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Full Length Research Paper

Phylogeny of *Symphytum* L. (Boraginaceae) with special emphasis on Turkish species

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The genus *Symphytum* L. (Boraginaceae tribe Boraginae) comprises perennial mesophytic species. The distribution area of the genus is mainly Euro-Siberian and Mediterranean regions. The whole genus comprises approximately 40 species, of which Turkey holds the largest number with 18 species. Boissier (1897), Kusnetsov (1910), Bucknall (1913) and Pawlowski (1971) made subgeneric classifications within the genus *Procopiania* Guşul. distributed in the Aegean archipelago, Southern Greece and Western Anatolia with three species. In 1967, Runemark included *Procopiania* into *Symphytum.* However, Pawlowski (1972) did not follow Runemark's combinations but treated the Greek species as *Procopiania*. Also, Wickens (1978) in his treatment of Turkish taxa completely synonymised *Procopiania* with *Symphytum*. Therefore, to reinvestigate the sectional delimitation, a phylogenetic analysis was conducted using ITS total and *trnL-F*. Morphological analysis was performed by using cluster analyses. The results indicate monophyly, a single origin of *Symphytum* including *Procopiania*. The clades within the genus mostly coincided with sections proposed by former researchers. The proposal for the synonym of subspecies of *S. asperum* and *S. sylvaticum* were crosschecked and the species distribution to sections were revised with the help of both phylogenetical and morphological analyses, as well as the experience gathered while collecting live materials.

Key words: *Symphytum*, phylogeny, morphology, ITS total, *trnL-F*.

INTRODUCTION

The genus *Symphytum* is a mesophytic genus with approximately 40 species, with a centre of origin in the Pontic province of the Euro-Siberian region. With 18 species Turkey holds the largest number (Tarıkahya, 2010). The genus belongs to Boraginae tribe of Boraginaceae family that is in Eusterid I (APG II, 2003). Boraginae is one of the biggest tribes in Boraginaceae, with approximately 170 species (Hilger et al., 2004). The phylogenetic relationship of the tribe Boraginae was investigated by Hilger et al. (2004) and in this research *Symphytum* was resolved as monophyletic. The old world genus *Symphytum* has got its valid name since "Species Plantarum". Afterwards, Boissier (1897) and Kusnetsov (1910) divided the genus into two species groups based on corolla properties. Bucknall (1913), however, did not accept this classification but decided to create his own classification. For the time being some researchers either when preparing country floras, revisions or taxonomical researches, made certain rearrangements on the taxonomy of the species belonging to this genus. As summarized by Sandbrink et al. (1990), the genus consists of 9 sections; 7 of these were constructed by Bucknall (1913), one by Pawlowski (1971) and one by Wickens (1969).

Moreover, Wickens in his treatment of Turkish taxa proposed *Procopiania* as a section in *Symphytum*. *Procopiania* Guşul. was described as a separate genus by Guşuleac in 1928 with *P. cretica* (Willd.) Guşul. formerly

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belonging to *Trachystemon* D.Don. Runemark (1967) did not agree with Guşuleac's approach and included *Procopiania* into *Symphytum.* He supported his hypothesis by morphological, palynological and chromosomal investigations. However, Pawlowski (1971) did not accept Runemark's combinations, but treated Greek species as *Procopiana*. Stearn (1986) also followed his approach while revising Greek species of *Symphytum*. Furthermore, in the report of Hilger et al. (2004), the tribe Boragineae was investigated and only two species from *Symphytum* included to this research. In the phylogenetic tree, *S. creticum* Runemark *and S. tuberosum* L. were resolved as sisters.

The objective of this research was to find out the phylogenetic relationships within *Symphytum*. Two gene regions: the internal transcribed spacer (ITS) and the intron and spacer of the chloroplast (*trnL-F*) were used as markers for this purpose. Also, phenetic analyses of morphological data were performed to examine the subgeneric classifications and to make taxonomical rearrangements.

MATERIALS AND METHODS

From 2006 to 2008, approximately 450 specimens belonging to 60 populations were collected from field in Turkey. Beside Turkish *Symphytum* species, phylogenetic analyzed involved 10 *Symphytum* taxa growing outside of Turkey and 25 species from related genera. Synonym taxa of *S. asperum*, *S. sylvaticum* and *S. officinale* were included in the analyses as separate taxa to check their status with molecular markers. European species were mostly gathered from Berlin (B) and Munich (M) herbaria. Sequences of related genera were gathered from Berlin Free University's DNA bank. For the molecular studies, our sampling included 56 taxa (31 *Symphytum*) for ITS and 49 taxa (24 *Symphytum*) for the combined ITS--trn*L-F* marker analysis of Boraginaceae. *Ogastemma pusillum* and *Echiochilon fruticosum* were chosen as outgroups (Appendix 1).

DNA extraction, amplification and sequencing

DNA extraction, PCR, purification and sequencing followed standard protocols as described in detail in Gottschling and Hilger (2001). The *trnL-trnF* spacer was amplified with primers C and F (Taberlet et al., 1991), and for ITS, the primers P5 and P4 (White et al., 1990) were used. The same primers were used for amplification and sequencing. Cycle sequencing was carried out with the BigDye® Terminator v1.1 Cycle Sequencing Kit (Perkin Elmer, Foster City, California, USA) with a total reaction volume of 5 µL. Initial denaturation (1 min at 96°C) was followed by 30 cycles of denaturation (30 s at 96°C), annealing (20 s at 52°C) and elongation (4 min at 60°C). Samples were sequenced on an Applied Biosystems 3130xl Genetic Analyzer.

Editing, alignment and gap coding

The initial sequence data were edited using *ChromasPro* V. 1.33 (Technelysium Pty. Ltd., 2003-2005). ITS and *trnL-trnF* spacer aligning was straightforward and was performed manually in a *Multicolor Sequence Alignment Editor* (Hepperle, 2001). The complete data matrix is available on request in NEXUS format. Gaps were coded as missing data.

Comparative sequence analysis

Phylogenetic reconstruction based on ITS1 and *trnL-trnF* was done by running parsimony analyses as implemented in PAUP4.0beta (Swofford, 2002). The maximum of trees retained was limited to 10,000. For the heuristic search, sequences were added randomly in 1,000 replicates during which 10 trees were saved in each replicate. Gaps were treated as missing. Bayesian Inference analyses were performed assuming default prior parameter distributions set in MrBayes version 3.1.2 (Ronquist and Huelsenbeck, 2003). MrBayes was run for 1,100,000 generations for ITS and for 1,200,000 generations for ITS--*trnL-F* combined data sets, with four chains, sampling trees every 10 generations. The burn-in stage needed to reach stationarity was determined by plotting the likelihood scores against the number of generations. To ensure that the two runs converged onto a stationary distribution, analyses were run until the average standard deviation of split frequencies was 0.01.

Morphological analysis

Sixty-four macroscopic and 9 palynological characters were selected for the data matrix (Appendix 2). Five individuals from 60 populations belonging to 17 species were included for analyses. Measurements were done with the samples collected from field between 2006 to 2008. At least one sample for each taxon was deposited at Hacettepe University Herbarium (HUB). *Symphytum officinale* could not be collected from field during our investigation, therefore morphological analyze did not involve this species. Palynological data were gathered from Harmata (1981). Within species, qualitative characters were summarized according to the dominancy, while metric characters were summarized as mean values. Similarities between the taxa were compared using cluster analysis (CA). The SIMINT similarity with Euclidian distance combined with unweighted pair group average linking (UPGMA) was used for the CA. Analyses were performed with NTSYS program written for the IBM PC by Rohlf (2000).

RESULTS

Phylogeny

The parsimony analysis of the ITS region yielded a well-resolved topology, but analysis of *trnL-trnF* region did not. Therefore, the tree topology was gathered by combining *trnL-trnF* data with ITS. For the maximum parsimony analysis of ITS, both spacer regions, as well as the 5.8 rDNA sequence, have been analyzed. In total, 694 characters were included in the analysis, of which 290 were parsimony informative (41.8%). For ITS-*trnL-F* combined analysis, in total, 1644 characters were included of which 406 were phylogenetically informative (24.7%). There was a concordance in parsimony and Bayesian analysis in means of intrageneric clades. *Ogastemma* and *Echiochilon* were chosen as outgroups. The results of all phylogenetic analyses indicate monophyly of *Symphytum* with a hundred Bootstrap and 1.0 Posterior Probability (PP).

According to Bayesian inference of ITS data, *Symphytum* was grouped with *Pulmonaria* L. (Figure 1) and for Parsimony analysis, *Symphytum* was clustered with *Pulmonaria*, *Moritzia* DC. *Thaumatocaryum* Baill., *Brunnera* Steven,

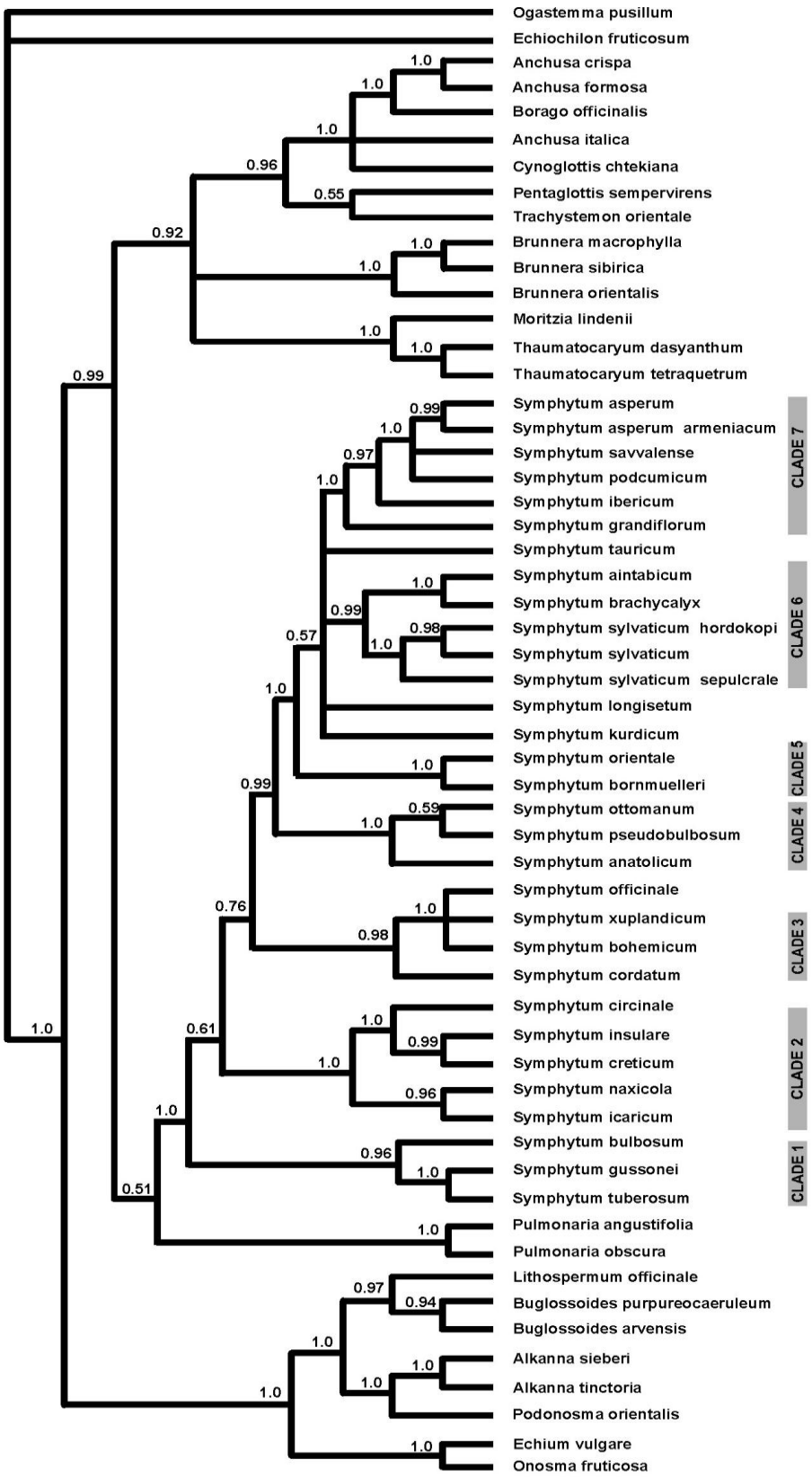

Figure 1. Bayesian analysis of ITS data. Numbers above branches correspond to their posterior probabilities.

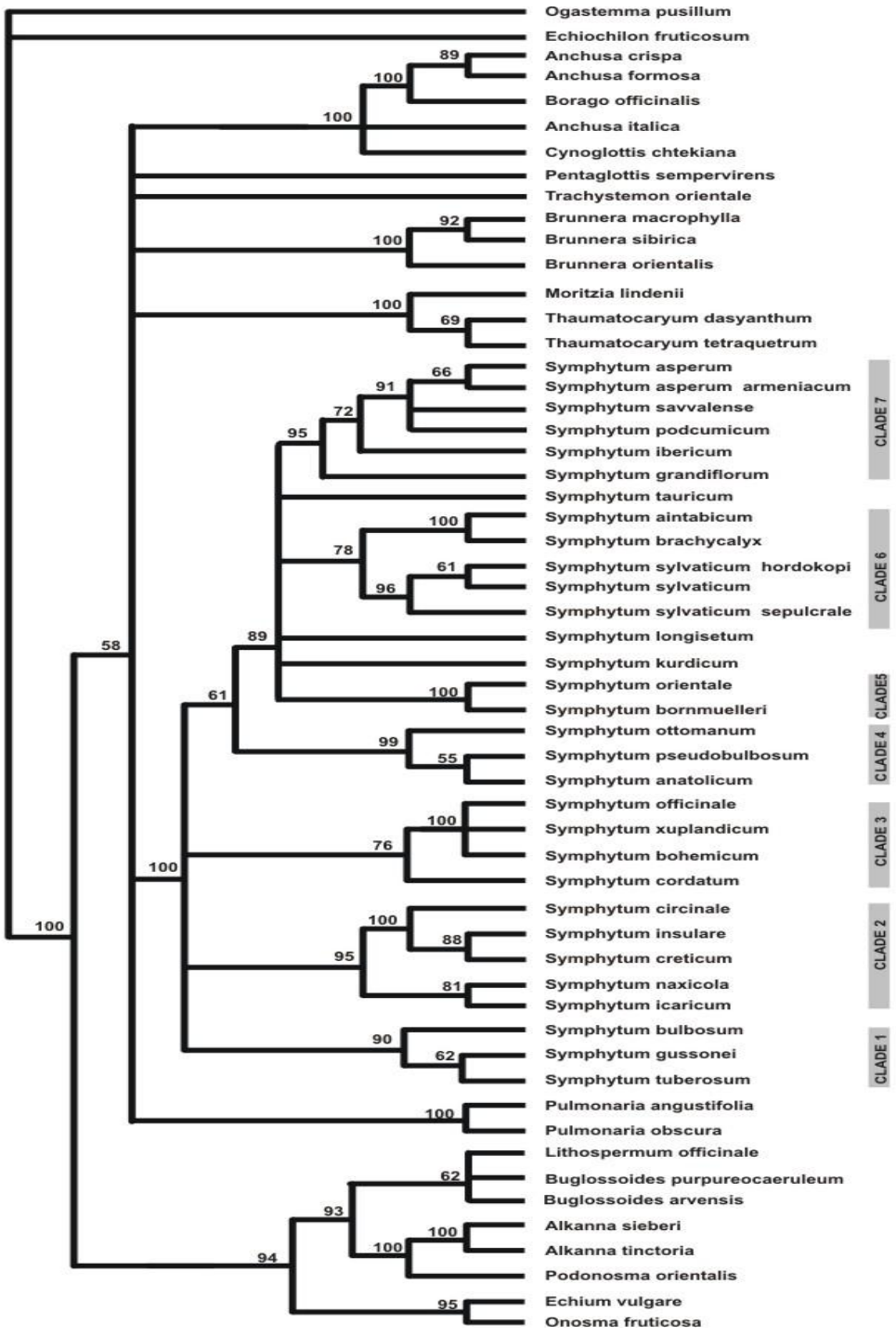

Figure 2. Parsimony analysis of ITS data. Bootstrap support values are shown above the branches.

Trachystemon D.Don., *Pentaglottis* Tausch, *Cynoglottis* (Gusul.) Vural et Kit Tan, *Anchusa* L. and *Borago* L. (Figure 2). *Symphytum* was grouped with *Brunnera*, *Thaumatocaryum, Moritzia, Trachystemon, Pentaglottis, Borago, Anchusa* and *Cynoglottis* according to the Bayesian analysis *trnL-F* and ITS sequence data (Figure 3) and to Parsimony analysis of combined *trnL-F* and ITS sequence data, *Symphytum* was clustered with *Theumatocaryum* and *Moritzia* (Figure 4). The following clades were also observed:

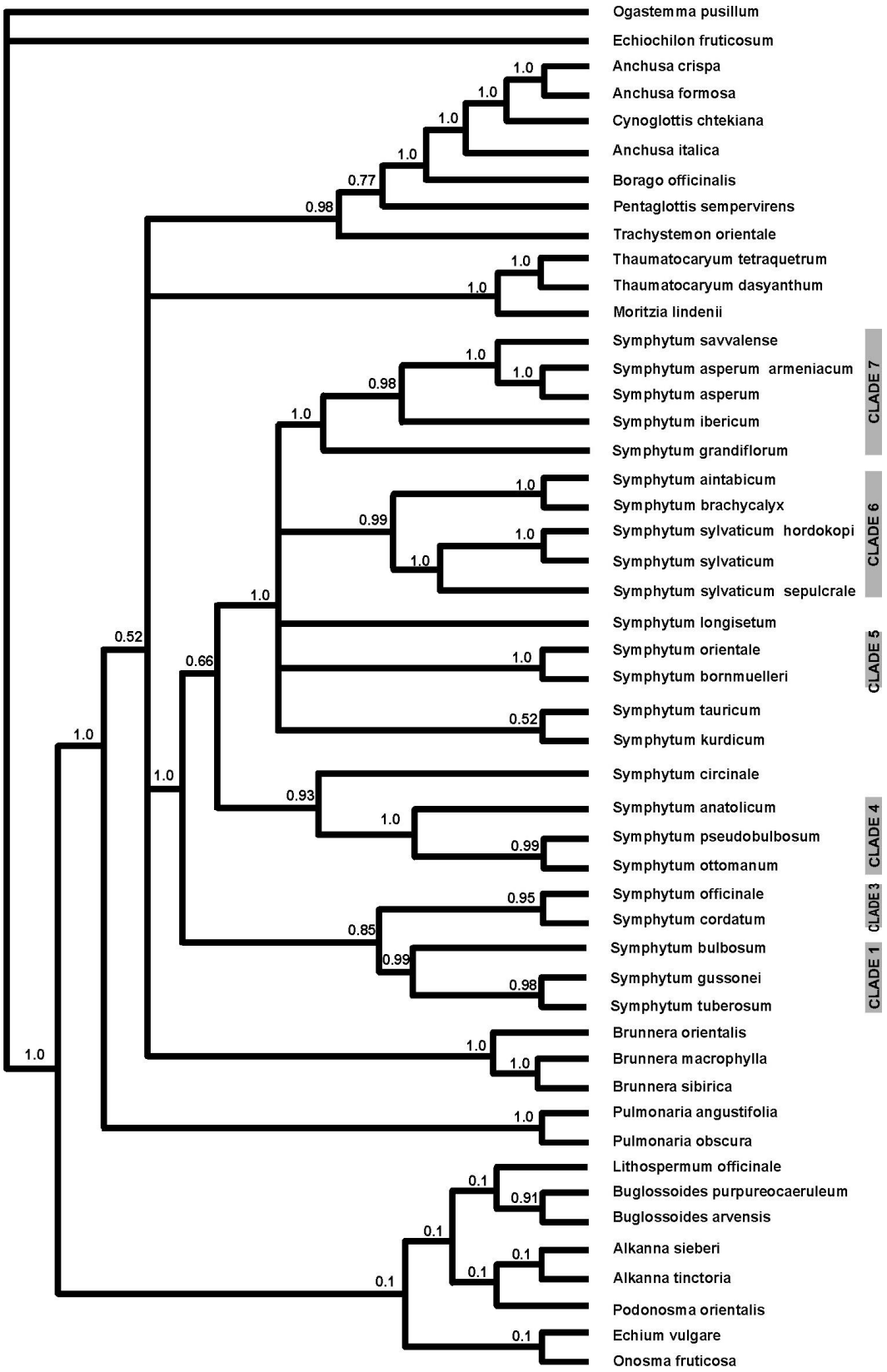

Figure 3. Bayesian analysis of combined ITS and *trnL-F* data. Numbers above branches correspond to their posterior probabilities.

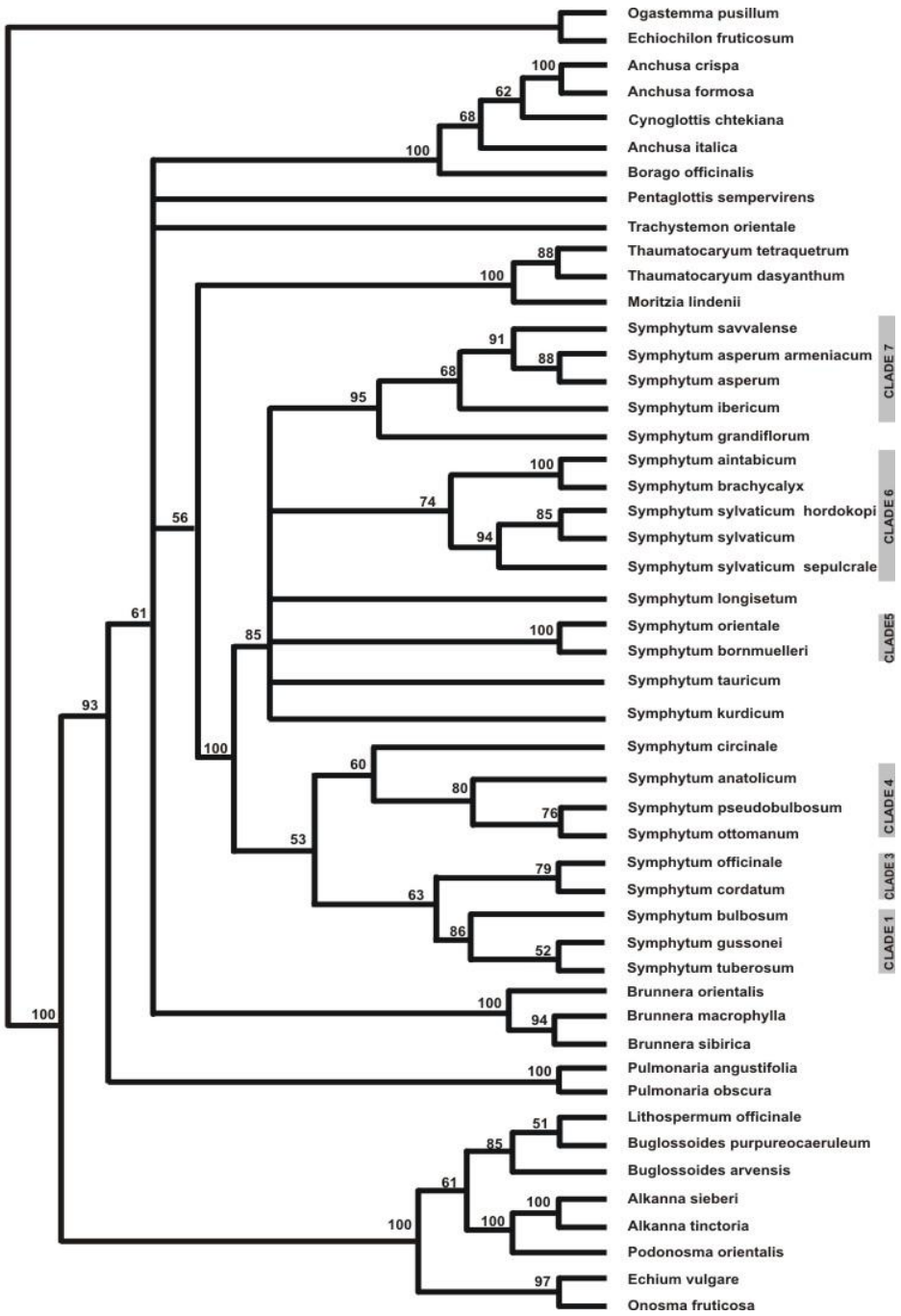

Figure 4. Parsimony analysis of combined ITS and *trnL-F* data. Bootstrap support values are shown above the branches.

Clade 1 (ITS: BS90/PP0.96; *trnL-F*-ITS: BS86/PP0.99): *S. bulbosum*, *S. gussonei* and *S. tuberosum* formed this clade. This group of plants was proposed to the section *Tuberosa* by Bucknall (1913) because of having tuberous underground organs.

Clade 2 (ITS: BS95/PP1.0): *S. icaricum* and *S. naxicola* formed a group which was proposed to the Graeca section by Pawlowski (1971). *S. circinale*, *S. insulare* and *S. creticum* formed another group in this clade which was proposed as *Procopiania* section by Wickens (1969).

Clade 3 (ITS: BS76/PP0.98; *trnL-F*--ITS: BS79/PP0.95): *S. bohemicum* is synonym of *S. officinale* (Pawlowski, 1972) and the analyses presented in this paper confirmed the synonym. *S. xuplandicum* is a hybrid species of *S.*

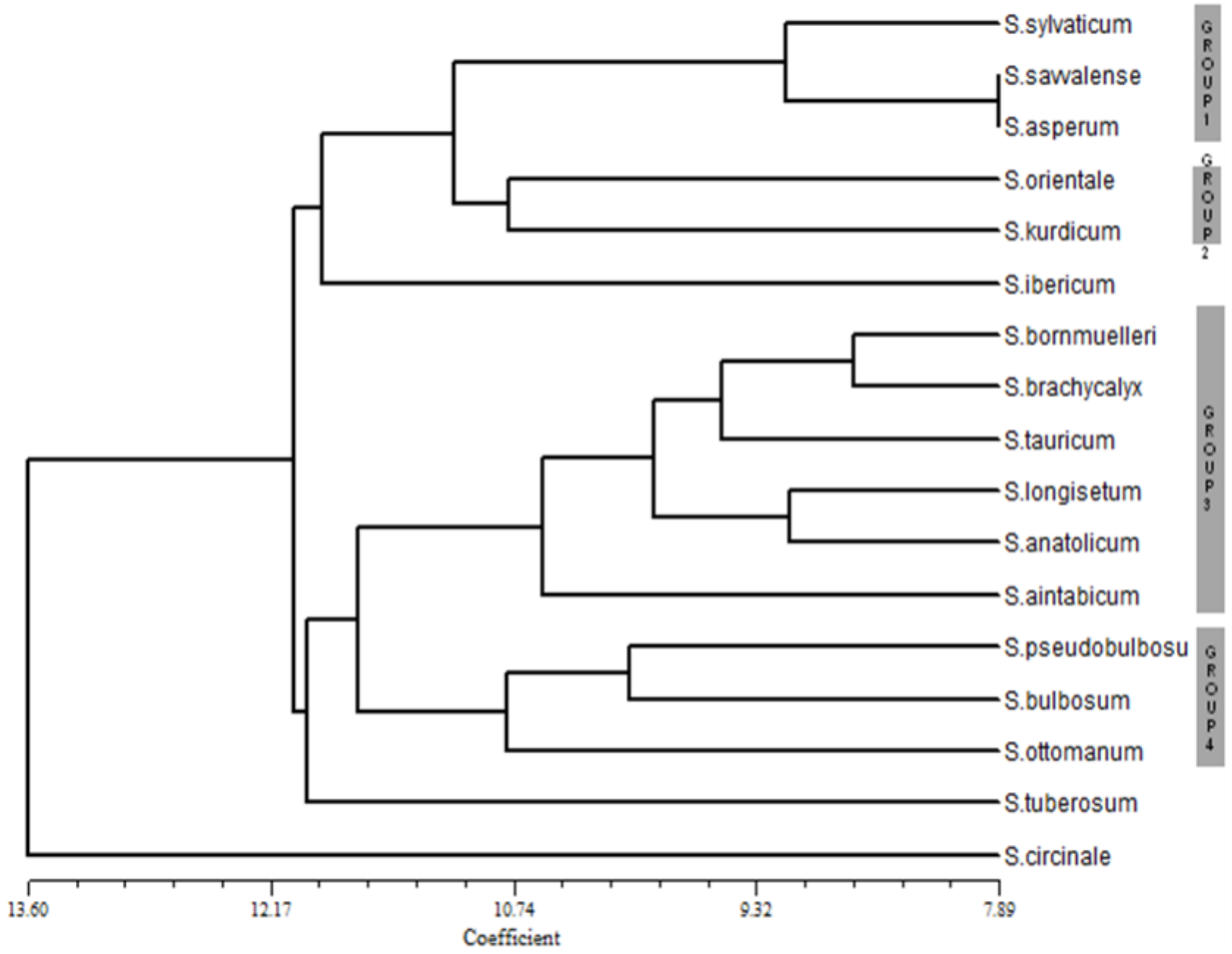

Figure 5. Cluster analysis of morphological data.

officinale and *S. asperum*. According to Bucknall's classification system *S. cordatum* belongs not only to a different section but also to a different division (Bucknall, 1913).

Clade 4 (ITS: BS99/PP1; *trnL-F*--ITS: BS80/PP1.0): This clade contains *S. anatolicum*, *S. ottomanum* and *S. pseudobulbosum*. Bucknall proposed *S. anatolicum* and *S. ottomanum* to the *Albida* section in 1913 with *S. tauricum* and *S. sylvaticum* that are neither phylogenetically nor morphologically closely allied.

Clade 5 (ITS: BS100/PP1; *trnL-F*--ITS: BS100/PP1.0): *S. orientale* and *S. bornmuelleri* belonged to this clade. Bucknall proposed *S. orientale* to *Orientalia* section and *S. bornmuelleri* to *Suborientalia* section (1913).

Clade 6 (ITS: BS78/PP0.99; *trnL-F*--ITS: BS74/PP0.99): *S. brachycalyx* was proposed to *Suborientalia* section by Bucknall (1913). Sandbrink et al. (1990) placed *S. aintabicum* to Bucknall's *Suborientalia* section as well. In Turkey, *S. aintabicum* grows in *S. brachycalx* distribution range. These two clustered with *S. sylvaticum*, which was proposed to *Albida* section by Bucknall (1913). Subcategories of this species were suggested as synonym of *S. sylvaticum* (Tarıkahya and Erik, 2010) and this approach was supported with the recent molecular analysis.

Clade 7 (ITS: BS95/PP1.0; *trnL-F*-ITS: BS95/PP1.0): This group of plants spread in the Caucasus range. *S. asperum* and *S. armeniacum* were proposed to *Coerulea* section by Bucknall (1913). *S. armeniacum* was first proposed as subspecies of *S. asperum* by Kurtto (1982) then Greuter et al. (1984) regarded this taxon as synonym of *S. asperum*. Phylogenetical analyses supported this synonymy. Sandbrink et al. (1990) placed *S. savvalense* in this section as well. *S. grandiflorum* was suggested for *Cordata* section by Bucknall (1913). According to Sandbrink et al. (1990) *S. ibericum* belongs to Bucknall's *Cordata* section. *S. podcumicum* is also a Caucasian species clustered in this clade.

Morphology

According to the cluster analysis of morphological data, *S. circinale* did not form a group with other species. There are two main clusters and four species groups (Figure 5) as follows:

Group 1:
S. sylvaticum, *S. savvalense* and *S. asperum* belonged to this group. These species are characteristic with having robust stem, pleiocormus under the ground and many blue flowers in the inflorescence. *S. sylvaticum* was proposed to *Albida* section by Bucknall (1913). However this species is now combined with *S. sepulcrale* under the name *S. sylvaticum* (Tarıkahya and Erik, 2010). *S. asperum* was proposed to *Coerulea* Section by Bucknall and *S. savvalense* was proposed to the same section by Sandbrink et al. (1990). *S. sylvaticum* and *S. savvalense* are endemic to Turkey.

Group 2: This group of species was proposed for *Orientalia* section by Bucknall because of morphological similarities of calyx, stem and flowers (Bucknall, 1913).

Group 3: This group of plants grows in Mediterranean region or Mediterranean-like habitats. *S. bornmuelleri, S. brachycalyx* were proposed to *Suborientalia* section by Bucknall (1913), and *S. longisetum* and *S. aintabicum* were proposed to the same section by Sandrink et al. (1990). Except *S. brachycalyx* and *S. tauricum*, all species of this group are endemic. The characteristic properties of this group are having slender stem and branched roots.

Group 4: These species are characterized with exserted corolla scales and they grow in Marmara region in Turkey. All species of this group were proposed to different sections by Bucknall (1913): *S. pseudobulbosum* to *Orientalia*, *S. bulbosum* to *Tuberosa* and *S. ottomanum* to *Albida* section. However, *S. tuberosum*, *S. officinale*, *S. circinale* and *S. ibericum* did not form a group with Turkish species.

DISCUSSION

Phylogeny

The results of our research indicated the monophyly of *Symphytum* with a hundred Bootstrap and 1.0 posterior probability (Figures 1 to 4). Bucknall's divisions (*Ramosa* and *Simplicia*) and subdivisions (*Ramosa, Segmentata*, *Dentata*) were not supported in molecular level. Our results indicate that *Tuberosa* Buckn. section coincides with Clade I. Pawlowski's *Graeca* section and Wickens' *Procopiania* section was grouped together as subclades in Clade 2. All plants belonging to this clade are spread in Aegean basin. In addition, Cordata Buckn. section comprises two species of which only *S. cordatum* has been investigated on a molecular base. Sandbrink et al. (1990) added several species to this section including *S. ibericum* which is grouped with the other Caucasian species in Clade 7. This clade is concordant with *Coerulea* Buckn.

The molecular data currently available do not coincide with the Bucknall's *Orientalia*, *Suborientalia* and *Albida* sections. Section *Orientalia* is polyphyletic as *S. pseudobulbosum* is retrieved in Clade 4, while *S.orientale* is retrieved in Clade 5. In *Suborientalia* section; *S. bornmuelleri* is grouped in Clade 5 while others grouped in Clade 6. The same situation is seen at *Albida* section; *S. sylvaticum* belongs to Cluster 6, while others belong to Cluster 4.

Morphology and geographical distribution

In Group I, two of the three species are endemic (*S. savvalense* and *S. sylvaticum*). *S. asperum* has the widest distribution range. It grows naturally in Georgia, Caucasus and in North Iran. This group of plants is very similar in habit but they also have some particular characters to be named as separate species:

-Corolla ca.10 mm, tube 4 to 5 mm, corolla distinctly concolor**:** ***S. savvalense***
- Corolla longer than 10 mm, tube 5 to 9 mm, corolla discolor, lower stem leaves are asperous, corolla 14 to 20 mm: ***S. asperum***
- Lower stem leaves are asperous puberulous, corolla 10 to 14(-17) mm: ***S. sylvaticum***

S. asperum grows in Artvin, Kars, Ardahan in Turkey and it has a big variation within the species. *S. sylvaticum* grows in a bit west in Giresun, Rize and Trabzon (endemic). These two species are most probably allopatric species. *S. savvalense* is only present in Artvin Şavval hill in *S. asperum*'s distribution area. This species is most probably sympatric with *S. asperum*.

In Group II there are morphological similarities between *S. orientale* and *S. kurdicum*. However, distribution areas were far away from each other. In Group III, *S. brachycalyx* had the widest distribution area: from Taurus range till Philistine in south. *S. longisetum* grows only in Mersin on *S. brachycalyx*'s distribution range, but morphologically, it is more closely related to *S. anatolicum* which grows in the Aegean region of Turkey. *S. anatolicum* can be considered as the transition species between Greek species and Turkish species. *S. bornmuelleri* is an endemic species that is distributed in the north of central Anatolia that has morphological affinities with *S. brachycalyx*. *S. aintabicum* has a small distribution area in east of Mediterranean region. *S. tauricum* grows in Russia, Ukraine, Romania and Bulgaria, but only in Sinop in Turkey. *S. brachycalyx, S. anatolicum* and S. *tauricum* seems to be the dominant species of this group. Most probably other species of this group are variated from these species in particular habitats. Finally, in Group IV, only *S. pseudobulbosum* is endemic. *S. bulbosum* has the widest distribution range in this group (very common in south Europe). Also according to phylogenetic tree, it seems to be the ancestral species of this group.

Conclusion

Due to phylogeny inference, *Procopiania* is in group and

should not be considered as a separate genus. Sections are revised due to molecular data, morphological investigations of several plant material and field observations. *Tuberosa* Buckn., *Suborientalia* Buckn. *Cordata* Pawl., *Graeca* Pawl. and *Procopiania* Wick. sections were reserved, as well as *Coerulea* Buckn. section. However in this section, *S. armeniacum* is synonym of *S. asperum* (Greuter et al., 1984) and *S. sepulcrale* is synonym of *S. sylvaticum* (Tarıkahya and Erik, 2010). Hence, due to morphological affinities, *S. sylvaticum* should be transferred from section *Albida* to *Coerulea*, while *S. pseudobulbosum* should be transferred from the section *Orientalia* to section *Albida*. Distribution of investigated species to sections is as follows:

Officinalia Buckn.- *S. officinale*
Coerulea Buckn.- *S. asperum*, *S. sylvaticum*, *S. savvalense*
Albida Buckn.- *S. tauricum*, *S. anatolicum*, *S. ottomanum*, *S. pseudobulbosum*, *S. longisetum*
Orientalia Buckn.- *S. orientale*, *S. kurdicum*
Suborientalia Buckn.- *S. brachycalyx*, *S. bornmuelleri*, *S. aintabicum*
Tuberosa Buckn.- *S. tuberosum*, *S. bulbosum*, *S. gussonei*
Cordata Buckn.- *S. ibericum*, *S. cordatum*
Procopiania Wick.- *S. circinale*, *S. creticum*, *S. insulare*
Graeca Pawl.- *S. icaricum*, *S. naxicola*

This investigation covered 24 species growing in Turkey and adjacent areas. Sections were revised with using molecular and morphological data. Field excursions also gave the clues for solving the relationships of the species. We believe that our investigation will give an important gateway to the researchers who intend to write the monograph of the genus.

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APPENDIX

Appendix 1. Voucher information and GenBank accession numbers of taxa sampled for the genus *Symphytum* and outgroup representatives. Order of GenBank accession numbers and presence: ITS, *trnL-trnF*. + denotes the presence and – denotes lacking sequence.

Alkanna sieberi A.DC.:*A. Kagiampaki 2000/10* (BSB) Greece FJ763199, FJ763263, ***Alkanna tinctoria*** Tausch: *H.H.Hilger s.n.* (BSB) Spain FJ763250, FJ763304, ***Anchusa crispa*** Viv: *Selvi & Bigazzi 99.005* (FI) GQ285227, GQ285252, ***Anchusa formosa*** Selvi, Bigazzi&Bacch.: *Bigazzi & Selvi 97.006* (FI) GQ285226, GQ285251, ***Anchusa italica*** Retz: *H.H.Hilger s.n.* (BSB) Spain GQ285233, GQ285268, ***Brunnera macrophylla*** (Adams) I.M.Johnst.: *H.H. Hilger s.n.* (BSB) Germany-cultivated GQ285223, GQ285247, ***Brunnera orientalis*** (Schenk) I.M.Johnst.: *F. Selvi & M. Bigazzi 00.28* (BSB, FI) Turkey AF531087, GQ285253, ***Brunnera sibirica*** Steven: *M. Weigend 9066* (BSB) GQ285234, GQ285273, ***Borago officinalis*** L.: *O. Mohr 600* (BSB) Germany FJ763248, FJ763302, ***Buglossoides arvensis*** (L.) I.M.Johnst.: *Weigend 2000/991* (BSB) Germany FJ763192, FJ763256, ***Buglossoides purpurocaerulea*** (L.) I.M.Johnst.: *M Weigend 8136* (BSB) Germany AJ555897_1, EU044902, ***Cynoglottis chetikiana*** Vural & Kit Tan subsp. ***paphlagonica***: *Selvi & Bigazzi 98.008* (BSB, FI) GQ285228, GQ285254, ***Echiochilon fruticosum*** Desf.: *Kagiampaki s.n.* 05.12.2007 (BSB) Libya AJ555908, FJ763310, ***Echium vulgare*** L.: *O. Mohr 597* (BSB) Germany AJ555896, FJ763301, ***Lithospermum officinale*** L.: *A. Werres & M. Ristow* (BSB) Germany FJ763189, FJ763254, ***Moritzia lindenii*** (A.DC.) Benth. ex Gürke : *Eriksen 59018* (MO) Ecuador GQ285231, GQ285255, ***Ogastemma pusillum*** (Coss. & Durieu ex Bonnet & Barratte) Brummitt: *Lady Rosemary Fitzgerald 73c* (READING) Saudi Arabia AJ555900, FJ763265, ***Onosma fruticosa*** Labill.: *Hilger 2000/10* (BSB) Cyprus FJ763196, FJ763260, ***Pentaglottis sempervirens*** (L.) L.H.Bailey: *Selvi & Bigazzi 6/2000* (FI) GQ285225, GQ285250, ***Podonosma orientalis*** (L.) Feinbrun: *F. Selvi, L. Cecchi, A. Coppi 07/16* (FI) Syria FJ763253, FJ763307, ***Pulmonaria angustifolia*** L.: *M. & K. Weigend 1999/45* (BSB) GQ285232, GQ285266, ***Pulmonaria obscura*** Dumort.: *H.H. Hilger s.n.* (B) Germany FJ763200, FJ763264, ***Symphytum aintabicum*** Hub.-Mor. & Wickens: *B.Tarıkahya 2434* (HUB) Turkey + +, ***Symphytum anatolicum*** Boiss.: *B.Tarıkahya 2277* (HUB) Turkey + +, ***Symphytum asperum*** Lepech. (var. ***armeniacum*****)**: *B.Tarıkahya 2540* (HUB) Turkey GQ285238, +, ***Symphytum asperum*** Lepech.: *B.Tarıkahya 2542* (HUB) Turkey + +, ***Symphytum bohemicum*** F.W.Schmidt *J.Štěpánek jun. et L. Štěpánek* (B) Czech Republic + -, ***Symphytum bornmuelleri*** Buckn.: *B.Tarıkahya 249*5 (HUB) Turkey GQ285237, GQ285276, ***Symphytum brachycalyx*** Boiss.: *B.Tarıkahya 2468* (HUB) Turkey GQ285236 +, ***Symphytum bulbosum*** K.F.Schimp.: *B.Tarıkahya 2369* (HUB) Turkey GQ285235, GQ285275, ***Symphytum circinale*** Runemark: *B.Tarıkahya 2288* (HUB) Turkey + +, ***Symphytum cordatum*** M.Bieb.: *A. Kagalo, N. Systschak 3820* (B) Ukrain + +, ***Symphytum creticum*** (Willd.) Runemark: *H. Hilger s.n.* (B) Greece AY383246 -, ***Symphytum grandiflorum*** DC.: *S.Charkevicz* (B) Caucasus + +, ***Symphytum gussonei*** F.W.Schultz: *s.n.* (B) + +, ***Symphytum ibericum*** Steven: B.Tarıkahya 2359 (HUB) Turkey + +, ***Symphytum icaricum*** Pawl.: *Christodoulakis 3420* (UPA) Greece + - , ***Symphytum insulare*** (Pawl.) Greuter & Burdet: *N.Böhling 8245* (B) Greece + -, ***Symphytum kurdicum*** Boiss. & Hausskn.: *M. Fırat 10410* (HUB) Turkey + +, ***Symphytum longisetum*** Hub.-Mor. & Wickens: *B. Tarıkahya 2396* (HUB) Turkey + +, ***Symphytum naxicola*** Pawl.: *N. Böhling 698* (B) Greece + -, ***Symphytum podcumicum*** Yu.M.Frolov: *Ju.Frulov s.n.* (B) Caucasus + -, ***Symphytum pseudobulbosum*** Azn:. *B.Tarıkahya 2235* (HUB) Turkey + +, ***Symphytum officinale*** L.: *Willing 17537D* (B) Germany + +, ***Symphytum orientale*** L: *B.Tarıkahya 2364* (HUB) Turkey + +, ***Symphytum ottomanum*** Friv.: *B.Tarıkahya 2526* (HUB) Turkey + +, ***Symphytum savvalense*** Kurtto: *B.Tarıkahya 2577* (HUB) Turkey + +, ***Symphytum sylvaticum*** Boiss. ssp. ***sepulcrale*** (Boiss. & Bal.) Greuter & Burdet var. ***hordokopii*** (Kurtto) R.Mill.: *B.Tarıkahya 2336* (HUB) Turkey + +, ***Symphytum sylvaticum*** Boiss. subsp. ***sylvaticum*** *B.Tarıkahya 2339* (HUB) Turkey + +, ***Symphytum sylvaticum*** Boiss. subsp. ***sepulcrale*** (Boiss. & Bal.) Greuter & Burdet var. ***sepulcrale***: *B.Tarıkahya 2357* (HUB) Turkey + +, ***Symphytum tauricum*** Willd.: *B.Tarıkahya 2489* (HUB) Turkey + +, ***Symphytum tuberosum*** L.: *B.Tarıkahya 2210* (HUB) Turkey + +, ***Symphytum x uplandicum*** Nyman: AY092903 -, ***Thaumatocaryon dasyanthum*** I.M.Johnst.: *R.C. Molon et al. 12177* (NY) Brasil GQ285230, GQ285271, ***Thaumatocaryon tetraquetrum*** (Cham.) I.M. Johnst.*: Hatschbach s.n.* (M) Brasil GQ285229, GQ285260, ***Trachystemon orientalis*** (L.) G.Don: *sn.* (B) Germany GQ285224, GQ285249.

Appendix 2. Morphological characters used for cluster analyses.

A- Habit

semi-decumbent (0), erect (1), prostrate (2)

B- Vegetative reproduction
tuber (0), adventive roots(1), pleiocorm (2), stolon (3), bulb (4), rhizome (5), lacking vegetative reproduction organ (6)

C- Root
unbranched (0), branched(1)
not fleshy (0), fleshy (1)
not woody (0), woody (1)

D- Hairs
dense (0), not dense (1), scattered (2), glabrous (3)
short hooked hairs; absent (0), present (1)
long simple hairs; absent (0), present (1)
long simple hairs arising from tubercles with one row of cells; absent (0), present (1)
simple strigose hairs; absent (0), present (1)
simple hairs; absent (0), present (1)
simple hairs arising from tubercles with one row of cells; absent (0), present (1)
hooked hairs; absent (0), present (1)
long hooked strigose hairs; absent (0), present (1)
short simple strigose hairs; absent (0), present (1)
short simple hairs arising from tubercles with one row of cells; absent (0), present (1)
short strigose hooked hairs; absent (0), present (1)
medium strigose hooked hairs; absent (0), present (1)
long hooked hairs absent (0), present (1)

E- Shoots
non flowering shoots; absent (0), present (1)

F- Stem
stout (0), slender (1), medium (2)
height (cm)

G- Lower cauline leaves
base; assymetric (0), rounded (1), cordate (2), subcordate (3), truncate(4), attenuate (5), cuneate (6)
lamina; elliptic (0), oblanceolate (1), ovate (2), ovate-elliptic (3), ovate-cordate (4), ovate-rhomboid (5), spatulate (6), ovate-lanceolate (7), triangular-ovate (8), elliptic-oblanceolate (9), oblong (10), ovate-oblong (11)
length (cm)
width (cm)
apex; acute (0), acuminate (1), rounded (2)
petiole length (cm)

H- Cauline leaves
venation; Semicraspedodrom (0), brochidodrom-semicraspedodrom (1), brochidodrom (2)
base; assymetric (0), rounded (1), cordate (2), subcordate (3), truncate (4), attenuate (5), cuneate (6)
lamina; elliptic (0), oblanceolate-elliptic (1), ovate (2), ovate-elliptic (3), ovate-lanceolate (4), rhomboid (5), spathulate-elliptic (6), linear-elliptic (7), elliptic-obovate (8), deltoid (9), lanceolate (10), ovate-oblong (11)

length (cm)
width (cm)
apex; acute (0), acuminate (1)
petiole length (upper sessile) (cm)

I- Inflorescence
flower number for branch
J- Flower:
pedicel length (mm)

K- Calyx
length (mm)
division (teeth/teeth+tube)
teeth apex; acute (0), subacute (1), obtuse (2), rounded (3)
shape of teeth; linear (0), triangular (1), lanceolate (2), triangular-lanceolate (3), narrowly triangular (4), narrowly triangular-lanceolate (5), ovate (6), triangular -ovate (7), subulate (8), oblong (9), triangular -oblong (10)

L- Corolla
color; cream (0), blue (1), pink (2), white (3), yellow (4)
limbus; not deeply divided (0), deeply divided (1)
tube length (mm)
limbus length (mm)
tube/limbus rate
length (mm)
Stamens not exserted (0), exserted (1)

M- Fornicle
shape; linear (0), triangular (1), lanceolate (2), triangular -lanceolate (3), narrowly triangular (4), narrowly triangular-lanceolate (5), subulate-lanceolate (6), oblong-lanceolate (7), subulate (8), oblong (9), narrowly triangular-oblong (10), narrowly triangular-subulate (11), linear-lanceolate (12), linear-subulate (13), oblong-subulate (14)
length (mm)
apex; acute (0), subacute (1), obtuse (2), emarginate (3)
longer/shorter than stamens; equal (0), short (1), long (2)

N- Stamen and pistil
filament length (mm)
anther length (mm)
width of filament compared to anther; narrow (0), equal (1), wide (2)
base of filament; glabrous (0), hairy (1)
style length (mm)

O- Nutlet
position; erect (0), curved (1)
shape; ovoid (0), narrowly ovoid (1), ovoid-oblique (2), narrowly ovoid-oblique (3), slightly narrowed ovoid (4)
length (mm)
width (mm)
base; not constricted (0), slightly constricted (1), constricted (2)
color; brown (0), dark brown (1), light brown (2), green (3), dark green (4), greenish brown (5)
surface; dull (0), slightly shining (1), shining (2)

P- Pollen properties
polar axis length (P) (µm)
equatorial axis length (E) (µm)
P/E
aperture number
colpus length (µm)
aperture length (µm)
mesocolpium (µm)
exine thickness at pole (µm)
exine thickness at equator (µm)