



Evolutionary origin and systematic position of *Euphorbia normannii* (Euphorbiaceae), an intersectional hybrid and local endemic of the Stavropol Heights (Northern Caucasus, Russia)

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Abstract

The Caucasus is one of the richest areas in the world in terms of animal and plant diversity, harbouring 6400 plant species. As a part of the Northern Caucasus, the Stavropol Heights are renowned for their local endemism, highlighted by six species of flowering plants endemic to this area. One of them is the annual species *Euphorbia normannii*, described in 1891, but with uncertain taxonomic position. We here used nuclear ribosomal internal transcribed spacer and plastid *trnT-trnF* sequences to infer the phylogenetic position of *E. normannii*. The nuclear data inferred its position within *E. sect. Myrsiniteae*, whereas the plastid data placed it within *E. sect. Pithyusa*, thus indicating a hybrid origin. Relative genome size (RGS) data indicate that *E. normannii* has the highest RGS compared to three other annual species belonging to both sections (*E. aleppica*, *E. gaillardotii* and *E. falcata*). Our data are inconclusive whether *E. normannii* is of allopolyploid or homoploid hybrid origin, or whether later hybridisation and plastid capture from *E. sect. Pithyusa* was responsible for the incongruent phylogenetic signal. Morphologically, *E. normannii* is distinct, as are all three before-mentioned annuals, which fall in predominately perennial sections. However, the species most similar to *E. normannii* is *E. falcata* from *E. sect. Pithyusa* and therefore we propose inclusion of *E. normannii* in this section.

Keywords Caucasus · Endemism · Genome size · Morphology · Phylogenetics · Taxonomy

Introduction

Euphorbia L. (Euphorbiaceae) is one of the largest genera of flowering plants. Most of its species occurring in temperate Eurasia belong to *E. subgen. Esula* Pers., which contains roughly 480 species and represents the earliest diverging subgenus within *Euphorbia*. In the most recent taxonomic revision, 21 sections were recognised within this subgenus, based mainly on nuclear and plastid DNA sequence phylogenies (Riina et al. 2013). Several species, for which phylogenetic data were not available, were classified into sections

based on morphological grounds. One of these species is *E. normannii* Schmalh. ex Lipsky, which was described by Lipsky (1891) with a very short diagnosis in Russian. During his exploration of the Northern Caucasus in 1889–1890, he collected an unusual annual *Euphorbia* near Nevinno-mysskaya village (now the town of Nevinno-myssk). The collections appeared to be identical with specimens collected in 1879 and 1883 in the vicinity of Stavropol by A. Normann and “kept together with *E. falcata*” in his herbarium. However, “I. F. Schmalhausen proposed to distinguish [these specimens] as a separate species *E. normannii*” (Lipsky 1891). One year later, Schmalhausen (1892) published a detailed Latin description of *E. normannii* along with an illustration of its seeds and listed three localities, all from the Stavropol Heights (Stavropol’skaya vozvyshennost’) in the Northern Caucasus in Russia.

The Caucasus is one of the richest areas in the world in terms of animal and plant diversity and is considered one of the 25 Global Biodiversity Hotspots of high conservation priority (Myers et al. 2000). It harbours 6400 plant species (Mittermeier et al. 2005) of which over 2700 taxa are

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endemic to this area (Solomon et al. 2014). Species diversity and endemism are exceptionally high for a temperate zone and relate to the geographic position of the Caucasus at the junction of two distinct biogeographic regions, the Euro-Siberian and Irano-Turanian (Mittermeier et al. 2005). Even if most endemic species occur in the Greater and Lesser Caucasus (Mittermeier et al. 2005), the Stavropol Heights in the north of the main Caucasian chain are also renowned for their local endemism (Menitsky 2004; Ivanov et al. 2010).

The formation of the Stavropol Heights began in the Late Miocene as a peninsula of the south coast of the Paratethys (Popov et al. 2004), but its area expanded later (Panina 2009) and nowadays consists mainly of clay, limestone and sandstones. The plateau, which is on average 300 to 600 m high, with the highest summit (Strizhament hill) reaching 831 m, is intertwined with wide river valleys and streams. The natural vegetation of this area with temperate continental climate consists mainly of steppes and localised deciduous forests, which have been largely transformed to agricultural landscapes. Besides *E. normannii*, Ivanov et al. (2010) listed five angiosperms as endemic to the Stavropol Heights, namely *Erodium stevenii* M. Bieb., *Euphorbia aristata* Schmalh., *Hieracium stauropolitanum* Üksip, *Psephellus annae* Galushko and *Vincetoxicum stauropolitanum* Pobed. Most of these species have no close relatives in the Caucasian flora (Ivanov et al. 2010).

Prokhanov (1949) included *E. normannii* in *Euphorbia* subgen. *Paralias* (Raf.) Prokh. sect. *Cymatospermum* Prokh. subsect. *Oleraceae* Prokh. and considered it to be “closely related to the common *E. falcata* L., from which it is easily distinguished by the seeds with two rows of pits at each face and not one”. This section, for which later the name *E. sect. Peplus* Lázaro got established (Prokhanov 1964), included annuals with various ornamentation of seed surface. However, Frajman and Schönswetter (2011) showed that *E. sect. Cymatospermum* was polyphyletic and its representatives were included in eight different sections by Riina et al. (2013). *Euphorbia falcata* was thus included in *E. sect. Pithyusa* (Raf.) Lázaro based on its phylogenetic position, whereas *E. normannii*, for which no phylogenetic data existed, was placed in *E. sect. Arvales* (Geltman) Geltman on morphological grounds. *Euphorbia normannii* shares free styles and in cross section subquadrangular, irregularly tuberculate–rugulose seeds with *E. arvalis* Boiss. & Heldr. and *E. franchetii* B. Fedtsch., which were, based on DNA sequence data, included in *E. sect. Arvales*. However, based on ITS sequence data (A. Kryukov, unpublished) Geltman (2015) suggested that *E. normannii* should rather be included in *E. sect. Myrsiniteae*. *Euphorbia* sect. *Myrsiniteae* and *E. sect. Pithyusa* include 14 and 50, mostly perennial, but also one (*E. aleppica* L.) and two (*E. falcata* and *E. gaillardotii* Boiss. & Blanche) annual species, respectively. Both sections share several morphological

characteristics, like being glaucous and having palmate leaf venation, and were resolved as sisters by the plastid sequence data, whereas the relationships between them based on the ITS sequences remained unresolved, and *E. sect. Myrsiniteae* was rather inferred as sister to *E. sect. Lagascae* Lázaro (Riina et al. 2013). The placement of annual *E. aleppica* in *E. sect. Myrsiniteae* and of *E. falcata* and *E. gaillardotii* in *E. sect. Pithyusa* was very surprising, as these annuals are morphologically very divergent from perennials included in these sections (Frajman and Schönswetter 2011; Pahlevani et al. 2011; Riina et al. 2013).

The aim of this paper is to infer the phylogenetic position of *E. normannii* using nuclear ribosomal ITS and plastid *trnT*–*trnF* sequences, and to provide a hypothesis of its hybrid origin based on the inferred phylogenies. In addition, we compare morphological characteristics and relative genome size (RGS) data of *E. normannii* with other annual species from *E. sect. Myrsiniteae* and *E. sect. Pithyusa* and provide a revised taxonomic treatment including the species description of *E. normannii*, updated compared to the one provided by Schmalhausen (1892).

Materials and methods

Plant material

Plant material for RGS estimation, molecular and morphometric analyses of *E. normannii* was sampled from herbarium vouchers deposited at LE, whereas molecular and RGS analyses of other species were based on silica gel dried leaf material (Online Resource 1). In total, three specimens of *E. normannii* from three different localities were studied phylogenetically, for one specimen RGS was estimated and 16 were included in the morphological analyses. (For details, see “Specimens studied” in the Taxonomic treatment.) For morphological characters of *E. normannii* and of closely related/morphologically similar *E. aleppica*, *E. falcata* and *E. gaillardotii*, several specimens deposited at LE were studied.

DNA extraction, sequencing and analyses of sequence data

Extraction of total genomic DNA and sequencing were performed as described by Frajman and Schönswetter (2011), with the exception that the sequencing was carried out at Eurofins Genomics (Ebersberg, Germany). Contigs were assembled, edited and sequences aligned using Geneious Pro 5.5.9 (Kearse et al. 2012). Base polymorphisms were coded using NC-IUPAC ambiguity codes. GenBank numbers of sequences are given in Online Resource 1. The sampling of species was based on

preliminary phylogenetic analyses, in which *E. normanii* was added to the alignments of Frajman and Schönswetter (2011). As *E. normanii* was shown to belong either to *E. sect. Myrsiniteae* or to *E. sect. Pithyusa*, we included several published sequences from these sections and sequenced some additional accessions, to achieve a representative taxonomic and phylogenetic coverage of both sections, mostly based on phylogenies inferred by Riina et al. (2013). As *E. sect. Lagascae* was inferred as sister to *E. sect. Myrsiniteae* by ITS sequences, we also included this section in our ITS phylogenetic analyses, but not in the plastid analyses, as in the plastid tree the former section was inferred as distantly related to *E. sect. Myrsiniteae* and *E. sect. Pithyusa* (Riina et al. 2013). Likewise, we did not include *E. sect. Arvales* in our analyses, as it is only distantly related to *E. sect. Myrsiniteae* and *E. sect. Pithyusa* (Riina et al. 2013). However, we included five species from *E. sect. Helioscopia* to root the trees (see Online Resource 1 for details). In total, seven ITS and five *trnT-trnF* sequences were generated in this study and 36 ITS and 33 *trnT-trnF* sequences were included from previous studies (Frajman and Schönswetter 2011, 2017; Riina et al. 2013; Falch et al. 2019). GenBank numbers are given in Online Resource 1, and the ITS and plastid alignments are available in Online Resource 2 and 3, respectively.

Maximum parsimony (MP) and MP bootstrap (MPB) analyses were performed using PAUP 4.0b10 (Swofford 2002). The most parsimonious trees were searched for heuristically with 1000 replicates of random sequence addition, TBR swapping and MulTrees on. The swapping was in the case of ITS performed on a maximum of 1000 trees (nchuck = 1000). 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 3.2.1 (Ronquist et al. 2012) applying the GTRG substitution model proposed by the Akaike information criterion implemented in MrAIC.pl 1.4 (Nylander 2004). Values for all parameters, such as the shape of the gamma distribution, were estimated during the analyses. The settings for the Metropolis-coupled Markov chain Monte Carlo process included four runs with four chains each (three heated ones using the default heating scheme), run simultaneously for 10,000,000 generations each, sampling trees every 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. In addition, a NeighborNet was produced with ITS sequences using SplitsTree4 12.3 (Huson and Bryant, 2006).

Relative genome size measurements

RGS was measured for one specimen of *E. normanii*, three populations of *E. aleppica* (*E. sect. Myrsiniteae*), twelve populations of *E. falcata* and one of *E. gaillardotii* (*E. sect. Pithyusa*), as well as two populations of *E. phymatosperma* (*E. sect. Lagascae*; see Online Resource 1 for details). We only included the annual species from the sections closely related to *E. normanii*, as the perennials for which RGS data exist are phylogenetically divergent from *E. normanii*. The perennials have RGS at least three times higher from *E. aleppica* in *E. sect. Myrsiniteae* (Falch et al. 2019) and ranging between the values recorded for *E. falcata* (in case of *E. niciciana* (Borbás) Rech. fil. and *E. seguieriana* Necker; Frajman et al. 2019) and the values that are about five times higher in *E. sect. Pithyusa* (Frajman, unpublished data).

The RGS was estimated with a CyFlow space flow cytometer (Partec, GmbH, Münster, Germany) using 4',6-diamidino-2-phenylindole (DAPI) and the reference standard *Bellis perennis* L. ($2C = 3.38$ pg; Schönswetter et al. 2007) following Suda and Trávníček (2006) and modifications described by Cresti et al. (2019). The RGS was calculated as the ratio between the values of the mean relative fluorescence of the sample and the standard.

Morphological analyses and geographical distribution data

The morphological description of *E. normanii* was produced based on 16 herbarium specimens deposited in LE. Plant height, stem, leaf and some of the raylet leaf characters, number and length of axillary and terminal rays as well as number of branchings of rays were scored manually. Raylet leaf, cyathium, capsule and seed characters were studied and measured using a stereo microscope Stemi 305 (Carl Zeiss) with measuring ruler. Photographs of seeds were taken using a stereo microscope SreREO Lumar.V12 (Carl Zeiss), with a camera AxioCam MRc5. In addition, morphological characters of *E. aleppica*, *E. falcata* and *E. gaillardotii* were studied on herbarium specimens deposited at LE and supplemented with the species' descriptions by Prokhanov (1949) and Radcliffe-Smith (1982). The geographical distribution data of *E. normanii* were extracted from the herbarium labels of the specimens deposited in LE and MW.

Results

ITS and plastid phylogenies

The ITS alignment was 731 characters long, and 187 characters (25.6%) were parsimony informative. Homoplasy index was 0.36 (0.42 after exclusion of uninformative

characters), and the retention index was 0.85. In total, 67,688 most parsimonious trees were found and their length was 606. Bayesian and maximum parsimony reconstructions resulted in congruent topologies (Fig. 1a). *Euphorbia normannii* was positioned within *E. sect. Myrsiniteae* (PP 1, MPB 96%), as sister to the rest of the taxa, which formed a clade (PP 0.98, MPB 93%), in which *E. aleppica* was sister to other taxa. *Euphorbia sect. Lagascae* was sister to *E. sect. Myrsiniteae* (PP 1, MPB 82%), and their sister was *E. sect. Pithyusa* (PP 1, MPB 97%), in which annual *E. falcata* and *E. gaillardotii* were included. The NeighborNet (Fig. 1b) revealed several conflicting splits. *Euphorbia normannii* was positioned along a long split with other species of *E. sect. Myrsiniteae*, from which it was clearly divergent, and shared a common short split with the species of *E. sect. Pithyusa*, of which the annual *E. falcata* was closest to *E. normannii*.

Euphorbia sect. Lagascae was intermediate between *E. sect. Helioscopia* and *E. sect. Myrsiniteae*.

The *trnT-trnF* alignment was 1676 characters long; 91 characters (5.4%) were parsimony informative. Homoplasy index was 0.07 (0.09 after exclusion of uninformative characters), and the retention index was 0.98. Sixty-six most parsimonious trees were found, and their length was 154. Bayesian and maximum parsimony reconstructions resulted in congruent topologies (Fig. 2). *Euphorbia normannii* was positioned within *E. sect. Pithyusa* (PP 1, MPB 94%), as sister of most of the perennial taxa (PP 0.99, MPB 70%), with exception of *E. cassia* Boiss. and *E. pithyusa* L., which were, along with the annual *E. falcata*, positioned in a basal polytomy; *E. gaillardotii* was in the same clade with *E. cassia* (PP 1, MPB 85%). *Euphorbia sect. Pithyusa* was sister (PP 1, MPB 100%) to *E. sect. Myrsiniteae* (PP 1, MPB 98%), in which the annual *E. aleppica* was sister to the perennial taxa.

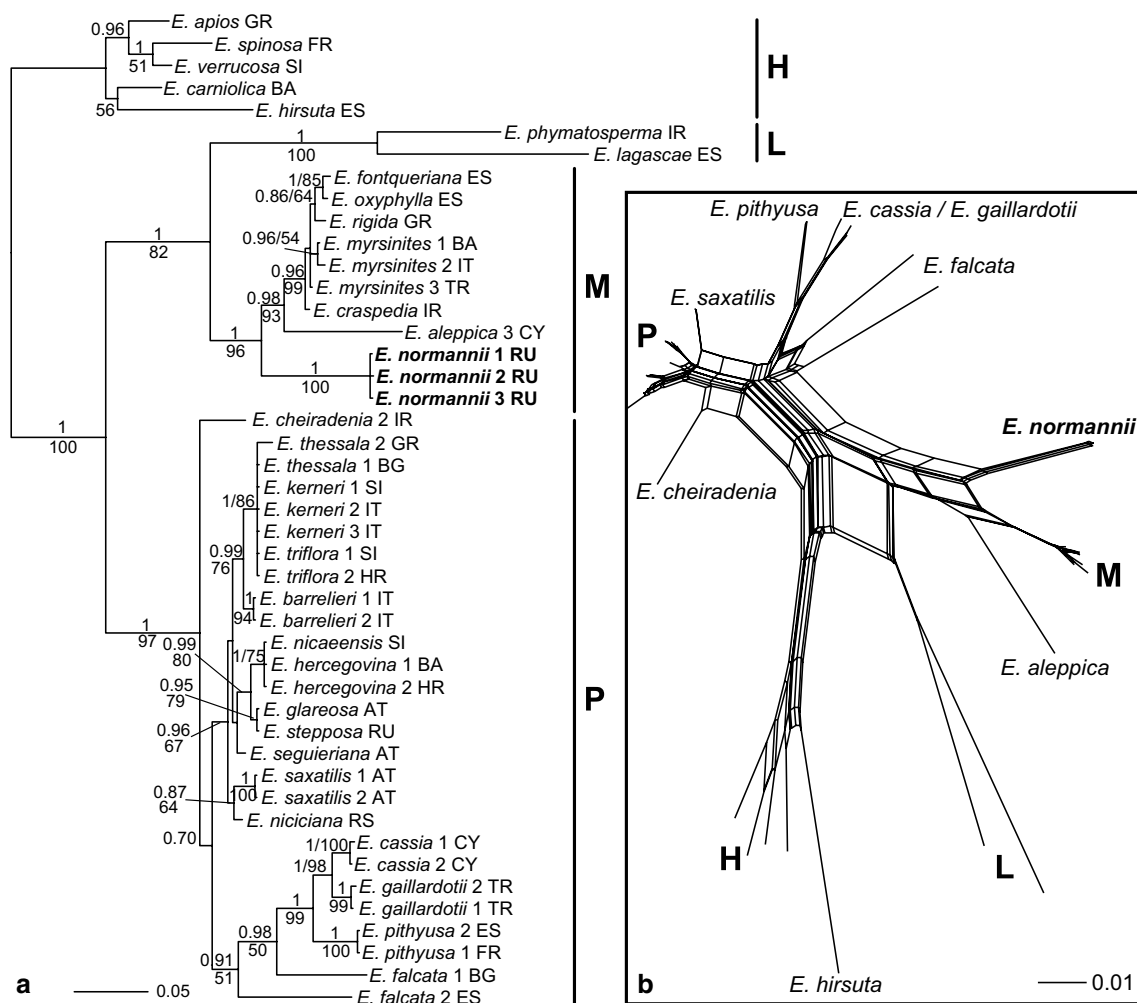


Fig. 1 Bayesian consensus phylogram (**a**) and NeighborNet (**b**) inferred from ITS sequences, showing the phylogenetic position of *Euphorbia normannii*, including different species from the sections *E. sect. Helioscopia* (H), *E. sect. Lagascae* (L), *E. sect. Myrsiniteae*

(M) and *E. sect. Pithyusa* (P). Numbers above branches in **a** are posterior probabilities above 0.65; those below branches maximum parsimony bootstrap values. Population numbers correspond to Online Resource 1. Two-letter country codes follow the accession names

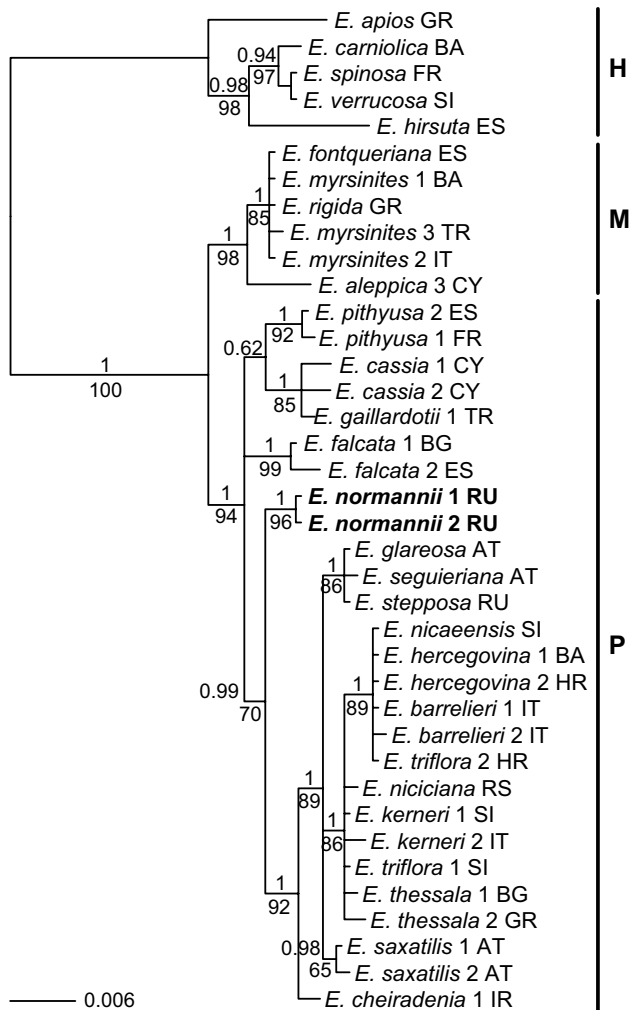


Fig. 2 Bayesian consensus phylogram inferred from plastid *trnT*–*trnF* sequences showing the phylogenetic position of *Euphorbia normanii*, including different species from the sections *E. sect. Helioscopia* (H), *E. sect. Myrsiniteae* (M) and *E. sect. Pithyusa* (P). Numbers above branches are posterior probabilities; those below branches maximum parsimony bootstrap values. Population numbers correspond to Online Resource 1. Two-letter country codes follow the accession names

Relative genome size

The RGS of *E. aleppica* ranged between 0.334 (population 1) and 0.373 (population 3), that of *E. falcata* between 0.450 (population 3) and 0.477 (population 14), *E. gaillardotii* had RGS 0.260, *E. normanii* 0.560, and *E. phymatosperma* between 0.233 (population 2) and 0.235 (population 3; Online Resource 1, Fig. 3).

Morphological and distributional data

The morphological description of *E. normanii* (Fig. 4) is provided in the Taxonomic treatment below. As in many

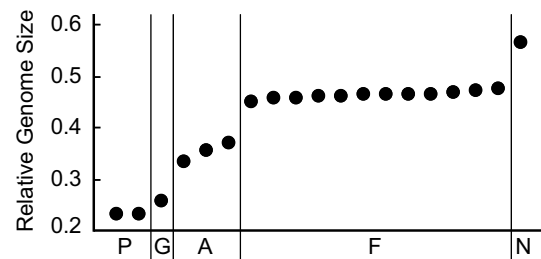


Fig. 3 Mean relative genome size (RGS) of *Euphorbia aleppica* (A), *E. falcata* (F), *E. gaillardotii* (G), *E. normanii* (N) and *E. phymatosperma* (P)

annual *Euphorbia* species, the general habit of *E. normanii* is very variable. The stems are sometimes very short, ca. 5 cm long, and the main part of the plants is formed by the long and branched rays of the synflorescence. *Euphorbia normanii* clearly differs not only from the perennial species of *E. sect. Myrsiniteae*, but also from the annual *E. aleppica* (Online Resource 4). In general habit and other characters, such as the shape of the leaves, it is very similar to *E. falcata* from *E. sect. Pithyusa*, but differs from this species in several other characters (see Table 1, Fig. 5 and Online Resource 4 for details).

The distribution of *E. normanii* based on available herbarium specimens can be summarised to five localities, four in the southern part of the Stavropol Heights and one from the vicinity of the town of Nevinnomyssk to the south of the Stavropol Heights (Fig. 6; Taxonomic treatment).

Discussion

Our phylogenetic data show that *E. normanii*, a regional endemic from the Stavropol Heights area in Russia, has a distinct phylogenetic position within *E. subgen. Esula*, which, however, differs strongly between the nuclear ITS and the plastid *trnT*–*trnF* sequences. In the ITS phylogenetic tree *E. normanii* was inferred as sister to the members of *E. sect. Myrsiniteae*, but shared also some common splits with the members of *E. sect. Pithyusa*, especially with annual *E. falcata*, in the NeighborNet (Fig. 1). On the other hand, in the plastid tree (Fig. 2) *E. normanii* was clearly positioned within *E. sect. Pithyusa* as sister to most perennial species of the section, which all together formed a polytomy with *E. falcata*, *E. cassia*/*E. gaillardotii* and *E. pithyusa*. This incongruent phylogenetic placement strongly suggests that *E. normanii* is of hybrid origin.

Hybridisation, leading to hybrid origin of species, is not a rare phenomenon in plants. It can occur at the homoploid level, i.e. via hybridization not involving whole-genome duplication and thus no increase in ploidy (e.g. Tzvelev 1992; Rieseberg 1997; Frajman et al. 2009; Abbott et al.

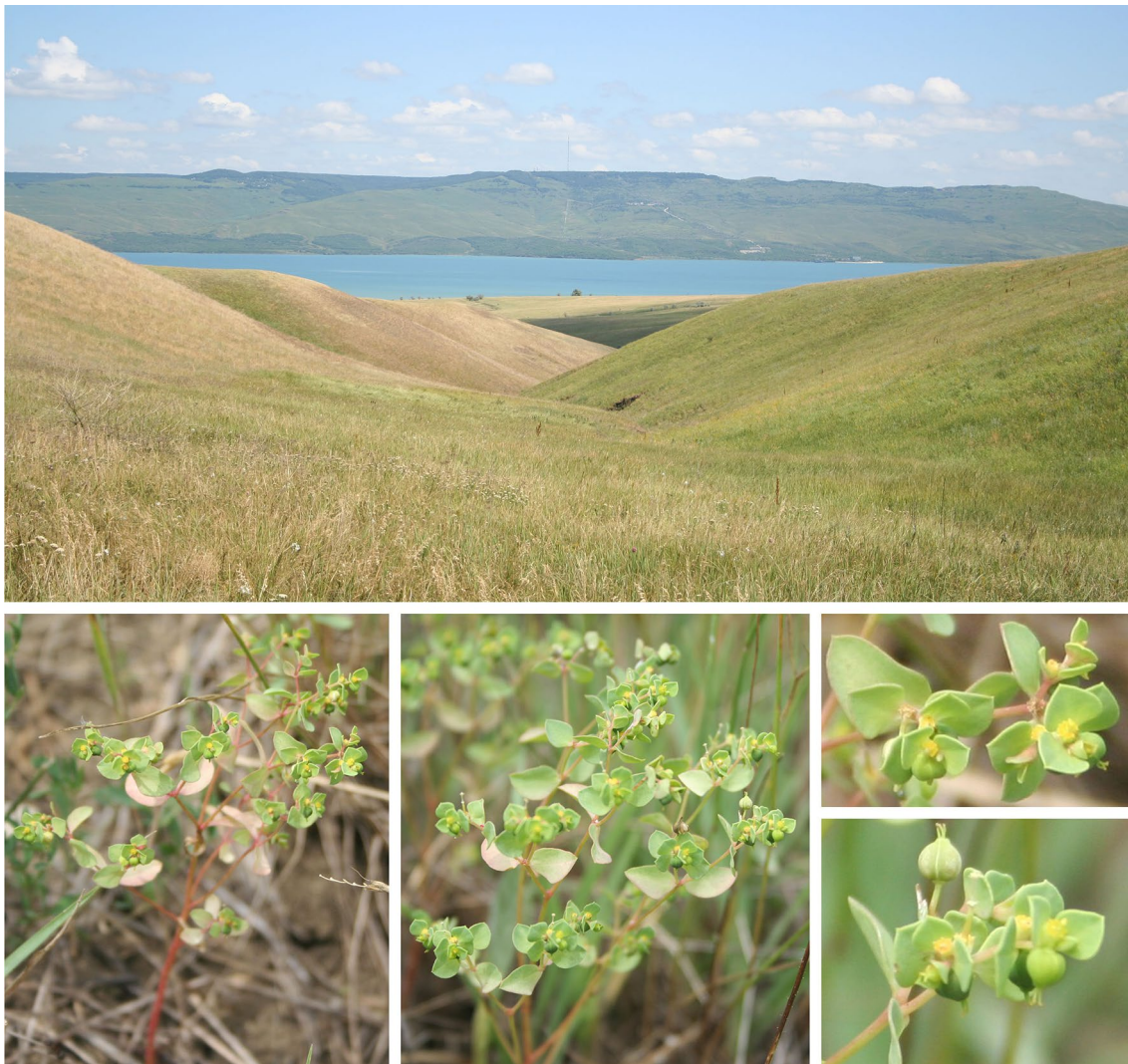


Fig. 4 Typical steppe habitat of *Euphorbia normanii* near its *locus classicus* in the Stavropol Heights (above); habit with inflorescence details (below)

2010; Nieto Feliner et al. 2017). More common is allopolyploidisation, accompanied by multiplication of chromosome sets (e.g. Rieseberg 1997; Soltis et al. 2009, 2016; Wood et al. 2009; Husband et al. 2013; Madlung 2013). In combination with plastid DNA sequences, ITS has often been used to infer origins of hybrid plant species (e.g. Sang et al. 1995; Popp et al. 2005; Frajman et al. 2009, 2018; Kuzmanović et al. 2017). However, since the homoeologous ITS repeats in a hybrid often are homogenised towards one of the parental types by concerted evolution (Wendel et al. 1995), the inference of hybrid origin is only possible if the homogenisation is directed towards the paternal lineage (Smedmark and Eriksson 2002; Popp et al. 2005), which was likely the case in *E. normanii*. A likely annual member of *E. sect. Myrsiniteae* thus acted as the paternal parent, and an annual

member of *E. sect. Pithyusa* as the maternal parent in the origin of *E. normanii*.

The distribution area of *E. normanii* today lies within the distribution of *E. sect. Pithyusa*. The annual *E. falcata* as well as the perennial *E. glareosa* Pall. ex M. Bieb. (sensu stricto, cf. Geltman 2005), *E. stepposa* Zoz ex Prokh. and *E. seguieriana* from this section co-occur in the Stavropol Heights, and *E. petrophila* C.A. Mey. is found in their vicinity. The closest localities of the annual *E. gailardotii* are in Asia Minor (Radcliffe-Smith 1982). On the other hand, the nearest localities of members of *E. sect. Myrsiniteae* are at least 200 to 300 km away, rendering current hybridization in situ impossible. Perennial *E. pontica* Prokh. and *E. rigida* M. Bieb. occur in the north-eastern Black Sea coast area, whereas annual *E. aleppica* has been reported from a few localities in Transcaucasia and

Table 1 Comparison of morphological characters of *Euphorbia normanii* and other annual species from *E. sect. Myrsinitae* and *E. sect. Pithyusa*

Character	<i>E. normanii</i>	<i>E. falcata</i>	<i>E. aleppica</i>	<i>E. gaillardotii</i>
Cauline leaves	Narrowly oblanceolate, entire, at base cuneate, with shortly acute or obtuse apex, 1.5–3 cm long and 2–4 mm wide, early deciduous	Oblong-spatulate or narrowly oblanceolate, at base cuneate, shortly acute or obtuse at apex, entire or sometimes minutely dentate in upper part, 0.5–3.2 cm long and 3–7 mm wide, more or less persistent	Numerous, narrowly linear, truncate at base, shortly acute at apex, entire, minutely papillose, 0.5–4.5 cm long and 0.2–1.5 mm wide	Oblanceolate, reflexed, tapered at base, acute at apex, minutely denticulate to subentire, 2–4 (5.5) cm long and 5–15 mm wide
Raylet leaves	Oblong-elliptic to rhombic-elliptic, sometimes almost falciform, 0.5–1.6 cm long and 3–5 mm wide	Triangular-elliptic and rhombic-elliptic to almost reniform, acute at apex, often aristate or acuminate, 0.5–1.8 cm long and 5–10 mm wide	Ovate-rhombic or rhombic-elliptic, long acuminate, entire or irregularly crenate, 0.5–3 cm long and 6–18 mm wide	Ovate-rhombic to suborbicular, 0.5–2.5 cm long and 5–20 mm wide
Cyathial glands	Trapezoid or oblong-elliptic, 0.5–0.7 × 0.3–0.4 mm, with two tiny horn-like white appendages ca. 0.1 mm long	Trapezoid or elliptic, 0.3–0.6 × 0.2–0.3 mm sometimes with horn-like appendages ca. 0.2 mm long, more often without them	Elliptic or semilunate, 0.8–1.2 × 0.2–0.3 mm, with distinct horn-like appendages ca. 1 mm long	Elliptic or trapezoid, 1.2–1.5 × 0.3–0.6 mm, without appendages
Capsule	Slightly trilobate, 1.6–2.0 × 1.4–1.8 mm	Minutely trilobate, 1.5–2 × 1.2–1.6 mm	Distinctly trilobate, 1.5–2 × 2–2.5 mm	Distinctly trilobate, ca. 1.8 × 2.2 mm
Seeds (Fig. 5)	Oblong-ovoid, subtetrahedral, greyish, 1.3–1.5 mm long and 0.7–1 mm wide, irregularly longitudinally sulcate-foveolate; caruncle small, early caducous	Oblong, compressed tetrahedral, 1.2–1.7 mm long and 0.7–1 mm wide, on facets with 5–10 regular transverse grooves; caruncle small, early caducous	Ovoid-subglobose, subtetrahedral, minutely tuberculate, 1.3–1.5 mm long and 1–1.2 mm wide, ecarunculate	Ovoid-ellipsoid, subtetrahedral, shallowly and irregularly pitted, 1.3–1.5 mm long, 0.8–0.9 mm wide

Fig. 5 Seeds of *Euphorbia normannii* (a), *E. falcata* (b), *E. aleppica* (c) and *E. gaillardotii* (d)

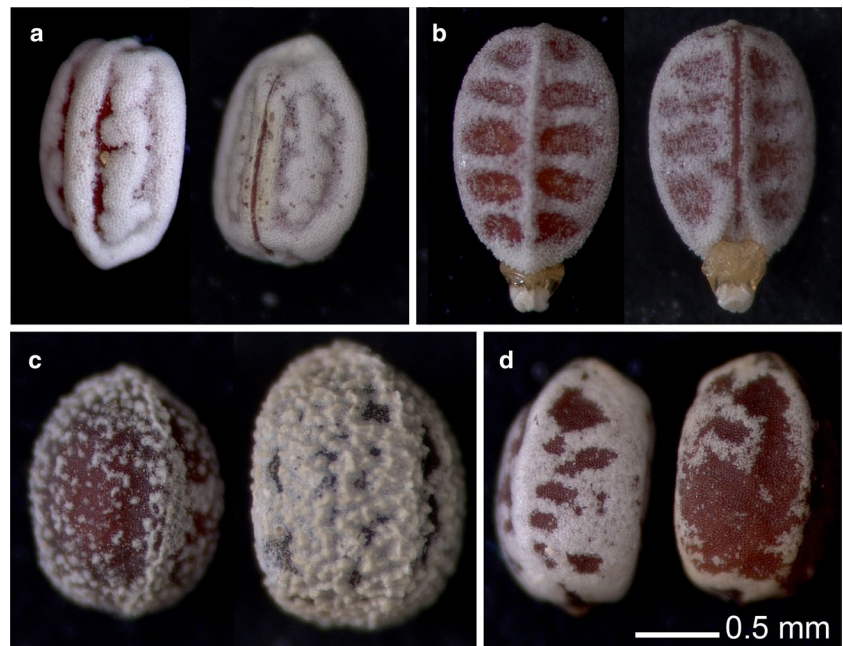
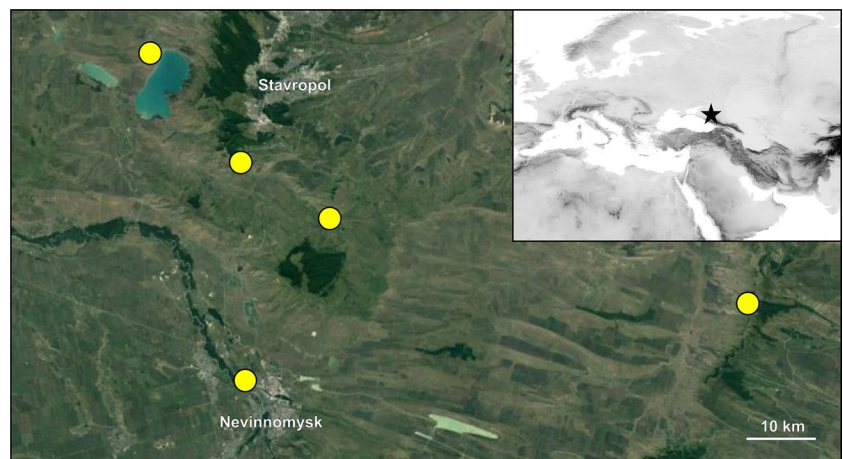


Fig. 6 Distribution of *Euphorbia normannii* shown on an aerial photograph of the Stavropol Heights (adopted from Google Earth); in the inset, the position of the Stavropol Heights in western Eurasia is indicated



Crimea 400 to 600 km away. However, it is possible that both sections came in contact in the Stavropol area in the past, since the distribution of species can change through time, which was especially evident during the Pleistocene climatic oscillations and in the face of the Holocene climate warming (e.g. Hewitt 1999; Petit et al. 2003; Magri et al. 2007; Schönswetter et al. 2007). Alternatively, it is either possible that *E. normannii* originated elsewhere, had a larger distribution in the past and persisted until today only in the Stavropol Heights, or, alternatively, that some other, nowadays extinct, member of the sections *Myrsiniteae* and/or *Pithyusa* was involved in its origin.

Based on morphological similarity as well as a close phylogenetic position in the plastid tree and shared

common splits in the ITS NeighborNet, we suggest that *E. falcata* was involved in the origin of or hybridisation with *E. normannii*. It is unlikely that perennial species would have been involved in hybridisation and origin of *E. normannii*, as they are morphologically very divergent (see e.g. Frajman and Schönswetter 2017; Frajman et al. 2019). In the case of *E. sect. Myrsiniteae*, we cannot clearly point to the parental species of *E. normannii*, given the high morphological dissimilarity of *E. aleppica*, but inferred phylogeny (Fig. 1) indicates that both annuals diverged early in the evolution of the section and obviously attained very distinct morphology different of that of perennials (see Pahlevani et al. 2011).

Without establishing the chromosome number and thus the ploidy level of *E. normanii*, it is also impossible to clearly infer whether it is of homoploid or of allopolyploid hybrid origin. The RGS data suggest that an allopolyploid origin is likely, as *E. normanii* has the highest RGS of all closely related annual species (Fig. 3). Its RGS is neither the double of that of *E. aleppica* nor of *E. falcata*, likely due to genome downsizing accompanying the process of diploidisation following polyploid speciation (Verma and Rees 1974; Leitch and Bennett 2004; Renny-Byfield et al. 2013). For *E. aleppica*—as well as for all perennial species of *E. sect. Myrsiniteae*—the chromosome number $2n=20$ has been established, whereas for *E. falcata* $2n=14, 16, 28, 32, 36$ and for *E. gaillardotii* $2n=18$ have been reported (Rice et al. 2015, and references therein). Given that polyploid, likely tetraploid, individuals have been reported for *E. falcata*, it appears likely that *E. falcata* participated also in the allotetraploid origin of *E. normanii*. A scenario alternative to and equally likely as the hybrid origin of *E. normanii* is its non-hybrid origin within *E. sect. Myrsiniteae* and a later hybridisation with co-occurring *E. falcata* (or any other related, now extinct species), from which *E. normanii* captured the plastome. Chloroplast capture (Rieseberg and Soltis 1991; Tsitrone et al. 2003) has been proposed as cause of discordant nuclear and plastid phylogenies in several plant groups (e.g. Okuyama et al. 2005; Frajman et al. 2009).

In conclusion, our data indicate that hybridisation was involved in the evolutionary history of *E. normanii*, a stenoendemic species of the Stavropol Heights in Russia, highlighting the importance of the Caucasus as an important biodiversity hotspot and centre of plant endemism. In particular, it emphasises a high conservation value of the Stavropol Heights, a small area harbouring six endemic angiosperms.

Taxonomic treatment

Based on the incongruent phylogenetic position of *E. normanii* across plastid and nuclear phylogenies, we could include this species both in *E. sect. Myrsiniteae* and in *E. sect. Pithyusa*. The absence of bracteoles subtending the male flowers is one of the main synapomorphies of *E. sect. Myrsiniteae* (Riina et al. 2013). However, in the cyathia of *E. normanii* we found filiform structures, which are very likely the bracteoles of male flowers. This, along with the similarity in habit of *E. normanii* with *E. falcata*, we propose its inclusion in *E. sect. Pithyusa*. We emphasise the importance of future studies of the internal cyathial structures and their ontogenetic development in *E. subgen. Esula*, along with the evaluation of their taxonomic value.

Euphorbia normanii Schmalh. ex Lipsky in Zap. Kievsk. Obshch. Estestvoisp. 11(2): 57. 1891. \equiv *Tithymalus normanii* (Schmalh. ex Lipsky) Prokh., in Komarov, Fl. URSS, 14: 466, nom. alt.—LECTOTYPE (designated by Geltman 2000: 103): [Russia, Stavropol Territory], Rybnoe Lake, 17 May 1879, 6 Jun 1883, *Normann* s.n. (KW!).

Description: Glabrous or rarely shortly pubescent annual 10–30 cm high, with solitary, erect or sometimes ascendent stem 5–10 cm high and 0.3–1.5(1.8) mm thick, arising from a vertical root, usually without axillary vegetative shoots. Axillary rays 2–8, 2–12(15) cm long, 1–3(4)-times dichotomous. Cauline leaves with 3 palmate veins, narrowly oblanceolate, entire, at base cuneate, with shortly acute or obtuse apex, 1.5–3 cm long and 2–4 mm wide, early deciduous. Ray leaves similar to the cauline leaves. Terminal rays 2–4, 2.5–6 cm long, 1–3 times dichotomous. Raylet leaves oblong-elliptic to rhombic-elliptic, sometimes almost falciform, 0.5–1.6 cm long and 3–5 mm wide. Cyathium campanulate, ca. 1×0.5 mm. Bracteoles of male flowers filiform. Cyathial glands trapezoid or oblong-elliptic, $0.5\text{--}0.7 \times 0.3\text{--}0.4$ mm, with two tiny horn-like white appendages ca. 0.1 mm long. Capsule broadly conical, slightly trilobate, $1.6\text{--}2.0 \times 1.4\text{--}1.8$ mm. Styles free, ca. 0.5 mm. Seeds oblong-ovoid, subtetrahedral, greyish, 1.3–1.5 mm long and 0.7–1 mm wide, irregularly longitudinally sulcate-foveolate. Caruncle conical 0.2–0.3 mm, early deciduous, and therefore seeds often appear ecarunculate.

Habitat: Steppes, usually on unstable, moving soil, or open steppe areas with sparse plant cover (Fig. 4). Sometimes growing together with *E. falcata*.

Distribution: Known from four localities in the southern part of the Stavropol Heights (vicinity of village Tatarka, village of Temnolesskaya, Lake Rybnoe—Sengileevskoe reservoir, Prikalaus Hills close to Aleksandrovskoe) and from the vicinity of the town of Nevinnomyssk south of the Stavropol Heights (Fig. 6).

Conservation status: Following criterion D for vulnerable species of the IUCN (2012) Red List categories, i.e. population size estimated to number fewer than 1000 mature individuals with a very restricted area of occupancy, we deem *E. normanii* vulnerable (VU) based on the current knowledge of its distribution.

Additional specimens studied: Russia, Stavropol Territory: Shpakovsky District, vicinity of Tatarka, $44^{\circ} 55' 46.3''$ N, $41^{\circ} 54' 30.1''$ E, slope of small depression amongst steppe vegetation, plot with sparse herb cover, 321 m a. s. l., 19 Jun 2015, *Geltman* 235 (LE 01057241, 01057242); south of

Tatarka (vicinity of Stavropol), valley of the river Egorlyk, on slope, 7 Aug 1949, *I.V. Novopokrovsky, A.I. Pojarkova* 296, 296a (LE 01057243, 01057247); In vicinia opp. Stavropol, ripa lacus Sengilejevski (v. Rybnoje), in glareosis, 28 Jul 1949, *E. Bobrov, Ja. Prokhanov s.n.* (LE 01057244, 01057245 MW 0690362); Kuban province, Nevinnomysskaya, close to the camp near the river Kuban, 26 Jun 1890, *V. Lipsky s.n.* (LE 01057246); Nevinnomysskaya, along the river Kuban, 3 Jun 1892, *V. Lipsky s.n.* (LE 01057248–01057253); Nevinnomysskaya, Rychev hill, 9 Jul 1932, [*V.A. Arsenyev*] *s.n.* (MW 0690361); Temnolesskaya, 2000' 17 Jun 1889, *I.Ya. Akinfiyev s.n.* (LE 01057254, 01057255); Prikalau Heights, 12–15 km NWW from Aleksandrovskoye, *Artemisia - Festuca valesiaca* steppes on slightly saline slopes and bottoms of a ravine, 29 Jun 1988, *Geltman et al.* 2050 (LE 01057256).

Information on Electronic Supplementary Material

Online Resource 1. Studied populations of *Euphorbia normanii* and related *Euphorbia* species, including voucher information, GenBank numbers and relative genome size (RGS) data.

Online Resource 2. Alignment of ITS sequences used in this study. Accession names are complemented with GenBank numbers and their voucher data available in Online Resource 1.

Online Resource 3. Alignment of plastid *trnT-trnF* sequences used in this study. Accession names are complemented with GenBank numbers and their voucher data available in Online Resource 1.

Online Resource 4. Herbarium specimens of *Euphorbia aleppica*, *E. falcata*, *E. gaillardotii* and *E. normanii* (all deposited at LE), showing morphological differences among the species.

Supplementary Information The online version of this article (<https://doi.org/10.1007/s00606-021-01741-8>) contains supplementary material, which is available to authorized users.

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Compliance with ethical standards

Conflict of interest There is no conflict of interests.

Ethics approval Ethical approval does not apply. Accepted principles of ethical and professional conduct have been followed.

Consent to participate No consent to participate is needed.

Consent for publication No consent for publication is needed.

Availability of data and material Data are available as Electronic Supplementary Material. Sequences have been submitted to GenBank.

Code availability No specific software application or custom code was generated.

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