Phytotaxa 706 (3): 173–185
https://www.mapress.com/pt/

ISSN 1179-3155 (print edition)
PHYTOTAXA
ISSN 1179-3163 (online edition)

Article

https://doi.org/10.11646/phytotaxa.706.3.1

Multilocus phylogenetic assessment of the taxonomic status of the Turkish endemic *Astrantia maxima* subsp. *haradjianii* (Saniculoideae)

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Abstract

Astrantia is a small genus within the family Apiaceae. Among its 15 recognized taxa, *Astrantia maxima* subsp. *haradjianii* is a Turkish endemic that has not previously been included in any phylogenetic analyses. This study aimed to evaluate the taxonomic status of subsp. *haradjianii*, which, despite exhibiting clear morphological distinctions, such as smaller umbel size, has been classified as a subspecies. To investigate its potential elevation to species rank, multiple populations of both subspecies were analyzed using phylogenetic methods based on nuclear ribosomal (complete internal transcribed spacer region) and combined chloroplast (rps16, rpl16, matK, rbcL, psbA-trnH, trnL, trnL-trnF, and rps16-trnK) DNA sequences. The phylogenetic reconstruction revealed that the subsp. *maxima* and subsp. *haradjianii* are closely related yet separated by distinct genetic divergence, which is particularly evident in the chloroplast dataset. Based on this phylogenetic evidence, the current subspecific classification of *A. maxima* subsp. *haradjianii* was reconsidered, and its elevation to species rank is proposed. Furthermore, a lectotype was designated for the name *Astrantia haradjianii*.

Key words: Apiaceae, morphology, phylogenetic, *stat. nov*., taxonomy, typification

Introduction

Astrantia Linnaeus (1753: 235) (Saniculeae, Saniculoideae, Apiaceae) comprises 10 species (POWO 2024). While the genus is predominantly distributed in Europe, *A. colchica* Albov (1895: 100), *A. trifida* Hoffmann (1816: 8), and *A. pontica* Albov (1895: 99) are found in Transcaucasia and/or the North Caucasus. *A. maxima* Pallas (Pallas 1793: 357) is native to Iran, the North Caucasus, Transcaucasia, and Türkiye. In Türkiye, the genus is represented solely by *A. maxima*, which includes two infraspecific taxa: *A. maxima* subsp. *maxima* and *A. maxima* subsp. *haradjianii* (Grintzesco 1910: 135) Rechinger f. (Rechinger 1959: 302). The latter is endemic to Türkiye (Hedge & Lamond 1972). Initially described as a variety (Grintzesco 1910), its status was later elevated to subspecies rank by Rechinger (1959). Kaya (2003) investigated the morphology and anatomy of *A. maxima* in Türkiye, identifying diagnostic differences between the two subspecies. These included variations in the size and surface ornamentation of the bracteoles, as well as differences in the stem transverse sections, such as the number of vascular bundles, width of the vascular bundle rays, cuticle papillae, and bundle sheaths (Kaya 2003).

Although no studies have directly investigated the phylogenetic position of *Astrantia* or the relationships among its species, Downie *et al.* (1998) identified the genus as a member of the subfamily Saniculoideae using sequences from the complete internal transcribed spacer (ITS) complete region. Valiejo-Roman *et al.* (2002) further suggested that *Astrantia* is phylogenetically a sister to *Actinolema* Fenzl. Based on Drude's classification, molecular divergence was detected between the sections *Macraster* Grintzesco (1910: 64) (=*Astrantia*; including *A. major* Linnaeus (1753: 235) and *A. maxima*) and *Astrantiella* Grintzesco (1910: 64) (including *A. minor* Linnaeus (1753: 235)) (Celastani 1905, Grintzesco 1910, Valiejo-Roman *et al.* 2002). Calviño *et al.* (2007) analyzed phylogenetic relationships using the trnQ-rps16 intergenic spacer, rps16 intron, and rps16-trnK intergenic spacer across five species, and their findings were consistent with those of Valiejo-Roman *et al.* (2002). The genus *Astrantia* was monophyletic, a sister to *Actinolema*, and divided into two sections: sect. *Astrantia*, which includes *A. colchica*, *A. major*, and *A. maxima*, and sect. *Astrantiella*, comprising *A. bavarica* F.W. Schultz (1858: 161) and *A. minor*. Kadereit *et al.* (2008) later sequenced samples from

Accepted by KM Prabhukumar: 17 Jun. 2025; published: 27 Jun. 2025

three different populations of *A. major*, proposing that the migration routes of the subfamily Saniculoideae extended from southern Africa to the Northern Hemisphere, likely originating in Southwest Asia. (Calvino *et al.* 2007, Kadereit *et al.* 2008). Kadereit *et al.* (2008: Fig. 2) suggested that this migration pathway passed through the distribution ranges of both *Actinolema*, and presumably, basal species of *Astrantia*. In contrast, Wörz (1999) conducted a cladistic analysis of *Astrantia* based on morphological characters.

In Türkiye, *Astrantia maxima* subsp. *haradjianii* is primarily distributed in central Anatolia, whereas subsp. *maxima* occurs in the northwestern regions (Fig. 1). According to Hedge & Lamond (1972), subsp. *haradjianii* differs from subsp. *maxima* in several morphological traits, including 3–5 partite basal leaves (vs. 3-partite), leaf size of 1–1.8 × 0.3–0.8 cm (vs. 1.8–3 × 0.7–1.2 cm), white and green bracteoles (vs. pink-tinged), and lateral umbels numbering (1–) 3–5 (vs. 0–2). Subsp. *haradjianii* also bears smaller, white flowers and more extensively branched umbels (Figs. 2–3). The observed morphological and anatomical variations, as documented by Kaya (2003) prompted a re-evaluation of the taxonomic status of subsp. *haradjianii*. While the anatomical and morphological features have been thoroughly examined (Kaya 2003), comprehensive phylogenetic studies remain lacking.

The present study aims to address the following questions: 1) Can two clades provisionally identified as *Astrantia maxima* subsp. *maxima* and subsp. *haradjianii* be distinguished using ITS and combined chloroplast markers? 2) If genetically distinct clades are identified, can they also be clearly differentiated based on morphological traits? 3) What taxonomic status should be assigned to these clades? Additionally, a lectotype is proposed for the name *A. haradjianii*.

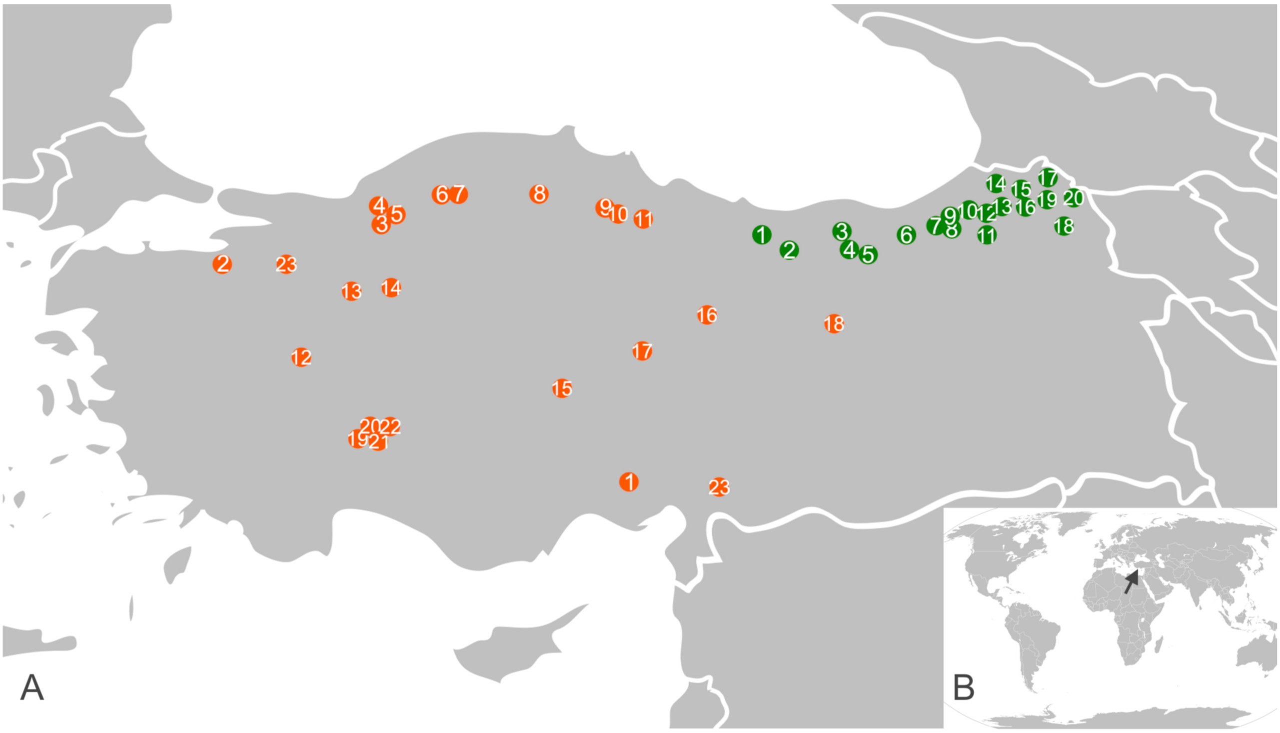

FIGURE 1. A. Distribution map of *A. haradjianii* and *A. maxima* in Türkiye. B. Türkiye in the world. Orange squares and green circles indicate *A. haradjianii* and *A. maxima*, respectively. The numbers in the circles indicate the locality numbers in Appendix 1.

Material and methods

Samples were collected during field expeditions conducted over several years as part of broader phylogenetic studies on taxa within Apiaceae. The collected specimens were deposited in the herbarium HUB. In addition, a range of herbarium specimens housed in HUB, ANK, GAZI, and G, were examined for morphological comparison. The localities of both the collected and examined herbarium specimens are listed in the Appendix, using the grid system adopted in Flora of Turkey and East Aegean Islands (Davis 1965).

Nuclear ribosomal DNA ITS (ITS1–5.8S–ITS2), and chloroplast markers (*rps16, rpl16, matK, rbcL, psbA-trnH, trnL, trnL-trnF, and rps16-trnK*) were used to reconstruct the phylogeny. Genomic DNA was extracted from four different subsp. *haradjianii* populations and three populations of subsp. *maxima*. DNA extraction was performed using the Qiagen DNeasy Plant Mini Kit (Qiagen GmbH, Hilden, Germany) following the manufacturer's instructions. The following primers were used: matK (Ford *et al.* 2009); rbcL (Fay *et al.* 1997); trnL, trnL–strnF (Taberlet *et al.* 1991); rps16 (Oxelman *et al.* 1997), rpl16 (Downie *et al.* 2000), and rps16-trnK (Calviño 2007). The primer sequences and polymerase chain reaction conditions for the remaining markers followed those described by Doğru-Koca (2024). The GenBank accession numbers for all the sequences are provided in Tables 1 and 2.

TABLE 1. GenBank barcodes used to reconstruct the phylogenetic hypothesis for the chloroplast dataset. Those in bold were obtained in this study.

Taxa	rps16	rpl16	matK	rbcL	psbA-trnH	trnL	trnL-trnF	rps16-trnK
***Astrantia maxima* (ADK3297)**	**PQ457053**	**PQ457046**	**PQ457025**	**PQ457032**	**PQ457039**	**PQ457067**	**PQ457067**	**PQ457060**
***Astrantia maxima* (ADK6321)**	**PQ457054**	**PQ457047**	**PQ457026**	**PQ457033**	**PQ457040**	**PQ457068**	**PQ457068**	**PQ457061**
***Astrantia maxima* (ADK6331)**	**PQ457055**	**PQ457048**	**PQ457027**	**PQ457034**	**PQ457041**	**PQ457069**	**PQ457069**	**PQ457062**
***Astrantia haradjianii* (ADK3532)**	**PQ457056**	**PQ457049**	**PQ457028**	**PQ457035**	**PQ457042**	**PQ457070**	**PQ457070**	**PQ457063**
***Astrantia haradjianii* (ADK6312)**	**PQ457057**	**PQ457050**	**PQ457029**	**PQ457036**	**PQ457043**	**PQ457071**	**PQ457071**	**PQ457064**
***Astrantia haradjianii* (ADK6330)**	**PQ457058**	**PQ457051**	**PQ457030**	**PQ457037**	**PQ457044**	**PQ457072**	**PQ457072**	**PQ457065**
***Astrantia haradjianii* (BÖ2162)**	**PQ457059**	**PQ457052**	**PQ457031**	**PQ457038**	**PQ457045**	**PQ457073**	**PQ457073**	**PQ457066**
Astrantia maxima	DQ832355	GQ243955	-	-	-	-	-	DQ832493
Astrantia bavarica	DQ832353	-	-	-	-	-	-	DQ832491
Astrantia colchica	DQ832354	-	-	-	-	-	-	DQ832492
Astrantia major subsp. *major* 1	AF110594	AF094451	-	HG416969	HG800499	-	HQ323834	-
Astrantia major subsp. *major* 2	DQ832443	-	-	KF602128	-	-	-	-
Astrantia major subsp. *major* 3	-	-	U58585	U50230	-	-	-	-
Astrantia minor	DQ832444	-	-	-	-	-	HQ323835	-
Actinolema macrolema 1	EU168944	-	-	-	-	-	-	DQ832473
Actinolema macrolema 2	DQ832337	-	-	-	-	-	-	-
Actinolema eryngioides	DQ832336	GQ243948	-	-	-	-	-	DQ832472
Eryngium campestre	EU070486	MW036660	JN894266	JN892017	HE602478	KY697527	KY697527	EU070548

TABLE 2. GenBank barcodes used to reconstruct the phylogenetic hypothesis for the ribosomal dataset. Those in bold were obtained in this study.

Taxa	ITS1	ITS2	Taxa	ITS1	ITS2
Astrantia maxima (ADK3297)	**PQ435317**	**PQ435317**	*Alepidea amatymbica*	AM158945	AM158945
Astrantia maxima (ADK6321)	**PQ435318**	**PQ435318**	*Arctopus monacanthus*	EU168964	EU168964
Astrantia maxima (ADK6331)	**PQ435319**	**PQ435319**	*Arctopus monacanthus* 2	AM158944	AM158944
Astrantia haradjianii (ADK3532)	**PQ435320**	**PQ435320**	*Arctopus echinatus*	AM158943	AM158943
Astrantia haradjianii (ADK6312)	**PQ435321**	**PQ435321**	*Arctopus dregei*	AM158942	AM158942
Astrantia haradjianii (ADK6330)	**PQ435322**	**PQ435322**	*Petagnaea gussonei* 1	EU169015	EU169015
Astrantia haradjianii (BÖ2162)	**PQ435323**	**PQ435323**	*Petagnaea gussonei* 2	EU070742	EU070742
Astrantia major subsp. *major* 1	AF031962	AF031962	*Eryngium maritimum*	EU070674	EU070674
Astrantia major subsp. *major* 2	AF077876	AF077876	*Eryngium planum*	EU070696	EU070696
Astrantia minor	AF337183	AF337191	*Eryngium scaposum*	EU070714	EU070714
Actinolema macrolema 1	EU168962	EU168962	*Eryngium yuccifolium*	EU070736	EU070736
Actinolema macrolema 2	AF337176	AF337184	*Sanicula crassicaulis*	AF031984	AF031984
Eryngium campestre	KY053513	KY053513	*Sanicula arctopoides*	AF031972	AF031972
Steganotaenia araliaceae	AM748814	AM748814	*Sanicula europea*	OQ064674	OQ064674
Alepidea peduncularis	EU168963	EU168963	*Sanicula epipactis*	AF077892	AF077892

Final sequence alignments were generated using MAFFT ver. 7 (Katoh *et al.* 2019) and manually adjusted using BioEdit (Hall 1999). The ITS alignment matrix included 22 populations representing 21 taxa and comprised 646 characters. The chloroplast alignment consisted of 18 populations representing 9 taxa and contained 5528 characters. The chloroplast and nuclear ribosomal datasets were analyzed separately. The best-fitting nucleotide substitution models were determined using JModelTest (Guidon & Gascuel 2003, Darriba *et al.* 2012) based on the Akaike information criterion. The GTR+G model was selected for the ITS dataset, and the TPM1uf+G model was chosen for the combined chloroplast regions. Phylogenetic relationships were inferred using both Bayesian inference (BI) and maximum likelihood (ML) analyses. BI was conducted with MrBayes v.3.2.7a (Ronquist *et al.* 2012), while ML analyses were performed using RaxML GUI 2.0b08 (Silvestro 2012, Stamatakis 2014). BI analyses for the ITS and combined chloroplast datasets were conducted using four chains, with 15 and 50 million generations, respectively, and sampling every 1000 generations. ML analyses for both datasets were performed with 100 search replicates and 1000 bootstrap (BS) replicates, applying a burn-in value of 10%. Effective sample sizes greater than 200 were confirmed using Tracer (Rambaut & Drummond 2018), indicating adequate convergence of the BI runs. The supporting BS values are presented following the posterior probability (PP) values above the branches in the resulting phylogenetic trees. The tree based on the ITS sequences was rooted with *Steganotaenia araliaceae*, while the chloroplast was rooted with *Eryngium campestre*, following the approaches of Calviño *et al.* (2007) and Kadereit *et al.* (2008). The phylogenetic trees were visualized using FigTree v1.4.3.

Results

Phylogenetic hypothesis

The maximum clade credibility trees based on the ribosomal ITS sequences and the combined eight chloroplast markers, reconstructed using BI and ML analyses, are shown in Figs. 4 and 5, respectively. A topological incongruence was observed between the phylogenetic trees generated by the two methods, raising questions about the monophyly of the genus *Astrantia*.

According to the BI analyses of both the ribosomal and chloroplast datasets, *Astrantia* appears to be monophyletic, with PP values of 0.68 and 0.51, respectively. In both trees, *Actinolema* is recovered as the sister genus of *Astrantia* (Fig. 4A, 5A, and clade A). However, the ML analysis produced a different topology, in which *Actinolema* is nested within *Astrantia*. In this topology, the *A. bavarica* and A. *minor* clade is a sister to the *Actinolema* clade.

In the BI-based phylogenetic tree constructed from the ITS sequences, *Astrantia* and *Actinolema* formed a maximally supported clade (PP = 1, BS = 100) and were clearly separated from other related genera within the subfamily Saniculoideae, including *Arctopus* L.*,* *Alepidea* F.Delaroche*, Actinolema, Petagnaea* Caruel*, Sanicula* L., and *Eryngium* Tourn. ex L. The populations of *Actinolema macrolema* formed a distinct monophyletic basal clade. The monophyly of *Astrantia* was weakly supported (PP = 0.68) and lacked BS support. Within *Astrantia*, the clade was divided into two subclades (Fig. 4A, clade a). *A. major* and *A. minor* were grouped within one subclade, which was also weakly supported (PP = 0.63), and not supported by BS analysis (Fig. 4b). In the other clade, the *A. maxima* subsp. *maxima* and *A. maxima* subsp. *haradjianii* populations were polytomous (Fig. 4C). This clade was supported by maximum PP and high BS values (PP = 1, BS = 97). However, according to the ML hypothesis, the *A. maxima* subsp. *haradjianii* populations formed a monophyletic clade with moderate BS support (BS = 83) (Fig. 4B). Two populations of *A. maxima* subsp. *maxima* were positioned polytomously with the subsp. *haradjianii* clade, while the remaining population was resolved as basal within the *A. maxima* clade.

Concerning the chloroplast data set, the BI-based phylogenetic tree showed that the *Actinolema* species formed a basal monophyletic clade (Fig. 5). The genus *Astrantia* was divided into two subclades. The basal clade (Fig. 5, clade A) comprised *A. bavarica* and *A. minor*, with strong PP support (PP = 0.99) but only moderate BS support (BS = 77). The remaining species, *A. colchica*, *A. mojor*, and *A. maxima*, formed a clade with high PP but a weak BS support (PP = 0.92, BS = 62) (Fig. 5, clade B). Within this clade, *A. colchica* was positioned basally. *A. major* and *A. maxima* formed a distinct monophyletic subclade (Fig. 5, clade C), which was supported by high PP (PP = 0.89), although the BS value remained low (BS = 54). All the populations of *A. major* and *A. maxima* formed distinct clades, clades D and E, respectively, as shown in Fig. 5. The *A. major* clade was strongly supported, with both high PP and BS values (PP = 0.99, BS = 91). The *A. maxima* clade was strongly supported, showing maximum PP and a high BS value (PP = 1, BS = 88). One population of *A. maxima* subsp. *maxima* was positioned at the basal lineage of this clade. Subsequently, three populations of *A. maxima* subsp. *maxima* populations formed a monophyletic clade with maximum PP and strong BS value (PP = 1, BS = 85). Similarly, the four populations of *A. maxima* subsp. *haradjianii* clustered into a well-supported monophyletic clade with maximum support values (PP = 1, BS = 99).

Taxonomic treatment

Typification:—In June 1908, Haradjian collected a new variety of *Astrantia maxima* from the Amanos Mountains in Türkiye. The original material is preserved on two herbarium sheets housed in two different herbaria: G [G00359811] (high-resolution image available at: https://www.ville-ge.ch/musinfo/bd/cjb/chg/adetail.php?id=269207&base=img&lang=en) and K [K000697275] (high-resolution image available at https://data.kew.org/records/occurrences/search?taxa=Astrantia+maxima#tab_recordImages).

According to Article 9.3 of the International Code of Nomenclature (Turland *et al.* 2018), a taxonomic name must be typified by a specimen or illustration. Accordingly, the original material housed at G has been designated herein, for the first time, as the lectotype of *Astrantia maxima* var. *haradjianii*. This specimen was selected because it bears more stems and umbels, and its 4 partite basal leaves more accurately reflect the protologue's original description.

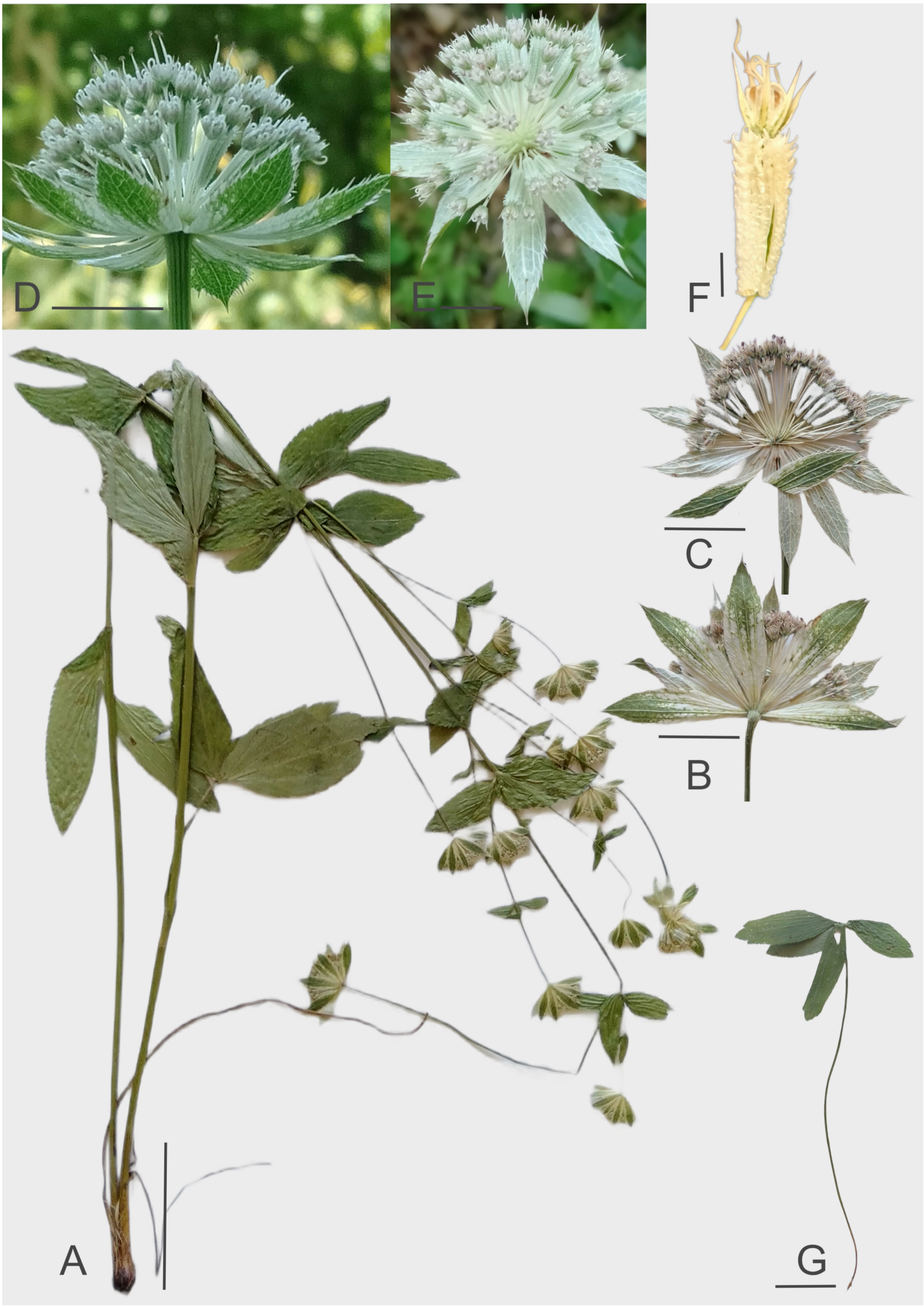

FIGURE 2. A. Habit of *A. haradjianii* (ADK 6312, in HUB), B–E. Umbels, F. Flower and mericarp, G. Basal leaf. Scales: A. 10 cm, B–G. 1 cm, F. 1 mm. Photographs were taken by the author.

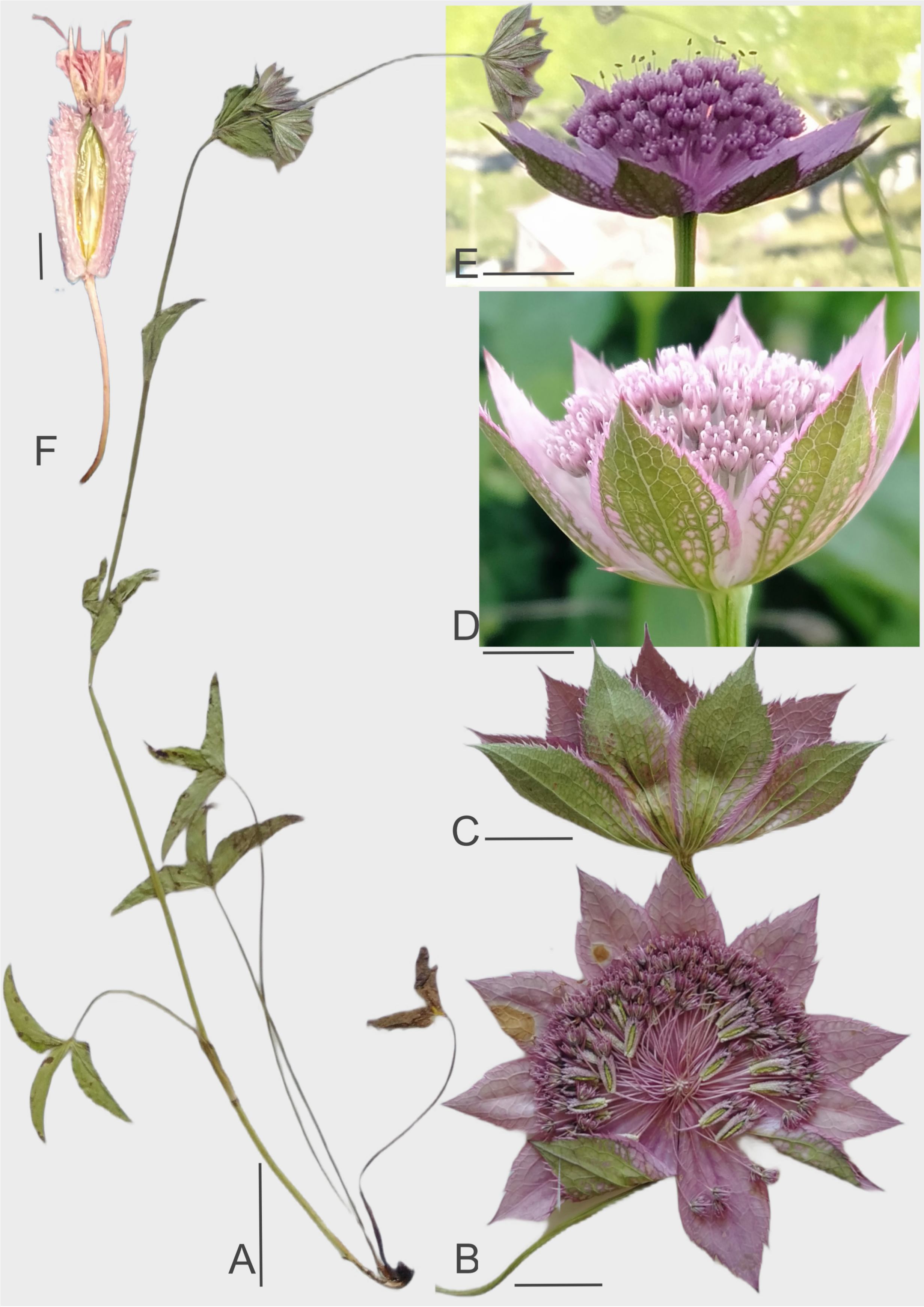

FIGURE 3. A. Habit of *A. maxima* (ADK 6321, in HUB), B–E. Umbels, F. Flower and mericarp. Scales: A. 10 cm, B–G. 1 cm, F. 1 mm. Photographs were taken by the author.

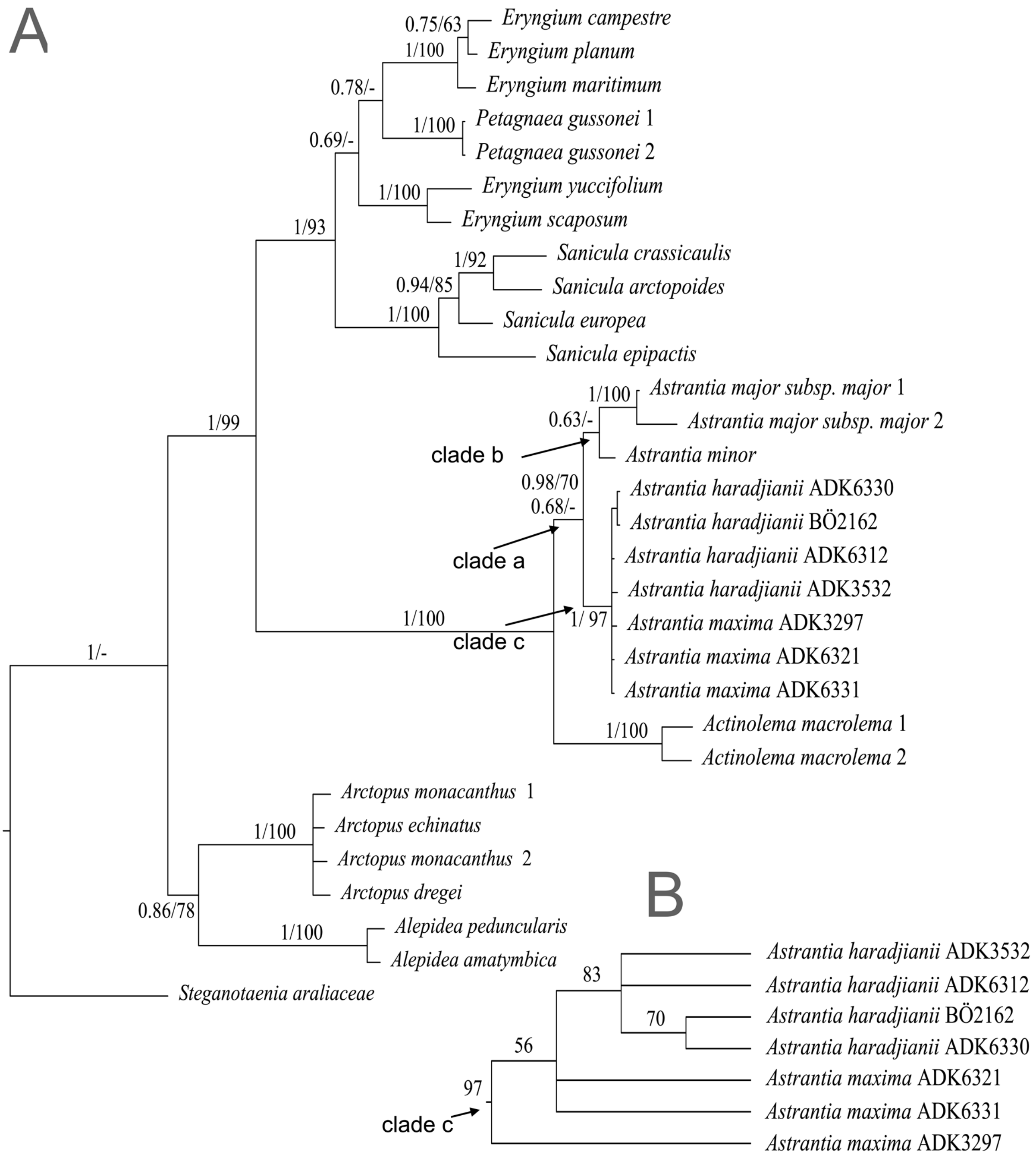

FIGURE 4. A. BI 50% majority-rule hypothetical tree based on the ITS sequence data of the genus *Astrantia* and adjacent genera. Branch support values are arranged as a PP from the BI/BS value from the ML. B. A part of the ML hypothesis with the same dataset. The voucher numbers for the specimens studied herein are at the terminals.

Astrantia haradjianii (Grintz.) Doğru-Koca, ***stat. nov.*** (Fig. 2)

≡ *Astrantia maxima* var. *haradjianii* Grintz. in Annuaire Conserv. Jard. Bot. Genève 13-14: 130 (1910). ≡ *Astrantia maxima* subsp. *haradjianii* (Grintz.) Rech. fil. in Ark. Bot. ser. 2, 5:302 (1959).

=*Astrantia maxima* var. *quadriloba* Grintz. in Annuaire Conserv. Jard. Bot. Genève 13-14: 134 (1910).

Lectotype (**designated herein**):—Türkiye. C6 Adana, Syria bor. mont. Amanus in reg. Hasan Veyli, 1220–1520 m, Haradjian PL Syr. bor. 2316 (G! [G00359811]; isolecto. K [K000697275] photo!).

Diagnosis (Figs. 2–3):—Astrantia haradjianii is closely related to *A. maxima* but differs several morphological traits. In *A. haradjianii*, the bracteoles only partially cover the flowers, with the flowers extending to at least two-thirds of the length of the bracteoles or longer. In contrast, the bracteoles of *A. maxima* more strongly enclose the flowers, which are typically no more than two-thirds of the length of the bracteoles. Additionally, the bracteoles of *A. haradjianii* are white and green, whereas those of *A. maxima* are pink to pinkish. The floral parts, including the pedicels, are white in *A. haradjianii*, while they are pinkish in *A. maxima*. Although the umbel size generally differs between the two species, with *A. haradjianii* producing smaller umbels, there is some overlap between the largest umbels of *A. haradjianii* and the smallest of *A. maxima*.

Description (Fig. 2):—Perennial herb with rhizomes crowned by a fibrous collar. Stem erect, 40–70 cm, either branched from the base or simple; terete, ridged, hollow, glabrous. Basal leaves long-petiolate, petiole up to 20 cm; lamina 3–5 partite, segments ovate to elliptic-ovate, 3–7 × 1–4 cm, unequally serrate or crenate, with mucronate teeth. Cauline leaves sessile, sometimes amplexicaul, shapes similar to the basal leaves. Bracteoles white and green, 9–12, elliptic, oblong-elliptic or oblanceolate, 10–30 × 3–10 mm, slightly covering the flowers; nerved, with the margin setose-serrulate toward the apex. Inflorescence a simple umbel; lateral umbels (1)–3–5. Pedicels 3–12 mm, white, sometimes minutely hairy. Sepals white, persistent, linear-setaceous, 2–3 mm. Petals white, 1–2 mm. Fruit oblong-cylindrical, 5–8.5 × 1.8–2.5 mm.

Flowering and Fruiting:—From June to September.

Distribution:—Türkiye (Fig. 1).

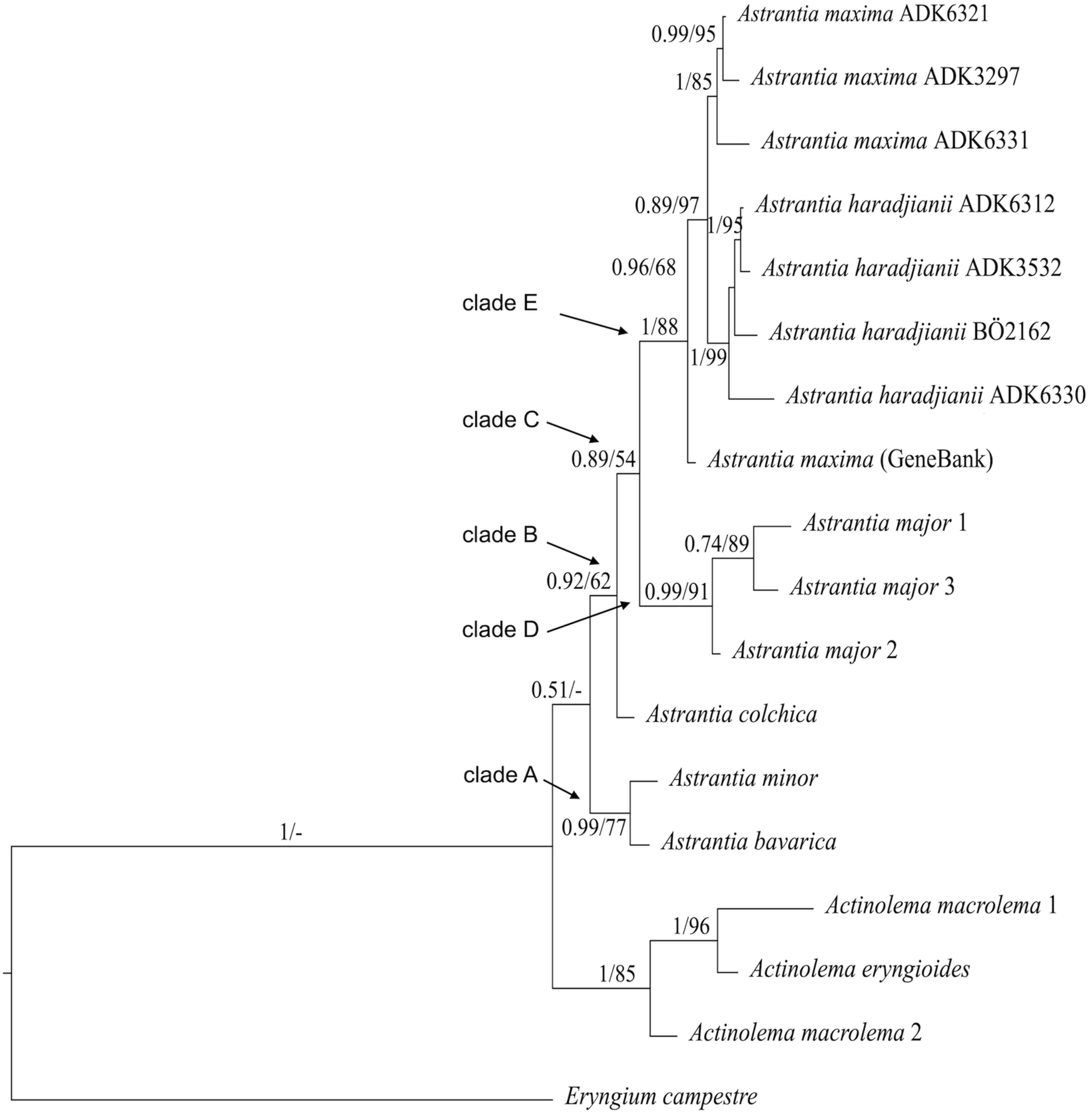

FIGURE 5. BI 50% majority-rule hypothetical tree based on the combined chloroplast sequence data (*rps16, rpl16, matK, rbcL, psbA-trnH, trnL, trnL-trnF, rps16-trnK*) of the genus *Astrantia* and adjacent genera. Branch support values are in the order of the BI/ML BS value. The voucher numbers for the specimens studied herein are at the terminals.

Identification key for Turkish taxa

- Bracteoles white and green; flowers at least two-thirds of the length of the bracteoles or longer.....................*Astrantia haradjianii*
- Bracteoles pink or pinkish; flowers at most two-thirds of the length of the bracteoles or shorter..........................*Astrantia maxima*

Discussion

Phylogenetic data on *Astrantia* remain limited, and there are still phylogenetically unknown species primarily distributed mainly in Transcaucasia, the North Caucasus (e.g., *Astrantia ossica* Woronow ex Grossh. (1905: 70), *Astrantia pontica*, *Astrantia trifida*), Austria, Italy, and the former Yugoslavia (e.g., *A. pauciflora* Bertoloni (1813: 76) and *A. carniolica* Wulfen (1778: 31)). According to Valiejo-Roman *et al.* (2002), *A. maxima* and *A. major* are closely related species, with *A. minor* forming a sister clade to this group based on the ITS data. Herein, however, the ITS-based phylogenetic hypothesis revealed a different relationship: *A. major* and *A. minor* were recovered as closely related (Fig. 4, clade A), with *A. maxima* forming a sister lineage to this clade. However, in the chloroplast-based phylogenetic reconstruction, *A. maxima* and *A. major* were found to be closely related, consistent with the findings of Valiejo-Roman *et al.* (2002) (Fig. 5).

The BI-based phylogenetic tree constructed from the ITS dataset did not resolve *A. maxima* subsp. *maxima* and subsp. *haradjianii* as distinct lineages. In contrast, the ML analysis of the same dataset recovered a monophyletic clade for the subsp. *haradjianii* populations. The chloroplast data, however, provided greater phylogenetic resolution. Both algorithms clearly distinguished the two subspecies based on the combined chloroplast dataset. This discrepancy is likely due to differences in the dataset size and phylogenetic resolution: the ITS matrix included 21 taxa and 646 characters, while the combined chloroplast data set comprised 9 taxa and 5528 characters, offering substantially more phylogenetic signal.

One sequence of *Astrantia maxima* subsp. *maxima* obtained from GenBank contained only the rps16, rpl16, and rps16-trnK markers (Plunkett *et al.* 1996, Cavino *et al.* 2007). The rpl16 sequence was obtained from a specimen collected in Georgia (Plunkett *et al.* 1996), while the rps16 and rps16–trnK sequences were derived from a specimen housed at the Bakuriani Botanical Garden in Georgia (Cavino *et al.* 2007). Based on the chloroplast dataset, this sequence occupies a basal position within the A. *maxima* clade. It is phylogenetically distinct from the populations of *A. maxima* subsp. *maxima* collected in Türkiye. In addition to *A. maxima*, *A. colchica*, *A. pontica*, *A. trifida*, *A. ossica*, and *A. major* also occur in Georgia (POWO 2024). According to Grintzesco (1910), all of these species, except *A. ossica*, are classified within sect. *Macraster*. *A. maxima* differs from other species of sect. *Macraster* by its broadly ovate-elliptic bracteoles, rather than merely ovate-elliptic, and ciliate-serrate margins, as opposed to having only 2–5 ciliated teeth restricted to the upper part) (Grintzesco 1910). Regarding the incongruence of the *A. maxima* sequence in GenBank, the weak diagnostic characters suggest a misidentification. Alternatively, the discrepancy may be due to limited resolution resulting from the restricted dataset, which includes only rps16, rpl16, and rps16-trnQ sequences.

Conclusion

Astrantia maxima was phylogenetically investigated for the first time using multiple populations representing its two subspecies. This study aimed to evaluate the potential species status of subsp. *haradjianii*, which has been classified at the subspecies level, even though there are clear morphological differences, such as smaller umbels. The phylogenetic analyses presented in this study indicate that the morphologically divergent populations of subsp. *maxima* and subsp. *haradjianii* are closely related but genetically distinct, particularly based on the chloroplast organelle. While the chloroplast dataset clearly supported the separation of the two subspecies, the ITS-based phylogenetic tree lacked sufficient resolution. Hence, the divergence of these two subspecies must have been so recent that their polytomous relationship is still evident in the ribosomal tree.

Astrantia haradjianii can be easily distinguished by its white and green bracteoles and white flowers, in contrast to the pink or pinkish bracteoles and flowers of *A. maxima*. It also consistently exhibits smaller umbels than *A. maxima*. Based on these findings, the taxonomic status of subsp. *haradjianii* is revised and elevated to species rank, supported primarily by the phylogenetic hypothesis derived from a combined large chloroplast dataset.

Acknowledgments

I wish to thank Nilay Şen and Dila Cana for their help during the field expeditions.

References

Albov, N.M. (1897) *Prodromus Florae Colchicae*. Romet, 294 pp.

Bertoloni, A. (1813) Correspondance: Extraits d'une lettre renfermant le caractere de quelques nouvelles plantes, ecrite a Desvaux par le docteur Bertholoni. *J. Bot. Agric.* 2: 75–77.

Calviño, C.I., Tilney, P.M., van Wyk, B.E. & Downie, S.R. (2006) A Molecular Phylogenetic Study of Southern African Apiaceae. *American Journal of Botany* 93: 1828–1847.
https://doi.org/10.3732/ajb.93.12.1828

Calviño, C.I. & Downie, S.R. (2007) Circumscription and phylogeny of Apiaceae subfamily Saniculoideae based on chloroplast DNA sequences. *Molecular Phylogenetics and Evolution* 44: 175–191.
https://doi.org/10.1016/j.ympev.2007.01.002

Calestani, V. (1905) Contributo alla sistematica. *Webbia: Journal of Plant Taxonomy and Geography* 1: 89–280.
https://doi.org/10.1080/00837792.1905.10669550

Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. (2012) jModelTest 2: More models, new heuristics and parallel computing. *Nature, Meth.* 9: 772.
https://doi.org/10.1038/nmeth.2109

Davis, P.H. (Ed.) (1965) *Flora of Turkey and East Aegean Islands, vol. 1*. Edinburgh University Press, Edinburgh, pp. 567.

Doğru-Koca (2024) Genetic divergences provide evidence to accept a new Mediterranean genus *Antalia* (Apiaceae) and insights into allopatric divergence extended to the Pliocene. *Botanical Journal of Linnean Society* 206: 95–113.
https://doi.org/10.1093/botlinnean/boae019

Downie, S.R., Ramanath, S., Katz-Downie, D.S. & Llanas, E. (1998) Molecular systematics of Apiaceae subfamily Apioideae: Phylogenetic analyses of nuclear ribosomal dna internal transcribed spacer and plastid *rpoc1* intron sequences. *American Journal of Botany* 85: 563–591.
https://doi.org/10.2307/2446441

Downie, S.R., Katz-Downie, D.S. & Watson, M.F. (2000) A phylogeny of the flowering plant family Apiaceae based on chloroplast dna *rpl16* and *rpoc1* intron sequences: towards a suprageneric classification of subfamily Apioideae. *American Journal of Botany* 87: 273–292.
https://doi.org/10.2307/2656915

Fay, M.F., Swensen, S.M. & Chase, M.W. (1997) Taxonomic affinities of *Medusagyne oppositifolia* (Medusagynaceae). *Kew Bulletin* 52: 111–120.
https://doi.org/10.2307/4117844

Ford, C.S., Ayres, K.L., Toomey, N., Haider, N., Van Alphen Stahl, J., Kelly, L.J., Wikström, N., Hollingsworth, P.M., Duff, R.J., Hoot, S.B., Cowan, R.S., Chase, M.W. & Wilkinson, M.J. (2009) Selection of candidate coding DNA barcoding regions for use on land plants. *Botanical Journal of the Linnean Society* 159: 1–11.
https://doi.org/10.1111/j.1095-8339.2008.00938.x

Grintzesco, J. (1910) Monnographie du genre *Astrantia*. *Annuaire du Conservatoire et du jardin botaniques de Genève* 13–14: 66–194.

Guindon, S. & Gascuel, O. (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* 52: 696–704.
https://doi.org/10.1080/10635150390235520

Hall, T. (1999) BioEdit: An important software for molecular biology. *GERF Bulletin of Biosciences* 2: 60–61.

Hedge, I.C. & Lamond, J.M. (1972) *Astrantia* L. *In:* Davis, P.H. (Ed.) *Flora of Turkey and East Aegean Islands, vol 4*. Edinburgh University Press, Edinburgh, pp. 290–291.

Hoffmann, G.F. (1816) *Astrantia trifida* Hoffm. *In: Genera Plantarum Umbelliferarum,* ed. 2.1. praef. 8 in nota.

Kadereit, J.W., Repplinger, M., Schmalz, N., Uhink, C.H. & Wörz, A. (2008) The phylogeny and biogeography of Apiaceae subf. Saniculoideae tribe Saniculeae: From south to north and south again. *Taxon* 57: 365–382.

Katoh, K., Rozewicki, J. & Yamada, K.D. (2019) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics* 20: 1160–1166.
https://doi.org/10.1093/bib/bbx108

Kaya, A. (2003) The genus *Astrantia* L. in Turkey: morphology and anatomy. *Acta Botanica Croatica* 62: 89–102.

Linnaeus, C. (1753) *Species plantarum, vol. 1*. Holmiae [Stockholm]: impensis Laurentii Salvii, pp. 560.

Oxelman, B., Liden, M. & Berglund, D. (1997) Chloroplast rps16 intron phylogeny of the tribe Sileneae (Caryophyllaceae). *Plant Systematics and Evolution* 206: 393–410.
https://doi.org/10.1007/BF00987959

Pallas, P.S. (1793) *Nova Acta Academiae Scientiarum Imperialis Petropolitanae.* vii. Imperatorskaja Akademija Nauk, Sankt-Peterburg, 357. t. 11.

Plunkett, G.M., Soltis, D.E. & Soltis, P.S. (1996) Evolutionary patterns in Apiaceae: inferences based on *matK* sequence data. *Systematic Botany* 21: 477–495.
https://doi.org/10.2307/2419610

POWO, (2024) *Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew.* Available from: https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:39706-1 (accessed 20 October 2024)

Rambaut, A. (2016) *FigTree v1.4.3: Tree Figure Drawing Tool*. Institute of Evolutionary Biology, University of Edinburgh. Available from: http://tree.bio.ed.ac.uk/software/figtree (accessed 20 October 2024)

Rambaut, A., Drummond, A.J., Xie, D., Baele, G. & Suchard, M.A. (2018) Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology* 67: 901–904.
https://doi.org/10.1093/sysbio/syy032

Rechinger, K.H. (1959) *Astrantia maxima* subsp. *haradjianii* (Grintz.) Rech.f. *Arkiv för Botanik* ser. 2, 5: 302.

Ronquist, F., Teslenko, M., Van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Schard, M.A. & Huelsenbeck, J.P. (2012) Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542.
https://doi.org/10.1093/sysbio/sys029

Schultz, F.W. (1858) Eine lange verkannte und verwechselte Pflanze Bayerns. *Flora* 41: 161–163.

Silvestro, M. (2012) raxmlGUI: A graphical front-end for RAxML. *Organisms Diversity & Evolution* 12: 335–337.
https://doi.org/10.1007/s13127-011-0056-0

Stamatakis, A. (2014) RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312–1313.
https://doi.org/10.1093/bioinformatics/btu033

Turland, N.J., Wiersema, J.H., Barrie, F.R., Greuter, W., Hawksworth, D.L., Herendeen, P.S., Knapp, S., Kusber, W.-H., Li, D.-Z., Marhold, K., May, T.W., McNeill, J., Monro, A.M., Prado, J., Price, M.J. & Smith, G.F. (eds.) (2018) *International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017. Regnum Vegetabile* 159. Glashütten: Koeltz Botanical Books.
https://doi.org/10.12705/Code.2018

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: 1105–1109.
https://doi.org/10.1007/BF00037152

Valiejo-Roman, C.M., Terentieva, E.I., Samigullin, T.H. & Pimenov, M.G. (2002) Relationships among genera in Saniculoideae and selected Apioideae (Umbelliferae) inferred from nrITS sequences. *Taxon* 51: 91–101.
https://doi.org/10.2307/1554966

Woronow, J.N. (1905) *Astrantia ossica*. *Trudy Botanicheskogo Sada Imperatorskogo Yur'evskogo Universiteta* 6: 70.

Wörz, A. (1999) Systematics and evolution of the genus *Astrantia* L (Apiaceae-Saniculoideae). *Botanische Jahrbucher fur Systematik Pflanzengeschichte und Pflanzengeographie* 121: 507–536.

Wulfen, N.J.von J. (1778) *Astrantia carniolica*. *In: Florae Austriaceae, vol. 5*. Opera AcSumptibus, Vienna, pp. 500.

Appendix. Examined and collected specimens of *A. maxima* from Türkiye. The underlined populations were molecularly studied. The numbers between parentheses indicate the points in Fig 1. The bold numbers display Davis' grid system (Davis 1965)

Astrantia haradjianii:—**A2** (1) Bursa: Ulu Da., Sestim (as *A. minor* L.). **A3** (2) Bolu: Abant Lake, 21 vii 1941, B. Post; (3) Abant National Park, Bolu Mount, vii 1978, O. Polunin 15081, (E, photo!); (4) around Abant Lake, 07 vii 2008, ADK 3532 (HUB!). Abant, meadow, ca. 1100 m, *Fagus orientalis* forest, 14 vii 1978, Y. Akman 9823 (ANK!). Mudurnu, Vakıftaş, *Pinus nigra*, ca. 1400 m, 14 vii 1978, Y. Akman 9824 (ANK!). Between Mudurnu-Uluhan, 1400 m, 22 vi 1974, Y. Akman 9360 & Quezel (ANK!). Yedigöller National Park, Tombullar area, ca. 1500 m, 12 vii 1977, R. İlarslan 137 (ANK!). **A4** (5) Zonguldak: Keltepe above Yenice, 1350 m, D. 37803 (E, photo!); (6) Keltepe, 20 km südwest Karabük, 1300 m, Kühne 1131 (E, photo!). Keltepe, around Gölyanı, 1850 m, 11 ix 1997, *Abies* forest, N. Adıgüzel 3053 & A. Kaya (GAZI!). Keltepe, around Forest Ministery regional buildings, ca. 1200 m, 12 vii 1984, M. Demirörs 2000 (ANK!). **A5** (7) Çorum: Osmancık, Gökbel, around Dipsiz Göl, under forest, 1473 m, ADK 6312 (HUB!). (8) Amasya: Merzifon, 1904, Manissadjianae 167 (E, photo!), (9) Amasia, in regione superiore mts. Sana-Dagh, 1300-1400 m, Bornmüller 15 vii 1889 (JE, photo!). Kastamonu: Yaralıgöz Mount, calcerous, 1500 m, 27 vii 1990, E. Yurdakul 3417 (ANK!). Pınarbaşı, Between Kastamonu-Pınarbaşı, Kurtgirmez Mount, forest road, 1200 m, 24 viii 2002, M.U. Özbek 1425 (GAZI!). (10) Between Kastamonu-Küre, forest edges, ca. 1200 m, 09 vii 2024, ADK 6330 (HUB!). **A6** (11) Samsun: Ladik, Zefe village, Akdağ, 1350 m, Tobey 1180 (E, photo!). Tokat: Artova, Aktaş district, Çal hill, ca. 1300-1400 m, 16 vii 1979, R. İlarslan 642 (ANK!). **A7** (12) Gümüşhane: Kızılcık village, around Kuşakkaya, 1600-2000 m, *Pinus sylvestris* forest, 11 vii 1989, Z. Aytaç 2814 (GAZI!). **B2** (13) Kütahya: Murat Mount., Kesik Soğuk, 1370 m, GuichardTUR/144/62 (E, photo!). **B3** (14) Bilecik: 11 km from Söğüt to Eskişehir, 1020 m, Hub.-Mor. 13629; (15) Eskişehir, Sündiken Mount, Karakütük river, c. 1600 m, 20 vi 1972, T. Ekim 761 (E, photo!). Türkmen Mount, Efsun hill, quercetum and *Fagus* forest, *Pinus* forest, ca. 1550 m, 9 vii 19977, T. Ekim 2661 (ANK!). **B5** (16) Kayseri: Erciyas Mount., 1500 m, Zederbauer. **B6** (17) Sivas: 36 km N. of Sivas, 1700 m, Sorger 69-51-22. (18) Şarkışla, Diğnendim hill, 1600-1900 m, under *P. sylvestris*, 16 vii 2008, B. Özüdoğru 2162 (HUB!). **B7** (19) Tunceli: S. of Ovacık, 1750 m, D. 31549 (E, photo!). **C3** (20) Isparta: Eğridir, 1445 m, Gökşin 8778; (21) Yukarı Gökdere, kasnak forest, calcareous, mixed forest, 1100 m, 11 vii 1974, H. Peşmen & A. Güner 1793 (HUB!, ANK!). (22) Anamas, Yaka village, Kapızderesi, calcareous valley, 1250-1450 m, 05 viii 1974, H. Peşmen & A. Güner 1848 (HUB!). Ibid, A. Güner 1498 (HUB!). **C6** (234 Adana, Syria borealis, Mount. Amanus in reg. Hasan Veyli, 1220-1520 m, Haradjian PL Syr. bor. 2316 (G!, K). (24) Adana/Maraş: Dumanlı Mount., 700-1200 m, Haradj. 3686.

Astrantia maxima:—**A6** (1) Ordu: Çambaşı, Semen, 2400 m, Tobey 1404! A7 (2) Giresun: Balabandağları above Tamdere, 1800 m, D. 20499. (3) Trabzon: Hamsiköy, c. 1350 m. Balls 1638 (ANK!). (4) Gümüşane: Karagöl Da., Kalis-baschi-forest, Sint. 1894: 7259. **A7** Giresun: Balaban Mounts, Tamdere, ca. 1800 m, 4 viii, Davis 20499 & AR. Çetik (ANK!). Kulakkaya, Tamdere, near the river, 6 vii 1958, H. Birand 10731 (ANK!). **A8** (5) Trabzon: Altındere, Haliya plataeu, 1945 m, 18 vi 2007, ADK 3297 (HUB!). (6) N. side of Soğanlı Mount. above Çaykara, 2000-2200 m, Davis 32148& Hedge (ANK!). (7) Between Gümüşhane-Trabzon, 35 km from Gümüşhane, Zigana Mount, *Carpinus* forest, ca. 1400 m, 28 vii 1956, K. Karamanoğlu 642 (ANK!). Zigana Pass, ca. 1700 m, *Pinus sylvestris*, 26 vi 1977, Y. Akman 1940 (ANK!). (8) Rize: İkizdere, Anzer yaylası, around Ballıköy, 2165 m, slopes, 21 vii 2023, ADK6321 (HUB!). Around Ballı village, 2000 m, meadow, 27 viii 1985, A. Güner 6199 & M. Vural (HUB! GAZI!). (9) Çamlıhemşin, Çat village, above Vanksi plataeu, alpinic meadow, metamorphic rocks, 2100-2300 m, 22 viii 1981, A. Güner 4259 (HUB!). (10) between Yukar Amlakıt plateau-Kışyatağı, *Rhododendron caucasicum* shrub, granit main rock, 2200-2350 m, 20 ix 1979, A. Güner 2185 (HUB!). (11) opposite Ortayayla village, 2300 m, meadow, 14 viii 1985, A. Güner 6819 & M. Vural (HUB! GAZI!). Djimil (Cimil), 2000 m, Bal. 1389. (12) Erzurum: nr. Oltu (Grossheim 7: map 6); Olur, plataeu of Kekikli village, front of Şirişin, 1750-2300 m, 01 viii 1984, N. Demirkuş 2334 (HUB!). Şenkaya, Gülveren village, 2500 m, 9 ix 1993, *Pinus sylvestris* openings, Y. Altan 6683 (ANK!). Çoruh: nr. Artvin (Grossheim 7: map 6). **A9** (13) Artvin: Şavşat, Karagöl, 1750 m, alpine meadow, 28 vii 2004, H. Altınözlü 4177 (HUB!). (14) around calcareous well, plantation area, 1950-2100 m, 28 vii 1982, N. Demirkuş 1594 (HUB!). Ardanuç, around Kutu, under *Picea* forest, ca. 1600 m, 22 vii 1961 (ANK!). (15) Çoruh: Ardanuç to Kordevan Mount, 1400 m, D. 30148 (ANK!). (16) Kars: E. of Kağızman, c. 20 km from Akçay to Cumaçay, 2200 m, D. 46733. (17) Posof, from Asmakonak village to Posof, along forest road, 1700-2000 m, 25 vii 1985, N. Demirkuş 2930 (HUB!). (18) 35-40 km before Kars, Balçeşme village meadow, N. Demirkuş 1311 (HUB!). (19) Ardahan: Ardahan-Hoçvan, S of Kısırdağ, 17 vii 1981, N. Demirkuş 1163 (HUB!). (20) road to Ardahan, 1 km S of Çam pass, 24 km SE of Şavşat, 28 ix 2005, B. Güner, 101 & S. Knees, M. Gardner *et al.* (NGBB!). Kısır Mount., Gülyüzü valley, valley slopes, *Cephalaria procera* step, 2100 m, 29 vii 2002, N. Adıgüzel 4423 & H. Ocakverdi, M. Pınar (GAZI!). (21) From Şavşat to Ardahan, Kocabey village plateau, c. 2100 m, 10 vii 2024, ADK6331 (HUB!). **B9** (22) Kars: Çıldır, Kenarbel village, around Ercan, 1800-1900 m, 4 viii 1996, humid slopes, G. Akgül (GAZI!). W. of Iğdır (Grossheim 7: map 6).