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Aethionema aytachii (Brassicaceae): A new species from central Anatolia, Turkey

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Abstract: *Aethionema aytachii* Ertuğrul & Hamzaoğlu, a new species from central Anatolia that grows on marly hills in the Ayaş district of Ankara Province (Turkey), is described and its relationships and distinguishing characters from the closest relative *A. dumanii* are discussed. The shape of pollen grains of *A. aytachii* is tricolpate, and its seed-coat sculpture is verrucate. Sequence data of the internal transcribed spacer region (ITS) of the new species was used to determine about its phylogenetic relation within *Aethionema*.

Key words: Cruciferae, internal transcribed spacer region (ITS), phylogeny, pollen and seed micromorphology

1. Introduction

Brassicaceae is a large family of some 345 genera and 4020 species (Al-Shehbaz compilation) distributed on all continents except Antarctica. It is centered primarily in the temperate areas, especially in the Mediterranean basin and in south-western and central Asia (Kandemir et al., 2017).

Aethionema W.T.Aiton is a taxonomically complex genus of some 57 species; the center of its greatest diversity is Turkey and less so in neighboring countries (Iran, Caucasian republics, Greece), but with individual species distributed eastward as far as Kazakhstan and westward into Spain and Morocco (Hedge, 1965; Moazzeni et al., 2016; authors' compilation). The genus is sister to the rest of the family and was placed in a unigeneric tribe Aethionemeae (Al-Shehbaz, 2012). The source of its complexity is the presence of few macro morphological characters (e.g., fruit and leaf characters) that can be used in the delimitation of species. *Aethionema* was previously known to be represented in Turkey by 40 species (Ertuğrul, 2012), but several new species have since been described, and it is currently estimated to include as many as 53 species in the country (Karabacak et al, 2013; Yıldırımlı and Kılıç, 2016; Kandemir et al., 2017; Yıldırımlı and Kılıç, 2019).

During ongoing systematic and phylogenetic studies on the genus by one of us (K.E.), independent extensive fieldwork by the first two authors resulted in the collection of numerous samples of many species. Among these were some specimens that did not belong to any of the known species. As a result of comprehensive studies, it was concluded that these represent a new species hereafter recognized as *A. aytachii* Ertuğrul & Hamzaoğlu.

2. Materials and methods

Some *Aethionema* specimens were collected from Aysantı Pass, in the Ayaş district of Ankara Province, by the first and second authors in 2019. These were compared against the treatments of the genus in the *Flora of Turkey and the East Aegean Islands* (Hedge, 1965; Davis et al., 1988; Adıgüzel 2000) and other related floras and checklists (e.g., Chaytor & Aktyroyd, 1993; Hedge, 1968; Busch, 1939; Ertuğrul, 2012) and the recently described new species (Yıldırımlı and Kılıç, 2016, 2018, 2019), as well as the study of *Aethionema* collections in the herbaria ANK, E, G, GAZI, HUB, K, and KNYA (acronyms follow Thiers, 2021). Our specimens were critically compared with *A. armenum* Boiss. And *A. dumanii* Vural & Adıgüzel, the two species that appeared most closely related to it.

For molecular phylogenetic studies, we used silica gel dried leaves collected from the type localities of *A. aytachii*, *A. dumanii*, *A. turcicum* H.Duman & Aytaç, *A. grandiflorum* Boiss. & Hohen, and *A. armenum*. Total genomic DNA extraction followed the 2X CTAB method of Doyle & Doyle (1987) as modified in Soltis et al. (1991) and Cullings (1992). Sequencing and amplification of both DNA strands was performed using ITS1 and ITS4 primers (White et al., 1990). Direct sequencing of amplified DNA was performed using the Big Dye Terminator Cycle Sequencing v.3.1 (Macrogen, Netherlands) software program, following the manufacturer instructions. The complete ITS gene sequences of nine *Aethionema* taxa and two *Noccaea* Moench. Species (as the out-group) were used. Sequences of *A. armenum* and the out-group were taken from GenBank (National Center for Biotechnology Information), but all other samples in this study are new (Table 1). Editing of the nucleotide sequences and visual alignments were performed using Bioedit v.7.0.5.3 (Hall, 1999). Parsimony analysis was conducted using PAUP v.4.0b10 (Swofford, 2002). Bootstrap (BS) analyses (Felsenstein, 1985) were conducted with 1000 replicates of the heuristic search using the default options. For the strict consensus tree, the retention index (RI) and consistency index (CI) were given, with the exclusion of the uninformative characters. We used MrBayes 3.2 (Ronquist et al., 2012) to perform the Bayesian

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Table 1. Voucher specimens for the ITS study.

Taxa	Collection number	GenBank	Author, year
Aethionema aytachii	*K.Ertuğrul 5757*	MW791188	Uysal et al., 2021
A. turcicum	*K.Ertuğrul 5754*	MW791189	Uysal et al., 2021
A. dumanii	*K.Ertuğrul 5755*	MW791190	Uysal et al., 2021
A. armenum	*K.Ertuğrul 5756*	MW791191	Uysal et al., 2021
A. armenum	*T.Uysal 4096*	MW791192	Uysal et al., 2021
A. grandiflorum	*K.Ertuğrul 5815*	MW791193	Uysal et al., 2021
A. armenum		MT799720	Bozkurt et al., 2020
Noccaea iberidea		MN871751	Özüdoğru et al., 2019
N. oppositifolia		MG944851	Özüdoğru et al., 2018

phylogenetic analyses. In the Bayesian analyses, random starting trees were used, which were run for 1×10^5 generations, comprising 2 independent runs that consisted of four metropolis-coupled chains. Tracer v.1.5.0 software was used to analyze the trace files created by the Bayesian Markov chain Monte Carlo studies (Rambaut and Drummond, 2007) and, after checking them for convergence, the first 1000 samples (20%) were discarded as burn-in. FigTree v1.4.0 software (http://tree.bio.ed.ac.uk/software/figtree/) was used as the graphic viewer of the phylogenetic tree.

Pollen was obtained from herbarium specimens and prepared following Wodehouse (1935). The pollen slides were observed using a Leica DM 1000 light microscope (LM) (Leica Microsystems, Wetzlar, Germany), and measured using Kameram 21 software (Argenit, Istanbul, Turkey). The measurements were based on at least 30 or more pollen grains from each specimen. The seeds were first investigated using a Leica Z6 Apo 16 stereoscopic microscope, and at least 15 mature seeds were measured. For the scanning electron microscopy (SEM) analyses, mature seeds or dried non-acetolysed pollen were placed directly onto aluminium stubs and coated with gold using a sputter-coater. They were photographed using a Zeiss Evo LS 10 SEM (Carl Zeiss NTS GmbH, Oberkochen, Germany). For pollen and seed terminology, Punt et al. (2007) and Pınar et al. (2007) were followed, respectively.

3. Results

3.1 *Aethionema aytachii* Ertuğrul & Hamzaoğlu sp. Nov.

Plants and dehiscent fruit of *Aethionema aytachii* and indehiscent fruit of *A. aytachii* are shown in Figures 1 and 2A, 2B.

Type: TURKEY. B4 Ankara: Ayaş, around Aysantı Pass, marly hills along roadsides, 1190 m, 31.v.2019, *K.Ertuğrul* 5757 & *T.Körüklü* (Holotype KNYA; Isotypes GAZI, ANK).

Paratypes: TURKEY. B4 Ankara: Ayaş, Aysantı Pass, marly hills on roadside, 1190–1250 m, 14.vi.2019, *H.Demirelma* 3371 (KNYA); ibid.18.v.2019, *E.Hamzaoğlu* 7549 (KNYA); ibid. 1.viii.1985, Z. Aytaç 1967 (GAZI).

Diagnosis: *Aethionema aytachii* resembles *A. dumanii* in having densely flowered racemes that elongate in fruit, and from which it differs by the densely (vs. loosely) overlapping stem leaves, heterocarpic (vs. homocarpic) fruits, inner filaments 2–2.5 (vs. ca. 1.7) mm long, fruiting pedicels 1.5–3.5 (vs. 6–7) mm long, and styles exerted from (vs. included in) the apical fruit sinus. From *A. armenum*, *A. aytachii* differs by its densely (vs. loosely) overlapping stem leaves, petals 5.8–7 (vs. 4–4.2) mm long, heterocarpic (vs. homocarpic) fruits, inner filaments dilated (vs. slender) at base, and style clearly exceeding (vs. equalling or shorter) than the apical fruit sinus (Table 2).

Description: Perennial, stem ascending, 3–9 cm tall, branched. Leaves alternate, falcate, margins involute, sessile, rounded at base, subapiculate or acute at apex; lowermost leaves ovate-oblong, 4–7 × 0.5–1.5 mm; stem leaves oblong to narrowly so, 4–6 × 1–2 mm. Raceme 10–20-flowered, compact, elongated in fruit. Pedicel 1.5–2.1 mm long in flowers, 1.5–3.5 mm long in fruit, erect at base, sometimes recurved distally. Sepals saccate, green with a white scarious margin, 2–2.5 × 0.8–1.5 mm. Petals 5.8–7 × 1.5–2.5 mm, pink, 3-veined at base, claw not distinct. Inner (median) filaments free, dilated at base, 2–2.5 mm long, outer (lateral) filaments 1.5–1.8 mm long; anthers triangular to oblong, 0.5–0.6 mm long, apex obtuse in inner filaments, acute in outer ones. Fruit lax, cordate at base, heterocarpic; indehiscent fruit orbicular, 4–5 × 5–5.5 mm, unilocular, 1-ovuled, septum 3–4 × 1–1.5 mm, wings 2–2.1 mm wide, irregularly crenate–dentate, sinus 0.5–1 mm deep, style 1–1.5 mm long; dehiscent fruit obovate, 6.5–7.1 × 5–5.1 mm, bilocular, 1- or 2- ovuled per locule, septum 4–5.5 × 1.5–2 mm, wings 1.5–2.1 mm wide, undulate along margins, sinus ca. 1 mm deep, style 0.5–1 mm long. Seeds (2–) 3 (–4), ovate, light-brown, 1.71–1.31 × 0.70–0.86 mm in indehiscent fruits, 1.3–1.4 × 1.3–1.4 mm in dehiscent fruits.

3.2. Etymology

The species was dedicated to Prof. Dr. Zeki AYTAÇ (25.01.1956), a Turkish botanist who has provided many contributions to plant taxonomy. The Turkish name of this new species was suggested as 'Ayaşkayagülü' (Menemen et al., 2016).

3.3. Molecular analyses and results

Seven accessions of closely related *Aethionema* species and two out-group *Noccaea* species were used for phylogenetic comparison and reconstruction. The total

Figure 1. Plants of *Aethionema aytachii*.

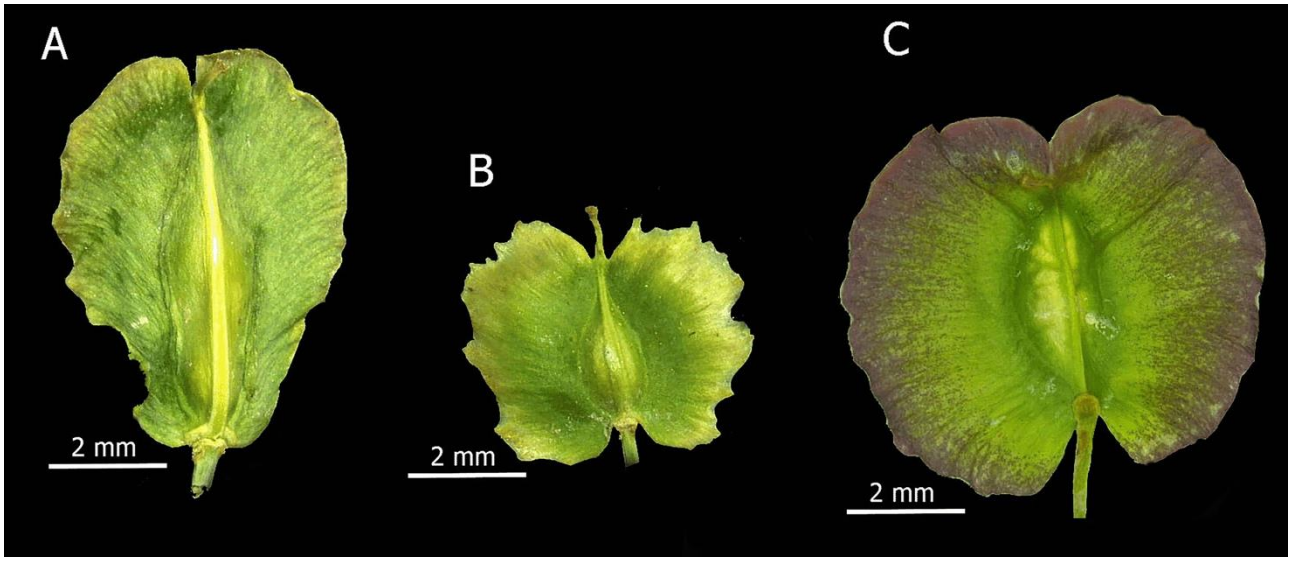

Figure 2. A. Dehiscent fruit of *Aethionema aytachii*, B. indehiscent fruit of *A. aytachii*, and C. Fruit of *A. dumanii*.

length of studied DNA segments was 601 bp, 123 of which were parsimony informative. The topologies obtained from both the parsimony and Bayesian inference analyses were identical, and the combined tree is shown in Figure 3. The constructed tree shows higher resolution in the Bayesian than parsimony values. The *Aethionema* taxa grouped into 3 main clades in the concatenated tree (PP: 1, BS: 100; Figure 3). The first clade comprised *A. aytachii* sister to *A. dumanii* and together sister to *A. turcicum*. The second clade included three different populations of *A. armenum*. The third clade was *A. grandiflorum*. Clearly, *A. aytachii* is closest to *A. dumanii* and *A. turcicum* than to *A. armenum*.

3.4. Pollen morphology

The pollen grains of *Aethionema aytachii*, *A. armenum*, and *A. dumanii* were radially symmetrical, isopolar, and tricolpate, as in about 97% of the Brassicaceae. However, the pollen grains of *A. aytachii* were sometimes (4%) syncolpate. Pollen shape was oblate in *A. aytachii* and *A. armenum* and subprolate in *A. dumanii*. The pollen size showed some differences among three taxa in the polar (P) and equatorial (E) views. In *A. aytachii*, it was P: 12.95 ± 1.16 µm, E: 19.31 ± 1.15 µm, while in *A. armenum,* it was P: 11.24 ± 0.76 µm, E: 16.51 ± 2.44 µm, and in *A. dumanii* P: 16.78 ± 1.56 µm, E: 13.71 ± 1.24 µm. The outline of the pollen grains was elliptic in equatorial view and triangular

Table 2. Morphological comparison of *Aethionema aytachii*, *A. dumanii*, and *A. armenum*.

Species/characters	*A. aytachii*	*A. dumanii* (Vural and Adıgüzel, 1995)	*A. armenum* (Hedge, 1965)
Stem	Ascending	Erect-ascending	Erect-ascending
Stem (cm)	3–9.5	10–20	6–21
Stem leaves	Densely spread	Loosely spread	Loosely spread
Leaves shape	Oblong-ovate to narrowly oblong	Oblong-linear	Oblong-linear
Petals	Pink, 5.8–7 × 1.5–2.5 mm	Pink, ca. 6 × 2.5 mm	Pink or white, 4–4.2 × 1.3–2 mm
Fruits	Heterocarpic	Homocarpic	Homocarpic
Inner filaments	Dilated at base, 2–2.5 mm	Dilated at base, ca. 1.7 mm	Not dilated at base, 1.5–2 mm
Fruiting pedicels	Erect, rarely recurved, 1,5–3.5 mm	Erect, (5–) 6–7- (–8) mm	Erect to recurved, 3–4.8 mm
Siliculae	Indehiscent fruits orbicular, 4–5 × 5–5.5 mm, wings 2–2.1 mm and irregularly crenate–dentate, sinus 1 mm, style 0.5–1 mm long; dehiscent fruits obovate, 6.5–7.1 × 5–5.1 mm, wings 1.5–2.1 mm and undulate, sinus 0.5-1 mm, style 1–1.5 mm long	Orbicular, 6–7.5 (–9) × 7–9, wings 3–4 mm and undulate, irregular crenate–dentate, sinus 1.5–2 mm, style 1.5–2 mm long	Ovate to obovate, 4–5.5 (–7) × 3.5–4 (–5), wings 1–1.5 mm and crenate or entire, sinus 0.5–1 (–1.5), style ca. 0.5 mm long
Style	Clearly exceeds sinus	As long as sinus	As long as or shorter than sinus

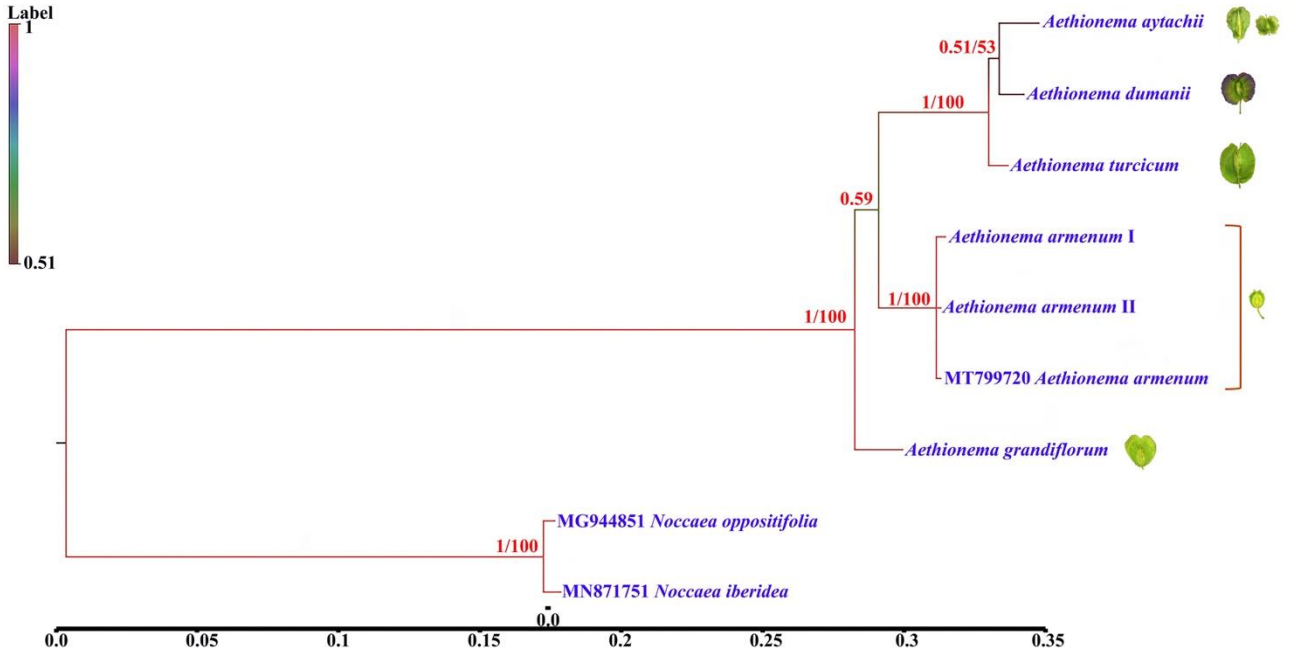

Figure 3. ITS majority rule consensus tree from Bayesian inference and Parsimony analysis, and numbers depict posterior probabilities and bootstrap values. (CI = 0.944; RI = 0.946; HI = 0.056).

in polar view. The colpus was long and sunken, margins distinct, regular, and ends ovate. The sculpture of the exine exhibited reticulate ornamentation. The muri shapes varied among the species, and that of *A. aytachii* was larger than the others (Figure 4, Table 3). Detailed pollen morphological characters of the examined species are given in Table 3.

3.5. Seed morphology

The seeds were ovate and light-brown in all species. In *Aethionema aytachii* the seed size from the indehiscent fruit was 1.17–1.31 × 0.70–0.86 mm in those from dehiscent fruit was 1.4–1.3 × 0.9–0.85 mm, while it was 1.19–1.31 × 0.64–0.90 mm in *A. armenum* and 1.23–1.52 × 0.73–0.96 mm in *A. dumanii*. The seed shape in *A. aytachii* and *A. armenum* was ovate, while those of *A. dumanii* were broadly oblong-ovate) (Table 4). The ornamentation of seeds surface in *A. aytachii* was verrucate, reticulate-verrucate in *A. armenum*, and reticulate in *A. dumanii* (Figures 5 and 6). The epidermal cells on the seeds were

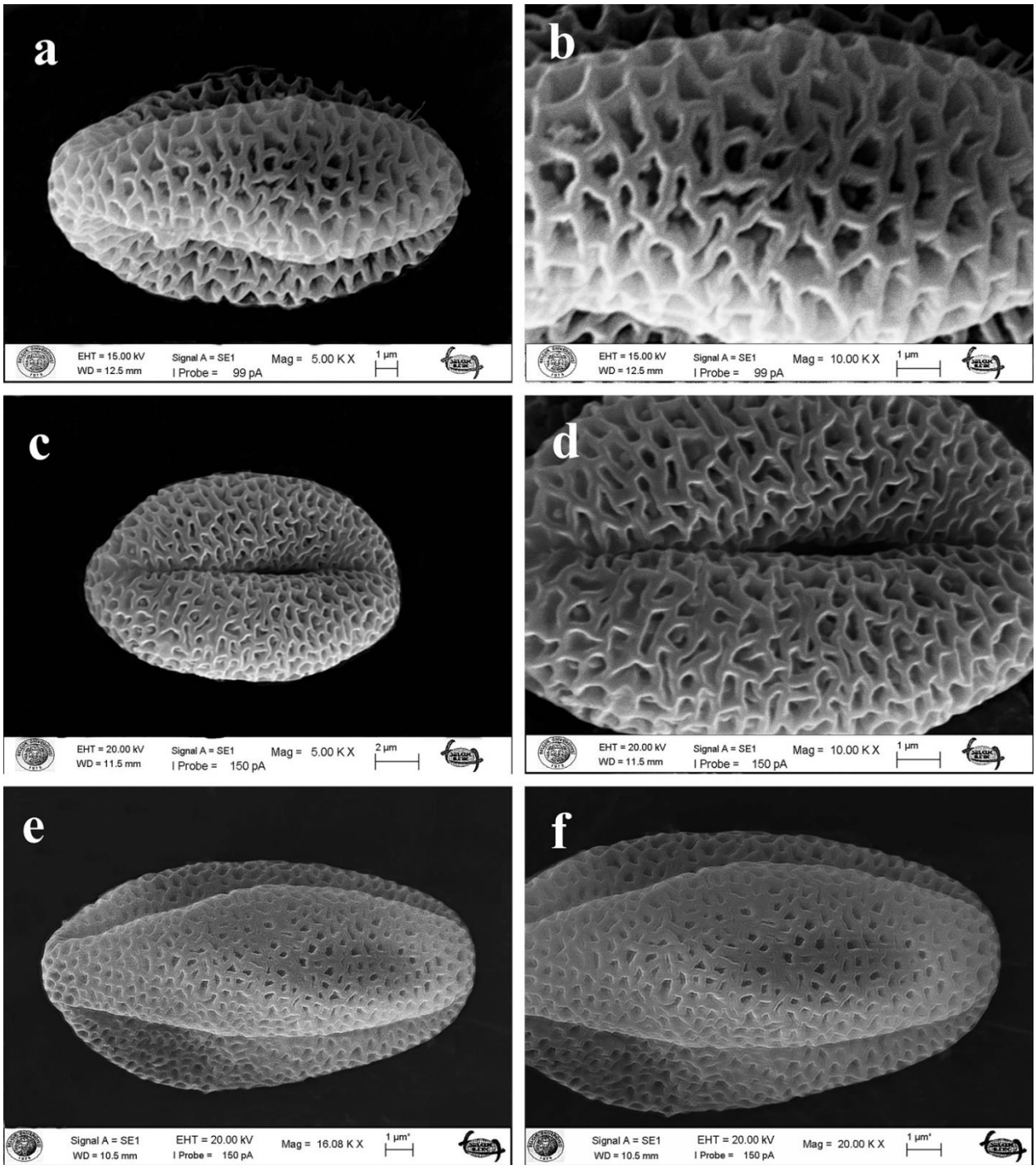

Figure 4. SEM micrographs of the pollen grains of *Aethionema aytachii* (a, b), *A. armenum* (c, d), and *A. dumanii* (e, f). a, c, e General view, and b,d,f exine sculpturing.

Table 3. Pollen morphological data of *Aethionema aytachii*, *A. armenum*, and *A. dumanii* (values in µm, mean ± standard deviation).

Species/pollen characters		*A. aytachii*	*A. armenum*	*A. dumanii*
Polar axes	Min-max	11.88–14.19	10.39–11.86	15.2–19.2
	Mean	12.95 ± 1.16	11.24 ± 0.76	16.78 ± 1.56
Equatorial axes	Min-max	18.06–20.32	14.55–19.26	11.54–15.41
	Mean	19.31 ± 1.15	16.51 ± 2.44	13.71 ± 1.24
Pollen shape		Oblate	Oblate	Subprolate
Aperture type		96% tricolpate and 4% syncolpate	Only tricolpate	Only tricolpate
Sculpture		Reticulate	Reticulate	Reticulate
Muri		1.63 ± 0.34	1.04 ± 0.19	0.59 ± 0.07
Colpus	Colpus length (Clg)	19.1 ± 0.67	15.07 ± 1.12	14.73 ± 1.04
	Colpus width (Clt)	2.62 ± 0.3	1.99 ± 0.17	1.38 ± 0.03
Exine thickness		1.08 ± 0.17	0.83 ± 0.14	0.93 ± 0.07
Intine thickness		0.63 ± 0.17	0.41 ± 0.01	0.56 ± 0.06
Apocolpium		2.01 ± 0.2	2.77 ± 0.83	2.13 ± 0.08

Table 4. Seed morphological data of *Aethionema aytachii*, *A. armenum*, and *A. dumanii* (values in mm).

Species/seed characters	*A. aytachii* (indehiscent fruit)	*A. aytachii* (dehiscent fruit)	*A. armenum*	*A. dumanii*
Seed length	1.17–1.31	1.3–1.4	1.19–1.31	1.23–1.52
Seed width	0.70–0.86	0.85–0.9	0.64–0.90	0.73–0.96
Seed shape	ovate	ovate	ovate	Broadly ovate-oblong
Seed color	Light brown	Light brown	Light brown	Light brown
Seed surface	Verrucate	Verrucate	Reticulate-verrucate	Reticulate

oval in shape, with striate ornamentation in *A. aytachii* and *A. armenum*.

3.6. Distribution, habitat, and ecology

Aethionema aytachii grows on marly hills around Aysantı Pass in the Ayaş district of Ankara Province at altitudes of 1190–1250 m, and it is associated with *A. dumanii*, *A. turcicum*, *Astragalus densifolius* Torr. subsp. *ayashensis* Aytaç & Ekim, and *Campanula damboldtiana* P.H.Davis & Sorger.

This region is one of the well-conserved marly steppe areas near Ankara, and it is part of the Irano-Turanian floristic region (Figure 7).

3.7. Conservation status

Aethionema aytachii is a locally endemic species and is known only from its type locality (Figure 7). The species is rare in the field, and its extent of occurrence (EOO) and area of occupancy (AOO) are less than 1 km^2. Due to agricultural activities, such as hobby gardening and road construction in this area, the new species is considered as "critically endangered" CR B1ab(I,ii) + 2ab(I,ii) (IUCN, 2017).

4. Discussion

Aethionema aytachii most closely resembles *A. dumanii* in having subapiculate or acute leaves, sepal size, pink petals, and dilation at the base of the inner filaments, but it differs by having dense stem leaves, heterocarpic fruit (Figure 2), fruiting pedicel measurements, and style/sinus ratio. According to Pınar et al. (2007), the four seed-ornamentation types in *Aethionema* are reticulate, ruminate, reticulate-verrucate, and verrucate. Using this seed-sculpture terminology, the seeds of *A. aytachii* are verrucate, and they are contrast reticulate-verrucate in *A. armenum* and reticulate of *A. dumanii*. The palynological data showed rather minor differences that need not be emphasized for the separation of these three species.

Mohammedin et al. (2017) showed some correlation between morphological characters (e.g., ovate vs. linear leaf shape, fruit type dehiscent vs. heterocarpic, presence vs. absence of spines, and plant duration annual vs. perennial) and molecular database on plastome coding regions and nuclear rDNA genes in the genus *Aethionema*. Their data showed that *A. dumanii* and *A. turcicum* fell in the same clade, and our results fully support that. According to the literature, it has been emphasized that in several species of *Aethionema*, both dehiscent and indehiscent fruits are developed (Appel and Al-Shehbaz, 2003), and heteromorphism is of independent origin (Lenser et al., 2016; Mohammedin et al., 2017). They speculated that there is a correlation between the annual habit and heterocarpy, but such hypothesis needs further testing because heterocarpy is found in four perennial species, including *A. aytachii*, *A. thomasianum* J.Gay (Italy, Spain), *A. rhodopaeum* D.Pavlova (Bulgaria), and the widespread *A. saxatile* (L.) W.T.Aiton (Turkey westward into S, C, and SW Europe and NW Africa). From the last

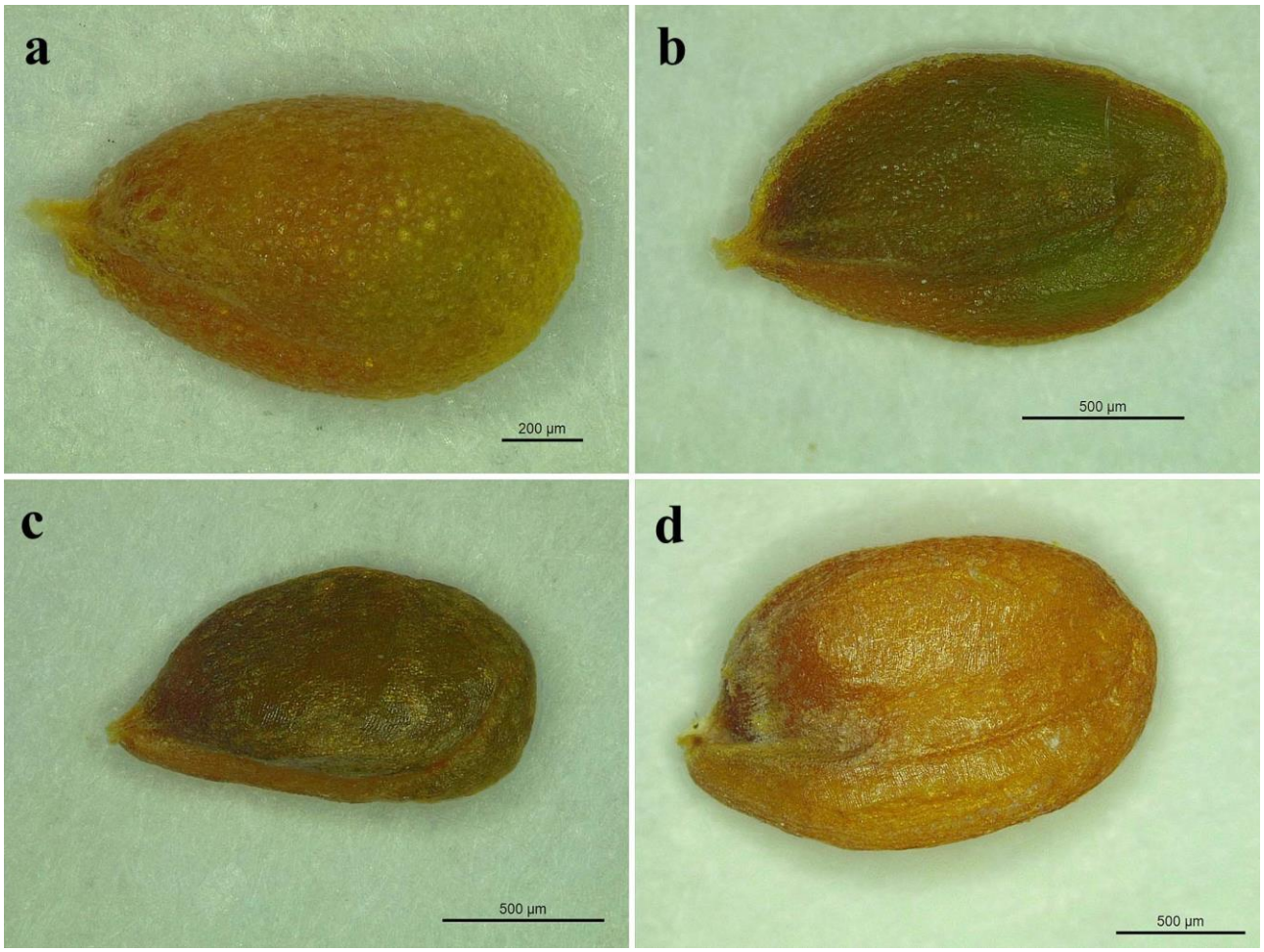

Figure 5. LM micrographs of the *Aethionema* species a. *A. aytachii* (seeds of indehiscent fruit), b. *A. aytachii* (seeds of dehiscent fruit), c. *A. armenum*, and d. *A. dumanii*.

three species, *A. aytachii* is readily distinguished by having much narrower leaves with length/width ratio of at least 4–6:1 (vs. 1–2.5:1). Heterocarpy was suggested to be a conservation strategy against risks arising from environmental conditions because the production of different morphs gives the plants some flexibility in response to environmental stimuli (Imbert, 2002; Lenser et al., 2016; Bhattacharya et al., 2019).

In conclusion, our study demonstrates that *Aethionema aytachii* is most closely related to *A. dumanii* from which it is readily distinguished by the production of heterocarpic vs. homocarpic fruits.

Examined specimens are as follows.

Aethionema armenum: Ayaş, around Aysantı Pass, marly hills to the right and left of the road, 1190 m, 31.v.2019, *K. Ertuğrul* 5756 & *T. Körüklü* (KNYA), Aysantı Pass, marly hills to the right of the road, 1190–1250 m, 14.vi.2019, *H. Demirelma* 3373, 3374 (KNYA).

Aethionema dumanii: Ayaş, around Aysantı Pass, marly hills to the right and left of the road, 1190 m, 31.v.2019, *K. Ertuğrul* 5755 (KNYA) Aysantı Pass, marly hills to the right of the road, 1190–1250 m, 14.vi.2019, *H. Demirelma* 3370 (KNYA), Eskişehir, Polatlı to Sivrihisar, 25. Km, 870 m, 10.vii.1993, *H. Duman* 5011 (holo. GAZI! Iso. ANK!)

Acknowledgments

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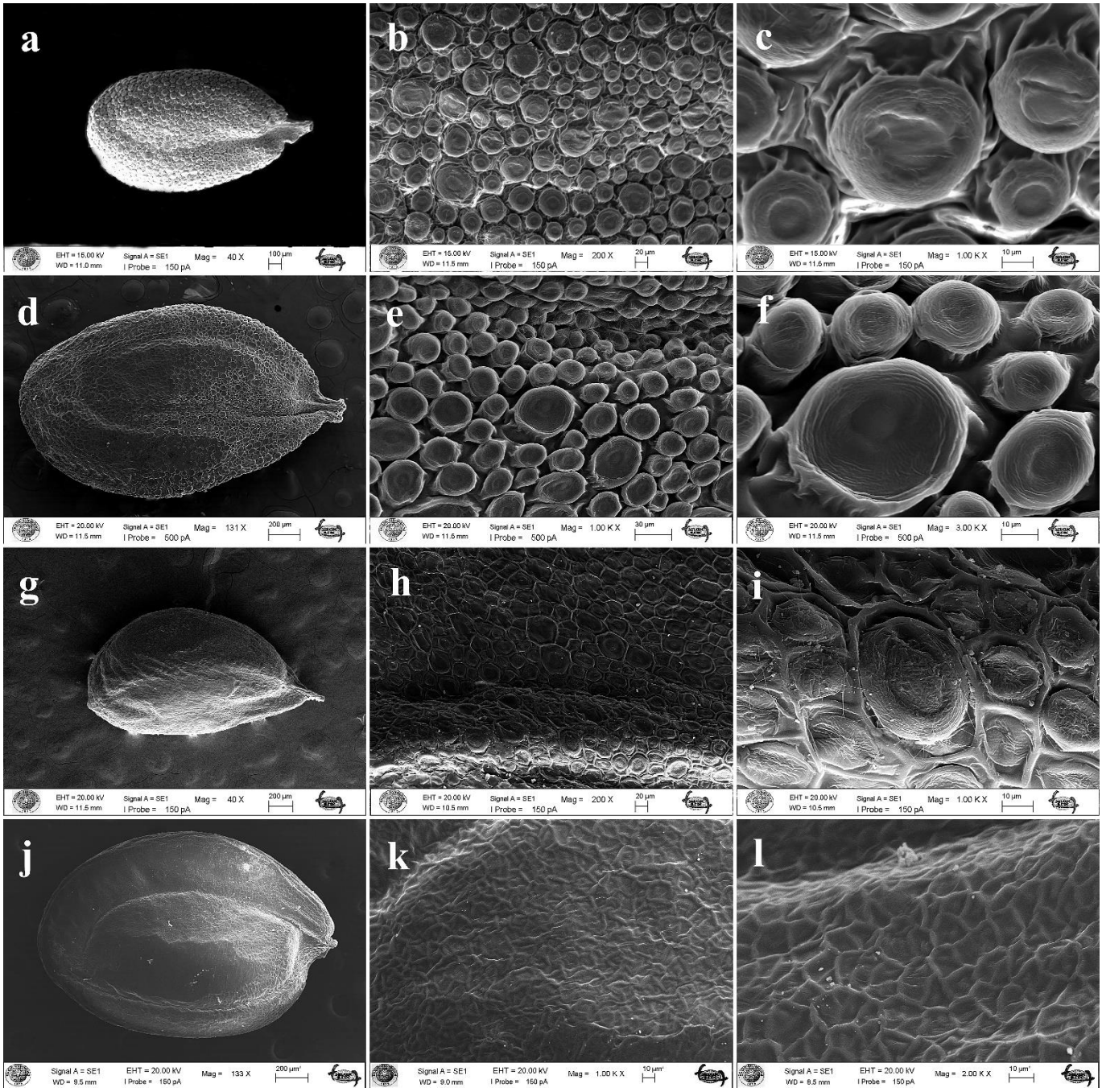

Figure 6. SEM micrographs of the seeds of *Aethionema aytachii* (indehiscent fruit) (a–c), *A. aytachii* (dehiscent fruit) (d–f), *A. armenum* (g–i), and *A. dumanii* (j–l). a, d, g, and j. General view, b, c, e, f, h, i, k, and l. Surface ornamentation.

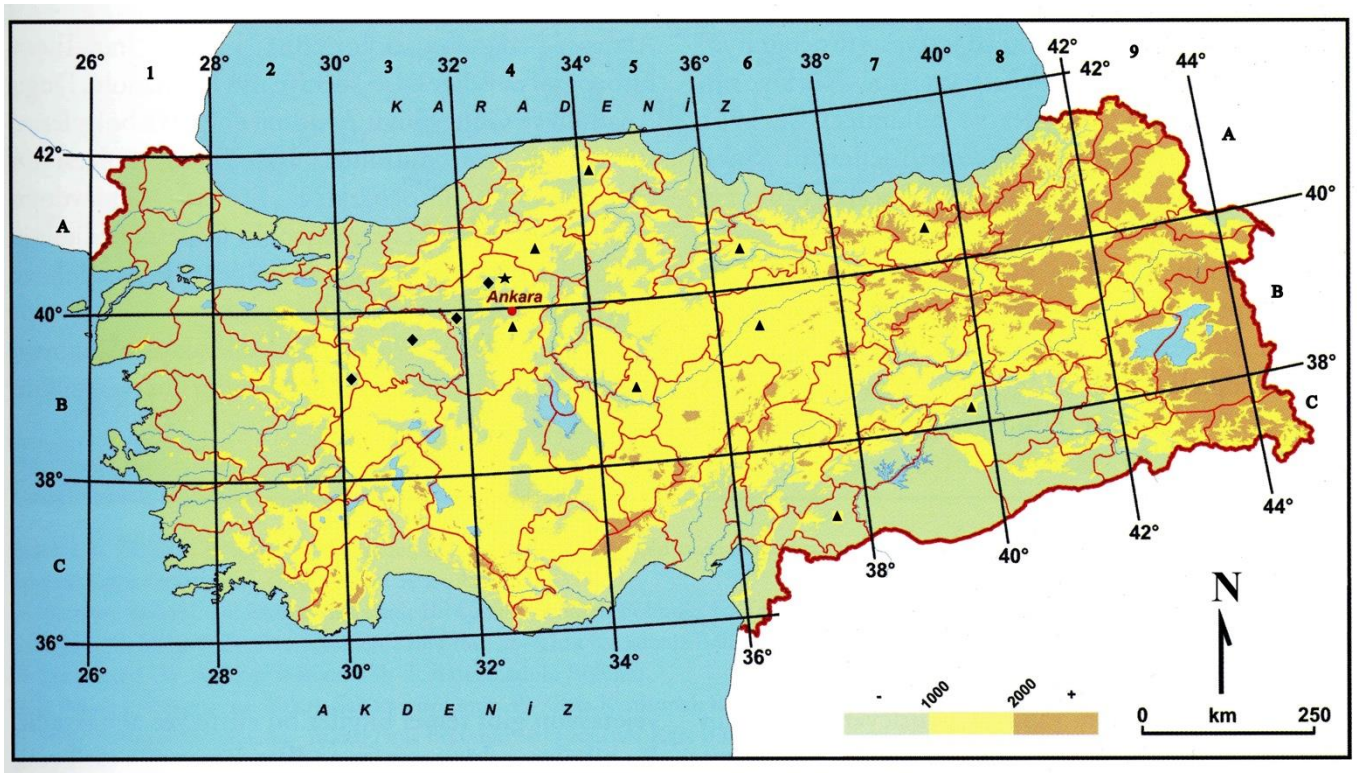

Figure 7. Distribution map of *Aethionema dumanii* (♦), *A. armenum* (▲), and *A. aytachii* (★).

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