Stachys vuralii (Lamiaceae), a new species from north Anatolia, Turkey

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Stachys vuralii Yıldız, Dirmenci & Akçiçek (Lamiaceae), a new species of the section Eriostomum is described from north Anatolia, Turkey. Detailed illustrations and taxonomic comments are provided along with a table listing the differential characters to the closely related S. byzantina and S. thirkei. Delimitations towards S. byzantina and S. thirkei and existence of putative hybrids are discussed. A phylogenetic analysis using ITS of nuclear ribosomal DNA confirmed the status of S. vuralii as a distinct species. The geographical location of S. vuralii is also presented.

With ca. 300 taxa, Stachys is among the largest genera of Lamiaceae. This cosmopolitan genus is centered in the warm temperate regions of the Mediterranean and SW Asia, with secondary centers in North and South America and southern Africa. The majority of the species grow in rocky places, mainly on limestone and other basic rocks (Bhattacharjee 1974, 1980, Harley et al. 2004).

In Turkey, 82 species (107 taxa) are found representing two subgenera and 15 sections. Fifty-one (48%) of these taxa are endemic to Turkey, mostly constituting part of the East Mediterranean floristic elements. After the generic revision by Bhattacharjee (1982), 12 new taxa have been described from Turkey (Bhattacharjee 1974, 1980, Davis et al. 1988, Yıldız & Tan 1988, Gemicî & Leblebici 1988, Sümbül 1990, Duman 2000, Dinç & Doğan 2006, İlçim et al. 2008, Daşkın et al. 2009, Akçiçek 2010).

Although various features, including pollen morphology, can be used for differentiating some Stachys taxa (Salmaki et al. 2008), utilization of ITS phylogeny in plants has been effective (Baldwin et al. 1995, Álvarez & Wendel 2003) and it has been used for Lamiaceae in multiple reports (e.g. Steane et al. 1999, Prather et al. 2002, Bräuchler et al. 2010, Dirmenci et al. 2010). In this study, we utilized ITS phylogeny along with morphological and karyological analyses to assess the taxonomic position of an unidentified specimen. Combined results suggested it was a new species in the genus Stachys, sect. Eriostomum. A comprehensive revision involv-
ing morphology and ITS phylogeny of *Stachys* sect. *Eriostomum* completed by us (unpubl. data) further confirmed these results.

**Material and methods**

**Specimen collection**

During an expedition to north Anatolia in the context of a revisionary study of *Stachys* sect. *Eriostomum* in August 2007, some unusual specimens of *Stachys* were collected in the Bartın province (Fig. 1). The specimens were examined using relevant literature (Koch 1848, Ball 1968, Knorring 1977, Bhattacharjee 1982, Rechinger 1982, Davis *et al.* 1988, Baden 1991, Duman 2000). Extensive herbarium studies were performed on relevant specimens collected previously from Turkey and the adjacent countries in addition to specimens housed in the herbaria ANK, AEF, BM, E, EGE, G, HUB, ISTE, ISTF, K, W, and WU. As a result, the Bartın specimens were confirmed to represent a new species with morphological affinities to *S. byzantina* and *S. thirkei*.

**Chromosome analysis**

Cytological observations on *S. vuralii*, *S. byzantina* and *S. thirkei* were made on mitotic metaphase cells of root tips obtained from germinating seeds. Root tips were pre-treated for 16 h in α-monobromonaphthalene at 4 °C and washed and fixed in Carnoy solution (3:1 absolute ethanol:glacial acetic acid) overnight. The root tips were hydrolyzed for 10 min in 1 N HCl at room temperature, washed with distilled water and stained in 2% aceto-orcein for 2 h. Stained root tips were then squashed in a drop of 45% acetic acid and permanent slides were made by mounting in Depex.

**Genomic DNA isolation, PCR and sequencing**

Total genomic DNA isolation was performed using Plant DNeasy kit (Qiagen GmbH, Hilden, Germany). PCR was run using the published ITS primers (White *et al.* 1990, Sang *et al.* 1995) with the following protocol in a Thermo Px2 Thermal Cycler (Thermo, U.S.A.): 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. The primers used to amplify the ITS regions were also used for sequencing at RefGen Inc. (Ankara, Turkey) using an ABI 3130XL Genetic Anaylzer (Applied Biosystems, Fostercity, CA) with a BigDye Cycle Sequencing kit (Applied Biosystems, Fostercity, CA). ITS sequences were generated in two independent sequencing reactions for each of the triplicates sampled for each species. No sequence divergence was observed within species, thus only one representative sequence of each species

![Fig. 1. Geographical distribution of *Stachys vuralii*, *S. byzantina* and *S. thirkei* in Turkey.](image-url)
was included in the phylogenetic analysis. The vouchers used for the genomic DNA extraction are as follows: S. byzantina (EA 4658, herb. Akçiçek), S. thirkei (EA 5209, herb. Akçiçek), S. vuralii (BY 16353, herb. Dirmenci).

Phylogenetic analysis

Alignment of the ITS sequences was generated using BioEdit (Hall 1999). ITS sequences of S. byzantina and S. thirkei were also searched with the BLAST program (Altschul et al. 1990) in the non-redundant nucleotide database of GenBank (NCBI), and the two most similar taxa to each were picked along with other (Sideritis) similar hits to construct a phylogenetic tree, which was inferred with the Neighbor-joining method (Saitou & Nei 1987) and constructed with MEGA4 software (Tamura et al. 2007). Stachys germanica subsp. heldreichii and S. tmolea both morphologically less similar to S. vuralii than S. byzantina and S. thirkei were included in the phylogenetic tree and sequence comparison.

Results

Stachys vuralii Yıldız, Dirmenci & Akçiçek, sp. nova (sect. Eriostomum) (Fig. 2)

Species Stachys byzantinae affinis, sed ab foliis oblongis versus ellipticis et discoloris (non oblonge-spathulatis versus lanceolatis et concoloris), rugosis, basi rotundatis ad subcordatis (non attenuatis vel raro rotundatis) calycis 5.5–8 mm longis (non 8–10 mm longis), calycis dentis valde recurvus et dense glandulosis (non erectis ad leviter recurvus et glandulosis vel raro spar- sim glandulosis) differt.

Holotype: Turkey. A4 Bartın: Road from Bartin to Cide, 3 km W of Kurucasile, 41°50′12″N, 32°42′10″E, 100 m, Pinus brutia forest clearings, growing in calcareous gravel along roadside. 41°50′N, 32°42′E, 100 m, 4.VIII.2007 Yıldız 16553, Dirmenci & Bräuchler (holotype GAZI; isotypes C, EGE, G, HUB, ISTE, K, M, W, herb. Bräuchler). Paratype: A4 Kastamonu: Road from Cide to Sinop, just W of Doganyol. 42°00′21″N, 33°27′33″E, 50 m, Cide, Doğanyol, Pinus brutia forest clearings, 50 m, 5.VIII.2007 Yıldız 16556, Dirmenci & Bräuchler (herb. Dirmenci, M and herb. Bräuchler keep isotypes and paratypes as well).

Etymology: This species is named in honour of the eminent Turkish botanist Prof. Dr. Mecit Vural who is an expert of conservation biology of endemic plants in Turkey.

Perennial mesophytic herb, many-stemmed from base. Flowering stems 30–100 cm, usually branched above, rarely simple, densely adpressed tomentose to adpressed lanate-villous. Leaves 5–8 pairs per stem, 2–7 × 0.7–3 cm, oblong to broadly elliptic, diminishing from base to inflorescence, discolored, greenish and shortly sericeo-tomentose above; white floccose-tomentose beneath, crenulate, obtuse, rarely acute, rounded to subcordate at base, usually with 0.5–2.5 cm long petiole, except uppermost. Floral leaves sessile, lanceolate to linear, lower 1–3 times longer than verticillasters, upper shorter than verticillasters. Verticillasters (2–)3–9, lower ones distant to 2.5 cm, uppermost (2–3) usually congested, (8–)10–18 flowered. Bracteoles lanceolate to linear lanceolate, 2.5–5 mm, as long as or shorter than calyx tube, tip not spinescent, densely villose, sparsely glandular hairy. Pedicels 0.5–1.5 mm. Calyx 5.5–8 mm, sub-bilobate, subcampanulate, densely villose and glandular papillate with sessile glands; mouth densely long villose; teeth 1.5–2.5 mm, triangular-lanceolate, 1/3–1/4 × tube, strongly recurved at and after anthesis, densely glandular papillate, tip spinescent. Corolla 10–12 mm, purplish-pink, tube slightly exserted from calyx, upper lip densely sericeous-tomentose outside, hairs longer than lip, lower lip 3-lobed, middle lobe much larger than lateral lobes; style not exceeding upper lip, glabrous, 2-lobed, lobes unequal; stamens 4, included in corolla; filaments villose towards thecae. Nutlets broadly obovate to ± rounded, faintly trigonous, 2–2.2 × 1.5–1.8 mm, slightly winged near base, glabrous, slightly tuberculate at apex, blackish-brown at maturity. Flowering and fruiting July–August.

Distribution and habitat ecology: Stachys vuralii is endemic to Bartın province (Fig. 1), north Anatolia, belonging in the Euro-Siberian element. It grows in Pinus brutia forest clearings at 100–230 m where a mixture of Euro-Siberian and Mediterranean elements is present. Stachys vuralii was growing with Clinopodium nepeta subsp. glandulosum, Cistus creticus, Arbutus sp., Rubus sp., Sideritis sp. and Pteridium aquilinum.
Karyology: *Stachys byzantina* (Fig. 3a), *S. vuralii* (Fig. 3b) and *S. thirkei* (Fig. 3c) have the somatic chromosome number (2n) of 30, suggesting the basic chromosome number (x) to be 15.

Phylogenetic analysis and DNA sequence comparison: As can be seen from the DNA (ITS) sequence comparison (Fig. 4), *S. vuralii* differed from *S. byzantina* and *S. thirkei* in eight and nine nucleotides, respectively. BLAST search results revealed that the most similar two GenBank records for *S. byzantina* (this study) were *Sideritis glauca* (gi15429097) and *Sideritis algarviensis* (gi15429090), while *Sideritis tragoriganum* (gi15429106) and *Sideritis murganetana* (gi15429103) were the most similar to *Stachys vuralii*. Likewise, the most similar two GenBank records for *Stachys thirkei* (this study) were *Stachys hirta* (gi15429110) and *Sideritis discolor* (gi114796956). Hence the BLAST analysis revealed that *Stachys vuralii* was distinct. A phylogenetic analysis using all of the above-mentioned taxa along with *Phlomis lychnitis* (gi61098615) as an outgroup further confirmed the result (Fig. 4).
Fig. 3. Somatic metaphase chromosomes. — a: Stachys byzantina 2n = 2x = 30. — b: Stachys vuralii 2n = 2x = 30. — c: Stachys thirkei 2n = 2x = 30.

Fig. 4. Sequence alignment and phylogenetic analysis of Stachys vuralii. The upper panel shows the ITS sequence alignment of S. vuralii with S. thirkei and S. byzantina and with the less closely related S. germanica subsp. heldreichii and S. tmolea. Differing nucleotides are shown for each taxon. The alignment was constructed using BioEdit (Hall 1999). The lower panel shows the phylogenetic relationship of S. vuralii. The phylogenetic tree was inferred using the Neighbor-joining method (Saitou & Nei 1987). The bootstrap values (1000 replicates) are shown next to the branches (Felsenstein 1985). The phylogenetic distances were computed using the Maximum Composite Likelihood method (Tamura et al. 2004). There were a total of 346 nucleotides in the final dataset. Phylomis lycnitis was used as an outgroup. Accession numbers of the sequences obtained from GenBank are shown in parentheses and the sequences obtained in the present study are also shown in the upper panel.
Table 1. Morphological comparison of *Stachys vuralii*, *S. byzantina* and *S. thirkei*.

<table>
<thead>
<tr>
<th>Characters</th>
<th><em>S. vuralii</em></th>
<th><em>S. byzantina</em></th>
<th><em>S. thirkei</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem indumentum</td>
<td>densely adpressed toadpressed lanate-villos</td>
<td>densely lanate-villos to floccose-tomentose</td>
<td>densely grey tomentose to sparsely tomentose</td>
</tr>
<tr>
<td>Leaf shape</td>
<td>oblong to broadly elliptic</td>
<td>oblong-spatulate to broadly lanceolate</td>
<td>oblong spathulate to broadly lanceolate</td>
</tr>
<tr>
<td>colour</td>
<td>discolourous</td>
<td>densely sericeous concolourous</td>
<td>concolourous</td>
</tr>
<tr>
<td>indumentum base</td>
<td>shortly sericeous tomentose above, white floccose tomentose below</td>
<td>grey-tomentose to sparsely tomentose above, densely tomentose below</td>
<td>grey-tomentose to sparsely tomentose below</td>
</tr>
<tr>
<td>Floral leaves</td>
<td>ovate to lanceolate, green</td>
<td>lanceolate, green</td>
<td>ovate, generally purplish at base</td>
</tr>
<tr>
<td>Calyx length (mm)</td>
<td>5.5–8 densely villous with glandular papillate</td>
<td>8–10 lanate-tomentose, eglandular or a few glandular</td>
<td>8.5–12 densely villose, glandular papillate</td>
</tr>
<tr>
<td>Indumentum</td>
<td>1/4–1/3 × tube, strongly recurved in flowering and</td>
<td>1/4–1/3 × tube, erect to slightly recurved, eglandular or a few glandular papillate</td>
<td>1/3–1/2 × tube, reflexed to clearly recurved in fruiting time, a few to many glandular papillate</td>
</tr>
<tr>
<td>Corolla length (mm)</td>
<td>10–12</td>
<td>12–14</td>
<td>12–15(–17.5)</td>
</tr>
</tbody>
</table>

Discussion

*Stachys vuralii* resembles *S. byzantina* and *S. thirkei*, but differs from both in several characters (Table 1). Bhattacharjee (1982) indicated a specimen (Davis 38839) of *Stachys* collected from the Zonguldak province as a potential hybrid involving *S. byzantina*. Investigation of the specimen revealed that it is similar to *S. vuralii* by its ±cordate leaf base, rugose leaf surface and discoloration, while it resembles *S. byzantina* in having a densely lanate-villos to floccose tomentum on the stem, leaves and inflorescence, and ±congested verticillasters. Also, no pollen or seeds in the Zonguldak specimen from the Davis collection were observed. The collection might represent a hybrid or intermediate of *S. vuralii* and *S. byzantina*.

A karyological analysis (revealing a basic chromosome number x = 15 for *S. thirkei*, *S. byzantina* and *S. vuralii*) did not differentiate these taxa. A phylogenetic analysis and DNA (ITS) sequence comparison of *S. vuralii* with *S. byzantina* and *S. thirkei* along with other species (including *S. hirta*, *S. germanica* and *S. tmolea*), however, clearly supported *S. vuralii* as a distinct species (Fig. 4).
Acknowledgements

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