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# *Marrubium zeydanlii* (Lamiaceae), a new species from Turkey

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**Abstract:** *Marrubium zeydanlii* (Lamiaceae) is described as a new species from Turkey. It is similar to *M. depauperatum* and *M. sivasense*; however, it is distinguished from M. *depauperatum* by having lanate hairs on the stem, oblanceolate dentate leaves, 4–6-flowered verticillasters. It is also distinguished from *M. sivasense* by having lanate hairs on the stem, oblanceolate leaves, and shorter petals. The pollen grains are radially symmetrical and isopolar, the shape is oblate-spheroidal with the polar axes 16.7–20 µm and the equatorial axes 17.6–19.3 µm. *trn*L-F region was used to identify the phylogenetic position of the newly described species. The results of the current study indicated that the new species show significant morphological and molecular differences from closely related species. Taxonomic descriptions and micromorphology of pollen are examined and also, some notes on the ecology, phytogeographic, conservation status, and distribution map of the new species are presented.

**Key words:** *Marrubium*, new species, pollen and nutlets morphology, phylogeny, Turkey

## 1. Introduction

The members of the genus *Marrubium* L. are annual or perennial, herbaceous and often woody at the base and generally distributed in the Mediterranean and Irano-Turanian phytogeographic regions (Cullen, 1982; Hedge, 1992; Martínez-Gor-dillo et al., 2017). The genus is represented by about 41 taxa in the world (Akgül et al., 2008). In Turkey, it is represented by 34 taxa and 17 of them are endemic and the endemism rate is 50% (Cullen 1982; Davis et al., 1988; Akgül, 2012, 2018; Fırat, 2016; Özhatay et al., 2017; Deshmukh et al., 2022). *M. depauperatum* Boiss. & Balansa (Cullen, 1982) is an endemic species for the flora of Turkey and was collected around Kayseri by Balansa in 1855. It was thought to be extinct later, was collected for the second time from the type location approximately 160 years later (Akgül et al., 2007[1]). This means that Turkey is one of the main centers of diversity for the genus (Bentham, 1834, 1848).

The number of calyx teeth and morphology are important characters in its classification. The genus is divided into two groups according to the number of calyx teeth, (7–)10–30 vs. 5(–7), in Flora of Turkey and the East Aegean Islands; 6 species are placed in the first group (*M. vulgare* L., *M. anisodan* C. Koch, *M. parviflorum* Fisch. & Meyer, *M. cuneatum* Russell, *M. yildirimlii* Akgül & B. Selvi, *M. amasiensis* Akgül & Ketenoğlu) *M. asumaniae* U. B. Deshmukh, E. S. Reddy, M. B. Shende (*M. lanatum* non Bentham) (Akgül, 2018; Deshmukh et al., 2022) and others are (17 species) in the second group (Cullen, 1982; Aytaç et al., 2012; Akgül and Selvi, 2014; Akgül et al., 2017). Another character of the genus is the indumentum. Basically, the hairs are stellate, but frequently the lateral branches are very small and the central branch is very elongate. At the same time, simple hairs seem to occur only in the hair tufts within the calyx (all or most species) and on the stems (*M. vanense* Hub.- Mor. and *M. vulcanicum* Hub.- Mor.).

When *Marrubium* was first revised, it was divided into two sections, *Lagopsis* Bunge ex Benth. and *Marrubium*. Later, many researchers worked on it and divided it into several sections (Boissier, 1879; Briquet, 1896; Seybold, 1982). However, the genus *Marrubium*

[1] Akgül G, Ketenoğlu O, Doğan M (2007). Rediscovery of *Marrubium depauperatum* Boiss. & Ball. after 152 years. In: International Symposium on 7th Plant Life of South West Asia (7th PLOSWA), Eskişehir, Turkey.

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was not divided into sections by Cullen, who prepared *Marrubium* account for the Flora of Turkey and the East Aegean Islands (Cullen, 1982; Akgül, 2004[2]).

Siadati et al. (2020) emphasized that indumentum was very important for the genus in their study. According to their study, two main hair types were observed in the genus, glandular (capitate, peltate, or branched) and nonglandular (simple or branched). Indumentum micromorphology is useful for taxon delimitation within *Marrubieae* at various ranks Siadati et al. (2020). Aytaş and Camili studied the anatomical and micromorphological characters of *Marrubium cephalanthum* Boiss. & Noe subsp. *montanum* Akgül & Ketenoglu (Aytaş and Camili, 2018).

The genus *Marrubium* pollen grains are tricolpate, radially symmetric and isopolar, and are prolate spheroidal and oblate spheroidal in shape (Erdtman, 1945, 1969; Akgül et al., 2008).

According to Siadati et al. (2019), the nutlets shape are usually ovate, rounded, broad ovate, elliptic, lanceolate, triangular, oblong and the type of sculpturing was more useful in taxon delimitation among *Marrubium* species and allies at the species rank.

This study was conducted to demonstrate the existence of a new *Marrubium* species.

## 2. Materials and methods

Flowering materials of the new species were collected twice during field trips in Sivrihisar of Eskişehir Province in September 2019 and August 2020. The literature, such as Flora of Turkey and the East Aegean Islands (Cullen, 1982), Flora Iranica (Seybold, 1982) and Flora Europeae (Tutin et al., 1964-1993) were used for description and the photo of *M. depauperatum* was examined from different herbaria photos by Balansa 1077! as Kew (K000929126!, K000929127!), JE (JE00011478!), P (P00743521!), GOET (typus, P00743519!), FI (FI011209!), (Thiers 2019). On the other hand, many specimens belonging to *M. depauperatum* and type material of *M. sivasense* Aytaç, Akgül & Ekici (Aytaç et al., 2012) were examined morphologically in different herbaria, such as GAZI, ANK, and HUB.

Pollen slides were prepared using the technique by Wodehouse (1935). LM studies were made using a Leica ICC50 HD microscope. Measurements were made for 30 or more pollen grains for each species. Dry pollen grains were mounted on stubs and coated with gold for the scanning electron microscope (SEM) investigation. Morphological observations were made with a JSM 5600 electron microscope. Shape classification followed Erdtman (1969), based on the P/E ratios.

The new material was collected during flowering and fruiting periods. Specimens were examined with a stereoscopic microscope and SEM. The nutlets were investigated by using a stereoscopic microscope and SEM.

The SEM photomicrographs were taken with the JEOL JSM 6060 SEM at the Ankara University Electron Microscopy Unit. In this study, the terminology of Punt et al. (2007) was used. The specimens were deposited in the GAZI herbarium.

Authors of botanical names were abbreviated according to Authors of Plant Names (Brummitt and Powell, 1992).

DNA extraction, PCR amplification and sequencing: The plastid *trn*L-F region was used to identify the phylogenetic position of the newly described species. Novel sequences were generated for two species, namely for *M. sivasense* and *M. zeydanlii* Aytaç, Kaptaner İğci & T. Ertuğrul sp. nov., while 54 sequences were downloaded from GenBank (Appendix I). Voucher specimens for these taxa were preserved in the Gazi University Herbarium (GAZI). Thirteen sequences of *Acanthoprasium* (Benth.) Spach, *Ballota* L., *Moluccella* L., *Otostegia* Benth. and *Roylea* Wall. ex Benth. were included as outgroups based on the phylogeny of Siadati et al. (2018). Appendix 1 includes National Center for Biotechnology Information (NCBI/GenBank) numbers for new and published sequences.

Genomic DNA was extracted from herbarium material using Qiagen DNeasy Plant Mini Kit (Qiagen, Germany) following the manufacturer's instructions. The plastid *trn*L-F region was amplified using the C and F primers of Taberlet et al. (1991) with the following thermocycling program: 4 min initial denaturation at 94 °C; followed by 28 cycles of 1 min denaturation at 94 °C, 1 min annealing at 50 °C, 1 min extension at 72 °C and final extension step of 7 min at 72 °C (Martínez-Azorín et al., 2011). All reactions were carried out in 50 µL volumes, containing 25 µL PCR Master Mix (Thermo Fisher), 23 µL distilled water, 0.5 µL of each primer, and 1 µL of template DNA. DNA products were analyzed by electrophoresis in 1% agarose gel. For purification and sequencing, products were sent to the ECGR Lab (Hacettepe University, Department of Biology, Ankara, Turkey).

Phylogenetic analyses: Sequences were assembled, aligned, edited, and trimmed using Geneious Pro 4.8.4 (Kearse et al., 2012). The alignment included 58 sequences belonging to 36 species and six genera (Appendix 1). jModelTest v2.1.6 (Guindon and Gascuel, 2003; Darriba et al., 2012) as implemented on the CIPRES Science Gateway (Miller et al., 2010) was used with the Akaike information criterion (AIC) test to determine the best nucleotide substitution model for the *trn*L-F plastid dataset.

To evaluate phylogenetic relationships, we constructed both the Maximum likelihood (ML) and Bayesian Inference (BI) analyses. ML analyses were conducted using

[2] Akgül G (2004). Türkiye *Marrubium* L. (Lamiaceae) cinsinin revizyonu (Revision of the *Marrubium* L. {Lamiaceae} species in Turkey). PhD, Ankara University, Institute of Sciences, Ankara, Turkey (in Turkish).

RAxML version 8.2.12 (Stamatakis, 2014) as implemented on the CIPRES Science Gateway (http://www.phylo.org/[3]). Default ML search options were employed, (except the "Let RAxML halt bootstrapping automatically" option). BI analyses, on the other hand, were performed on the CIPRES Science Gateway ((Miller et al., 2010); http://www.phylo.org/) using MrBayes 3.2.7a (Ronquist et al., 2012). For each analysis, four parallel Monte Carlo Markov chains (MCMC) were run with three heated chains and one cold chain, for ten million generations, sampling every 100 generations. The first 25% of samples (the first 25,000 trees) were discarded as "Burn-in", and maximum credibility trees were constructed from the remaining trees. This was repeated twice to ensure convergence. The phylogenetic tree image was rendered in the Interactive Tree of Life (iTOL) (Letunic and Bork, 2016).

## 3. Results

***Marrubium zeydanlii*** Aytaç, Kaptaner İğci & T. Ertuğrul **sp. nov.** (Figure 1,3)

**Type:** Turkey, B3 Eskişehir: Sivrihisar, Acıkır Basin, Buzluca village, gypsum steppe, 840 m, 03.09.2019, Kaptaner İğci 1303, Aytaç, Özüt & Zeydanlı (holotype GAZI, isotypes: ANK, HUB).

**Paratype:** Turkey, B3 Eskişehir: Sivrihisar, Gülçayır to Buzluca 1.5 km, steppe, 850 m, 10.08.2020, Aytaç 10797 & S. H. Genç (GAZI).

### 3.1. Diagnosis

*Marrubium zeydanlii* is similar to *M. depauperatum* and *M. sivasense*, but it is distinguished from *M. depauperatum* by having 4–5 (–6) flowered verticillasters (versus 1–2), lanate hairs on the stem and leaves (versus stellate-pilose), leaves oblanceolate (versus oblong cuneate). On the other hand, it is distinguished from *M. sivasense* by having lanate hairs on the stem and calyx (versus adpressed and greyish stellate-pubescent above); leaves oblanceolate (versus elliptic to oblong), margin entire below, ± dentate (3–5 teeth) at apex (versus crenate to crenulate); calyx teeth equal (versus subequal); corolla 6–7 mm, densely pilose-stellate (versus 5–7 mm, stellate-pubescent outside).

### 3.2. Description

Erect, perennial, woody and branched from base. Sterile shoots 1–3 cm and leaves very small, 3–5 × 2–3 mm, obovate, densely white lanate, and ± crenate. Stem 30–45 cm, quadrangular, and densely white lanate. Fertile stem leaves are 3–5 pairs, petiole 2–3 mm long, ± involute, 15–25 × 3–5 mm, oblanceolate, obtuse, entire below, ± dentate (3–5 teeth) at apex, and densely white lanate and stellate on both sides (the stellate hairs on the leaf surface are covered by lanate hairs). Number of verticillasters per stem is 5–15 and 4–5 (–6) flowered; sessile; floral leaves as long as or longer than verticillaster, oblanceolate, lanate - stellate, shortly (1–2 mm) petiolate and bracteoles linear 1–1.5 mm shorter than calyx. Pedicel shorter than bracteoles (0.5–1 mm), and lanate-stellate. Calyx obconical, 5 mm, densely whitish lanate with stellate-pubescent and tufts of long simple hairs inside at mouth; calyx teeth equal, straight. Corolla whitish, densely stellate-pubescent outside, sparsely pilose within upper lip, 6–7 mm, upper lip deeply bifid, two times longer than lower lip, less stellate-pilose inside upper lip; tube including calyx and glabrous at base. Stamen 4, including in corolla tube. The nutlet ovate-oblong, 2–2.5 × 0.7–1.9 mm, dark brownish.

Flowering and fruiting time: August–September.

### 3.3. Pollen morphology

The pollen grains are radially symmetrical and isopolar. The shape is oblate-spheroidal with the polar axes 16.7–20 µm and the equatorial axes 17.6–19.3 µm. The pollen grains are inoperculate, 99% tricolpate and 1% syncolpate. The colpus is 10–12.5 µm × 1.4–2.2 µm. The apertural membrane is generally psilate or rarely granulate-rugulate. The exine is tectate and 1.5–1.9 µm in thickness. The ectexine is slightly thicker than endexine. The intine thickness ranges from 0.3 to 0.5 µm. Psilate-foveolate-perforate ornamentation was observed. The foveolate are ≥1 µm in diameter, distributed regularly over the whole surface and 7–10 foveolate per 5 µm². The size of perforations is ≤ 1 µm in diameter, they are distributed regularly over the whole surface, and there are 3–5 perforations per 5 µm² (Figure 2).

### 3.4. Etymology and proposed Turkish name for the new species

This species is named in honour of Uğur Zeydanlı, Ph.D, who is one of the collectors of the new species within the framework of the Tanap Project. *Marrubium* is called in Turkish "Bozotu". We propose "Zeydanlı bozotu" as a vernacular name for *M. zeydanlii* (Menemen et al., 2016).

### 3.5. Phylogeny

GTR+I was selected as the best substation model for our *trn*L-F plastid data set. The *trn*L-F alignment was 829 base pair (bp) long, in which 42 were variable, 36 were parsimony informative.

The results of our phylogenetic analyses were mostly congruent with Siadati et al. (2018). *Ballota* was monophyletic (98% BS, 1.00 PP) and sister to monophyletic *Marrubium* (97% BS, 0.99 PP) with strong support (93% BS, 0.99 PP) (Figure 4). While the two newly sequenced *M. zeydanlii* and *M. sivasense* were monophyletic (50% BS and 66% BS, respectively) (Figure 4), the monopoly of these species was not resolved in the BI phylogenetic tree (results not shown). Yet, although morphologically newly described *M. zeydanlii* closely resembles *M. sivasense;* these species were distantly related to each other in both

[3] http://www.phylo.org/sub_sections/portal.php.

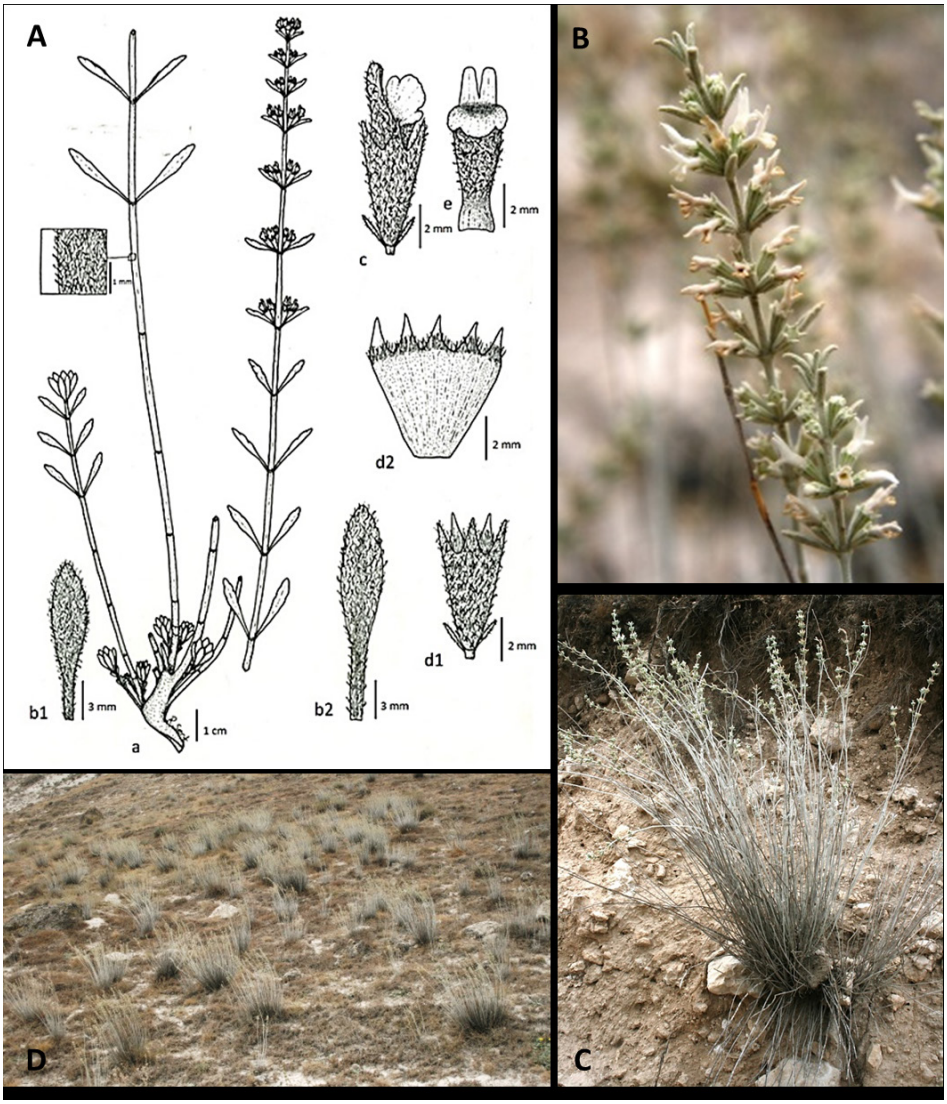


**Figure 1.** *Marrubium zeydanlii* **A**-habitus: a-general habitus, b1-upper surface of leaves, b2-lower surface of leaves, c-flowers, d1-calyx, d2-dissected calyx (inner surface), e- corolla, **B**,**C**-habitus in the field, **D**-habitat.

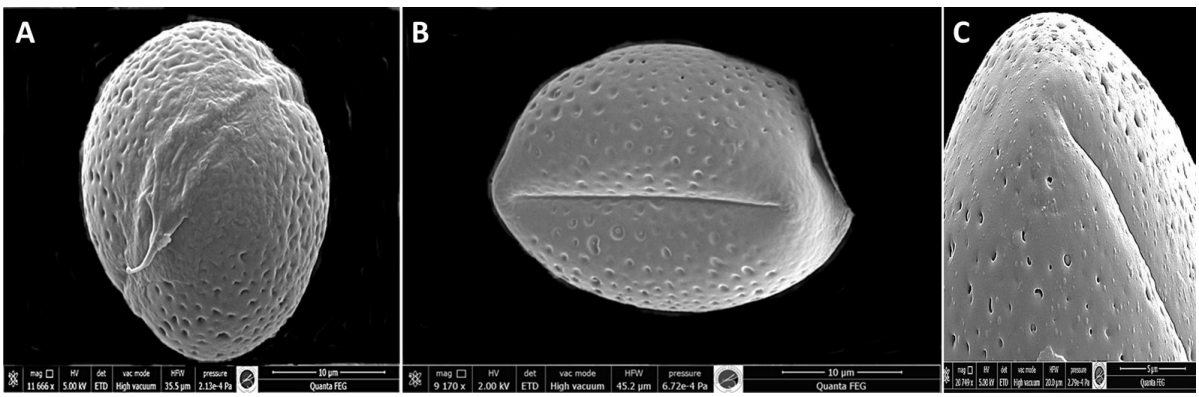


**Figure 2.** Pollen micrographs of *M. zeydanlii*: **A**-Equatorial view and apertures, **B**-Polar view, **C**-Ornamentation.

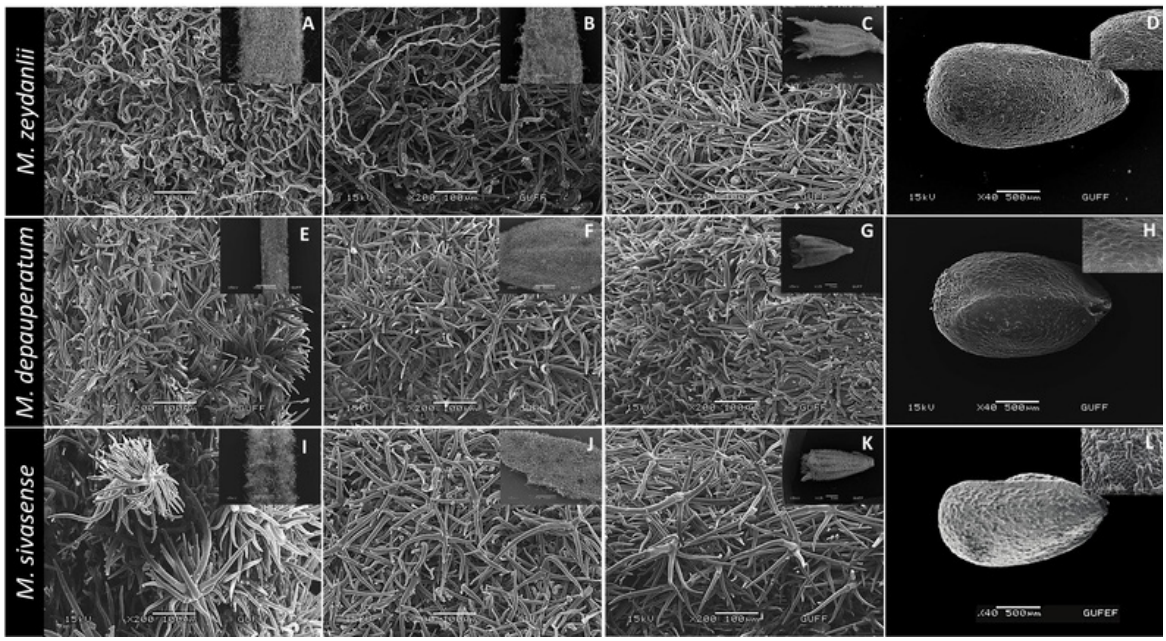


**Figure 3.** SEM micrographs of *M. zeydanlii* : A-stem, B-leaves, C-calyx, D-nutlet; *M. depauperatum*: E-stem, F-leaves, G-calyx, H-nutlet; *M. sivasense*: I-stem, J-leaves, K-calyx, L-nutlet.

the ML and BI analyses; and therefore, our phylogenetic analyses confirm that the *M. zeydanlii* described here as a new species represents an independent taxon.

### 3.6. Distribution and ecology

The new species is growing in Central Anatolia, around Eskişehir (B3) province, endemic and belonging to the Irano-Turanian phytogeographical region (Figure 5). The new taxon also grows on gypseous steppes with *Artemisia santonicum* L., *Centaurea virgata* Lam., *Verbascum salviifolium* Boiss., *Heliotropium europaeum* L., *Salvia wiedemannii* Boiss., *Capparis sicula* Veill. subsp. *sicula* and *Eryngium creticum* Lam.

### 3.7. Suggested conservation status

*Marrubium zeydanlii* is locally endemic in Sivrihisar (Eskişehir) and is known from two localities. Its extent of occurrence (EOO) and area of occupancy (AOO) are 44 km² and the number of members is around 5000. So, the new species is considered as "EN" (B1 ab (i, ii, iii, v) + B2 ab (i, ii, iii, v) (IUCN, 2017). This taxon is under pressure from agriculture and overgrazing. The opening of new agricultural areas and the continuation of grazing cause a decrease in the habitat (AOO), distribution area (EOO), population number of the species and habitat degradation. In addition, mildew fungus is abundant on individuals and continues its negative effects.

### 3.8. Discussion and conclusion

*M. zeydanlii* is similar to *M. sivasense* and *M depauperatum,* and both of them are growing in Central Anatolia. *M. depauperatum* generally prefers calcareous bedrock, *M. sivasense* and *M. zeydanlii* prefer gypsiferous limy bedrock. *M. depauperatum* is the member with the least flowered in its verticillasters 1 (–2). Likewise, *M. sivasense* is of similar character, but its verticillasters are 3–10 flowered; *M. zeydanlii* carries 4–6 flowers in its verticillaster. The stem leaves are elliptic to oblong in *M. depauperatum* and *M. sivasense,* oblanceolate in *M. zeydanlii*. On the other hand, the stellate hairs on both side of the leaf are covered by lanate hairs in *M. zeydanlii*, not covered in the other two species. The corolla is sparsely pilose within the upper lip of *M. zeydanlii*, glabrous in *M. sivasense* and stellate-pilose within the upper lip of *M. depauperatum*. Other distinctive characters are given in Table.

The palynological and nutlet structures of *M. zeydanlii* and its other relatives *M. sivasense* and *M. depauperatum* are mostly similar and it is seen that they cannot be used in species separation (Akgül et al., 2008; Aytaç et al., 2012).

The phylogenetic trees based on the *trn*L-F region presented evidence for the divergence of *M. zeydanlii* from the *M. sivasense* sequences. As seen in Figure 4, both species fall into different clades. It is maybe due to previous evolutionary processes (i.e. reticulation events), such as, hybridization, incomplete lineage sorting (ILS) or introgression (Solís-Lemus and Ané, 2016). Therefore, future studies with additional markers from different genomes are needed to reveal the correct phylogenetic placement of *M. zeydanlii* and *M. sivasicum*. Yet, as a result of the evaluation of the morphological and molecular data, it was evident that the *M. zeydanlii* distributed in

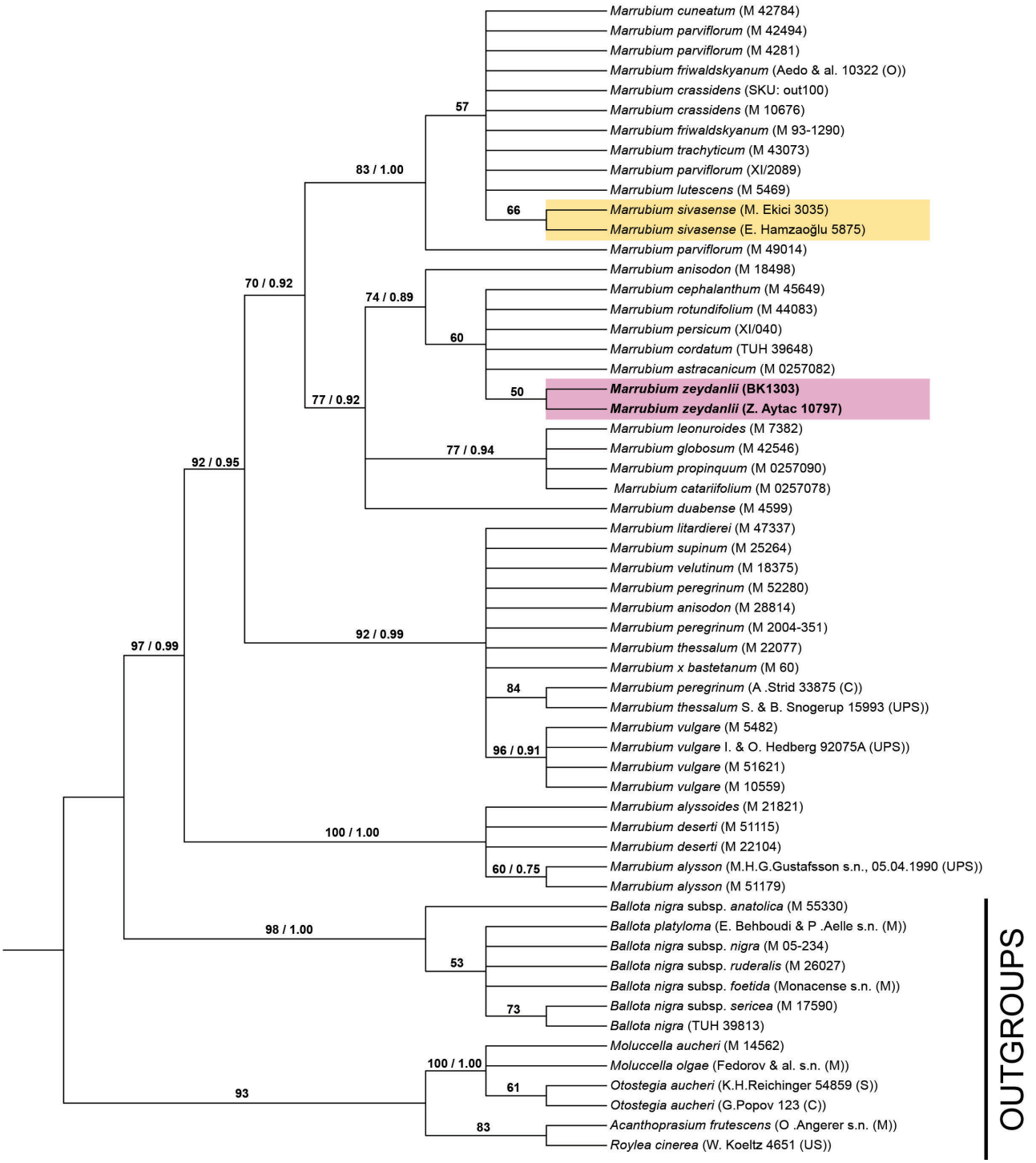


**Figure 4.** Maximum Likelihood (ML) cladogram of *Marrubium* based on *trn*L-F region dataset. Posterior probabilities (PP) > 0.5 are provided after the bootstrap (BS) values >50%. *M. zeydanlii*, *M. sivasiense* and the outgroups are indicated.

Sivrihisar (Eskişehir) represents a new species of the genus *Marrubium*. Therefore, with the inclusion of the newly described species, the genus is now represented by 35 species in Turkey.

In light of morphological, phylogenetic, and palynological information, it was concluded that this taxon should be evaluated as a new species within the *M. sivasense* and *M. depauperatum* groups.

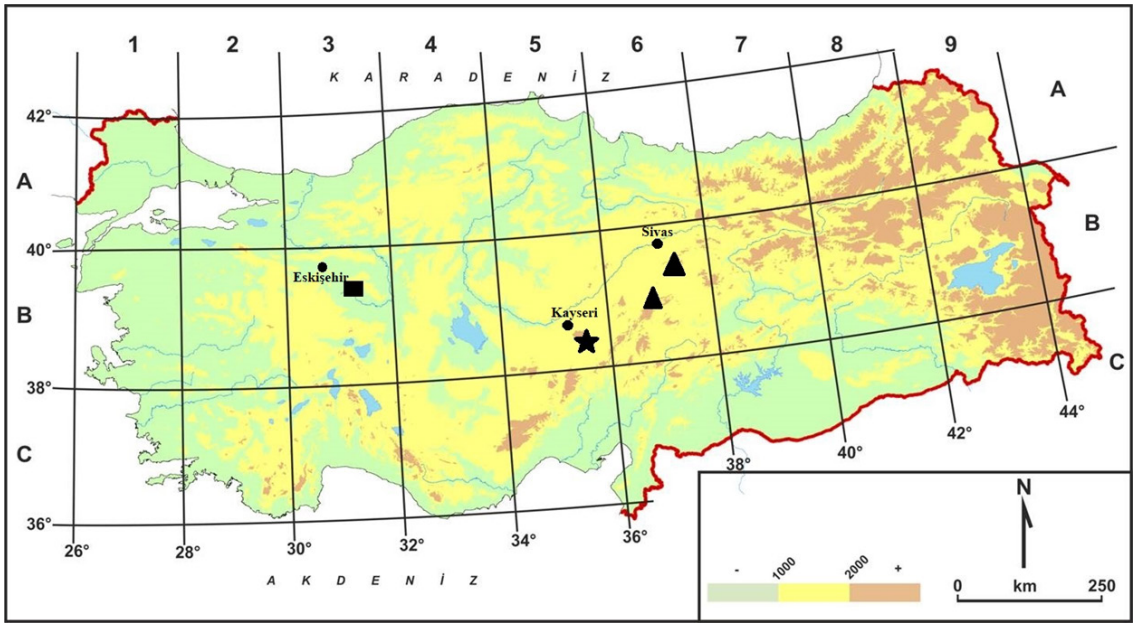


**Figure 5.** Distribution map of: *Marrubium zeydanlii* (■), *M. depauperatum* (★), and *M. sivasense* (▲).

**Table.** Comparison of *M. zeydanlii, M. depauperatum*, and *M. sivasense*.

| Characters | *M. zeydanlii* | *M. sivasense* | *M. depauperatum* |
|---|---|---|---|
| Indumentum of stem | densely white lanate | greenish white lanate below, adpressed and greyish stellate-pubescent above | stellate-pubescent |
| Cauline leaves | oblanceolate | elliptic to oblong | oblong-cuneate |
| Indumentum of cauline leaves | the stellate hairs on the leaf surface are covered by lanate hairs on both sides | densely adpressed greyish stellate-lanate on both sides. | densely adpressed greyish stellate-pubescent on both sides |
| Margin of cauline leaves | entire below, dentate at apex | crenate to crenulate | entire below, serrate above |
| Verticillasters | 4–5 (–6) flowered | 3–10 flowered | 1– (–2) flowered |
| Bracteoles (mm) | 1–1.5 | 1.6–2 | <1 |
| Calyx | densely lanate with rarely stellate-pubescent | completely stellate-pubescent | completely adpressed stellate-pubescent |
| Corolla | 6–7 mm, densely stellate-pilose outside, sparsely pilose within the upper lip | 5–7 mm, stellate- pubescent outside, glabrous in upper inside | 3–4 mm, densely stellate-pilose outside, stellate-pilose within the upper lip |

Key to *Marrubium zeydanlii* and related taxa:

1. Verticillasters 1(–2) flowered ..... *M. depauperatum*
1. Verticillasters 3–10 flowered
2. Stem lanate; stem leaves oblanceolate; calyx teeth equal; corolla sparsely pilose in inner part ..... *M. zeydanlii*
2. Stem adpressed stellate; stem leaves elliptic to oblong; calyx teeth subequal; corolla glabrous in upper inside ..... *M. sivasense*

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Thanks to Rukiye Sert for drawing the *M. zeydanlii* illustration and thanks to the curators of the ANK, K, JE, P, and GOET Herbaria and Samet H. Genç for IUCN comments.

**Additional examined specimens:**

*M. depauperatum*: B5 Kayseri: 36 km E.S.E of Kayseri, 1450 m, 1855, Balansa 1077: [FI (FI011209), GOET

(GOET004276), JE (JE00011478), K (K000929126), P (P00743519) photo!]. B5 Kayseri: Pınarbaşı, Elbaşı-Ekrek, stony places, 1450 m, 09.09.2003, Akgül 2683 (topotype ANK!).

*M. sivasense*: B6 Sivas: Between Kangal and Sivas, 2 km, steppe, 1550–1600 m, 09.07.2002, Ekici 3035, Aytaç & Akan (holotype GAZI! and isotype GAZI!). Sivas: Between Kangal and Gürün, east of Mancılık village, steppe, 1640 m, 10.07.2007, Aytaç 9074 (paratype GAZI!). Sivas: Hamzaoğlu 5875 (GAZI!). Sivas: 5 km. southwest of Kangal, steppe, 1605 m, 24.07.2008, Güner 15140 et al. (GAZI!).

## References


Akgül G, Ketenoğlu O, Pınar NM, Kurt L (2008). Pollen and nutlet morphology of the genus *Marrubium* (Lamiaceae) in Turkey. Annales Botanici Fennici 45: 1–10. doi: 10.5735/085.045.0101

Akgül G (2012). *Marrubium* L. In: Güner A, Aslan S, Ekim T, Vural M & Babaç MT (editors). Türkiye Bitkileri Listesi (Damarlı Bitkiler) (List of plants in Turkey {Vascular plants}). İstanbul, TR: Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırmaları Derneği Yayını, pp. 559–561 (in Turkish).

Akgül G, Selvi B (2014). A new species of *Marrubium* L. (Lamiaceae) from southwestern Anatolia, Turkey. The Herb Journal of Systematic Botany 21 (2): 15–22.

Akgül G, Ketenoğlu O, Doğan M (2017). A new species of *Marrubium* L. (Lamiaceae) from Turkey. The Herb Journal of Systematic Botany 24 (2): 37–46.

Akgül G (2018). A new species of *Marrubium* L. (Lamiaceae), *M. lanatum* Akgül from Niğde, Turkey. The Herb Journal of Systematic Botany 25 (2): 23–30.

Aytaç Z, Akgül G , Ekici M (2012). A new species of *Marrubium* (Lamiaceae) from Central Anatolia, Turkey. Turkish Journal of Botany 36 (5): 443-449. doi:10.3906/bot-1101-9

Aytaş Akcin T, Camili B (2018). Anatomical and micromorphological investigations on Turkish endemic *Marrubium cephalanthum* subsp. *montanum* (Lamiaceae). Journal of International Environmental Application & Science 13 (2): 97–104.

Bentham G (1834). Labiatarum Genera Et Species. James Ridgway & Sons, London (in Latin).

Bentham G (1848). Labiatarum. In: De Candolle AP (editor) Prodromus systematis naturalis regni vegetabilis. Paris, France: Treuttel & Würtz, pp. 536–549 (in Latin).

Boissier PE (1879). Flora Orientalis 4. Basel, Switzerland: Reg. Acad. Scient., pp. 692–705 (in Latin).

Briquet J (1896). Labiatae. In: Engler A, Krause K, Pilger RKF, Prantl K (editors). Die Natürlichen Pflanzenfamilien nebst ihren Gattungen und wichtigeren Arten, insbesondere den Nutzpflanzen, under Mitwirkung zahlreicher hervorragender Fachgelehrten begründet-4. Leipzig, Germany: W. Engelmann, pp. 183–375 (in German).

Brummitt RK , Powell CE (editors) (1992). Authors of plant names. A list of authors of scientific names of plants, with recommended standard form of their names including abbreviations. Kew, UK: Royal Botanic Gardens.

Cullen J (1982). *Marrubium* L. In: Davis PH (editor). Flora of Turkey and the East Aegean Islands, Vol. 7. Edinburgh, UK: Edinburgh University Press, pp. 165–178.

Darriba D, Taboada GL, Doallo R, Posada D (2012). jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9 (8): 772. doi: 10.1038/nmeth.2109

Davis PH, Mill RR , Tan K (editors) (1988). Flora of Turkey and the East Aegean Islands (Suppl. 1). Edinburgh, UK: Edinburgh University Press, pp. 202–203.

Deshmukh UB, Reddy ES, Shende MB (2022). *Marrubium asumaniae*, a new name proposed for a *Marrubium* species (Lamiaceae) in the flora Turkey. Phytotaxa, 543 (2):161-162. doi: 10.11646/phytotaxa.543.2.6

Erdtman G (1969). Handbook of Palynology. Morphology-Taxonomy- Ecology. An Introduction to the Study of Pollen Grains and Spores. Copenhagen, Denmark: Verlag Munksgaard.

Erdtman G (1945). Pollen morphology and plant taxonomy IV. Labiatae, Verbenaceae and Avicenniaceae. Svensk Botanisk Tidskrift 39: 279–285.

Fırat M (2016). *Marrubium eriocephalum* (Lamiaceae); a species new to the flora of Turkey, with contributions to its taxonomy. PhytoKeys 58: 9-20. doi: 10.3897/ phytokeys.58.5890

Guindon S, Gascuel O (2003). A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. Systematic Biology 52: 696-704.

Hedge IC (1992). A global survey of the biogeography of the Labiatae. In: Harley RM & Reynolds T (editors) Advances in Labiatae Science. Kew, UK: Royal Botanic Gardens, pp. 7–17.

International Union for Conservation of Nature (IUCN) (2017). IUCN Red List Categories and Criteria: Version 3.1. 2nd ed. Gland, Switzerland and Cambridge, UK: IUCN Species Survival Commission.

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M et al. (2012). Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28 (12): 1647-1649.

Letunic I, Bork P (2016). Interactive tree of life (iTOL) v3: an online tool for the display and annotation of phylogenetic and other trees. Nucleic Acids Res. 44(W1): W242-5 Available at https://itol.embl.de [accessed 13 April 2022].

Martínez-Gor-Dillo M, Bedolla-Garcia BN, Cornejo-Tenorio G, Fragoso-Martínez I, García-Peña MR et al. (2017). Lamiaceaede México, Botanical Sciences 95 (4): 780-806.

Martínez-Azorín M, Crespo MB, Juan A, Fay MF (2011). Molecular phylogenetics of subfamily Ornithogaloideae (Hyacinthaceae) based on nuclear and plastid DNA regions, including a new taxonomic arrangement. Annals of Botany 107: 1-37.

Menemen Y, Aytaç Z, Kandemir A (2016). Türkçe bilimsel bitki adları yönergesi (Directive of Turkish scientific plant names). Bağbahçe Bilim Dergisi 3 (3): 1–3 (in Turkish).

Miller MA, Pfeiffer W, Schwartz T (2010). Creating the CIPRES Science Gateway for inference of large phylogenetic trees [online]. In Proceedings of the Gateway Computing Environments Workshop (GCE), 1–8, New Orleans, Louisiana, USA. Available at http://www.phylo.org/sub_sections/portal.php.

Özhatay N, Kültür Ş, Gürdal B (2017). Check-List of Additional Taxa to The Supplement Flora of Turkey VIII. Journal of Pharmacy of Istanbul University 47 (1): 31–46. doi: 10.5152/IstanbulJPharm.2017.006

Punt W, Hoen PP, Blackmore S, Nilsson S, Le Thomas A (2007). Glossary of pollen and spore terminology. Review of Palaeobotany Palynology 143 (1-2): 1-81. doi: 10.1016/j.revpalbo.2006.06.008

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A et al. (2012). MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space. Systematic Biology 61 (3): 539-542. doi: 10.1093/sysbio/sys029

Seybold S (1982). *Marrubium*-Arten (Labiatae), in Rechinger KH. *Flora Iranica: Flora Des Iranischen Hochlandes und der Umrahmenden Gebirge.* Graz, Austria: Akademische Druck-u Verlagsanstalt, pp 88-104 (in German).

Siadati S, Salmaki Y, Mehrvarz SS, Heubl G, Weigend M (2018). Untangling the generic boundaries in tribe Marrubieae (Lamiaceae: Lamioideae) using nuclear and plastid DNA sequences. Taxon 67 (4):770-783. doi: 10.12705/674.6

Siadati S, Mehrvarz SS, Salmaki Y (2019). Nutlet micromorphology of the genus *Marrubium* L. and allies and its systematic implication (Lamiaceae: tribe Marrubieae). Nova Biologica Reperta 6 (3): 338-346. doi: 10.29252/nbr.6.3.338

Siadati S, Salmaki Y, Brauchler C (2020). Trichome morphology provides phylogenetically informative signal for generic delimitation in tribe Marrubieae (Lamiaceae). Flora 273 (151720). doi:10.1016/j.flora.2020.151720

Solís-Lemus C ,Ané, C (2016). Inferring phylogenetic networks with maximum pseudolikelihood under incomplete lineage sorting. PLoS GENETICS, 12 (3): e1005896. doi.org/10.1371/journal.pgen.1005896

Stamatakis A (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics, 30 (9): 1312-3, doi:10.1093/bioinformatics/btu033

Taberlet P, Gielly L, Pautou G ,Bouvet J (1991). Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Molecular Biology, 17 (5): 1105-1109.

Thiers B (2018). Index Herbariorum: a Global Directory of Public Herbaria and Associated Staff. New York botanical Garden's virtual herbarium [online]. Available at http://sweetgum.nybg.org/ih [accessed 13 April 2021].

Tutin TG, Heywood VH, Burges NA, Valentine DH, Walters SM et al. (1964-1993). Flora Europaea. Cambridge, UK: Cambridge University Press.

Wodehouse RP (1935). Pollen Grains: Their structure, identification, and significance in science and medicine. New York, USA: McGraw Hill.

**Appendix.** Taxon sampling of the phylogenetic analyses based on the *trn*L-F intergenic spacer region. Information is presented in the following order: taxon name, collection number and GenBank accessions numbers.

*Marrubium* L: *M. alyssoides* Pomel, M 21821, MH685076.1; *M. alysson* L., M.H.G. Gustafsson s.n., 05.04.1990 (UPS), FJ854284.1/FJ854172.1; *M. alysson*, M 51179, MH685077.1; *M. anisodon* K.Koch, M 28814, MH685079.1; *M. anisodon*, M 18498, MH685078.1; *M. astracanicum,* M 0257082, MH685080.1; *Marrubium* x *bastetanum* Coincy, M 60, MH685081.1; *M. catariifolium* Jacq., M 0257078, MH685082.1; *M. cephalanthum* Boiss. & Noë, M 45649, MH685083.1; *M. cordatum* Nábelek, TUH 39648, MH685084.1; *M. crassidens* Boiss., M 10676, MH685085.1; *M. crassidens,* SKU:out100, KF577402.1; *M. cuneatum* Banks & Sol., M 42784, MH685086.1; *M. deserti* (de Noé) Coss., M 22104, MH685054.1; *M. deserti*, M 51115, MH685055.1; *M. duabense* Murata, M 4599, MH685087.1; *M. friwaldskyanum* Boiss., M 93-1290, MH685088.1; *M. friwaldskyanum*, Aedo & al. 10322 (O), HQ911699.1/HQ911769.1; *M. globosum* Montbret & Aucher ex Benth, M 42546, MH685089.1; *M. leonuroides* Desr., M 7382, MH685090.1; *M. litardierei* Marmey, M 47337, MH685091.1; *M. lutescens* Boiss. & Heldr., M 5469, MH685092.1; *M. parviflorum* Fisch. & C.A.Mey., XI/2089, MH685093.1; *M. parviflorum*, M 49014, MH685094.1; *M. parviflorum*, M 42494, MH685095.1; *M. parviflorum,* M 4281, MH685096.1; *M. peregrinum* L., A. Strid 33875 (C), FJ854285.1/HQ911770.1; *M. peregrinum*, M 2004-351, MH685097.1; *M. peregrinum*, M 52280, MH685098.1; *M. persicum* C.A.Mey, XI/1040, MH685100.1; *M. propinquum* Fisch. & C.A.Mey., M 0257090, MH685101.1; *M. rotundifolium* Boiss., M 44083, MH685102.1; *M. sivasense* Aytaç, Akgül & Ekici, E. Hamzaoglu 5875, ON097159; *M. sivasense*, M. Ekici 3035, ON097160; *M. supinum* L., M 25264, MH685103.1; *M. thessalum* Boiss. & Heldr., M 22077, MH685104.1; *M. thessalum*, S. & B. Snogerup 15993 (UPS), FJ854286.1/FJ854173.1; *M. trachyticum* Boiss., M 43073, MH685105.1; *M. velutinum* Sm., M 18375, MH685106.1; *M. vulgare* L., M 51621, MH685107.1; *M. vulgare*, M 10559, MH685108.1; *M. vulgare*, M 5482, MH685109.1; *M. vulgare*, I. & O. Hedberg 92075A (UPS), EU138443.1/EU138366.1; *M. zeydanlii* Aytaç, Kaptaner İğci & T. Ertuğrul sp. nov., BK1303, ON097161; *M. zeydanlii*, Z. Aytac 10797, ON097162; OUTGROUPS. *Acanthoprasium* (Benth.) Spach: *A. frutescens* (L.) Spenn., O. Angerer s.n. (M), MH685045.1; *Ballota* L.: *B. nigra* L., TUH 39813, MH685069.1; *B. nigra* subsp. *anatolica* P.H.Davis, M 55330, MH685065.1; *B. nigra* subsp. *foetida* (Vis.) Hayek, Monacense s.n. (M), MH685066.1; *B. nigra* subsp. nigra, M 05-234, MH685068.1; *B. nigra* subsp. *ruderalis* (Sw.) Briq., M 26027, MH685071.1; *B. nigra* subsp. *sericea* (Vandas) Patzak, M 17590, MH685070.1; *B. platyloma* Rech.f., E. Behboudi & P. Aelle s.n. (M), MH685072.1; *Moluccella* L.: *M. aucheri* (Boiss.) Scheen, M 14562, MH685046.1; *M. olgae* (Regel) Ryding, Fedorov & al. s.n. (M), MH685047.1; *Otostegia* Benth.: *O. aucheri* Boiss., G. Popov 123 (C), EU138446.1/EU138369.1; *O. aucheri*, K. H. Reichinger 54859 (S), EU138447.1/EU138370.1; *Roylea* Wall. ex Benth.: *R. cinerea* (D. Don) Baill., W. Koeltz 4651 (US), HQ911682.1/HQ911752.1.