One New Species of the Genus Edraianthus, and a Change in Taxonomic Status for Edraianthus serpyllifolius f. pilosulus (Campanulaceae) from the Balkan Peninsula

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Abstract—A new species, Edraianthus pulevicii (Campanulaceae), endemic to the Durmitor mountain range (SE Dinaric Alps, Montenegro, Balkan Peninsula) is described and illustrated. Additionally, specific rank is proposed for E. serpyllifolius f. pilosulus from the Komovi Mts. (NW Prokletije mountain range, Montenegro, Balkan Peninsula), E. pilosulus. Both are morphologically similar to E. serpyllifolius s.s., all having spathulate leaves. Edraianthus pulevicii differs from E. serpyllifolius s.s. in having distinctly crenate as well as much longer and broader basal and cauline leaves with indumentum on the upper side, hairs on the leaf margin and leaf surface oriented towards the leaf base, broader and longer bracts, and revolute calyx lobes reflexed at the apex. Edraianthus pilosulus is morphologically similar to both E. serpyllifolius and E. pulevicii but differs from the former in having more or less dense hairs on the upper side of the leaves, and from the latter in having considerably smaller basal and cauline leaves with no or sparse crenation, hairs on the leaf margin and the leaf surface oriented towards the point, and narrower bracts. A new systematic treatment is supported by chloroplast DNA sequence data and AFLP fingerprinting data. A distribution map and a key to Edraianthus taxa with spathulate leaves are provided and the name Edraianthus sutjeskae is validated.

Keywords—Campanulaceae, cryptic speciation, Dinaric Alps, Edraianthus, endemism, Prokletije mountain range

Edraianthus A. DC. (Campanulaceae) includes approximately 13 herbaceous species (e.g., Kuzmanov 1976; Lammers 2007a; 2007b). The majority of the taxa are considered to be stenoenemics (Turrill 1929). As one of the taxonomically and biogeographically most interesting and polymorphic genera of the Balkan flora, there are already three monographs of the genus (Wettstein 1887; Janchen 1910; Lakušić 1974). Recently, the genus was the subject of extensive molecular phylogenetic and phylogeographic studies which brought some new insights into phylogenetic relationships and systematics both among the genera closely related to Edraianthus and within the genus itself (Stefanović et al. 2008). Due to its spathulate or spathulate-lanceolate leaves, it has rather long, linear to linear-lanceolate, ciliate basal leaves and long bracts. Lakušić (1974) originally placed this species in section Captitati, but afterwards (1988) moved it to subgenus Visiania. According to the results of the molecular phylogeny of Edraianthus based on noncoding plastid DNA sequences, Stefanović et al. (2008) and Surina et al. (unpublished data) found that neither section Spathulati nor subgenus Visiania is monophyletic. Two separate lineages, one from the Durmitor Mts. and the other from the Komovi Mts. (NW Prokletije mountain range, NE Montenegro), sister to the E. graminifolius group, were identified. Due to its spathulate or spathulate-lanceolate leaves and solitary flowers, these populations are morphologically most similar to E. serpyllifolius, a taxon restricted to the subalpine and alpine belt of the Central and SE Dinaric Alps from Croatia to Albania. Additionally, results of the AFLP fingerprinting data confirmed their unique systematic position.

In light of those findings, a new species, E. pulevicii sp. nov., is described here, a change of taxonomic status of E. serpyllifolius f. pilosulus Beck, E. pilosulus stat. nov., is proposed, and the name Edraianthus sutjeskae Lakušić is validated.

Materials and Methods

Morphological analyses were carried out on herbarium specimens deposited in Natural History Museum Rijeka (NHMR), WU and BEOU,
and field collections were preserved in a solution of 96% ethanol and glycerol (1:1). Three taxa recognized morphologically with spatulate leaves were studied: *E. serpyllifolius* f. *pilosulus* from Durmitor (83 specimens) and the Komovi Mts. (25 specimens) in Montenegro, *E. serpyllifolius* s. s. from the Herzegovinian Mts. in Bosnia and Herzegovina and Mt. Orjen in Montenegro (191 specimens), and *E. sutjeskae* from Sutjeska canyon in Herzegovina. To restore the turgescent state of the herbarium material, leaves were rehydrated with diluted detergent for several hours. The leaves were afterwards rehydrated in FDA (formaldehyde dimethyl acetal) for 24 hr and dried in CO₂. The material was mounted on stubs with double sided sticky tape, sputter-coated with gold, and investigated using JEOL JSM-6390 SEM.

To ascertain the chromosome number of *E. serpyllifolius* f. *pilosulus* from the Durmitor Mts., living plants were transferred to the Botanical Garden of the University of Vienna. A voucher is deposited in NHMR. Actively growing root tips were collected and pretreated with 0.1% colchicine for 24 hr at room temperature, and stored at −20°C until use. For further chromosome preparation and analysis we followed Weiss et al. (2002).

DNA extractions were made from silica gel-dried leaf material gathered in the field following the CTAB protocol of Doyle and Doyle (1987) and field collections were preserved in a solution of 96% ethanol and glycerol (1:1). Three taxa recognized morphologically with spatulate leaves were studied: *E. serpyllifolius* f. *pilosulus* from Durmitor (83 specimens) and the Komovi Mts. (25 specimens) in Montenegro, *E. serpyllifolius* s. s. from the Herzegovinian Mts. in Bosnia and Herzegovina and Mt. Orjen in Montenegro (191 specimens), and *E. sutjeskae* from Sutjeska canyon in Herzegovina. To restore the turgescent state of the herbarium material, leaves were rehydrated with diluted detergent for several hours. The leaves were afterwards rehydrated in FDA (formaldehyde dimethyl acetal) for 24 hr and dried in CO₂. The material was mounted on stubs with double sided sticky tape, sputter-coated with gold, and investigated using JEOL JSM-6390 SEM.

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DNA extractions were made from silica gel-dried leaf material gathered in the field following the CTAB protocol of Doyle and Doyle (1987)
with the following modifications: after precipitation with isopropanol and subsequent centrifugation, the DNA pellet was washed with 70% ethanol, dried at 37°C, and resuspended in TE buffer. The quality of the extracted DNA was assessed on 1% TAE-agarose gels. AFLPs were amplified following Dixon et al. (2008).

Raw AFLP data were collected and aligned with the internal size standard using ABI Prism GeneScan analysis software 3.7.1. (Applied Biosystems, Foster City, California). The GeneScan files were imported into Genographer v. 1.6.0 (available at http://hordeum.oscs.montana.edu/genographer) for scoring the fragments.

In the 40 individuals successfully analyzed, we scored 337 AFLP fragments, of which four were excluded after comparison between the replicated individuals. The error rate (Bonin et al. 2004) before the exclusion of unreliable characters was 0.2%. The results of the scoring were exported as a presence/absence matrix. Using the program SplitsTree 4 (Huson and Bryant 2006), a neighbor-net diagram was produced from Nei-Li distances (Nei and Li 1979). To obtain bootstrap support values for branches, 1,000 pseudoreplicates were employed. Details on the plant material are provided in the Appendix.

Results

Populations of *E. serpyllifolius f. pilosulus* from the Durmitor and Komovi Mts. are both morphologically very similar to *E. serpyllifolius* s. s., all having spathulate leaves and solitary flowers densely subtended by small leaf-like bracts. However, detailed morphological analyses showed considerable differences in several morphological characters among the populations (Table 1). *Edraianthus serpyllifolius f. pilosulus* from the Durmitor Mts. differs from *E. serpyllifolius* s. s. in having distinctly crenate as well as much longer and broader basal and cauline leaves with indumentum on upper side, which in *E. serpyllifolius* s. s. is lacking completely (Mededović 1981; see also Šoljan 1983), hairs on the leaf margin and leaf surface oriented towards the leaf base, broader and longer bracts and revolute calyx lobes reflexed at the apex (Table 1, Figs. 1, 2G, H). Like all other taxa within *Edraianthus* (e.g. Löve 1973; Mededović 1980; Van Loon and Kieft 1980, Mededović 1981; Tessitore et al. 1994), this population has the chromosome number $2n = 32$.

*Edraianthus serpyllifolius f. pilosulus* from the Komovi Mts. (Figs. 1, 2E, F; Table 1) is morphologically similar to both *E. serpyllifolius* s. s. (Figs. 1, 2B, C; Table 1) and *E. serpyllifolius f. pilosulus* from the Durmitor Mts. but differs from the former in having more or less dense hairs on the upper side of the leaves and much longer corolla lobes, and from the latter in having considerably smaller basal and cauline leaves with no or sparse crenation, hairs on the leaf margin and the leaf surface oriented towards the apex, and narrower bracts. According to our observations, the presence or absence of indumentum is the most constant and reliable morphological feature to differentiate *E. serpyllifolius f. pilosulus* from the Komovi Mts. and *E. serpyllifolius* s. s.

Morphological differentiation is congruent with the results of cpDNA sequence data (see Stefanović et al. 2008) and AFLP fingerprinting data. In the neighbor-net network of AFLP phenotypes (Fig. 3), four groups with high bootstrap support were formed: *E. serpyllifolius* s. s. from the Herzegovinian Mts. and Mt. Orjen (SE Dinaric Alps), *E. sutjeskae* from the Sutjeska canyon, and two well separated groups of *E. serpyllifolius f. pilosulus* from the Durmitor and Komovi Mts. Therefore, a new species is proposed, *E. pulevicii* sp. nov. for the population from the Durmitor Mts., and a change in taxonomic status of *E. serpyllifolius f. pilosulus*, *E. pilosulus* stat. nov., from the Komovi Mts.

Taxonomic Treatment


Fig. 1. Bracts, sepals and petals of *Edraianthus* taxa with spathulate leaves: *E. serpyllifolius f. pilosulus* from the Durmitor and Komovi Mts. (Kom Vasojevicki), and *E. serpyllifolius* s. s. from the Mt. Orjen. Scale: 1 cm.
**Edraianthus pulevicii** ab *E. serpyllifolium* s. s. foliis distincte crenatis, basalibus et caulinae multo longioribus et latioribus, supra pilosis, margine pilis retroflexis obsitis, bracteis latio-ribus et dentibus calycinis margine et apice revolutis differt, ab *E. pilosulum* foliis rosulatis et caulinae non crenatis, sicut eis.
with setulose or ciliate appendages. Corolla campanulate, (15–)21.6–28) mm, glabrous or hirsute on veins, divided to 1/2.5–1/2, bluish to violet-blue. Style 1, (11.5–)16.7–(26.4) mm long, stigma (2)–3–(4) lobed, decurrent; stamens 5, inserted on disc, anthers (5)–6.4–(8.7) mm long, filaments (0.9)–2.4–(3) mm long, lower part distinctly dilated to deltoid shape. Seeds numerous, elliptic-oval, light brown (1.3–)1.5–(1.7) × (0.7)–1–(1.2) mm. Figures 1, 2G, H, and 4.

**Chromosome Number**—2n = 32


**Phenology**—Flowering specimens have been observed from July to August (September).

**Etymology**—The new species is named in honor of Prof. Vukić Pulević, a Montenegrin botanist who significantly contributed to the knowledge of the Dinaric and the Balkan flora.

**Distribution and Habitat**—Until now the newly described species has only been found in the Durmitor mountain range (SE Dinaric Alps, Montenegro, Fig. 5). It inhabits shaded and moist rock crevices of limestone, snow bed margins, screes and rocky grasslands in the subalpine and alpine belt between 2,100 and 2,500 m.


**Conservation Status**—Since both taxa are known only from a few adjacent localities in the Durmitor mountain range (E. *pulevicii*) and Komovi Mts. (*E. pilosulus*), and their estimated area of occupancy is smaller than 10 km² (Fig. 5), they both meet the criteria for being critically endangered (CE; IUCN Red List of Threatened Species v. 3.1, available at http://www.iucnredlist.org/info/categories_criteria2001).

**Edraianthus sutjeskae** Lakusišć ex Surina & D. Lakušić—**TYPE**: BOSNIA AND HERZEGOVINA. Dinaric Mts., Sutjeska Gorge, Vratar, 11 August 1925, K. Maly (lectotype, here designated: SARA 40751!).

**Edraianthus sutjeskae** was originally described in 1974 by Lakusišć in his monograph of *Edraianthus*. Although the protologue included a Latin diagnosis and a photogram, the name of the taxon was not validly published since neither type specimen, collector’s name, nor collection date were designated, thus violating Art. 37.1. of the Vienna code (McNeill et al. 2006). Later, during the siege of Sarajevo from 1991–1995, the herbarium at the Institute of Botany, University of Sarajevo (IBUS), where the specimens of *E. sutjeskae* were stored, was completely destroyed.

**Discussion**

Earlier monographers (Wettstein 1887; Janchen 1910) and systematists (e.g. Kuzmanov 1976; Greuter and Raus 1983) did not observe taxonomically significant polymorphism in leaf, involucrum and calyx shape, and indumentum density in *E. serpyllifolius*. Plants with conspicuous indumentum from the Komovi Mts. were collected by I. Szyszylowicz in 1886 (Beck and Szyszylowicz 1888) and treated only as a form: *E. serpyllifolius* f. *pilosulus* (Beck 1893). However, this taxon, according to the cited locality (Komovi Mts.), had already been recog-
nized by R. Visiani (in Nyman 1882), probably on the basis of material collected by J. Pančić in 1873 from the same locality (Pančić 1875, as E. serpyllifolius subsp. thyminifolius sive descr. In his monograph of the genus Edraianthus, Lakušić (1974) originally ranked this taxon as a subspecies of E. serpyllifolius. Subsequently, he realigned it within newly proposed genera, first as Protodraianthus pilosulus (Lakušić 1988) and than as Viniaria pilosula (Lakušić 2001). However, in neither case was an adequate explanation and/or diagnosis offered, nor were the rules of nomenclature followed, so neither name was validly published. A recently published molecular phylogeny of the genus (Stefanović et al. 2008) does not support Lakušić’s systematic treatment.

The data on the geographical distribution of E. pilosulus (= E. serpyllifolius  f. pilosulus auct. non Beck) are a bit confusing. Based on estimates from the literature, E. pilosulus is distributed on mountain ranges of Vojnik, Komovi, and Durmitor in Montenegro, and Maglič, Volujak, Zelengora, Črvanj, and Čvrsnica (Mt. Mali Vran) in Bosnia and Herzegovina (e.g. Rohlena 1942; Lakušić 1974; Međedović 1981; Bjelčić et Mayer 1983). However, our intensive and detailed field investigations did not confirm any of these localities, except for the Komovi Mts., nor did the results of molecular analyses (see Stefanović et al. 2008; Fig. 3).

Although genetically well differentiated, a population of E. sutjeskai with rather elongated cauline and rosette leaves, and sometimes sparsely developed indumentum (Fig. 2C, D) is morphologically similar to E. serpyllifolius s. s. Based only on morphological data, as Međedović (1981) already stated, the distinction of the two taxa is hardly justified. Chloroplast sequence data (Stefanović et al. 2008) render this population as a sister taxon to E. serpyllifolius s. s., while some karyological and palynological peculiarities of this taxon were observed by Med edović (1981). Unpublished AFLP fingerprinting data (Surina et al.) suggest the possibility of its hybrid origin. Nevertheless, its phylogenetic and taxonomic status remain uncertain.

**Key to Edraianthus taxa with spathulate leaves**

1. Margins and upper surfaces of basal leaves with hairs (indumentum) oriented towards leaf base; bract margins hirsute to hisutose-lomentos; calyx lobes revolute and regularly reflexed at the apex .............................................. E. pulevicii

2. Margins and upper surfaces of basal leaves with straight hairs, or hairs oriented towards the apex, or basal leaves without indumentum; bract margins ciliate; calyx lobes flat ......................................................................................................................... 2

3. Bract margins with distinctly prolonged cilia, bract apex acuminate; stems (10–23–52(-72) mm, basal leaves (10–13–20(-25) mm .............................. 3

3. Basal leaves glabrous on upper surface ................................................................................................................. E. pilosulus

Conflicting patterns of genetic and morphological variation in the E. serpyllifolius group are either a result of ancient interspecific hybridization events followed by genome introgression, and range shifts, contractions and expansions as responses to climate change, lineage sorting, or they might be due to extensive morphological homoplasy. Further research is underway to unravel the putative hybrid origin and systematic position of selected Edraianthus taxa.

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**Literature Cited**


Appendix 1. List of morphological characters and their acronyms used in the present analysis.


Appendix 2. Information on taxa sampled, localities, labels, collector, date and the herbarium used in the AFLP fingerprinting.


Edraianthus pilosulus: Prokletije mountain range, Komovi Mts. – Kom Kuk (Montenegro), Knv, B. Surina, 26.7.2007, 488 (NHMR).