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Relations of the western Balkan endemic *Campanula* L. (Campanulaceae) lineages based on comparative floral morphometry

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Abstract

In the north-eastern quadrant of the Mediterranean Region many endemic *Campanula* (Campanulaceae) taxa and lineages arose, of which the Isophylla group and the Rotundifolia complex (sect. Heterophylla), essentially separated on the basis of their leaf shapes, and some of the endemic western Balkan, Dinaric lineages (i.e. 'isophylloid' aggregates Waldsteiniana and Pyramidalis) are members. The aim of this study is to revise the mutual morphological floral relations of these endemic lineages, on the basis of 14 taxa, 33 populations and 409 flower samples, and measure simple flower traits and their ratios. For data interpretation, a phenetic approach using multivariate analyses was applied, as a first attempt in understanding biometrical floral relations between the similarly distributed *Campanula* lineages, and to provide a fundamental background for the cladistic and molecular analyses that will follow. Two 'natural' *Campanula* groups, isophyllous/isophylloid and heterophyllous, are identified according to their floral properties. The floral pattern of *Campanula waldsteiniana* and *C. tommasiniana* are clearly separated, by the corolla shapes, into isophyllous and heterophyllous, respectively, while *C. pyramidalis* follows the isophyllous pattern, but is plainly recognizable.

Key words: *Balkans,* Campanula*, Isophylla, Heterophylla, floral variability, multivariate analysis*

Introduction

In the north-eastern quadrant of the Mediterranean Region many endemic *Campanula* taxa and lineages arose (Fedorov & Kovanda, 1976; Geslot, 1984) among which the western Balkan, Dinaric groups (subsections, aggregates) have hardly ever been investigated separately. To elucidate some of the complicated relationships in this floristically rich – yet still poorly known – European region, we initiated a broad study of the endemic *Campanula* lineages. Comparative floral morphometry is applied as a first step in solving at least some of the many open questions within that extraordinarily diverse taxon, as already performed for some other *Campanula* taxa and groups in Europe (Carlström, 1986; Kovanda & Anchev, 1989; Eddie & Ingrouille, 1999; Oganesian, 2001; Saez & Aldasoro, 2003; Lakušić & Conti, 2004).

Our interest was focused on several indigenous *Campanula* lineages, whose members are restricted to the mountains around the Adriatic basin, and hardly ever spread further to the south or deeper into the continent. Amongst these are the rather well defined series of the Isophylla group (Damboldt, 1965a) with its possible relatives, but also the extremely polymorphous members of the Rotundifolia complex (Kovanda, 1970) of the large subsection Heterophylla (Wit.) Fedorov. In spite of the fact that many species of these groups are well known in horticulture worldwide (Lewis & Lynch, 1998), their mutual relationships and evolutionary patterns in the wild are poorly known. Moreover, their systematic relations in the local floras are still based on premises deriving from old investigations and conclusions, mainly preformed outside the Region (for data and literature see Kovačić, 2004, 2006).

In the classic research by Damboldt (1965a), the Isophylla group was morphologically well-portrayed. That lineage was considered, until today, to be a natural group of the relic, Tertiary schizo-endemics (true vicariants), an assemblage of about 12 disjunct taxa, mainly distributed in the sub-Mediterranean area of the Adriatic coastal mountains (this, however, was never critically tested after 1965). Damboldt (1965a) excluded from the Isophylla group a number of small lineages or single species, primarily because

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of their morphological differences. For these expelled (and not further investigated) *Campanula* taxa Eddie et al. (2003) and Eddie & Kovačić (2004; personal communication) used the temporary term 'Isophylloids'. This taxonomically and evolutionally inconsistent, provisional assembly of taxa is apparently not closely related to any of the many recognized and accepted series, groups, aggregates, or complexes within the large *Campanula* genus. Nevertheless, the Isophylloids possess some of the features of the 'true' Isophylla group *sensu* Damboldt (1965a) (i.e. apparent isophylly, similar distribution, ecology and phenology, chromosome number $2n = 34$). For the time being, until further research is carried out, we assembled under the name of Isophylloids some rather isolated aggregates and species of the amphi-Adriatic region: first of all, the members of the aggregates Waldsteiniana and Pyramidalis (*sensu* Geslot, 1984), then the Italian Dolomite endemic *C. morettiana* Reichenb., and *C. sartorii* Boiss. & Heldr. from the Greek island of Andros. As illustrated by the recent molecular data of Eddie et al. (2003), the classical dichotomy between the sections *Campanula sensu stricto* (or *Medium* (A.DC.) Boiss) and *Rapunculus* (Fedorov & Kovanda, 1976) is arbitrary. Thus, the classic rapunculoids, such as *C. raineri* Perpenti (E Insubria), *C. arvatica* Lag. *sensu lato* (incl. *C. adsurgens* Leresche & Levier, NW Spain), and even the paleoendemic *C. carpatica* Jacq. from the Carpathians and *C. zoysii* Wulf. from the eastern Alps, are to be considered isophylloid as well. All these species have been, at one time or another, classified as isophyllous taxa (Wohlfahrt & Koch, 1902; Fiori & Paoletti, 1903; Fiori, 1927; Hayek, 1931; Crook, 1951), and most of them were excluded from the Isophylla group only by Damboldt (1965a), without further explanations.

According to Damboldt (1965a), his 'Isophylla *s.s.* group' was parallel to the much larger and far more widespread subsect. Heterophylla. Within this subsection, based on previous authors, Kovanda (1970) recognized five heterophyllous groups (series) gathered around the extremely polymorphous *C. rotundifolia* L. (as *Rotundifolia* complex). However, Kovanda suggested that, in the mountains of central Europe, western Balkans and in the amphi-Adriatic region, a number of neoendemic taxa of the series Saxicolae, Lanceolatae, Alpicolae and Scheuchzerianae are developing, replacing a typical, northern *C. rotundifolia* (series Vulgares). Many members of the Rotundifolia group are so difficult to recognize that they are disregarded or included in the better differentiated 'collective species', i.e. *C. velebitica* Borbás (cp. Kovačić, 2004).

As two major lineages, Isophylla and Heterophylla, are separated fundamentally on the basis of their leaf-shapes, i.e. the non/existing heterophylly, their overall floral structure is essentially identical. The Campanulaceae share a basically similar floral structure (clearly specialized pollination mechanism via modifications of the style), although size and shape are considerably different. For example, the isophylloid Waldsteiniana aggregate consists of only two species separated exclusively by their corolla shapes. The stenoendemic *C. waldsteiniana* Schultes is centred in the Croatian Adriatic Dinarides (Mts Velebit, Plješivica, Velika Kapela), while the subendemic *C. tommasiniana* C. Koch inhabits only the slopes of the Croatian Mt Učka (Istria peninsula). The relationships between agg. Waldsteiniana and all other campanulas remain rather controversial (Fiori, 1927; Hayek, 1931; Gadella, 1964); it was excluded from the Isophylla *s.s.* lineage by Damboldt (1965a, b), while sharing some morphological characteristics with the heterophyllous campanulas (week heterophylly, similar seed coat structure). However, *C. waldsteiniana* and *C. tommasiniana* are well distinguished, and do not hybridize with any other taxa in the Region. Damboldt (1965b) came to the conclusion that flower traits in general, and corolla shape in particular, should be given much more taxonomic importance in the *Campanula* genus. Based on this observation, we examined in detail the mutual morphological floral relations of the *Campanula* lineages having a similar amphi-Adriatic distribution. We also included an Illyrian-Adriatic/Balkan endemic *C. pyramidalis* L., a typical member of the Pyramidalis aggregate, the only one of three (according to Geslot, 1984) distributed in the Adriatic Dinarids.

Together with its closest relatives, the southern Balkan/southern Italian *C. versicolor* Andrews and a Serbian subendemic *C. secundiflora* Vis. & Pančić, *C. pyramidalis* forms a unique group of campanulas most likely indigenous to the Balkans, and also members of the provisional, isophylloid assembly.

As our preliminary comparative results indicated a high degree of both phenotypic plasticity and polymorphism among the western Balkan campanulas (cp. Kovačić & Nikolić, 2004a, b), leading to serious difficulties in identifying taxa, a phenetic approach, using multivariate analysis, was adopted in an attempt at understanding the biometrical floral relations of the several, similarly distributed, *Campanula* lineages.

Materials and methods

A total of 409 *Campanula* flowers were gathered in 29 localities (Figure 1), representing 208 specimens belonging to 31 populations (Table I); they were mostly perennials, sharing trilocular fruits, similar shape of seeds, porrate pollen grains, and a basic

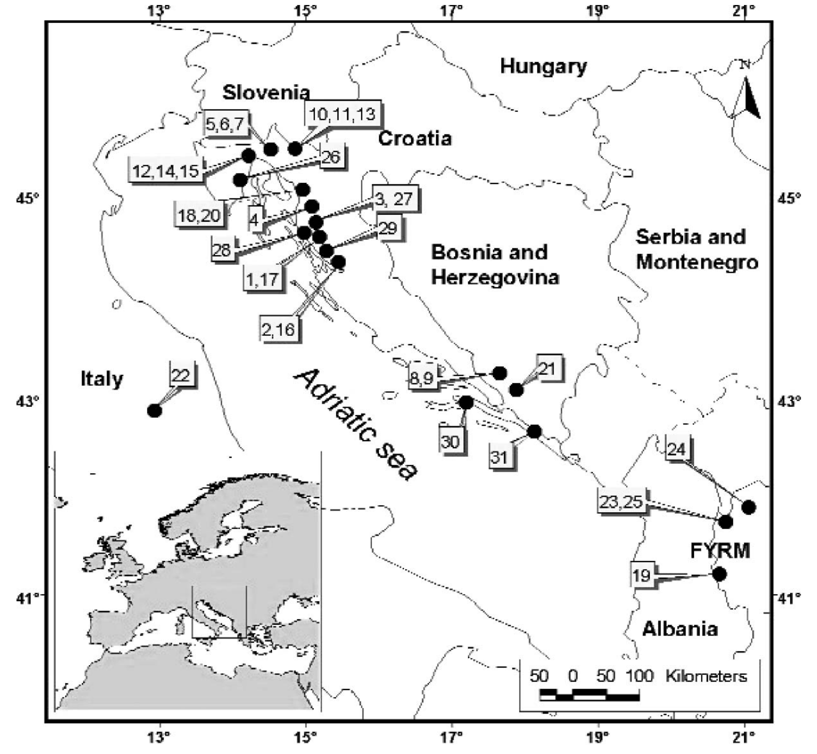

Figure 1. Investigated area with sampling localities.

chromosome number n = 17. Of the isophyllous lineage we included two taxa, which were poorly known or entirely unknown to Damboldt, as the representatives of two highly related lineages of the Garganicae Series (s. Trinajstić, in Lovašen-Eberhardt & Trinajstić, 1978): *C. fenestrellata* Feer and *C. garganica* Ten.. *Campanula fenestrellata* Feer subsp. *debarensis* (Rech. f.) Damboldt (in this research represented by one population) is biogeographically sharply isolated from its main Garganicae core, while *C. reatina* Lucchese (one population) is the latest recognized member of the Isophylla group (Lucchese, 1993). Of the isophylloid lineages, we included *C. waldsteiniana* (three populations), *C. tommasiniana* (two populations), and *C. pyramidalis* (six populations). Of the heterophyllous lineage, with a similar western-Balkan distribution as the isophyllous/isophylloid group, we included nine "rotundifolius" taxa *a priori* classified as *C. albanica* Wit. (one population), *C. cespitosa* Scop. (one population), *C. hercegovina* Deg. & Fiala (two populations), *C. jordanovii* Anchev & Kov. (one population), *C. justiniana* Wit. (four populations), *C. marchesettii* Wit. (one population), *C. rotundifolia s.l.* (two populations), *C. scheuchzeri* Vill. (one population), and *C. velebitica s.l.* (seven populations). The total sample per taxon was highly unbalanced (Table I), while it was sometimes impossible to find more than several individuals per population. The majority of the sampling localities are situated in the coastal part of the western Balkan Dinarid Alps, the so-called Adriatic or Coastal Dinarides, along the same NW/SE direction as the Adriatic Sea. Only the central Italian subendemic *C. reatina* originates from its *locus classicus* in the Apennines (Figure 1).

Most of the sampled flowers, collected at full anthesis during 2001, 2002 and 2003 at the native localities, were fixed in formal-acetic alcohol (FAA). Flower samples of five populations (the Italian *C. reatina*, and the Macedonian *C. fenestrellata* subsp. *debarensis*, *C. albanica*, *C. jordanovii* and *C. velebitica s.l.*) were first pressed. In the laboratory, dry flowers were briefly boiled, and wet flowers briefly soaked in distilled water, cut between the corolla limbs, and immersed in glycerine between two glass slides. Dissected flowers were then scanned, at high resolution, using standard PC tools, and the transparency scale adjusted. For each scanned flower, 11 biometrical variables (morphological traits) were measured using the CARNOY 2.0 image analyser (Laboratory of Plant Systematics, K.U. Leuven, Belgium; available from: http://www.kuleuven.ac.be/bio/sys/carnoy/) (Table II). Five ratios were calculated on the basis of eight simple variables, and tested for their significance in separating taxa compared to the original (simple) measurements (Figure 2). The ratios were further combined with the total length of style (STYL) and stamens (ANTL, FILL), as well as the position of the calyx teeth (SEPO). The calyx teeth position (SEPO) was defined, before dissection (Figure 2), as: 1 = firmly adherent, 2 = appressed, 3 = patent/reflexed, and 4 = reversed/deflexed. The

Table I. Investigated *Campanula* species with their taxonomic affiliations, origin of sampled populations, and sampling details.

Number	*Campanula* lineage	*Campanula* species	Population sampling locality	No of sampled individuals	No of sampled flowers
1	Rotundifolia	*velebitica*	Mali Alan (CRO)	12	22
2	Rotundifolia	*velebitica*	Buljma Mt (CRO)	4	8
3	Rotundifolia	*velebitica*	Visibaba Mt (CRO)	11	22
4	Rotundifolia	*velebitica*	Šugarska duliba (CRO)	2	4
5	Rotundifolia	*rotundifolia*	Kuk-Platak (CRO)	2	4
6	Rotundifolia	*marchesettii*	Kuk-Platak (CRO)	4	8
7	Rotundifolia	*scheuchzeri*	Kuk-Platak (CRO)	3	5
8	Rotundifolia	*rotundifolia*	Čvrsnica Mt (B&H)	3	6
9	Rotundifolia	*hercegovina*	Čvrsnica Mt (B&H)	7	14
10	Rotundifolia	*justiniana*	Čabranka River (CRO)	6	11
11	Rotundifolia	*justiniana*	Snježnik Mt (CRO)	15	31
12	Rotundifolia	*justiniana*	Učka Mt (CRO)	6	12
13	Rotundifolia	*cespitosa*	Gerovo (CRO)	6	12
14	Waldsteiniana	*tommasiniana*	Učka Mt-rocks (CRO)	12	23
15	Waldsteiniana	*tommasiniana*	Učka Mt-forest (CRO)	11	21
16	Waldsteiniana	*waldsteiniana*	Buljma Mt (CRO)	11	22
17	Waldsteiniana	*waldsteiniana*	Alaginac Pass (CRO)	6	12
18	Waldsteiniana	*waldsteiniana*	Zavižan Mt (CRO)	12	24
19	Isophylla	*fenestrellata* ssp *debarensis**	Debar Gorge (MAK)	9	18
20	Rotundifolia	*velebitica*	Zavižan Mt (CRO)	2	4
21	Rotundifolia	*hercegovina**	Drežnica River (B&H)	8	15
22	Isophylla	*reatina*	Turano Valley (ITA)	3	5
23	Rotundifolia	*jordanovii**	Jakupica Mt (MAK)	3	6
24	Rotundifolia	*albanica**	Ljuboten Mt (MAK)	7	13
25	Rotundifolia	*velebitica**	Jakupica Mt (MAK)	10	22
26	Pyramidalis	*pyramidalis*	Brseč (CRO)	4	8
27	Pyramidalis	*pyramidalis*	Ravni dabar (CRO)	2	4
28	Pyramidalis	*pyramidalis*	Karlobag (CRO)	5	9
29	Pyramidalis	*pyramidalis*	Tribanj (CRO)	6	12
30	Pyramidalis	*pyramidalis*	Pelješac (CRO)	12	24
31	Pyramidalis	*pyramidalis*	Dubrovnik (CRO)	4	8
	Total			208	409

Herbaria samples are marked with an asterisk (*). Numbers indicate localities on Figure 1. Abbreviations: CRO = Croatia, B&H = Bosnia & Herzegovina, MAK = Former Yugoslav Republic of Macedonia, ITA = Italy.

variables were chosen, and adjusted, according to some of the most recent morphological investigations regarding the campanuloids (Eddie & Ingrouille, 1999; Saez & Aldasoro, 2003; Lakušić & Conti, 2004). After measurements, all flowers (dry and wet) were stored in FAA. The flower samples and vouchers of the populations are kept in the Botanical Garden of the Faculty of Science, University of Zagreb (Croatia) and Herbarium Croaticum (ZA), respectively.

Statistical methods

A forward stepwise discriminate function analysis was performed on the five floral ratios (PETLl/PETW, PETLc/PETLl, SEPLl/SEPW, SEPLc/SEPLl and PETL/SEPL; Figure 2). The aim was to establish which variables are best suited for discriminating the 'natural' *Campanula* groups (dependent or grouping variable), prearranged from the specimens classified *a priori* as members of the same taxa, using standard floristic keys. In this manner, the input data-matrix did not contain the missing values. Wilks' lambda coefficient, with its values ranging from 1.0 (no discriminatory power) to 0.0 (perfect discriminatory power), was used as a standard statistical tool to denote the statistical significance of discriminatory power on the current model. Partial Wilks' lambda coefficient was used to measure the exclusive contribution of each respective variable to discrimination of the groups. Wilks' lambda values were further converted to standard F-values, and corresponding p-levels calculated for each F. The tolerance value, which gives an indication of the redundancy of the respective variable, was defined as $1-R^2$ of the respective variable to all other variables in the model.

For additional insight into the relations among the floral ratios, the Canonical correlation analysis was performed, and the significances of all canonical roots and variables tested by the Chi-square test. Coefficients of the canonical roots and variables were calculated, and the matrix standardized in order to compare the scales of different floral ratios with the

Table II. Simple floral variables and their ratios used in this study.

Number		Abbreviation
	Simple variables	
1	Free calyx lobe (tooth) width (mm)	SEPW
2	Free calyx lobe (tooth) length (mm)	SEPLl
3	Synsepal calyx length (mm)	SEPLc
4	Total calyx length (2 + 3) (mm)	SEPL
5	Free corolla limb width (mm)	PETW
6	Free corolla limb length (mm)	PETLl
7	Synpetal corolla length (mm)	PETLc
8	Total corolla length (5 + 6) (mm)	PETL
9	Style length (mm)	STYL
10	Anther length (mm)	ANTL
11	Filament length (mm)	FILL
12	Sepal position (state 1 – 4, Figure 2)	SEPO
	Ratio variables	
13	Calyx lobe length/width (mm)	SEPLl/SEPW
14	Synsepal calyx length/calyx lobe length (mm)	SEPLc/SEPLl
15	Corolla limb length/width (mm)	PETLl/PETW
16	Synpetal corolla length/corolla limb length (mm)	PETLc/PETLl
17	Total corolla length/total calyx length (mm)	PETL/SEPL

Abbreviations: PETLl = free corolla limb length, PETLc = synpetal corolla length, PETL = total corolla length, SEPW = free calyx lobe width, SEPLl = free calyx lobe length, SEPLc = synsepal calyx length, SEPL = total calyx length, STYL = style length, ANTL = anther length, FILL = filament length; SEPO = sepal position.

eigen-values and cumulative properties in total variability of each canonical variable (root). Standardized coefficients for the canonical variables, and means of the canonical variables were calculated, and the results presented, in plains of the most informative roots, as 2D-scatter-plots.

Discriminate analysis was further applied as a tool of the predictive classification of the *Campanula* specimens. Once a model has been finalized and the discriminate functions derived, one can predict which group a particular specimen affiliates to, and how accurate the predeterminations were. For each group in our sample, we determined the position of the point that represents the means of all variables in the multivariate space, defined by the variables in the model (centroids). To establish the fitting of a sample in a given predetermined taxon, squared Mahalanobis distances between the group centroids were calculated, where the groups represent the taxa recognized *a priori* using standard keys. The Mahalanobis distance is similar to the standard measure of the Euclidean distance, except that it takes into account the correlations between the variables. The larger the differences in this spreadsheet, the farther apart the groups are from one other; the current model gains more discriminatory power in separating two groups. For each specimen, a Mahalanobis distance from each of the group centroids was calculated. The specimen was classified as belonging to a particular taxon to which it was closest, that is, for which the Mahalanobis' distance was the smallest. The final classification matrix portrayed a number of specimens that were correctly classified, and a number of those that were misclassified.

The analyses described above included predefined 'natural' groups (or taxa) of specimens. However, it also allowed a search for natural groups without any previous information on their relationships, and the possibility to decide about a group's characteristics and composition *a posteriori*, i.e. after analysing the results. When it is so complicated to correctly classify samples, it is acceptable to define a group of individuals regardless of a predeterminated taxon, by assembling a natural group of samples that can be recognized on the basis of their similar floral traits. An assembly of this kind could potentially not only point towards phenotypic similarities based on flower structures, but also have some phylogenetic significance. For this purpose, a Principal Component Analysis (PCA) was performed, as a linear dimensionality reduction technique that identifies orthogonal directions of maximum variance among the original data, by projecting the records into a lower dimensionality space formed by a subset of the highest-variance components. The relations between groups originated by PCA are depicted in the 2D-scatter-plots, where the specimens in the plains of the most informative principal axes are presented, and spatial distributions analysed.

Some of the analyses were reiterated with different sets of variables included or excluded (i.e. STYL, ANTL, FILL and SEPO) to test the impact of these variables on the group structure based on the ratios. When necessary data were standardized by the *Z*-score method.

All calculations were made using Statistica 6.0 and NT-SYS 2.10s packages (Rohlf, 2000). The statistical background and computational approaches for the applied methods are described in Legendre & Legendre (1998) and Quinn & Keough (2004).

Results

The multivariate methods (discriminate, canonical and principal components) were used to establish a variability pattern of the mutual relations on an individual and population level of 14 *Campanula* taxa, based on simple measured floral variables and their ratios.

In the forward stepwise discriminate procedure, the calculated Wilks' lambda coefficients are between 0.063 and 0.153, indicating that all ratios have a certain discriminating potential. The maximum *F*-value was calculated for the ratio between the lengths of synpetal and free corolla lobes (PETLc/PETLl). Thus, the Forward stepwise analysis started with that value. Table III gives the summary of the analysis of five calculated steps performed on the whole sample. The ratio between the synpetal and free corolla parts (PETLc/PETLl) is, with $F = 57.7$, by far the most discriminating of all calculated ratios, followed by the ratio between total corolla length and calyx length (PETL/SEPL, $F = 21.6$), and corolla limb length and width (PETLl/PETW, F = 15.5).

During Canonical correlation analysis, five independent orthogonal functions (roots) were calculated, all significant in separating samples (Table IV). The First canonical variable (Root 1), assigned mostly with the positive values of PETLc/PETLl and PETL/SEPL, contributes to the total variability with as much as 72.4%. The Second canonical variable (Root 2) is again influenced by PETLc/PETLl (this time negative) and by PETLl/PETW, while the Third (Root 3) relies mostly on the highly positive SEPL/SEPW ratio. Consequently, the first three canonical variables contain almost 94% of the total variability, while the Fourth and the Fifth are much less significant. After establishing the most significant variables in discriminating taxa along the

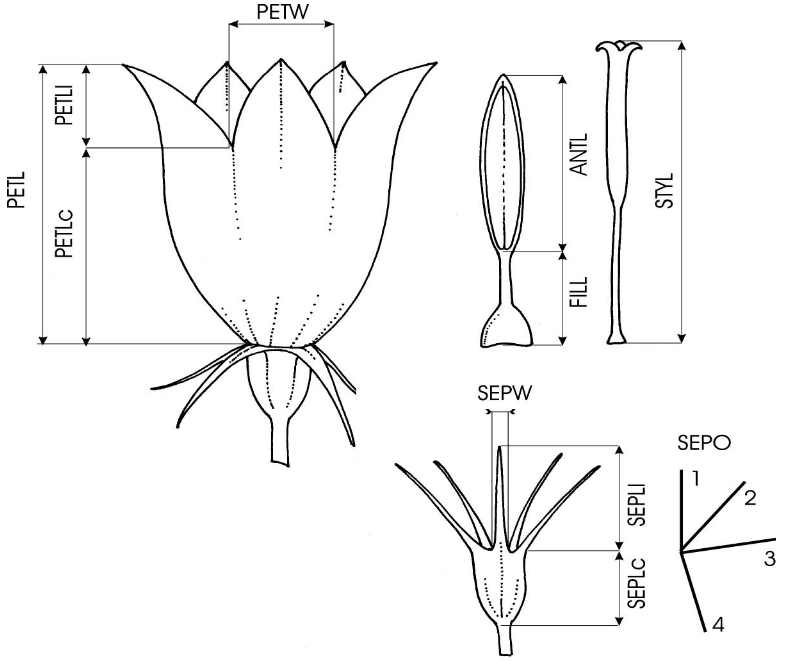

Figure 2. Schematic representation of the measured flower characteristics: PETW = free corolla limb width, PETLl = free corolla limb length, PETLc = synpetal corolla length, PETL = total corolla length, SEPW = free calyx lobe width, SEPLl = free calyx lobe length, SEPLc = synsepal calyx length, SEPL = total calyx length, STYL = style length, ANTL = anther length, FILL = filament length; SEPO = sepal position.

Table III. Discriminant function analysis summary for total sample based on the ratios between variables. Five variables in model, 14 groups (taxa); Wilks' lambda = 0.05251; approximate F (65.1851) = 24.658; $p < 0.01$.

Ratio	Wilks' lambda	Partial Wilks' lambda	*F*-value	Tolerance	1 − Tolerance
PETLc/PETLl	0.15325	0.34261	57.71107	0.87339	0.12661
PETL/SEPL	0.09019	0.58215	21.58788	0.96846	0.03154
PETLl/PETW	0.07955	0.65999	15.49458	0.96093	0.03906
SEPLl/SEPW	0.06633	0.79159	7.91840	0.86719	0.13281
SEPLc/SEPLl	0.06281	0.83589	5.90514	0.93152	0.06848

Abbreviations as in Table II.

calculated canonical roots, the nature of this discrimination was established for every root separately, by calculating the canonical means for each *a priori* classified taxon (Table V).

As shown in the 2D-scatter-plot (Figure 3), Root 1 clearly discriminates the samples preclassified as the isophyllous/isophylloid taxa (*C. pyramidalis*, *C. fenestrellata* subsp. *debarensis*, *C. reatina* and *C. waldsteiniana*, Figure 3/group A), as well as the relict heterophyllous species *C. cespitosa*, and the samples preclassified as *C. rotundifolia s.l.* (Figure 3/group B). The samples of *C. tommasiniana* are further separated along Root 2 by their highly negative coefficient for PETLl/PETW (Figure 3/group C), though somewhat dispersed and overlapping with *C. cespitosa* samples. According to the eigen-values and cumulative properties, the discriminating power of Roots 3, 4 and 5 is much smaller than that of the first two canonical variables ($<13\%$).

The analyses were, at first, correlated to the *a priori* determined taxa, where some serious difficulties in separating closely related ones, especially among the heterophyllous series, were encountered. The lack of recognizable heterophyllous taxa inside the B-group cloud, and a partial overlapping of groups B and C (Figure 3), could result from incorrect predeterminations. The squared Mahalanobis distances between the group centroids show that the isophyllous/isophylloid taxa are almost completely correctly classified, i.e., the Mahalanobis distance between a particular specimen and the *a priori* determined species' centroid is the shortest. The percentage of correct predeterminations for *C. tommasiniana*, *C. fenestrellata* subsp. *debarensis* and *C. pyramidalis* samples are shown to be up to 80%. On the other hand, the *a priori* classifications of the heterophyllous taxa are most often found to be incorrect, i.e., the percentage of correct predeterminations for *C. albanica*, *C. marchesettii, C. rotundifolia s.l.* and *C. hercegovina* samples are less than 25%.

In order to establish whether there are some 'natural' groups within our overall sample, and therefore classify it, PCA was applied to the whole data set. In accordance with five calculated ratios of the measured flower traits, five PC-axes with their eigen-values were designed, representing the proportion of variance of the main components. Cumulative variability contained in the first three PC-axes was high (79.3%), with the standard largest contribution coming from the First axis (46.3%, Table VI).

Table IV. Standardized coefficients for canonical variables of five studied ratios.

Ratio	Root 1	Root 2	Root 3	Root 4	Root 5
PETLc/PETLl	0.8040	−0.6821	−0.0924	0.0331	0.1485
PETL/SEPL	0.5102	0.4234	−0.3752	0.6585	−0.1366
PETLl/PETW	−0.2640	−0.5708	0.2064	0.7546	0.1805
SEPLc/SEPLl	−0.2011	−0.3568	−0.2567	−0.1092	−0.9099
SEPL/SEPW	0.0569	0.2901	0.8022	0.0408	−0.6485
Eigen-values	4.2895	0.9211	0.3450	0.2878	0.0820
Cumulative properties	0.7239	0.8793	0.9376	0.9861	1.0000

Abbreviations as in Table II.

Table V. Means of canonical variables of all *a priori* classified taxa.

Campanula	Root 1	Root 2	Root 3	Root 4	Root 5
debarensis	−2.86665	−1.12552	1.61697	1.46530	0.17071
rotundifolia	2.86102	−0.17704	0.46744	0.45628	−0.09426
reatina	−2.73529	−0.91163	−0.67738	−0.10489	−0.83453
waldsteiniana	−2.57293	0.13188	−0.22201	−0.28308	−0.34525
pyramidalis	−2.57089	−0.09002	−0.37766	0.05312	0.22311
tommasiniana	2.16920	−2.12842	−0.09434	−0.14447	−0.14882
cespitosa	2.21928	−1.16346	−0.18641	−0.69228	0.10832
velebitica	1.80662	0.73377	−0.52131	0.58483	0.02294
marchesettii	1.77442	0.56808	0.76734	0.43167	−0.17042
justiniana	0.93508	1.02180	0.88464	−0.48087	−0.17657
hercegovina	0.47953	0.31806	−0.04357	−0.38418	0.13676
jordanovii	0.22125	0.34222	0.53705	−1.04119	0.86887
albanica	0.16500	−0.45078	0.14431	−0.81213	0.85778
scheuchzeri	0.14315	1.41895	−0.31546	−0.05610	0.29987

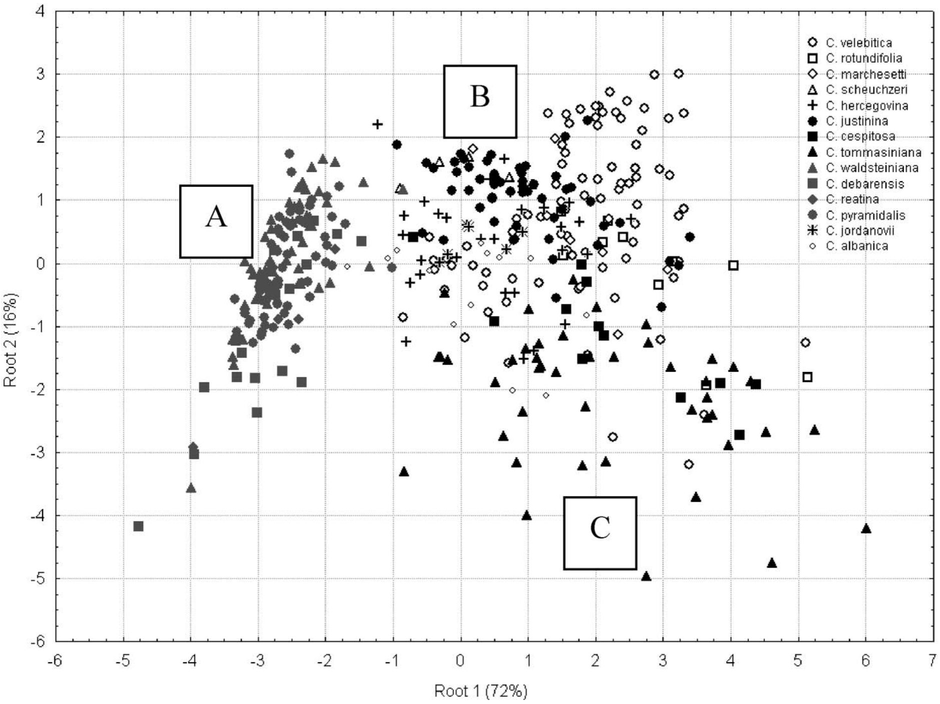

Figure 3. 2D scatter-plot of non-standardized canonical scores in the plain of the first two canonical variables (Root 1, Root 2).

Table VI. Eigen-values of the correlation matrix, and related statistics for the total sample based on five ratios.

PC axis	Eigen-value	% Total	Cumulative	Cumulative %
1	2.3159	46.3176	2.3159	46.3176
2	0.9300	18.5993	3.2458	64.9170
3	0.7177	14.3537	3.9635	79.2707
4	0.6464	12.9286	4.6100	92.1993
5	0.3900	7.8007	5.0000	100.0000

The Second, Third and Fourth PC-axes contributed to the total variability much less (with 18.6%, 14.3% and 12.9%, respectively). The Fifth axis carries only 7.8% of the total variability. The contribution of each ratio to every calculated PC-axis is shown in Table VII.

The distribution of the specimens along the First PC-axis mostly depends upon PETLc/PETLl and SEPL/SEPW, while along the Second PC-axis upon PETL/SEPL and SEPLc/SEPLl. The Third PC-axis is again assigned by PETL/SEPL and PETLc/PETLl, while the last two axes have a low influence on the object distributions. As an example, a 2D-scatter-plot depicting the dispersion of objects in the plains of the First and Third axes is shown in Figure 4.

Along the First axis, which primarily relies on PETLc/PETLl and SEPL/SEPW, most of the isophyllous/isophylloid samples are clearly separated from the heterophyllous samples. Along the Factor 3 axis, which relies mostly on PETL/SEPL and PETLc/PETLl, the inner structure of the heterophyllous cloud is better defined. Group C, obtained by the Canonical analysis (Figure 3, group C), and consisting mainly of the samples preclassified as *C. tommasiniana* and *C. cespitosa*, is just weakly recognizable, and overlaps with other heterophyllous samples, as well as a central group of *C. justiniana* samples. Interestingly, the samples predetermined as *C. velebitica* are widely dispersed along the Third axis, but mainly gathered in the fully negative quadrant of this plain.

As the heterophyllous samples are not easy to assign to the preclassified taxa, we took the remaining four simple variables (STYL, ANTL, FILL and SEPO), and tested their effect on group separation. Cumulative variability contained in the first four PC-axes was 78%, and thus very similar to the calculations made without the simple variables (Table VIII). The First and Second axes' contribution to the total variability was almost equally high: 26.7% and 24%, while the Third and Fourth axes contributed with 14.8% and 12.5%, respectively. The rest of the axes (5–9) have a low influence on object distribution, in total only 22%. The contribution of each ratio to every calculated PC-axis is shown in Table IX. The distribution of the objects along the First PC-axis mostly depended on SEPLc/SEPLl, PETLc/PETLl and the other three ratios in descending order. The Second PC-axis relied on ANTL and STYL, the Third axis mostly on SEPO, and the Fourth on FILL. The rest of the axes had little influence on object distribution.

Table VII. Factor coordinates of five ratios based on correlations for the whole sample.

Ratio-variable	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
PETLc/PETLl	−0.7679	−0.0615	0.4880	−0.1481	−0.3828
SEPL/SEPW	−0.7211	0.4518	0.0821	−0.3756	0.3578
PETLl/PETW	0.6983	0.3886	−0.1793	−0.5075	−0.2678
SEPLc/SEPLl	0.6301	−0.4991	0.4399	−0.3436	0.2057
PETL/SEPL	−0.5671	−0.5674	−0.4972	−0.3284	−0.0380

Abbreviations as in Table II.

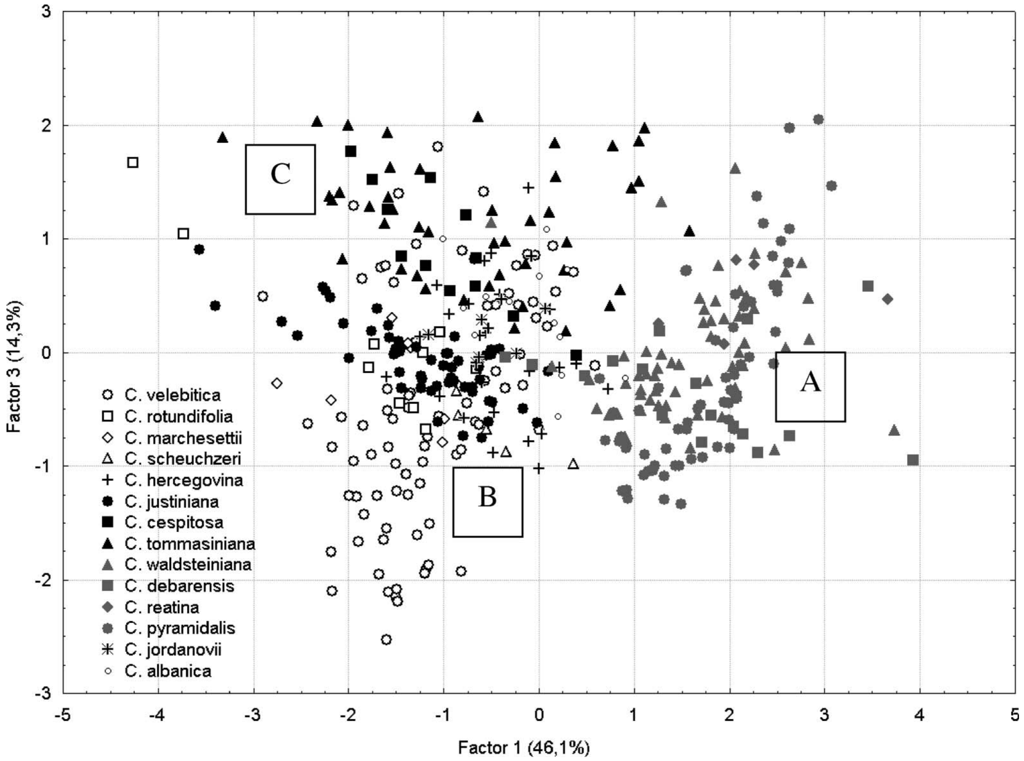

Figure 4. 2D-scatter-plot for the total sample based on five ratios in the plain of the First and Third PC axes.

Table VIII. Eigen-values of the correlation matrix, and related statistics for the whole sample based on five ratios with STYL, ANTL, FILL and SEPO.

Number	Eigen-value	% Total	Cumulative	Cumulative %
1	2.4033	26.7033	2.4033	26.7033
2	2.1606	24.0072	4.5639	50.7105
3	1.3286	14.7626	5.8926	65.4731
4	1.1241	12.4897	7.0167	77.9629
5	0.6446	7.1626	7.6613	85.1254
6	0.5412	6.0129	8.2024	91.1383
7	0.3694	4.1041	8.5718	95.2425
8	0.2698	2.9982	8.84166	98.2406
9	0.1583	1.7594	9.0000	100.0000

Table IX. First five factor coordinates of five ratios with STYL, ANTL, FILL and SEPO, based on correlations for the whole sample.

	PC axis				
Variable	1	2	3	4	5
SEPLc/SEPLl	−0.731	0.122	0.238	0.253	0.358
PETLc/PETLl	0.701	−0.084	−0.080	0.578	0.209
PETLl/PETW	−0.688	−0.163	−0.243	−0.238	0.363
SEPL/SEPW	0.676	−0.224	−0.500	−0.070	0.208
PETL/SEPL	0.586	0.297	0.417	−0.313	0.491
FILL	−0.241	0.542	−0.328	0.663	0.076
SEPO	0.152	0.428	0.731	0.175	−0.120
STYL	0.138	0.836	−0.310	−0.209	−0.169
ANTL	−0.040	0.893	−0.211	−0.228	0.096

Abbreviations as in Table II.

If we add the four simple variables (STYL, ANTL, FILL and SEPO) to the five ratios and then perform the PCA, groups of preclassified samples look somewhat different (Table IX). In the 2D-scatter-plot, showing the dispersion in the plain of the first two axes (Figure 5), the isophyllous/isophylloid group is once again separated from the heterophyllous group along the First axis (relying on the ratios,

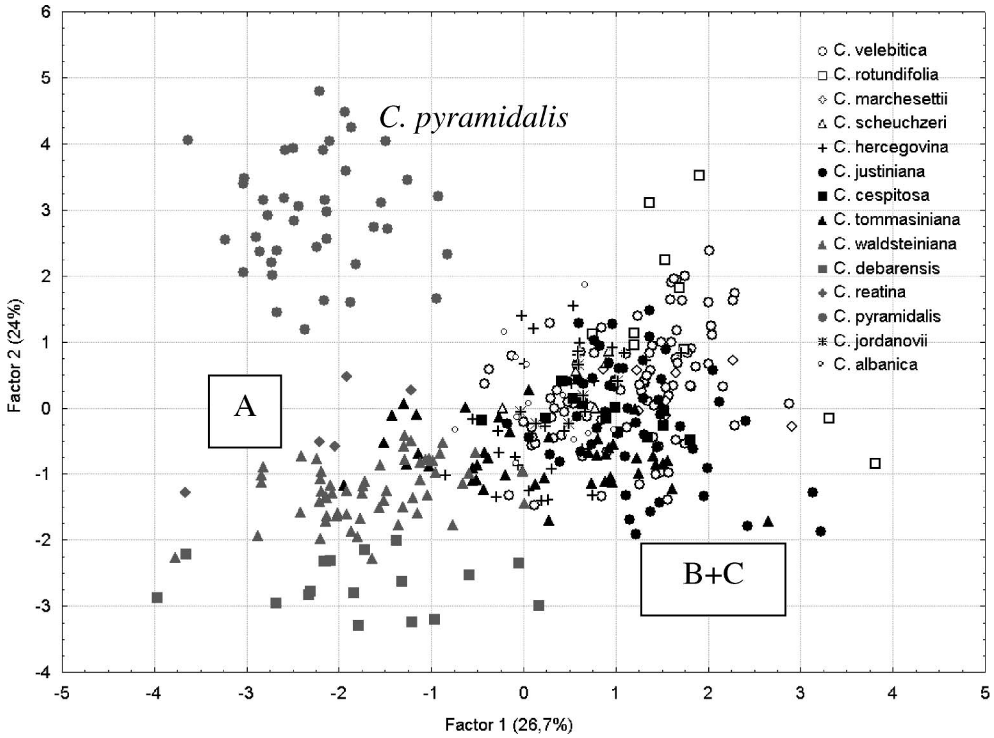

Figure 5. 2D-scatter-plot for the total sample based on five ratios with STYL, ANTL, FILL and SEPO in the plain of the First and Second PC axes.

mainly SEPLc/SEPLl and PETLc/PETLl). The heterophyllous samples are so densely assembled that this compact cloud reveals very little of its inner structure. On the contrary, the inner structure of the isophyllous/isophylloid group is clear. The samples preclassified as belonging to *C. pyramidalis* are fully separated along the Second axis (relying on the simple variables, mainly ANTL and STYL), while the three remaining isophyllous/isophylloid taxa are also clearly identifiable, though somewhat overlapping with *C. tommasiniana* samples.

Discussion

Several "natural" *Campanula* groups, significantly different in their flower traits, are more or less clearly detected in this research. The ratios between variables divided the samples preclassified as isophyllous/isophylloid (*Campanula fenestrellata* subsp. *debarensis*, *C. reatina*, *C. pyramidalis* and *C. waldsteiniana*) from the heterophyllous ones, to which adjoins the samples of isophylloid *C. tommasiniana*. The discriminate procedure specified that all of the chosen floral ratios have a certain discriminating potential, with the ratio between the synpetal and free corolla parts (PETLc/PETLl) being the most discriminating trait of all, followed by the ratio between total corolla and calyx lengths (PETL/SEPL) and corolla limb length and width (PETLl/PETW). The results of the canonical correlation analysis again indicate that the canonical variables rely almost exclusively on the ratios based on the corolla variables (PETLc/PETLl, PETL/SEPL and PETLl/PETW), while the ratios between the calyx parts (SEPLl/SEPW, SEPLc/SEPLl) are comparatively weaker in discriminating the studied taxa.

It is not surprising that the samples of an alleged isophylloid *C. tommasiniana* are (weakly) recognized as a separate cloud, often close or overlapping with the heterophyllous samples, especially the relict *C. cespitosa*, *C. justiniana* and *C. jordanovii*. The squared Mahalanobis' distances between the group centroids also confirmed the isophyllous and isophylloid taxa as being almost completely correctly classified, while the *a priori* classifications of the heterophyllous taxa are most often found to be incorrect. PCA on the basis of the ratios established the existence of 'natural' groups within the total sample, revealing that the object distributions along the PC-axes mostly depended upon PETLc/PETLl and PETL/SEPL, but also SEPL/SEPW and SEPLc/SEPLl. Again, most of the isophyllous taxa are clearly separated from the heterophyllous samples. The samples preclassified as *C. tommasiniana* (often again with heterophyllous *C. cespitosa* or *C. justiniana*), though not clearly separated and overlapping with the other samples, are evident in a weakly coherent cloud. Every isophyllous/isophylloid taxon could be more or less clearly recognized in the collective cloud, sometimes even completely separated from the others. On the contrary, although it is quite easy to recognize the main core of the heterophyllous samples, there are serious difficulties in detecting some particular taxa; while there are large discrepancies in the number of samples per *a priori* classified taxon, it seems that the Rotundifolias share an almost identical floral pattern. Most of the heterophyllous samples, assumed to belong to a particular *a priori* identified taxon, are scattered, dispersed and overlapping with the others in the heterophyllous cloud; only a fraction of the preclassified heterophyllous samples (i.e. *C. velebitica*, *C. hercegovina*, *C. rotundifolia* or *C. scheuchzeri*) form a weakly coherent group. The impossibility of recognizing particular taxa inside a common heterophyllous cloud could be, of course, the result of incorrect *a priori* determinations. By adding four simple variables (STYL, ANTL, FILL and SEPO) to the ratios, a clearer picture emerged. The strongest influence came from the ratios, but the simple variables are also highly significant in separating 'natural' groups, and even some preclassified taxa. The isophyllous/isophylloid group is yet again separated from the heterophyllous based on floral ratios, with a perfectly clear inner structure: the samples preclassified as belonging to *C. pyramidalis* are sharply separated on the basis of the lengths of their anthers and styles (ANTL, STYL), but the three remaining taxa are also clearly identifiable, mostly on the basis of the position of their calyx-lobes (SEPO). The majority of the samples preclassified as *C. tommasiniana* are separated on the basis of SEPO, and, to a lesser extent, of SEPL/SEPW, but they overlap with isophyllous/isophylloid, as well as with heterophyllous samples. The heterophyllous samples are, as usual, not very well (or correctly) separated inside their rather compact cloud, but much better than without the simple variables. Even though it should be emphasized that the simple variables, STYL, ANTL, FILL and SEPO, are generally dependent on flower maturity, and, therefore, should be taken with caution, it is clear that the heterophyllous taxa cannot be successfully separated only on the basis of their floral variables, while they are often so polymorphous and variable that they cannot be recognized even on the basis of the entire plant morphology. Unlike the scattered and disjunct Isophyllas, which can be further separated on the basis of their floral – mostly corolla - variables, the Heterophyllas of the Adriatic Dinarides often share wild habitats (e.g. *C. scheuchzeri*, *C. marchesettii*, *C. velebitica s.l.* and *C. witasekiana*), where they probably hybridize (Kovanda, 1999). This could be one of the reasons for their strong morphological overlapping, while the second reason could lie in the fact that many local populations of the large *C. rotundifolia* complex often function as the incipient (small) species. The older members of the Rotundifolia group (*C. cespitosa* and *C. justiniana*), are shown to be somewhat better recognizable, while the *C. velebitica* group (*C. velebitica* and its nearest relatives *C. albanica* and *C. jordanovii*) can barely be separated according to their flower properties. As expected, the two members of the Waldsteiniana agg. are clearly separated by their corolla shapes: according to our results, the star-shaped flowers of *C. waldsteiniana* resemble, to some degree, those of the isophyllous Garganicae members, while the narrow tubular corolla of *C. tommasiniana* somewhat follows the heterophyllous floral pattern of the older Rotundifolia members. We assume that *C. tommasiniana* may have the same ancestors as the rotundifolious taxa (maybe the older members, such as *C. cespitosa* and *C. justiniana*, as some of the results indicate), but this could also be coincidental, while it does not explain the star-shaped corolla of *C. waldsteiniana*. It is not known how, when and why this strange floral discrepancy among two close relatives occurred: at this early stage of our research we can conclude that the agg. Waldsteiniana members are, from a floral point of view, indeed placed between the isophyllous and heterophyllous *Campanula* lineages. There is also a possibility (Damboldt, 1965b) that some isolated, relict species, such as the NE Italian subendemics *C. morettiana* or *C. raineri*, could be related to the Waldsteiniana core, but this hypothesis requires further research.

It is interesting to observe closely the apparently most distinct species among the ones studied here, and another isophylloid, *C. pyramidalis*. Though the samples preclassified as belonging to this taxon are in most of the cases rather dispersed, the clouds consisting of the *C. pyramidalis* objects are compact enough to indicate that this flower pattern is not easily confused with any other, isophyllous or heterophyllous. When not separated, the *C. pyramidalis* samples are gathered together with the isophyllous/isophylloid taxa, indicating that these lineages could be related after all, especially if we take into consideration the crossing experiments made by Musch & Gadella (1972), and some of the recent molecular and isoenzyme data (Liber et al., 2004; Tkalec et al., 2004). Besides the confirmed floral similarities with the isophyllous/isophylloid species, *C. pyramidalis* differs greatly in the seed coat structure, often biennial life cycle, phenology (late anthesis), and overall external morphological features. Yet, one of its closest relatives, the Serbian bellflower (*C. secundiflora*), has somewhat similar growth to the isophyllous species. The relatively successful crossing experiments of *C. pyramidalis* with the typical isophyllous species *C. isophylla* and a paleoendemic rapunculoid *C. carpatica* (Gadella, 1964) indicated that the evolutionary lineage of agg. Pyramidalis may have developed from the same ancient stock. The possible relations of the isophylloid taxa with the ancient Section Rapunculus of the *Campanula* genus were already suggested by Damboldt (1965b), who also obtained several sterile hybrids between the 'campanuloid' *C. tommasiniana* and the 'rapunculoid' *C. morettiana*. As already mentioned, the recent molecular results of Eddie et al. (2003) revealed that the classical dichotomy of the *Campanula* genus in two sections is, to a large extent, unnatural. Perhaps time of flowering was the first (temporal) barrier established between the *C. pyramidalis* ancestors and the future isophyllous lineages. Although a change in developmental timing is one of the major ways by which evolution can proceed, there is no doubt that the driving force of floral evolution has been the selection pressure of pollinators, especially for the *Campanula* species in Europe (Eddie, 1984). This aspect could be important when studying the evolution of the peculiar floral shapes in agg. Waldsteiniana, a study that shall soon be undertaken.

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