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Stachys istanbulensis (Lamiaceae) a new species from Turkey: evidence from morphological, micromorphological and molecular analysis

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Abstract: *Stachys* species show high morphological diversity and their main diagnostic characteristics are calyx and corolla length, ratio of tooth to calyx, and verticillaster arrangement. This manuscript describes and provides detailed morphological, micromorphological and molecular information about a new species, *Stachys istanbulensis* Ö. Güner, and its close relatives. The new species resembles *S. recta* complex, but is distinguished by its procumbent flowering stems, shorter calyx and teeth, congested verticillasters, creamy yellow corolla and oblong-obovoid nutlets. Moreover, the periclinal surfaces of *S. istanbulensis* nutlets are usually wrinkled, while those of *S. recta* are smooth. In addition, nrITS DNA region sequences were used to find the relationships of the new species to its close relatives and determine its correct phylogenetic position within section *Olisa*. Therefore, the newly described *S. istanbulensis* and its close relatives *S. recta* and *S. atherocalyx* comprise a clade (III). With this addition, the number of Turkish *Stachys* species has reached 94 (121 taxa), and 65 (53.7%) of these taxa are endemic.

Key words: Classification, endemic, İstanbul, phylogeny, systematic, taxonomy

1. Introduction

Stachys L., one of the largest genera in Lamiaceae, consists of 370 species with more than 475 taxa (POWO, 2022). The genus has a subcosmopolitan distribution and many of its species grow in warm temperate regions of the Mediterranean and southwestern Asia. *Stachys* species are also found in Europe, North and South America, but are absent from Australia and New Zealand. The genus consists of annual, perennial herbs and subshrubs, which grow in various environmental conditions such as forests openings, rocky places, limestone, steppe, fallow fields, meadows and stream banks (Bhattacharjee, 1980).

In the most comprehensive revision of *Stachys* to date, Bhattacharjee (1980) revised the Old World species. She described new sections and species, accepted *Betonica* L. as a subgenus and assigned types to previously identified sections of the genus. *Stachys* has been reported to be polyphyletic because some of its species are more closely related to *Gomphostemma, Phlomidoschema, Prasium* and *Sideritis* (Lindqvist and Albert, 2002). Recent phylogenetic studies (Scheen et al., 2010; Roy et al., 2013) based on nuclear ribosomal and plastid DNA data have shown it to be paraphyletic, subdividing it into two clearly supported clades. The first lineage forms its center of diversity in the eastern part of the Mediterranean region, migrating over time to Western Asia, Western Europe and Macaronesia, and sub-Saharan Africa, while the second clade includes the Hawaiian mints, *Suzukia*, all New World *Stachys* species and some Old World species. Moreover, Scheen et al. (2010) reestablished *Betonica* as a genus.

Turkey is the richest country in the world in terms of the number of *Stachys* taxa, making it the center of diversity for this genus. Other territories where *Stachys* is actively studied include the former Soviet Union (which has 50 native species), Greece (38), Iran (32) and Iraq (24) (Hedge, 1986; Constantinidis et al., 2015; Güner, 2022). *Stachys*, the most diverse and speciose genus of the family Lamiaceae in Turkey (Güner and Ferrer-Gallego, 2021), is represented in the country by 93 recognized species (120 taxa), 64 (53.3 %) of which are endemic (Akçiçek and Güner, 2022). They are primarily distributed in the Mediterranean floristic region of Turkey. Some *Stachys* species with high morphological diversity, such as *S. annua* (L.) L., *S. cretica* L., *S. iberica* M.Bieb. and *S. lavandulifolia* Vahl, which are represented by a few infraspecific taxa, are widely distributed in Turkey, while *S. kurdica* Boiss. & Hohen. has a narrower distribution in Southeast Turkey, but still exhibits high morphological variability (Bhattacharjee, 1982; Güner et al., 2019).

Alongside morphological studies (Dinç and Doğu, 2015; Akçiçek et al., 2016; Doğu and Bağcı, 2021; Fırat, 2021;

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Güner et al., 2021), recent works have used karyological (Martin et al., 2011, 2016), micromorphological (Salmaki et al., 2008; Satıl et al., 2012; Karaismailoğlu and Güner, 2019, 2021) and phylogenetic (Lindqvist and Albert, 2002; Dündar et al., 2013; Roy et al., 2013; Salmaki et al., 2013) analyses to identify and distinguish *Stachys* species exhibiting high morphological variability. The nuclear ribosomal internal transcribed spacer (nrITS) and plastid genome have proven useful in recent phylogenetic studies on the genus (Bendiksby et al., 2014; Berumen-Cornejo et al., 2017). nrITS sequences have been used to separate *Stachys* taxa in revision studies and new species descriptions. (Scheen et al., 2010; Roy et al., 2013; Salmaki et al., 2019). Based on morphological, micromorphological and phylogenetic investigation, *Stachys istanbulensis* Ö.Güner is described as a new species. In addition, the position of the new species within the section *Olisia* Dumort. and closely related species were determined.

2. Materials and methods

2.1. Morphological studies

Stachys specimens collected from İstanbul Province in Turkey were compared with descriptions from the relevant literatures, including Florula Belgica (Dumortier, 1827), Mountain Flora of Greece (Strid and Tan, 1991), Flora Europaea (Ball, 1972), Flora of Turkey (Bhattacharjee, 1982; Davis et al., 1988) and the recent literature (Akçiçek, 2020). After the specimens were found to be distinct from these descriptions, type specimens of *Stachys* in the herbaria of ANK, BM, HUB, E, EGE, GAZI, ISTE, ISTF, ISTO, K, W, and WU were examined (acronyms according to Thiers, 2022).

2.2. Micromorphological studies

For investigation of micromorphological features, nutlet and pollen grain samples were fixed on a stub with silver epoxy, coated with platinum and gold and examined using a JEOL Neoscope-5000 scanning electron microscope. Photographs were taken using an incorporated Zeiss LS-10 camera. The terminology of Erdtman (1952) and Faegri and Iversen (1975) were used to describe the micromorphological features of the pollen. Nutlet characters were described according to various works (Bojňanský and Fargašová, 2007; Stearn, 1992; Salmaki et al., 2008; Satıl et al., 2012; Karaismailoğlu and Güner, 2019; Celep et al., 2022).

2.3. Phylogenetic studies

Internal transcribed spacer (ITS) sequences of *Stachys istanbulensis* sp. nov. (GenBank: ON922908) were obtained for this study, and compared to sequences of 17 *Stachys* species and one outgroup (*Sideritis*) obtained from NCBI. DNA was isolated using DNeasy Plant Mini Kit (Qiagen, Germany), following the manufacturer's instructions. ITS regions were amplified using primers ITS4 and ITS5 (White et al., 1990). PCR conditions set to 95 °C for 5 min initial denaturation, 35 cycles of 94 °C for 1 min denaturation, 50 °C for 1 min annealing, 72 °C for 1 min extension, and 72 °C for 10 min final extension. PCR products were visualized in agarose gel. The amplified fragments were sequenced using the same PCR primers by Genoks. The obtained sequences were assembled, edited using BioEdit v.7.2.0 (Hall, 2018) and submitted to a search for similarity in GenBank (the numbers are given in Bayesian tree) using the Blast program. Phylogenetic trees were constructed using the maximum parsimony (MP) and Bayesian interference methods. The GTR+I+G model was selected with jModelTest 2.1.10 (Darriba et al., 2012) for the Bayesian analysis, performed using MrBayes v.3.2 (Ronquist et al., 2012). The BI analyses were conducted using four simultaneous runs of Metropolis-coupled Markov chain Monte Carlo (MCMC) sampling for 106 generations, and one tree was sampled every 1000 generations, with the default of three "heated" and one "cold" chain and a random starting tree (Rambaut and Drummond, 2007). Twenty-five percent of trees were discarded as burn-in, and the remaining trees were used to construct a 50% majority-rule consensus tree and posterior probabilities (PP). The MP analysis involving a heuristic search was conducted in PAUP* version 4.0.164 (Swofford, 2002). Characters were unordered and equally weighted. All gaps were treated as missing data. Bootstrap values were calculated from 1000 replicates with random addition sequence and tree bisection-reconnection (TBR) branch swapping. Consistency index (CI), retention index (RI), and rescaled consistency index (RC) were also calculated. Trees were visualized in FigTree v.1.4.3.

3. Results

3.1. Morphological results

Stachys istanbulensis Ö.Güner **sp. nov**. (Figure 1).

Type: Turkey. İstanbul, Arnavutköy, Karaburun, Black Sea shore, sandy places, 09.06.2019 *Ö. Güner* 3103 (holotype GAZI, isotypes ANK, HUB).

Diagnosis: *Stachys istanbulensis* differs from *S. recta* complex with its procumbent flowering stems, shorter calyx and teeth, congested verticillasters, creamy yellow corolla and oblong-obovoid nutlets.

Description: Suffrutescent perennial herbs. Flowering stems procumbent, usually unbranched, 15–35 cm long, sparsely and retrorsely pubescent with sessile glands. Basal leaves oblong-ovate, 1–1.8 × 0.5–1 cm, margin faintly crenate, apex obtuse, cuneate to attenuate at base, hairy as the flowering stem, petiole 0.3–1.7 cm long. Cauline leaves oblong-lanceolate to linear-lanceolate, sometimes oblanceolate, 0.5–2.3 × 0.2–0.8 cm, margin faintly serrate to subentire, apex acute, usually not spinescent, sometimes

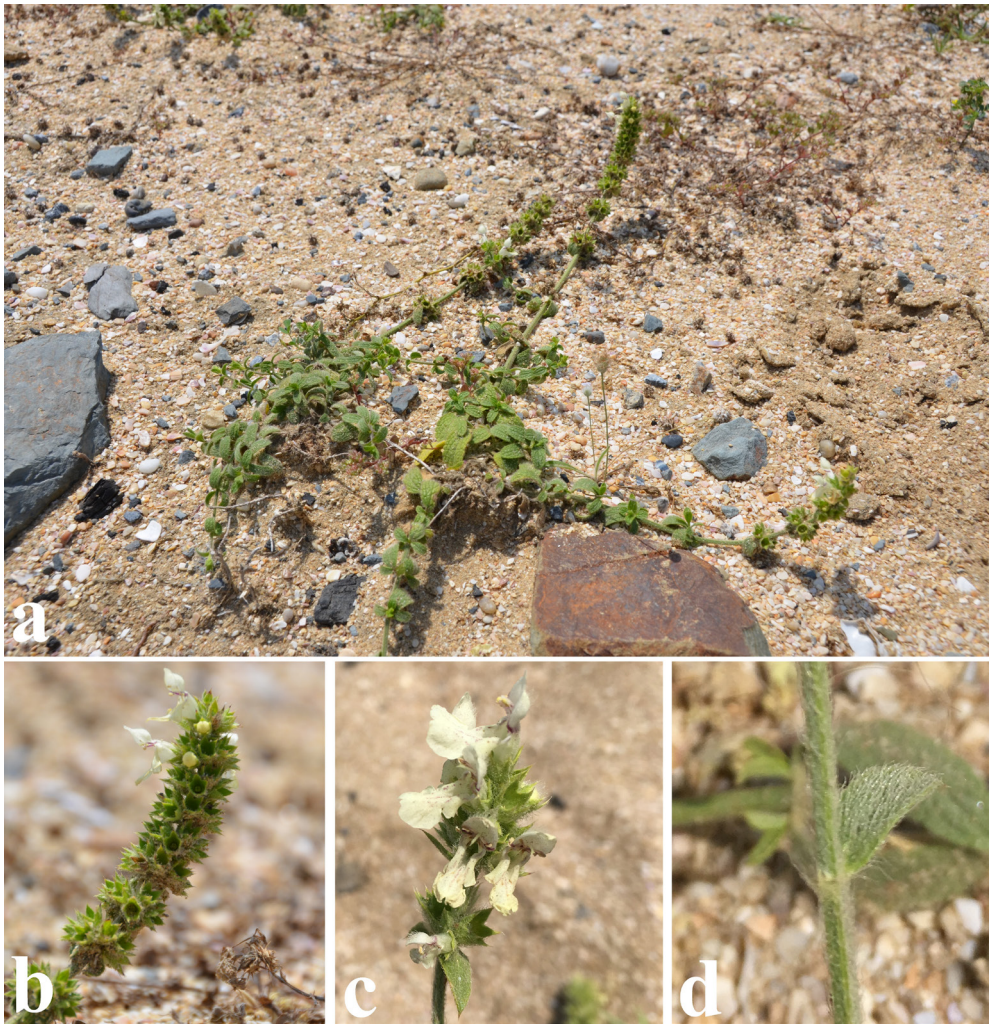

Figure 1. The new species *Stachys istanbulensis* **a**- habitus, **b, c**- verticillasters, **d**- stem and cauline leaf.

up to 0.5 mm spinescent, attenuate at base, hairy as the flowering stem, sessile or with petioles up to 3 mm. Floral leaves usually ovate, sometimes ovate-lanceolate, 5–17 × 2–5 mm, margin entire, apex acute, usually not spinescent, sometimes up to 0.5 mm spinescent, lower exceeding the verticillasters, upper ones equaling or slightly shorter than verticillasters. Verticillasters 4–7(-10), usually congested, sometimes flowers remote, 4–6 flowered. Bracteoles few, inconspicuous, setaceous, 1–2 mm long. Pedicels subsessile or absent. Calyx subbilabiate, campanulate, 6–7 mm long, hairy as flowering stem, with sparse sessile glands; teeth ± erect, 2–3 mm long, triangular-lanceolate, slightly shorter than tube, mucro (0,3-) 0.5–1 mm long. Corolla creamy-yellow with purple to pink streaks and spots inside, 11–14 mm long, tube subexserted from calyx; limb bilabiate, upper lip 5–6 mm long, c. ⅔ as long as lower lip, flattened at apex, lower lip 3-lobed, middle lobe larger than 2 lateral lobes, 7–9 mm long. Stamens 4, exserted ½-way along upper corolla lip. Nutlets oblong-obovoid, trigonous, 2–2.2 × 1.8 mm, minutely reticulate on surface, slightly winged near base, blackish-brown.

Etymology: The epithet is derived from İstanbul Province, where the species was discovered.

Proposed Turkish name: "kum deli çayı" (Menemen et al., 2016).

Distribution, habitat and phenology: *Stachys istanbulensis* naturally grows in the northwestern part of Turkey, also called the Marmara region (Figure 2). The new species is distributed on the sands throughout the Karaburun public beach in İstanbul, which is its only known population, and is well adapted to live on the maritime sands of the Black Sea shore. *Stachys istanbulensis* shares habitat with the herbs *Festuca beckeri* (Hack.) Trautv., *Jasione montana* L., *Plantago* sp. and *Catapodium marinum* (L.) C.E.Hubb. This new species flowers in May and fruits in mid–May and June.

Figure 2. Habitat of *Stachys istanbulensis* in İstanbul Province, Turkey.

Conservation status: *Stachys istanbulensis* is locally endemic to Turkey and found in a narrow coastal area in sandy soil on Karaburun beach, which is used as a public recreational area. Karaburun beach is a popular tourist destination, being the most visited Black Sea seaside resort in the European part of İstanbul Province. The beach is exposed to intense human pressure, especially between May and September, which poses a threat to the only known population of this species. Fewer than 100 mature individuals were observed in the area, which covers less than 10 km². The new species is assessed as critically endangered - CR [B1ab(i,ii,iii)+2ab(i,ii,iii)] according to criterion of the IUCN Red List Criteria (IUCN 2017).

3.2. Micromorphological results

Nutlets: The nutlets of *S. istanbulensis* are oblong-obovoid and size 1.8–1.9 × 2–2.2 mm. No tubercle-like surface ornamentation or trichome structure was observed. The nutlets have a reticulate surface texture and narrowly winged structure, with the wing being more prominent at the base, becoming indistinct towards the apex. The cell anticlinal walls are elevated, while the periclinal walls are concave and thickened without a flat surface. The apex is rounded with a mucro and the base is obtuse. The nutlets are blackish brown in color (Figure 3).

The nutlets of *S. recta* are obovoid and size 1.7–1.8 × 2.1–2.2 mm. No tubercle-like surface ornamentation or trichome structure was observed. The nutlets have a reticulate surface texture and no wings. The anticlinal walls are elevated and flat, while the periclinal walls are concave. The surface of the periclinal walls is smooth. The apex is rounded and the base is obtuse. The nutlets are blackish-brown in color (Figure 3).

Pollen: The pollen grains of *S. istanbulensis* are monad, radially symmetrical and isopolar. Polar length of the pollen grains ranges from 34.03 to 44.00 µm and equatorial length ranges from 19.40 to 26.88 µm. The mean length of P is 41.83 ± 2.57 µm, and the mean of E is 23.97 ± 2.16 µm. The sculpturing pattern is reticulate. The pollen grains of *S. recta* are monad, radially symmetrical and isopolar. P of the pollen grains ranges from 39.22 to 44.86 µm and E ranges from 21.77 to 28.57 µm. The mean length of P is 42.58 ± 1.73 µm, and the mean of E is 24.86 ± 1.93 µm. P/E averages 1.72 ± 0.10, creating prolate pollen grain. The sculpturing pattern is reticulate (Figure 4).

3.3. Phylogenetic results

Sequences of 17 *Stachys* and one outgroup, *Sideritis macrostachys,* were obtained from NCBI. *Stachys istanbulensis* was newly sequenced for the present study and was deposited to NCBI. The ITS region ranged in size from 600 bp to 652 bp. The 608 alignment positions yielded 454 constant, 85 parsimony uninformative, and 111 parsimony informative characters. The consistency index (CI), homoplasy index, and retention index are 0.77, 0.22, and 0.77, respectively (Figures 5 and 6).

Molecular phylogenetic trees based on ITS data constructed using the BI (Figure 5) and MP (Figure 6) methods, include ten species of *Olisia*, three species of section *Eriostomum* (Hoffmanns. & Link) Dumort., two species of section *Fragilicaulis* R.Bhattacharjee, three species of section *Setifolia* R.Bhattacharjee, and one

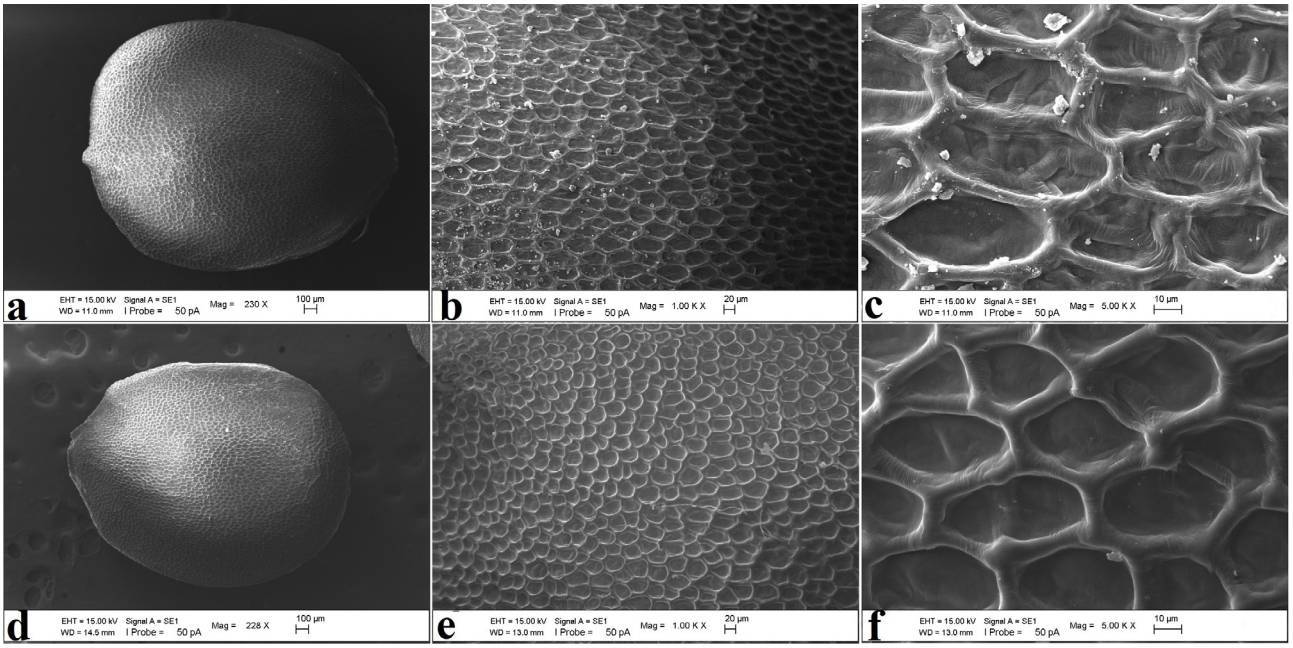

Figure 3. Micromorphological comparison of nutlets of *Stachys istanbulensis* (a, b, c) and *S. recta* subsp. *subcrenata* (d, e, f).

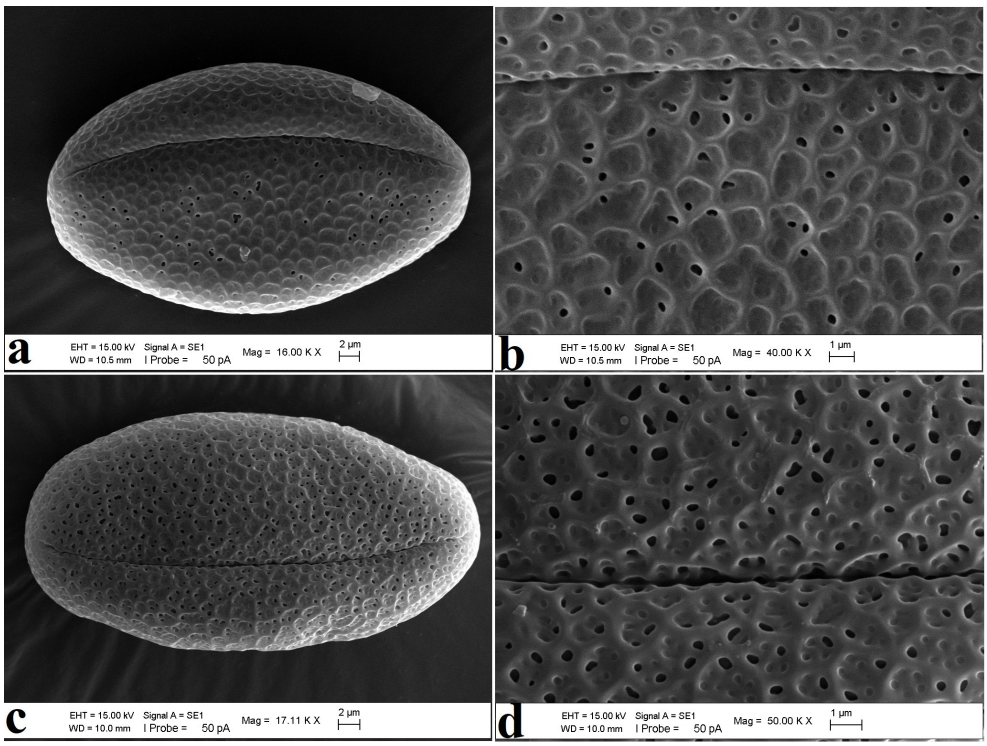

Figure 4. Micromorphological comparison of pollens of *Stachys istanbulensis* (a, b) and *S. recta* subsp. *subcrenata* (c, d).

species from *Sideritis* as an outgroup. In the results of this study the Bayesian tree is nearly identical to the MP trees and the phylogenetic tree is divided into five main clades.

4. Discussion

The *Stachys recta* complex, *S. atherocalyx* K.Koch and the new species *Stachys istanbulensis* belong to subsection

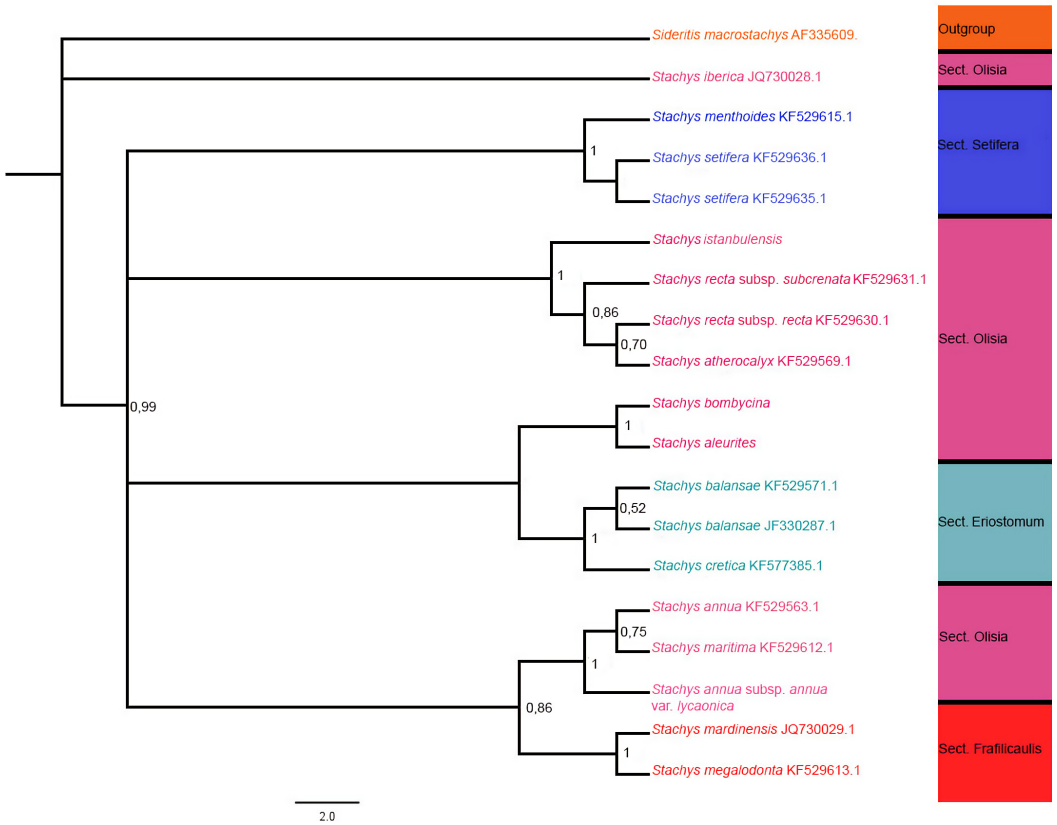

Figure 5. Phylogenetic tree of nrITS sequences based on Bayesian analysis. Posterior probability values are indicated above branches. The newly described *Stachys istanbulensis* and its close relatives *S. recta* and *S. atherocalyx* comprise a clade (III).

Rectae R.Bhattacharjee of section *Olisia*. The section appears to be polyphyletic due to the independent origination of *S. iberica*, *S. recta*, *S. annua*, *S. maritima* Gouan and *S. bombycina* Boiss. The present data placed species of this section into five clades that have far relationships with each other. *Olisia* is a widely variable section having its greatest diversity in Europe and Anatolia. As a whole, the section shows affinities with other sections (*Setifolia, Fragilicaulis* and *Eriostomum*). Morphological data of Bhattacharjee (1980) also suggested that the section is divided into five subsections. Moreover, the phylogenetic results (Salmaki et al., 2013) placed species of the section into different clades.

Stachys recta, with its eight recognized subspecies, is a highly polymorphic taxon with a wide distribution from West Europe to East Asia (POWO, 2022). The species displays a remarkable diversity in floral, nutlet and vegetative features among its subspecies (Figure 7). *Stachys recta* subsp. *recta* is described by flowering stems erect or slightly ascending, distant verticillasters and regular calyx, and its native range is from Europe to Caucasus. (Chrtekjun, 1992). Its closest relative in Turkey is *S. atherocalyx*, from which it is distinguished by its campanulate calyx, triangular-lanceolate calyx teeth, and broadly lanceolate lower and median cauline leaves. *Stachys recta* subsp. *subcrenata* (Vis.) Briq is the only representative of *S. recta* in Turkey and is separated from subsp. *recta* by its longer calyces (7–9 mm) and narrowly lanceolate, faintly serrate to entire margined median cauline leaves (Table 1).

The new species *Stachys istanbulensis* is characterized by having procumbent flowering stems in maritime sands and congested verticillasters. The new species is related to the *S. recta* complex but can be clearly distinguished by its procumbent and unbranched flowering stems, lack of spinescent leaves, sessile calyx glands, creamy yellow corolla and oblong-obovoid nutlets (Table 1). The new species is closely allied to *S. recta* subsp. *subcrenata*, from which it mainly differs in its unbranched flowering stems, shorter calyx and corolla tube which is long-exserted from the calyx.

Stachys istanbulensis is only distributed in maritime sands and dunes. While the majority of *Stachys* taxa grow in forests, fallow fields, rocky slopes and on limestone, few prefer maritime sands. *Stachys annua* subsp. *ammophila* (Boiss. & Blanche) Sam. near the Mediterranean, and *S. maritima* Gouan and *S. recta* subsp. *subcrenata* near

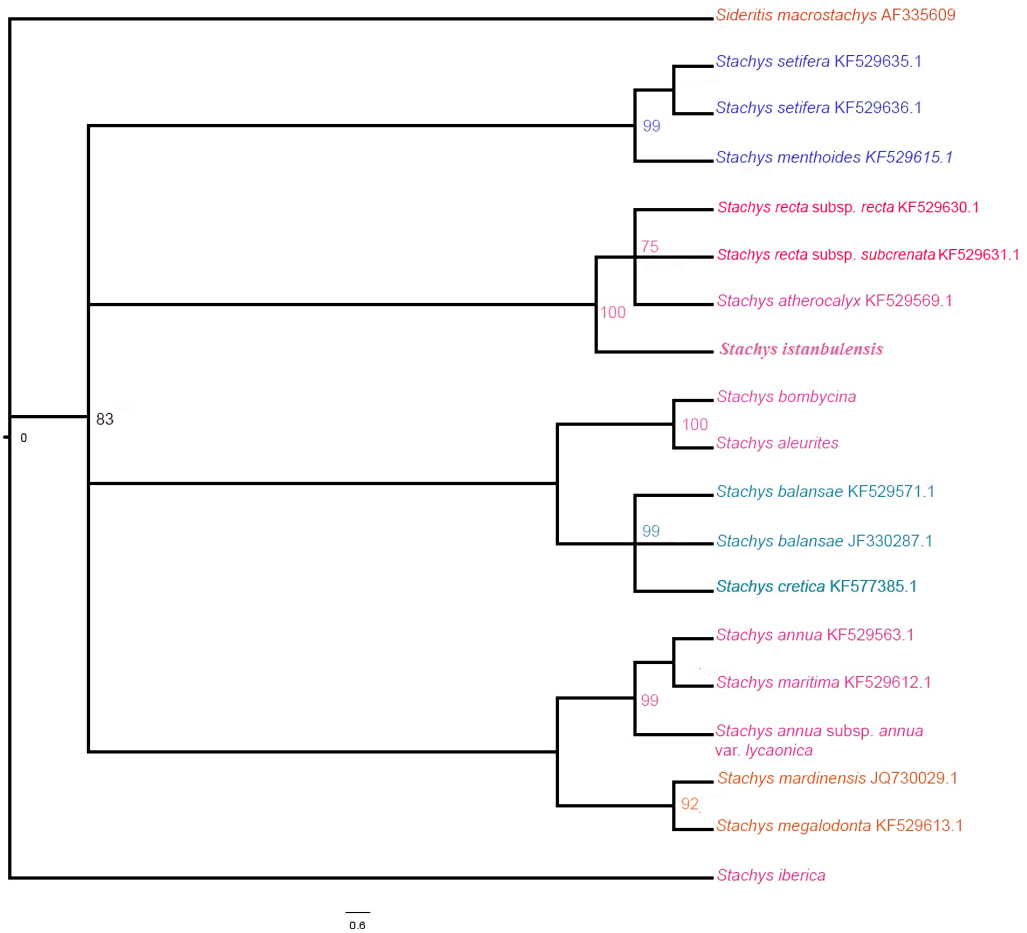

Figure 6. Phylogenetic tree of nrITS sequences based on MP analysis. MP bootstrap values are indicated above branches. Values at nodes indicate bootstrap support values that are ≥ 50%.

the Black Sea are known to grow in sandy soil in coastal areas. However, *S. annua* subsp. *ammophila* prefers to live in loamy and sandy soils near rocky slopes, sometimes in fallow fields. *Stachys cretica* L. subsp. *gulendamiana* Akçiçek, which is found in maritime sand along the Aegean Sea (Akçiçek and Güner, 2022), is a recently described taxon, which, alongside the species described in this paper, brings the number of taxa growing in maritime sands to five. The author has observed that the flowering stems of species growing in this habitat are usually procumbent or decumbent and rarely erect to decumbent (Figure 8).

Micromorphological and molecular analyses also provide data on the morphological distinction between *Stachys* species. Nutlet characters are important tools in elucidating the relationships between taxa (Salmaki et al., 2008; Açar, 2019; Açar and Satıl, 2019). Observation of the nutlet structures found both significant similarities and differences. The common features of both taxa include their obovoid nutlet shape, lack of tubercles on the surface structures, lack of trichomes, reticulate surface texture, rounded apex, obtuse base, and blackish brown nutlet color. Important differences are as follows: the mucronate apex and wing structure is found in nutlets of *S. istanbulensis* but not of *S. recta* subsp. *subcrenata*, while the periclinal walls are thickened in *S. istanbulensis* but flat in *S. recta* subsp. *subcrenata*. Moreover, the pollen grains are similar to each other in the species examined. However, there are a few differences in the measurements of palynological characters in terms of their size.

In the phylogenetic analysis, the Bayesian tree is divided into five main clades (Figure 5). The first clade includes *S. iberica,* a species also belonging to subsection *Rectae*, which has four subspecies and is found in Iran, Iraq, Krym, Lebanon-Syria, Transcaucasus and Turkey. The second clade contains *S. menthoides* Kotschy & Boiss. and *S. setifera* C.A.Mey representing the section *Setifolia.* The new species *S. istanbulensis* and its morphologically close relatives, *S. recta* and *S. atherocalyx,* which are placed in section *Olisia,* form the third clade. The new species is closely related to these two species but clearly differs in the previously mentioned morphological characters. Salmaki et al. (2013) suggested that there is a close

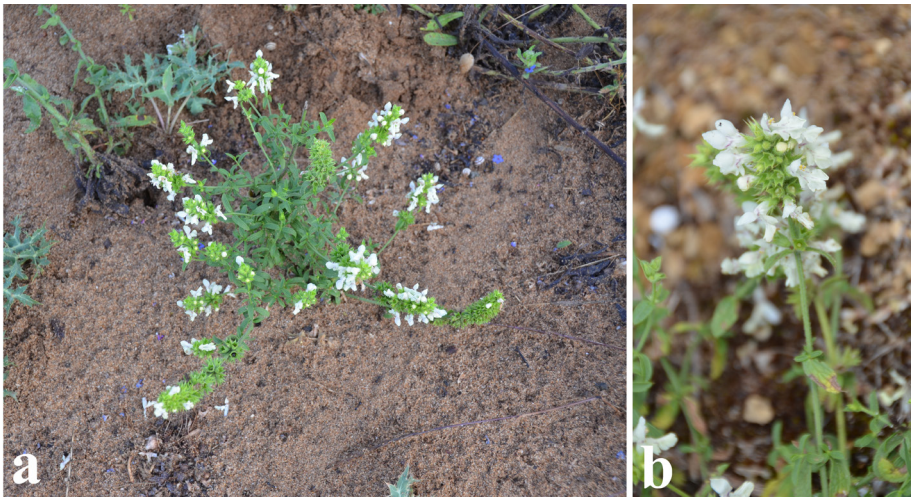

Figure 7. *Stachys recta* subsp. *subcrenata*, **a-** habitus, **b-** verticillasters.

Table 1. Morphological comparison of *Stachys istanbulensis, S. recta* subsp. *subcrenata and S. recta* subsp. *recta*

Characters	*Stachys istanbulensis*	*Stachys recta* subsp. *subcrenata*	*Stachys recta* subsp. *recta*
Habitat and Altitude (a.s.l.)	Maritime sands	Maritime sands	Rocky slopes, forest openings, steppe, fallow fields, meadows
Flowering stems	Procumbent; 15–35 cm long; usually unbranched; sparsely sessile glands	Erect or decumbent, 10–60 cm long, branched; sparsely sessile glands	Erect or slightly ascending, 20–40 cm long; branched; usually eglandular
Cauline leaves	Faintly crenate, apex usually not spinescent	Faintly crenate-serrate to subentire, 1–1.2 mm long spinescent	Densely serrate, 1–1.2 long mm spinescent
Floral leaves	Usually ovate, sometimes ovate-lanceolate; apex usually not spinescent	Linear-lanceolate to ovate-lanceolate, apex 1–1.2 mm long spinescent	Oblanceolate to ovate, sometimes obovate, apex 1–1.2 mm long spinescent
Verticillasters	Usually congested, sometimes lowers remote	Remote, 2–5 cm distant, few ± approximate above	Usually distant
Pedicel	Subsessile or absent	1–1.2 mm long	1–1.2 mm long
Calyx	Subbilabiatae, 6–7 mm long, with sparsely sessile glands; Teeth triangular or triangular-lanceolate, 2–3 mm long, mucro (0.3-)0.5–1 mm long	Subbilabiatae, 7–10(-11) mm long, often glandular; Teeth triangular-lanceolate, 3–4(-5) mm long, mucro (1-)1.2–2 mm long	Regular, 5–8(-9) mm long, eglandular, without glandular hair; Teeth triangular, 3–4 (-5) mm long
Corolla	Creamy yellow, tube subexserted calyx, upper corolla lip flattened at apex	White, tube exserted calyx, upper corolla lip not flattened at apex	White, tube exserted calyx, upper corolla lip flattened at apex
Stamens	Exserted ½-way along upper corolla lip	Exserted more than ½-way along upper corolla lip	Exserted more than ½-way along upper corolla lip
Nutlets	Oblong-obovoid	Obovoid	Obovoid

relationship between *S. recta* and *S. atherocalyx* based on plastid data. Moreover, *S. atherocalyx* and *S. recta* form a well-supported subclade in the newly identified clade in the combined nuclear tree (Salmaki et al., 2019). While *S. recta* grows in most of Europe and Caucasia, *S. atherocalyx* is distributed in Iran, Caucasia, Crimea, the North part of

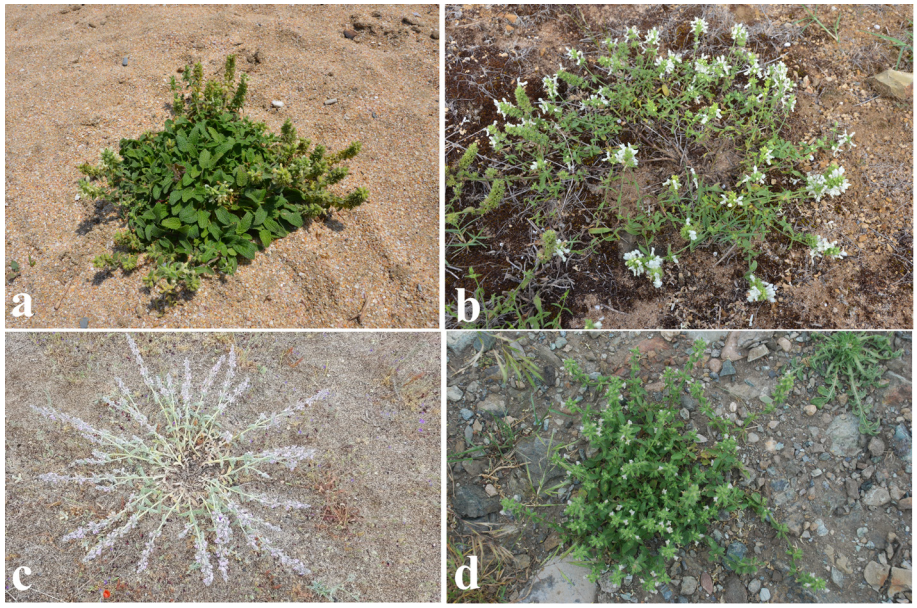

Figure 8. Procumbent or decumbent flowering stems of various *Stachys* taxa in maritime sands in Turkey. **a**- *S. maritima*, **b**- *S. recta* subsp. *subcrenata*, c- *S. cretica* subsp. *gulendamiana,* **d**- *S. annua* subsp. *ammophila.*

the Balkan peninsula and Romania. *Stachys recta* forms a morphologically fairly well distinguishable group in Europe. According to many studies to date (Bhattacharjee, 1982; Chrtekjun, 1992; Salmaki et al., 2013; 2019; Akçiçek, 2020), it has been accepted that these species most resemble each other but they are two different species. Moreover, *Stachys istanbulensis* is outside the variation limits of *S. recta* complex and is a new species also different from *S. atherocalyx*

Clade IV is divided into two subclades. The first consists of *S. bombycina* and S. *aleurites,* which are placed in *Olisia,* whereas the second subclade includes *S. balansae* and *S. cretica,* which are placed in section *Eriostomum*. Based on nrITS DNA region sequences, Dündar et al. (2013) also showed that these species were found in section *Eriostomum*. Clade V is divided into two subclades. *Stachys annua, S. maritima*, *S. mardinensis* (Post) R.R.Mill and *S. megalodonta* Hausskn. & Bornm. ex P.H.Davis were grouped together with moderate posterior probability (0.86) in the phylogenetic analyses. The first subclade comprises *S. annua* and *S. maritima. Stachys annua*, with its 3 recognized subspecies, is a polymorphic taxon with a wide distribution in Turkey and is similar to *S. maritima* which is adapted to live on the maritime sands (Akçiçek, 2020). Salmaki et al. (2013) suggested that there is a close relationship between *S. annua* and *S. maritima* based on plastid data. The second subclade includes *S. mardinensis,* which is closely related to *S. megalodonta*, and this group is also well-supported. These species grow SE Turkey and are found within same complex in the section *Fragilicaulis* (Güner et al., 2021).

Molecular phylogenetic analyses show that some sections in the traditional classification of *Stachys* are nonmonophyletic. As observed in previous studies (Scheen et., 2010; Dündar et al., 2013; Salmaki et al., 2013), section *Olisia* was shown to be polyphyletic and species of the section are divided into four separate clades in the current analysis. In these analyses, *S. recta* complex and *S. atherocalyx* share same clade in section. In addition, the present ITS nrDNA molecular data support that the new species, *S. istanbulensis*, belongs to the section *Olisia* since it displays a close relationship with *S. recta* complex and *S. atherocalyx* of this section. Sequence analyses of the ITS region shows that *S. istanbulensis* is genetically distinct from these two closely related species, confirming that it is a distinct, new species.

The most inclusive revision of section *Olisia* in Turkey was undertaken by Akçiçek (2020), who made some taxonomic changes and produced a key for identification of all taxa found in Turkey. *Stachys willemsei* Kit Tan & Hedge, less known and not mentioned in many studies, is similar to *S. iberica* in section *Olisia* and endemic to Turkey. The closest affinities of *S. willemsei* seem to be *S. iberica* from which it can be distinguished by its taller habit, smaller sessile flowers and nonexserted stamens. Moreover, *S. istanbulensis* is an endemic species to İstanbul and grows on the maritime sands of the Black Sea shore (Figure 9). With the new current species from Turkey, the

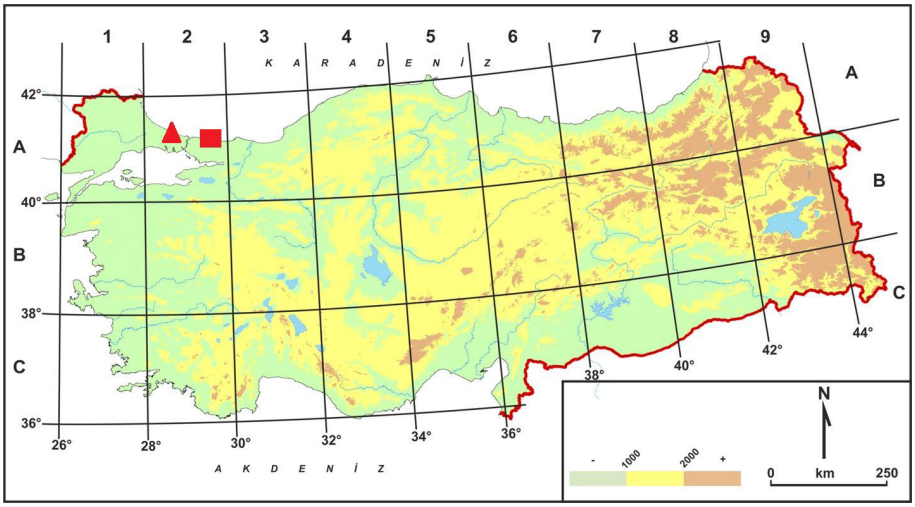

Figure 9. Geographical distribution of *Stachys istanbulensis* (▲) and and *S. recta* subsp. *subcrenata* (■) in Turkey

species number of the section is raised to 16 (22 taxa). With this addition, the number of Turkish *Stachys* species has reached 94 (121 taxa), and 65 (53.7%) of these taxa are endemic.

Key to Turkish species of *Stachys* section *Olisia* subsection *Rectae*

1. Flowering stems glabrescent; verticillasters (1-)2 flowered 2

1. Flowering stems adpressed hirsute or adpressed pilose; verticillasters 4–8(-10) flowered 3

2. Cauline leaves laciniate to pinnatipartite; pedicels 2–5 mm long ..... ***angustifolia***

2. Cauline leaves linear to linear-lanceolate; pedicels 1.5–2 mm long ..... ***hakkariensis***

3. Flowering stems sparsely and/or antrorsely adpressed hirsute; cauline leaves acute at apex spinescent, corolla white or creamy yellow; 4

3. Flowering stems adpressed pilose; cauline leaves acute to obtuse, not spinescent; corolla mauve 6

4. Flowering stems procumbent; verticillasters congest; corolla creamy yellow ..... ***istanbulensis***

4. Flowering stems decumbent to erect; verticillasters usually remote; corolla white 5

5. Calyx tubular to subcampanulate; teeth lanceolate-subulate, 4–6 mm long, never more than 1 mm broad; verticillasters congested spicate, few remote below ..... ***atherocalyx***

5. Calyx campanulate; teeth triangular-lanceolate, 2–4 mm long, more than 1 mm broad; verticillasters remote, few ±approximate above ..... ***recta***

6. Pedicel of fruiting calyx 2–4(-6) mm long; calyx teeth as long as tube; verticillasters remote throughout, 2–6 flowered ..... ***sparsipilosa***

6. Pedicel of fruiting calyx 0.5–1.5 mm long; calyx teeth ½ x tube; verticillasters remote below; few approximate above, (2-)4–8(-10) flowered ..... ***iberica***

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Additional specimens examined: *Stachys aleurites:* Turkey: Antalya, Konyaaltı beach road, calcareous rocks near the coast, 10–30 m, 24 May 2013, *Akçiçek* 5601 & *Ö. Güner* (Hb. Akçiçek). *Stachys annua:* Hakkari, Cilo mountain, 11 km from Kırıkdağ to Cehennemdere, Dizderesi, 1760 m, 9 June 2013, *Akçiçek* 5561, *Dirmenci* & *Ö. Güner* (Hb. Akçiçek). *Stachys atherocalyx*: Turkey: Erzurum, 14 km from Erzurum to Pasinler, meadows. 1800 m, 28 August 2013, *Dirmenci* 3954 (Hb. Dirmenci!); Uzundere, Azor plateau, roadside banks, 1750 m, 29 June 2014, *Akçiçek* 5641, *Dirmenci* & *Ö. Güner* (Hb. Akçiçek); 5 km from Tortum to Uzundere, stony ground, 1600 m, 29 June 2014, *Akçiçek* 5643, *Dirmenci* & *Ö. Güner* (Hb. Akçiçek). *Stachys bombycina*: Turkey: Antalya, Kemer, Küçükçaltıcak, under *Pinus brutia* canopy, 24 May 2013, *Akçiçek* 5550 & *Ö. Güner* (Hb. Akçiçek). *Stachys recta* subsp. *recta*: Austria: *P. Schönswetter* 2517 (WU!). *Stachys recta* subsp. *subcrenata*: Turkey: Istanbul, Uskumruköy, 3 August 1902, Aznavour s.n. (G!); Çatalca, Terkos lake, Durusu, dunes, 12 June 1972, *Demiriz* (ISTF 26395!):

Istanbul, Riva, 12 June 1893, Aznavour s.n. (G!); Şile, Riva stream, dunes, 2 m, 20 June 2014, *Ö. Güner* 2412 (Hb. Akçiçek); ibid, 26 June 1981, *M. Temel* (ISTF 34872!); ibid, 17 May 1970, Demiriz (ISTF 29404!): Kocaeli, Yuvacık dam, between Camidüzü and Serindere, 501 m, 2006, *A. Efe, N. Aksoy* & *D. Oral* (ISTO 34406!).

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