

CONTRIBUTION TO THE SYSTEMATIC KNOWLEDGE OF ENDEMIC *AUBRIETA PINARDII* BOISS. (BRASSICACEAE) FROM TURKEY

EMRAH ŞIRİN* AND MEHMET CENGİZ KARAIŞMAILOĞLU¹

Department of Biology, Faculty of Science, Selçuk University, Konya, Turkey

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Abstract

The aim of this study was to document the taxonomical, morphological, anatomical, palynological and cytological characters, and geographical distribution of endemic *Aubrieta pinardii* Boiss. (Brassicaceae) from Turkey. The description of the taxon was revised as a consequence of comprehensive assessments of many specimens. The surface pictures belonging to seed and pollen of the taxon were obtained by Scanning Electron Microscope. The seed surface ornamentation was rugose. The pollen was radially and isopolar and prolate in forms, with polar axes of $19.52 \pm 0.29 \mu\text{m}$ and equatorial axes of $13.04 \pm 0.22 \mu\text{m}$, with oval outlines in the equatorial axes, and elliptical in the polar axes. They were three-colpate and colpus sizes varied between $12.98 \mu\text{m}$ and $13.29 \mu\text{m}$ in length, and between 1.33 and $2.09 \mu\text{m}$ in width. Also, the anatomical structures of the root, stem and leaf of species were studied. In cytological studies, the chromosome number of species was found as $2n = 16 (x = 8)$. This was the first work including taxonomical, morphological (macro and micro), anatomical and cytological data of endemic *Aubrieta pinardii*.

Introduction

The family Brassicaceae has 365 genera and 3250 species worldwide, and it is well-known as a major family having commercial importance (Simpson, 2006; Tekin *et al.*, 2013; Karaismailoğlu, 2017a). Turkey with over 650 species is one of the most diversity centers of the family (Al Shehbaz *et al.*, 2007; Güner *et al.*, 2012). The genus *Aubrieta* has 22 species in the world. In Turkey, it has 10 species, six of which (*A. alshehbazii* Dönmez, Uğurlu & M.A. Koch, *A. anamasica* Pesmen & Güner, *A. ekimii* Yüzb., Al-Shehbaz & M.A. Koch, *A. olympica* Boiss., *A. pinardii* Boiss. and *A. vulcanica* Hayek & Siehe) are endemic (Güner *et al.*, 2012; Yüzbaşıoğlu *et al.*, 2015; Dönmez *et al.*, 2017; Karaismailoğlu 2017a). This endemism percentage (50%) indicates that Turkey is one of the gene centers of the genus (Karaismailoğlu, 2016, 2017a). Most of the *Aubrieta* taxa are perennials plants growing on stones in mountainous or subalpine regions between South Europe and Middle Asia (Gustavsson, 1986; Phitos, 2002; Karaismailoğlu, 2016, 2017a; Koch *et al.*, 2017).

The first exhaustive taxonomic research of *Aubrieta* was given by Boissier (1867). Afterwards, Mattfield (1937) worked on the taxonomical appearance of the genus. Al-Shehbaz (2010) informed the difficulties in determining reliable morphological characteristics that show variation. The taxonomy of Arabideae including genus *Aubrieta* has been compelling due to excessive similarity in macromorphological characters (Koch *et al.*, 2017).

A number of studies on some *Aubrieta* taxa, like morphology of some Bulgarian taxa (Ančev and Goranova, 2009), morphology, anatomy and cytology of *A. canescens* subsp. *canescens* (Karaismailoğlu, 2016) and molecular data such as plastid matK and chloroplast gene ndhF of *A. deltoidea* (Koch *et al.*, 2001) were conducted previously. Apart from these, there were no systematic studies directly related to the genus. The genus *Aubrieta* need to be studied in detail, owing to the paucity of information on its taxa.

*Corresponding author, email: emrahsirin@selcuk.edu.tr

¹Siirt University, Faculty of Arts and Sciences, Department of Biology, Siirt, Turkey.

In this study, taxonomical, macromorphological, micromorphological, anatomical, palynological, and cytological characters of endemic *Aubrieta pinardii* Boiss. have been revealed for the first time, and that have contributed to the systematics of the genus.

Materials and Methods

Specimens were collected by the second author and housed in the herbarium of the Science Faculty of Selçuk University (KNYA) (Locality: Konya, Beyşehir, Başarakavak crossroads, stony places, 1350 m, 11 April 2018, E. Şirin 708 H. Günel). At least ten seeds or anthers for each species were dehydrated in alcohol series (70%, 80%, 96% and 100%) in SEM analyses for cleaning process. Seeds were coated with gold under ZEISS EVO LS-10 model SEM high-vacuum mode for observing their surface at 30X, 1000X and 2000X magnifications. The terminology of micro characteristics was performed according to Stearn (1992) and Koul *et al.*, (2000). In addition, the stereo microscope images of the seeds were photographed with the LEICA DFC295 digital camera attached to the LEICA S8AP0 microscope.

For the anatomical examinations, cross-sections from the root, stem, and leaf were collected using a fully automatic microtome (Thermo Shonda Met Finesse). Later, they were treated through a ethyl alcohol and xylene series and stained with hematoxylin or methylene blue in a dying apparatus (ASC 720 Medite) and covered with Entellan to examine their anatomical structures (Karaismailoğlu, 2015a, 2015b, 2016, 2019). The anatomical characters were observed with utilizing an Olympus CX21FS1 microscope and Kameram Imaging Software.

Primary root meristems obtained by germinating seeds were utilized for chromosomal analyses. The protocol of Karaismailoğlu (2016) was followed with some modifications in preparation of the slides. The root tips were pretreated in 5% α -bromonaftol solution for 4 h, allowed to stand 24 h in Carnoy (3:1 = ethyl alcohol:glacial acetic acid), hydrolyzed in 1 N HCl for 6-8 min at 60°C, and stained with aceto-orcein for 3 h. Eventually, preparations were coated with Entellan to make them permanent. The best metaphase images were photographed with an Olympus CX21FS1 light microscope (Tokyo, Japan) attached to a digital camera.

Results and Discussion

Taxonomic description: Densely caespitose herbs. Stems 7–11 cm; flowering stems 3–5.5 cm; covered with mixture of stalked pubescent, 3–5-rayed dendroid, simple long setose and forked trichomes. Leaves numerous, similar to each other, shortly petiolate, slightly clasping the stem, narrowly oblanceolate, with one pair of teeth on each side or entire, 14–18 × 1–4 mm, obtuse at apex; stalked, 3–6-rayed and densely mixed with stellate, forked and simple bristles on both surface. Racemes close cluster of 3–6 flowers, densely pubescent. Sepals lanceolate, 8–10 × 2–3 mm, pedicellate, pubescent, green outside, glabrous inside, inner sepals saccate, apex acute. Petals violet, 16–18 × 7–9 mm, well-differentiated into an obovate limb and a claw 7–9 mm. Filaments unwinged, white in lower half, violet towards apex; median ones 8–19 mm, lateral 6–7 mm; anthers narrowly elliptic, 0.5–1 mm. Fruit siliquiform, 23–30 × 1.5–2 mm (excluding style), mostly straight, compressed parallel to the septum; uniformly pubescent with short-stalked 3–6-rayed trichomes, mature valves slightly reticulate-veined; style 2.5–4 mm; stigma capitate, entire. Seeds biseriate, c. 15–20 in each locule, broadly elliptic to broadly oblong, pale brown to black, wingless, surface ornamentation rugose, not mucilaginous when wetted, 1.3–1.7 × 0.6–0.7 mm (Figs 1-2).

Pollen morphology: Pollens were radially and isopolar and prolate in forms, with polar axes of $19.52 \pm 0.29 \mu\text{m}$ and equatorial axes of $13.04 \pm 0.22 \mu\text{m}$, with oval outlines in the equatorial axes, and elliptical in the polar axes (amb) (Fig. 3). They were three-colpate. Also, colpus sizes varied between 12.98 μm and 13.29 μm in length, and between 1.33 and 2.09 μm in width. The

margins were organized. The exine thickness varied between 1.05 and 1.48 μm , and it was usually thicker in the apertural sections. Additionally the intine thickness ranged from 0.32 to 0.48 μm . *A. pinardii* was of coarse reticulate ornamentation type with somewhat meandering muri. The lumina comprised of polygonal or irregular cells; its diameter ranged from 0.48 to 1.52 μm .

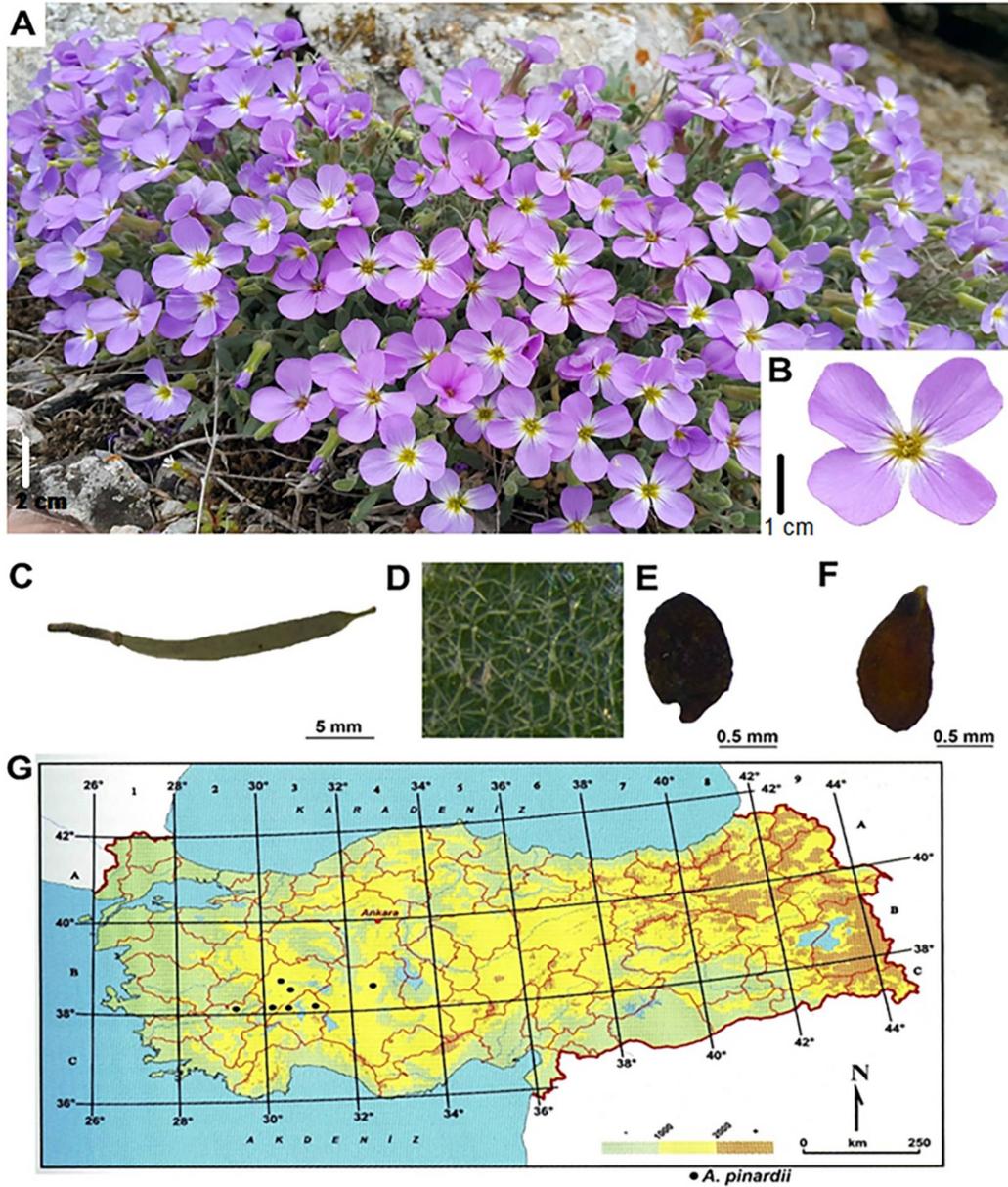


Fig. 1. *Aubrieta pinardii*. a: Habit, b: a flower, c: a fruit, d: surface of the fruit, e and f: seed, g: distribution map (localities were taken from Dönmez *et al.* 2017).

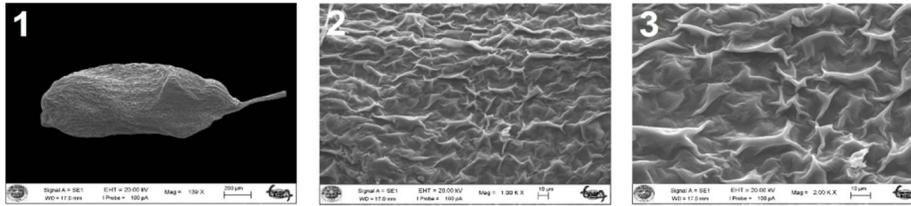


Fig. 2. SEM pictures of *A. pinardii* seeds: 1. Overview, 2 and 3. surface.

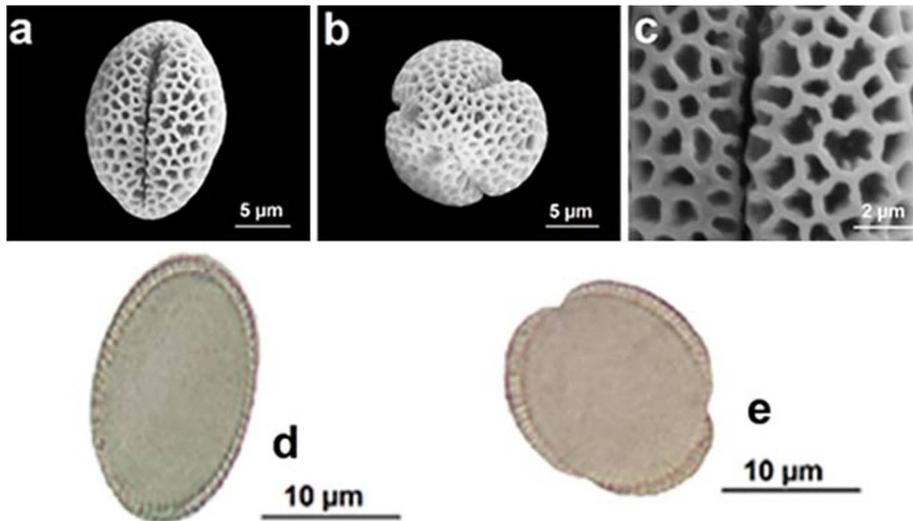


Fig. 3. Pollen morphology of *A. pinardii*. a: polar axis, b: equatorial axis, c: surface of pollen (SEM), d: polar axis, e: equatorial axis (Light microscope).

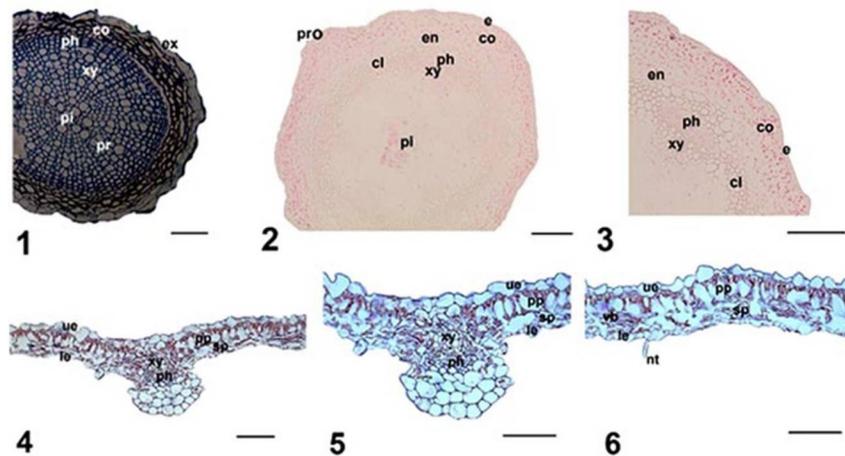


Fig. 4. The anatomical structures of *A. pinardii*. 1: root, 2-3: stem, 4-6: leaf (ex: exodermis, co: cortex, ph: phloem, xy: xylem, pi: pith region, pr: pith ray, e: epidermis, pro: protrusion, en: endodermis, cl: chlorenchyma, ue: upper epidermis, le: lower epidermis, pp: palisade parenchyma, sp: spongy parenchyma, vb: vascular bundle, nt: non-glandular trichome, scale bars=100 μ m).

Root, stem and leaf anatomy: An exodermis, consisting of flat cells with 1-3 layered was placed on the outermost surface of *A. pinardii* (Fig. 4). The thickness of this layer ranged from 21.43 μm to 50.65 μm . Under exodermis, cortex contained of multilayer scleranchymatic cells between 15 μm and 38 μm in diameter. Endodermis layer is not pronounced. The most enclosed space in the roots was shaped by secondary xylem. Pith rays extended from large parenchymatic cells (Fig. 4). The palisade parenchyma in the mesophyll layer covers more space than spongy parenchyma.

In cross-sections of the stem, 1 layered epidermis consisting of flat or rectangular cells was detected in outermost (Figure 4). The dimensions of epidermis cells were recorded as 8-18 μm in length, and 3-6 μm in width. Under the epidermis layer, there was a cortex with 5-9 layered, consisting of ovoid or flat shaped cells. Its thickness was in 70.59 and 81.96 μm . The xylem and phloem elements were indistinct. The vascular bundle was open collateral type. The vessel member diameter showed variations between 11.72 and 26.08 μm . Innermost, there was also a layer consisting of large parenchymatous cells (Fig. 4).

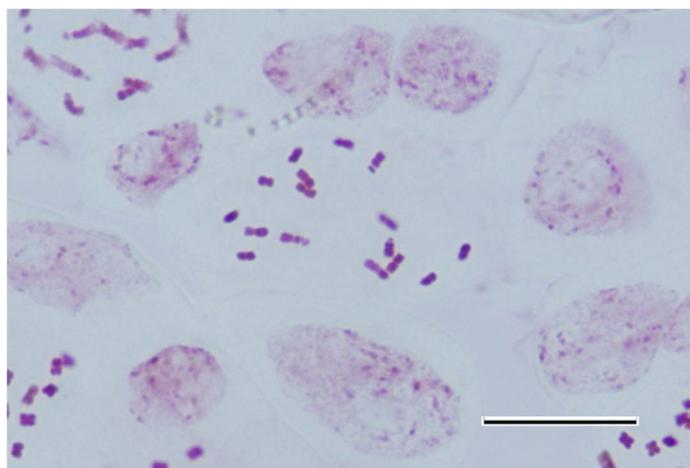


Fig. 5. The chromosomes of *A. pinardii* in metaphase ($2n=16$).

In the abaxial and adaxial surfaces of the leaf, single-layer epidermis cells consisting of irregularly flat or polygonal cells were detected. The leaf was bifacial. The mesophyll layer was noticed as having 2-4 spongy layers and a thickness of 35-55 μm , and 1 layered palisade parenchyma with a thickness of 65-85 μm . The leaves were of collateral vascular bundles, which were surrounded by parenchymatic cells (bundle sheet) (Fig.4).

Cytology: The chromosome number of *A. pinardii*, $2n = 16$, counted in root tips, is also reported and illustrated for the first time (Fig. 5).

Aubrieta is problematic in terms of its systematics, and frequent field examinations are necessary to define the limit of taxa belonging to the genus (Cullen, 1965). Description of *A. pinardii* was very narrow-scope in Flora of Turkey and it has been updated and revised with intensive field works (Table 1). The taxonomic characterization of the species was updated by addition of more characteristics from a significant number of plants taken from the native populations, in comparison with characters in Flora of Turkey (Cullen, 1965). Morphological characters reported for the first time are stem and leaves indumentum and measurements,

filaments, anthers, style, stigma and seeds features (Table 1). In this study, information on the distribution of *Aubrieta pinardii* have been provided for the first time.

Table 1. New characters for the examined taxon (*A. pinardii*) and their comparison with the relevant descriptions in the Flora of Turkey (Cullen, 1965).

Characters	According to Cullen (1965)	The obtained outcomes from this investigation
Stems (cm)	–	7–11
Flowering stems (cm)	–	3–5.5
Stem indumentum	–	pubescent with stalked, 3–5-rayed dendroid, simple long setose and forked trichomes
Stem leaves (mm)	–	14–18 × 1–4 mm
Stem leaves indumentum	–	stalked 3–6-rayed and densely mixed with stellate, forked and simple bristles
Sepals	8–10 mm	lanceolate, 8–10 × 2–3 mm, pubescent, green outside, glabrous inside, inner sepals saccate, apex acute
Petals	purple, 18–19 mm	violet, 16–18 × 7–9 mm, well differentiated into an obovate limb and a claw 7–9 mm.
Filaments	–	unwinged, white in lower half, violet towards apex; median ones 8–19 mm, lateral 6–7 mm
Anthers	–	narrowly elliptic, 0.5–1 mm
Fruits	siliquiform, 22–35 × 2–2.5 mm, with an indumentum of stellate hairs	siliquiform, 23–30 × 1.5–2 mm (excluding style), mostly straight, compressed parallel to the septum; uniformly pubescent with short-stalked 3–6-rayed trichomes, mature valves slightly reticulate-veined
Style–stigma	–	style 2.5–4 mm; stigma capitate, entire
Seeds	–	biseriate, c. 15–20 in each locule, broadly elliptic to broadly oblong, pale brown to black, wingless, not mucilaginous when wetted, 1.3–1.7 × 0.6–0.7 mm

A. pinardii seems morphologically similar to *A. vulcanica* and *A. parviflora*, however, *A. pinardii* differs from these species by its petal lengths and indumentum of inner sepals (Cullen, 1965), toothed leaves and similar to them stellate hairs on fruits. The results obtained from morphological studies are consistent with description given in the Cullen (1965), Yüzbaşıoğlu *et al.* (2015) and Dönmez *et al.* (2017). Seed coat patterns are used for solving classification problems, establishing evolutionary relationships, elucidating the adaptive significance of the seed coat, and serving as genetic markers for the identification of genotypes in segregating hybrid progenies (El-Naggar, 2005; Bona, 2013; Karaismailoğlu and Erol, 2018; Gabr, 2018; Ozcan and Akinci, 2019). Seed micromorphology of *Aubrieta* taxa from Turkey was studied before by Yüzbaşıoğlu *et al.* (2015; *A. ekimii*), Karaismailoğlu (2016; *A. canescens* subsp. *canescens*) and Dönmez *et al.* (2017; *A. alshehbazii*). Our study is the first report on the seed micromorphology of *A. pinardii*. The surface ornamentation of species has recorded as rugose, which is commonly noticed in many genera in the family (Murley, 1951; Koul *et al.*, 2000; Zeng *et al.*, 2004; Moazzeni *et al.*, 2007; Karaismailoğlu, 2016, Karaismailoğlu and Erol, 2018).

The data about pollen morphological characters can permit us to better recognize the pragmatism of pollen works in separating the correlated taxa. The pollen shape observed in *A.*

pinardii is the most common type in its family and is consistent with the findings of Khalik and Maesn Van Der (2002), Mutlu and Erik (2012), and Karaismailoğlu (2017b and 2019). The aperture and exine features of *A. pinardii* were explained as the important standard for the categorization of phylogenetic correlation in many studies (Kuprianova, 1967; Cronquist, 1968; Takhtajan, 1980; Karaismailoğlu 2019).

The pollen surface ornamentation is useful for delaminating some closely-related taxa belonging to various genera in the family (Khalik and Maesn Van Der, 2002; Karaismailoğlu, 2017b, 2019). The coarse reticulate type of pollen ornamentation found in the studied species is in accordance with the findings of Anchev and Deneva (1997), Mutlu and Erik (2012), and Karaismailoğlu (2017b, 2019). Mutlu and Erik (2012) have informed that pollen including coarse reticulate (lumina of more than 1 µm) are generally found in humid areas unlike others, which is supported by the findings of this study.

The systematic use of anatomical features is useful in the taxonomy of the family Brassicaceae (Metcalf and Chalk, 1957). The anatomical characteristics of the root, stem, and leaf of *A. pinardii* have been given in this study for the first time (Figure 4). *A. pinardii* cortex cells are subsequent to a thin epidermis layer in the stem which seems to be similar to the relevant images of some *Alyssum*, *Erysimum*, *Aubrieta* species (Orcan and Binzet, 2003; Cansaran *et al.*, 2007; Karaismailoğlu, 2016) and *Pachypragma macrophyllum* (Karaismailoğlu, 2019).

Chromosome numbers in the Brassicaceae are mostly different for taxa within genera and they are important in terms of the evaluation of systematics and evolution in this family (Karaismailoğlu, 2018). Chromosome numbers of seven out of 12 species of *Aubrieta* in the world is known so far (Warwick and Al-Shehbaz, 2006). The chromosome counts of *Aubrieta canescens* subsp. *canescens* in Turkey reported as $2n = 16$ (Karaismailoğlu, 2016) is consistent with our study.

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