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The first record of *Ephedra distachya* L. (Ephedraceae, Gnetophyta) in Serbia - Biogeography, coenology, and conservation -

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ABSTRACT: During floristic investigations of eastern Serbia (foothills of the Stara Planina Mountains near Minićevo, Turjačka Glama hill), *Ephedra distachya* (Ephedraceae) was discovered as a species new for the vascular flora of Serbia. An overview of the family, genus, and species is given in the present paper. In addition, two phytocoenological relevés recorded in the species habitat are classified at the alliance level. The IUCN threatened status of the population in Serbia is assessed as Critically Endangered.

Keywords: *Ephedra distachya*, Ephedraceae, new record, Stara Planina Mountains, flora of Serbia

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INTRODUCTION

In spite of continuous and intensive investigations of the Serbian flora at the end of the 20th century (Josifović 1970-1977; Sarić & Diklić 1986; Sarić 1992; Stevanović 1999), numerous new species and even higher taxa were recorded in the past two decades (Stevanović 2015). Last year, *Ephedra distachya* L. – a relict species new for the flora of Serbia – was accidentally discovered in its natural habitat in eastern Serbia. It belongs to an ancient group of gymnosperms (Ephedraceae, Gnetophyta), which was also not previously recorded for the flora of Serbia. In view of the importance of the taxa in question, the form of this paper is adapted to the publications mentioned as a supplement (The Flora of Serbia and Red Data Book of the Flora of Serbia).

MATERIAL AND METHODS

The voucher specimens of *E. distachya* are deposited in the General Herbarium of the Balkan Peninsula of the Natural History Museum in Belgrade (BEO). The morphological descriptions of taxa follow Bobrov (1934), Amaral Franco J (1986), Markgraf *et al.* (1993), Stevenson (1993), and Fu *et al.* (1999). The distribution of *E. distachya* in the Southeast Europe is mapped on a 50×50 km MGRS grid system (Lampinen 2001) based on the species distribution map in the Atlas Florae Europaeae (Jalas & Suominen 1973) and supplemented and/or confirmed by chrorological records from Stoyanov (1963), Horeanu & Viţalariu (1992), Christensen (1997), Sanda *et al.* (2001), Tzonev *et al.* (2005, 2006), Stoyanov (2006), Frink & Szabó (2009), Szalczer & Szalczer (2009), Făgăraş (2011), Peev *et al.* (2012), Miho *et al.* (2013), Caković *et al.* (2014), Biserkov *et al.* (2015), Nikolić (2015), Strat (2016), and Giannopoulos *et al.* (2017). Phytocoenological relevés were sampled using the Braun-Blanquet (1964) method. The threatening status of *E. distachya* according to the IUCN criteria (IUCN 2012) was estimated using a software application adapted to MS Office (Niketić 1999, upgraded 2016).

OVERVIEW OF THE FAMILY AND THE GENUS

Ephedraceae Dumort., Anal. Fam. Pl. 11, 12 (1829); ***Ephedra*** L., Sp. Pl. 2: 1040 (1753), lectotype: *E. distachya* L., loc. cit. (Hitchcock & Green 1947: 115).

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Family description. Ephedraceae encompasses dioecious, rarely predominantly monoecious (with unisexual strobili), much-branched shrubs, subshrubs, climbers, or small trees from (2)7 cm to 4.5(5) m high; not resiniferous. Roots fibrous. Stem arising from a rhizome or a woody and branched caudex (bark grey or reddish-brown); erect to procumbent, photosynthetic, finely sulcate, often with whitish dotted lines along the ridges; branchlets terete, opposite, verticillate or dichotomously divided, yellowish green to olive-green when young, terete, finely sulcate; internodes 1–10 cm long. Leaves simple, opposite and decussate or verticillate (3–4 per node); usually reduced and scarious, 1–2(-3) mm long, non-photosynthetic (yellowish or brownish) or partially photosynthetic, sometimes connate at the base (forming a basal sheath), with obtuse to setaceous apex; persistent or partially to entirely deciduous. Strobili terminal or axillary; sessile or on short shoots; unisexual, rarely male strobili with sterile female parts. Male strobili ('cones') compound, lanceoloid or ellipsoid to ovoid or obovoid, solitary or 2–10 whorled at branch nodes; each compound strobilus with 2–8(-12) sets of decussate or three-whorled free scarious bracts; proximal bracts empty, distal bracts subtending an axillary male strobilus ('flower') composed of a pair of fused bracteoles ('perigon') surrounding the antherophore ('androecium') with a column (sometimes divided at apex) bearing 2–10(-15) sessile or long stipitate sporangiophores ('stamens') with microsporangia ('anthers') often exserted slightly beyond the cone bract; microsporangia 2–3-lobed, all lobes dehiscing by means of apical horizontal slits; pollen elongatedly ellipsoidal, with 6–12 longitudinal furrows, not winged. Female strobili compound, ellipsoid to ovoid, obovoid, or nearly globose, solitary or 2–10 whorled at branch nodes; compound strobili sessile or short- to long-stalked, with 2–10 sets of decussate or three-whorled free scarious or papery to fleshy bracts; proximal bracts empty, distal bracts subtending an axillary female strobilus ('flower') composed of a pair of fused bracteoles ('perigon') enclosing (1-)2–3 orthotropous ovules; in some species, each ovule completely surrounded by a utricle-like envelope of fused bracts (also reminiscent of an additional outer integument); ovules with a single membranous integument prolonged into a slender apical tube (tubillus) which protrudes out of the envelope producing a pollination droplet. Seeds 1–2(-3) per compound female strobilus (false fruit), ellipsoid to globose, yellow to dark-brown, smooth or furrowed; bracts usually becoming succulent during ripening (forming white, yellow, red, or purple false berries) or remaining dry and subcoriaceous, winged or not. Cotyledons two; germination epigeal.

According to Mundry & Stützel (2004), individual male strobili ('flowers') are homologous to reduced compound strobili of the extinct Cordaitales (hypothetically a sister group of gymnosperms) in being reduced to a single terminal synangium. On the other hand, the ephedral 'flowers' are actually analogous to angiosperm pseudanthia.

Pollination. Anemophily, rarely entomophily. The primarily entomophilous *E. foeminea* Forssk. is pollinated by nocturnal insects. In this species, production of a pollination droplet is synchronised with the cycles of the full moon, which is a unique situation in the plant world (Rydin & Bolinder 2015).

Species diversity and general distribution. The monotypic family Ephedraceae comprises 55–65 species of the genus *Ephedra* L., which are autochthonous in arid regions of the Old and New World (Bolinder *et al.* 2016). The vast majority of species are distributed in Central (25 species) and Western (17 species) Asia; secondary centres of diversity are situated in North (13 species) and South (12 species) America. Species are also present on the Canary Islands, in the Mediterranean region, from Southern to Eastern Europe, on the Arabian Peninsula, in the Indo-Himalayan region, in Eastern Asia, and in North and South Africa.

Biogeographical history. The earliest known fossil records of Ephedraceae and extinct relatives were dated to the Early Cretaceous. Some authors suppose that the family originated in the Permian, when other modern groups of gymnosperms, including Gnetales, appeared (Wilson 1959). The diversity of Ephedraceae reached a peak during the Middle Cretaceous (Rydin *et al.* 2006), but it declined dramatically at the end of the period, causing a bottleneck effect during the Palaeocene (Rydin *et al.* 2004, 2010). A second radiation of the genus is estimated to have occurred during the Oligocene in the Mediterranean region, but later in Asia and America (during the Miocene and Pliocene) (Rydin *et al.* 2006). Modern species probably originated from extinct and morphologically very similar Cretaceous sister lineage(s). Migration and long-distance dispersal, not vicariance, are responsible for the current distribution pattern with a low level of genetic and morphological diversity (Rydin *et al.* 2004, 2010). According to Bolinder *et al.* (2016), an evolutionary shift from entomophily to anemophily in the Oligocene can explain secondary diversification. Thus, the majority of recent species are primarily pollinated by the wind and only two species are entomophilous. One of them, *E. foeminea*, as a sister to all other species of the genus, has male strobili with ovules that are sterile but produce pollination drops. These relict plants therefore are morphologically bisexual but functionally unisexual, and insects are the main pollen vectors. Bisexual structures and entomophily as the primary mode of pollination in the Ephedraceae can be regarded as retained primitive characteristics (Bolinder *et al.* 2016).

Taxonomic and phylogenetic position. It has been shown that the family *Ephedraceae* forms a strongly monophyletic group (Bowe *et al.* 2000; Rydin *et al.* 2002) within Gnetales, with a unique combination of morphological and biochemical characters: small decussate or whorled ephemeral scarious leaves on photosynthetic stems; photosynthetic vessels and tracheary system; partially reduced flower-like strobili; inaperturate polyplicate pollen; bracts becoming fleshy and/or winged during ripening; presence of ephedrine; etc. (Ickert-Bond & Wojciechowski 2004). On the other hand, synapomorphic characteristics between Ephedraceae and the remaining members of Gnetales (*Welwitschia*, *Gnetum*) are not so numerous: reduced compound stobili; bracts enveloping around the antherophore and ovules; and production of a pollination droplet (Judd *et al.* 2008). Gnetales is a sister group to the conifers (Bowe *et al.* 2000) or the rest of the gymnosperms (Lee *et al.* 2011), which is not in concordance with the previous anthophyte hypothesis (Doyle & Donoghue 1987) and possible relationship with angiosperms.

Infrageneric variability. The traditional sectional classification of the genus *Ephedra* (Stapf 1889) encompasses three sections: *E.* sect. *Ephedra* (syn. *E.* sect. *Pseudobaccatae* Stapf), with mostly succulent unwinged bracts; *E.* sect. *Alatae* Stapf, with dry winged bracts; and *E.* sect. *Asarca* Stapf, with ovulate strobili and scariously bordered bracts which are neither winged nor fleshy. The type section is the most species-rich and, together with *E.* sect. *Alatae*, is widespread within the range of the genus in the Old and New World. *Ephedra* sect. *Asarca* is known from North America with two species. Studies treating phylogeny (Ickert-Bond & Wojciechowski 2004; Rydin *et al.* 2010) did not support this subdivision, and it is also not correlated with other morphological traits (bracts, sporangiophores, and habitus). Among the Old World species, three highly phylogenetically and morphologically supported monophyletic groups were separated. The sister clade of the rest of the genus represents members of the traditional subsection *E.* subsect. *Scandentes* Stapf (including the Mediterranean *E. foeminea* and *E. fragilis*), which is in concordance with the known morphology of this relict group. Representatives of the second clade (predominantly Asian species) are members of the type group (*Distachyae*), together with representatives of the *Sarcocarpae* group and one species from *E.* sect. *Alatae*. The third Old Word clade (*E.* subsect. *Leptocladae*) is comprised of three species from Somalia, Israel, the Himalayas, and Mongolia. The New World clade includes two strongly supported subclades of the North and South American species.

Pharmacological properties. Many *Ephedra* species contain in their aerial parts ephedrine and related alkaloids (norephedrine, pseudoephedrine, methylephedrine, methylpseudoephedrine) as characteristic constituents. These alkaloid-rich *Ephedra* species (alkaloid content ≥ 3%) are mainly distributed in Eurasia, especially in Eastern Asia (Sticher *et al.* 2015). They include *E. sinica* Stapf, *E. intermedia* Schrenk & C.A. Mey., and *E. equisetina* Bunge. According to the European Pharmacopoeia (Ph. Eur. 2013), these three species are the only sources for the *Ephedra* herb, *Ephedrae herba*. It consists of dried herbaceous stems of these species and should contain a minimum of 1% of ephedrine (Ph. Eur. 2013). In traditional medicine in China and Japan, *Ephedrae herba* is used as a stimulative, antitussive, and antiasthmatic agent (Abourashed *et al.* 2003; Kakiuchi *et al.* 2011). The pharmacological action of *Ephedrae herba* is due to the presence of ephedrine and pseudoephedrine. It has been used chiefly as a source of these alkaloids, which can also be of synthetic origin, and is employed for the relief of nasal congestion associated with colds (Sweetman 2011). It should be noted that there are also *Ephedra* species with low content of ephedrine-type alkaloids (Sticher *et al.* 2015), and these species include *E. distachya*. For example, the mean content of ephedrine and pseudoephedrine was 0.36% in samples of *E. distachya* from France and 0.17% in ones from Turkey (Ni *et al.* 2013).

OVERVIEW OF THE SPECIES

Ephedra distachya L., Sp. Pl. 2: 1040 (1753), lectotype: The George Clifford Herbarium 456 "Ephedra 1"–*BM000647523* BM (Nouviant 1996: 132) ≡ *Chaetocladus distachys* (L.) J. Nelson, Pinaceae: 162 (1866).

= *E. monostachya* L., Sp. Pl. 2: 1040 (1753) ≡ *E. vulgaris* var. *monostachya* (L.) C.A. Mey., Vers. Monogr. *Ephedra*: 84 (1846) ≡ *E. distachya* subsp. *monostachya* (L.) Riedl, Sci. Pharm. 35: 228 (1967).

= *E. vulgaris* Rich., Comm. Bot. Conif. Cycad.: 26 (1826), nom. illeg. (nom superfl. for *E. distachya*)

= *E. minor* Host, Fl. Austriac. 2: 671 (1831).

= *E. media* C.A. Mey., Monogr. Ephedra: 80 (1846).

= *E. helvetica* C.A. Mey., Monogr. Ephedra: 87 (1846) ≡ *E. distachya* subsp. *helvetica* (C.A. Mey.) Asch. & Graebn., Syn. Mitteleur. Fl. 1: 260 (1897).

= *E. botryoides* Fisch. in C.A. Mey., Monogr. Ephedra: 90 (1846).

= *E. arborea* Lag. ex Bertol., Fl. Ital. 10: 393 (1857).

= *E. dubia* Regel, Trudy Imp. S.-Peterburgsk. Bot. Sada 6: 482 (1879).

Common names. joint-pine, joint fir, sea grape, vilina brada, metlina, kositernica, vlasac.

Species description. Dioecious subshrub 7–25(-100) cm high, with numerous stems from creeping rhizome. Stems often procumbent; bark dark grey; branchlets (0.7-)1–1.2(-1.5) mm in diameter, glaucous or more rarely yellowish green, often become reddish, ascending, straight or often curved or twisted at apex, usually

papillose-scabrid on the ridges, difficult to decompose; internodes up to 3 cm long. Leaves opposite, reduced and scarious, 1–2 mm long, brownish yellow on young branchlets, the oldest ashen-whitish, connate for 1/3–2/3 of their length, with two triangular lobes, apex obtuse or subacute. Male strobili ('cones') compound, ovoid to lanceoloid, solitary or in cluster of (3)4–8, at apex of short branchlets; with four decussate pairs of bracts; each compound strobilus pedunculate, with 4–8 pairs of male strobili ('flowers'); antherophore ('androecium') ca. 2 mm long, with 6–8 sessile or shortly stipitate sporangiophores ('stamens') with microsporangia ('anthers') exserted beyond the cone bract; microsporangia 2(3)-lobed. Female strobili compound, narrowly ovoid, terminal solitary or 2–5 whorled at branch nodes; pedunculate [peduncle up to 0.3–1(-2.5) cm long], with 3(-4) pairs of decussate, broadly ovate, obtusate, scarious bracts; distal bracts connate for ca. 1/3–1/2 of their length, enclosing the straight tubillus, which is 1-1.5 mm long; micropylar tube relatively short, (0.7-)1–1.2(-2) mm long, straight to twisted. Seeds 1–2(-3) per compound female strobilus (false fruit), 4–5 × 2–3 mm, ovoid or oblong-ovoid, convex on the back below, dark-brown, smooth, glossy; bracts usually becoming succulent during ripening forming red false berry, which is 5–7 × 2.5–4 mm, globose, not winged; seeds protrude clearly from the bracts. Cotyledons two; germination epigeal.

Phenology and pollination. Pollination May–June, seed maturity July–September. The main pollen vector is the wind, and pollen grains are adapted for their farther dispersal. Although female plants produce pollination drops, no insects were observed visiting either female or male cones (Bolinder *et al.* 2016).

Chromosome number. 2n = ?24, 28, ?36 (Fu *et al.* 1999).

General distribution. Scattered in S, SW (Spain, France), SC, and SE Europe, Turkey (Anatolia), and Syria. More abundant to the East, viz., in E Europe, N Caucasia, Transcaucasia, N Iran, S Siberia, and WC Asia (Kazakhstan, Uzbekistan, Turkmenistan) (Jalas & Suominen 1973; Meusel & Jäger 1978; Ickert-Bond & Wojciechowski 2004; Bell & Bachman 2011). In continental Asia, extending far to the north. Some records indicating distribution of the species in the Eastern part of C Asia (NW China) (Fu *et al.* 1999) actually refer to the C Asiatic *E. pseudodistachya* Pachom. (Kakiuchi *et al.* 2011). In S and SW Europe, *E. distachya* is predominantly a coastal species on dunes (Atlantic Ocean, Mediterranean Sea). Some (semi)halophytic succulents (*Camphorosma monspeliaca*, *Salsola soda*, and *Salsola kali*) that are often recorded in *E. distachya* communities have similar ranges and ecological preferences. Moreover, some steppe plants (*Silene otites*, *Gypsophila paniculata*, *Polygonum arenarium*, *Glaucium corniculatum*) also have a similar distribution pattern. *Ephedra distachya* is recorded as an invasive weed in the USA (California) (USDA, NRCS 2017) and Australia (Randall 2007).

Floristic element. Atlantic-(sub)Mediterranean-Pontic-South Siberian-Oriental-Turanic; merid-submerid-temp; plan-mont.

Biogeographic history. Fossil pollen deposits of *E. distachya* have been found in many places in the European boreo-temperate region as far as the Alps in the south, and also on the Balkan and Iberian Peninsulas (Godwin 1975; Bozilova & Tonkov 2000; Valero-Garcés *et al.* 2004; Ivanov *et al.* 2007). They originate from the Late Glacial and Pre-Boreal period, when this species was an important element of the cold-steppe flora. In the continental part of SE Europe and the Iberian Peninsula, the cold-continental dry steppes were transformed into warm-continental steppes during the Holocene (Molnár *et al.* 2012). In these regions, populations of *E. distachya* therefore represent relict remnants of the former cold-steppe flora. A similar history and distribution is shared by some other relict cold-steppe plants, species such as *Krascheninnikovia ceratoides* and *Bassia prostrata*.

Taxonomic and phylogenetic position. In the traditional classification (Staph 1889), *E. distachya* is included in the type species group, *Distachyae*, which belongs to *E.* sect. *Ephedra* (syn. *E.* sect. *Pseudobaccatae* Stapf). Current generic phylogenies (Ickert-Bond & Wojciechowski 2004; Rydin *et al.* 2010) classify *E. distachya* in a large predominantly Asiatic clade, which mostly includes representatives of the traditional group *Distachyae*. A particular subclade of the group, the *E. distachya* / *E. sinica* complex, was subjected to molecular analysis by Kakiuchi *et al.* (2011). Within this complex, *E. distachya* is (almost completely) monophyletic and represents a sister species to several other taxa (including *E. dahurica* Turcz., *E. regeliana* Florin, *E. intermedia*, *E. lomatolepis* Schrenk, and *E. pseudodistachya*). On the other hand, some species, such as *E. likiangensis* Florin and *E. monosperma* J.G. Gmel. ex C.A. Mey., are phylogenetically older than *E. distachya*. Metabolomic analysis of *Ephedra* species also showed clear differentiation of *E. distachya* from the E Asian *E. dahurica* and *E. intermedia* (Kim *et al.* 2005).

Infraspecific variability. Plants from the Alpine valleys are currently classified as *E. distachya* subsp. *helvetica* (C.A. Mey.) Asch. & Graebn. They are morphologically almost identical to typical *E. distachya* (Caruso *et al.* 2012) and only differ in having a long and twisted micropylar tube and shortly stipitate sporangiophores. However, some intermediate plants (*E. negrii* Nouviant and *E. delacourii* Nouviant), currently included in

the typical subspecies, are also known from the Alps (Nouviant 1998). In their phylogenetic tree topologies and nucleotide variations, *E. distachya* subsp. *helvetica* is not clearly separated from the type subspecies (Ickert-Bond & Wojciechowski 2004; Kakiuchi *et al.* 2011; Ni *et al.* 2013). In view of this fact, the current taxonomic status of *E. distachya* subsp. *helvetica* can be considered as doubtful. The status of another doubtful plant, *E. distachya* subsp. *monostachya* (L.) Riedl, known from the Mediterranean region, E Europe, and Siberia, was also not supported by molecular analysis (Kakiuchi *et al.* 2011).

Habitat. Unlike most species of the genus, it grows at lower altitudes, up to 1200 (1400) m (Amaral Franco 1986; Bell & Bachman 2011). Besides having an unusual distribution pattern, the plant is also adapted to wide range of extreme arid and sun-exposed conditions (Table 1) and often inhabits areas where other plants are not present (Bell & Bachman 2011). It is recorded on different types of geological substrates (sand, loess, limestone, chalk, marl, gypsum, sandstone, gravelly plains) with saline, non-saline, or nitrophilous edaphic conditions, usually in eroded, sandy, or rocky places. The main part of the species' population inhabits dry continental deserts and semideserts in Central Asia and Eastern Europe, on saline, non-saline, nitrophilous, and gypsum soils, also in arid conditions on sandy or rocky ground (Rhind 2010; Breckle *et al.* 2013). In the continental part of the plant's western Pontic range, semideserts are replaced by dry grasslands, sandy-steppe growth, and rocky-steppe vegetation, sometimes on loess and clay cliffs, usually as extrazonal desert communities (Sanda *et al.* 2008; Tzonev 2009; Molnár *et al.* 2012). On the shores of the Black Sea and the Mediterranean, then westward to the Atlantic coast, the species grows as a coastal psammophyte on stabilised grey hind dunes and in small depressions behind dunes with a predominance of dwarf chamaephytes, nanotherophytes, lichens, and mosses (Houston 2008; Schneider-Binder & Kuhlke 2015; EVC 2017). Although many authors assigned this azonal vegetation type to the class of mobile coastal dunes (*Ammophiletea*) or to the class *Koelerio-Corynephoretea*, these habitats syntaxonomicaly belong to the class *Helichryso-Crucianelletea maritimae* (EVC 2017). In the central part of the Iberian Peninsula, the plant is also characteristic of thermo to supra-Mediterranean gypsicolous tomillar ("gypsum steppes") and semi-desert halo-nitrophilous scrub (Agudo *et al.* 2007). Finally, in the foothills of the Alps, "*E. distachya* subsp. *helvetica*" grows on xerophilous rocky-steppic grasslands within the zone of *Pinus nigra*, as well as on walls and calcareous cliffs (Wilhalm 2007; Caruso *et al.* 2012; Info Flora 2017).

Regional threatened status. The species is included in the IUCN Red List of Threatened Species (Bell & Bachman 2011) under the category of "Least Concern". In the Red Lists of Slovakia (Feráková *et al.* 2015) and Hungary (Király *et al.* 2007), *E. distachya* was estimated as "Critically Endangered" and "Near Threatened", respectively.

RESULTS AND DISCUSSION (*EPHEDRA* IN SERBIA)

During floristic investigations in the foothills of the Stara Planina Mountains in Eastern Serbia (near Minićevo), *E. distachya* was recorded for the vascular flora of Serbia. The presence of the species in Serbia was also reported by Bell & Bachman (2011), but without any concrete sources. The authors in fact attributed a single common record for ex-Yugoslavia from Flora Europaea (Markgraf *et al.* 1993) to all former Yugoslav countries, including Slovenia, Bosnia and Herzegovina, Serbia, and Macedonia, from which it has never been previously recorded. Therefore this source should not be considered as relevant.

The species was found in a carbonate hilly area on the ridge of SE slopes of Turjačka Glama hill, near the top of the hill (Fig. 1a). Viewed more broadly, the locality belongs to the foothills of the Stara Planina Mountains on the Serbo-Bulgarian border and also to the Timok river basin. The investigated territory belongs to the continental climate zone. Despite intense searching for them in the remaining limestone hills and similar habitats in the investigated area, no further specimens have been found. The absence of species at neighbouring localities can be explained by microclimatic factors, since they are somewhat closer to the mountains and exposed to moderate droughts and temperatures in summer.

The nearest population from the vicinity of Belogradchik in NW Bulgaria (Stoyanov 2006) is not so far away, ca. less than 50 km. On the Balkan Peninsula, the species mainly occurs on sand dunes near the sea coasts. Relict continental Balkan populations are for the most part only found scattered in Bulgaria, but a single one is also recorded in NE Albania (Jalas & Suominen 1973). The new locality in Serbia represents the most continental occurrence of the species on the Balkan Peninsula (from the geographical, not the climatic point of view) (Fig. 2).

Collected specimens: Flora serbica: E Serbia, Minićevo, Ošljane, Turjačka Glama hill, 520 m, Exp. S, limestone, rocky steppic fragment on the ridge, 22.373500 E, 43.660379 N, leg & det. M. Niketić 13.06.2017. *201706003* BEO; Flora serbica: E Serbia, Minićevo, Ošljane, Turjačka Glama hill, 520 m, Exp. S, limestone, rocky steppic fragment on the ridge, leg & det. M. Niketić, G. Tomović 30.06.2017. *201706016* BEO.

Habitat. The observed species habitat (Fig. 1b–d) represents a single rocky steppic fragment on the margins of

Table 1. Syntaxonomic review of *Ephedra distachya* habitats adapted to the current classification (Mucina *et al*. 2016). An asterisk (*) refers to *E. distachya* as a diagnostic species of phytocoenological units. Communities with *E. distachya* as a dominant species are bolded.

Type of vegetation	Class	Order	Alliance
Grassland and drift-scrubs on base-rich stabilised hind dunes (maritime; azonal)	*Helichryso-Crucianelletea maritimae**	*Artemisio-Koelerietalia*	*Euphorbio portlandicae-Helichrysion stoechadis*
		Crucianelletalia maritimae	*Crucianellion maritimae*
		Medicago-Seselietalia tenderiensis	*Scabiosion ucranicae** *"West Pontic Ephedra-Carex fixed dunes" (proposed as Annex I to Council Directive 92/43/EEC on the Conservation of Natural Habitats and Wild Fauna and Flora) (Tzonev et al.* 2004*)*
Rupicolous vegetation of salt-sprayed coastal cliffs (maritime; azonal)	*Crithmo-Staticetea*		
Dry grasslands on sandy soils and on rocky outcrops (continental; intrazonal in nemoral forest)	*Koelerio-Corynephoretea canescentis*	*Festucetalia vaginatae*	*Festucion beckeri*
			*Festucion vaginatae**
		Corynephoretalia canescentis	*Koelerion glaucae*
Dry grassland and steppe vegetation (continental and maritime; zonal. On the Balkan Peninsula intra- and extrazonal)	*Festuco-Brometea*	*Festucetalia valesiacae*	*Festucion valesiacae*
			Artemisio-Kochion
		Stipo pulcherrimae-Festucetalia pallentis	*Pimpinello-Thymion zygoidi*
Saline steppes and secondary saline steppic grasslands (continental; intrazonal in steppe zone)	*Festuco-Puccinellietea*	*Puccinellietalia*	*Puccinellion limosae*
Mediterranean scrub on base-rich substrates (continental; zonal)	*Ononido-Rosmarinetea*	*Gypsophiletalia*	*Lepidion subulati**
Mediterranean semidesertic halo-nitrophilous scrubs (continental; intrazonal in Mediterranean zone)	*Pegano harmalae-Salsoletea vermiculatae*	*Salsolo vermiculatae-Peganetalia harmalae*	*Salsolo vermiculatae-Peganion harmalae*
Vegetation of semideserts (continental; zonal)	*Artemisietea lerchianae*	*Artemisietalia tschernievianae*	*Euphorbion seguieranae*
Hypersaline scrub (continental; intrazonal in vegetation of semideserts)	*Kalidietea foliati*	*Kalidietalia foliati*	*Kalidion caspici*

Community	Area	Reference
	shores of the Atlantic Ocean (FRA)	Houston (2008)
	shores of the Atlantic Ocean (FRA) and W & C Mediterranean	EVC (2017)
Alysso borzaeani-Ephedretum distachyae ***Ephedro distachyae-Sileneetum subconicae*** ***Scabioso argenteae-Ephedretum distachyae*** *Scabioso argenteae-Caricetum colchicae* subass. ***ephedretosum*** *Poo bulbosae-Caricetum colchicae* *Secali sylvestri-Alyssetum borzaeani*	shores of Adriatic, Aegean, Black, and Azov Seas (ALB, GRC, BGR, ROU, MDA, UKR)	Dubyna *et al.* (1995);Tzonev *et al.* (2005); Doniţa *et al.* (2005); Sanda *et al.* (2008); Fagaras (2011); CEC (2013); Doody (2015); Fagaras *et al.* (2015); Schneider-Binder & Kuhlke (2015); Šilc *et al.* (2016)
	ITA, ROU	Caruso *et al.* 2012; Schneider-Binder & Kuhlke (2015)
*Festucetum beckeri** *Secali-Stipetum borysthenicae* *Centaureo odessanae-Stipetum capillatae*	ROU, UKR	Dubyna *et al.* (1995); Sanda *et al.* (2008)
Festuco vaginatae-corinophoretum	ROU	Sanda *et al.* (2008)
*Koelerio glaucae-Stipetum borysthenicae**	ROU	Schneider-Binder & Kuhlke (2015)
Astero oleifolius-Ephedretum distashyae *Stipetum capillatae* *Festuco rupicolae-Caricetum humilis*	ROU, HUN	Sanda *et al.* (2008); Bartha (2007); Frink & Szabó (2009)
Hedysaro bulgaricum-Camphorosmetum monspeliacae	BGR, HUN	Tzonev 2009; Molnár *et al.* (2012)
Alysso caliacrae-Atremisietum lerchianae	BGR	Tzonev *et al.* (2006)
	UKR	Mihajlovna (2014)
Lino differentis-Lepidietum subulati	ESP	Agudo *et al.* (2007)
*Artemisio herbae-albae-Santolinetum squarrosae**	ESP	Agudo *et al.* (2007)
	Aralo-Caspian region	Rhind (2010)
Haloxylon aphyllum-Ephedra distachya ***Calligonum aphyllum-Ephedra distachya***	Aral Sea	Breckle *et al.* (2013); Pankratova (2013)

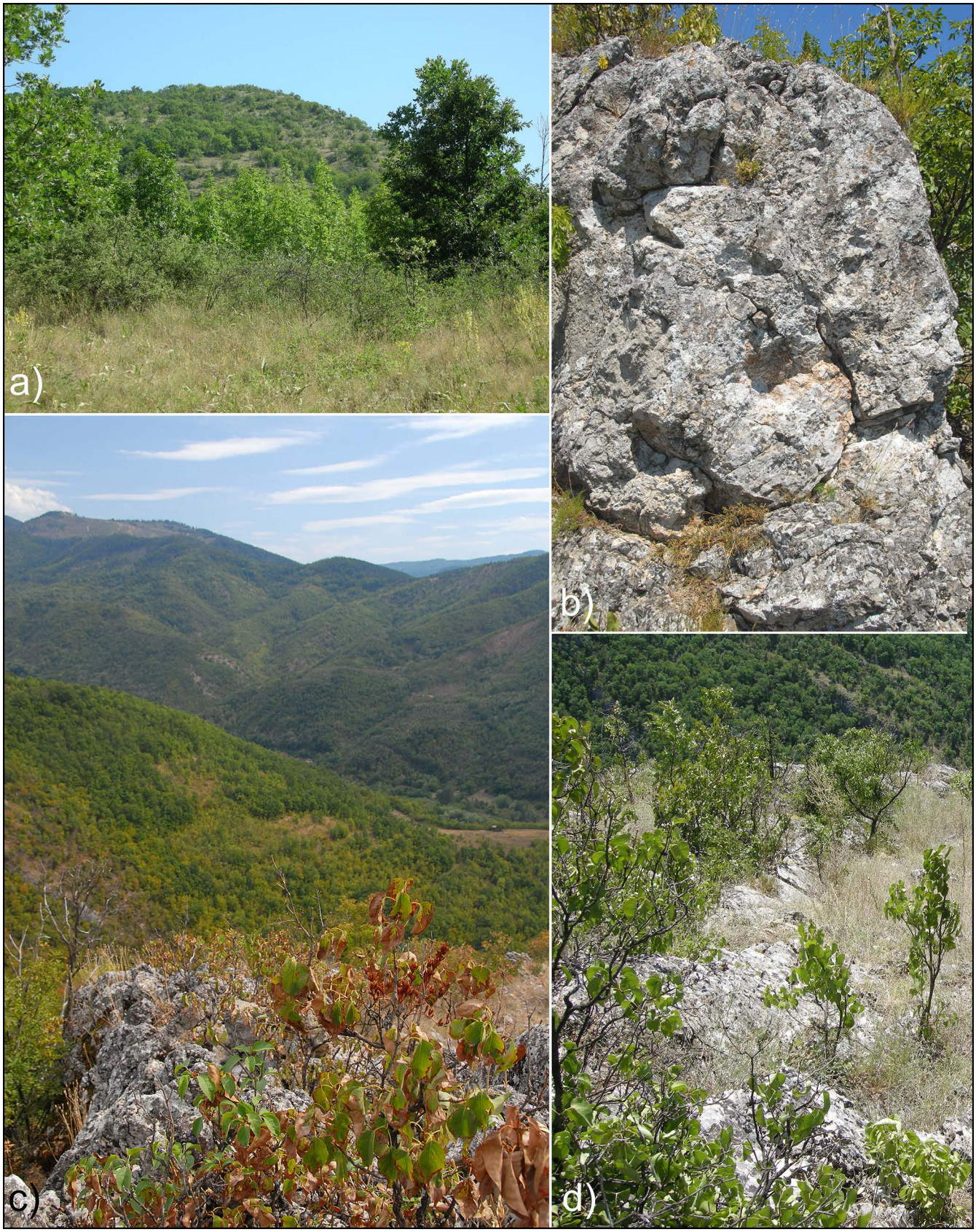

Fig. 1. Locality and habitat of *Ephedra distachya* in Serbia: a) Turjačka Glama hill near Minićevo (E. Serbia); b) rock crevices; c–d) rocky steppe and shrub vegetation (photo M. Niketić).

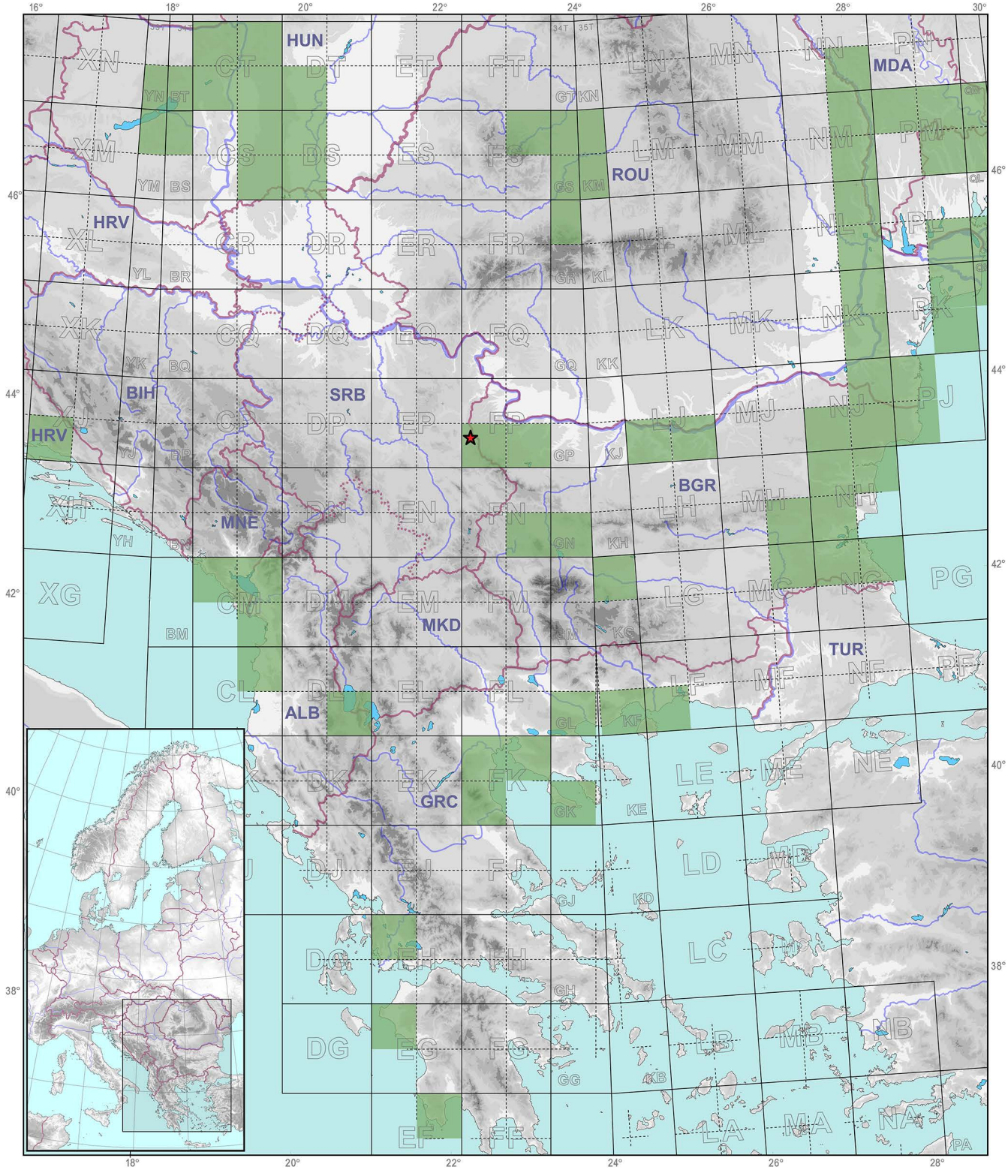

Fig. 2. Map of the distribution of *Ephedra distachya* in southeastern Europe. Resolution 50×50 km, MGRS grid system based on Jalas & Suominen (1973) and supplemented and/or confirmed by chrorological records from Stoyanov (1963), Horeanu & Viţalariu (1992), Christensen (1997), Sanda *et al.* (2001), Tzonev *et al.* (2005, 2006), Stoyanov (2006), Frink & Szabó (2009), Szalczer & Szalczer (2009), Făgăraş (2011), Peev *et al.* (2012), Miho *et al.* (2013), Caković *et al.* (2014), Biserkov et al. (2015), Nikolić (2015), Strat (2016), and Giannopoulos *et al.* (2017). The new locality in Serbia is marked with a star.

shrub vegetation (*Syringo-Carpinetum orientalis* Jakucs 1959). The surrounding zonal forest vegetation belongs to a xerophilous variant of *Quercetum frainetto-cerridis* Rudski (1940) 1949 with a high presence of *Quercus pubescens*. The geological substrate consists of massive Cretaceous limestone and rock debris. Phytocoenological analysis of the habitat showed that the sampled relevés do not fit completely into the existing classification and could thus belong to a new plant community with *E. distachya* and *Asperula purpurea* as dominant species (Table 2). However, a small population of *E. distachya* (with an occupancy area of less than 200 m^2), a single locality, and only two relevés are not sufficient for an original diagnosis of a new association (Weber *et al*. 2000). The recorded relevés should be included in the following syntaxonomic classification at the alliance level:

Festuco-Brometea Br.-Bl. et Tx. ex Soó 1947

Stipo pulcherrimae-Festucetalia pallentis Pop 1968, nom. cons. prop.

Saturejion montanae Horvat in Horvat et al. 1974

This is the first occurrence of *E. distachya* within the alliance of xerophilous rocky steppic grasslands on calcareous substrates of the Central Balkans (*Saturejion montanae*) (absent in Table 1). Sub-Mediterranean and Pontic-Mediterranean representatives are the most numerous in the floristic spectrum, some (sub)endemic plants (*Satureja kitaibelii*, *Silene flavescens*, *Erysimum welcevii*, *Hypericum rumeliacum*) are also frequent, but *E. distachya* is the only member of the Eurasiatic (sub-Turanic) range type. Hemicryptophytes are the most numerous in the overall sample, but chamaephytes are common among the dominant and most abundant representatives. It is particularly interesting that grasses are almost completely absent (*Melica ciliata* is present as a rare exception in a single relevé), which indicates a strong Mediterranean impact on this primary steppe community.

This habitat is physiognomically (steep slopes with carbonate rocks) similar to the xerophilous rocky steppic grasslands *Alysso caliacrae-Atremisietum lerchianae* Tzonev et al. 2006 (*Pimpinello-Thymion zygoidi*) near the coast of the Black Sea, but their relevés shared only two species (*E. distachya* and *Linaria genistifolia*). The two plots from Turjačka Glama are floristically much more similar to relevés of the associations *Stipetum capillatae* (Hueck 1931) Krausch 1961 and *Festuco rupicolae-Caricetum humilis* Klika 1939 from Transylvania (Frink & Szabó 2009), as well as to *Asperulo purpurei-Micromerietum cristatae* Niketić, prov. ["*Galio-Micromerietum cristatae*"] from the Jelašnička klisura gorge in the vicinity of Niš (E Serbia) (Niketić 1986). Relevés from the latter locality belong to a very exposed and more chasmophytic vegetation type (also included in *Saturejion montanae*) with a small percentage of coverage and have a common ediphicator, *Asperula purpurea*. It is also noticeable that *E. distachya* on Turjačka Glama often grows solitarily at

Table 2. Phytocoenological review of the *Ephedra distachya* habitat in Serbia (E Serbia, Minićevo, Ošljane, Turjačka Glama hill).

Relevé	1	2
Date	30.06.2017.	30.06.2017.
Relevé area	10 m^2	7 m^2
Altitude	520 m	510 m
Exposition	S	S-SE
Slope	0-90°	20-30°
Total cover	20-25%	20%
Species number	14	19
Number of *Ephedra* individuals	26	46
Dominant species		
Asperula purpurea	1.2	1.1
Ephedra distachya	1.1	1.1
Silene flavescens	2.2	+
Sedum urvillei	1.3	+
Allium flavum	+	+
Allium moschatum	+	+
Verbascum anisophyllum	+	+
Diagnostic species of *Saturejion montanae*		
Satureja kitaibelii		+
Stachys recta	+	1.1
Teucrium montanum		+
Erysimum welcevii		+
Minuartia glomerata		1.2
Hypericum rumeliacum	+	
Diagnostic species of *Festuco-Brometea*		
Linaria genistifolia		+
Melica ciliata		+
Asperula cynanchica		+
Convolvulus cantabrica		+
Other species		
Euphorbia cyparissias	+	+
Campanula sibirica	+	
Coronilla varia	+	+
Syringa vulgaris	1.2	+
Carpinus orientalis	+	
Fraxinus ornus	1.1	+

Fig. 3. *Ephedra distachya* in the habitat: orange (a), yellow (b), reddish (c), and green (d) individuals (photo M. Niketić).

the most exposed rocky and arid sites of relevés, where adjacent plants cannot survive. The observed habitat conditions are quite unique for the investigated taxon, although it is still unknown whether they are of the same type in the nearest population in NW Bulgaria.

Estimation of threatened status. A small area (less than 200 m^2) near Minićevo in the foothills of the Stara Planina Mountains with ca. 70 individuals of the species represents the single known habitat of *E. distachya* in Serbia. Average height of the plants is 5–10 cm; only a few individuals are about 20 cm high. On this occasion, no fertile individuals were found, probably due to seasonal weather fluctuations, since the past year was extremely dry. It was also observed in Greek populations that seeds were not fully developed and strobili never became fleshy during a long dry period, even though pollination and early seed development were successful (Bolinder *et al.* 2016). At the end of the summer, due to severe drought and temperature stress, 10 individuals lost their chlorophyll, becoming yellow (Fig. 3). The small size of the population, irregular reproduction, and continuous reforestation of the habitat represent major threatening factors. According to the IUCN (2012), the conservation status of *E. distachya* in Serbia should be assessed as Critically Endangered [CR B1ab(i,ii,iii,iv,v)+2ab(i,ii,iii,iv,v); C2a(i, ii); D]. In the event of expansion of the neighbouring population in NW Bulgaria, the threatened category in Serbia might be deemed lower (IUCN 2012), but there is little likelihood of this.

CONCLUSIONS

During floristic investigations of Eastern Serbia in 2017 (foothills of the Stara Planina Mountains near Minićevo, Ošljane) in 2017, the species *Ephedra distachya*, genus *Ephedra*, and Gnetophyta ancient group of gymnosperms were recorded for the first time for the flora of Serbia. The species was found in a carbonate hilly area on the ridge of SE slopes of Turjačka Glama hill near the top of the hill in an area smaller than 200 m^2. For now, only 70 individuals of *E. distachya* represent the single known population in Serbia, and no further specimens have been found in the remaining limestone hills and similar habitats in the area. No fertile individuals were found in the population, probably owing to the fact that the seasonal weather was extremely dry. The nearest population from the vicinity of Belogradchik in NW Bulgaria, situated about 50 km away, also belongs to the continental climate zone. On the Balkan Peninsula, *E. distachya* mainly occurs on sand dunes near the sea coasts. Continental populations are only scattered in the Balkans, and the new locality in Serbia represents the most continental occurrence of the species on the peninsula. The species habitat represents a single rocky steppic fragment on the margins of shrubby vegetation. Two newly sampled relevés belong to the *Saturejion montanae* alliance with 23 species, including *E. distachya* and *Asperula purpurea* as dominant. Sub-Mediterranean and Pontic-Mediterranean representatives are the most numerous in the floristic spectrum, as are hemicryptophytes and chamaephytes. The most similar habitat was recorded in E Serbia (Jelašnička klisura gorge, ass. *Asperulo purpurei-Micromerietum cristatae*). The observed habitat conditions are quite unique for this species, for which the small size of the population, irregular reproduction, and continuous reforestation represent major threatening factors. Consequently, the IUCN threatened status of the population in Serbia was estimated as Critically Endangered. Further monitoring and specific protection measures are necessary to preserve this small population of *E. distachya* in Serbia.

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Botanica SERBICA

REZIME

Prvi nalaz *Ephedra distachya* L. (Ephedraceae, Gnetophyta) u Srbiji - Biogeografski, cenološki i konzervacioni osvrt -

Marjan Niketić

Tokom florističkih istraživanja na području istočne Srbije (podnožje Stare planine, Turjačka Glama kod Minićeva) vrsta *Ephedra distachya* (Ephedraceae) po prvi put je konstatovana za vaskularnu floru Srbije. Predstavljen je opšti pregled familije, roda i vrste. Na staništu vrste zabeležena su dva fitocenološka snimka, klasifikovana na nivou sveze. Na osnovu IUCN kriterijuma populacija u Srbiji procenjena je kao krajnje ugrožena.

Ključne reči: *Ephedra distachya*, Ephedraceae, novi nalaz, Stara Planina, flora Srbije