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On the border of extinction and speciation, *Polygala seyfegoluensis* (*Polygalaceae*), a new species from marshy habitat, Central Türkiye: Under the threat of climate change, water retreat, and subsequent degradation

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Abstract: *Polygala seyfegoluensis* Dönmez, Uğurlu & Yüzb. (*Polygalaceae*) is herein described and illustrated as a new species from Kırşehir Province around Seyfe Lake in Central Türkiye. This new species is similar to *P. pruinosa* and *P. supina* is distinctly characterized by its prostrate habit, nearly glabrous and smaller flower parts, and an unwinged capsule. A phylogenetic analysis was conducted to show the placement of the new species within selected *Polygala* taxa using the *trnL-F* and *rbcL* markers. Based on the field observation, an evolutionary scenario is proposed for the new species under the driving forces of climate change, the shrinking of Seyfe Lake, and its surrounding man-made habitat. The evolutionary significance of the style and stigma of the new species and the closely related taxa has been discussed since the breeding biology. Considering the ecological issues, population size, and the accounted individuals of the new species, it is here assessed as Critically Endangered. Additionally, lectotype is selected here with a new description for the closest relative of the new species, *Polygala pruinosa*.

Key words: Conservation, endemic, marshy plants, taxonomy

1. Introduction

Polygala L. is a nearly cosmopolitan genus with a global distribution—excluding Antarctica, the Arctic, and South Africa—and the Americas (North, Central, and South) are known as the diversification center of the genus (Paiva, 1998; Bernardi, 2000). The genus *Polygala* has 553 species (Pastore et al., 2019; Çeçen et al., 2023) but recently New World *Polygala* taxa is accepted under the new genus (Pastore et al., 2023). Molecular phylogenetic studies indicated polyphyly (Eriksen, 1993; Persson, 2001; Monro, 2003; Forest et al., 2007) and New World and Old World clades are nested separately in *Polygala* (Pastore et. al., 2019).

Traditionally, the genus has been divided into three subgenera: subg. *Polygala*, including majority of the species (Pastore, 2018), subg. *Chodatia* Paiva with 23 species, and *Brachytropis* DC., which represent only *Polygala microphylla* L. The most species-rich subgenus *Polygala* is distributed in Europe and West Asia (McNeill, 1968; Lyskov et al., 2019). Despite the extensive polymorphism of the subg. *Polygala*, some of the floral characters (8 stamens, two petaloid inner, and three smaller outer sepals) are useful for distinguishing them. Moreover, the capsule forming stipitate or sessile and seeds with caruncular appendages are well-known characters in the subgenus (Chodat, 1891; Eriksen, 1993; Paiva, 1998; Uğurlu Aydın, 2020).

Research on the Turkish *Polygala* has been a comprehensive project, and all the taxa have undergone extensive field collection. Traditional taxonomy, along with anatomical, palynological, cytological, molecular, and phytochemical perspectives, has been employed in the investigation (Dönmez et al., 2015; Çalış et al., 2022, 2023; Ünlü et al., 2022; Çeçen et al., 2023).

During fieldwork to research an unusual area for *Polygala* taxa in 2021, specimens were collected in the meadow just beneath Seyfe Lake Kırşehir, Türkiye. The specimen was interesting because it had prostrate habit, fleshy leaves, and small flowers at first glance. The abundance and the population size of the specimens were examined in the field, and the samples for the relevant research methodology were taken and photographed. The area is visited after two years and the population structure has been investigated in detail. Further examination of the specimens revealed that plants are superficially similar to *P. supina* Schreb. in respect to small flower and fruit size and

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indumentum. However, the main morphological features resemble *P. pruinosa* Boiss., which naturally grows in the region at steppe areas. According to the critical evaluations of the characters, the specimens discovered from the unusual habitat for *Polygala* taxa represent an undescribed species. Based on morphological and molecular data, the plant is described here as a new species, *P. seyfegoluensis* Dönmez, Uğurlu & Yüzb. with morphological and SEM photos, and an evolutionary history is proposed.

Polygala pruinosa, the closest relative of the new species, is distributed mainly in Türkiye and scattered in some of the neighbouring countries and it has several syntypes. Based on the studies on the original herbarium materials and the protologue, a lectotype is selected here for this species.

2. Materials and methods

2.1. Morphological and SEM studies

All *Polygala* specimens mentioned below, belonging to *P. seyfegoluensis*, *P. pruinosa*, and *P. supina* from herbaria and online databases, were morphologically examined in detail (see Supplementary Information). The leaves, capsules, and seeds were washed with distilled water and mounted on aluminium stubs coated with gold-palladium mixture in a sputter coater and examined using scanning electron microscopy (SEM). The photomicrographs were taken with a ZEISS EVO 50 EP scanning electron microscope.

2.2. DNA extraction, PCR amplification, and DNA sequencing

Total genomic DNA was extracted from silica-dried plant leaves using the DNeasy Plant Mini Kit (Qiagen, Redwood City, CA, USA), following the manufacturer's protocol. Target DNA regions including three plastid DNA regions, *trnL* intron and *trnL-trnF* spacer (hereafter *trnL-F*) and *rbcL* gene, were amplified flowing the relevant literature (Eriksen, 1993; Forest et al., 2007; Pastore et al., 2019). The *trnL-F* region was amplified using c and d primers (for the intron) and e and f primers (Taberlet et al., 1991) and *rbcL* gene was amplified using universal primers (Kress et al., 2007). For plastid regions, polymerase chain reaction (PCR) was performed in a 20 µL volume containing 10 µL of 10X HS Prime PCR Master Mix with the addition of 5–10 ng of genomic DNA and 8 µL H_2O. The target regions were amplified using the following parameters: 94 °C for 3 min, 35 cycles at 94 °C for 1 min, 55 °C for 1 min (for *rbcL*), 50 °C for 1 min (for *trnL-F*), and 72 °C for 1 min, and a final extension of 72 °C for 3 min. The amplified products were sent to Macrogen company (Amsterdam, Netherland) for Sanger's sequencing using the same primers as those for the amplification. Sequences newly produced for this study were submitted to GenBank (Table 1).

The contigs were created using forward and reverse sequences of *rbcL* and *trnL-F* datasets from 24 specimens in Geneious Prime 2021.2.2 (www.geneious.com). Multiple sequence alignment of each marker and concatenation of DNA regions into a single combined matrix was done using Clustal Omega implemented in Geneious Prime 2021.2.2. The aligned sequences were trimmed from both ends to obtain an equal length aligned DNA data for further analysis.

2.3. Phylogenetic analysis

The aligned concatenated sequences were used to generate the phylogenetic tree. The phylogenetic relationship was constructed both with Bayesian analyses and maximum likelihood (ML) inference. The best model according to the Akaike information criterion (Posada and Buckley, 2004) was GTR + G for dataset, and Geneious Prime was used for both analyses. The consensus tree of BI inference was run in MrBayes version 3.2.6 (Ronquist et al., 2012) with one million generations, two parallel runs, and four chains. The ML analysis was carried out with 10,000 bootstrap replications in RAxML (Stamatakis, 2014). In the Bayesian analysis, effective sample sizes (ESS > 200) were checked using Tracer version 1.6. Trees and all parameter values were sampled every 1000 generations, and the first 20% was discarded as burn-in. The 50% majority rule consensus trees were visualized in FigTree 1.4.4.[1] To assess branch support, bootstrap (bs) and posterior probability (pp) were calculated. We considered a branch supported if bs >70% and pp >0.80. One of the New World *Polygala* taxa, *P. rugelii*, was used as an outgroup.

3. Results

3.1. Taxonomic treatment

Polygala seyfegoluensis Dönmez, Uğurlu & Yüzb., **sp. nov.** (*Polygala* L. sect. *Polygala* subsect. *Polygala*) (Figures 1–2).

Holotype: Türkiye. Kırşehir: Mucur, SW peak of Seyfe Lake, between Yazıkınık and Seyfe villages, in *Juncus* association, dried marshy place, 39°10′46″N, 34°21′47″E, 1117 m, 10 July 2021, *A.A.Dönmez* 20983-*S.Yüzbaşıoğlu* (HUB). **Isotypes**: HUB, ISTF. **Paratypes**: Türkiye. Kırşehir: Mucur, between Yazıkınık and Seyfe villages, in *Juncus* association, dried marshy place, 39°10′46.0″N, 34°21′155.0″E, 1117 m, 5 August 2023, *A.A.Dönmez* 21692-*Z. Uğurlu Aydın* (HUB); 39°10′18.1″N, 34°22′41.3″E, 1117 m, 5 August 2023, *A.A.Dönmez* 21694-*Z. Uğurlu Aydın* (HUB); 39°10′0.1″N, 34°23′34.3″E, 1117 m, 5 August 2023, *A.A.Dönmez* 21696-*Z. Uğurlu Aydın* (HUB).

3.2. Diagnosis

Polygala seyfegoluensis is similar to *P. pruinosa* and *P. supina*. The new species differs from *P. pruinosa* by its prostrate habit (not erect or ascending), glabrous to rarely pilose indumentum (moderately to densely puberulous

1 Rambaut A (2018). Published at http://tree.bio.ed.ac.uk/software/figtree/[accessed January 4, 2023]

Table 1. Taxon list with information on voucher specimens and GenBank accessions for three DNA regions (asterisk indicates new sequences produced for this study).

Taxa	Voucher specimen	GenBank accession numbers		
		rbcL	***trnL-F***	***trnL intron***
Polygala hybrida DC.	*A.A.Dönmez* 21057	OL632206	OM676349	OM732501
P. major Jacq.	*A.A.Dönmez* 20470	OL542851	OM676344	OM732496
P. major	MW0428474	-	MK108141	MK108141
P. major	MHA-1HU	-	MK108138	MK108138
P. major	MW0799480	-	MK108129	MK108129
P. major	MHA-1IT	-	MK108136	MK108136
P. major	MW0617491	-	MK108142	MK108142
P. major	MHA-1RM	-	MK108140	MK108140
P. major	MHA-1SL	-	MK108139	MK108139
P. anatolica Boiss.	*A.A.Dönmez* 21061	OL542850	OM676346	OM732498
P. anatolica	ERE111558	-	MK108157	MK108157
P. anatolica	MW0689676	-	MK108152	MK108152
P. anatolica	MW0689688	-	MK108156	MK108156
P. anatolica	MW0689668	-	MK108145	MK108145
P. anatolica	*A.A.Dönmez* 19820	OL542852	OM676348	OM732500
P. anatolica	*A.A.Dönmez* 20382	OL542844	OM76347	OM732499
P. pruinosa subsp. *pruinosa*	*A.A.Dönmez* 20418	OL542843	OM676341	OM732492
P. pruinosa subsp. *pruinosa*	*A.A.Dönmez* 20876	OQ642073*	OQ676417*	OQ676414*
P. supina	*A.A.Dönmez* 19772	OL542841	OM676339	OM732490
P. supina	*A.A.Dönmez* 20722	OL542839	OM676337	OM732488
P. monspeliaca L.	*A.Cantaş* 003	OL542846	OM676343	OM732495
P. hohenackeriana Fisch. & C.A.Mey	*A.A.Dönmez* 11415	OQ642074*	OQ676418*	OQ676415*
P. seyfegoluensis	*A.A.Dönmez* 20983	OQ642072*	OQ676416*	OQ676413*
P. rugelii Stuttlew. ex Chapm.	*Abbott* 14273	KJ773774	GQ888853	GQ889187

in *P. pruinosa*), smaller flower length (not more 5 mm), smaller inner sepals (not more than 5 × 4.2 mm), smaller capsule (not more than 4.2 × 3.5 mm), nearly unwinged capsule, smaller seed length (not more than 3.2 × 1.2 mm). *Polygala seyfegoluensis* also differs from *P. supina* by lanceolate (obovate at lower) leaves (not ovate to orbicular), smaller inner sepals (not more than 5 × 4.2 mm), smooth upper sepal base (not saccate), smaller seed length (not more than 3.2 × 1.2).

3.3. Description

Perennial herb, 5–10 cm. *Stems* prostrate, striate, with small turions at base; all axes and leaves glabrous to sparsely 0.1–0.2 mm antrorse hairs. *Leaves* sessile, lanceolate, obovate at lower, obtuse at apex, rarely with small mucro; stem leaves 9–12 × 2–3 mm, entire or slightly revolute at margin. *Inflorescence* 2.5–5 × 1.5–3 cm, usually branched, rarely simple raceme, apically not comose, dense, with (5-) 10–25 spreading or nodding flowers. *Pedicels* 1.5–2 mm, horizontally spreading or bent downwards. *Bracts* shorter than pedicels, deciduous with distinct scar, leaf-like, widely ovate, 0.8–1.2 mm; *bracteoles* one pair, deciduous, lanceolate, 0.6–0.8 mm. *Flowers* pink, suffused with white, glabrous, 4–5 × 2.5–4 mm. *Sepals* 5, persistent, glabrous; *outer sepals* 3, leafy, scarious at margin, *lower two (outer) sepals* smaller than the upper, narrowly ovate, 1.8–2 × 0.5–0.7 mm, obtuse at apex; *upper sepal* ovate, entire, not saccate, scarcely gibbous, obtuse at apex, 2.5–3 × 1.5–2 mm, scarious at margin, sometime ± ciliate; *wings (inner sepals)* 2, petaloid, ovate, pinkish, 4–5.5 × 3.5–4.2 mm, slightly longer than fruit, glabrous, inner two veins mostly anastomosing to midvein, apex obtuse, rarely with small mucro. *Petals* 3, deciduous, 4.5–5 mm, equal or

slightly shorter than the inner sepals; glabrous; *keel (lower petal)* 2.5–3 × 1.8–2.2 mm, concave, crest fringed; *wings (upper petals)* 2.5–3 × 1.8–2 mm. *Stamens* 8, concealed by concave part of keel; *filaments sheath* glabrous, ciliate on margin, adnate to upper of corolla; anthers distinctly stipitate, 0.2–0.4 mm, opening by an oblique pore at apex. *Capsule* sessile, orbicular to obovate, 4–4.2 × 3.2–3.5 mm, bilocular; wingless or very narrowly winged, not more than 0.3 mm; *style* deciduous. *Seeds* narrowly ovate, 2.8–3.2 × 1.1–1.6 mm, covered with sparse stiff hairs; *caruncle* laterally elongate, 1.4–2 mm long.

3.4. Eponymy

The epithet of *Polygala seyfegoluensis* is derived from the Seyfe Lake in Kırşehir, Türkiye, which is drastically shrinking due to climate change.

3.5. Phenology

Flowering in June–July.

3.6. Distribution and ecology

The species is known from a single location of the Seyfe Lake in Kırşehir, Türkiye (Figure 3). Seyfe Lake is a hydrographically closed basin surrounded by high mountains and covers an area of 152,200 ha. The basin extends from northwest to southeast, with elevations ranging between 1115 and 1350 m. Seyfe Lake has three different protection statuses, both nationally and internationally, due to its natural resource value. The basin includes an area of 10,700 ha designated as the Nature Protection Area and 23,585 ha designated as the First Degree Natural Site Area. In addition, 10,700 ha of land are on the Ramsar Convention list (Tapan, 2008; field observation of the authors 2023).

According to Dönmez (2013), based on the calculations using satellite images and meteorological data between 1975 and 2001, the area of the lake decreased by 1/4 in the last 25 years. A similar study investigating temporal changes on the surface area of the Seyfe Lake was conducted by Yurteri and Kurttaş (2021). It was determined that the lake surface area decreased by 93.78% during these 35 years' period (1985–2020). It is seen that this shrinkage in wetlands has accelerated in recent years, which causes agricultural lands to move towards the lake.

The basin is located in the semiarid climate zone, and its morphology was shaped under the influence of this climate. Being a closed basin, it experiences significant fluctuations in water levels during the rainy and dry seasons. The lake bottom is composed of fine materials such as clay, silt, and sand. This material prevents water from leaking underground during rainy periods and causes the lake area to expand towards the south (Çiftçi et al., 2021). There are wide steppe areas extending to the lake shore in the north of the lake. Other shores are often surrounded by fields (Çiftçi et al., 2021; Tapan, 2008).

Depending on precipitation, evaporation, resources, and human interventions, the area covered by the lake changes.

Figure 1. A general habitat and habit of *Polygala seyfegoluensis* (from A.A. Dönmez 20983-Holotype), Habitat (A), Inflorescence and flowers (B), Capsules (C), Habit and leaves (D) (Note: the branch is raised for taking photograph, arrowhead showing the lever).

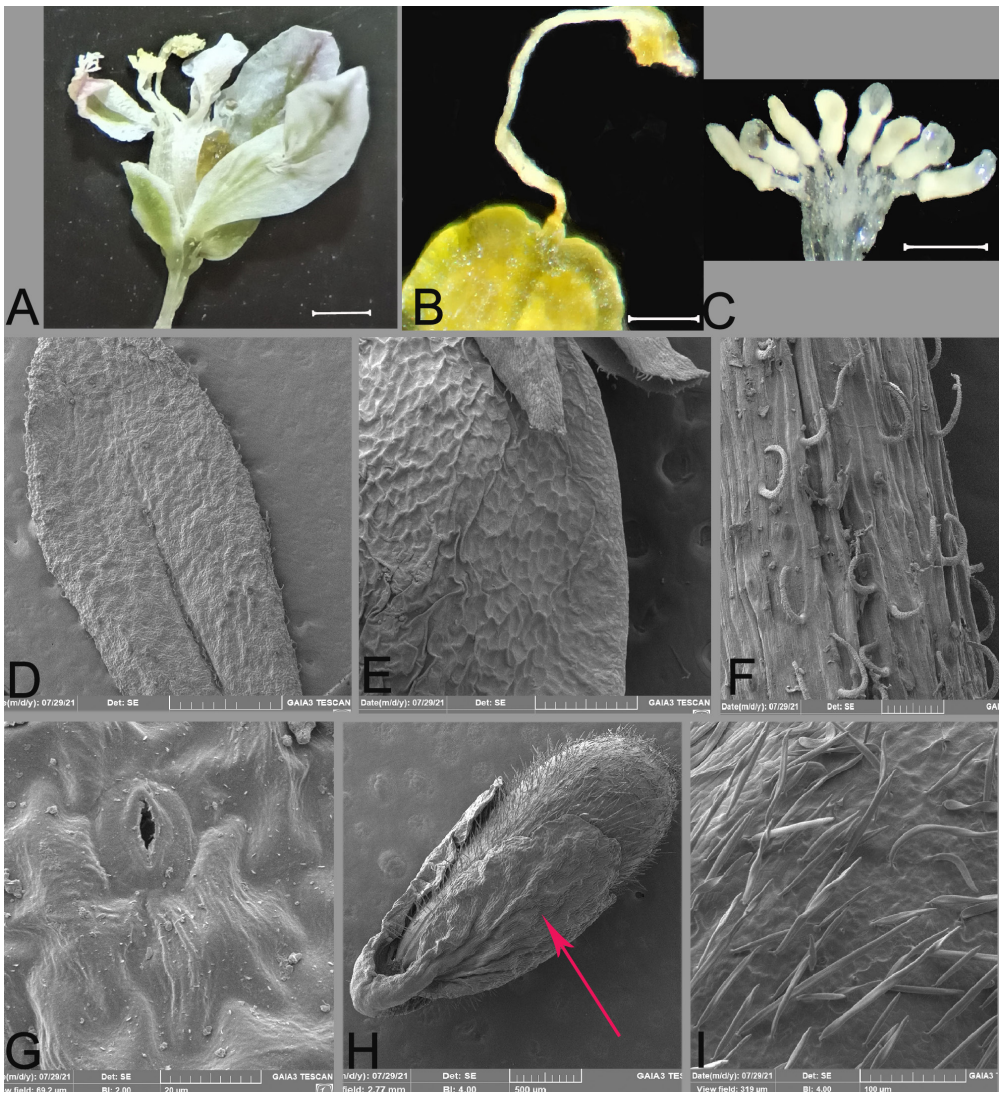

Figure 2. *Polygala seyfegoluensis* (from A.A. Dönmez 20983-Holotype). Partly dissected flower (A), Style (B), Stamens (C), Leaf surface (D), Capsule wing (E), Stem surface (F), Stoma (G), Seed and elaiosome (arrow) (H), Seed surface (I). (Scale bars A-B = 1 mm, C = 0.5 mm, D-E= 500 µm, F= 100 µm).

Three types of soils are common around Seyfe Lake: alluvial, hydromorphic, and halomorphic. Hydromorphic soils are characterized by their high moisture content. These soils, dominant in the environment, are rich in $CaCO_3$. Different vegetation structures adapted to the marsh and salt structure surrounds the lake. In general, it has been determined that halophytic (salty-salt-loving), pure steppe, and meadow vegetation exist (Çeşmeci, 2010). In recent years, the decrease in the amount of water, both due to natural factors and, especially, human effects, has caused changes in vegetation types (Yiğitbaşoğlu, 1995; Eyüboğlu, 2022). Dönmez (2013) categorized the basin into three main groups concerning land use: 68% agricultural land, 19% pasture (grassland) land, and 5% wetland. It is seen that agricultural lands cover a large area in the basin. Human influence in the basin has been on the rise since the early 1900s. Areas around the lake formed by the withdrawal of the lake are used for agriculture. The meadow habitat of *Polygala seyfegoluensis* is under threat of being converted into fields for agricultural purposes.

The population of the species is restricted to steppe patches between agricultural fields and the lake. In the past, these sites were partly transformed from steppes to agricultural fields. It is most likely that the species was present in agricultural fields in the past. Therefore, it is inferred that the area of occupancy of the species has decreased due to the conversion of steppes to agricultural fields.

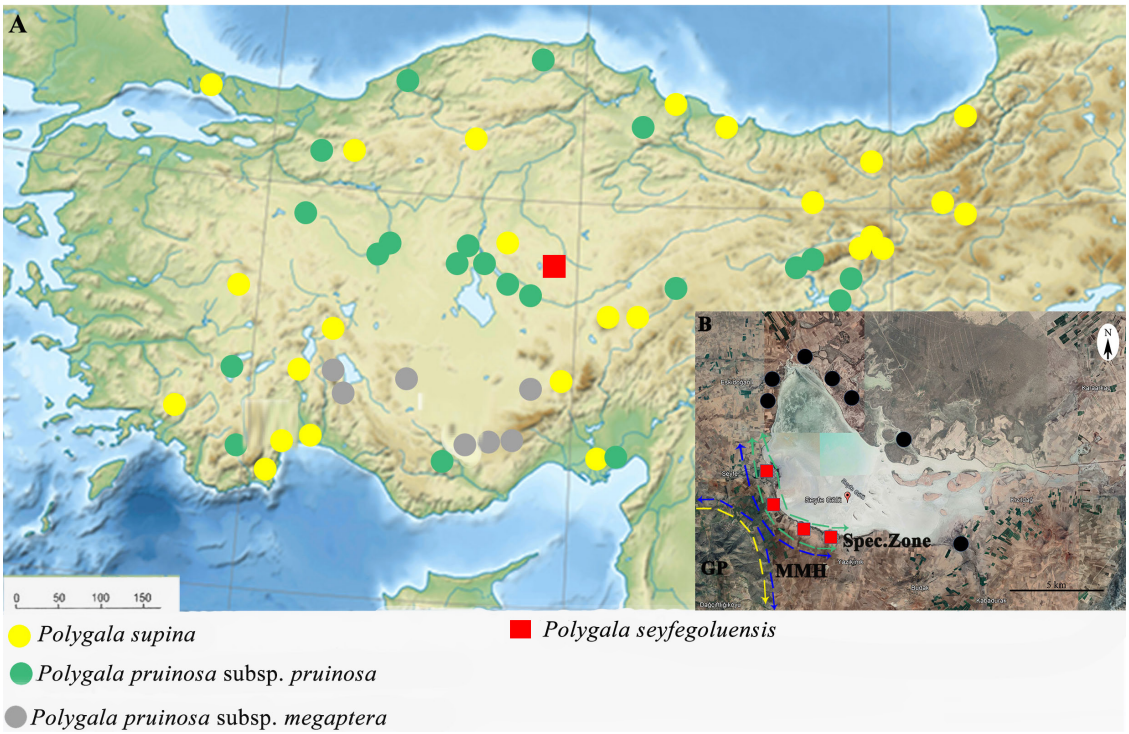

Figure 3. A distribution map of *Polygala seyfegoluensis*, *P. supina*, and *P. pruinosa* in Türkiye (A) with visiting locations around Seyfe Lake (B) (red square: the samples collected, black circle: visited but not individuals found). GP: gene pool, MMH: Man-made habitat.

The main threat to the species is habitat loss due to conversion to agricultural areas. Today, it is observed that the lake area has become a salt desert covered with salt and open to wind erosion, threatening agricultural areas and narrow distribution areas of the species. Finally, extreme seasonal events due to climate change may impact the population of the species.

Polygala seyfegoluensis is distributed southwest of Seyfe Lake, Kırşehir and it is endemic to Türkiye. It is only known from the type locality with three separate small populations. Its area of occupancy is estimated at less than 10 km^2. Based on available data, CR ab(iii,v) 2ab(iii,v) threat category has been proposed for this new species (IUCN 2022).

3.7. Molecular phylogenetic study

A total of 61 sequences were used in the current analysis to construct the phylogenetic tree, of which six are newly sequenced for this study. The concatenated alignment was used for maximum likelihood (ML) and Bayesian analyses, and the topologies of the trees are congruent concerning the relationships between the new species and the remaining *Polygala* taxa. Hence, we present only the Bayesian topology (Figure 4), with the posterior probability over 0.50 for each node. The new species form a distinct clade from the rest of the related *Polygala* taxa with low posterior probability (pp) and bootstrap support (<50). However, it is separated from *P. supina* and some related taxa (*P. anatolica*, *P. hohoneckeriana*, *P. hybrida*, *P. monspeliaca*, and *P. major*) with strong posterior probability (pp) and moderate bootstrap support (0.88/75). The other morphologically related taxa, *P. pruinosa*, clustered distantly from the remaining *Polygala* taxa in a monophyletic clade with moderate support (0.64/74).

3.8. Description and lectotypification

The Turkish *Polygala* taxa are mainly herbaceous plants and their morphology is strongly affected by environmental conditions. Due to the plasticity of the traditionally used diagnostic morphological characters, delimiting the taxa is particularly challenging. Therefore, the type of materials and the protologue, along with relevant materials of various taxa, are crucial for accurate identification. Given that the descriptions of closely related species, namely *P. pruinosa* and *P. supina*, are rather outdated, a new description and lectotypification have been conducted here for *P. pruinosa*.

Polygala pruinosa Boiss., Diagn. Pl. Orient. ser. II, I: 58 (1853).

Lectotype: (Selected here) Türkiye: Cadmos herbidis supra Demisleh, [Babadağ, above Denizli], *P.E. Boissier,* s.n. (G!) (Barcode: G00150161; Figure 5).

= *Polygala ramulosa* Boiss. & Bal. in Boiss., Diagn. ser. 2(6): 24 (1859).

Figure 4. Bayesian 50% majority-rule consensus tree of concatenated dataset. The topology of the Bayesian tree is congruent with that of the ML tree. Numbers above and below the branches are pp and bs values, respectively.

= *Polygala guneri* Yild., Ot Sist. Bot. Dergisi 19(2): 41 (2012): syn. nov. Type. Türkiye. B3 Eskişehir: Sivrihisar, Aşağıkepen village, gypseous soil, steppe slopes, 955–1025 m, 1 June 2012, *Ş. Yıldırımlı* 38551 (holo. Y.Otk.; iso. HUB!, Y.Otk.). Paratype. B3 Eskişehir: Sivrihisar, Afyon road, around Aşağıkepen village, gypseous slopes, 990–1030 m, 31 May 2008, *Ş. Yıldırımlı* 34762 & *G. Yıldırımlı*.

Paralectotypes: Türkiye: Aucher pl. exs. No (Barcode: G00150147), Pamphylia et Pisidia Pisidia (Heldr. not found).

Perennial herb, 5–15 (-30) cm. *Stems* ascending to erect, much branched at base, striate, moderately to densely puberulous. *Leaves* sessile, lanceolate to oblanceolate, rarely narrowly elliptic, entire or revolute at margin, obtuse, rarely acute at apex; stem leaves (5-)10–15 (-20) × 1–2 (-3) mm. *Inflorescence* 2–3 (-5) cm, pale bluish or violet, terminal raceme, rarely branched, sometimes comose at early flowering stage, with 5–15 (-30) flowers. *Pedicels* 2–2.5 mm, glabrous. *Bracts* shorter than pedicels, deciduous with distinct scar, leaf-like, deciduous, glabrous, lanceolate, 1.5–2 × 0.2–0.5 mm; *bracteoles* one pair, deciduous, lanceolate, 1–1.5 × 0.2–0.3 mm. *Flowers* 6–8 (-10) mm, pale blue to violet, rarely pinkish, patent to deflexed. *Sepals* 5, persistent, glabrous or rarely hairy on margin and veins; *outer sepals* 3, two of them (*upper sepals*) similar to each other, unequal, 3–4 × 0.3–0.6 mm, lanceolate or narrowly elliptic, carinate, acute; *lower sepal* scarcely gibbous, ovate to lanceolate, acute at apex, 4–5 × 0.7–1.2 mm; *wings (inner sepals)* 2, petaloid, ovate, pinkish, pale blue-violet, rarely pinkish, greenish in dry; 8–10 × 4–5 mm, longer than capsule, elliptic, glabrous, rarely ciliate at margin and veins, inner two veins mostly anastomosing to midvein apex obtuse. *Petals* 3, deciduous, bluish to violet, rarely pinkish; *keel (lower petal)* (2-) 4–5 mm, concave, crest fringed; *wings (upper petals)* (2-) 4–6 × 3–4 mm, elliptic. *Stamens* 8, concealed by concave part of keel; *filaments sheath* glabrous, adnate to upper of corolla; anthers distinctly stipitate, 0.2–0.3 mm, opening by an oblique pore at apex. Ovary 2–3 × 2–2.5 mm. *Capsule* sessile, obovate, emarginated at apex, 5–6 × 3.5–4 mm,

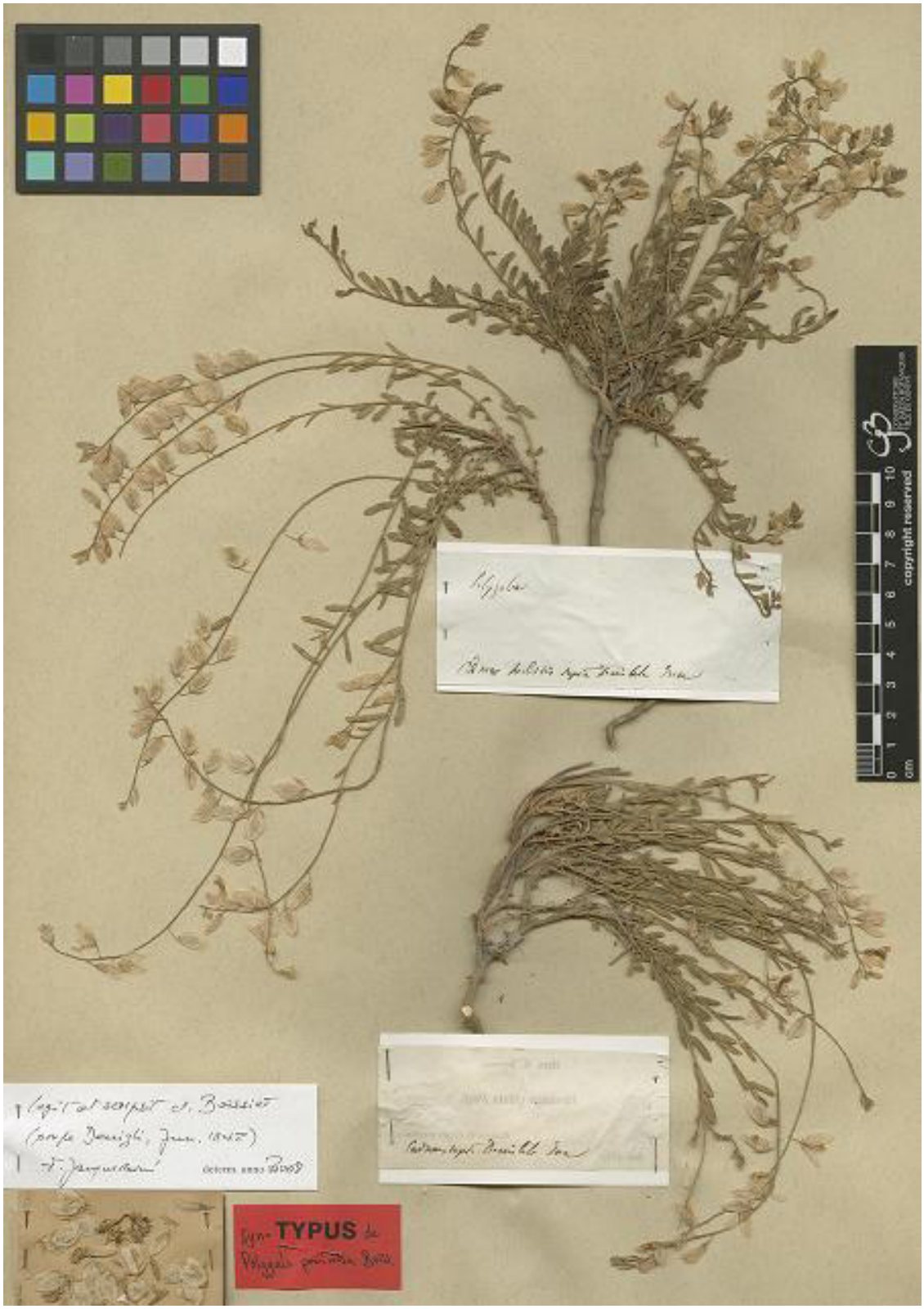

Figure 5. Lectotype of *Polygala pruinosa*.

wings 0.5–3 mm width. *Seeds* oblong-elliptic, 3–4 × 1.3–1.6 mm, with long stiff hairs; *caruncle* 1.5–1.9 mm, vento-laterally elongated.

4. Discussion

Due to ongoing revisionary research on the Turkish *Polygala* taxa, numerous samples collected from field and herbarium materials have been examined. The specimens from the discovered marshy habitat, evaluated as a new species based on the available data presented here, are described under the name *P. seyfegoluensis*. Morphological comparisons of the new species with closely related taxa, *P. pruinosa* and *P. supina*, have been conducted using diagnostic characters (Table 2).

While the specimens are superficially similar to *P. supina*, the altitude and marshy habitat are not typical for this species. Specimens of *P. supina* are usually found in high mountain meadows, close to springs, and in dry forest areas, but not in marshy habitats.

The prostrate habit of *P. supina* is one of its characteristic features, while the rest of the *Polygala* taxa in Türkiye and neighboring countries are ascending or erect. One of the close relatives of the new species, *P. pruinosa*, is widely branched at the base with thick rootstocks in dry habitats. Some individuals of the species sporadically grow long creeping branches in habitats with well-drained soils and pastures. However, this kind of life form is rare and not common in the native areas. *Polygala seyfegoluensis* has a prostrate habit in its native area and is similar to *P. supina* in this respect.

Although the appearance varies among taxa and habitats, nearly all *Polygala* taxa have indumentum to varying degrees. Among closely related species, *P. pruinosa* is the most densely hairy, with color ranging from grey to whitish, but not green like *P. supina*. Due to small hairs or a glabrous indumentum, *P. seyfegoluensis* has a green appearance. Unlike other Turkish *Polygala* taxa, the new species has fleshy leaves, a characteristic feature observable

Table 2. Comparison of *Polygala seyfegoluensis* with *P. pruinosa* and *P. supina*.

Character	*Polygala seyfegoluensis* (AADönmez 20983)	*P. pruinosa*	*P. supina*
Habit	Prostrate	Ascending to erect	Prostrate
Indumentum	Glabrous to pilose	Moderately to densely puberulous	Glabrous to pilose
Leaf texture	Fleshy	Leafy	Leafy
Stem leaves shape	Lanceolate	Lanceolate to oblanceolate, rarely narrowly elliptic	Ovate to orbicular
Inflorescence	Branched	Simple, rarely branched	Branched
Flower length (mm)	4–5	6–8 (-10)	4–5
Inner sepals in fruit (mm)	4–5 × 3.5–4.2	8–10 × 4–5	6–8 × 3.5–5
Upper sepal base	Not saccate	Not saccate	Saccate
Corolla/inner sepal ratio	Equal or slightly longer	Shorter, rarely equal	Shorter, rarely equal
Inner sepal/mature capsule ratio	Slightly longer than capsule	Distinctly longer than capsule	Slightly longer than capsule
Capsule shape	Orbicular	Obovate	Orbicular
Capsule (mm)	4–4.2 × 3.2–3.5	5–6 × 3.5–4	4–6 × 3.5–4.5
Capsule wing length (mm)	0.1–0.3	0.5–3	0.1–0.3
Seed length (mm)	2.8–3.2 × 1.1–1.2	3–4 × 1.3–1.6	3–3.3 × 1.1–1.2

on the stem and inflorescence. Due to salinity and other ecological and genetic factors in the marshy habitat, the plants, whether halophytes or nonhalophytes, belong to various families and have fleshy leaves (Greenway and Munns, 1980; Flowers et al., 2010).

Most of the flowers of *Polygala* taxa are showy, and *P. myrtifolia* has been widely used in horticulture due to its spectacular pinkish flowers. Despite the small sizes of the flowers of Turkish *Polygala* taxa, their brilliant colors attract insects, making them important contributors to the landscape, especially when the taxa have terminal inflorescence. In terms of flower size, *P. supina* has smaller flowers, while the rest of the Turkish *Polygala* taxa usually have longer ones. The new species has also smaller flowers and the size is nearly equal to *P. supina*. Inflorescences of both species have smaller flower sizes and they are axillary. In consideration of pollination, both of the species has prostrate habit, and flowers are slightly concealed by the floral leaves. Therefore, we conclude that such kind of habitus and inflorescence will drive a new pollination syndrome with the different insect groups closely living on the soil surface. The variation of pollination based on prostrate versus aerial inflorescence needs further investigation for a precise description of the mechanisms.
The pouch of the outer upper sepal is a commonly used characteristic trait for distinguishing certain species at Eurasian floras (Cullen, 1965; McNeill, 1968; Townsend, 1980). The pouched outer upper sepal is most prominent in *P. supina* but not in the other species accepted in the pouched groups in the identification keys. They exhibit variations in the shapes, as seen in *P. vulgaris* and *P. pruinosa*. In the case of *P. seyfegoluensis*, the sepal is neither straight as is seen in *P. monspeliaca* or *P. major* nor gibbous. Hence, the character state is evaluated as "not saccate" for clearly distinguishing from one of the closely relative taxa (Table 2).

Alongside the diagnostic characters given in Table 2 for distinguishing *P. seyfegoluensis* from two closely relative species, the wing of the capsule is also narrower than *P. pruinosa.* The new species has a prostrate habit and dispersal of the capsule and seeds are not eligible for wind distribution. We can conclude that this undeveloped capsule wing is a useless structure for seed dispersal and it is not well developed during the evolutionary life history of the species.

Many of the flowering plant clades have elaborate flowers for successful pollination (Yeo, 1993; and references therein; Endress and Matthews, 2006). Among them, *Polygalaceae* has a specialized flower (Eriksen, 1993; Castro et al., 2008; De Kock et al., 2018), and secondary pollen presentation has been demonstrated in some of the taxa (Howell et al., 1993; Castro et al., 2008; Xu et al., 2022). Secondary pollen presentation has been generally accepted as a special mechanism to increase pollen transfer for elaborate flowers. Experimental studies on *Polygala vayredae* Costa have shed light on floral traits variation, legitimate pollination, nectar robbing (Castro et al., 2009), and their effects on fitness (Castro et al., 2008). In terms of floral morphology and anatomy, the androecium and gynoecium of *Polygala seyfegoluensis* has similar structures. Similarly, the style is many times longer than the ovary and twisted in accordance with the lower petal and the anthers (Figure 2B).

Martinez et al. (2022) attributed evolutionary significance to the style types of New World Clade (NWC) *Polygala* taxa, whether having a superior appendage or the ancestral taxa having the appendage. In terms of the appendage, the absence of it in *P seyfegoluensis* suggests that it is a recently evolved species. Besides this, none of the *Polygala* taxa, including *P. pruinosa* naturally growing in the SW Asian area, has the appendage. In consideration of the appendage, Martinez et al. (2022) have not found a significant relationship among the NWC *Polygala* taxa in terms of the distribution pattern of geniculate and straight styles. On the other hand, the geniculate style type has also historically been used to define certain taxa at various taxonomic ranks. Martinez et al. (2022) focused on the style type, and they accepted that the geniculate style within *Polygala* sect. *Clinclinia* has the ancestral style, which was likely geniculate and later evolved into the straight form in some of the other taxa.

In terms of style shape, the *Polygala* taxa naturally growing in the SW Asian area do not exhibit a geniculate versus straight style type. The style shape of the SW Asian taxa is long and curved inside the slightly connoted upper petals. To understand the evolutionary significance of the style shape, further field observations focused on functional adaptation in terms of breeding biology are necessary, rather than relying on various evolutionary assumptions.

The stigma of *Polygala seyfegoluensis* is elongated, and the upper part is sterile, serving as the secondary pollen presentation (Figure 2B). However, dissection of the tightly packaged flowers showed that the stigma is surrounded by the sticky pollen of the closely attached anthers of the same flowers. Moreover, based on our field observations on *Polygala* taxa, there is no specified insect for pollination. Justifying the breeding strategy of the specialized and small-flowered *Polygala* taxa is challenging. Further field observation and experimental studies will enlighten the pollination strategy of the taxa.

Seeds of *Polygala* taxa have an elaiosome (Figure 2H), and the main function of the elaiosome is to feed the embryo during germination. The elaiosome is also food for small insects, like ants, and helps to distribute the seed. However, functional studies indicate that elaiosomes have other roles, acting to facilitate hydration by absorbing

water from the soil and transferring it to the rest of the seed during germination, a property likely to be important in dry environments (Bond and Slingsby, 1983).

Several phylogenetic studies have been conducted to resolve relationships at a generic level and to infer the delimitation of species complex in *Polygala* (Persson, 2001; Lyskov et al., 2019; Pastore et al., 2019; Martinez et al., 2022). Among them, the species delimitation of *P. major* was evaluated by Lyskov et al. (2019) using molecular and morphological datasets, and some *Polygala* taxa were synonymized under *P. major*. The phylogenetic placement of *P. major* and *P. anatolica* in the current study is consistent with the findings of Lyskov et al. (2019). All *P. major* specimens are nested together with *P. anatolica* with strong support (1/83). Moreover, one of the *P. major* specimens (MK108141), collected from Ukraine, is placed with *P. anatolica*, collected from Türkiye, in the same clade with strong support (1/100). Our result clearly supports the taxonomic treatment and is congruent with the synonym of *P. anatolica* based on molecular data. The phylogenetic tree presented here shows that the new species is nested as a separate taxon, but it is placed distant from two morphologically similar *Polygala* taxa, and the phylogenetic position is not resolved. The relationship between the new species and morphologically related species needs a phylogenomic approach, and further investigation is required.

In terms of the abundance of rivers and lakes, Türkiye is a rich country. Unfortunately, due to climate change and human activities, nearly all of the inland water resources are under various threats, including drainage, shrinkage, and pollution. Although Seyfe Lake is a Nature Conservation Area, it is also under threat and is surrounded by agricultural areas. Ecological changes and long-term human activities will drive the species to extinction. In the case of *Polygala seyfegoluensis*, these ecological pressures will lead to the speciation of this new species. We assume that *P. seyfegoluensis* has been generated from *P. pruinosa*, which is naturally growing just beneath Seyfe Lake (Figure 1B), and we admit that the area is a gene pool (GP) for the new species. *P. pruinosa* is a steppe plant, and some of the seeds that arrived around the lake have adapted to the new conditions. However, the transition zone between the steppe and the lake has been used by humans for agricultural purposes. While the shrunk area of the lake is used by humans, the population that is adapted to the marsh habitat has survived over time. The agricultural area, as a man-made habitat (MMH), has an isolation function for the new species from the GP (Figure 3). The isolated new population, generated from *P. pruinosa*, became a distinct species over time. Due to apomorphic characters such as prostrate habit, smaller flowers, and unwinged fruit, *Polygala seyfegoluensis* is superficially similar to *P. supina*. Alongside many new species, strict monitoring and conservation measurements should be developed according to the specific threats to their habitats.

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Supplementary Information
Additional specimens examined
***Polygala supina*:**
TÜRKİYE. Zonguldak, Eğerci, Aksu village, N41°04′51.2″, E031°45′2.9″, 2. 6. 2013, *A.A. Dönmez 8978* (HUB!); Kırklareli, Istranca Mountain, on road, 700 m, 20. 6. 1999, *A.A. Dönmez 7055 - B. Mutlu* (HUB!)*;* Bolu: Gölcük, Sünnet Lake, 13. 7. 2009, *A.A Dönmez 15917* (HUB!).
***Polygala kurdica*:**
IRAQ. 'Kurdish Hills' (locality not specified), *Mrs. A. Low 323* (BM [photo!], MSU); northern slopes of Qopi Qaradagh, *Hadac et al. 5189* (PR).
Polygala pruinosa:
A3 Bolu: Göynük, above Karaaliler village, *Pinus nigra* openings, limestone, 1650 m, N40°19′30.2″ E31° 03′00.6″, 18.07.2021, *A.A.Dönmez* 20876-*S.Yüzbaşıoğlu*. A3 Zonguldak: 10 km W of Kozlu, 5–10 m, *P.H.Davis* 37544! A3 Ankara: Nallıhan-Beypazarı road, 43 km to Nallıhan, red soil, 593 m, N44°37′40″, E36°37′133″, 6.5.2019, *A.A.Dönmez* 20358.A4 Kastamonu: Kastamonu, 900 m, *P.H.Davis* 21600! A5 Amasya: Amasya, 4–600 m, *Bornm.* 181! 2727!. A6 Tokat: Tokat, *Noë.*. B2 Kütahya: Gediz, 850 m, *P.H.Davis* 36906!B3 Eskişehir: Eskişehir: Sündiken Mt., steppe, 1000 m., T. Ekim 722 (ANK!) Eskişehir, 800 m, *Krause* 40 (ANK!). B4 Ankara: B4 Ankara: Bala, Beynam forest, 06.06.1969, Ö. *İnceoğlu* (HUB!); Beynam forest, 1200 m, *Markgraf* 11048 (ANK!). B4 Ankara: Bala, Kırşehir road, between İsmetpaşa-Büyükboyalık villages, steppe, 1179 m, N37°32′14.3″ E33°10′54″, 10.07.2021, *A.A.Dönmez* 20978-*S.Yüzbaşıoğlu*.B5 Nevşehir: Hacıbektaş, 1220 m, N38°45′07.5″, E034°21′54.5″, 26.08.2021, *A.A.Dönmez* 21147*,* 21156, 21159-*Z. Uğurlu Aydın*. B5 Kırşehir: Mucur, *P.H.Davis* 21826! B6 Erzincan: Refahiye, 15th km from Sakaltutan Pass to Refahiye, 1700 m, N44°15′688″, E37°49′884″, 26.5.2019, *A.A.Dönmez* 20418. B6 Tunceli: Pertek, 1400 m. *P. H. Davis* 29138 (ANK!, E!). B7 Erzincan: Kelkit road, across Dereyurt village, 1454 m, N44°07′403″, E37°53′530″, 01.06.2019, *Ali A. Dönmez* 20423-*Z. Uğurlu & X.Wang*; Erzincan: Kesiş Dağ, 2500 m., *P. H. Davis & Hedge* 31692 (ANK!, E!); Kemaliye, between Yaka-Yeşilyamaç villages, 850 m., 02.05.1980 *Ş. Yıldırımlı* 2023 (HUB!): Kemah, Uluçınar village, 1500 m., 28.05.1979, *Ş. Yıldırımlı* 1677 (HUB!). B7 Elazığ: from Elazığ to Pertek, 1000 m, *P.H.Davis* 29170 (ANK!, E!). B7 Adıyaman: Between Kuyulu-Akpınar villages, marly slopes, 600 m, N37°31′15.9″, E38°13′13″, 14.06.2021, *A.A.Dönmez* 20844-*E.O.Karahan.* C2 Denizli: Çal, from Çal to Çivril, 1 km from Haşatbeli Pass to Çivril, 978 m, 18.04.2019, *A.A.Dönmez* 20301-*K. Şenova*. C2 Antalya: Elmali, *Bourgeau* 106!. C3 Burdur: Bucak, 1000 m, *Little* 208!. C4 Karaman: Hotamış, between Hotamış-Akşehir, marly slopes, 1006 m, N37° 33′04.6″, E033°27′06.1″, 10.05.2021, *A.A.Dönmez* 20818-*E. O. Karahan*: Ayrancı-Erdemli road, 20 km from Ayrancı Dam to Kavaközü village, marly slopes, 1550 m, N37°10′59.5″, E33° 44′31.3″, 14.06.2021, *A.A.Dönmez* 20821-*E. O. Karahan*: Ermenek road, around Akın village, 1160 m, N36° 53′44.5″, E033°01′00.5″, 14.06.2021, *A.A.Dönmez* 20827-*E. O. Karahan*.: Karamanbeli Pass, above marbel area, limestone, 1903 m, N36°49′28″, E32° 57′01″, 13.07.2021, *A. A. Dönmez* 21011-*S. Yüzbaşıoğlu*: above Kayaönü village, marly soil, macquies, 1660 m, N36° 38′21″, E32°57′30″, 13.07.2021, *A.A.Dönmez* 21015, 21016-*S.Yüzbaşıoğlu*: around Çevrekavak village, 1624 m, N36°39′08.6″, E32°38′17.5″, 04.07.2021, *A.A.Dönmez* 20967-*E.O.Karahan.* C4 Antalya: Antalya: Elmalı, Çığlıkara, Kabakçılar district, 1750 m., R. Çetik 1882 (ANK!); Ovacik and Söğüt Cumasi, 1100–1300 m, *P.H.Davis* 15232 (ANK!, E!). C5 Adana: Korsantı, Söğüt road, Terrarosa, 900 m., *Ender Yurdakulol* 1759 (ANK!): Seyhan, Burujik (Bürücek), 1000 m, *Balls* 1246 (E!). C6 Maraş: 25 km W of Fevzipaşa, 750 m, *It. Leyd.* 1959: 1352!. C8 Mardin: Haylak (Halak), *Chiovenda.* Mentioned syntypes (Boissier)Thessaliae, Aucher pl. exs. No: 909, Lydiae et Cariae regione montana in dumosis Ego; in Armenia. Aucher pl. exs. No: 4202. The specimens mentioned in the protologue: Tokat et Kastamonu, Safranbolu, E. Wiedemann, 99 (G!).; Lydiae et Cariae regione montana in dumosis Ego; in Armenia. Location not provided. Türkiye: Village de Gulek-Boghas, au Nord de Tarsous, 1855, Balansa, B. 417, (Barcode: G00150151).