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A new annual Satureja (Lamiaceae) species from Turkey with molecular evidence, and lectotypification of two species

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Abstract: A new species, Satureja hasturkii, is described using morphological characters and molecular studies in this study. The new species is closely related to S. hortensis but differs from it by very lax thyrsoid inflorescence, longer pedicels, shorter calyx, upper and lower calyx teeth, and corolla. In order to support the morphological data, molecular studies are also done using nrITS and cptrnL-F gene regions. nrITS data gives better results in phylogenetic analysis. It is pointed out that the closest relatives of S. hasturkii are S. hortensis and S. laxiflora. The fact that having annual life forms of these three species also explain their molecular closeness. With this new species, the number of Satureja species in Turkey increased to 17. Moreover, lectotypes were determined for Satureja macrantha and S. wiedemanniana here.

Key words: Endemic, Erzincan, lectotype, Mentheae, Satureja

1. Introduction

Satureja L. s.l. (Lamiaceae, Mentheae, Menthinae) is a taxonomically complex genus. Many taxonomists have worked on the genus for many years, and some taxonomists have accepted it as a larger genus combining many closer genera, while some taxonomists have accepted it as a genus with a narrower sense and with fewer species. It can summarize some significant studies on Satureja chronologically as follows. Bentham (1832-36, 1848, 1876) divided the genus into 3 sections: Sect. Satureja, Sect. Tragoriganum Benth., and Sect. Pycnothymus Benth. Kuntze (1891) included the genus Satureja in the genus Clinopodium L. Contrary to Kuntze (1891), Briquet (1895-97) accepted Satureja more broadly and included a total of 13 genera with Clinopodium s.l. and Micromeria Benth. s.l. and 130 species in Satureja. He also divided the genus into the 14 sections. Again, Greuter (1985) adopted Briquet's (1895-97) concept and stated that Micromeria s.l. and Clinopodium s.l. should be included in Satureja. On the other hand, Doroszenko (1985) argued that Satureja complex should be narrower and reported that this genus consists of 17 genera in four groups: the satureioid genera, the calaminthoid genera, the micromerioid genera, and American genera. In some country floras, especially Acinos Mill., Calamintha Mill., Clinopodium, and Micromeria are

given as separate genera (Borisova, 1954; Tutin et al., 1972; Davis, 1982; Rechinger, 1982; Harley et al., 2004, Jamzad, 2010).

Some important phylogenetic studies conducted in recent years also provide important information about the taxonomy of the genus (Wagstaff et al., 1995, Cantino and Wagstaff, 1998). cpDNA data was used in Wagstaff et al. (1995); according to their parsimony analysis, they suggested that Satureja should be divided into several genera. Cantino and Wagstaff (1998) also used chloroplast DNA data and some distinctive morphological characters and divided the complex (consisting of Satureja and its allies) into five well-known genera: Cyclotrichium Manden. & Scheng., Obtegomeria Doroszenko & P.D.Cantino, Gardoquia Ruiz & Pav., Xenopoma Willd., and Clinopodium. According to recent molecular studies with Satureja group, it is more systematically correct to examine the genus Satureja and its allies in tribe Mentheae (Bräuchler et al., 2005, 2006, 2010; Trusty et al., 2004). These studies presented that Satureja is a distinct genus and had closer molecular structure with Micromeria and Clinopodium, and Gontscharovia Boriss. (monotipic genus: G. popovii (B.Fedtsch. & Gontsch.) Boriss.) is a sister genus of Satureja (Bräuchler et al., 2010). On the other hand, there are some important infrageneric studies that

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have been conducted on the genetic structure of *Satureja* species to resolve of phylogenetic relationship (Öz Aydın and Köçkar, 2008; Bezić et al., 2009; Bräuchler et al., 2010; Hadian et al., 2010; Noormand-Moaied et al., 2021).

With the taxonomic studies outlined above, the genus *Satureja* has been accepted as a narrower genus concept of Old World *Satureja* species and is represented by approximately 43 species worldwide, most of the species are distributed in the Mediterranean countries, and the rest of them from Western Europe and North Africa to the Altai-Western Himalayas and Xinjiang (POWO, 2022; WCVP, 2022). Although *Satureja* species are concentrated in the Mediterranean basin and the Caucasus-East of Turkey-West Iran and Northern Iraq border, if evaluated on a country basis, Turkey is the country with the highest species number with 17 species, 7 of which are endemic (Davis, 1982; Dirmenci et. al., 2019).

Satureja members are mostly composed of perennials and subshrub. Only two species, S. hortensis L. and S. laxiflora K.Koch, are annual. Its species are a well-known Lamiaceae genus as "dağ kekiği", "taş kekiği", "kaya kekiği", "sivri kekik", and "çipriska" in Turkey (Satıl et al., 2008; Çarıkçı et al., 2020; Selvi et al., 2022). It is consumed as spice and tea due to its strong aromatic structure and has some species having commercial value. Especially in Turkey, it is consumed both by collecting it from its natural environment (S. cuneiifolia Ten., S. boissieri Hausskn. ex Boiss., S. cilicica P.H.Davis etc.) and by culturing (S. hortensis) (Satıl et al., 2008).

In this study, a new species is described from eastern Turkey that will contribute to the diversity of annual species in the genus. Information was provided from molecular data as well as morphological data in the identification of this new species. The aim of the study is 1-to determine the phylogenetic relationship of the new species and describing it as a new species, 2-to evaluate the phylogenetic diversity of annual species, 3-to determine the phylogenetic relationship of the genus comprehensively with GenBank data, 4-to determine the lectotype specimens for *S. macrantha* C.A.Mey. and *S. wiedemanniana* (Avé-Lall.) Velen.

2. Materials and methods

2.1. Plant materials

The new species (in İliç district of Erzincan) (Figure 1) and some of Satureja hortensis specimens were collected during some field trips between 2019 and 2022, and at least ten different herbarium specimens collected from the same region and at least ten specimens from five different regions from S. hortensis individuals collected from different regions were examined for morphological studies. All the specimens were identified using the relevant literature (Borisova, 1954; Davis, 1982; Rechinger, 1982; Dirmenci et al., 2019). All morphological measurements have been made on dried herbarium specimens kept at Balıkesir University, GAZI, and K herbaria using stereo microscope, and the specimens' morphology compared with the materials stored in the herbaria ANK, B, BM, E, EGE, GAZI, HUB, INONU, ISTE, ISTF, ISTO, K, KNYA, L, LE, MA, W, and WU. For lectotypification studies, S. macrantha and S. wiedemanniana syntype specimens in BM, G, K, LE, and W herbaria were also examined.

2.2. Total DNA extraction and PCR reactions

Total DNA extraction of *Satureja hortensis* (4 samples) and the new species (3 samples) was conducted



Figure 1. Distribution map of Satureja hasturkii.

using powdered plant materials of fresh leaf materials using the Hibrigen Plant Genomic DNA Extraction Kit (Kocaeli, Turkey) following the manufacturer's instructions. ITS regions of studied taxa were amplified using ITS4 (TCCTCCGCTTATTGATATGC) and ITS5a (GGAAGGAGAAGTCGTAACAAG) primers (White et al., 1990), and the primers used for the trnL-F region as follows: trnL-c (CGAAATCGGTAGACGCTACG) trnL-f (ATTTGAACTGGTGACACGAG) and (Taberlet et al., 1991). PCR conditions were set to 95 °C for 5 min initial denaturation, 35 cycles of 94 °C for 1 m denaturation, 50 °C for 1 m annealing, and 72 °C for 1 min extension, 72 °C for 10 min final extension. After visualizing of PCR products on agarose gel, appropriate PCR products were sent to LetGen Biotechnology (İzmir, Turkey) for purification and sequencing.

2.3. DNA editing and phylogenetic analysis

All Satureja taxa (23 specimens) and outgroup specimens (16 samples) were edited with BioEdit v. 7.2 (Hall, 1999), aligned with Clustal W (Thompson et al., 1994), and adjusted manually and converted to nexus file using MEGA software v.11.0.10 (Tamura et al., 2021). The aligned ITS and *trn*L-F datasets were analyzed with three phylogenetic algorithms: Bayesian inference (BI), maximum likelihood (ML) and maximum parsimony (MP). MCMC Bayesian analysis was run for 10 million generations with four runs and trees were sampled every 1000 generations using MrBayes v.3.2 in CIPRES (Miller et al., 2010). MP analyses were performed in PAUP* v.4.0b10 (Swofford, 2003) with a heuristic search option, tree bisection reconnection (TBR) branch swapping algorithm, and 10 random-addition sequence replicates. Branch clade credibilities were finally estimated with the bootstrap (BS) approach, based on 10000 replications. All phylogenetic trees were visualized using FigTree v.1.4.3 (Rambaut, 2010). All the accessions of Satureja and the outgroups are given in Figures 6 and 7 and can be downloaded from GenBank. An asterisk was placed next to taxa sequenced, edited, and uploaded to the GenBank for the first time in this study.

3. Results

3.1. Taxonomic treatment

In addition to describing the new species, it was determined that *Satureja macrantha* and *S. wiedamanniana* needed lectotype determination within the scope of lectotypification studies carried out in Kew herbarium by Tuncay Dirmenci. Lectotype has been designated here for these two species.

Satureja macrantha C.A. Mey. Index Seminum (LE, Petropolitanus) 11(Suppl.): 67 (1846).

Lectotype (designated here by Dirmenci): Azerbaijan. In montium lapidosis calcareis ad Ghierus, Akarschai & Pachlutschinari, 03 Sept. 1829, *Szovits* 587 (LE01175439, isolectotypes LE01175438, LE01175440) (Figure 2). Satureja macrantha was identified based on "Syntypes" collected from two different locations: "Hab. In provincia Karabagh prope Schuscha: Hoh., et prope loca Ghierus, Akartchai et Pachlutschinari (Szov.)., sita aut in aedem terra aut in prov. Aserbeidshan" locations were given in its protologue (Meyer 1846:68)". These two locations were given in the Flora of Turkey, too (Davis, 1982:323) and the specimen collected by Szovits was specified as isotype and it was stated that it was kept in herbarium G.

During some investigations carried out in the G-BOISS herbarium, it was observed that there were two sheets specified as typus (G00786052, G00786038). One of the specimens barcoded as G00786052 was collected by Szovits from Armenia. The same specimen was also found in the BM herbarium (coded as BM18580). There was no collector number and other information on the label of the specimens coded as G00786052 and BM18580. Moreover, on the label of G00786038, information was not fully understood. However, G00786038 was not compatible with the location information given in the protologue, and it is suspected that these specimens in G-BOISS and BM herbaria are type specimens. On the other hand, some specimens named as Satureja macrantha were found in the LE herbarium in 2011. There were 4 sheets in the LE herbarium that can be compatible with the protologue. One of these specimens was collected from Schuscha by Hohanacker (LE01175437), this example did not have the original label of Hohanacker on it and the name was written afterwards with pencil and also there was no collector number or other detailed information on the label of this specimen. The other specimens on the three sheets (LE01175438, LE01175439 and LE01175440) with similar morphological features are thought to have been collected by Szovits. All these three sheets contained protologue location belonging to Szovits, and collector number of Szovits 587. The label of LE01175439 with three different individuals on the sheet had detailed information (In montium lapidosis calcareis ad Ghierus, Akarschai & Pachlutschinari, 03 Sept. 1829, Szovits 587) compatible with the protologue (Figure 2). Although all the examples mentioned above (in BM, G, LE) reflect the general morphological features of S. macrantha, it was concluded that it would be more appropriate to choose the specimen LE01175439, which is in the LE herbarium, as lectotype (Figure 2), and the other two specimens, LE01175438 and LE01175440, collected by Szovits (Szovits 587) as isolectotypes.

Satureja wiedemanniana (Ave-Lall.) Velen, Sitzungsber. Königl. Böhm. Ges. Wiss., Math.-Naturwiss. Cl. 39: 24 (1894).

Lectotype (designated here by Dirmenci): Turkey. Tokat, in Natolia (Anatolia) montibus Mahmudagh, *Wiedemann* 311, G-BOISS [G00786084] (Figure 3).



Figure 2. Lectotype specimen of *Satureja macrantha* (*Szovits* 587, LE01175439 Specimen LE 01175439 // Virtual herbarium of Komarov Botanical Institute RAS — http://re.herbariumle.ru/01175439

Satureja widemanniana (Ave-Lall.) Velen. was previously described as Satureja cuneifolia Ten. var. wiedemanniana (Ave-Lall., 1846:64) based on syntypes collected from two locations. Their locations in the protologue were given as "in Natoliae montibus Mahmudagh et Kiskischdagh, prope Kaisarieh: Wied." These two locations are given as the syntypes locations of the species in the Flora of Turkey and the collection year was given together with the collector, too: "Syntypes: [Turkey A6 Tokat] in Natolia montibus Mahmudagh (G) et Kiskischdagh (K), prope Kaisareh (Gaziura = Turhal?), [1835], *Wiedemann* [311 & 312-probably mixed] (G)" In addition, it was stated that



Figure 3. Lectotype specimen of *Satureja wiedemanniana* (*Wiedemann* 311, G00786084). http://www. ville-ge.ch/musinfo/bd/cjb/chg

the collector numbers on the specimens may have been mixed. The specimens collected from Mahmudagh were in the G herbarium, and the Kiskischdagh specimens were in the K herbarium (Davis, 1982:317). As a result of the investigations, some specimens that may be compatible with the specimens specified in the protologue of *Satureja wiedemanniana* have been identified. These specimens are "W18860002001, Anatolia, *Wiedemann* s.n", "GOET004328, Anatolia, *Wiedemann* s.n.", "G00786084, Mahmudagh, *Wiedemann* 311" and "G00786087, Kischkisch dagh, *Wiedemann* 312". In addition, although it is stated that the Kischkisch dagh specimen is found in the K herbarium in the Flora of Turkey, this specimen was not found in K herbarium. Although the features of the samples mentioned here are compatible with the characteristics of *Satureja wiedemanniana* stated in the protologue, only the location information of the sample "G00786084, Mahmudagh, *Wiedemann* 311" is compatible with the protologue. For this reason, it was chosen as the Lectotype [G00786084] (Figure 3). There is only a sheet containing three specimens preserved in the G-BOISS herbarium.

Satureja hasturkii H.Duman & Dirmenci (Figs. 4-5).

Type: Turkey. Erzincan: İliç, Yakuplu Village, Around Bayramdere, 1200 m, moving gravel slopes, 37 S 466902 E, 4363929 N, 27.08.2021, *H.Duman 10682* (Holotype: GAZI; isotypes: K, E, BM, ANK, NGBB).

Paratypes: Erzincan: İliç, Yakuplu Village, around Fındıkdere, 1200 m, moving gravel slopes, 37 S 467726



Figure 4. *Satureja hasturkii* (from *H.Duman* 10682-Holotype), A- Habit, B1- Flower, C1- Calyx, D1- Dissected corolla, E- Nutlet, F- A leaf. *Satureja hortensis* (from *Z.Aytaç* 10978) B2- Flower, C2- Calyx, D2- Dissected corolla.



Figure 5. Satureja hasturkii A- general inflorescence, B- a part of inflorescence, C- a flower; Satureja hortensis: D- a verticillaster, E- a part of inflorescence, F- a flower.

E, 4362268 N, 28.08.2021, *H.Duman 10688* (GAZI, ANK, HUB); ibid., 14.10.2021, *H.Duman* 10689a (GAZI, Balıkesir Herb.); ibid., 31.08.2022, *H.Duman 10717* (ANK, GAZI, HUB, EGE, ISTE, NGBB)

Diagnosis: Characters of *Satureja hasturkii* are similar to *S. hortensis* (Figures 4A–4F and 5A–5F), but it differs from *S. hortensis* by its stems usually glabrous below, sparsely puberulent towards above (not puberulent to retrorsely pubescent throughout), axillary leaves absent (not usually present), inflorescence very laxly thyrsoid (not raceme or cymes pedunculate) (Figure 4A), calyx 1.8-2.5 mm long (not 3.5-4.2 mm long) (Figures 4B1 B2, C1 C2), upper teeth triangular and 0.5-0.8 mm long (not linear-lanceolate and 1.5-2 mm long), lower teeth lanceolate, 0.7-1 mm long and shorter than tube (not linear, 2.5-2.7 mm, as long as or longer than tube, to × 2 tube) (Figures 4C1 and C2) corolla 3-4 mm long (not 4-6 mm long) (4B₁, B₂), stamens 2 (not 4) (Figure 4D1, D2).

Description: Annual. Stems 20-45 cm tall, slender, broadly dichotomously branched almost from the base, usually glabrous below, sparsely puberulent towards above. Leaves \pm linear to oblanceolate, 5-18 × 1.2-3 mm, axillary leaves absent, subglabrous to puberulent with sessile glands and minutely glandular papillae on both surfaces, rarely punctate, attenuate at base, entire, obtuse at apex. Inflorescence very laxly thyrsoid, opposite branches, 4-10 cm width on per main flowering branches; cymes pedunculate, peduncles and pedicels filiform;

peduncles 10–30 mm long. Bracts 2, oblanceolate, 1–3 mm long, shorter than pedicels, puberulent with sessile glands, ciliate. Flowers distinctly pedicellate, pedicel 5–20 mm long. Calyx 1.8–2.5 mm long, subbilabiate, tubular-campanulate, puberulent to hirsute with sessile glands and minutely glandular papillae, veins distinct; upper lip teeth 0.5–0.8 mm long, triangular; lower teeth 0.7–1 mm long, lanceolate, as long as slightly longer than upper teeth; hairy between the teeth. Corolla 3–4 mm long, white to bluish-white, puberulent to pubescent, included in the calyx or slightly exserted, scarcely long hairy at mouth. Style included in corolla. Stamens 2, included in corolla, only posterior anther present, anther short, c. 1 mm, filaments 0.3–0.4 mm long, fertile, thecae parallel. Nutlet c. 1×0.7 mm, oblong, black, minutely tuberculate.

Habitat and ecology: The new species is endemic to the west of Munzur Mountain and occurs in serpentine screes thermophilous deciduous woodland zone, between 1150 and 1250 m. *Satureja hasturkii* grows in scree on stony slopes with *Satureja hortensis*, *Heldreichia bupleurifolia* Boiss. subsp. *rotundifolia* (Boiss.) Parolly var. *atalayi* (Kit Tan) Parolly, Nordt. & Mumm., *Ricotia aucheri* (Boiss.) B.L.Burtt, *Glaucium grandiflorum* Boiss. & A.Huet, *Cleome ortithopodioides* L.

Distribution and conservation status: *Satureja hasturkii* is known only from the types localities, and it is an Irano-Turanian element. In types locations where its total distribution area is less than 10 km², there are two

populations 500 m apart from each other and the total number of individuals is less than 250 [B2ab(i,ii,v), C2a(i)]. Therefore, it should be classified as a critically endangered (CR) species according to the World Conservation Union (IUCN) (IUCN Species Survival Commission 2014).

Etymology: The species epithet is dedicated to Can Serdar Hastürk, who is the environmental manager of Anagold Mining Company.

3.2. Molecular results

In this study, focusing on a new Satureia species, as mentioned above, two main regions from nuclear genome (nrITS) and chloroplast genome (trnL-F) were examined. All the sequences of new species (marked with * sign in Figure 6) and one of S. hortensis (Sat3 S. hortensis 1040-marked with * sign in Figure 6) were obtained for the first time in this study. For phylogenetic analysis, all nrITS sequences were obtained from 39 accessions (33 accessions from NCBI), representing 23 accessions belonging to Satureja specimens and accessions of close and far outgroup specimens (Figure 6). After editing, cutting, and alignment of the sequences of belonging to 39 specimens, 631 nucleotide characters were totally obtained. These 631 nucleotide positions yielded 399 constant (proportion = 0.63), 66 parsimony uninformative, and 166 parsimony informative characters. According to parsimony analysis of nrITS sequences, the consistency index (CI), homoplasy index, and retention index are 0.51, 0.49, and 0.82, respectively.

MP and ML (PAUP*) and Bayesian (MrBayes) analysis gave phylogenetic trees (Figures 6 and 7) in almost the same topology; on the other hand, Bayesian inference tree had better resolution. Therefore, Bayesian majority rule consensus tree is shown in Figure 6, and bootstrap values can be seen based on parsimony and likelihood analysis in the same figure.

Forty-four accessions were examined for *trn*L-F analysis. A total of 866 nucleotide characters were obtained for 44 specimens (Figure 7). Among these, 748 characters were constant which equaled about 0.86 of all nucleotide characters, and 73 characters were parsimony uninformative, and 45 characters were parsimony informative. As seen from the given information, nrITS data have more parsimony informative characters. According to parsimony analysis of *trn*L-F sequences, the consistency index (CI), homoplasy index, and retention index are 0.90, 0.09, and 0.92, respectively.

4. Discussion

A new *Satureja* species is described to the science based on morphological and molecular evidence. It is closely relative to *S. hortensis* which is a very widespread species from SE to SW Europe as far as Siberia. The inflorescence of *S. hortensis* varies greatly from dense and multiflowered to sparsely regular and 2-flowered verticillasters (Figures 5D and 5E). Individuals that are very close to *S. hortensis* with laxly inflorescence and two-to-few-flowered verticillasters



Figure 6. Bayesian 50% majority rule consensus tree inferred from ITS dataset. Numbers above branches are maximum parsimony bootstrap (left), maximum likelihood bootstrap (medium) and Bayesian posterior probability (right) values.

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Figure 7. Bayesian 50% majority rule consensus tree inferred from *trnL-F* dataset. Numbers above the branches are maximum parsimony bootstrap (left) and Bayesian posterior probability (right) values.

(Figures 5E and 5F) are named S. laxiflora, which is distributed from W Asia to Caucasus. In Turkey, specimens of S. hortensis with these variations like S. laxiflora are frequently encountered. In the Flora of Turkey, all abovementioned variations were accepted as S. hortensis. Moreover, according to Katar et al. (2017), chemical differences can be observed in different populations belonging to S. hortensis in different ecological conditions. Nikrouz-Gharamaleki et al. (2019) discussed Iranian S. hortensis specimens' based on morphological characteristics and they concluded that different S. hortensis specimens distributed in Iran have some variations and all of them are described as S. hortensis just like described in the Flora of Turkey. Here, too, it was concluded that all specimens from Turkey should be considered to be S. hortensis in a broad sense. Satureja hortensis (incl. S. laxiflora) and S. hasturkii are both species of the genus with annual life cycle. They show similarity in terms of lifespan, general morphological appearance, and leaf characteristics. However, there are important differences in their inflorescence, calvx, and corolla characteristics. The different characteristics of the two species are given in the diagnosis and Table 1. Although it is stated in the records of *S. hortensis* POWO (2022) that it spreads in Southern Europe, Central Asia and Western Himalayas, its distribution in Turkey and the Caucasus is suspected. However, the distribution of *S. hortensis* in the Flora of Turkey is given as mentioned before. Distribution of *S. laxiflora*, which has another annual lifespan, is given from Turkey, Iran, Iraq, and the Caucasus (POWO 2022). There are no definite geographical and morphological boundaries between these two species. Again, the new species described in this study, with annual lifespan, is the third species in the genus, and is morphologically separated from these two species by distinct characters.

In addition to the diagnostic characters, the *Satureja hasturkii* differs significantly from *S. hortensis* and all other *Satureja* species in another characteristic, too. The number of stamens is two in *S. hasturkii* and only posterior stamens are present (*H.Duman* 10682, 10688, 10689a, and 10717) (Figure 4D1). It is not present in any remains of the anterior stamens. So far, there are no reports of it, and this is the first two-stamened species in the genus *Satureja*.

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	S. hasturkii	S. hortensis
Stem indumentum	Usually glabrous below, puberulent towards above	Puberulent to retrorsely pubescent throughout
Axillary leaves	Absent	Usually present
Inflorescence	Lax thyrsoid, opposite branched, 4-10 cm width on per main flowering branches	Raceme, 1-2 cm width on main flowering stems and branches
Peduncle	10-30 mm long	Usually to 10 mm long
Pedicel	5-20 mm long	2-10 mm long
Calyx Upper teeth Lower teeth	1.8-2.5 long Triangular, 0.5-0.8 mm long Lanceolate, 0.7-1 mm long shorter than tube	3.5-4.2 mm long Linear-lanceolate, 1.5-2 mm long Linear, 2.5-2.7 mm, usually longer than tube, to × 2 tube
Corolla	White to bluish white, 3-4 mm long	White, lilac or purple, (4-)6-8(-10) mm long
Stamens	2	4

Table 1	Comparison	of diagnostic	characters used to	distinguish the	e Satureia	hasturkii and S horten	sis
Laure 1.	Comparison	of diagnostic	characters used to	uistinguisti the	e Sutureju i	<i>nusiurkii</i> and <i>s. noriens</i>	,15



Figure 8. NeighborNet graph based on nrITS sequences of examined taxa.

0.01

0.02

0.03

Fit: 99.9

The close affinity and differentiation of the new species from *S. hortensis* is supported by the nrITS phylogenetic tree obtained in this study (Figure 6). Bordbar et al. (2020) and Bräuchler et al. (2010) also presented the close affinity between *S. hortensis* and *S. laxiflora*. Although the genus *Satureja* has not been fully resolved and has some polytomies in the phylogenetic tree obtained from *trn*L-F data (Figure 7), *S. hortensis* is the closest species to the new species as seen in the tree obtained according to ITS data (Figure 6). As seen in Figure 6, annual clade of *Satureja* genus (incl. *S. hortensis, S. laxiflora* and *S. hasturkii*) is separated from other *Satureja* members with values of 62/64/0.89 (MP, ML, PP). This annual clade is divided into two subclades. Among these clades, the values of the branch containing *S. hasturkii* are 100/100/1 (MP, ML, PP) and the values of the branch consisting of *S. hortensis*

and *S. laxiflora* are 96/96/1. The fact that the values are so high supports that *S. hasturkii* is different from other annual species. As seen also from Figure 8, *S. hasturkii* (indicated as *Satureja* sp. nova) is a conspicuous member of the annual group, but distinctly distinguished from other species.

Fifty percent majority-rule consensus trees obtained from MrBayes (3.2.7a) based on nrITS and cp*trn*L-F data with bootstrap (executed in PAUP*) and posterior probabilities values are given in Figures 6 and 7, respectively. These trees give us information about monophyletic structure of *Satureja* genus. As seen from Figures 6 and 7, nrITS and *trn*L-F trees have some similarities as *Satureja* members are completely separated from the different outgroup members analyzed. On the other hand, nrITS tree (Figure 6) has a better resolution especially for *Satureja* taxa. Again, according to the nrITS tree, *Gontscharovia popovii* (B.Fedtsch. & Gontsch.) Boriss. is a close species to the genus *Satureja*, and this relationship was also reported by Bräuchler et al. (2010).

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On the contrary, *G. popovii* appeared together with *Satureja* members in *trn*L-F tree, and polytomies are also seen within the genus *Satureja* (Figure 7). Therefore, nrITS data gives us better results in this study.

Consequently, *Satureja hasturkii* has a close relationship with *S. hortensis* and *S. laxiflora* according to nrITS data (Figure 6). Moreover, *S. hasturkii* is similar to *S. hortensis* as seen from morphological observation.

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