



# Molecular reappraisal confirms that the *Campanula trichocalycina-pichleri* complex belongs to *Asyneuma* (Campanulaceae)

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**ABSTRACT** The phylogenetic position of the *Campanula trichocalycina-pichleri* complex, a group consisting of two species distributed respectively in the Apennines and the Balkans, represents a long-standing taxonomic problem resulting from a conflict among limited number of morphological characters. Traditionally, this complex was treated either as a separate genus, *Podanthum*, or as part of *Campanula* or *Asyneuma*. However, these competing taxonomic hypotheses have never been tested using explicit phylogenetic approaches. We present here the results of molecular phylogenetic analyses based on non-coding chloroplast sequences (*trnL-F*). Molecular evidence indicates that *Campanula trichocalycina* and *C. pichleri* form a strongly supported lineage, positioned as sister-group to *Asyneuma* s.str. Consequently, we treat this complex as congeneric with *Asyneuma*. We also discuss relative importance of floral morphology versus fruit dehiscence for circumscription of *Asyneuma* and *Campanula* as well as proposed taxonomic status of *Asyneuma comosiforme*, a species recently discovered not to be closely related to other members of *Asyneuma* and segregated from this genus.

**KEY WORDS:** Balkans, Campanulaceae, molecular phylogeny, *Asyneuma*, *Campanula*, *Physoplexis*, *Petromarula*, *trnL-F*.

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## INTRODUCTION

*Campanula trichocalycina* Tenore was originally described based on specimens collected on Cervialto (actually Cervialto) of the Monti Picentini, Campania region, Italy (CASALE & GUSSONE 1812, TENORE 1815, lectotype designated by LAKUŠIĆ & CONTI 2004). Subsequently, VISIANI (1872) described species *Campanula pichleri* Visiani on the basis of herbarium specimen collected by Thomas Pichler on Mt. Orjen (Montenegro) in 1870 (lectotype designated by LAKUŠIĆ & CONTI 2004). Pichler had mistakenly determined this plant as "*Campanula alpini* L." [i.e. *Adenophora lilifolia* (L.) Besser]. In 1871 Rupert Huter designated the same plant on herbarium labels as

a new species under the name "*Adenophora pichleri*"; however, Huter did not publish a valid description for this plant. The combination *Adenophora pichleri* was published by PANTOCZEK (1874), but never used later on.

When BOISSIER (1875) described a new genus *Podanthum* Boiss., he included Visiani's *Campanula pichleri* as a synonym in Tenore's *Campanula trichocalycina* but under the name *Podanthum trichocalycinum* (Ten.) Boiss. This view was also accepted by ASCHERSON & KANITZ (1877). In 1908 K. MALÝ introduced a new combination, *Asyneuma trichocalycinum* (Ten.) K. Malý. Since then *Campanula pichleri* has been used exclusively as a synonym for *Asyneuma trichocalycinum* (BORNMÜLLER 1921; HAYEK 1930; DAMBOLT 1968, 1970; BJELČIĆ 1983)

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or has been completely ignored (FEDOROV & KOVANDA 1976; GREUTER *et al.* 1984; PIGNATTI 1982; HARTVIG 1991). In general, Boissier's taxonomic opinion (BOISSIER 1875) that *C. pichleri* is a synonym of *C. trichocalycina* (under the name *Podanthum trichocalycinum*) has not been substantially changed until recently, when, based on morphometric studies, two sibling species have been separated under the names *Asyneuma trichocalycinum* from the Apennines and *Asyneuma pichleri* (Visiani) D. Lakušić & F. Conti from Balkan peninsula (LAKUŠIĆ & CONTI 2004). This view was also accepted by LAMMERS (2007b).

According to RECHINGER & SCHIMAN-CZEIKA (1965) *Asyneuma trichocalycinum* (including *A. pichleri*) belongs to section *Clinocarpium* (Boiss.) Rech. f. & Schiman-Czeika, together with *A. asperum* (Boiss.) Rech. f. & Schiman-Czeika, *A. persicum* (DC.) Bornm., *A. multicaule* (Boiss.) Rech. f. & Schiman-Czeika, and *A. macrodon* (Boiss. & Hausskn.) Bornm. In comparison to other species of Campanulaceae, species from section *Clinocarpium* are perennial branched plants, characterized by flowers with deeply divided corolla (corolla divided at least to  $\frac{3}{4}$  of its length or nearly to the base) and by pending capsules that open with basal pores. Species included in this section are distributed from the mountains in Northern Africa, Sicily and Southern Italy, across the Balkan Peninsula, Asia Minor, Caucasus, Iranian, and Iraqi mountains, up to Afghanistan (BOISSIER 1875, RECHINGER & SCHIMAN-CZEIKA 1965).

Depending on various views of relationships within Campanulaceae, species from the section *Clinocarpium* used to be included into the genus *Asyneuma* Griseb. & Schenk (MALÝ 1908; BORNMÜLLER 1921; HAYEK 1930; ROHLENA 1942; RECHINGER & SCHIMAN-CZEIKA 1965; OBRADOVIĆ 1974; BJELČIĆ 1983; LAKUŠIĆ & CONTI 2004; LAMMERS 2007b) or into *Campanula* L. (TENORE 1815; DAMBOLT 1968, 1970, 1978a; FEDOROV & KOVANDA 1976; GREUTER *et al.* 1984; PIGNATTI 1982; HARTVIG 1991). In most of the contemporary national and regional Floras and Checklists (FEDOROV & KOVANDA 1976; GREUTER *et al.* 1984; PIGNATTI 1982; HARTVIG 1991) the consideration of the latest monography of genus *Asyneuma* (DAMBOLT 1968, 1970, 1978a) is generally accepted, and taxa from section *Clinocarpium* (except *A. macrodon*) are classified within *Campanula*.

It has been proven difficult to ascertain the phylogenetic positions of *Campanula trichocalycina* and *C. pichleri* (as belonging to *Campanula*, *Asyneuma*, or its own genus *Podanthum*) relying exclusively on a limited number of morphological characters. In the present study we aim to resolve this long-standing taxonomic problem by employing molecular data, chloroplast DNA sequences, using a broad sampling of taxa from across Campanulaceae.

## MATERIAL AND METHODS

**Taxon sampling.** Names of all species used in this study, along with their sources, voucher information, and corresponding DNA extraction numbers, are provided in Appendix 1. Sampling for the *Campanula trichocalycina-pichleri* complex included three individuals of *C. trichocalycina* and eight individuals of *C. pichleri*, broadly covering the geographic distribution and morphologic diversity of these two closely related species. As representatives of putatively closely related *Asyneuma*, we used five species from this genus, distributed from the Balkans via the Pontic region to the Caucasus. A total of 18 accessions were sampled from these *Asyneuma* placeholders, ranging from one to eight individuals per species. Formal nomenclature for *Asyneuma* follows the DUMBOLT (1976), HAYEK (1930) and LAKUŠIĆ & CONTI (2004).

To select the appropriate outgroups, we relied on the results from broad molecular systematic studies of the bellflower family (e.g., EDDIE *et al.* 2003; ROQUET *et al.* 2008). According to these previous analyses, both *Asyneuma* and the *Campanula trichocalycina-pichleri* complex are found to be nested within the *Rapunculus* clade (EDDIE *et al.* 2003). We have chosen 20 diverse taxa from this group to represent more closely related outgroups. Additionally, 22 members of the *Campanula* s.str. clade (EDDIE *et al.* 2003) were included in the analyses as more distant outgroups, used to root the phylogenetic trees.

**Molecular phylogenetic analyses.** To infer the phylogenetic position of *Campanula trichocalycina-pichleri* complex, we targeted a non-coding region containing the *trnL*-UAA intron, 3' *trnL*-UAA exon, and intergenic spacer between this exon and *trnF*-GAA (hereafter called *trnL-F*) from the chloroplast genome (cpDNA). In addition to the DNA samples used in previous studies (STEFANOVIĆ *et al.* 2008), total genomic DNA was isolated from newly obtained specimens as well (see Appendix 1). DNA extractions, polymerase chain reaction (PCR) reagents and conditions, amplification, purifications and sequencing procedures follow STEFANOVIĆ *et al.* (2008). Newly obtained sequences have been submitted to GenBank (accession numbers GQ254891-GQ254931). Sequences were aligned manually using Se-AL v.2.0a11 (RAMBAUT 2002). Gaps in the alignments were treated as missing data. Phylogenetic analyses were conducted under parsimony and Bayesian optimality criteria.

Parsimony searches were conducted in PAUP\* 4.0 b10 (SWOFFORD 2002). Under this criterion, nucleotide characters were treated as unordered and all changes were equally weighted. Search for most parsimonious (MP) trees was performed using a two-stage strategy. First, the

search involved 1000 replicates with random stepwise addition (RAS) of taxa, tree bisection-reconnection (TBR) branch swapping saving no more than 10 trees per replicate and MULTREES option off. In the second stage, all trees in memory were analyzed with the same settings except the MULTREES option on. Both stages were conducted to completion or until 500,000 trees were found. Relative support for clades was inferred by nonparametric bootstrapping (FELSENSTEIN 1985) as implemented in PAUP\* using 500 heuristic bootstrap replicates, each with 20 RAS cycles, TBR branch swapping and MULTREES option off (DEBRY & OLMSTEAD, 2000). Nodes receiving bootstrap support (BS) <70%, 70-80, and >80% are considered weakly, moderately, and strongly supported, respectively.

The Transversion model of DNA substitution (TVM; variable base frequencies, variable transversions, but equal transitions probabilities), with rate variation among nucleotides following a discrete gamma distribution (TVM + G), was selected as the best-fit by both the hierarchical likelihood ratio test (hLRT) and Akaike Information Criterion (AIC), as implemented in ModelTest v.3.7 (POSADA & CRANDALL 1998). Bayesian phylogenetic inferences (YANG & RANNALA, 1997) were conducted using MrBayes version 3.1.2 (RONQUIST & HUELSENBECK 2003). Four runs starting from random trees were carried out using the TVM + G substitution model. All model parameters were treated as unknown variables with uniform prior probabilities and were estimated as part of the analysis together with tree topologies. Metropolis-coupled Markov chain Monte Carlo algorithm was used with four simultaneous chains, set at three million generations and sampled every 100 generations. Convergence of the chains was determined by examining the plot of all parameter values and  $-\ln L$  scores against generation using Tracer version 1.3 (RAMBAUT & DRUMMOND 2004). Stationarity was assumed when all parameter values and the  $-\ln L$  had stabilized. Only the nodes receiving  $\geq 0.95$  posterior probabilities (PP) were considered to have statistically significant support (RANNALA & YANG 1996).

## RESULTS

The parsimony analysis resulted in >500,000 equally parsimonious trees of 423 steps each, with a consistency index (CI) of 0.82 and a retention index (RI) of 0.95. The strict consensus is presented in Fig. 1. Four Bayesian runs carried out the same data set converged on similar  $-\ln L$  scores and reached stationarity at no later than 200,000 generations. The burn-in of data points, accumulated before asymptotic plateaus were reached, left a total of 112,000 combined trees (4 x 28,000) that were summarized as a majority-rule consensus tree (Fig. 2). Phylogenetic trees resulting from these two optimality criteria are topo-

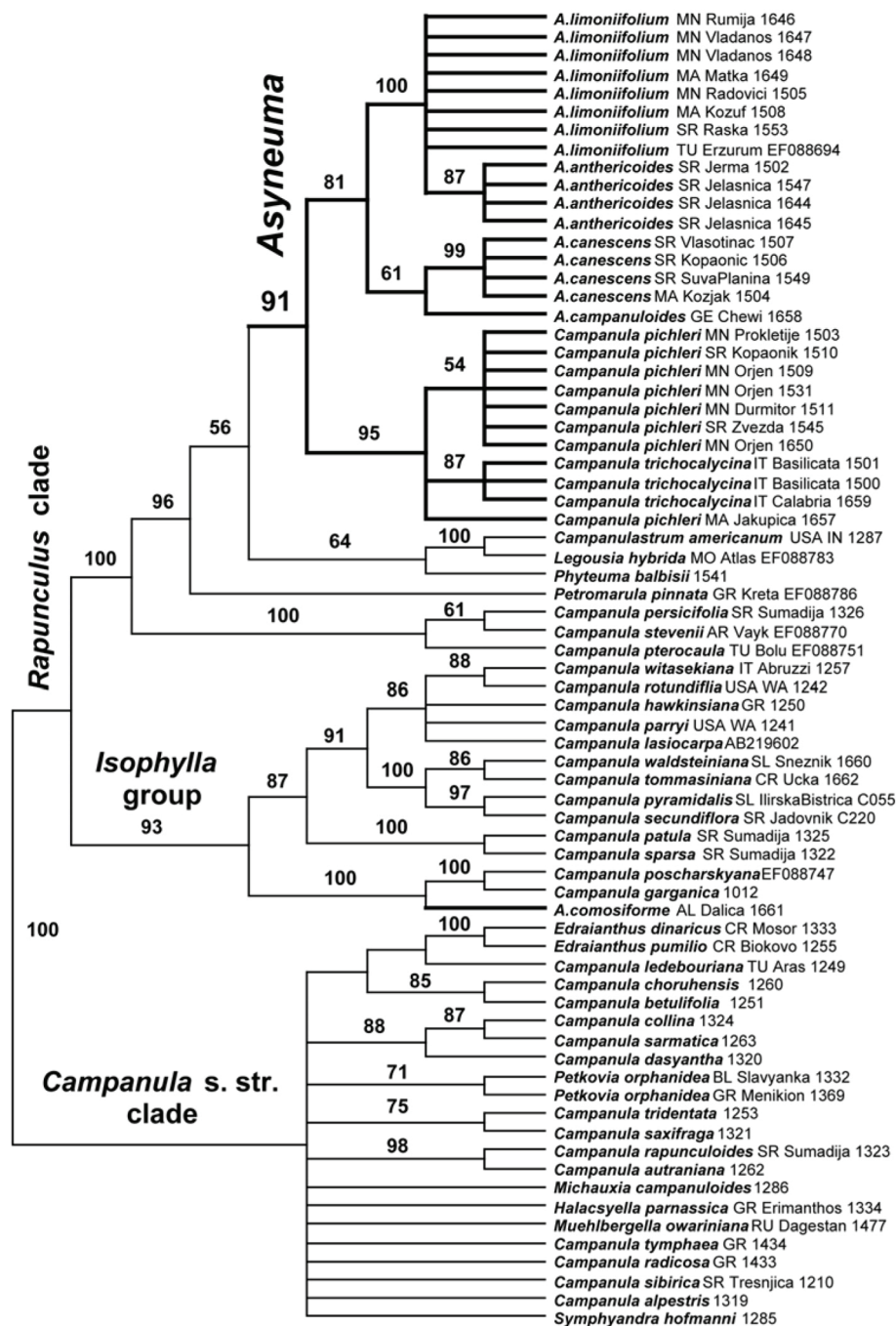
logically essentially identical. These analyses also show congruent support values for clades; therefore, our results and discussion are based on both trees (Figs. 1 and 2).

According to our results, all individuals representing the *Campanula trichocalycina-pichleri* complex were found in a single strongly supported (95% BS;  $\geq 0.95$  PP) clade. Within this clade, individuals belonging to *C. trichocalycina* form a distinct lineage. However, relationships of *C. pichleri* representatives to each other and to *C. trichocalycina* are neither well resolved nor supported, indicating that the reciprocal monophyly between these two species may have not been yet achieved. As expected based on recent molecular analyses (FRAJMAN & SCHNEEWEISS 2009), we also found that *Asyneuma* is not monophyletic. One species traditionally assigned to *Asyneuma*, the enigmatic Albanian stenoendemic *A. comosiforme* Hayek, bears no close relationships with the rest of the genus. This species is found elsewhere on the tree, within the *Isophylla* group (PARK *et al.* 2006), as strongly supported sister-group to the *Campanula garganica* clade (cf. FRAJMAN & SCHNEEWEISS 2009). *Asyneuma* s.str. is resolved as monophyletic according to both optimality criteria (Figs. 1 and 2), but with mixed support. While parsimony provided relatively strong support (81% BS; Fig. 1), Bayesian analysis did not show this clade significantly supported (i.e., <0.95 PP; Fig. 2). According to both analyses, the sister-group relationship between the *Campanula trichocalycina-pichleri* complex and *Asyneuma* s.str. clade has received strong support, 91%BS and  $\geq 0.95$  PP, respectively (Figs. 1 and 2).

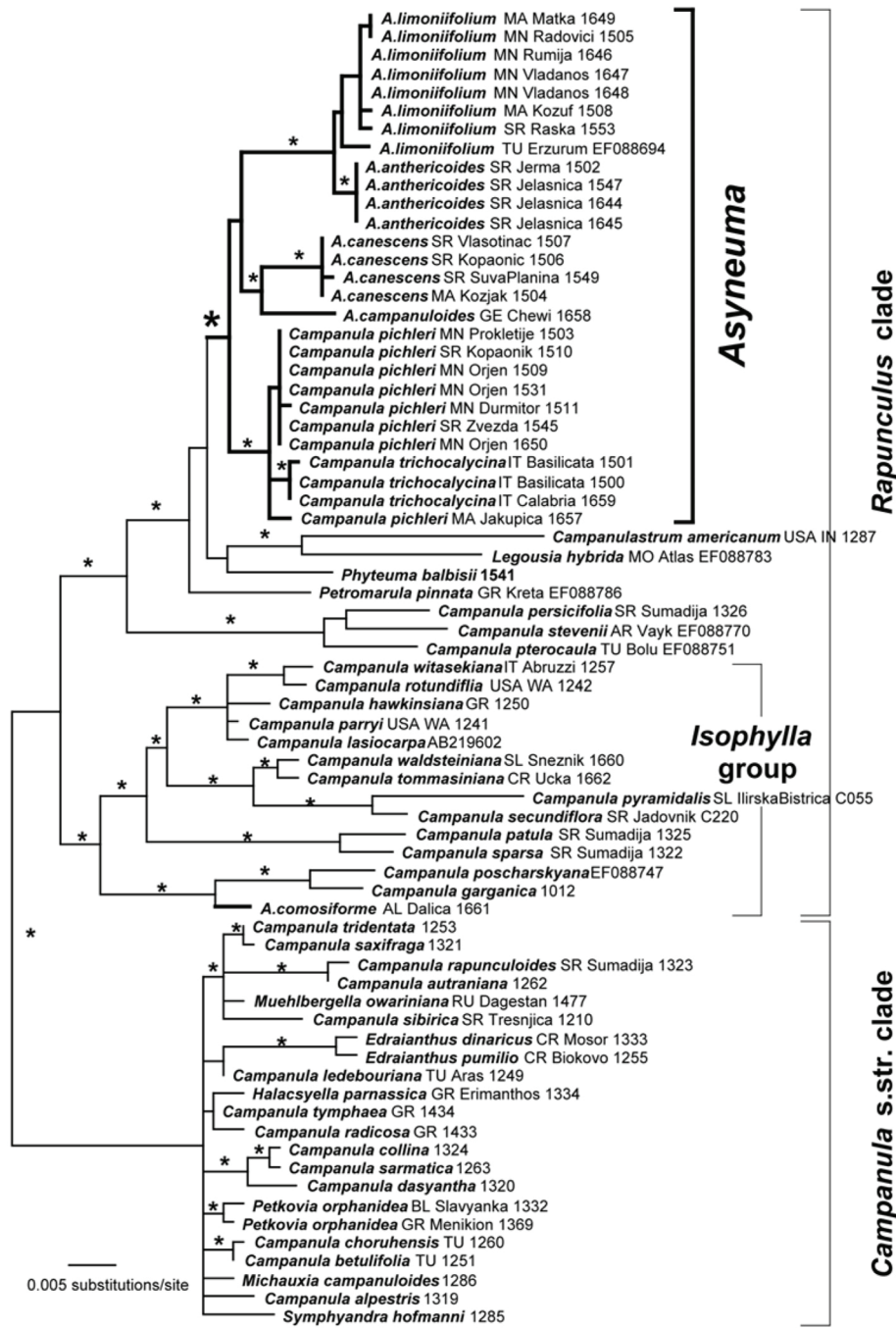
The newly circumscribed *Asyneuma*, which include the *Campanula trichocalycina-pichleri* complex and exclude *A. comosiforme*, is found nested deeply within the *Rapunculus* clade (EDDIE *et al.* 2003), with several closely related genera (i.e. *Petromarula*, *Phyteuma*, *Campanulastrum*, *Legousia*; Figs. 1 and 2) as potential sister-groups (alone or in combination with each other). The backbone relationships within the *Campanula* s.str. clade (EDDIE *et al.* 2003), used here as a functional outgroup, remain highly unresolved, as reported previously (e.g. EDDIE *et al.* 2003, ROQUET *et al.* 2008, STEFANOVIĆ *et al.* 2008).

## DISCUSSION

**Transfer of the *Campanula trichocalycina-pichleri* complex to *Asyneuma*.** Our results re-present the first attempt to address the issue of the precise phylogenetic position of *Campanula trichocalycina-pichleri* complex as well as preliminary circumscription of *Asyneuma* using molecular data. We found the *Campanula trichocalycina-pichleri* complex resolved as strongly supported sister-group to the *Asyneuma* s.str. clade (Figs. 1 and 2). This particular phylogenetic position of the *Campanula trichocalycina-pichleri* complex is compatible with all three previously proposed taxonomic scenarios: its retention



**Fig. 1.** The strict consensus of all recovered equally parsimonious trees ( $L = 423$ ;  $CI = 0.82$ ;  $RI = 0.95$ ) showing the phylogenetic position of the *Campanula trichocalycina-pichleri* complex based on cpDNA *trnL-F* sequences. Tree is rooted using representatives from the *Campanula s. str.* clade as the functional outgroups. Following species names, two-letter country abbreviation (AL: Albania; AR: Armenia; BL: Bulgaria; CR: Croatia; GE: Georgia; GR: Greece; IT: Italy; MA: Macedonia; MN: Montenegro; MO: Morocco; RU: Russian Federation; SL: Slovenia; SR: Serbia; TU: Turkey; US: United States of America) and locality are provided when known while numbers correspond to DNA or GenBank accessions (compare with Table 1). Numbers above branches indicate bootstrap support.



**Fig. 2.** Majority-rule consensus tree with mean branch lengths from the Bayesian analysis of cpDNA *trnL-F* sequences under the TVM + G model of DNA evolution showing the phylogenetic position of the *Campanula trichocalycina-pichleri* complex. Asterisks indicate branches with Bayesian posterior probability  $\geq 0.95$ ; all other branches have posterior probability  $< 0.95$ . Following species names, two-letter country abbreviation (see Fig. 1) and locality are provided when known while numbers correspond to DNA or GenBank accessions (compare with see Appendix 1).

within *Campanula*, realignment within *Asyneuma* s.str. (i.e. without *A. comosiforme*) or segregation into a separate genus, *Podanthum*. However, retention of this complex in *Campanula* would make this large and paraphyletic genus even less natural. Namely, given the phylogenetic position of a number of other segregate genera, such as *Petromarula*, *Phytreauma*, *Legousia*, and *Campanulastrum*, *Campanula* would be rendered polyphyletic. It is conceivable that the genus *Podanthum* (BOISSIER 1875) could be resurrected when additional samples of different *Asyneuma* species are taken into consideration, both at morphological and molecular level.

Nevertheless, for the time being, we favor the transfer of the *Campanula trichocalycina-pichleri* complex to *Asyneuma*, a solution strongly supported by currently available molecular data.

The opinion that *Campanula trichocalycina* and *C. pichleri* do not belong to *Campanula* was first expressed by BOISSIER (1875), who placed them into a new genus *Podanthum*. Subsequently, the segregation of these two species from *Campanula* was also accepted by ASCHERSON & KANITZ (1877), MALÝ (1908), BORNMÜLLER (1921), HAYEK (1930), ROHLENA (1942), RECHINGER & SCHIMAN-CZEIKA (1965), OBRADOVIĆ (1974) and BJELČIĆ (1983) but instead they were transferred to *Asyneuma*, along with several additional species belonging to the section *Clinocarpium*. Diversity of approaches taken to clarify the taxonomic position of species from the section *Clinocarpium*, within *Asyneuma* or *Campanula*, or as a new separate genus, stem from different views about the choice of a particular character used and its perceived significance. On one side, those who treated species from section *Clinocarpium* as part of the genus *Asyneuma* gave priority to the morphology of the corolla. Namely, in all species of this section corolla is deeply divided, all the way to the base or a minimum of  $\frac{3}{4}$  of its length. In contrast, authors who included these species in *Campanula*, gave priority to capsule morphology. According to the last comprehensive monographic works on *Asyneuma* (DAMBOLT 1968, 1970, 1978a), because of pending capsules with basal pores, species from section *Clinocarpium* (except *A. macrodon*) belong to *Campanula*. For this reason, the taxa with corolla divided to the base (*C. trichocalycina* and *C. pichleri*) are included in genus *Campanula*. Most of the contemporary national and regional Floras and Checklists (e.g., FEDOROV & KOVANDA 1976, PIGNATTI 1982, GREUTER *et al.* 1984, HARTVIG 1991) generally follow this view.

The relative importances of corolla and capsule morphology for a separation of *Asyneuma* and *Campanula* were evaluated recently in a morphometric studies by LAKUŠIĆ and CONTI (2004). These authors concluded that the corolla morphology has higher differential significance in defining *Asyneuma*. Therefore, regardless of the way the

fruits open, all the species from section *Clinocarpium* that have corolla divided for a minimum of  $\frac{3}{4}$  of its length to all the way to the base should be included in *Asyneuma*, and not in *Campanula*. This argumentation was accepted by Lammers in his recent taxonomic treatments of Campanulaceae (LAMMERS 2007a, b), which retain *Campanula* and *Asyneuma* as separate genera. Compared to the flower morphology (and in particular corolla morphology), relatively lower taxonomic importance of the capsule dehiscence within Campanulaceae was inferred also with respect to the phylogenetic position of *Edraianthus* A.DC (STEFANOVIĆ *et al.* 2008).

**Taxonomic status of *Asyneuma comosiforme*.** Our results also indicate that *Asyneuma* is not monophyletic as currently circumscribed and support recent conclusion of FRAJMAN & SCHNEEWEISS (2009) that *Asyneuma comosiforme* does not belong to *Asyneuma*. Instead, this species is found closely related to isophyllous *Campanula* species, sister to the *Garganica* clade (following the circumscription of PARK *et al.* 2006). Taken together, these molecular results question the relative importance of flower morphology and suggest that deeply divided corolla should not be relayed upon as the sole character to differentiate *Asyneuma* from *Campanula* and other closely related genera.

Having pedicellate flowers with corolla divided to the base and hanging capsules that dehisce basally with three pores, *A. comosiforme* seems close to species of *Asyneuma* section *Clinocarpium*. However, as already noted in its protologue (HAYEK & JANCHEN in HAYEK 1921), given its general habit, this species resembles more some other taxa from Campanulaceae, such as *Physoplexis comosa* (L.) Schur. Furthermore, the overall habit similarities, including distinctly petiolate cauline leaves and capsules opening by basal pores, are shared also with the *Isophylla* group, a clade of isophyllous *Campanula* species distributed mainly in the Balkans (PARK *et al.* 2006), whom *A. comosiforme* is most closely related with (FRAJMAN & SCHNEEWEISS 2009). While this newly recovered phylogenetic position of *A. comosiforme* and its molecular distinctiveness are strongly supported (FRAJMAN & SCHNEEWEISS 2009 and present study), its taxonomic status remains questionable. If this species is transferred to *Campanula*, as suggested by FRAJMAN & SCHNEEWEISS (2009), it becomes quite difficult to find a character (or a character combination) to clearly separate *Asyneuma* and *Campanula* on morphological grounds. Taking this into account, we suggest that this taxon should be treated as a separate genus, perhaps under the name *Hayekia* R. Lakušić *nom. inval.*, as already proposed by LAKUŠIĆ (2001). In addition, the segregation of Hayek's *A. comosiforme* into a monotypic genus could be further supported similarly as taxonomic

solutions adopted for monotypic genera *Petromarula* Vent. ex R.Hedw. and *Physoplexis* Schur. Thus, according to FRAJMAN & SCHNEEWEISS (2009) and our results, *A. comosiforme* relationship to its closest relatives from the *Isophylla* group is similar to that of *Petromarula pinnata* (L.) A. DC. with respect to its closely related *Asyneuma* s.str. species as well as *Physoplexis comosa* with respect to *Phyteuma* L. species. We propose this taxonomic status to be adopted when revisions of *Campanula* are conducted, as called for by recent broad molecular studies of this genus (PARK *et al.* 2006; ROQUET *et al.* 2008; see the latter for suggested taxonomic options) and family as a whole (EDDIE *et al.* 2003).

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## REZIME

## Molekularni podaci potvrđuju da kompleks *Campanula trichocalycina-pichleri* pripada rodu *Asyneuma* (Campanulaceae)

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Zbog malog broja distinktivnih morfoloških karaktera, filogenetska pozicija kompleksa *Campanula trichocalycina-pichleri*, grupe koja se sastoji od dve sestrinske vrste rasprostranjene na Apeninskom i Balkanskom poluostrvu predstavlja taksonomski problem već duže vreme. Različiti autori vrste ovog kompleksa najčešće uključuju u rodove *Campanula* ili *Asyneuma*, a redje i u zaseban rod *Podanthum*. Ove suprotstavljene hipoteze do sada nisu bile testirane uz pomoć modernog filogenetskog pristupa. U ovom radu, prikazani su rezultati molekularno-filogenetske analize zasnovane na ne-kodirajućoj hloroplastnoj sekvenci (*trnL-F*), koji ukazuju da *Campanula trichocalycina* i *C. pichleri* formiraju jasno odvojenu liniju koja se pozicionira kao sestrinska grupa sa *Asyneuma* s.str. Shodno tome, predlaže se da se ovaj kompleks tretira kao kongenerični sa *Asyneuma*. Takođe se diskutuje relativna važnost morfologije cveta nasuprot morfologiji plodova pri definisanju rodova *Asyneuma* i *Campanula*. Posebna pažnja je posvećena razmatranju pitanja taksonomskog statusa *Asyneuma comosiforme*, vrste za koju je novijim istraživanjima pokazano da nije blisko srodna sa ostalim predstavnicima roda *Asyneuma*.

**KLJUČNE REČI:** Balkan, Campanulaceae, molekularna filogenija, *Asyneuma*, *Campanula*, *Physoplexis*, *Petromarula*, *trnL-F*.



**Appendix 1.** - Species names, sources, voucher information (Herbarium acronyms according to HOLMGREN *et al.* 1990), and corresponding DNA extraction numbers of analysed species in this study.

Species name	DNA accession	Country, locality	Voucher	GeneBank ( <i>trnL-F</i> )
<i>Asyneuma anthericoides</i> Bornm.	1502	Serbia, Jerma	Vukojčić <i>et al.</i> 15060 (BEOU)	GQ254891
<i>Asyneuma anthericoides</i> Bornm.	1547	Serbia, Jelašnička klisura	Stevanović <i>et al.</i> 5430 (BEOU)	GQ254892
<i>Asyneuma anthericoides</i> Bornm.	1644	Serbia, Jelašnička klisura	Stevanović 26807 (BEOU)	GQ254893
<i>Asyneuma anthericoides</i> Bornm.	1645	Serbia, Jelašnička klisura	Stevanović 24071 (BEOU)	GQ254894
<i>Asyneuma campanuloides</i> Bornm.	1658	Georgia, Chewi	No voucher	GQ254895
<i>Asyneuma canescens</i> Griseb. & Schenk	1507	Serbia, Vlasinsko jezero	Randjelović & Stanković 12802 (BEOU)	GQ254896
<i>Asyneuma canescens</i> Griseb. & Schenk	1506	Serbia, Kopaonik	Lakušić 19974 (BEOU)	GQ254897
<i>Asyneuma canescens</i> Griseb. & Schenk	1549	Serbia, Suva planina	Vukojčić & Tomović 6344 (BEOU)	GQ254898
<i>Asyneuma canescens</i> Griseb. & Schenk	1504	Macedonia, Kozjak	Tomović & Zlatković 19244 (BEOU)	GQ254899
<i>Asyneuma comosiforme</i> Hayek & Janch.	1661	Albania, Đalica, Gryka e Shijes near Bicay	Surina 1093 (NHMR)*	GQ254900
<i>Asyneuma limoniifolium</i> (L.) Bornm.	1649	Macedonia, Matka	Stevanović 26808 (BEOU)	GQ254901
<i>Asyneuma limoniifolium</i> (L.) Bornm.	1646	Montenegro, Rumija	Stevanović 26811 (BEOU)	GQ254902
<i>Asyneuma limoniifolium</i> (L.) Bornm.	1647	Montenegro, Valdanos	Lakušić 26809 (BEOU)	GQ254903
<i>Asyneuma limoniifolium</i> (L.) Bornm.	1648	Montenegro, Valdanos	Lakušić 26810 (BEOU)	GQ254904
<i>Asyneuma limoniifolium</i> (L.) Bornm.	1505	Montenegro, Radovići	Lakušić 15169 (BEOU)	GQ254905
<i>Asyneuma limoniifolium</i> (L.) Bornm.	1553	Serbia, Raška	Vukojčić & Niketić 7020 (BEOU)	GQ254906
<i>Asyneuma limoniifolium</i> (L.) Bornm.	1508	Macedonia, Kožuf	Tomović & Zlatković 19135 (BEOU)	GQ254907
<i>Asyneuma limoniifolium</i> (L.) Bornm.	n/a	Turkey, Erzurum	Nisa 1006 (MA)	EF088694
<i>Campanula alpestris</i> All.	1319	Cultivated	SS-05-167 (TRTE)	EF213141
<i>Campanula autraniana</i> Albov	1262	Cultivated	SS-04-20 (TRTE)	GQ254908
<i>Campanula betulifolia</i> K. Koch	1251	Turkey	SS-04-09 (TRTE)	EF213142
<i>Campanula choruhensis</i> Kit Tan & Sorger	1260	Turkey	SS-04-18 (TRTE)	GQ254909
<i>Campanula collina</i> M. Bieb.	1324	Cultivated	SS-05-185 (TRTE)	EF213143

Species name	DNA accession	Country, locality	Voucher	GeneBank ( <i>trnL-F</i> )
<i>Campanula dasyantha</i> M. Bieb.	1320	Cultivated	SS-05-169 (TRTE)	EF213144
<i>Campanula garganica</i> Ten.	1012	Cultivated	SRD-485 (IND)	EF213145
<i>Campanula hawkinsiana</i> Hausskn. & Heldr. ex Hausskn.	1250	Greece	SS-04-08 (TRTE)	EF213146
<i>Campanula lasiocarpa</i> Cham.	n/a	Japan, Hokkaido	Senni S1471 (MAK)	AB219602
<i>Campanula ledebouriana</i> Trautv.	1249	Turkey, Aras	SS-04-07 (TRTE)	GQ254910
<i>Campanula parryi</i> A. Gray	1241	USA, WA	No voucher	EF213147
<i>Campanula patula</i> L.	1325	Serbia, Šumadija	SS-05-174 (TRTE)	EF213148
<i>Campanula persicifolia</i> L.	1326	Serbia, Šumadija	SS-05-171 (TRTE)	EF213149
<i>Campanula pichleri</i> Vis.	1657	Macedonia, Jakupica	Frajman & Schönswetter 11690 (WU)	GQ254911
<i>Campanula pichleri</i> Vis.	1509	Montenegro, Orjen	Stevanović 1789 (BEOU)	GQ254912
<i>Campanula pichleri</i> Vis.	1531	Montenegro, Orjen	Stevanović & Lakušić 20987a (BEOU)	GQ254913
<i>Campanula pichleri</i> Vis.	1650	Montenegro, Orjen	Stevanović & Lakušić 20987b (BEOU)	GQ254914
<i>Campanula pichleri</i> Vis.	1511	Montenegro, Durmitor	Lakušić 1495/96 (BEOU)	GQ254915
<i>Campanula pichleri</i> Vis.	1510	Serbia, Kopaonik	Niketić et al. 18960 (BEOU)	GQ254916
<i>Campanula pichleri</i> Vis.	1545	Serbia, Zvezda	Stevanović & Ostojić 16259 (BEOU)	GQ254917
<i>Campanula pichleri</i> Vis.	1503	Montenegro, Prokletije	Lakušić 8/88 (BEOU)	GQ254918
<i>Campanula poscharskyana</i> Degen	n/a	Cultivated	Alarcón 178 (MA)	EF088747
<i>Campanula pterocaula</i> Hausskn.	n/a	Turkey, Bolu	Nydegger 19005 (MA)	EF088751
<i>Campanula pyramidalis</i> L.	C055	Slovenia, Ilirska Bistrica	Surina (NHMR)	GQ254919
<i>Campanula radicata</i> Bory & Chaub.	1433	Greece	Baden et al. 969 (C)	EF213151
<i>Campanula rapunculoides</i> L.	1323	Serbia, Šumadija	SS-05-172 (TRTE)	EF213152
<i>Campanula rotundifolia</i> L.	1242	USA, WA	No voucher	GQ254920
<i>Campanula sarmatica</i> Ker Gawl.	1263	Cultivated	SS-04-21 (TRTE)	GQ254921
<i>Campanula saxifraga</i> M. Bieb.	1321	Cultivated	SS-05-166 (TRTE)	EF213155
<i>Campanula secundiflora</i> Vis. & Pancic	C220	Serbia, Jadovnik	Lazarević 16192, 16191 (BEOU)	GQ254922

Species name	DNA accession	Country, locality	Voucher	GeneBank ( <i>trnL-F</i> )
<i>Campanula sibirica</i> L.	1210	Serbia, Trešnjica	No voucher	EF213157
<i>Campanula sparsa</i> Friv.	1322	Serbia, Šumadija	SS-05-175 (TRTE)	EF213159
<i>Campanula stevenii</i> M.Bieb.	n/a	Armenia, Vayk	Oganessian s.n. (ERE)	EF088770
<i>Campanula tommasiniana</i> Koch ex F.W.Schultz	1662	Croatia, Učka	Surina 1165 (NHMR)	GQ254923
<i>Campanula trichocalycina</i> Ten.	1501	Italy, Pollino	Conti & Lakušić 19981 (BEOU)	GQ254924
<i>Campanula trichocalycina</i> Ten.	1500	Italy, Volturino	Conti 19979 (BEOU)	GQ254925
<i>Campanula trichocalycina</i> Ten.	1659	Italy, Serra Dolcedorme	Dixon 11179 (WU)	GQ254926
<i>Campanula tridentata</i> Schreb.	1253	Cultivated	SS-04-11 (TRTE)	EF213160
<i>Campanula tymphaea</i> Haussshn.	1434	Greece	Strid et al. 30314 (C)	EF213161
<i>Campanula waldsteiniana</i> Roem. & Schult.	1660	Slovenia, Snežnik	Surina 1165 (NHMR)	GQ254927
<i>Campanula witasekiana</i> Vierh.	1257	Italia, Abruzzi	SS-04-15 (TRTE)	EF213164
<i>Campanulastrum americanum</i> (L.) Small	1287	USA, IN	SS-04-163 (TRTE)	GQ254928
<i>Edraianthus pumilio</i> A.DC.	1255	Croatia, Biokovo	SS-04-13 (TRTE)	EF213288
<i>Edraianthus dinaricus</i> (Kerner) Wettstein	1333	Croatia, Mosor	E. Janchen 3083 (BEOU)	EF213189
<i>Halacsyella parnassica</i> (Boiss. & Spruner) Janch.	1334	Greece, Erimanthos	Horandl et al. 7584 (C)	EF213284
<i>Legousia hybrida</i> (L.) Delarb.	n/a	Morocco, Atlas	Romo et al. R10113 (BC)	EF088783
<i>Michauxia campanuloides</i> L'Hér. Ex Ait.	1286	Cultivated	SS-04-154 (TRTE)	GQ254929
<i>Muehlbergella oweriniana</i> (Rupr.) Feer	1477	Russia, Dagestan	Radzki s.n. (LENUD)	EF213283
<i>Petkovia orphanidea</i> (Boiss.) Stef.	1332	Bulgaria, Slavyanka	Pashaliev SOM151369 19982 (SOM)	EF213339
<i>Petkovia orphanidea</i> (Boiss.) Stef.	1369	Greece, Menikion	Franzen et al. 18623 (C)	EF213341
<i>Petromarula pinnata</i> (L.) DC	n/a	Greece, Kreta	Shay 82-1059 (B)	EF088786
<i>Phyteuma balbisii</i> A.DC.	1541	Cultivated	SS-06-248 (TRTE)	GQ254930
<i>Symphyantra hofmanni</i> Pant.	1285	Cultivated	SS-04-153 (TRTE)	GQ254931