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Morphological variation within the *Edraianthus graminifolius* complex (*Campanulaceae*) from the central Balkan Peninsula – Evidence from multivariate statistical analysis

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ABSTRACT

The *Edraianthus graminifolius* complex is one of the most interesting groups within the genus *Edraianthus* DC (Campanulaceae). The plants inhabit a variety of habitats and there is a micro-geographic and ecological differentiation of populations, accompanied by pronounced morphological variation. Hence, it is not surprising that this complex is taxonomically a very controversial group. On the one hand it is described to comprise only two to four taxa, including subspecies, and on the other, a classification comprising 23 taxa of the rank of species and subspecies has been proposed.

The basic aim of the present study therefore was to quantify the morphological and anatomical differences within and between populations of this complex from the central and western parts of the Balkan Peninsula (Serbia, Montenegro, Bosnia and Herzegovina, and Croatia), and to determine whether there are, in fact, clear morphological differences between these populations.

For these purposes, several multivariate statistical analyses (PCA – principal component analysis, CDA – canonical discriminant analysis, clustering UPGMA analysis based on Mahalanobis distances, and CA – correspondence analysis) of vegetative and generative plant organs have been carried out.

The morpho-anatomical analysis of 704 individuals from 44 populations has confirmed that there are several morphologically distinct groups of populations in the central Balkans, between which no strict morphological discrimination exists. Some of these distinct groups of populations correspond to taxa *E. caricinus* Schott, Nyman, & Kotschy, *E. montenegrinus* Horak, *E. jugoslavicus* Lakušić and *E. vesovicii* R. Lakušić, while others were identified for the first time in this study.

Introduction

The genus *Edraianthus* A. DC. (*Campanulaceae*) is distributed mainly in the Balkan Peninsula, particularly in its western part, while disjunct parts of its range are found in Sicily and in the Apennines and S. Carpathians (Wettstein, 1887; for a map of distribution of the genus *Edraianthus* see Fig. 1 in Stefanović et al., 2008). It is one of the taxonomically and biogeographically most interesting and polymorphic genera of the Balkan flora. Because of its (sub)endemic and relict character, as well as of an outstanding phenotypic plasticity related to its wide distribution, it has attracted the attention of botanists and has been the object of comprehensive investigations that resulted in three monographs (Janchen, 1910; Lakušić, 1974; Wettstein, 1887). Recently, the genus was the subject of extensive phylogenetic, phylogeographic and cytogenetic studies at the molecular level that brought some new insights into the phylogenetic relationships and systematics, both among the genera closely related to *Edraianthus* (Park et al., 2006; Roquet et al., 2008; Siljak-Yakovlev et al., 2010) and within the genus *Edraianthus* itself (Lakušić et al., 2009; Stefanović et al., 2008; Surina et al., 2009, 2011).

Within the genus *Edraianthus*, the *E. graminifolius* complex is taxonomically the most intriguing group, primarily due to the wide distribution of its populations and the outstanding morphological variability. It is distributed from Italy (Sicily, Apennines) to the Carpathians in Romania, reaching southwards to Sterea Ellas Mt. and the southern Pindus Mts. in Greece, thus covering almost the entire range of the genus, with populations occurring at an extremely wide range of altitudes (Stefanović et al., 2008). Its populations inhabit predominantly rock crevices, screes, rocky grounds and grasslands on a variety of limestone and dolomitic bedrocks

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Table 1
Synopsis of the most influential precladistic classifications and floristic treatments of the *E. graminifolius* complex. Janchen – classification scheme according to Janchen (Janchen, 1910); Lakušić – classification scheme according to Lakušić (Lakušić, 1974; modified 1987, 1988); Kuzmanov – taxa accepted by the Flora Europaea (Kuzmanov, 1976), Greuter – taxa accepted by the Med-Checklist (Greuter et al., 1984); Lammers – taxa accepted by the World checklist and bibliography of Campanulaceae (Lammers, 2007).

Taxa	Janchen	Lakušić	Kuzmanov	Greuter	Lammers
E. graminifolius	+	+	+	+	+
E. graminifolius subsp. *graminifolius*		+	+	+	+
E. graminifolius subsp. *coeruleus*	+				
E. graminifolius subsp. *siculus*		+		+	+
E. graminifolius subsp. *apenninus*		+			
E. niveus	+	+	+	+	+
E. australis		+		+	+
E. caricinus		+			+
E. caricinus subsp. *caricinus*		+			
E. caricinus subsp. *baldaccii*		+			
E. jugoslavicus		+			
E. jugoslavicus subsp. *jugoslavicus*		+			
E. jugoslavicus subsp. *subalpinus*		+			
E. croaticus		+			
E. murbeckii		+			
E. montenegrinus		+			
E. horvatii		+			
E. vesovicii		+			
E. zogovicii		+			
E. albanicus		+			
E. kitaibelii		+			
E. kitaibelii subsp. *kitaibelii*		+			
E. kitaibelii subsp. *carpaticus*		+			
E. kitaibelii subsp. *bihariense*		+			
Total	**3**	**23**	**3**	**5**	**6**

(but very rarely on siliceous ones) in the montane, sub-alpine and alpine zones, as well as on cliffs in canyons and gorges over a wide range of altitude, from 300 to 2900 m.

The most significant floristic works, *Flora Europaea* (Kuzmanov, 1976), *Med-Checklist* (Greuter et al., 1984) and the fundamental *World checklist and bibliography of Campanulaceae* (Lammers, 2007) mainly follow Janchen's (1910) concept of infrageneric classification of *Edraianthus* that comprises two to four taxa, including subspecies (Table 1). In contrast, Lakušić (1974; modified 1987, 1988) proposed a classification, based not only on morphology but also on their chorology and ecology, which comprises a significantly larger number of taxa than in Janchen's classification (Table 1). Within *E. graminifolius* s.l. Lakušić distinguished 23 taxa of the rank of species and subspecies. According to his classification, *E. graminifolius* L. s.str. is restricted to the Apennine Peninsula, while in the Balkans he recognized several chorologically well separated species: *E. caricinus* Schott, Nyman & Kotschy (coastal Dinarides), *E. croaticus* Kern (NW continental Dinarides), *E. montenegrinus* Horak (SE continental Dinarides), *E. jugoslavicus* Lakušić, (central part of the Balkan Peninsula), *E. kitaibelii* A. DC. (S Carpathians), *E. horvatii* Lakušić (N Scardo-Pindus mountains), *E. australis* (Wettst.) Lakušić (C. and S. Scardo-Pindus mountains). Moreover, Lakušić (1974, 1987, 1988) recognized several locally distributed endemic species, such as *E. niveus* G. Beck (Vranica and Zec), *E. murbeckii* Wettst. em. R. Lakušić (Prenj and Velež), *E. vesovicii* R. Lakušić (Gusinjske Prokletije), *E. zogovicii* R. Lakušić (Komovi) and *E. albanicus* (Degen & Kümmerle) R. Lakušić (Nemerečka).

Recent studies on the molecular phylogeny of *Edraianthus*, based on chloroplast DNA sequence analysis, have recognized 17 molecularly distinct groups within the *E. graminifolius* complex (Stefanović et al., 2008). Some of them correspond to previously established taxonomic positions while others were identified for the first time. This molecular study, however, did not differentiate precisely between the studied populations within the *E. graminifolius* complex and, in most cases, provided only weak or non-existing statistical support for the identified lineages.

Given these sharp differences in opinion, the basic aim of the present study was to quantify morphological and anatomical variation within and between populations of the *E. graminifolius* complex from the central and western part of the Balkan Peninsula (Serbia, Montenegro, Bosnia and Herzegovina, and Croatia) on the basis of multivariate statistics, and to determine whether there are clear morphological differences between the populations. Thus, without presuming to resolve definitively the taxonomic questions within this complex, our objective was to determine whether there is a basis for either taxonomic concept – one large species or many single species.

Materials and methods

Plant material

Plant material was sampled from 44 populations of the *E. graminifolius* complex from the central and western Balkan Peninsula (Table 2, Fig. 1), from elevations between 100 and 2500 m. From each population, 15 plant samples were used for morphological and anatomical analysis. Voucher specimens are deposited in the Herbarium of the Institute of Botany and Botanical Garden, Faculty of Biology, University of Belgrade (BEOU). Nomenclature follows the *Flora Europaea* (Kuzmanov, 1976) and *Med-Checklist* (Greuter et al., 1984). In addition, informal names (*E. graminifolius* "*jugoslavicus*", *E. graminifolius* "*montenegrinus*", *E. graminifolius* "*caricinus*" and *E. graminifolius* "*vesovicii*") were used to distinguish between taxa proposed by Lakušić (1974).

Morpho-anatomical analysis

Morphometric analyses were performed on dissected plant organs, well preserved in glycerol:96% ethanol (50:50, v/v). The anatomical structure of rosette leaves was analyzed on cross sections obtained using a cryostat, Leica CM 1850, at −21 °C. Sections were cleared in parazone, thoroughly washed in water and stained in safranine (1%, w/v in 50% ethanol) and alcian blue (1%, w/v, aqueous).

Epidermal peels for analysis of stomata were performed by warming leaves in glacial acetic acid:20% H_2O_2 (1/1, v/v) for