

A NEW TAXON OF *SALVIA* (LAMIACEAE) FROM TÜRKİYE

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Abstract

Salvia divaricata Montbret & Aucher ex Benth. subsp. *artvinense* Eminagaoglu, Ozcan & Akyıldırım is described as a new endemic subspecies from Ardanuç (Artvin, Türkiye). It is related to *Salvia divaricata* Montbret & Aucher ex Benth. and *S. tomentosa* Mill. from which it differs in stem, leaf characters and flower color and numbers. A key is given to distinguish the new subspecies from the other species in the genus *Salvia*. Morphology, stem, petiole and leaf anatomy, and nutlet micromorphology were investigated. Nearly rounded stem, hemispherical petiole, bifacial leaf with diacytic stomata, and ovoid to rotund nutlet with glabrous and distinctly rough to protuberances ornamentation were determined for this subspecies. Taxonomic interpretations of the new subspecies are given using morphological, anatomical and phylogenetic analyses.

Introduction

Lamiaceae is one of the largest family with nearly 210 genera and more or less 1000 species of annuals shrubs, perennials and herbaceous (Celep *et al.*, 2015). *Salvia* L. belongs to the Nepetoideae subfamily. It was determined as a paraphyletic group, well supported by both morphological and molecular data (Walker *et al.*, 2004; Walker and Sytsma, 2007) and 987 living species of *Salvia* are distributed on the world (Hassler, 2020). Previously, 86 *Salvia* species were determined in Turkey by Hedge (1982a) and later, the number of species was raised to 100 (Davis *et al.*, 1988; Vural and Adiguzel, 1996; Donmez, 2001; Celep *et al.*, 2015), 53 of which are endemic in Turkey (Guner *et al.*, 2012; Celep *et al.*, 2015). About half of the species generally found in Central Anatolia and 23 species are present in NE Anatolia (Guner *et al.*, 2012).

Boissier (1879) determined 75 *Salvia* species from Turkey. He found out seven sections (*Salvia* (Syn. *Euphace*) Benth. (1876:1195), *Hymenosphace* Benth. (1876:1195), *Drymosphace* Benth. (1876:1195), *Aethiopsis* Benth. (1876:1195), *Plethiosphace* Benth. (1876:1195), *Horminum* Benth. (1876:1195) and *Hemisphace* Benth. (1876:1196), which had been all previously recognized by Bentham (1833). The section *Salvia*, represented by 24 species in Turkey (Celep *et al.*, 2015), has about 600 species in the world (Santos, 1995). Turkish *Salvia* are shrubs or perennial herbs with a relatively primitive staminal structure. The characteristic features of this section are leaves pinnatisect or simple, stems herbaceous or suffruticose, calyx little enlarging after anthesis and not diverging lips, upper lip of corolla is more or less straight and corolla tube is annulate. Staminal connectives are equal or slightly longer than filaments and lower theca is fertile (Hedge, 1972).

Türkiye is one of the major diversity centers for the genus *Salvia* and comprises approximately 90 species in our country. Endemism ratio is 45% (Hedge, 1982; Davis *et al.*, 1988; Hamzaoglu *et al.*, 2005; Aktaş *et al.*, 2009). Recent studies showed that Artvin has the highest plant species

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in Turkey. There are lots of floristic studies and new plant records in Artvin (Anşin *et al.*, 1997; Eminağaoğlu and Anşin, 2004; Eminağaoğlu, 2009; Eminağaoğlu, 2012; Eminağaoğlu *et al.*, 2012a, b; Eminağaoğlu and Ozcan 2013, 2014, 2018; Eminağaoğlu, 2015; Yüksel and Eminağaoğlu, 2017; Eminağaoğlu *et al.*, 2018; Yüksel and Akyıldırım Beğen, 2018; Akyıldırım Beğen and Yüksel, 2018; Eminağaoğlu and Eminağaoğlu, 2018).

During fieldwork in Ardanuç in 2013, it was observed that the specimens were different from other *Salvia* species with morphology and habitat characteristics. Detailed studies with herbarium samples and all data about these specimens confirmed the existence of this new subspecies of *Salvia*, *Salvia divaricata* subsp. *artvinense* in Türkiye.

Materials and Methods

Plant material

Specimens of *Salvia divaricata* subsp. *artvinense* were collected from Ardanuç province (Artvin) during field studies within the scope of the project “Determination of Native Plants in Artvin” (Eminağaoğlu, 2015). Morphological characteristics of new subspecies were recorded both in the field and in the laboratory. All specimens collected from area were evaluated and compared with literature (Komarov, 1934-78; Grossheim, 1939-1967; Hedge, 1982a; Eminağaoğlu and Anşin 2003, 2004) and different resources including Flora Orientalis (Boissier, 1879), Flora Europaea (Hedge, 1972) and Flora of the USSR (Pobedimova, 1954). Also, *Salvia* specimens have been checked in ANK, GAZI, ISTE and ISTO herbaria. Samples are deposited in Artvin Coruh University Herbarium (ARTH).

The morphological features of the new subspecies were studied with stereo-binocular microscope. Furthermore, photos and notes made in the field were used for description and drawings from different parts of the samples by using stereo microscope (Figs 1, 2).

Anatomical preparations

Stem and leaf parts of living specimens were used in anatomical observations. Different plant parts were stored in 70% alcohol for anatomical studies. Transverse sections of stem and leaf, and peripheral sections of upper and lower epidermis of leaves were taken by hand using commercial razor blades, and Haematoxylin solution for about 15 min was used for staining. Sections were washed in water several times to remove the excess stain (Algan, 1981). Semi-permanent slides were mounted in glycerin, and well stained sections were examined under a light microscope and photographed using an Olympus BX53 microscope with digital camera attachment DP73.

Micromorphological examinations

Micro and macromorphological features of the seeds were studied using a stereomicroscope (Leica M60 with a digital camera attachment DFC295) and a scanning electron microscope (Zeiss Evo LS10, ACU Science-Research Center). The seeds were first examined using a stereomicroscope to determine size, shape, color and maturity, and were then photographed. For scanning electron microscopy, mature seeds were placed on stubs using double-sided adhesive tape, and coated with gold in a Cressington sputter coater 108 auto coating apparatus for 2-3 minutes. Seeds were examined and photographed from the middle part of the lateral region. The terminology for cypselar characteristics proposed by Stearn (1985) was adopted to describe the fruit coat, size and shape, cell arrangements and primary sculpturing.

DNA extraction, polymerase chain reaction amplification (PCR) and sequencing

DNA extraction of *Salvia* leaves was applied using kit procedure (PureLink Genomic DNA kits) according to the manufacturer's protocol. trnL (UAG) (5- CTGCTTCCTAAGAGCAGCGT-3) / rpL32 (5_ CAGTTCCAAAAAACGTACTT C-3) primers (Shaw *et al.*, 2007) were used for PCR amplification. PCR were performed in 50 µl volume containing Taq DNA polymerase buffer with 6 µl MgCl₂ (2 mM), 5 µl 10x TAE Buffer, 0,5 µl dNTP (0.2 mM), 0,5 µl each forward and reverse primer (0.3 mM), 0,2 µl 5 U of Taq DNA polymerase and 2 µl of genomic DNA (20–100 ng). Thermocycling was performed with BioRad T100 Thermal Cycler. The temperature profile of amplification included an initial denaturation step of 95 °C for 5 min followed by 37 cycles of 94 °C for 30 s, 51°C for 1 min, 72 =°C for 1 min and a final elongation period of 72 °C for 5 min, then stored at + 4 °C. PCR products were resolved in 1 % agarose gel by electrophoresis at 85 volts, after a single band was observed, PCR products (50–250 ng/ul) were cleaned and then sequenced both forward and reverse direction at the Macrogen.

Phylogenetic studies

The sequences were aligned in BioEdit v.7.0.9.0 (Hall, 1999) and DnaSP v.5.10 (Librado and Rozas, 2009). This programs were used to determine haplotypes and to estimate haplotype and nucleotide diversities within each species. We used MEGA 7.0 (Tamura *et al.*, 2013) to calculate the genetic distances among sequences of the *Salvia* species, based on the Kimura 2- parameter (K2P) model of DNA substitution (Kimura, 1980) and their reliability has been tested with 10,000 bootstrap replications (Felsenstein, 1985). Phylogenetic trees were constructed using tree analyses: maximum parsimony (MP). *S. hylocharis* (KC473351) and *S. coccinea* (KC473345) species were used as an outgroup in the phylogenetic analysis. List of *Salvia* species were given in Table 1. All *Salvia* species on the table are genetically the closest species to *S. divaricata* subsp. *artvinense* selected by Blast (Basic Local Alignment Search Tool-Genebank).

Table 1. Information and GenBank accession numbers of *Salvia* species.

| Taxon | Voucher | Location | GeneBank accession |
|--|---------------------|----------------|--------------------|
| <i>Salvia divaricata</i> subsp. <i>artvinense</i> | <i>O.Emin</i> 16896 | Artvin, Turkey | |
| <i>S. divaricata</i> | W620659 | NPGS | KU578226.1 |
| <i>S. tomentosa</i> | W020129 | Sichuan, China | KU578214.1 |
| <i>S. aucheri</i> | W020132 | Sichuan, China | KU578248.1 |
| <i>S. coccinea</i> | NA67377 | NPGS | KC473345 |
| <i>S. sclarea</i> | W620660 | NPGS | KC473391 |
| <i>S. hylocharis</i> | PI440651 | Sichuan, China | KC473351 |

Results and Discussion

Taxonomic treatment

Salvia divaricata subsp. *artvinense* Eminagaoglu, Ozcan & Akyıldırım, **subsp. nov.** (Fig.1).

Type: TURKEY. Artvin, Ardauç, roadside, sloping area, 41°09'26" N, 42°00'01" E, 492 m, 19 June 2013, *O.Emin* 16896 (ARTH 13579, 13580).

Morphological description: Erect, perennial herbs; 40–54 cm tall, with several stems arising from a woody rootstock; stems glabrous; Leaves simples, developed towards the base, spreading, petioles 1.1–6 cm long, ciliate; basal leaf blades ovate, 3.2–11 × 0.8–3.5 cm, apex and base

obtuse, margin crenate, upper surface sparsely eglandular pilose, beneath densely tomentose (Fig. 3). Inflorescences an elongate, widely branched panicle, peduncles 4.5–9 cm long. Verticillasters 2-4 flowered, clearly distant. Bracts and bracteoles absent or deciduous early at the development of the inflorescence. Pedicels 1.8–3 cm long, glabrous, rigid, erecto-patent. Calyx tubular-campanulate, green, tube 12–17 mm long, densely glandular pilose and sometimes a few eglandular villous hairs; upper lip obsolete tridentate; calyx lobes acuminate, posterior lobe 3-veined, lobes 5–6 mm long.



Fig. 1. *Salvia divaricata* subsp. *artvinense*. A-B. Habit. C. Inflorescence peduncle and floral bracts. D. Calyx lobes and corolla color. E. Nutlets. Scale bar: 1000 µm.

Corolla white-yellow or dirty yellow, tube straight below, widening above, 25–30 mm long; posterior lobe of the corolla galeate, with the external surface pubescent, 2–3 cm long; anterior lobe 5–7 (–8) mm long, reflexed, internal basal surface tomentosa pubescent. Stamens included;

filaments 0.8–1 cm long; connectives 3–4 mm long. Style 2.5–3.5 cm long, pubescent. Stigma forked. Seed 69-78,5 x 55-62 mm, pale or dark, black, brown, hilum 15-19 mm.

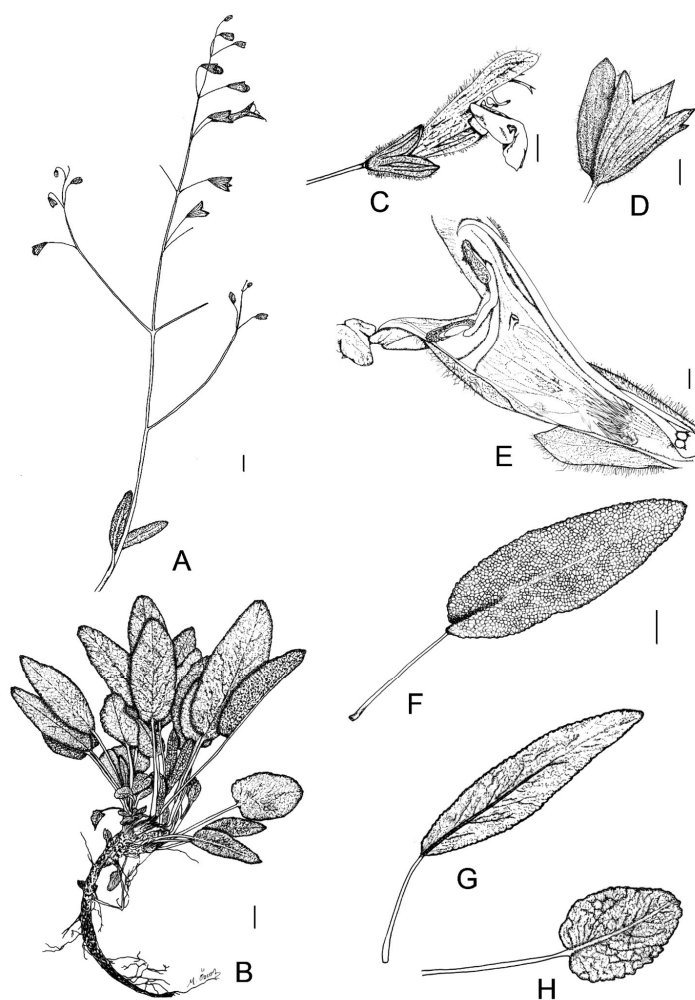


Fig. 2. Drawings. A. inflorescens. B. basal leaves. C. flower. D. calyx. E. dissected flower. F-G. different type leave. Scale bars: A, B, D, F, G, H = 1 cm. C = 5 mm. E = 1mm, illustrations were drawn from the holotype (*O.Emin 16896*) by Dr. Melahat Ozcan.

Distribution and ecology: *Salvia divaricata* subsp. *artvinense* is endemic to Artvin, Turkey and only known from the type locality. This species has small population size in the field observation. Partly a mixture of terrestrial and Mediterranean climate; the summers are warm and dry, while the winters are partly warm and less rainy, characteristic plants such as *Alyssum artvinense* Busch., *Micromeria elliptica* K.Koch, *Hedysarum hedysaroides* (L.) Schinz & Thell., *Satureja hortensis* L., *Colutea armena* Boiss. & Huet., *Cotinus coggyria* Scop., *Teucrium polium* L., *Thymus praecox* Opiz, *Capparis sicula* subsp. *herbacea* (Willd.) Inocencio, D.Rivera, Obón & Alcaraz.

Phenology: It has been registered flowering in June and fruiting in September.

Conservation status: Only one populations with nearly 45 individuals of *Salvia divaricata* subsp. *artvinense* in Artvin were determined. The area of occupancy was 8 km² (less than 10 km²), extent of occurrence was 82 km² (less than 100 km²) and continuing decline was observed, and number of mature individuals was 45 (less than 250). The population of the species is threatened by extinction because of road construction activities. Therefore, the threat category should be assessed as Critically Endangered [CR: B1+2b (i,ii); C2a(i)] status (IUCN, 2021).

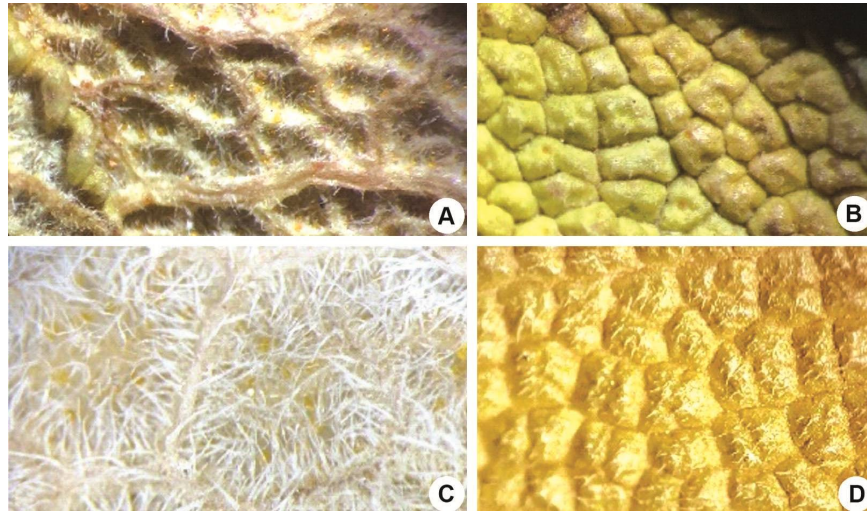


Fig. 3. Difference of leaf types between *S. divaricata* (A-B) and *S. divaricata* subsp. *artvinense* (C-D).

Remarks: *Salvia divaricata* subsp. *artvinense* resembles *S. divaricata*, *S. tomentosa* (Sec. *Salvia*), in habit, pedicels long; narrow leaves shapes. However, yellow-white corolla color in *Salvia divaricata* subsp. *artvinense* differs significantly, whereas the lilac color and taller plant size, smaller pedicel size and lilac corolla of *S. divaricata* and *S. tomentosa* are very distinct morphological characters. In addition; *S. divaricata* subsp. *artvinense* spreads below 600 m, but *S. divaricata* is over 1400 m.

Additional specimens examined (paratypes) *Salvia divaricata* subsp. *artvinense*: TÜRKİYE. **Artvin:** Ardanuç, 1-2 km to road, 41°09'26" N, 42°00'01" E, 483 m, 19 June 2013, *O.Emin* 16896 (ARTH! 13579); Artvin: Ardanuç, near road, 41°09'27" N, 42°00'09" E, 486 m, 02 June 2017, *O.Emin* 22364 (ARTH! 13580); *S. divaricata*: TURKEY. **Sivas:** between Zara-Divriği, 1630 m, 15 July 2007, *Z.Aytaç* 9543 (GAZİ!); **Erzincan:** İliç, Pınar village, 03 July 1977, *G.Arar, M.A. Ömür, S.Boldağ* (İSTE 116!); **Nevşehir:** Cappadoce Oriental, 1836, *Coquebert de Montbret, A.F.E.*, 2379 (K, K000929686!); **ARMENIA.** Type Specimen; 1837 *Aucher-Eloy, P.M.R.* 1528 (CJB G00156024!); *S. tomentosa*: TÜRKİYE. **Van:** *İ. Karakısa* 1585 (VAN!).

Key to the species of *Salvia* (sect. *Salvia*) based on morphological characters

- | | |
|---|---------------------|
| 1a. Stem quadrangular, verticillate 4-10 flowers | <i>S. tomentosa</i> |
| 1b. Stem rounded, verticillate 2-4 flowers | 2 |
| 2a. Pedicels 15-30 mm; leaves narrowly oblong, 3.2-7.8 x 0.8-2.7 cm | 3 |

- | | |
|--|---|
| 2b. Pedicels 1.5-7 mm; leaves oblong to ovate, 1.4-13.5 x 0.6-6 cm | 4 |
| 3a. Corolla lilac | <i>S. divaricata</i> |
| 3b. Corolla white-yellow | <i>S. divaricata</i> subsp. <i>artvinense</i> |
| 4a. Calyces 12-16 mm; leaves cuneate at base | <i>S. aramiensis</i> |
| 4b. Calyces 6-8 mm; leaves \pm cordate or rounded at base | <i>S. aucheri</i> |

Anatomical characteristics

Stem: Anatomical studies reveal that stem is almost rounded and has collenchymatic tissue in the corners. The epidermis contains a single layer of cells. There are simple and multicellular trichomes above the epidermis. Parenchymatic cortex has been observing 6-8 series. Vascular bundles of corners are larger than others and it is possible to observe small vascular bundles at the interfascicular areas arranged in a one circle. Sclerenchymatous caps were present above vascular bundles. Cambium is indistinguishable. Large pith formed of cylindrical and thin walled big parenchymatic cells are present in the stem (Figs. 4A, 6C).

Leaf: Midrib is hemispherical in outline. 2-3 layers of collenchyma are present under a single epidermal layer. One large vascular bundle can be seen in the midrib region. Simple densely trichomes or glandular trichomes are observed in upper and lower surfaces. Simple trichomes contain 2-4 stalk cells and glandular trichomes with in two shape as capitate and peltate. Capitate trichomes have one-two stalk cells and one or two head cells, while peltate trichomes contain one large head. This type trichomes are only present in abaxial surfaces of leaf. There is a single-layered epidermis. In terms of size, upper epidermal cells are much larger than those of the abaxial ones. Lamina is bifacial (dorsiventral) and mesophyll composed of 3-4 layers of spongy parenchyma and two layers of palisade parenchyma (Fig. 2C, D). Leaf is amphistomatic, and diacytic type stomata are observed in the depths (Fig. 5A; upper epidermis, 5B; lower epidermis).

Petiole: It is hemispherical or more or less triangular in outline. Mechanical tissue develops in the corners of triangular outline. Petiole is also surrounded by collateral tissue. One large vascular bundle in the center and three-four accessory bundles in the corners are present (Fig. 4Bb). Several simple trichomes with up to four stalk cell and capitate trichomes with different stalk cells encircled the petiol (Figs. 4B, 6A, B).

Mericaip micromorphology

Mericaip color was brown to blackish. Their size varied from 2.57 mm to 4.13 mm in length and 2.0 mm to 3.26 in width. They ranged in length to width ratio from 1.27 to 1.36. Shape of the mericarps was broadly ovoid to rotund. The nutlet surfaces are glabrous, distinctly rough with protuberances and undulate. Epidemal cells are irregular and anticlinal walls are not distinct and represented by undulate channel. The attachment scar diameter ranges from 0.79 mm to 1.0 mm. Their color is dark brown to brackish (Fig. 7).

Molecular analysis

A dataset of cpDNA sequences with 800 bp was analyzed for 7 taxa. Both Bayesian and MP analyses produced the same topology. The Bayesian inference tree with both posterior probability and maximum parsimony bootstrap support values is shown in Fig. 8. Both Bayesian and MP analyses showed that the ingroup formed a well-supported clade with 0.63 posterior probability and 65% bootstrap value, respectively (Fig. 8). The new subspecies is resolved as sister to the *S. divaricata* confirming its novelty in the genus.

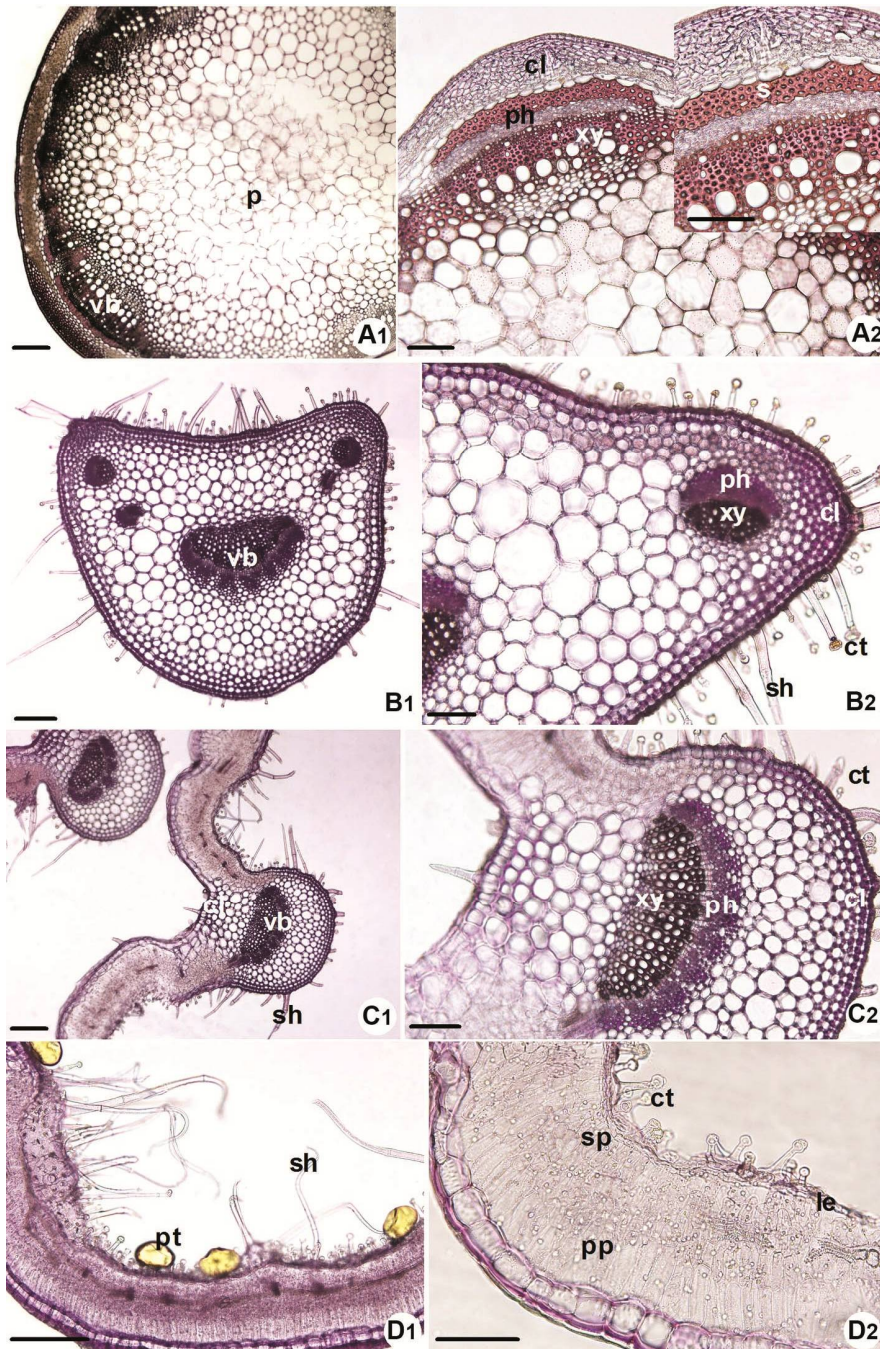


Fig. 4. Cross sections of *S. divaricata* subsp. *artvinense*. A. stem. B. petiole. C. leaf midrib. D. leaf lamina. 1: general appearance, 2: magnified part. Scale bars: 1 = 200 μm . 2 = 100 μm . cl: collenchyma, ct: capitate trichome, p: pith, ph: phloem, pp: palisade parenchyma, pt: peltate trichome, s: sclerenchyma, sh: simple trichome, sp: spongy parenchyma, ue: upper epidermis, xy: xylem, vb: vascular bundle.

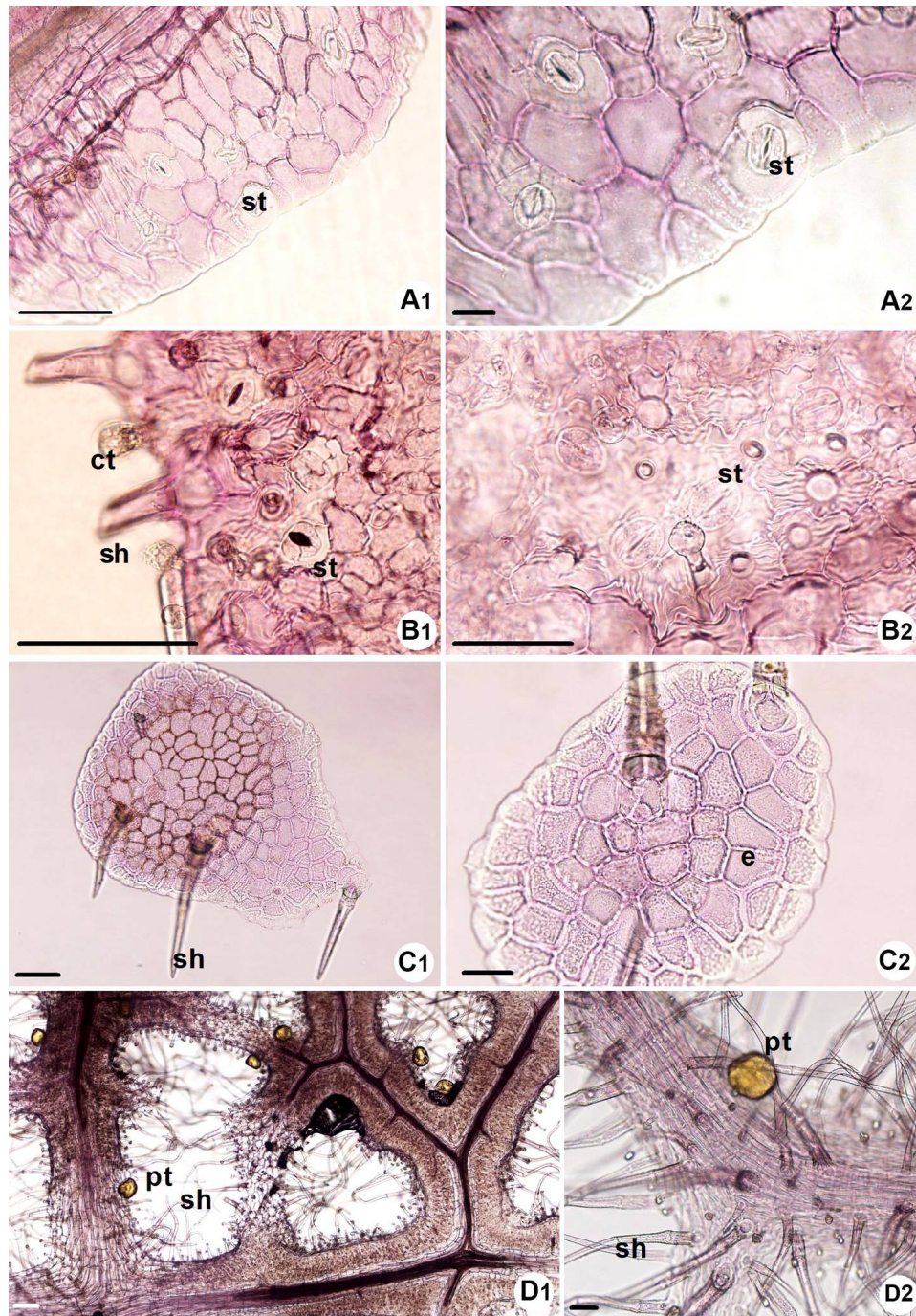


Fig. 5. Peripheral sections of *Salvia divaricata* subsp. *artvinense*. A. upper epidermis. B. lower epidermis. C. trichomes from upper epidermis. D. trichomes from lower epidermis. See Fig. 1 for abbreviations. Scale bars: A, B, C2, D2 = 50 μ m. C1, D1 = 100 μ m.

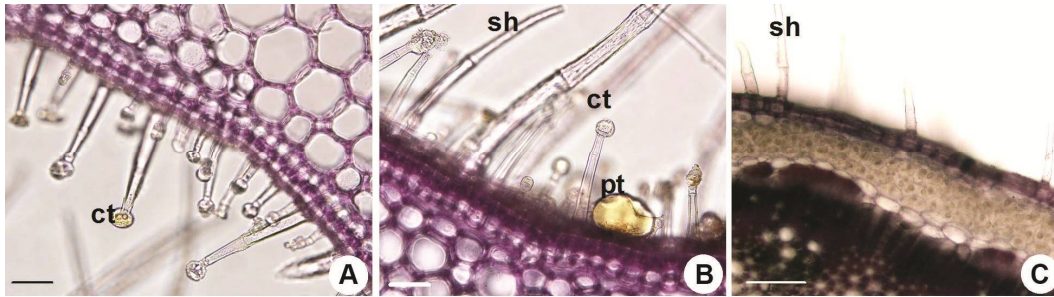


Fig. 6. Some trichomes from different parts of *Salvia divaricata* subsp. *artvinense*. A-B. petiole. C. stem. Scale bar: 100 μ m.

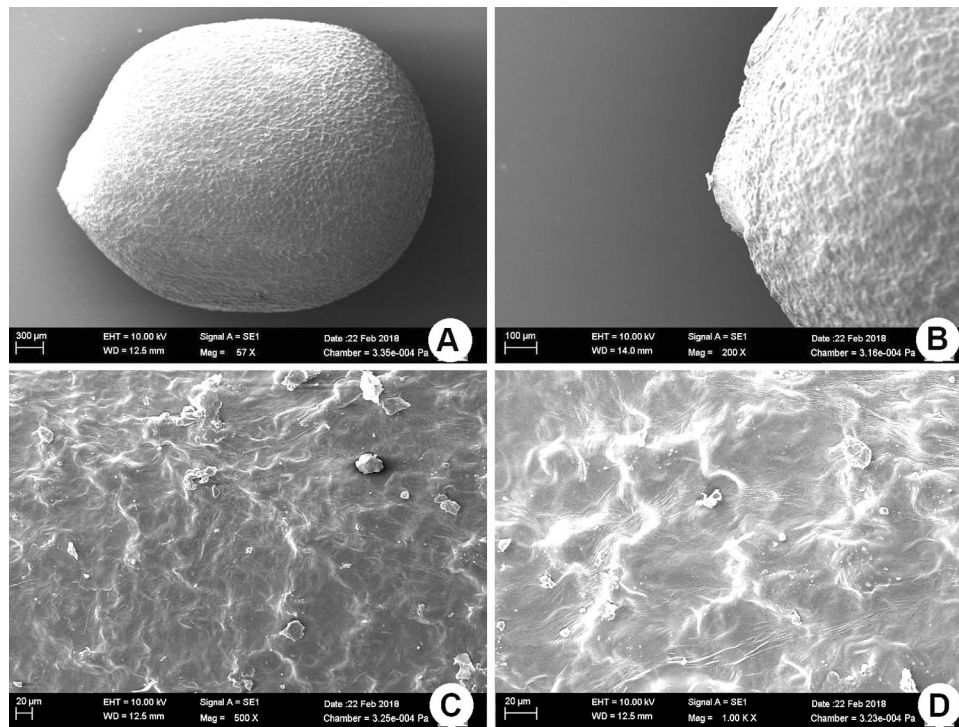


Fig. 7. Nutlet of *S. divaricata* subsp. *artvinense*. A. general appearance. B. 200X. C. 500X. D. 1000X.

The new subspecies *Salvia divaricata* subsp. *artvinense* is similar to *S. divaricata*, *S. aucheri*, *S. tomentosa* and *S. aramiensis* (Sect. *Salvia*), with some morphological characters but differentiated by yellow-white corolla color. *S. aucheri* and *S. aramiensis* can be separated from *S. divaricata* subsp. *artvinense* by various morphological characters including taller plant size, smaller pedicel size and lilac corolla color. *S. divaricata* subsp. *artvinense* has narrowly oblong leaves, tubular campanulate calyx shape and 2-4 flowers per node like *S. divaricata*. In contrast, *S. divaricata* has lilac-pink corolla color, smaller leaf size (4-7 x 0, 8-2 cm) and less pubescence structure upper and lower surface of leaves. *S. divaricata* subsp. *artvinense* spreads below 600 m, but *S. divaricata* is over 1400 m. Their differences are given in Table 2.

Table 2. Morphological comparisons of related *Salvia* species.

| Character | <i>S. divaricata</i> subsp. <i>artvinense</i> | <i>S. tomentosa</i> | <i>S. divaricata</i> | <i>S. aucheri</i> | <i>S. aramiensis</i> |
|--|---|---|------------------------------------|---------------------------------------|-----------------------------|
| Life form | herb | subshrub | herb | herb | suffruticose |
| Stem | erect rounded | quadrangular decumbent | erect | erect | clump-forming |
| Plant size (m) | 0.4-0.6 | 0.4-0.75 | 0.4-0.5 | 0.3-1 | 1 |
| Underground perennation structures | present | -- | present | absent | absent |
| Pubescence on the stem surface | glabrous (above) pilose (below) | glabrous above shortly patent hairs | glabrous (above) Pilose (below) | glabrous (above) Pilose (below) | finely pilose |
| Leaf | | | | | |
| Size (cm) | 3.2–11 × 0.8–3.5 | 5–5.5 × 1.5–2 | 3-8 x 0.8-2.5 | 4-10x 2-5 | 1.4-5 x 0.6-1.7 |
| Shape | narrowly oblong - ovate | elliptical | narrowly oblong | elliptic to ovate-elliptic, | narrowly oblong to ovate |
| Base | obtuse | rounded or cuneate | obtuse | -- | clustered |
| Pubescence in the upper surface | densely pilose | strongly rugose, sparsely covered | pilose | -- | -- |
| Pubescence in the lower surface | densely tomentose | short fine appressed white hairs | tomentose | adpressed white pubescent | -- |
| Petiole length (cm) | 1.1–6 | 5-7 | 1-5 | 1.5-4 | 1.7-5.5 |
| Inflorescence | | | | | |
| Pedicele size (mm) | 18–30 | 5-10 | 15-30 | 3-4 | 1.5-4(-7) |
| Number of flowers per node | 2-4 | 4-10 | 2-4 | 2 | 4-10 |
| Flower | | | | | |
| Calyx length (mm) | 12–17 | 12-15 | 15 | 6-8 | 12-16 |
| Calyx shape | tubular-campanulate | --- | tubular- campanulate | ovate- to tubular- campanulate | campanulate |
| Corolla color | white-yellow | | pink-lilac | lilac | lilac |
| Corolla tube length (mm) | 25–30 | 24-36 | 34 | 25 | 25-30 |
| Flowering time | 6-8 | | 6-7 | 7-10 | 5-6 |
| Elevation range | 490-550 m | 90-1600m | 1500-1800m | 550-1350m | 250-600m |

In the Flora of USSR, some diagnostic characters of *S. trigonocalyx* missed, flower colors were not given in its description by Woronow (1912). Additionally, *S. trigonocalyx* taxon was indicated as synonym of *S. tomentosa*, recently (Hassler, 2020; Banki *et al.*, 2021).

Till now, *S. divaricata* was only collected from Sivas and Erzincan (Türkiye). This species was not reported from Artvin in recent investigations. By taking the morphological differences observed in stem, leaves, inflorescences and flower colour, *S. divaricata* subsp. *artvinense* was distinguished as a new subspecies, after its comparison with all the revised herbarium specimens and data provided in the bibliography.

Metcalf and Chalk (1972) has previously reported in the family Lamiaceae has a quadrangular stem and a well developed collenchyma in the corners. Özdemir and Şenel (1999) and Polat *et al.* (2017) also reported rectangular stem in *S. sclarea* L. and in *S. divaricata* Montbret & Aucher *ex* Benth, respectively. On the contrary, Aktaş *et al.* (2009) reported rounded stem without collenchyma in the corners for *S. tchihatcheffii*. Cortex consists of three different cell

types as parenchyma, 6-8 layers and collenchyma, only in the corners. Simple and multicellular trichomes are observed in epidermal layers. Cambium is indistinguishable. Large pith formed of thin walled big parenchymatic cells in the pith of stem. Our findings about stem characteristics are similar to the reports for *S. sclarea* (Özdemir and Şenel, 1999) and *S. divaricata* (Polat *et al.*, 2017).

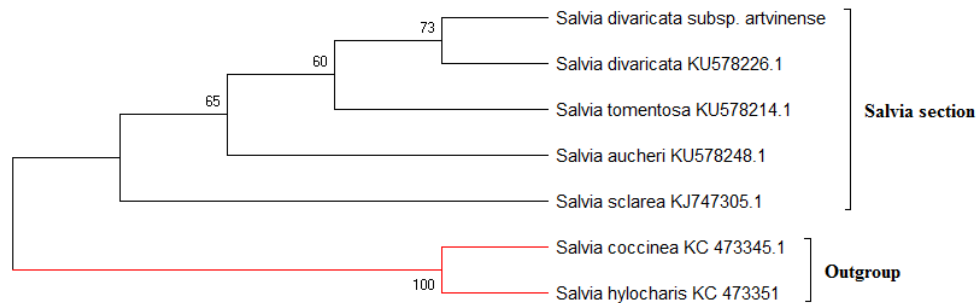


Fig. 8. Molecular phylogenetic relationship within *Salvia* with the maximum parsimony bootstrap support values.

Petiole anatomical structure is very important for the family Lamiaceae. Several studies were conducted on *Salvia* spp. (Özdemir and Şenel, 1999; Polat *et al.*, 2017). Özdemir and Şenel (1999) previously reported eglandular and glandular trichomes, parenchymatic cortex and two large vascular bundles with small bundles in the petiole of *S. sclarea*. Polat *et al.* (2017) also mentioned many glandular and eglandular hairs on the uniseriate epidermal cells. Similarly, we found a lot of unicellular simple trichomes and peltate and capitate glandular trichomes in the epidermal layers of petioles. The petiole is triangular in outline and composed of large parenchymatic cortex. In contrast to *S. sclarea* (Özdemir and Şenel, 1999), one big vascular bundle in the middle of petiole and four small bundles on the edges can be seen in *S. divaricata* subsp. *artvinense*. These results are in accordance with some reports in literatures (Nakipoğlu and Oğuz, 1990). Polat *et al.* (2017) also reported single median vascular bundle with a crescent appearance but two small bundles on the edges differently from us. In addition, median vascular bundle of *S. divaricata* subsp. *artvinense* is smaller than *S. divaricata* and do not have crescent appearance.

S. divaricata subsp. *artvinense* has bifacial (dorsiventral mesophyll) leaf and diacytic type of stomata. Stomata are present both upper and lower epidermal surfaces (amphistomatic leaf). Metcalfe and Chalk (1972); Özdemir and Şenel (1999) and Polat *et al.* (2017) also mentioned these characters in some *Salvia* species. Like stem and petiole, leaves also have different types of trichomes. Eglandular trichomes in all parts are unicellular, but glandular trichomes with in the shapes of capitate and peltate. Peltate trichomes with fragrant essential oil are present only in lower parts of leaves in *S. divaricata* subsp. *artvinense* and they have previously been reported in *S. sclarea* (Özdemir and Şenel, 1999), in *S. tchihatcheffii* (Aktaş *et al.*, 2009) and Polat *et al.* (2017).

Mericaip length, shape of mericaip and exocarp cells and structure of anticlinal walls have been reported significant diagnostic characters by Büyükkartal *et al.* (2011). Hedge (1982) reported rounded, trigonous or rounded-trigonous in the mericaip of some Turkish *Salvia* species. Özkan *et al.* (2009) determined three sculpturing in 12 *Salvia* taxa as foveate, reticulate and verrucate. Büyükkartal *et al.* (2011) reported colliculate pattern in some *Salvia* taxa. Seed of this new species was ovoid shape, however their surface ornamentation was determined as ruminate.

Our molecular analysis based on cpDNA sequences confirmed that *S. divaricata* subsp. *artvinense* distinguishable from all other species in the series (Fig. 8). In the phylogeny, *S. divaricata* was resolved as sister to *Salvia divaricata* subsp. *artvinense*. All data confirmed that *S. divaricata* subsp. *artvinense* is distinguishable from all other species in the series (Table 2).

The population of the species is in danger of extinction due to road construction activities. For this reason, it should be produced by being transported to the botanical garden (Eminağaoğlu and Eminağaoğlu, 2018), protected and transported to another suitable habitat.

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