growing on serpentines have been suggested to have smaller genomes than those growing on non-toxic soils (Pustahija & al., 2013). However, in our study, the RGS values of *E. serpentini* were scattered among or higher than those of *E. montenegrina* (suppl. Table S1) and even slightly higher than in the latter species (Fig. 6), thus indicating that there is no detectable impact of the serpentine substrate on the genome size of *E. serpentini*. In the same line, serpentine and non-serpentine populations of *Armeria maritima* (Mill.) Willd. exhibit no RGS divergence, pointing to the existence of intrinsic mechanisms, which could confer heavy metal tolerance (Vekemans & al., 1996; Pustahija & al., 2013). Nevertheless, only diploids were detected in *E. serpentini*, contrary to *E. montenegrina*, where the majority of populations were tetraploid.

The genetic differentiation inferred by AFLPs was more pronounced within both *E. montenegrina* and *E. serpentini* than within *E. verrucosa* (suppl. Fig. S3), which is in line with previous studies that have unravelled deeper genetic divergence in plant lineages from the southern, as compared to the northern Balkan Peninsula (e.g., Kutnjak & al., 2014; Caković & al., 2015; Đurović & al., 2017; Falch & al., 2019). Finally, tetraploids likely originated several times in the evolutionary history of *E. montenegrina*. Tetraploid populations appear in different clusters with diploids in the AFLP tree (suppl. Fig. S3) and are also disjunctly distributed among diploid populations (Fig. 1). Since the monoploid RGS of diploids and tetraploids did not differ significantly, no genome downsizing (Verma & Rees, 1974; Leitch & Bennett, 2004) happened after the origin of the tetraploids. It has been suggested that different ploidy levels are non-randomly distributed along the altitudinal gradient and that higher-ploid plants tend to inhabit higher elevations compared to their lower-ploid counterparts (Löve & Löve, 1943; Brochmann & al., 2004). We have shown that in *E. montenegrina*, the elevational distribution of diploid populations overlaps entirely with that of tetraploids, although tetraploids extend their distribution higher compared to diploids (Fig. 7). Tetraploid *E. montenegrina* is often found above the timberline, which is rarely the case for diploid populations and exceptional in *E. verrucosa*; in *E. serpentini*, all known populations grow below the timberline.

**Widespread but genetically uniform: rapid range expansion of *Euphorbia verrucosa*.** — We have observed pronounced genetic, karyological and morphological diversification between and partly within geographically restricted *E. montenegrina* and *E. serpentini*. In contrast, populations of *E. verrucosa*, which inhabits large areas spanning from the northern Balkan Peninsula to the northern Iberian Peninsula, were genetically only weakly differentiated (suppl. Fig. S3). All investigated populations were diploid (Fig. 6), and K-means clustering (Fig. 4) indicated that the populations (north-)west of the Alps were segregated from all other populations at $K = 2$ and that with increasing $K$ only the Eastern Cluster was split into smaller groups of geographically coherent populations. Stronger genetic differentiation of the populations south-east of the Alps is in line with the hypothesis of the origin and Pleistocene persistence of *E. verrucosa* in the Balkan Peninsula, from where the species likely spread rapidly after the last glacial maximum in response to the Holocene climate warming.
All three ITS ribotype groups of *E. verrucosa*, one of them similar to the ribotypes of *E. montenegrina*, are present in the Balkan Peninsula, from where one group is spanning to the areas east and north of the Alps, one to the northern Apennine Peninsula and one to the Pyrenees (Fig. 2). Given the probable Pleistocene origin of *E. verrucosa* (Cresti & al., 2019) and its ecological adaptation to open mesophilic grasslands, it is likely that the species extended its range from its Balkan Pleistocene refugium only during the last 6000 years, when the progressive decline of mixed temperate forest due to forest clearance for agriculture started in central Europe (Lechterbeck & al., 2014; Roberts & al., 2018). In contrast, *E. montenegrina*, which mostly thrives in (sub)alpine grasslands above the timberline, and *E. serpentina*, which inhabits naturally forest-free serpentine outcrops, and scrublands or open pine forests over serpentine, could have persisted in their current areas over longer periods and responded to Pleistocene glaciations with altitudinal migrations and increased genetic divergence due to isolation in grasslands separated by forested areas.

**The Iberian Peninsula only partly mirrors the Balkans.**

— *Euphorbia flavicoma* had its Pleistocene refugium in the Iberian Peninsula (Cresti & al., 2019). The present study, based on an increased geographical sampling of both *E. flavicoma* and *E. verrucosa* also in areas with parapatric occurrence, confirmed the genetic (Figs. 2, 3), RGS (Fig. 6) and morphological (Fig. 8) differentiation between both species. In addition, our AFLP data (Fig. 2) unravelled a pronounced divergence among the populations of *E. flavicoma*, which parallels that within *E. montenegrina*/*E. serpentina* in the Balkan Peninsula. This suggests that also *E. flavicoma* has survived Pleistocene glaciations in geographically distinct refugia in the Iberian Peninsula, a scenario proposed for other Iberian species by Gomez & Lunt (2007). From the Iberian Peninsula, only one lineage extended its range to north-easterly adjacent areas in France, which parallels the expansion of *E. verrucosa*, but not of *E. montenegrina* and *E. serpentina*, from the Balkan Peninsula. However, whereas *E. verrucosa* colonised large areas across Europe, *E. flavicoma* only expanded to a relatively small area in southern France. Such a moderate expansion out of Iberia has also been observed in *Pinus pinaster* and *Quercus suber* (Bucci & al., 2007; Magri & al., 2007).

*Euphorbia flavicoma* is more thermophilous than *E. verrucosa*, inhabiting Mediterranean scrublands, dry, warm grasslands and open forests. Its ecological preferences and lack of such habitats in extra-Mediterranean Europe thus likely limited its wider dispersal out of Iberia. Mediterranean climate, which is nowadays prevalent in large areas of Iberia, is in adjacent France restricted to the south. In contrast, continental and oceanic climate types extend from the Balkan Peninsula across large parts of Europe (Peel & al., 2007), which likely contributed to wider-ranging colonisation of central Europe from the Balkan, rather than from the Iberian Peninsula, not only in *E. verrucosa* but also in other plant species.

Even if the AFLP data (Fig. 3) partly indicate correlation of genetic differentiation with taxonomic entities within *E. flavicoma*, our scarce geographic sampling within the Iberian Peninsula precludes conclusions on whether taxonomic boundaries among subspecies (Benedí & al., 1997; Simon & Vicens, 1999) are phylogenetically supported. Our AFLP data suggest that *E. f.* subsp. *occidentalis* is monophyletic, but only three populations from the western half of the subspecies’ suggested distribution (Simon & Vicens, 1999) were studied. *Euphorbia flavicoma* from the Sierra Bermeja in Andalusia is the weakly supported (BS 55%) sister of *E. f.* subsp. *occidentalis*, and thus divergent from *E. f.* subsp. *flavicoma* in Catalonia. Since the intervening areas between Andalusia and Catalonia were not sampled, the addition of intermediate populations could blur the observed genetic divergence.

The tetraploid populations of *E. f.* subsp. *flavicoma* from France form a cluster sister to diploid Iberian populations (Fig. 3), probably supporting a single, autoploid origin of the tetraploids, as proposed by Simon & al. (1997). It remains, however, unclear, if all French populations, including also the type of *E. flavicoma*, are tetraploid. Contrary to the AFLP patterns, the ITS sequences of the tetraploid populations were strongly divergent from the diploid populations and positioned along the same split with geographically close populations of *E. verrucosa* (Fig. 2). This pattern could indicate that *E. verrucosa* was involved in their origin, rendering them allotetraploid. Under this scenario, however, the monoploid RGS of tetraploid *E. flavicoma* is expected to be intermediate between diploid *E. flavicoma* and *E. verrucosa*, which is not the case (Fig. 6). The RGSs of diploid and tetraploid *E. flavicoma* do not differ significantly, thus rather supporting the autoploid origin of tetraploids, which are also morphologically similar to the diploids.

It remains unclear whether the strongly pubescent populations of *E. f.* subsp. *giselae* from south-eastern France (from where the type of this subspecies is) share their origin with the Andalusian populations, as suggested by Simon & Vicens (1999), or if the name *E. f.* subsp. *bermejense* should rather be applied to the latter, following Hidalgo-Triana & al. (2016). Our populations 14 and 15 from Sierra Bermeja and Sierra de las Nieves in Andalusia were only slightly pubescent; thus, they correspond morphologically to *E. f.* subsp. *flavicoma* rather than *E. f.* subsp. *bermejense* or *E. f.* subsp. *giselae*. However, since they are genetically divergent from north-eastern populations of *E. flavicoma* subsp. *flavicoma* (Fig. 3), we suggest that probably all Andalusian populations merit an independent subspecific status, but further studies with denser sampling in the Iberian Peninsula are needed to confirm this. In our study, the ITS sequence of a plant belonging to *E. f.* subsp. *bermejense* (population 12) did not differ from that of *E. f.* subsp. *flavicoma* (populations 13 and 14) co-occurring in the Sierra Bermeja (Fig. 2). *Euphorbia f.* subsp. *bermejense*, which is stenoendemic to one serpentine locality in the Sierra Bermeja (Hidalgo-Triana & al., 2016), is likely merely an ecotype, whose specific morphology results from adaptation to a specific environment. Similar patterns were unravelled in different plant groups, also such growing on serpentine (e.g., Berglund & al., 2004; Roda & al., 2013; Trucchi & al., 2017; Stevanoski & al., 2020).
Occurrence in a serpentine habitat was likely also a basis for the taxonomic segregation of *E. flavicoma* as a subspecies in *E. flavi- coma* by Pignatti (1973, 1982), and several records of *E. flavicoma* in Italy thus actually correspond to *E. verrucosa*. This was confirmed by a visit to localities in Liguria, from which *E. flavicoma* has been reported (Simon & Vicens, 1999; Barberis & al., 2019); all visited Ligurian populations actually belong to *E. verrucosa* (B. Frajman, pers. obs.), as confirmed by RGS data and for some populations also ITS sequences. According to our knowledge, *E. flavicoma* does not occur in Italy nor in Croatia, from which it was reported by Simon & Vicens (1999); it is thus restricted to the Iberian Peninsula and southern France.

### TAXONOMIC TREATMENT

The here proposed taxonomic treatment and the description of *Euphorbia flavicoma* largely correspond to those of Cresti & al. (2019), but additional samples were included in the morphometric study, and the distribution of this species has been clarified. The description and distribution of *E. verrucosa* have been largely revised, after the recognition and new circumscription of *E. montenegrina* and *E. serpentina*. For the treatment of *E. gasparrini*, which also belongs to this alliance, see Cresti & al. (2019).

**Identification key.** — The most discriminating characters in the key are underlined. Images of all four species are shown in Figs. 9 and 10.

1. Stems 10–30(36) cm high, usually pubescent. Middle stem leaves usually slightly glaucous, (9)12–18(28) × 3–7(8) mm. Ray leaves (7)8–16(19) × (3)4–8(9) mm. Raylet leaves (4)5–9(11) × (2)3–7(8) mm. Cyathial glands (0.3)0.5–0.8 (0.9) × (0.7)0.9–1.5 mm, (0.7)0.8–1.2(1.3) times longer than wide. Capsule (1)2–4 × (1)3–4.5 mm, with warts (0.1)0.2–0.7(1) × 0.2–0.4(0.5) mm, (0.4)0.5–2(3) times longer than wide. Seeds brownish ............... *E. flavicoma*

2. Leaves glabrous to densely hairy, with 0–32(63) hairs per mm² at the upper surface of the distal half of the leaf. Cyathial involucre (1)1.4–2.6(3.3) × (1)1.2–2(2.9) mm, (0.4)0.5–1.6(1.8) times longer than wide. Cyathial glands (0.3)0.4–0.7(1.0) × (0.6)1.2–1.4 mm. Capsule (1)1.5–3(0.7) × (1)1.7–3.5(4.2) mm. Warts on the capsule 0.2–0.6(0.8) × 0.1–0.3(0.5) mm, (0.8)1.1–3.1(4.8) times longer than wide……………………………………….. *E. verrucosa*

3. Stem 13.9–24.6 cm high, 1–2 mm thick. Middle stem leaves lanceolate 17.2–27.2 × 5–11.2 mm, 2.1–5.2 times longer than wide. Leaf margin sometimes minutely serrulate; the teeth 0.07–0.27(0.3) mm long and (0.1)0.13–0.36(0.46) mm wide at the base. Terminal rays 4.3(5) × 3.3–34(35) mm long, 0.3–1.7(1.8) times longer than ray leaves. Warts on the capsule (0.6)0.7–1.4(1.5) × 0.1–0.3(0.7) mm, (1.2)1.4–6(13.0) times longer than wide ………………….. *E. serpentina*

4. Stems 26 mm long, 2(3) times longer than wide, 2 mm thick. Middle stem leaves oblong to elliptic-lanceolate (14)14.5–30(38) × (7.3)7.5–12(13.2) mm, (1.3)1.5–3.1 times longer than wide. Leaf margin coarsely serrulate; the teeth 0.07–0.27(0.3) mm long and (0.1)0.13–0.36(0.46) mm wide at the base. Terminal rays 4.2–26(26) mm long, (0.3)0.5–2(4.9) times longer than ray leaves. Warts on the capsules (0.6)0.7–1.4(1.5) × 0.1–0.3(0.7) mm, (2.2)2.6–5.5(9.6) times longer than wide………………….. *E. montenegrina*

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**Euphorbia flavicoma** DC., Cat. Pl. Horti Monsp.: 110. 1813
≡ *Galarhoeus flavicomics* (DC.) Fourr. in Ann. Soc. Linn. Lyon, n.s., 17: 149. 1869 ≡ *Tithymalus flavicomics* (DC.) Bubani, Fl. Pyren. 1: 108. 1813 ≡ *E. epithymoides* var. *flavicoma* (DC.) Fiori in Fiori & Béguinot, Fl. Italia 2: 277. 1901 ≡ *E. brittingeri* subsp. *flavicoma* (DC.) Ladero in Anales Inst. Bot. Canavilles 31: 124. 1974 – Lectotype (designated by Simon & Vicens, Estud. Biosk. Euphor- bia Mediter. Occid.: 326. 1999): [France], "Env. de Montpellier" (G barcode G00312001).

**Description.** – Pubescent perennial, (10)13.2–40(52.8) cm tall, with usually several stems (9)4.02.8–28.36(36) cm high and (1.1)1.5–2(3) mm thick, arising from a woody stock. Middle stem leaves oblong to elliptic-lanceolate, (9)11.7–17.5(28) × (2.9)3.5–7.1(7.9) mm, (1.9)2–4(5.4) times longer than wide, widest at 0.4–0.6(0.7) of the length, with an attenuate to rounded basis and an acute to obtuse apex with the tip angle (47)70–127(152°). Leaf margin minutely serrulate with (0)2–15 (18) teeth along a 5 mm section just below the tip; the teeth 0.03–0.11(0.15) mm long and 0.1–0.23(0.3) mm wide at the base. Leaves glabrous or hairy with 0–11(57) hairs per mm² at the upper surface of the distal half of the leaf. Terminal rays five, rarely three or four, (6)11–40(45) mm long, 1–2 times dichotomous, (0.6)1–3(4.9) times longer than ray leaves. Ray leaves elliptic-ovate, rarely oblong, (7)8.6–15.7(18.3) × (2.8)4.2–7.8(9) mm, (1.2)1.5–3.4(4.4) times longer than wide,

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Fig. 9. Habit and inflorescence details of: A, Euphorbia flavicoma; B, E. verrucosa. The upper right photo in B shows E. verrucosa (left) and E. montenegrina (right) grown together in the Botanical garden in Innsbruck. — Photos: B. Frajman.
Fig. 10. Habit and inflorescence details of: A, E. serpeniti; B, E. montenegrina. — Photos: B. Frajman.
widest at (0.3)0.4–0.5(0.6) of the length with a rounded basis and an acute to obtuse apex with the tip angle (45°)9–146 (153°). Raylet leaves elliptic-ovate to suborbicular, (4.2)5.2–9(11) × (2.3)2.9–6.6(7.6) mm, (1.2)1.3–1.8(2.1) times longer than wide, widest at 0.4–0.6(0.7) of the length with a rounded basis and an obtuse, rarely acute apex with the tip angle (81°)106–149(164°). Cyathial involucre campanulate, (1.2)1.4–2.2(2.5) × (1.1)1.5–2.4(2.5) mm, (0.7)0.8–1.2(1.3) times longer than wide, with transversely elliptic glands, (0.3)0.5–0.8 (0.9) × (0.7)0.9–1.5 mm, (0.7)0.8–1.2(1.3) times longer than wide. Capsule subglobose, shallowly trilobate, (1.2)1.5–3.8 (4.2) × (1.3)2.7–4.4(4.6) mm, (0.6)0.8–1.0 times longer than wide, widest at (0.3)0.4–0.6(0.7) of the length, with warts (0.1)0.2–0.7(1.0) × 0.2–0.4(0.5) mm, (0.4)0.5–2.1(3.1) times longer than wide, widest at (0.1)0.2–0.6(0.7) of the length. Style (0.3)0.5–1.0(2.0) mm long. Seeds ovoid-ellipsoid to ellipsoid, smooth, brownish, 2.1–2.4 × 2.0–2.2 mm, 1.0–1.1 times longer than wide, widest at 0.4–0.5 of the length. Caruncle kidney-shaped or semi-hemispherical, 0.2–0.7 × 0.3–1.0 mm, 0.6–1.0 times longer than wide and widest at 0.4–0.7 of the length. 2n = 14, 28.

Distribution. – Iberian Peninsula (Portugal, Spain, southern and western France). Note: even if Portugal is not listed as distribution area of E. flavicoma by Benedí & al. (1997), it is included with one locality in the red list of the Portugal flora (https://lvf.flora-on.pt/).

Habitat. – Mesic to mediterranean grasslands, thermophilous forests and forest margins, scrublands from lowlands to 1900 m.

**Euphorbia montenegrina** (Bald.) K. Malý in Glasn. Zemaljsk. Muz. Bosni Hercegovini 20: 556. 1908 = E. verrucosa var. montenegrina Bald. in Mem. Reale Accad. Sci. Ist. Bologna, ser. 5, 9: 38. 1900 = Tithymalus montenegrinus (Bald.) Sojak in Čas. Národn. Muzej, Odd. Prir. 140: 174. 1972 – **Lectotype (designated here):** [Montenegro, Iter Albicanicum (Montenegrinum) sextum], “In reg. fagi m. Balj supra Andrijevca distr. Vasojevići”, 24 Jul 1914, A. Baldacci 383 (WU No. 4498); isolecotypes: not tracked, possibly at several herbaria.

=Euphorbia epiphytoides var. serratifolia Rholena in Sitzungsber. Königl. Böhm. Ges. Wiss., Math.-Naturwiss. Cl. 28(17): 55. 1903 (“1904”) – Type material cited in the protologue: [Montenegro], “Felsige Abhänge des Durmitor oberhalb Crno jezero, c. 2000 m”, J. Rholena s.n.

=Euphorbia montenegrina var. bertsicea Rech.f. in Repert. Spec. Nov. Regni Veg. 38: 374. 1935 – **Lectotype (designated here):** [Kosovo, Rechinger fil. et. Scheffer: Iter Balcanicum 1933], “BERTICUSCUS (Alpes boreales albanicae): In valle rivuli Do[e]! close to Bistrica, Ad casam Kurvala, alt. 1600 m, substr. serpentini.”, 17–19 Jul 1933, K.H. Rechinger 1116 (G barcode G0039033! [two sheets]).

Notes. – Additional original material of Euphorbia montenegrina var. bertsicea cited in the protologue: [Montenegro, Rechinger fil. et. Scheffer: Iter Balcanicum 1933], “BERTICUSCUS (Alpes boreales albanicae): prope Pagum Gusanje, In monte Greben, in rupestribus, alt. ca. 1700 m, substr. calc.”, 25–26 Jul 1933, Scheffer s.n. (G barcode G0039034). Euphorbia montenegrina var. bertsicea should differ from the type variety in being more densely hairy and in having longer and more coarsely dentate leaves. However, all these characters are very variable within E. montenegrina and do not merit taxonomic recognition.

**Description.** – Glabrescent to pubescent perennial, (10)18–45.8(52.8) cm tall, with several stems (16.8)18.8–43.0 (48.3) cm high and (1.5)2–3 mm thick, arising from a woody stock. Middle stem leaves elliptic to elliptic-lanceolate, (14.0)14.6–30.5(37.5) × (7.3)7.5–12.7(13.2) mm, (1.3)1.5–3.0(3.1) times longer than wide, widest at (0.3)0.4–0.6 of the length, with an attenuate basis and an acute apex with the tip angle 50–125 (128°). Leaf margin coarsely serrulate with 3–20(22) teeth along a 5 mm section just below the tip; the teeth (0.07)0.08–0.27 (0.3) mm long and (0.11)0.13–0.36(0.46) mm wide at the base. Leaves glabrous or rarely hairy with 0–5(6) hairs in 1 mm² at the upper surface of the distal half of the leaf. Terminal rays five, rarely three, four or six, (4.3)5.6–33(34.5) mm long, 1 time dichotomous, 0.3–1.8(1.9) times longer than rays leaves. Ray leaves suborbicular, (10.9)13.3–23.1(24.6) × (8.1)8.2–15(17.3) mm, (1.2)1.3–1.8(1.9) times longer than wide, widest at 0.4–0.6 of the length, attenuate at the basis with an acute apex with the tip angle (60)62–106(111°). Raylet leaves elliptic-ovate, (7.7)7.9–14.1(15.3) × (4.4)4.8–10.2(10.7) mm, 1.1–1.8(1.9) times longer than wide, widest at 0.5–0.6 of the length with a rounded basis and an obtuse, rarely acute apex with the tip angle (95)103–170(199°). Cyathial involucre campanulate, (1.5)1.6–3.9(4.0) × (1.4)1.5–2.5(2.6) mm, (0.9)1–2 times longer than wide, with transversely elliptic glands (0.4)0.5–1(1.2) × (0.8)0.9–1.8(2) mm, (0.3)0.4–0.7(0.8) times longer than wide. Capsule subglobose, shallowly trilobate, (1.6)1.8–3.8(4.4) × 1.7–4.1(4.6) mm, (0.7)0.9–1.1(1.2) times longer than wide, widest at (0.3)0.4–0.6(0.7) of the length, with warts 0.2–1.1 (1.3) × (0.07)0.1–0.3(0.4) mm, (1.2)1.4–6.0(13) times longer than wide, widest at 0.7–0.9(0.7) of the length. Style (0.4)0.5–1 (1.2) mm long. Seeds ovoid-ellipsoid to ellipsoid, smooth, greyish, 1.7–2.4 × (1.5)1.7–2(2.3) mm, 1–3(1.4) times longer than wide, widest at (0.3)0.4–0.5(0.6) of the length. Caruncle kidney-shaped, 0.4–0.7(0.8) × (0.6)0.7–1.0(1.2) mm, 0.5–0.7 (0.9) times longer than wide and widest at (0.2)0.3–0.7(0.8) of the length. 2n = 14, 28.

Distribution. – Central and southern Balkan Peninsula (Bosnia and Herzegovina, Montenegro, Kosovo, Albania, North Macedonia, Greece).

Habitat. – Mesic mountain meadows from 1200 to 2250 m, mostly over limestone.

**Euphorbia serpentina** Novák in Acta Bot. Bohem. 3: 35. 1924 – **Lectotype (designated here):** “Flora serbica No. 226. Serbia occidentalis. Zlatibor: ad declivia lapidosa supra rivi Crni Rzav ripam sinistrum apud Gmizovo Cuprijna, in serpentinicis, ca 950 m s.m.”, 18 Jul 1923, Dr. Frant. A. Novák 226 (PRC barcode PRC 455795!).
Description. – Glabrescent to pubescent perennial, 15.5–26.3 cm tall, with several stems 13.9–24.6 cm high and 1–2 mm thick, arising from a woody stock. Middle stem leaves lanceolate, 17.2–27.2 × 5–11.2 mm, 2.1–5.2 times longer than wide, widest at 0.5–0.6 of the length, with an attenuate basis and an acute apex with the tip angle 45.7–88.7°. Leaf margin serrulate with 0–20 teeth along a 5 mm section just below the tip; the teeth up to 0.14 mm long and 0.18 mm wide at the base. Leaves glabrous or rarely hairy with 0–4 hairs per mm² at the upper surface of the distal half of the leaf. Terminal rays 4–6, 4.2–26 mm long, 1 time dichotomous, (0.3)0.5–2.8(4.9) times longer than ray leaves. Ray leaves elliptic-ovate, 14–21.8 × 7.8–15.5 mm, 1.4–2.5 times longer than wide, widest at 0.5–0.6 of the length, attenuate at the basis with an acute apex with the tip angle 59–109°. Raylet leaves elliptic-ovate, 7.2–13.6 × 4.2–10.5 mm, 1.3–2.0 times longer than wide, widest at 0.5–0.6 of the length with a rounded basis and an obtuse, rarely acute apex with the tip angle 130–160°.

Cyathial involucre campanulate, 2.1–5.1 × 1.3–2.5 mm, 1.3–2.2 times longer than wide, with transversely elliptic glands 0.4–0.9 × 0.9–2.5 mm, 0.3–0.8 times longer than wide. Capsule subglobose, shallowly trilobate, (2.4)2.5–3.9(4.1) × (2.1)2.3–4.0(4.1) mm, (0.6)0.7–1.2 times longer than wide, widest at (0.3)0.5–0.6 of the length, with warts (0.6)0.7–1.4(1.5) × 0.1–0.3(0.7) mm, (2.2)2.6–5.5(9.6) times longer than wide, widest at 0–0.6(0.9) of the length. Style 0.8–0.9 mm long. Seeds ovoid-ellipsoid to ellipsoid, smooth, greyish, 1.8–2.4 × 1.6–2.1 mm, 1–1.2 times longer than wide, widest at 0.4–0.5 of the length. Caruncle kidney-shaped, 0.4–0.6 × 0.6–1 mm, 0.6–0.8 times longer than wide and widest at 0.4–0.6 of the length. 2n = likely 14 (based on RGS data).

Distribution. – Central Balkan Peninsula (Bosnia and Herzegovina, Serbia).

Habitat. – Open pine forests, scrubland and rocky grasslands over serpentine from 250 to 1350 m.

Euphorbia verrucosa L., Sp. Pl.: 459. 1753 = Tithymalus verrucosus (L.) Hill, Hort. Kew.: 172.3. 1768 = Galarrhoeus verrucosus (L.) Haw., Syn. Pl. Succ.: 148. 1812 = E. epithymoides var. verrucosa (L.) Fiori in Fiori & Béguinot, Fl. Ital. 2: 278. 1901 = E. flavicoma subsp. verrucosa (L.) Pigatti in Giorn. Bot. Ital. 107: 219. 1973 – Lectotype (designated by Geltman in Novosti Sist. Vyssh. Rast. 40: 121. 2008): “Tithymalys Myersinthes, fructu verrucarum simile Bauh. Ingolstadii, Florentiae”, Herb. Burser XVI(2): 38 (UPS No. V-1749111).

= Euphorbia verrucosa var. montana Gaudin, Fl. Helv. 3: 284. 1828 – Localities cited in the protologue: “In m. Iurae pascius, fere ubique: in m. Thoiry, Dolaz, Montendre, prope St. Cergues etc.”

= Euphorbia brittingeri Opiz ex Rchb., Fl. Germ. Excurs. 2: 757. 1832 = Galarrhoeus brittingeri (Opiz ex Rchb.) Gand., Fl. Eur. 20: 85. 1890 = Tithymalus brittingeri (Opiz ex Rchb.) Holub in Preslia 42: 94. 1970 = E. flavicoma subsp. brittingeri (Opiz ex Rchb.) O.BoIòs & Vigo in Butl. Inst. Catalana Hist. Nat., Secc. Bot. 38: 85. 1974 = Tithymalus flavicomus subsp. brittingeri (Opiz ex Rchb.) Soják in Čas. Nár. Mus., Odd. Přír. 148: 79. 1979 (“1980”) – Lectotype (designated by Cresti & al. in Bot. J. Linn. Soc. 189: 274. 2019): [Austria], “Linz”, May, 48?? (W No. 0077684!)

= Euphorbia verrucosa var. velutina Boiss. in Candolle, Prodr. 15: 129. 1862 ≡ E. flavicoma var. velutina (Boiss.) Breistr. in Bull. Soc. Bot. France, Lett. Bot. 128: 69. 1981 – Localities cited in the protologue: “In littore Genuensi (DC!) et prope Sasrrana (Bertol!)”.

= Euphorbia ericetorum Zumagl., Fl. Pedem. 2: 163. 1864 ≡ Galarrhoeus ericetorum (Zumagl.) Gand., Fl. Eur. 20: 85. 1890 ≡ Tithymalus ericetorum (Zumagl.) Soják in Čas. Nár. Mus., Odd. Přír. 149: 210. 1980 (“1981”).

= Euphorbia flavicoma [unranked] costeana Rouy, Fl. France 12: 149. 1910 ≡ E. costeana (Rouy) P.Fournier, Quatre Fl. France: 270. 1936 ≡ E. flavicoma subsp. costeana (Rouy) Greuter & Burdet in Willdenowia 11: 278. 1981 – Lectotype (designated here): [France: H. Coste – Plantes de France] “Euphorbia flavicoma DC. var. depurpateria Coste/Aveyron: Firmy, serpentine des Puy-de-Wolf. 450 m.”, 24 Apr 1907. H. Coste s.n. (LY barcode LY0001737?). [Image of lectotype: https://explore.recolnat.org/occurrence/F93DA16E775C414B96DA8C9B8D654E8C].

= Euphorbia verrucosa var. viridis Erdn. in Ber. Naturwiss. Vereins Schwaben 39-40: 569. 1911. Description. – Glabrous or sometimes pubescent perennial, (6.5)20–41.5(49) tall, with usually several stems (6.1)17.9–39.0 (42.5) cm high and (1)1.5–3 mm thick, arising from a woody stock. Middle stem leaves oblong-elliptic, (6.6)14.4–32.4 (39.2) × (3.1)5.8–10.3(13) mm, (1.9)2.1–4.0(4.5) times longer than wide, widest at (0.3)0.4–0.6 of the length, with an attenuate to rounded basis and an acute, rarely obtuse apex with the tip angle (46)55–108(120°). Leaf margin serrulate with 0–19 (27) teeth along a 5 mm section just below the tip; the teeth 0.04–0.1(0.6) mm long and up to 0.21(0.32) mm wide at the base. Leaves glabrous or hairy, with 0–32(63) hairs per mm² at the upper surface of the distal half of the leaf. Terminal rays five or rarely three, four or six, (4)7.1–36.5(59) mm long, 1–2 times dichotomous, (0.2)0.5–2.5(3.2) times longer than ray leaves. Ray leaves elliptic to ovate, (7.1)11.6–21.4(31.8) × (3.9)6.7–11.4(12.5) mm, (1.3)1.5–2.5(3) times longer than wide, widest at (0.3)0.4–0.6(0.7) of the length with an attenuate to rounded basis and a broadly acute, rarely obtuse apex with the tip angle (46)62–118(140°). Raylet leaves orbicular-ovate to broadly ovate, (3.7)7.2–14(19.6) × (2.7)4.5–9.5(13) mm, (1.1)1.3–1.8(2) times longer than wide, widest at (0.2)0.4–0.6(0.7) of the length, with rounded basis and broadly acute to obtuse apex with the tip angle (72)100–160(200°). Cyathial involucre campanulate, (1.0)1.4–2.6(3.3) × (1.0)1.2–2.0(2.9) mm, (0.6)0.9–1.6(1.8) times longer than wide, with transversely elliptic glands (0.3)0.4–0.7(1.0) × (0.6)1.2–1.4 mm, (0.3)0.4–0.7(0.8) times longer than wide. Capsule subglobose, shallowly trilobate, (1.1)1.5–3.0(3.7) × (1.0)1.7–3.5(4.2) mm, (0.7)0.8–1(1.1) times longer than wide, widest at 0.3–0.6(0.7) of the length, with warts 0.2–0.6(0.8) × 0.1–0.3(0.5) mm, (0.8)1.1–3.1(4.8) times longer than wide.
than wide, widest at 0–7(0.8) of the length. Styles (0)0.2–0.7 (0.9) mm long. Seeds ovoid-ellipsoid to ellipsoid, smooth, greyish, rarely brownish, (1.4)1.6–2.0(2.7) × (0.8)1.5–1.9(2.0) mm, (0.4)0.9–1.2(1.4) times longer than wide, widest at (0.3)0.4–0.5 (0.6) of the length. Caruncle kidney-shaped or semi-hemispherical, (0.3)0.4–0.5(0.7) × (0.5)0.6–1.0(1.1) mm, (0.4)0.5–0.8(0.9) times longer than wide and widest at 0–5(0.7) of the length. 2n = 14.

**Distribution.** Western, central and south-eastern Europe (north-eastern Spain, France, Switzerland, Germany, Austria, Hungary, northern Italy, Slovenia, Croatia, Bosnia and Herzegovina).

**Habitat.** Mesic grasslands and forest margins from lowlands to 1700 m.

**AUTHOR CONTRIBUTIONS**

BF designed and coordinated the study, collected plants and wrote major parts of the paper. DC, LC and DS performed field work, lab work and morphometric measurements. DC performed statistical analyses of morphological data and wrote corresponding parts, LC performed analyses of RGS and AFLP data and wrote corresponding parts. PS advised morphological data and wrote corresponding parts, LC performed analyses of AFLP data and wrote corresponding parts. BF designed and coordinated the study, collected plants and wrote major parts of the paper. PS advised morphological data and wrote corresponding parts, LC performed analyses of AFLP data and wrote corresponding parts. PS advised morphological data and wrote corresponding parts, LC performed analyses of RGS and AFLP data and wrote corresponding parts. PS advised morphological data and wrote corresponding parts, LC performed analyses of AFLP data and wrote corresponding parts.

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