

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

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12
13 **Abstract:** *Aethionema aytachii* Ertuğrul & Hamzaoglu, a new species from central
14 Anatolia that grows on marly hills in the Ayaş district of Ankara Province (Turkey), is
15 described and its relationships and distinguishing characters from the closest relative *A.*
16 *dumanii* are discussed. The shape of pollen grains of *A. aytachii* is tricolpate, and its seed-
17 coat sculpture is verrucate. Sequence data of the internal transcribed spacer region (ITS)
18 of the new species was used to determine about its phylogenetic relation within
19 *Aethionema*.

20
21 **Key words:** Cruciferae, ITS, phylogeny, pollen and seed SEM

1 **1. Introduction**

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

6 *Aethionema* W.T.Aiton is a taxonomically complex genus of some 57 species the center
7 of its greatest diversity is Turkey and less so in neighbouring countries (Iran, Caucasian
8 republics, Greece), but with individual species distributed eastward as far as Kazakhstan
9 and westward into Spain and Morocco (Hedge, 1965; Moazzeni et al., 2016; authors'
10 compilation). The genus is sister to the rest of the family and was placed in a unigeneric
11 tribe Aethionemeae (Al-Shehbaz, 2012). The source of its complexity is the presence of
12 few macro morphological characters (e.g., fruit and leaf characters) that can be used in
13 the delimitation of species. *Aethionema* was previously known to be represented in
14 Turkey by 40 species (Ertuğrul, 2012), but several new species have since been described,
15 and it is currently estimated to include as many as 53 species in the country (Karabacak
16 et al, 2013; Yıldırımli and Kılıç, 2016; Kandemir et al., 2017; Yıldırımli and Kılıç, 2019).
17 During ongoing systematic and phylogenetic studies on the genus by one of us (K.E.),
18 independent extensive fieldwork by the first two authors resulted in the collection of
19 numerous samples of many species. Among these were some specimens that did not
20 belong to any of the known species. As a result of comprehensive studies, it was
21 concluded that these represent a new species hereafter recognized as *A. aytachii* Ertuğrul
22 & Hamzaoğlu.

23

24

1 **2. Materials and methods**

2 Some *Aethionema* specimens were collected from Aysantı Pass, in the Ayaş district of
3 Ankara Province, by the first and second authors in 2019. These were compared against
4 the treatments of the genus in the *Flora of Turkey and the East Aegean Islands* (Hedge,
5 1965; Davis et al., 1988; Adıgüzel 2000), and other related floras and checklists (e.g.,
6 Chaytor & Aktyroyd, 1993; Hedge, 1968; Busch, 1939; Ertuğrul, 2012), and the recently
7 described new species (Yıldırım and Kılıç, 2016, 2018, 2019), as well as the study of
8 *Aethionema* collections in the herbaria ANK, E, G, GAZI, HUB, K, and KNYA
9 (acronyms follow Thiers, 2021). Our specimens were critically compared with *A.*
10 *armenum* Boiss. and *A. dumanii* Vural & Adıgüzel, the two species that appeared most
11 closely related to it.

12 For molecular phylogenetic studies, we used silica gel dried leaves collected from the
13 type localities of *A. aytachii*, *A. dumanii*, *A. turcicum* H.Duman & Aytaç, *A. grandiflorum*
14 Boiss. & Hohen, and *A. armenum*. Total genomic DNA extraction followed the 2X CTAB
15 method of Doyle & Doyle (1987) as modified in Soltis et al. (1991) and Cullings (1992).
16 Sequencing and amplification of both DNA strands was performed using ITS1 and ITS4
17 primers (White et al., 1990). Direct sequencing of amplified DNA was performed using
18 the Big Dye Terminator Cycle Sequencing v.3.1 (Macrogen, Netherlands) software
19 program, following the manufacturer instructions. The complete ITS gene sequences of
20 nine *Aethionema* taxa and two *Noccaea* Moench. species (as the out-group) were used.
21 Sequences of *A. armenum* and the out-group were taken from GenBank (National Center
22 for Biotechnology Information), but all other samples in this study are new (Table 1).
23 Editing of the nucleotide sequences and visual alignments were performed using Bioedit
24 v.7.0.5.3 (Hall, 1999). Parsimony analysis was conducted using PAUP v.4.0b10

1 (Swofford, 2002). Bootstrap (BS) analyses (Felsenstein, 1985) were conducted with 1000
2 replicates of the heuristic search using the default options. For the strict consensus tree,
3 the retention index (RI) and consistency index (CI) were given, with the exclusion of the
4 uninformative characters. We used MrBayes 3.2 (Ronquist et al., 2012) to perform the
5 Bayesian phylogenetic analyses. In the Bayesian analyses, random starting trees were
6 used, which were run for 1×10^5 generations, comprising 2 independent runs that
7 consisted of four metropolis-coupled chains. Tracer v.1.5.0 software was used to analyse
8 the trace files created by the Bayesian Markov chain Monte Carlo studies (Rambaut and
9 Drummond, 2007) and after checking them for convergence, the first 1000 samples (20%)
10 were discarded as burn-in. FigTree v1.4.0 software
11 (<http://tree.bio.ed.ac.uk/software/figtree/>) was used as the graphic viewer of the
12 phylogenetic tree.

13 Pollen was obtained from herbarium specimens and prepared following Wodehouse
14 (1935). The pollen slides were observed using a Leica DM 1000 light microscope (LM)
15 (Leica Microsystems, Wetzlar, Germany), and measured using Kameram 21 software
16 (Argenit, Istanbul, Turkey). The measurements were based on at least 30 or more pollen
17 grains from each specimen. The seeds were first investigated using a Leica Z6 Apo 16
18 stereoscopic microscope, and at least 15 mature seeds were measured. For the scanning
19 electron microscopy (SEM) analyses, mature seeds or dried non-acetolysed pollen were
20 placed directly onto aluminium stubs and coated with gold using a sputter-coater. They
21 were photographed using a Zeiss Evo LS 10 SEM (Carl Zeiss NTS GmbH, Oberkochen,
22 Germany). For pollen and seed terminology, Punt et al. (2007) and Pinar et al. (2007)
23 were followed, respectively.

24

1 **3. Results**

2 **3.1 *Aethionema aytachii* Ertuğrul & Hamzaoglu sp. nov. (Figure 1 and 2 A,B).**

3 **Type:** TURKEY. B4 Ankara: Ayaş, around Aysantı Pass, marly hills along roadsides,
4 1190 m, 31.v.2019, *K.Ertuğrul* 5757 & *T.Körüklü* (Holotype KNYA; Isotypes GAZI,
5 ANK).

6 **Paratypes:** TURKEY. B4 Ankara: Ayaş, Aysantı Pass, marly hills on roadside, 1190–
7 1250 m, 14.vi.2019, *H.Demirelma* 3371 (KNYA); *ibid.* 18.v.2019, *E.Hamzaoglu* 7549
8 (KNYA); *ibid.* 1.viii.1985, *Z. Aytaç* 1967 (GAZI).

9

10 **Diagnosis**

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

19

20 **Description**

21 Perennial, stem ascending, 3–9 cm tall, branched. Leaves alternate, falcate, margins
22 involute, sessile, rounded at base, subapiculate or acute at apex; lowermost leaves ovate-
23 oblong, 4–7 × 0.5–1.5 mm; stem leaves oblong to narrowly so, 4–6 × 1–2 mm. Raceme
24 10–20-flowered, compact, elongated in fruit. Pedicel 1.5–2.1 mm long in flowers, 1.5–

1 3.5 mm long in fruit, erect at base, sometimes recurved distally. Sepals saccate, green
2 with a white scarious margin, 2–2.5 × 0.8–1.5 mm. Petals 5.8–7 × 1.5–2.5 mm, pink, 3-
3 veined at base, claw not distinct. Inner (median) filaments free, dilated at base, 2–2.5 mm
4 long, outer (lateral) filaments 1.5–1.8 mm long; anthers triangular to oblong, 0.5–0.6 mm
5 long, apex obtuse in inner filaments, acute in outer ones. Fruit lax, cordate at base,
6 heterocarpic; indehiscent fruit orbicular, 4–5 × 5–5.5 mm, unilocular, 1-ovuled, septum
7 3–4 × 1–1.5 mm, wings 2–2.1 mm wide, irregularly crenate–dentate, sinus 0.5–1 mm
8 deep, style 1–1.5 mm long; dehiscent fruit obovate, 6.5–7.1 × 5–5.1 mm, bilocular, 1- or
9 2- ovuled per locule, septum 4–5.5 × 1.5–2 mm, wings 1.5–2.1 mm wide, undulate along
10 margins, sinus ca. 1 mm deep, style 0.5–1 mm long. Seeds (2–) 3 (–4), ovate, light-brown,
11 1.71–1.31 × 0.70–0.86 mm in indehiscent fruits, 1.3–1.4 × 1.3–1.4 mm in dehiscent fruits.
12

13 **3.2. Etymology**

14 The species was dedicated to Prof. Dr. Zeki AYTAÇ (25.01.1956), a Turkish botanist
15 who has provided many contributions to plant taxonomy. The Turkish name of this new
16 species was suggested as ‘Ayaşkayagülü’ (Menemen et al., 2016).
17

18 **3.3. Molecular analyses and results**

19 Seven accessions of closely related *Aethionema* species and two out-group *Noccaea*
20 species were used for phylogenetic comparison and reconstruction. The total length of
21 studied DNA segments was 601 bp, 123 of which were parsimony informative. The
22 topologies obtained from both the parsimony and Bayesian inference analyses were
23 identical, and the combined tree is shown in Figure 3. The constructed tree shows higher
24 resolution in the Bayesian than parsimony values. The *Aethionema* taxa grouped into 3

1 main clades in the concatenated tree (PP: 1, BS: 100; Figure 3). The first clade comprised
2 *A. aytachii* sister to *A. dumanii* and together sister to *A. turcicum*. The second clade
3 included three different populations of *A. armenum*. The third clade was *A. grandiflorum*.
4 Clearly, *A. aytachii* is closest to *A. dumanii* and *A. turcicum* than to *A. armenum*.

5

6 **3.4. Pollen morphology**

7 The pollen grains of *Aethionema aytachii*, *A. armenum*, and *A. dumanii* were radially
8 symmetrical, isopolar, and tricolpate, as in about 97% of the Brassicaceae. However, the
9 pollen grains of *A. aytachii* were sometimes (4%) syncolpate. Pollen shape was oblate in
10 *A. aytachii* and *A. armenum* and subprolate in *A. dumanii*. The pollen size showed some
11 differences among three taxa in the polar (P) and equatorial (E) views. In *A. aytachii* it
12 was P: $12.95 \pm 1.16 \mu\text{m}$, E: $19.31 \pm 1.15 \mu\text{m}$, while in *A. armenum* it was P: 11.24 ± 0.76
13 μm , E: $16.51 \pm 2.44 \mu\text{m}$, and in *A. dumanii* P: $16.78 \pm 1.56 \mu\text{m}$, E: $13.71 \pm 1.24 \mu\text{m}$. The
14 outline of the pollen grains was elliptic in equatorial view and triangular in polar view.
15 The colpus was long and sunken, margins distinct, regular, and ends ovate. The sculpture
16 of the exine exhibited reticulate ornamentation. The muri shapes varied among the
17 species, and that of *A. aytachii* was larger than the others (Figure 4, Table 3). Detailed
18 pollen morphological characters of the examined species are given in Table 3.

19

20 **3.5. Seed morphology**

21 The seeds were ovate and light-brown in all species. In *Aethionema aytachii* the seed size
22 from the indehiscent fruit was $1.17\text{--}1.31 \times 0.70\text{--}0.86$ mm in those from dehiscent fruit
23 was $1.4\text{--}1.3 \times 0.9\text{--}0.85$ mm, while it was $1.19\text{--}1.31 \times 0.64\text{--}0.90$ mm in *A. armenum* and
24 $1.23\text{--}1.52 \times 0.73\text{--}0.96$ mm in *A. dumanii*. The seed shape in *A. aytachii* and *A. armenum*

1 was ovate, while those of *A. dumanii* were broadly oblong-ovate) (Table 4). The
2 ornamentation of seeds surface in *A. aytachii* was verrucate, reticulate-verrucate in *A.*
3 *armenum*, and reticulate in *A. dumanii* (Figures 5 and 6). The epidermal cells on the seeds
4 were oval in shape, with striate ornamentation in *A. aytachii* and *A. armenum*.

5

6 **3.6. Distribution, habitat, and ecology**

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

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

13

14 **3.7. Conservation status**

15 *Aethionema aytachii* is a locally endemic species and is known only from its type locality
16 (Figure 7). The species is rare in the field, and its extent of occurrence (EOO) and area of
17 occupancy (AOO) are less than 1 km². Due to agricultural activities, such as hobby
18 gardening and road construction in this area, the new species is considered as “Critically
19 Endangered” CR B1ab(i,ii) + 2ab(i,ii) (IUCN, 2017).

20

21 **4. Discussion**

22 *Aethionema aytachii* most closely resembles *A. dumanii* in having subapiculate or acute
23 leaves, sepal size, pink petals, and dilation at the base of the inner filaments, but it differs
24 by having dense stem leaves, heterocarpic fruit (Figure 2), fruiting pedicel measurements,

1 and style/sinus ratio. According to Pinar et al. (2007), the four seed-ornamentation types
2 in *Aethionema* are reticulate, ruminant, reticulate-verrucate, and verrucate. Using this
3 seed-sculpture terminology, the seeds of *A. aytachii* are verrucate, and they are contrast
4 reticulate-verrucate in *A. armenum* and reticulate of *A. dumanii*. The palynological data
5 showed rather minor differences that need not be emphasized for the separation of these
6 three species.

7 Mohammedin et al. (2017) showed some correlation between morphological characters
8 (e.g., ovate vs. linear leaf shape, fruit type dehiscent vs. heterocarpic, presence vs. absence
9 of spines, and plant duration annual vs. perennial) and molecular data base on plastome
10 coding regions and nuclear rDNA genes in the genus *Aethionema*. Their data showed that
11 *A. dumanii* and *A. turcicum* fell in the same clade, and our results fully support that.
12 According to the literature, it has been emphasized that in several species of *Aethionema*,
13 both dehiscent and indehiscent fruits are developed (Appel and Al-Shehbaz, 2003), and
14 heteromorphism is of independent origin (Lenser et al., 2016; Mohammedin et al., 2017).
15 They speculated that there is a correlation between the annual habit and heterocarpy, but
16 such hypothesis needs further testing because heterocarpy is found in four perennial
17 species, including *A. aytachii*, *A. thomasianum* J.Gay (Italy, Spain), *A. rhodopaeum*
18 D.Pavlova (Bulgaria), and the widespread *A. saxatile* (L.) W.T.Aiton (Turkey westward
19 into S, C, and SW Europe and NW Africa). From the last three species, *A. aytachii* is
20 readily distinguished by having much narrower leaves with length/width ratio of at least
21 4–6:1 (vs. 1–2.5:1). Heterocarpy was suggested to be a conservation strategy against risks
22 arising from environmental conditions because the production of different morphs gives
23 the plants some flexibility in response to environmental stimuli (Imbert, 2002; Lenser et
24 al., 2016; Bhattacharya et al., 2019).

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

4 5 **Examined specimens:**

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

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

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17

1 **Table 1.** Voucher specimens for the ITS study.

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

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

Species/characters	<i>A. aytachii</i>	<i>A. dumanii</i> (Vural and Adıgüzel, 1995)	<i>A. armenum</i> (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

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

Species/pollen characters		<i>A. aytachii</i>	<i>A. armenum</i>	<i>A. dumanii</i>
Polar axes	Min-max	11.88–14.19	10.39–11.86	15.2–19.2
	Mean	12.95 \pm 1.16	11.24 \pm 0.76	16.78 \pm 1.56
Equatorial axes	Min-max	18.06–20.32	14.55–19.26	11.54–15.41
	Mean	19.31 \pm 1.15	16.51 \pm 2.44	13.71 \pm 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 \pm 0.34	1.04 \pm 0.19	0.59 \pm 0.07
Colpus	Colpus length (Clg)	19.1 \pm 0.67	15.07 \pm 1.12	14.73 \pm 1.04
	Colpus width (Clt)	2.62 \pm 0.3	1.99 \pm 0.17	1.38 \pm 0.03
Exine thickness		1.08 \pm 0.17	0.83 \pm 0.14	0.93 \pm 0.07
Intine thickness		0.63 \pm 0.17	0.41 \pm 0.01	0.56 \pm 0.06
Apocolpium		2.01 \pm 0.2	2.77 \pm 0.83	2.13 \pm 0.08

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

Species/seed characters	<i>A. aytachii</i> (indehiscent fruit)	<i>A. aytachii</i> (dehiscent fruit)	<i>A. armenum</i>	<i>A. dumanii</i>
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

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1 **Figure 1.** Plants of *Aethionema aytachii*.

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

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6 **Figure 3.** ITS Majority rule consensus tree from Bayesian inference and Parsimony
7 analysis, and numbers depict posterior probabilities and bootstrap values. (CI = 0.944; RI
8 = 0.946; HI = 0.056). (CI = 0.944; RI = 0.946; HI = 0.056).

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10 **Figure 4.** SEM micrographs of the pollen grains of *Aethionema aytachii* (A, B), *A.*
11 *armenum* (C, D), and *A. dumanii* (E, F). A, C. General view, and B, D. exine sculpturing.

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

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

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20 **Figure 7.** Distribution map of *Aethionema dumanii* (◆), *A. armenum* (▲), and *A. aytachii*
21 (★).

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