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RESEARCH ARTICLE

Anatomy, morphology, palynology and nutlet micromorphology of the rediscovered Turkish steno-endemic *Stachys longiflora* Boiss. and Bal. (Lamiaceae)

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Abstract

Stachys longiflora Boiss. & Bal, previously known only from the type gathering is a steno-endemic species from Turkey. The species was firstly collected by Balansa from Mersin-Guzeldere in 1855 and described by Boissier and Balansa in 1859. It was not collected again until 2011, when the author found it in Guzeldere (Mersin). Therefore, *S. longiflora* is treated under "Data Deficient" DD category in the Turkish Red Data Book. In this study, some anatomical and micromorphological characters, observations and measurements of *S. longiflora* are presented for the first time. The diagnostic morphological, anatomical, nutlet micromorphological and palynological characters of this steno-endemic species are discussed. Morphological characteristics of corollas, calyces, leaves, and stems are useful for specific delimitation in *Stachys*. Anatomical characters such as the shape of sclerenchymatic tissue and presence or absence of sclerenchymatic tissue in stems, mesophyll structures in leaves are of taxonomic significance. Moreover, the presence or absence of nutlets trichomes, shape and sculpturing pattern of nutlets are diagnostic characters. The emended and expanded description, ecology and phenology of this steno-endemic species are also presented along with its revised conservation status.

Keywords: Rediscovery, Stachys, steno-endemic, Turkey

Introduction

Stachys L. (Lamiaceae, subfamily: Lamioideae) a subcosmopolitan genus that is mainly centered in the temperate regions of Mediterranean and Southwest Asia and, secondarily, in North and South America and South Africa. It is one of the largest genera of Labiatae which contains about 370 species (435 taxa) around the world (Harley et al. 2004; Govaerts 2019). There are two centers of the genus in terms of the number of species. One of them is Southern and Eastern Anatolia, Caucasia, Northwestern Iran, Northern Iraq and the other is Balkan peninsula (Bhattacharjee 1974, 1980). Many species of this genus are distributed in rocky areas, especially on limestone rocks (Bhattacharjee 1974, 1980; Harley et al. 2004).

In the Flora of Turkey, *Stachys* was revised by Bhattacharjee (1982). Since then, 20 new species were described from Turkey. *Stachys* has 91 species (118 taxa) belonging to 15 sections and 2 subgenera in Turkey. Of the 118 taxa, 54(45.7 %) are endemic to Turkey (Bhattacharjee 1982; Davis et al. 1988; Duman 2000; Dinc & Dogan 2006; Ilcim et al. 2008; Akcicek 2010; Yildirimli 2010; Yilmaz et al. 2010; Dirmenci et al. 2011; Ozhatay et al. 2011; Dinc & Dogu 2016; Akcicek et al. 2016). The endemic taxa are mostly eastern Mediterranean elements. Turkey as a center of diversity of Stachys contains about 91 species (Celep & Dirmenci 2017).

Representatives of *Stachys* sect. *Fragilicaulis* R. Bhattacharjee distributed only Turkey, Iran, Iraq and Southern Caucasus. In Turkey, *Stachys* sect. *Fragilicaulis* which is divided into two subsection (*Fragiles* Rech. f., *Multibracteolatae* R. Bhattacharjee) is composed of 22 taxa, 15 of which are endemic. Members of *Stachys* subsect. *Fragiles* of which seven are endemic taxa were indicated as chasmophilous (dwelling in rock crevices or joints) and they are all distributed in the Taurus mountain range in

South Turkey. (Bhattacharjee 1982; Rechinger 1982; Davis et al. 1988; Duman 2000).

Stachys longiflora Boiss. & Bal. (subsect. *Fragiles*), a local endemic species for Turkey, was firstly collected by Balansa from the northern part of Mersin province 'in speluncis faucis Guzel Dere supra Sedichig in Cilicia littorali prope Mersina' in 1855 and described as a new species by Boissier and Balansa in 1859, but since that time any specimen has not been collected. Because the local name 'Guzel Dere' has been changed. The local name 'Guzel Dere' was only known by the vicinity people in that area, especially by the elderly. Nowadays, that area is known as 'Kizil Dere'. In 2011, in the scope of the taxonomical studies on the species belonging to subsect. *Fragiles*, some interesting specimens were collected on the field trip to Mersin-Kizil Dere region by the author.

The specimens collected from Kizil Dere (Mersin) were checked with descriptions given in both the "Flora of Turkey" and in the original description, also specimens compared with the herbarium specimens which kept in ANK, AEF, B, BM, E, G, GAZI, H, HUB, ISTE, ISTF, K and KNYA herbaria. As a result, the author identified the specimens collected from Kizil Dere-Mersin in 2011, as *S. longiflora*. Thus, *S. longiflora*, which had not been collected for the past 156 years, was rediscovered. The author has also determined that the local name 'Guzel Dere' is currently being used as 'Kizil Dere'.

This study aims to document the rediscovery of a population of the species, to give a detailed account of the anatomical, morphological, pollen and nutlet micromorphological characteristics of *S. longiflora*, and to evaluate their significance for the taxonomy of the genus.

Materials and Methods

The specimens that are belonging to *S. longiflora* were collected from Kizil Dere district of Mersin province. The collecting locality of the specimens is Turkey, C5 Mersin: Işıktepe village, Kizil Dere, limestone crevices and cave mouth, 250 m-350 m, 36°52´36 35N, 34°33´14 74E, 17 July 2011, S. Dogu 2730 and 07 October 2011, S. Dogu 2740, ibid.

Fifteen individuals of the *S. longiflora* were collected in Kizil Dere district, during the flowering and fruiting stage (Fig. 1). The voucher specimens were prepared according to standard herbarium techniques and stored in the Herbarium of the Selçuk University (KNYA). Other samples collected from natural habitats were fixed in 70% ethyl alcohol for anatomical studies. The studies were performed using 15 samples for species. Anatomical examinations, cross-sections of the stem and leaves were made together with the adaxial and abaxial surfaces of the leaves. An average of 25 slides was prepared for each type of section and stained with basic fuchsin for easy identification of the tissues. Well-stained sections covered with glycerin gelatin were made into permanent slides according to Vardar (1987). The stained preparations were examined with an Olympus BX50 microscope and photographed by Cameram imaging apparatus.

Some of the herbarium samples were used in the studies on pollen and nutlet morphological studies. The pollen morphology studies were made under the light and Scanning Electron Microscope (SEM). The studies of a Light Microscope (LM) were made on preparates which were prepared according to the Wodehouse (1935) technique. For this, the material on the microscope slide was mixed by a needle to distribute the pollens with dripping fused glycerin-gelatine with basic fuchsin. The preparate was preserved as covering with coverslip over it in which microscope slide was placed at the bottom until the gelatine freezes. Each of the measurements was made by pollen morphology with the help of ocular and objective micrometer on 30 pollen. For the SEM studies, pollen grains were put on aluminum stabs and were covered with gold using ZEISS LS-10 gold coating apparatus on low-vacuum mode at about 20 nm in thickness to observe their surface structures. Pollen grains were examined and micrographs were obtained at magnifications ranging from ×3000-×15,000 with a JEOL-JEM 2100 scanning electron microscope at ILTEK (Advanced Technology Search and Application Centre of Selçuk University, Konya) to determine the exine ornamentation. The terminology of pollen morphology is arranged according to Punt et al. (1994).

The studies about the nutlet morphology were made by stereomicroscope and SEM. Also, the mature nutlets were covered with gold on aluminium stab for determining the surface ornamentation for SEM studies. The measurements about the nutlet were made by the stereo binocular microscope on 20 mature nutlets.

Photographs of the preparates were taken with an Olympus BX-50 microscope. The stomatal index and stomatal index rate were calculated as described by Meidner and Mansfield (1968).

Results

Stachys longiflora Boiss. & Bal.

Type: TURKEY. C5 Mersin: in speluncis faucis Güzel Dere supra Sedichig in Cilicia littorali prope Mersina, 02 × 1855, Balansa 598. (holo: G!; iso: E!, K!).

Description: Perennial suffruticose herbs. Flowering stems 15 cm–65 cm, pendent, fragile at the base, usually

branched from base, very densely patent-pilose with short glandular and long eglandular hairs. Cauline leaves orbicular to widely ovate, (1.2–6.3) cm × (1–5.7) cm, margin dentate to crenate-dentate, apex narrowly to widely acute, usually cordate, rarely subcordate at base, thin and herbaceous, pilose, petiolate; petiole 1 cm–5 cm, eglandular pilose and short glandular hairs. Floral leaves similar to cauline leaves but smaller, (0.5–3.5) cm × (0.4–3.1) cm, margin dentate, apex acute, usually turuncate to subcordate, rarely widely cuneate at base, sparsely pilose, petiolate to subsessile; petiole 1 mm–13 mm. Verticillasters usually remote throughout, rarely uppermost approximate, 0.4 cm–2.1 cm distance, 1–2 flowered, ebracteolate. Pedicels 1 mm–7 mm, sparsely pilose with, capitate and peltate glandular hairs, rigid, erecto-patent. Calyx ± regular, infundibular to campanulate, mouth without hairy ring, 0.7–15 mm at flowering, up to 18 mm and scarcely broadening at fruiting, herbaceous, densely glandular and eglandular pilose with minutely sessile glands; teeth ± equal, 1/2 × tube, triangular-ovate to triangular lanceolate, glabrescent and spinescent tipped, mucro 0.5 mm–3 mm. Corolla white to pinkish, 20 mm–33 mm; lips streaked and spotted with pink inside, glandular with rarely sparsely pilose with peltate glands outside, minutely hairy inside; upper lip 2.1 mm and lower one 4.3 mm long; tube exserted, very narrowly cylindrical, exannulate, minutely hairy upper half inside and outside, Nutlets elongate, bright brown, elliptic-oblong, apex rounded (Fig. 1).

Phenology, habitat and ecology

Stachys longiflora is flowering in July-October and fruiting in August–November. This species grows on limestone crevices and mouths of caverns between attitudes of 250 m and 350 m. Other species in its habitat are *Teucrium odontites* Boiss. & Balansa, *T. lamiifolium* d'Urv., *T. kotschyanum* Poech, *Stachys rupestris* Bentham, *Origanum majorana* L., *Scutellaria rubicunda* Hornem subsp. *pannosula* (Rech.f.) J.R.Edm., *Ficus carica* L., *Pistacia terebinthus* L., *Hypericum lanuginosum* Lam., *Styrax officinalis* L.

Anatomical characteristics

Stem anatomy: General view of the stem is rectangular in the transverse section. The epidermis is covered by a thin and smooth cuticle and its consists of uniseriate squarish, rectangular, or oval cells. The epidermis contains sparsely eglandular and glandular trichomes. The collenchyma tissue is located at the corners of the stem and composed of 3–5 layered cells whose walls are thickened. There is a cortex layer between the collenchyma and endodermis tissue which is composed of 3–5 layers of rectangular and irregularly oval parenchymatic cells. The endodermis is regular, single-layered and composed of quadrate, subglobose and ovoid cells. However, endodermis tissue cells did not show a significant differentiation from the cortex parenchyma cells. Fibres of sclerenchyma are formed as bouquets which consist of 2–3 sheets and are seen as sickle in the corner. Vascular bundles are next to each other. The size of the vascular bundles at the corners is distinctly larger than the others. In the vascular tissue, xylem and phloem elements can be distinguished. The cambium between the xylem and phloem is inconspicuous. In the center of the stem, the pith is wide and filled with hexagonal, slightly pentagonal and orbicular parenchymatous cells with intercellular spaces (Fig. 2).

Mesophyll anatomy: In the cross-section, the lamina and mid-rib are seen to be covered by a regular layer of the epidermis. Both epidermis is composed of ovoid, quadrate and rectangular cells and both surfaces are covered with a thick layer of cuticle. The thickness of both epidermis cuticles is nearly equal. There are glandular

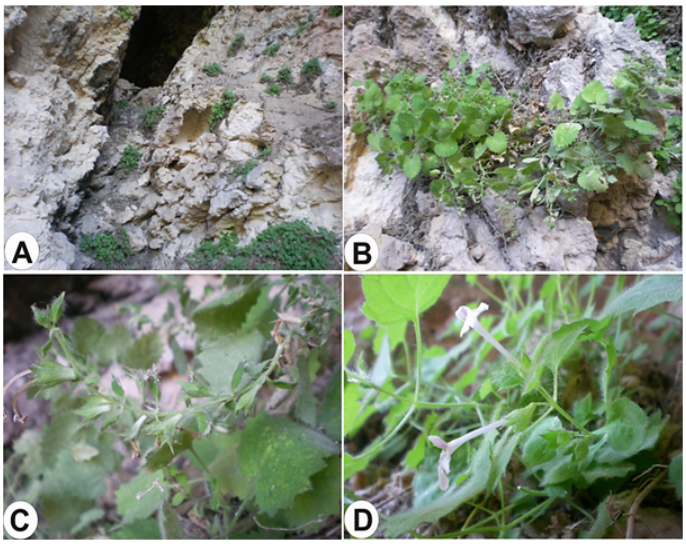

Figure 1. *Stachys longiflora* Boiss. & Bal. A: Natural habitat; B: General view in natural habitat; C: Inflorescence; D: Flowers. (All photographs by S. Dogu.)

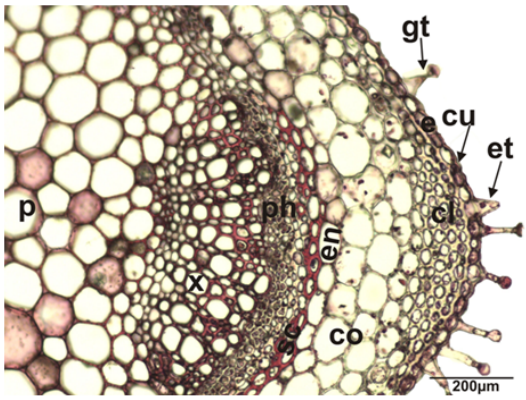

Figure 2. Transverse section of the stem of *Stachys longiflora*, **co:** cortex; **cl:** collenchyma; **cu:** cuticle; **e:** epidermis; **ph:** phloem; **p:** pith region; **sc:** sclerenchyma; **x:** xylem; **gt:** glandular trichome; **et:** eglandular trichome.

and non-glandular trichomes on both epidermis layers surrounding the abaxial and adaxial surfaces. Both leaf surfaces are covered by glandular and non-glandular hairs of the same type as seen on the stem. Epidermal cells whose thickened external walls are seen on both sides of the leaf. The leaf is dorsiventral and hypostomatic type. The mesophyll is composed of circular spongy parenchyma and cylindrical palisade parenchyma cells. Palisade parenchyma under the upper epidermis is composed of 1–3 layered cylindrical cells arranged perpendicular to the leaf lamina. The palisade parenchyma occupies about 60%–65% of the mesophyll. The spongy parenchyma cells are 2–3 layered under the palisade parenchyma tissue. There are more intercellular spaces between the spongy parenchyma cells. The mesophyll is thicker around the central vein. Mid-rib is triangle shaped and has a 3–4 layered collenchyma located below lower epidermal cells. The collateral vascular bundle is located in the central part of the midvein. Vascular bundles in the midvein are larger than the lateral ones and all vascular bundles are surrounded by a parenchymatic bundle sheath. The xylem layer is just below the collenchyma. 1–2 layered parenchyma and 3–4 layered collenchyma are located under the phloem (Fig.3).

Leaf surface anatomy: The leaf surface sections showed that the leaves are hypostomatic (the upper surface of the leaf lacks stomata) with diacytic type stomata and the wall of the upper epidermal cells is thicker than the one of the lower epidermal cells. The number of stomata is 98 per $mm^2 \pm 4$ per mm^2, and the size of the epidermal cells is 314 per $mm^2 \pm 7$ per mm^2 on the lower leaf surface. The stomatal index is 20.6. The stoma cells form a row below the epidermis (xeromorphic type). The sphaerocrystals were observed in some upper epidermal cells of leaves (Fig. 4).

Pollen Characteristics

Pollen grains of the S. longiflora are single, isopolar and tricolpate. The mean of a polar axis (P) and equatorial axis (E) is 33.12 µm ± 4.33 µm (29.84 µm–36.65 µm) and 15.0 µm ± 3.12 µm (13.91 µm–18.03 µm). The shape of pollen grains is prolate to perprolate (P/E=1.87 µm–2.4 µm). The colpi length (Cl) and colpus width (Clg) are 28.75 µm ± 3.98 µm and 2.25 µm ± 0.18 µm. The outline is elliptic in the equatorial view. The sculpturing of the exine in *S. longiflora* is micro-reticulate with perforations. The exine thickness is between 1.20 µm and 1.35 µm. The Intine thickness is 0.3 µm–0.5 µm. The ornamentation is retipilate. The width of the muri is 0.5 µm-0.6 µm and that of lumina is 0.8 µm–1.4 µm (Fig. 5).

Nutlet Micromorphology

Nutlets are elliptic-oblong-shaped. The apex of trigonous nutlets is obtuse in *S. longifolia*. Areoles are small and circular or square. Nutlets are bright brown and size ranges between 2.89 mm and 3.57 mm in length and 1.27 mm and 1.43 mm in width. Nutlet surfaces are reticulate-papillate. The reticulate pattern consists of small polygonal cells with papillate. The apex of the nutlet is long-haired which consists of 1–4 cells (Fig. 6).

Conservation Status

Stachys longiflora has not been collected since 1855, so it was treated as 'Data Deficiency' (DD) by Ekim et al. (2000). During a field study conducted in 2011, the author found the species at the Kizil Dere in Mersin province. According to the author's field observations, its current conservation status has been re-evaluated. The population size covers about 100 m by 50 m and is bisected by a paved road from Mersin to Asagi Keslik village. According to the IUCN criteria guidelines for

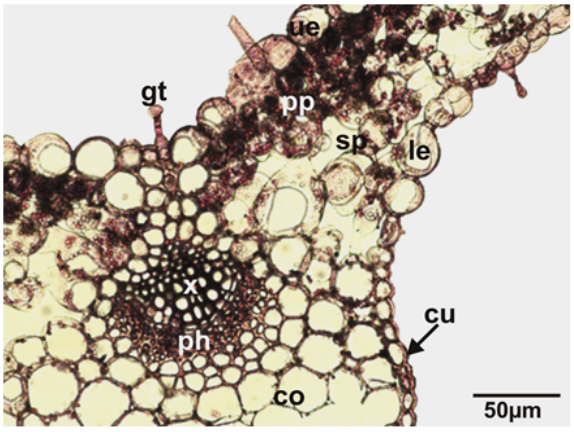

Figure 3. Transverse section of the leaves of *Stachys longiflora*. **cu:** cuticle; **gt:** glandular trichome; **le:** lower epidermis; **ue:** upper epidermis; **pp:** palisade parenchyma; **sp:** spongy parenchyma; **co:** collenchyma; **x:** xylem; **ph:** phloem.

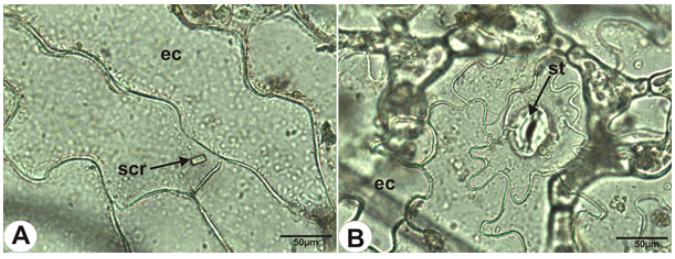

Figure 4. Upper and lower surface sections of the leaves of *Stachys longiflora*. **st:** stoma; **ec:** epidermal cell; **scr:** sphaerocrystal.

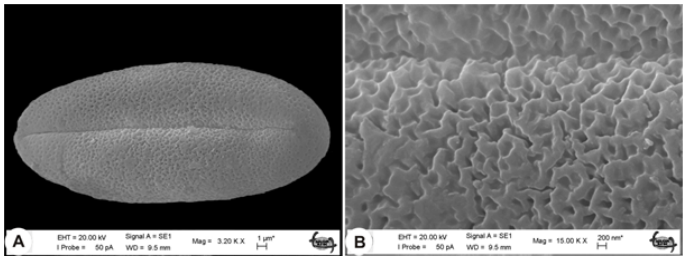

Figure 5. SEM micrographs of the pollen of *Stachys longiflora*. **A:** equatorial view; **B:** Exine sculpturing (S Dogu 2730).

their application (IUCN 2014), taking the criterion B1ab (iii) (EOO<100 km^2, fragmented locations due to road and declining area of occupancy) and D1 (number of mature individuals less than 100 in a population), the author strongly propose that *S. longiflora* should be recognized as Critically Endangered (CR) (Tab. 1 and Tab. 2).

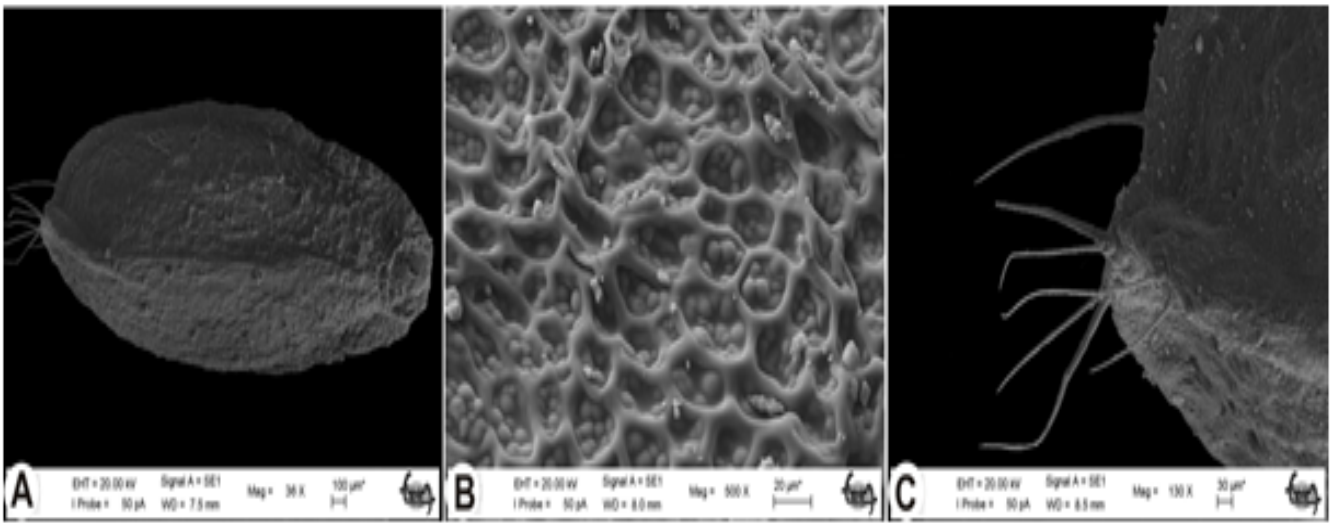

Figure 6. SEM micrographs of the nutlet of *Stachys longiflora*. **A:** general appearance; **B:** surface ornamentation; **C:** non-glandular trichomes (S Dogu 2740).

Table 1. Morphological characters of *Stachys longiflora* based on the present study and 'Flora of Turkey'.

	Present data (S Dogu 2730, 2740)	**Flora of Turkey (Bhattacharjee 1982)**
Stem (cm)	15-65	44-50
	branched from base	-
Cauline leaves (cm)	1.2-6.3 × 1-5.7	3-6 × 2.5-5.5
	orbiculare to widely ovate	ovate to ovate-orbicular
	margin dentate to crenate-dentate	margin broadly dentate
	apex narrowly to widely acute	apex acute
	usually cordate, rarely subcordate at base	cordate at base
	sparsely pilose	-
Petiole (cm)	1-5	3-4.5
Floral leaves (cm)	0.5-3.5 × 0.4-3.1	-
	margin dentate	-
	apex acute	-
	usually turuncate to subcordate, rarely widely cuneate at base	cuneate to truncate at base
	sparsely pilose	-
Petiole (mm)	1-13	2-9
Inflorescence (cm)	5-21	-
Verticillasters	usually remote throughout, rarely uppermost approximate	remote
	0.4 cm-2.1 cm distant	0.7 cm-1.2 cm distant
Pedicel (mm)	1-7	1-3
Calyx	infundibular to campanulate	infundibular
Calyx (in flower, mm)	0.7-15	10-13
Calyx (in fruit, mm)	up to 18	-
Calyx	densely glandular and eglandular pilose with minutely sessile glands	-
Calyx teeth	triangular-ovate to triangular lanceolate	-
Mucro (mm)	0.5-3	0.5-1
Corolla (mm)	20-33	22-32
	white to pinkish	purple
Lips	spotted with pink inside, glandular with rarely sparsely pilose with peltate glands outside	-
Upper lip (mm)	1.9-2.1	-
Lower lip (mm)	4.0-4.3	-

Table 2. Comparison of morphological characters of *Stachys longiflora* and closely related species.

	***S. longiflora* (Author data)**	***S. pseudopinardii* (Bhattacharjee 1982))**
Stem (cm)	brached from base, 15-65	unbrached, 30-55
	densely patent-pilose	sparsely patent-pilose
Cauline leaves (cm)	1.2-6.3 × 1-5.7	1-5 × 0.8-4.5
	apex narrowly to widely acute	apex obtuse
Petiole (cm)	1-4	2-5
Floral leaves (cm)	0.5-3.5 × 0.4-3	0.5-3 × 04-2.8
	usually truncate to subcordate, rarely widely cuneate at base	cordate to cuneate at base
Petiole (cm)	0.1-1.3	0.2-1.2
Verticillasters	1-2 flowered	4-6 flowered
Pedicels (mm)	1-7	2-2.5
Calyx	± regular	sub-bilabiate
Calyx teeth	± equal	unequal
Corolla (mm)	20-33	20-22

Discussion

Some *Stachys* taxa are sometimes highly polymorphic and morphological differences between similar species are often unclear. Particularly, due to the large differences in macromorphological characters under different ecological conditions, taxonomy of *Stachys* is very difficult (Abu-Asab & Cantino, 1994). Based on gross morphological characters, taxonomic delimitation and identification of species in the subsect (Rechinger 1982).

Taxa of the subsect. *Fragiles* are suffrutescent saxatile perennial herbs without basal rosettes. Flowering stems are pendent and below fragile. Indumentum is patent-pilose. Leaves are orbicular to ovate and ± cordate at base. Verticillasters are (2–)6–10(–16) flowered. Bracteoles are a few, inconspicuous and setaceous. Calyces are almost infundibular to subcampanulate, teeth triangular to triangular–lanceolate and softly spinescent at apex. The Corolla tube is long and exserted from the calyx tube. Nutlets are oblong-elliptic, apiculate and unwinged (Bhattacharjee 1980). *Stachys longiflora* exhibits these morphological features in general. Therefore, its taxonomic placement is correct in the subsect *Fragiles*.

Evaluation of obtained results revealed that stem, leaf and corolla characteristics of *S. longiflora* were found to be more variable than reported in 'Flora of Turkey' (Bhattacharjee 1982). Observations and measurements of some morphological characters of *S. longiflora* are presented here for the first time (Tab. 1). Bhattacharjee (1982) stated that *S. longiflora* was closely allied to *Stachys pseudopinardii* Bhattacharjee and Hub.-Mor., but a critical examination of *S. longiflora* reveals striking differences (Tab. 2). The stems of *S. longiflora* are brached from base, taller and densely patent-pilose, whereas those of *S. pseudopinardii* are unbrached from base, short and sparsely patent-pilose. The cauline leaves of *S. longiflora* are 1.2 cm-6.3 cm × 1 cm-5.7 cm and apex narrowly to widely acute, but those of *S. pseudopinardii* are 1 cm–5 cm × 0.8 cm–4.5 cm and apex obtuse. The floral leaves are 0.5 cm–3.5 cm × 0.4 cm-3 cm and usually truncate to subcordate, rarely widely cuneate at the base in *Stachys longiflora*, 0.5 cm-3 cm × 04 cm–2.8 cm and cordate to cuneate at the base in *S. pseudopinardii*. The verticillasters of *S. longiflora* are 1–2 flowered, whereas those of *S. pseudopinardii* are 4–6 flowered. Pedicels of *S. longiflora* are 1 mm–7 mm, but those of *S. pseudopinardii* are 2 mm–2.5 mm. The calyx of *S. longiflora* is ± regular and teeth ± equal, whereas those of *S. pseudopinardii* are sub-bilabiate and teeth unequal. Corolla of *S. longiflora* are 20 mm–33 mm, but those of *S. pseudopinardii* is 20 mm–22 mm (Tab. 2).

Metcalfe and Chalk (1950) stated that the stems of many genera and species of the family Lamiaceae are rectangular and the collenchymatic tissue covers a broad area at the corners, and a developed scleranchymatic tissue surrounds the vascular tissue. Stem of *S. longiflora* showed the typical four-angled and structure of Lamiaceae members, covered with a one-layered epidermis with trichomes, well-developed collenchyma at the angles of the stem and ring shaped sclerenchymatic tissue outside the vascular tissue. The same observations were reported during the anatomical studies on other family Lamiaceae species in Turkey (Uysal 1991, 2002, 2003; Kaya et al. 2000; Dinc & Ozturk 2008; Kahraman et al. 2009, 2010a, 2010b; Erdogan et al. 2012; Celep et al. 2014; Kaya et al. 2015; Atalay et al. 2016). However, Donmez et al. (2012), Temel et al. (2015) and Acar & Satil (2019) reported that *Stachys palustris* L., *Stachys byzantina* C. Koch, *Stachys bombycina* Boiss., *Stachys distans* Bentham var. *cilicica* R. Bhattacharjee and Hub.-Mor. and *Stachys distans* Benth. var. *distans* does not contain sclerenchymatic tissue surrounding the vascular tissue in the anatomical structure of the stems. The sclerenchyma forms an unceasing ring-shaped tissue or separate bundles in the stem anatomy of Labiatae members. The sclerenchyma forms a separate bundles

tissue in the stems of *S. longiflora*. These characters were reported to be used for separating sect. Ambleia species, especially close relatives (Dinc et al. 2008). Therefore, the sclerenchyma forms an unceasing ring-shaped tissue or separate bundles in the stem are useful characters for distinguishing the species.

Salmaki et al. (2011) reported that leaf anatomy provides valuable features that are useful in the classification of subgeneric classification as well as species delimitation in *Stachys*. These diagnostic characters have been described as the number of cell layers of palisade and spongy parenchyma, the shape of the transverse section, length of ventral/dorsiventral axis and type/thickness of collenchyma. Leaves of Lamiaceae members are generally dorsiventral or isobilateral (Metcalfe & Chalk 1950). This study showed that *S. longiflora* has a dorsiventral leaf. The same results have been also observed in the leaves of other *Stachys* taxa (Dinc & Ozturk 2008; Donmez et al. 2012; Temel et al. 2015; Kaya et al. 2015), but some *Stachys* specimens were observed to be isobilateral (Erdogan et al. 2012; Acar & Satıil 2019). According to the mesophyll structure, the leaves of *S. longiflora* have 1-3 layered palisade parenchyma under the upper epidermis and 2-3 layered spongy parenchyma under the palisade parenchyma. However, the leaves of *Stachys balansae* Boiss. & Kotschy ex Boiss. and *Stachys carduchorum* (R. Bhattacharjee) Rech.f. have 2 layered palisade parenchyma under the upper epidermis and 1 to 2 layered above the lower epidermis. In the center of mesophyll, there is 2 to 5 layered spongy parenchyma (Erdogan et al. 2012). Acar and Satil (2019) point out that leaves of *Stachys aleurites* Boiss. & Heldr. and *S. distans* var. distans has 2–3 layered palisade parenchyma under the upper epidermis and 4–5 layered spongy parenchyma under the palisade parenchyma those of *S. bombycina* has 1–2 layered palisade parenchyma under the upper epidermis and 3–4 layered spongy parenchyma under the palisade parenchyma, those of *S. distans* var. cilicica has 2 layered palisade parenchyma under the upper epidermis and 3–4 layered spongy parenchyma under the palisade parenchyma. Also, Kaya et al. (2015) reported that leaves of *Stachys rupestris* Bentham have 3–4 layered palisade parenchyma under the upper epidermis and 2–3 layered spongy parenchyma under the palisade parenchyma.

Moore et al. (1991) classified the pollen morphologies of *Stachys sylvatica* L., *Stachys palustris* L., *Stachys arvensis* (L.) L., *Stachys annua* (L.) L., *Stachys germanica* L., *Stachys alpina* L., *Stachys recta* L. and *Stachys officinalis* (L.) Trevisan under the group called *Stachys sylvatica* type. In this group, the pollen morphologies are trizonocolpate, pollen exines have reticulate ornamentation, and lumina are more or less uniform in size. Pollen morphologies of *S. longiflora* exhibit the features of *S. sylvatica* type as well. However, the pollen exine ornamentation is microreticulate in *S. longiflora*.

Salmaki et al. (2008) were examined nutlets of 31 taxa of *Stachys*, representing nine sections distributed in Iran, by Light and Scanning Electron Microscopy. Authors reported that, the basic shape of nutlets in most taxa is ovate, broad ovate, oval, triangular and oblong can also be found in few species. The nutlets of *Stachys longiflora* are elliptic-oblong and 2.89 mm–3.57 mm long and 1.27 mm × 1.43 mm wide, but the nutlet shapes of sect. *Fragilicaulis* species in Iran are ovate (Salmaki et al. 2008). The nutlet surface sculpturing pattern of *Stachys* taxa growing in Iran were studied and it was understood that these characters have a systematic value at the generic and specific levels (Salmaki et al. 2008). While the nutlets surface ornamentation of *S. longiflora* is a reticulate-papillate, sect. *Fragilicaulis* species in Iran are reticulate (Salmaki et al. 2008).

In the current study, trichomes were not found on the nutlets of any *Stachys* taxa (Salmaki et al. 2008; Satil et al. 2012). However, this study shows that the apex of the *S. longiflora* nutlets is long-haired which consists of 1-4 cells. This character is important for separating *S. longiflora* from the other members of sect. Fragilicaulis in Iran.

According to Bhattacharjee (1982), the flowering time of the species has been reported as of October. Field observations have shown that the species flowers from mid of June to early October. The altitude range belongs to *S. longiflora* has not been reported so far. Specimens were collected by the author only at an altitude of between 250 and 300 m. While the habitat of *S. longiflora* was reported in Flora of Turkey as "Mouth of limestone caves", the specimens were collected from the limestone crevices.

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