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Phylogenetic analysis of the genus *Stachys* sect. *Eriostomum* (Lamiaceae) in Turkey based on nuclear ribosomal ITS sequences

Ekrem DÜNDAR[1,*], Ekrem AKÇİÇEK[2], Tuncay DİRMENCİ[2], Şakir AKGÜN[1]
[1]Department of Biology, Faculty of Arts and Sciences, Balıkesir University, Balıkesir, Turkey
[2]Department of Biology Education, Necatibey Faculty of Education, Balıkesir University, Balıkesir, Turkey

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Abstract: Morphological revision and phylogenetic analysis of *Stachys* L. sect. *Eriostomum* (Hoffmanns. & Link) Dumort. (Lamiaceae) based on nuclear ribosomal internal transcribed spacer (nrITS) sequences were conducted. Morphological analysis confirmed the previous arrangements of 3 subsections, i.e. *Germanicae* R.Bhattacharjee, *Creticae* R.Bhattacharjee, and *Spectabiles* R.Bhattacharjee, while suggesting some status changes and new records that were also confirmed by the phylogenetic analysis. The phylogenetic analysis suggested, however, some arrangements different from those indicated by morphology. Two subsections, i.e. *Creticae* and *Germanicae-Spectabiles*, instead of 3 were observed in the phylogenetic tree. The tree also suggested that 9 *Stachys cretica* L. subspecies form a polyphyletic rather than a monophyletic group. While at least 2 clades were clearly identified in the subsection *Creticae*, no meaningful clades in the subsection *Germanicae-Spectabiles* were detected in the tree. Flower colour and altitude were not represented by any difference in nrITS DNA sequence of the taxa examined, while 2 distant populations of *Stachys spectabilis* Choisy ex DC. differed in that respect. A potentially new species of *Stachys* was observed in the tree. The tree clearly displayed the monophyly of *Eriostomum*, while confirming the recently suggested new status (as a genus) of the subgenus *Betonica* (L.) R.Bhattacharjee.

Key words: *Eriostomum*, nrITS phylogeny, Lamiaceae, phylogenetic analysis

1. Introduction

The genus *Stachys* L. is one of the largest genera of the Lamiaceae (also known as Labiatae), and it consists of approximately 300 species displaying a remarkable range of variation. It is mainly distributed in the warm temperate regions of the Mediterranean and south-west Asia, with secondary distributions in North and South America and Southern Africa. In terms of the number of species, there are 2 main centres of diversity. The first centre is confined to south and east Anatolia, Caucasia, north-west Iran, and north Iraq, while the second is confined to the Balkan Peninsula. The Asiatic centre is mainly composed of Mediterranean and Irano-Turanian phytogeographical elements. On the other hand, the European centre embraces the Mediterranean and Euro-Siberian phytogeographical regions. *Stachys s.l.* is divided into 2 subgenera [subgen. *Stachys* and subgen. *Betonica* (L.) R.Bhattacharjee]. A third subgenus, *Menitskia*, was suggested by Krestosvkaja (2003) as a monotypic subgenus in addition to Bhattacharjee's (1980) 2 subgenera.

The first revision of Turkish *Stachys* taxa was conducted by Bhattacharjee (1982) for the *Flora of Turkey*, which revealed 90 species (115 taxa) belonging to 15 sections and 2 subgenera. Of the 115 taxa, 54 (47%) are endemic to Turkey (Davis et al., 1988; Sümbül, 1990; Gemici & Leblebici, 1998; Duman, 2000; Dinç & Doğan, 2006; İlçim et al., 2008; Yıldırımlı, 2010; Dirmenci et al., 2011; Akçiçek et al., 2012). The endemic taxa consist of mostly East Mediterranean elements.

Eriostomum (Hoffmanns. & Link) Dumort. is the largest section of the subgenus *Stachys* (as well as of the whole *Stachys* genus) in Turkey. The name *Eriostomum* was first used by Hoffmannsegg and Link (1809) in the *Flora of Portugal* as a genus. Later, Dumortier (1827) arranged the genus *Stachys* into 3 sections, i.e. sect. *Olisia* Dumort., sect. *Stachydotypus* Dumort., and sect. *Eriostomum*, lowering *Eriostomum* to section level for the first time. Several years later, Reichenbach (1830–32) suggested 3 groups (*Campanistrum* (Habrl.) Rchb., *Chamaesideritis* Rchb., and *Eriostachys* Rchb.) when he constructed the infrageneric classification of *Stachys*, and placed the currently known *Eriostomum* species into *Chamesideritis* and *Eriostachys*. Later, Bentham (1834, 1848) made *Eriostomum* (17 species) a synonym of *Eriostachys*. After

* Correspondence: dundar@balikesir.edu.tr

that, Briquet (1897) accepted *Eriostomum* as a section and divided it into 4 subsections: subsect. *Germanicae* Boiss., subsect. *Temnocorydes* Briq., subsect. *Micranthae* Boiss., and subsect. *Biflorae* Briq. Finally Bhattacharjee (1980) accepted *Eriostomum* as a section and divided it into 3 subsections: subsect. *Germanicae* R.Bhattacharjee, subsect. *Creticae* R.Bhattacharjee, and subsect. *Spectabiles* R.Bhattacharjee.

The section *Eriostomum* comprises more than 40 species in the world; 23 of them (34 taxa) are distributed in Turkey (Bhattacharjee, 1980, 1982). This section, which is homogeneous in its overall character similarity, has a wide range throughout Europe, Asia, and part of North Africa. Species of the sect. *Eriostomum* are characterised by conspicuous bracteoles that are (at least the outer ones) half as long as or longer than the calyx tube, ovate-lanceolate to linear lanceolate, and herbaceous. The corolla lip contains densely sericeous hairs on upper parts and the calyx mouth is covered with dense hairs. With these aspects, it can easily be distinguished from the other sections of *Stachys*. The sect. *Eriostomum* is made up of species very similar to one another, but it shows a great morphological variability on both individual and population level. Of the 3 subsections with respect to the most recent morphological taxonomy (Bhattacharjee, 1980, 1982), the subsect. *Spectabiles* is mainly distributed in oriental and Irano-Turanian regions, while the subsect. *Creticae* and the subsect. *Germanicae* grow widely throughout Europe and Asia (Bhattacharjee, 1974; Falciani, 1997).

While there are a number of studies on the taxonomy, morphology, anatomy, trichome and seed micromorphology, and palynology of *Stachys* (Epling, 1934; Bhattacharjee, 1974, 1980; Nelson, 1981; Mulligan & Munro, 1989; Demissew & Harley, 1992; Turner, 1994; Falciani, 1997; Uysal, 2002, 2003; Dinç & Öztürk, 2008; Salmaki et al., 2008; Salmaki et al., 2011), a taxonomical status update of Turkish *Stachys* taxa complemented with molecular systematic studies has not been reported to date. Utilisation of nuclear ribosomal internal transcribed spacer (nrITS) sequence comparison in plants has been well established (Baldwin et al., 1995; Álvarez & Wendel, 2003; Gültepe et al., 2010; Chao et al., 2012; Lee et al., 2012; Terzioğlu et al., 2012; Tsai et al., 2012; Yousefzadeh et al., 2012). Phylogenetic analysis using nrITS has been proven effective for Lamiaceae in multiple reports (Steane et al., 1999; Prather et al., 2002; Dirmenci et al., 2010; Dirmenci et al., 2011; Akçiçek et al., 2012; Salmaki et al., 2012). Although chloroplast markers are also widely used in Lamiaceae phylogeny (Scheen et al., 2010; Bendiksby et al., 2011), these markers did not provide satisfactory resolution for our taxa (results not shown). In the present study, nrITS phylogeny was used along with morphological analyses to revise the taxonomic positions of the taxa belonging to the sect. *Eriostomum* of *Stachys* along with members from the other *Stachys* sections in addition to an out-group.

2. Materials and methods

2.1. Specimen collection and morphological evaluation

During field studies for the revision of *Stachys* sect. *Eriostomum* in Turkey between 2007 and 2010, a large number of specimens were collected from different regions of Turkey; they were properly dried and deposited at Balıkesir University Herbarium. All *Stachys* specimens were examined in light of the relevant literature (Boissier, 1879; Colmeiro, 1888; Fiori, 1926; Hayek & Markgraf, 1931; Rechinger, 1937; Palhinha, 1939; Savulescu, 1961; Halacsy, 1968; Ball, 1972; Knorring, 1977; Bhattacharjee, 1982; Rechinger, 1982; Davis et al., 1988; Jordanov, 1989; Baden, 1991; Strid & Tan, 1991; Duman, 2000) and compared with specimens at the following herbaria: ANK, BM, E, EGE, G, GAZI, HUB, ISTE, ISTF, ISTO, K, SO, W, and WU. Voucher information of the plant materials used in this work and their GenBank accession numbers (along with DNA sequences retrieved from NCBI GenBank) are listed in the Table.

2.2. Genomic DNA isolation, PCR, and sequencing

Total genomic DNA was isolated using a DNeasy Plant Kit (Qiagen GmbH, Hilden, Germany). PCR reactions were prepared using ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') and ITS5 (5'-GGA AGG AGA AGT CGT AAC AAG G-3') primers from previous reports (White et al., 1990; Sang et al., 1995) with the following protocol: 5 min 95 °C initial denaturation, 35 cycles of 30 s 94 °C denaturation, 30 s 50 °C annealing, and 1 min 72 °C extension, followed by a 10 min final extension at 72 °C. These primers amplify the whole region containing ITS1, 5.8S, and ITS2 sequences (White et al., 1990; Sang et al., 1995). The same primers were used both for amplification and for sequencing, which were conducted at RefGen Inc. (Ankara, Turkey) using an ABI 3130XL genetic analyser (Applied Biosystems, Foster City, CA, USA) with a BigDye cycle sequencing kit (Applied Biosystems). ITS sequences were generated from 2 independent sequencing reactions for each (of the triplicates for each) taxon. When no sequence difference was observed within triplicates of a taxon, only one representative DNA sequence was included in the phylogenetic analysis. An experimental detail to note is the extraordinary difficulty encountered when amplifying and sequencing the nrITS region of *Stachys* taxa (probably due to the secondary metabolites inhibiting the PCR reaction). Therefore, a total of around 400 sequencing attempts (an average of around 10 sequencings per taxon) were needed to obtain accurate nrITS sequences of the 43 taxa (Table). On the other hand, only 2 sequencings per taxon were enough for most non-

Table. Materials of *Stachys* and related taxa used in this study.

Taxon	Voucher information	GenBank accession no.
Stachys germanica L. subsp. *heldreichii* (Boiss.) Hayek	*Akçiçek* 5374 & *Dirmenci*	JF330310
S. bithynica Boiss.	*Akçiçek* 4780 & *Dirmenci*;	JF330299
	Akçiçek 5215 & *Dirmenci*	JF330300
S. tymphaea Hausskn.	*Akçiçek* 4598 & *Dirmenci*	JF330302
S. thracica Davidov	*Akçiçek* 5291 & *Dirmenci*	JF330314
S. alpina subsp. *macrophylla* (Albov) R.Bhattacharjee	*Akçiçek* 4771 & *Dirmenci*	JF330285
S. balansae Boiss. & Kotschy	*Dirmenci* 3547	JF330286
S. carduchorum (R.Bhattacharjee) Rech.f.	*Akçiçek* 5335 & *Dirmenci*	JF330287
S. rizeensis R.Bhattacharjee	*Akçiçek* 5235 & *Dirmenci*	JF330309
S. huber-morathii R.Bhattacharjee	*Akçiçek* 5175 & *Dirmenci*	JF330303
S. pinetorum Boiss. & Balansa	*Akçiçek* 4757 & *Dirmenci*	JF330308
S. obliqua Waldst. & Kit.	*Akçiçek* 4659 & *Dirmenci*	JF330307
S. minor (Boiss.) Akçiçek & Dirmenci	*Akçiçek* 5319 & *Dirmenci*	JF330306
S. sericantha P.H.Davis	*Akçiçek* 4624 & *Dirmenci*	JF330310
	Akçiçek 5496 & *Dirmenci*	JF330310
S. tmolea Boiss.	*Akçiçek* 4779 & *Dirmenci*	JF330315
S. cretica subsp. *cassia* (Boiss.) Rech.f.	*Akçiçek* 4758 & *Dirmenci*	JF330292
S. cretica subsp. *garana* (Boiss.) Rech.f.	*Akçiçek* 4763 & *Dirmenci*	JF330293
S. cretica subsp. *lesbiaca* Rech.f.	*Akçiçek* 4645 & *Dirmenci*	JF330295
S. cretica subsp. *trapezuntica* Rech.f.	*Akçiçek* 5489 & *Dirmenci*	JQ730032
S. cretica subsp. *bulgarica* Rech.f.	*Akçiçek* 5287 & *Dirmenci*	JF330289
S. cretica subsp. *vacillans* Rech.f.	*Akçiçek* 4628 & *Dirmenci*	JF330298
S. cretica subsp. *smyrnaea* Rech.f.	*Akçiçek* 4638 & *Dirmenci*	JF330297
S. cretica subsp. *mersinaea* (Boiss.) Rech.f.	*Akçiçek* 5376 & *Dirmenci*	JF330296
S. cretica subsp. *anatolica* Rech.f.	*Akçiçek* 4610 & *Dirmenci*	JF330291
S. cretica subsp. *kutahyensis* Akçiçek	*Akçiçek* 4609 & *Dirmenci*	JF330294
S. byzantina K.Koch	*E.Erdoğan* 1003	JF330290
S. vuralii Yıldız, Dirmenci & Akçiçek	*Yıldız* 16553, *Dirmenci* & *Bräuchler*	JF330317
S. thirkei K.Koch	*Akçiçek* 5211 & Dirmenci	JF330313
S. spectabilis Choisy ex DC.	*Dirmenci* 3583;	JF330312
	Dirmenci 3539	JF330311
S. longispicata Boiss. & Kotschy	*Akçiçek* 5183 & *Dirmenci*	JF330305
S. viticina Boiss.	*Akçiçek* 4746 & Dirmenci	JF330316
S. huetii Boiss.	*Akçiçek* 5134 & *Dirmenci*	JF330304
S. bayburtensis R.Bhattacharjee & Hub.-Mor.	*Akçiçek* 5136 & *Dirmenci*	JF330288
S. hirta L.	(Barber et al., 2002)	AF335643
S. sylvatica L.	*Akçicek* 5213 & *Dirmenci*	JQ730027
S. iberica M.Bieb.	*Akçiçek* 2848	JQ730028
S. viscosa Montbret & Aucher ex Benth.	*Akçiçek* 5152 & *Dirmenci*	JQ730024
S. lavandulifolia Vahl	*Akçiçek* 5335 & *Dirmenci*	JQ730026
S. diversifolia Boiss.	*A.Duran* 6318	JQ730030
S. mardinensis (Post) R.R.Mill.	*Yıldız* 9840	JQ730029
S. bombycina Boiss.	*Akçiçek* 5127 & *Dirmenci*	JQ730025
S. macrantha (K.Koch) Stearn	*Yıldız* 16657	JQ730031
Stachys sp.	*Akcicek* 4644 & *Dirmenci*	JQ730033
Sideritis syriaca Griseb.	(Barber et al., 2002)	AF335621
Sideritis syriaca Griseb	(Barber et al., 2002)	AF335620
Sideritis scardica Griseb.	(Barber et al., 2002)	AF335619
Sideritis athoa Papan. & Kokkini	(Barber et al., 2002)	AF335615
Sideritis montana L.	(Barber et al., 2002)	AF335612
Sideritis romana L.	(Barber et al., 2002)	AF335614
Sideritis tragoriganum Lag.	(Barber et al., 2002)	AF335639
Sideritis murgetana Obon & D.Rivera	(Barber et al., 2002)	AF335636
Sideritis sericea Pers.	(Barber et al., 2002)	AF335638
Sideritis marmorinensis Obon & D.Rivera	(Barber et al., 2002)	AF335635
Sideritis hirta Roth	(Barber et al., 2002)	AF335632
Sideritis brevicaulis Mend.-Heuer	(Barber et al., 2007)	DQ900751
Marrubium supinum L.	(Barber et al., 2002)	AF335642
Ballota hispanica (L.) Benth.	(Barber et al., 2002)	AF335641

Stachys taxa (data not shown). Detailed information of the specimen vouchers used for genomic DNA extraction is listed in the Appendix. ITS sequences for *Stachys cretica* L. subsp. *cretica* and *S. cretica* subsp. *salviifolia* (Ten.) Rech.f. could not be obtained despite multiple attempts.

2.3. Phylogenetic analysis

Alignment of the ITS sequences was generated using the ClustalW algorithm (Thompson et al., 1994) of BioEdit 7.0.4.1 (Hall, 1999). Ends of the alignment were trimmed to make all the sequences of equal length, which was a total of 549 positions in the final dataset. The phylogenetic tree was generated using the neighbour-joining method (Saitou & Nei, 1987), constructed using PAUP 4.0b10 (Swofford, 2001) and viewed using TreeGraph2 (Stöver & Müller, 2010). An independent bootstrap analysis (Felsenstein, 1985) was also run using PAUP 4.0B10 (Swofford, 2001) and the bootstrap values were integrated into the tree. ITS sequences of taxa from the other *Stachys* sections were also obtained through the same methods explained above, and were used to construct the tree. ITS sequences of *Stachys hirta* L. and *Sideritis* L. taxa used in the tree were retrieved from NCBI GenBank, and their accession numbers are listed in the Table along with *Marrubium supinum* L. and *Ballota hispanica* Benth., which were used as the out-group. The phylogenetic analysis contained a total of 58 nrITS sequences, 43 of which were generated through this work.

3. Results

3.1. Revised key and descriptions for the subsections

1. Calyx ± regular, teeth eglandular.... Subsect. **Spectabiles**
1. Calyx sub-bilabiate, teeth with glandular hairs
   2. Cauline leaves usually oblong-lanceolate to lanceolate or oblong-spathulate; narrowed towards base; attenuate to cuneate, rarely rounded or subcordate at …… Subsect. **Creticae**
   2. Cauline leaves usually ovate to ovate-lanceolate or oblong-ovate; broader towards base; cordate, subcordate, or rarely rounded at base …..... .....… Subsect. **Germanicae**

The descriptions of revised subsections belonging to the sect. *Eriostomum* in Turkey are as follows:

Subsect. *Germanicae* R.Bhattacharjee

Cauline leaves ovate to ovate-lanceolate or oblong-ovate, rarely oblong to oblong-lanceolate, broader towards base, cordate, subcordate, or rarely rounded at base. Calyx sub-bilabiate, teeth usually with glandular hairs.

Subsect. *Creticae* R.Bhattacharjee

Cauline leaves oblong-lanceolate to lanceolate or oblong spathulate, rarely oblong-ovate or elliptic, narrowed towards base, attenuate to cuneate, rarely rounded or subcordate at base. Calyx sub-bilabiate, usually with glandular hairs.

Subsect. *Spectabiles* R.Bhattacharjee

Cauline leaves ovate to ovate-lanceolate or oblong to oblong-ovate, cordate to subcordate or rounded, rarely cuneate at base. Calyx usually ±regular, teeth eglandular.

The subsect. *Spectabiles* is distinctly separated from all other subsections. The subsection has ±regular calyx, eglandular teeth (not calyx sub-bilabiate, teeth with glandular hairs). Subsect. *Creticae* can be distinguished from subsect. *Germanicae* by the following features: cauline leaves usually oblong-lanceolate to lanceolate or oblong-spathulate; usually attenuate to cuneate at base (not cauline leaves usually ovate to ovate-lanceolate or oblong-ovate; cordate, subcordate, or rarely rounded at base).

General view, flowers, and cauline leaves of the species thought to represent each subsection are shown in Figure 1.

3.2. Phylogenetic analysis

The phylogenetic tree clearly revealed that the sect. *Eriostomum* was a monophyletic taxon. Moreover, the other sections of *Stachys* sampled in the phylogenetic tree as in-groups [sect. *Stachys*, sect. *Olisia* Dumort, sect. *Fragilicaulis* R.Bhattacharjee, and sect. *Zietenia* (Gled.) Benth.] also appeared polyphyletic, intermingling not only with each other but also with *Sideritis* (Figure 2). The 3 subsections, on the other hand, were polyphyletic and only *Creticae* could be identified from the tree. The other subsections (*Germanicae-Spectabiles*) appeared randomly mixed with each other as were the other *Stachys* sections sampled. Factors potentially causing taxonomic differentiations such as altitude, geographical distance, and flower colour were evaluated in the tree. Pink flowered specimens and white flowered specimens of *Stachys bithynica* Boiss. from the same location (Mount Uludağ, Bursa) did not have any ITS nucleotide difference. Likewise, *S. sericantha* P.H.Davis specimens from 10 m and 1200 m from Antalya bore the same ITS sequence. Two distant populations of *S. spectabilis*, however, differed in that respect although they were closest to each other (Figure 2). *S. cretica* subspecies were grouped in 2 polyphyletic clades under the subsection *Creticae* while no clades could clearly be displayed under the subsections *Germanicae* and *Spectabiles*. An unidentified *Stachys* taxon (a potentially new species based on morphological observations) was placed next to *S. obliqua* Waldst. & Kit. The subgenus *Betonica* was very far from the whole genus *Stachys* and was placed very close to the out-group (*Marrubium supinum* L. and *Ballota hispanica* (L.) Benth.).

4. Discussion

The most recent arrangement of the sect. *Eriostomum* was conducted by Bhattacharjee (1980), who divided it into 3 subsections (subsect. *Germanicae*, subsect. *Creticae*, and

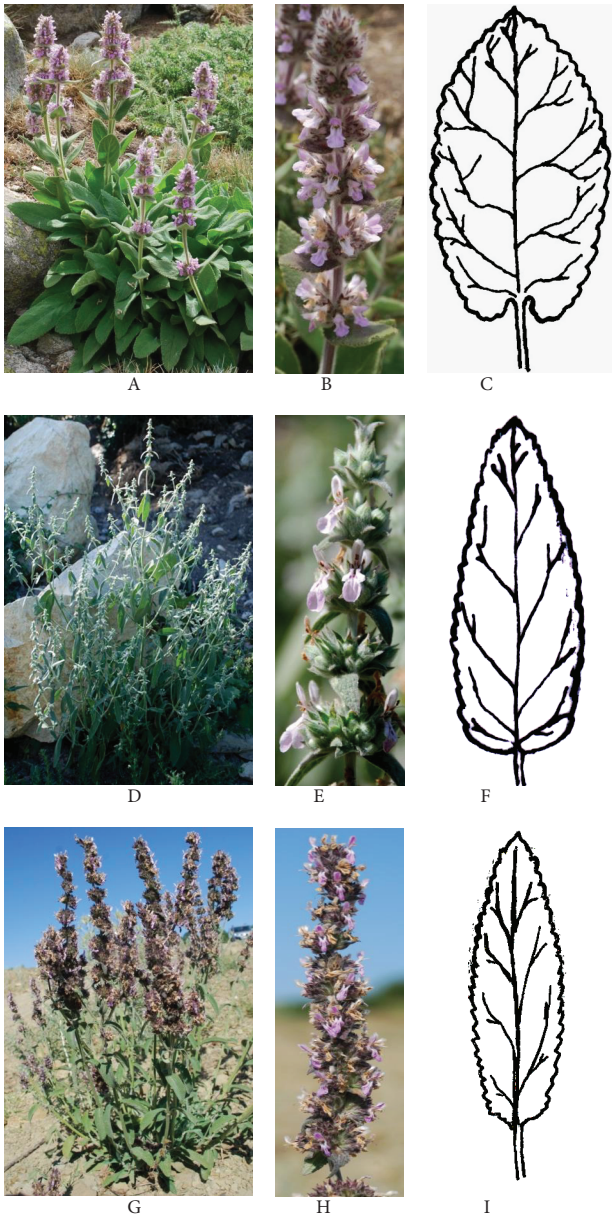

Figure 1. General views, flowers, and cauline leaf diagrams of the species that are thought to best represent the subsections they belong to. General views, flowers, and cauline leaf diagrams (respectively) of *S. bithynica* (subsect. *Germanicae*) (A, B, C), *S. viticina* (subsect. *Spectabiles*) (D, E, F), and *S. cretica* subsp. *bulgarica* (subsect. *Creticae*) (G, H, I).

subsect. *Spectabiles*) (Falciani, 1997). Our morphological revision of the Turkish members of this section mostly suggested keeping the current taxonomical positions for most of the taxa, while we recommend rearrangements for some of them.

In the subsection *Germanicae*, *Stachys bithynica* Boiss. is related to *S. balansae* Boiss. and *S. tymphaea* Hausskn. This species shows an affinity with the eastern species *S. balansae*, but it can be distinguished from *S. balansae* by the following features: cauline leaves oblong-ovate or ovate and densely sericeous-tomentose above, densely adpressed floccose white-tomentose beneath, glandular.

Stachys carduchorum (R.Bhattacharjee) Rech.f. resembles *S. balansae*, but it can be distinguished from subsp. *balansae* by the following features: cauline leaves ovate to broadly elliptic, glabrescent or sparsely pilose on both surfaces; nutlets obovoid, 2.5–3 × 1.8–2 mm.

In the subsection *Creticae*, *S. cretica* is very variable in density of indumentum, calyx teeth shape, tube/teeth ratio, length of calyx teeth mucros, and length/breadth ratio of leaves. A certain amount of morphological overlap occurs between subspecies in regions of contact (Bhattacharjee, 1982).

Stachys huetii Boiss. of the subsection *Spectabiles* is very similar to *S. bayburtensis* R.Bhattacharjee & Hub.-Mor. This species is distinguished by its simple or sparsely branched flowering stems and distant verticillasters throughout.

Despite the polyphyletic nature of *Stachys* and its close relative *Sideritis* (Bendiksby et al., 2011), the phylogenetic tree revealed the sect. *Eriostomum* was clearly a monophyletic taxon. Unlike the other sections of *Stachys* sampled for the analysis, Turkish members of the sect. *Eriostomum* can be considered a single natural taxonomic group.

As for the infrasectional classification, however, the phylogenetic tree suggested a significantly different taxonomy from that of morphological analysis. First of all, the tree suggests 2 subsections, while the morphological analyses suggested 3 subsections: *Germanicae*, *Creticae*, and *Spectabiles*. Although the subsection *Creticae* can clearly be identified from the tree (Figure 2), the other group almost equally consists of taxa belonging to the subsections *Germanicae* and *Spectabiles*. Subsects. *Germanicae* and *Spectabiles* are more similar to each other with respect to leaf basis shape (Figure 1) and habitats. Hence it would not be very unnatural to combine these 2 subsections.

The phylogenetic analysis also suggested several new status arrangements and confirmed some new records (*S. tymphaea* and *S. thracica* Davidov) for Turkey that were also confirmed by morphological analyses (Akçiçek et al., 2012). Akçiçek et al. (2012) have changed *S. germanica* L. subsp. *bithynica* (Boiss.) R.Bhattacharjee (a subspecies) to *S. bithynica* Boiss. as a species, and *S. balansae* Boiss. & Kotschy subsp. *carduchorum* R.Bhattacharjee (a subspecies) to *S. carduchorum* (R.Bhattacharjee) Rech.f. as a species. They also accepted the category of Haussknecht for *S. tymphaea* Hausskn. and changed the status of *S. libanotica* Benth. var. *minor* Boiss. to *S. minor* (Boiss.) Akçiçek & Dirmenci (Akçiçek et al., 2012). Our findings also suggest the 2

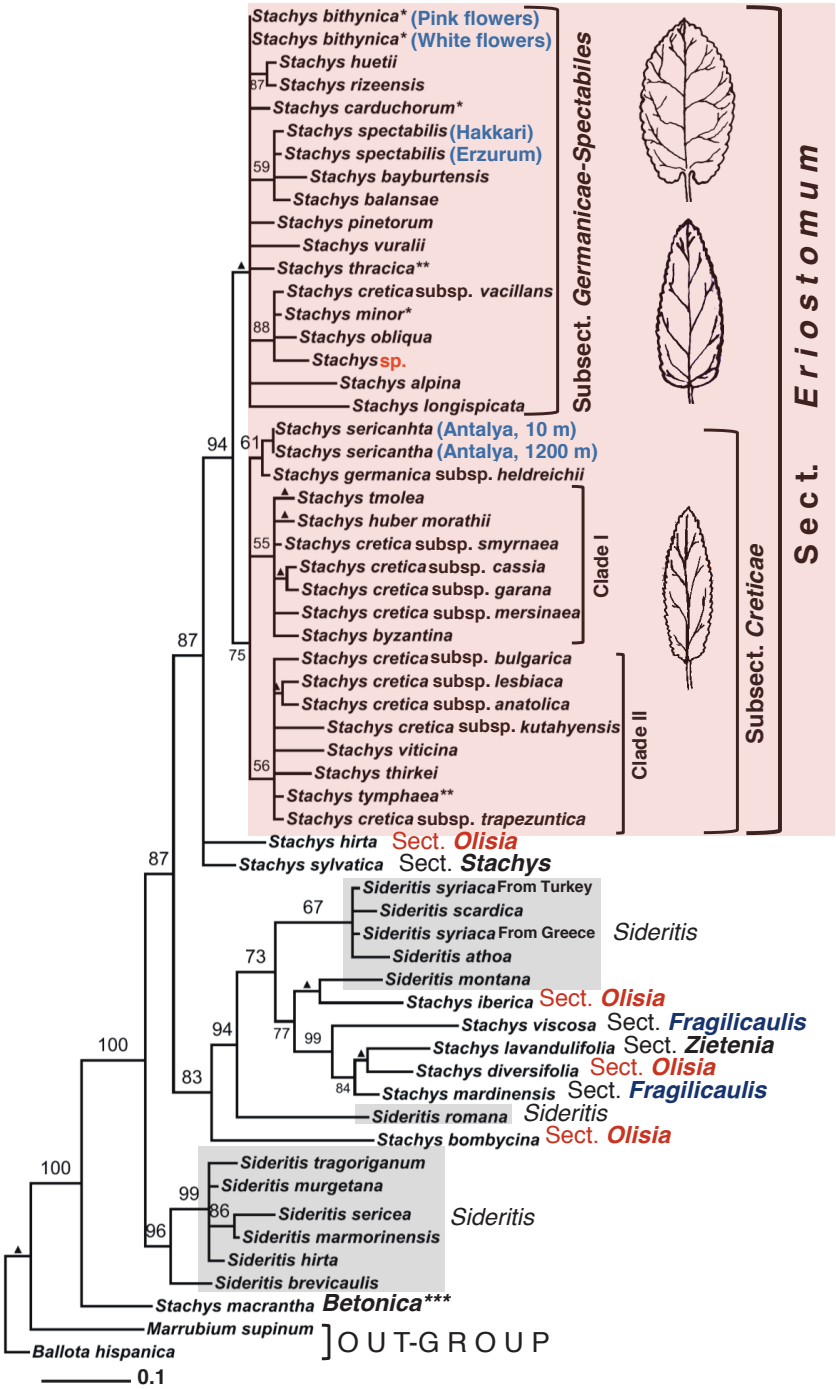

Figure 2. The neighbour joining (Saitou & Nei, 1987) tree generated using nrITS DNA sequences of sect. *Eriostomum* taxa and the related sequences retrieved from NCBI GenBank. Filled triangles mark the branches that collapsed in the bootstrap analysis. The tree was drawn to scale, with branch lengths in the same units as those of the phylogenetic distances used to infer the tree. There were a total of 549 positions in the final dataset. Leaf diagrams of the species (see Figure 1 for species names) thought to best represent their subsections are placed next to the respective subsections. * Taxa with status change. ** New records for Turkey. *** *Stachys* L. subgenus *Betonica* has been recently suggested to revert back to *Betonica* (Scheen et al., 2010; Bendiksby et al., 2011). Altitude, geographical places, and flower colour are noted for taxa that were evaluated for any sequence differences. *Ballota hispanica* and *Marrubium supinum* were used as an out-group. Accession numbers of the ITS sequences and voucher information of the specimens used for this study are shown in the Table. *Stachys* sp. was different from all other taxa examined regarding morphology as well, and it will be further analysed in detail as a potential new taxon.

geographical isolates of *S. spectabilis* (Erzurum and Hakkari) should be at least 2 different subspecies (if not 2 different species), although they interestingly do not have any distinct morphological differences, which further points out the complicated taxonomy of *Stachys*. Also interesting is the unexpected closeness of *S. huetii* and *S. rizeensis* (in the tree), which have been classified into different subsections based on morphological traits. On the other hand, 2 morphologically very similar species (*S. huetii* and *S. bayburtensis*) are not close to each other in the tree. *S. germanica* lost all its subspecies except subsp. *heldreichii* (Boiss.) Hayek, which was also suggested before (Akçiçek et al., 2012). The most similar finding of the phylogenetic analysis with the morphological classification is the clear separation of the subsection *Creticae* although *S. cretica* subspecies do not completely confirm with morphological grouping; while all of its subspecies fall in the same subsection (*Creticae*) they do appear polyphyletic if not independent species (Figure 2). It is possible to suggest 2 clades that *S. cretica* subspecies fall into, although these 2 clades do not distinctly look different from one another based on morphology. A potentially new species morphologically close to *S. obliqua* was confirmed in the tree. A detailed report about this new taxon will follow this publication.

While altitude difference of about 1200 m (in *S. sericantha*) and flower colour (in *S. bithynica*) did not cause any taxonomical difference, significant geographical distance (around 600 km) seems to be effective in *Eriostomum* to give rise to taxon differentiation as in the example of 2 isolates of *S. spectabilis* (Figure 2). *S. macrantha* (K.Koch). Stearn. (subgenus *Betonica*) clearly appears to be a member of a different genus and hence the tree confirms the recent suggestion of status change from subgenus *Betonica* to *Betonica* as a genus (Scheen et al., 2010; Bendiksby et al., 2011).

In summary, we have reported that the Turkish taxa of *Stachys* sect. *Eriostomum* are a monophyletic group with respect to morphological characters and ITS phylogenetics. Both approaches detected no synonyms, but suggested some status changes from subspecies level to species level, and some new records for Turkey. As for the intrasectional taxonomy, the phylogenetic analysis suggested a subsection classification different from that of morphological analysis. While the subsection *Creticae* can clearly be identified in the phylogenetic tree (Figure 2), it is hard to identify the other 2 sections, which appear to be combined. The subspecies of *S. cretica* also differed from morphological classification, being a polyphyletic rather than a monophyletic subspecies group. With this report, *Stachys* sect. *Eriostomum* has been revised for the first time based on morphological evaluation and ITS phylogenetic analysis.

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Appendix

Voucher specimens of the genus *Stachys* Sect. *Eriostomum* examined in the present study. These specimens were also used for genomic DNA extraction.

–*Stachys germanica* L. subsp. *heldreichii* (Boiss.) Hayek: Turkey. C2 Muğla: Ortaca, 5 m, 16.08.2009, *Akçiçek* 5374 & *Dirmenci*. –*S. bithynica* Boiss.: Turkey. A2 Bursa: Uludağ, 2050 m, 06.09.2007 *Akçiçek* 4780. –*S. tymphaea* Hausskn.: Turkey. A1 Kırklareli: İğneada-Limanköy road junction, 8 m, 31.05.2007, *Akçiçek* 4598 & *Dirmenci*. –*S. thracica* Davidov: Turkey. A1 Kırklareli: Armutveren, 380 m, 21.06.2009 *Akçiçek* 5291 & *Dirmenci*. –*S. alpina* L. subsp. *macrophylla* (Albov) R.Bhattacharjee: Turkey. B1 Balıkesir: Alaçam Mountains, 800 m, 25.07.2007, *Akçiçek* 4771 & *Dirmenci*. –*S. balansae* Boiss. & Kotschy: Turkey. B9 Ağrı: Tahir village, 2450 m, 12.08.2007, *Dirmenci* 3547. –*S. carduchorum* (R.Bhattacharjee) Rech.f.: Turkey. B9 Van: Çatak, Kavuşşahap Mountain, 2750 m, 24.07.2009, *Akçiçek* 5335 & *Dirmenci*. –*S. rizeensis* R.Bhattacharjee: Turkey. A8 Rize: Çamlıhemşin, 2500 m, 04.09.2008, *Akçiçek* 5235 & *Dirmenci*. –*S. huber-morathii* R.Bhattacharjee: Turkey. A5 Çorum: Kırkdilim gorge, 1150 m, 10.07.2009, *Akçiçek* 5175 & *Dirmenci*. –*S. pinetorum* Boiss. & Balansa: Turkey. Osmaniye: Amanos Mountains, 850 m, 09.07.2007, *Akçiçek* 4757 & *Dirmenci*. –*S. obliqua* Waldst. & Kit.: Turkey. B1 Balıkesir: Madra Mountain, 300 m, 29.06.2007, *Akçiçek* 4659 & *Dirmenci*. –*S. minor* (Boiss.) Akçiçek & Dirmenci. Turkey. C6 Hatay: Yayladağı, 500 m, 20.07.2009 *Akçiçek* 5319 & *Dirmenci*. –*S. sericantha* P.H.Davis: Turkey. C3 Antalya: Kemer, Ovacık village, 1200 m, 08.06.2007, *Akçiçek* 4624 & *Dirmenci*; Beldibi, 10 m, 12.06.2010, *Akçiçek* 5496 & *Dirmenci*. –*S. tmolea* Boiss.: Turkey. B1 Balıkesir: Kazdağı, 1750 m, 27.07.2007 *Akçiçek* 4779 & *Dirmenci*. –*S. cretica* L. subsp. *cassia* (Boiss.) Rech.f.: Turkey. C6 Osmaniye: Amanos Mountains, Yarpuz, 850 m, 09.07.2007, *Akçiçek* 4758 & *Dirmenci*. –*S. cretica* L. subsp. *garana* (Boiss.) Rech.f.: Turkey. C6 Kahraman Maraş: Başkonuş Mountain,

1250 m, 10.07.2007, *Akçiçek* 4763 & *Dirmenci*. –*S. cretica* L. subsp. *lesbiaca* Rech.f.: Turkey. A1 Çanakkale: Çan, 300 m, 11.06.2007, *Akçiçek* 4645 & *Dirmenci*. –*S. cretica* L. subsp. *trapezuntica* Rech.f.: Turkey. A7 Trabzon: Maçka, 400 m, 07.07.2010, *Akçiçek* 5489 & *Dirmenci*. –*S. cretica* L. subsp. *bulgarica* Rech.f.: Turkey. A1 Tekirdağ: Malkara, 250 m, 20.06.2009, *Akçiçek* 5287 & *Dirmenci*. –*S. cretica* L. subsp. *cretica*: Turkey. A1 Çanakkale: 23 km from Keşan to Gelibolu, Koru mountain, 50 m, 29.05.2007, *Akçiçek* 4543 & *Dirmenci*. –*S. cretica* L. subsp. *salviifolia* (Ten.) Rech.f.: Turkey. A1 Kırklareli: Pınarhisar, Poyralı village, 200 m, 31.05.2007, *Akçiçek* 4600 & *Dirmenci*. –*S. cretica* L. subsp. *vacillans* Rech.f.: Turkey. C3 Antalya: Kemer, Ovacık village, 1200 m, 08.06.2007, *Akçiçek* 4628 & *Dirmenci*. –*S. cretica* L. subsp. *smyrnaea* Rech.f.: Turkey. C2 Muğla: Marmaris, 120 m, 09.06.2007, *Akçiçek* 4638 & *Dirmenci*. –*S. cretica* L. subsp. *mersinaea* (Boiss.) Rech.f.: Turkey. C5 Mersin: Kuzucubelen, 550 m, 16.08.2009, *Akçiçek* 5376 & *Dirmenci*. –*S. cretica* L. subsp. *anatolica* Rech.f.: Turkey. B3 Afyonkarahisar: Şuhut, Kumalar Mountain, 1250 m, 07.06.2007, *Akçiçek* 4610 & *Dirmenci*. –*S. cretica* L. subsp. *kutahyensis Akçiçek*: Turkey. B2 Kütahya: 20 km from Tavşanlı to Harmancık, 850 m, 07.06.2007, *Akçiçek* 4609 & *Dirmenci*. –*S. byzantina* K.Koch: Turkey. A4 Kastamonu: Ilgaz Mountain, 1740 m, 10.07.2009, *E.Erdoğan* 1003. –*S. vuralii* Yıldız, Dirmenci & Akçiçek: Turkey. A4 Bartın: Road from Bartın to Cide, 3 km, W of Kurucaşile, 100 m, 04.08.2007, *Yıldız* 16553, *Dirmenci* & *Bräuchler*. –*S. thirkei* K.Koch: Turkey. A2 Bursa: İnegöl, 1150 m, 12.07.2008, *Akçiçek* 5211 & *Dirmenci*. –*S. spectabilis* Choisy ex DC.: Turkey. B8 Erzurum, Pasinler, 1680 m, 12.08.2007, *Dirmenci* 3539. C10 Hakkari: 10 km from Şemdinli to Yüksekova, 1700 m, 05.09.2007, *Dirmenci* 3583. –*S. longispicata* Boiss. & Kotschy. Turkey. C6 Kahraman Maraş: Göksun, 1360 m, 19.07.2008, *Akçiçek* 5183 & *Dirmenci*. –*S. viticina* Boiss.: Turkey. C5/6 Hatay: Yayladağı, 400 m, 08.07.2007, 400 m, *Akçiçek* 4746 & *Dirmenci*. –*S. huetii* Boiss.: Turkey. B8 Erzurum: Palandöken Mountain, 2500 m, 28.06.2008, *Akçiçek* 5134 & *Dirmenci*. –*S. bayburtensis* R.Bhattacharjee & Hub.-Mor. Turkey. A8 Bayburt: 36 km Bayburt to Aşkale, Kop mountain pass, 2030 m, 28.06.2008, *Akçiçek* 5136 & *Dirmenci*.

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