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Research Article

NOTES ON *Arabis kaynakiae* Daşkın (Brassicaceae), A CRITICALLY ENDANGERED SPECIES ENDEMIC TO TURKEY

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Abstract: This study reports on the evaluation of the taxonomical, macro- and micro morphological, anatomical, palynological and cytological features, together with the geographical distribution of *Arabis kaynakiae* Daşkın (Brassicaceae), a Critically Endangered (CR) species from Turkey. The description of the species was updated following detailed studies on new specimens found in Karaman/Ermenek. The surface microphotographs of seeds and pollen were obtained using Scanning Electron Microscopy. The seed surface ornamentation is papillate and its margins minutely tuberculate. Pollen grains appear tricolpate, isopolar, subprolate in shape, with polar axis of 19.62 ± 0.33 µm and equatorial axis of 12.94 ± 0.17 µm. The exine thickness ranges from 1.68 to 1.87 µm; however, the intine thickness ranges from 0.21 to 0.39 µm. *Arabis kaynakiae* has pollen of coarse reticulate type surface ornamentation with slightly undulated muri. Anatomical characters of root, stem and leaf of the species were also given. The cytological investigations revealed that *A. kaynakiae* is diploid with 2*n* = 2x = 16 chromosomes. The discovered population of *A. kaynakiae* increased the known distribution range of the species in Turkey, and an updated evaluation of the species considering the IUCN criteria was provided.

Özet: Bu çalışma, Türkiye'den kritik olarak tehlike altındaki endemik (CR) bir tür *Arabis kaynakiae* Daşkın'ın (Brassicaceae) taksonomik, makro ve mikro morfolojik, anatomik, palinolojik ve sitolojik özelliklerinin coğrafi dağılımıyla birlikte değerlendirilmesini rapor etmektedir. Türün deskripsiyonu Karaman/Ermenek'te bulunan yeni örnekler üzerinde yapılan detaylı çalışmaların ardından güncellenmiştir. Tohum ve polenlerin yüzey mikrofotoğrafları Taramalı Elektron Mikroskobu kullanılarak elde edilmiştir. Tohum yüzey ornamentasyonu papillattır ve kenarları az çok tuberkulattır. Polen taneleri trikolpattırlar, 19,62 ± 0,33 µm polar ekseni ve 12,94 ± 0,17 µm ekvator ekseni ile izopolar ve subprolat şeklindedirler. Eksinin kalınlığı 1,68 ila 1,87 µm arasında değişmiş; buna karşın, intin kalınlığı 0,21 ila 0,39 µm arasında değişmiştir. *Arabis kaynakiae* hafif dalgalı muri ile kaba ağsı tip yüzey ornamentasyonuna sahiptir. Türün kök, gövde ve yaprak anatomik karakterleri de verilmiştir. Sitolojik araştırmalar *A. kaynakiae*'nin 2n = 2x = 16 kromozomlu diploit olduğunu ortaya koymuştur. *A. kaynakiae*'nin Keşfedilen popülasyonu, Türkiye'de türün bilinen dağılım aralığını artırmış ve türün IUCN kriterleri dikkate alınarak güncellenmiş bir değerlendirmesi sağlanmıştır.

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Introduction

The genus *Arabis* L. (Brassicaceae) includes about 118 species distributed in all warm areas of the Northern hemisphere (Warwick & Al-Shehbaz 2006, Koch *et al.* 2010, Mutlu & Erik 2015). Koch *et al.* (2010) suggested that this number may be higher since exhaustive investigations of the Central Asian and Russian taxa are still lacking (Mutlu & Erik 2015). Turkey is an important diversity center for *Arabis* since 24 taxa are distributed in the country, 10 of which are endemic (Cullen 1965, Davis 1988, Parolly & Hein 2000, Duman 2001, Duman & Duran 2001, Mutlu & Dönmez 2003, Mutlu 2004, Mutlu & Erik 2012, Daşkın 2013, Mutlu & Erik 2015).

Arabis was investigated both from a morphological point of view (Cullen 1965, Duman & Duran 2001, Mutlu 2002, Mutlu & Erik 2012, 2015), and a biogeographical and phylogenetic point of view (Karl & Koch 2013). Micromorphological and palynological characteristics of *Arabis* in Turkey were reported by İnceoğlu & Karamustafa (1975), Bıçakçı & Güleryüz (1998), Mutlu (2002), Mutlu & Erik (2012), and Karaismailoğlu (2019b).

Arabis kaynakiae Daşkın is a recently described local endemic of Gülnar district of Mersin in southern Anatolia

(Daşkın 2013). The anatomy, palynology, cytology, and micromorphology of this rare species have not been studied so far and they remain largely unknown. We, therefore, aimed in the present study to present a detailed examination of *A. kaynakiae* and evaluate taxonomic significance of obtained data. New data on distribution of the species and its updated evaluation considering the International Union for Conservation of Nature (IUCN) criteria are also presented.

Materials and Methods

Arabis kaynakiae was collected from Karaman/Ermenek by the second author and collected specimens are deposited in the Herbarium of the Science Faculty of Selçuk University (KNYA). The morphometric analyzes were performed with 10 specimens in total according to Daşkın (2013).

Ten to twenty seeds and fifty pollen grains from ten different specimens were dehydrated in alcohol series (70%, 80%, 96% and 100%) for SEM analyses. Samples were covered with gold and microphotographs were taken with a Scanning Electron Microscope (ZEISS EVO LS-10). Terminology of SEM microphotographs is in accordance to Stearn (1992) and Koul *et al*. (2000). Images of the seeds were taken with a LEICA DFC295 digital camera attached to a LEICA S8AP0 stereo microscope.

Pollen preparations for light microscopy were performed according to the method of Wodehouse (1935). Pollen grains were stained with safranin, coated with a cover slip, analyzed with an Olympus CX21FS1 light microscope, and photographed by Kameram Imaging Software (Karaismailoğlu 2017, Karaismailoğlu & Erol 2019).

For anatomical investigations, cross-sections of root, stem and leaves for which different alcohol and xylene series were applied were obtained using a microtome (Thermo Shonda Met Finesse). Preparations were dyed with hematoxylin (ASC 720 Medite) and coated with Entellan to assess their anatomical characters (Karaismailoğlu 2015a, 2015b, 2016, 2019a, Karaismailoğlu *et al.* 2018). The Olympus CX21FS1 microscope and the Kameram Imaging Software were used for anatomical investigations.

Root tips obtained after seed germination were used for chromosome examinations. The protocol of Karaismailoğlu (2018) was followed with some modifications for slide preparations. The roots were pre-treated in a 5% a-bromonaphthalene solution for 4 h, kept in Carnoy's fixative (3:1 = ethyl alcohol: glacial acetic acid) for 24 h, hydrolyzed in 1 N HCl for 8-10 min at 60°C and dyed with aceto-orcein for 3 h. In the last step, preparations were coated with Entellan to make them permanent. The best metaphase plate was photographed under an Olympus CX21FS1 light microscope with a digital camera attached.

Results

The updated description of *Arabis kaynakiae* is as follows;

Perennial herb with a woody stock. Flowering stem erect, 5−8 cm long, canescent with stellate hairs, 2−4 leaved and unbranched. Rosette leaves widely obovate 8−15 × 2−8 mm, densely silvery-canescent with 2−4-furcate and stellate hairs, few toothed to almost entire, apex obtuse, attenuate at base to a 5-7 mm petiole, with conspicuous midvein. Stem leaves oblong-lanceolate, 8−15 × 2−5 mm, sessile, tomentose with 2−4- furcate and stellate hairs. Inflorescence 1.5−3 cm long, 7−10-flowered, covered with 2−4- furcate and stellate hairs, without bracts. Pedicels 2−6 mm long, glabrous, ascending-erect. Sepals 3−4 × 0.8−1.0 mm, ovate-lanceolate, greenish white, margins membranous, sparsely to densely 2−4-furcate to stellate hairy. Petals white or white-lilac, 7−10 × 2.5−3.0 mm, oblong to spathulate, obtuse. Filaments yellow, cylindrical, long ones 6.8–7.0 mm long, short ones 3.5−4.0 mm long. Anthers 1.5 mm long, yellow, ovate, apex apiculate. Fruiting pedicels green, glabrous, spreading to reflexed, 10−12 mm long. Siliques brownish-green, 25−30 × 0.9−1.0 mm, flattened, glabrous, the valves without median nerve. Style 0.9−1.0 mm long; stigma capitate. Seeds 1.4−1.7 × 1.1−1.2 mm, ovate to orbicular, pale brown to black, surface papillate and margins minutely tuberculate, unwinged; radicle accumbent (Figs. 1-2).

Arabis kaynakiae is morphologically similar to *A. androsacea* Fenzl, *A. carduchorum* Boiss. and *A. alanyensis* H. Duman. However, it mainly differs from these species by its canescent flowering stems, rosette leaves and flowering stem leaves with 2−4-furcate and stellate hairs, sparsely to densely hairy sepals with 2−4-furcate and stellate hairs, and papillate seeds (Daşkın 2013). The results obtained from morphological studies are mainly consistent with the description given in the protologue (Daşkın 2013), except some features of rosette leaves, inflorescence, flowering and fruiting pedicel, filament, anther, silique, seed, and radicle that are presented in this study (Table 1).

The morphological characteristics of *A. kaynakiae* pollen were evaluated in detail (Fig. 3). Pollen grains are tricolpate, and isopolar and subprolate (P/E=1.28) in shape, with polar axis (P) in size 19.62 ± 0.33 µm and equatorial axis (E) in size 12.94 ± 0.17 µm. They have an oval appearance in equatorial and elliptical appearance in polar axes (amb) (Fig. 3). Colpi dimensions are varying from 14.72 µm to 15.33 µm in length, and from 1.41 to 2.13 µm in width. The edges are regular. The exine thickness ranges from 1.68 to 1.87 µm and the intine thickness ranges from 0.21 and 0.39 µm. Pollen grains have coarse reticulate type surface ornamentation with slightly undulated muri. The lumina is consisted of polygonal cells and its diameter varies between 0.59 and 1.91 µm.

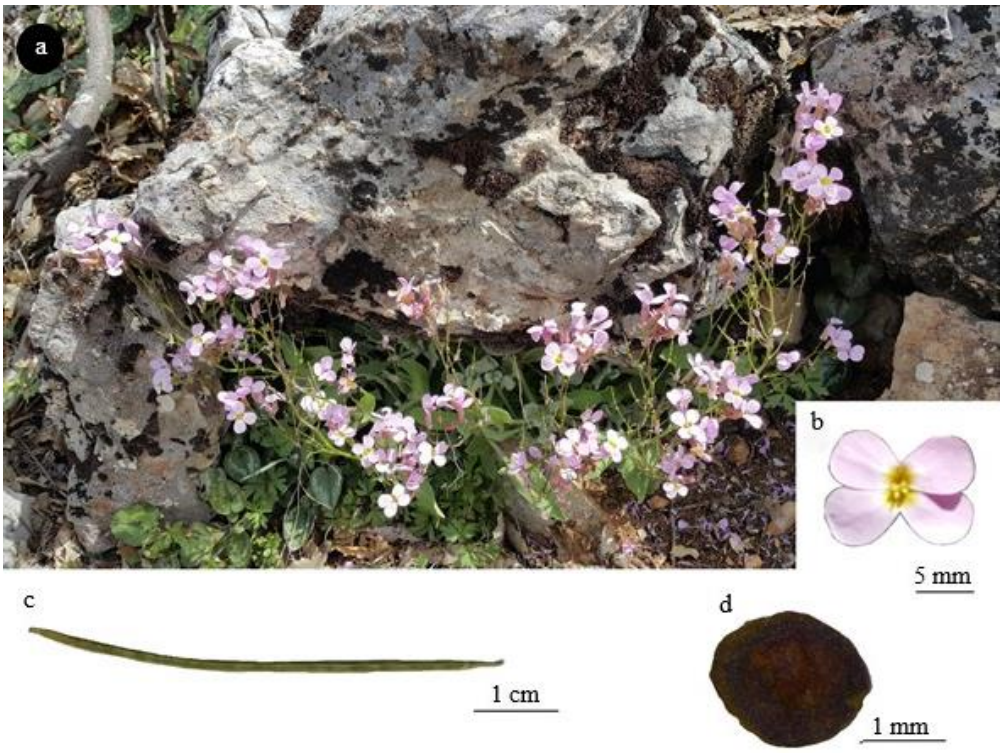

Fig. 1. *Arabis kaynakiae*. **a:** general appearance, **b:** flower, **c:** fruit, **d:** seed (Locality: Karaman, Ermenek, Tekeçatı high plateau, stony places, 1450 m, 8 April 2018, E. Şirin 684).

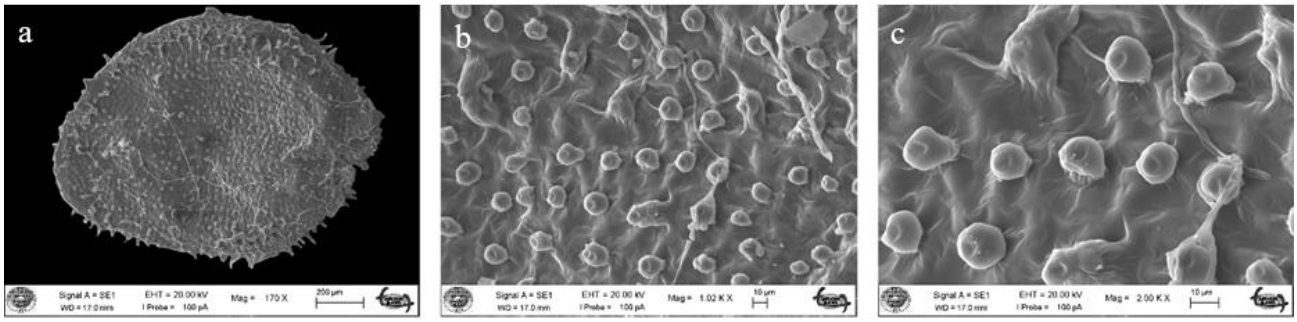

Fig. 2. Overview (**a**) and surface (**b-c**) SEM images of *A. kaynakiae* seeds.

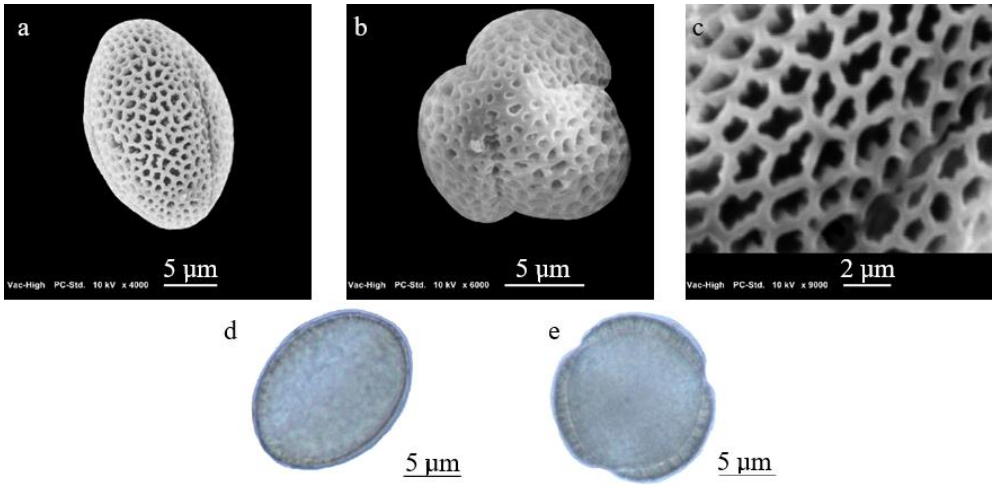

Fig. 3. SEM images of **a:** polar axis, **b:** equatorial axis and **c:** surface and light microscopy images of **d:** polar axis and **e:** equatorial axis of pollen grains.

Table 1. New characters of *A. kaynakiae* in addition to those reported by Daşkın (2013).

Characters	The obtained outcomes
Inflorescence	3-5 furcate to stellate hairy
Pedicel	glabrous
Seed and radicle	unwinged and accumbent
Rosette leaves	conspicuous midvein
Filaments	yellow and cylindrical
Anthers	ovate, apex apiculate
Fruiting pedicels	green, glabrous
Siliques	green - brownish

The anatomical structures of the root, stem, and leaf for *A. kaynakiae* are given for the first time in this study (Fig. 4). The outermost surface of the root consists of a multi-layered periderm. The cortex under periderm comprises multi-layered parenchymatic cells and its thickness is 194.71 ± 5.44 µm. The endodermis layer is not clear. There is no obvious cambium amid phloem and xylem. The largest part of the roots is composed of secondary xylem elements. Pith rays are prolonged from large parenchymatic cells. The stem cross-sections showed that there is 1-layered epidermis containing flat cells in outermost (Fig. 4a). Its thickness varies from 30.71 µm to 43.65 µm. There is a cortex consisting of 3-4 layers of flat cells underneath epidermis. The thickness of this layer ranges from 149.78 µm to 181.14 µm. Under cortex, there is a sclerenchymatic layer, surrounding the side facing the cortex of the vascular bundles. This structure consists of a 3-4-celled layer and its thickness varies between 103.15 µm and 121.43 µm. Phloem components are not clear. The types of vascular bundles are closed collateral. The vessel member diameters range from 10.92 to 38.17 µm (Fig. 4b). The abaxial and adaxial surfaces of the leaves consist of a 1-layered epidermis cells, irregularly flat or polygonal in shape. The leaves are equifacial. The mesophyll layer is noted as a 2-4 layered palisade parenchyma 145.13-169.87 µm thick on both sides and a 2-3 layered spongy parenchyma 41.66-65.19 µm thick in the middle region. Leaves have collateral vascular bundle type. Vascular bundles are enclosed with parenchymatic cells (Fig. 4c-e).

The chromosome number of *Arabis kaynakiae* was determined as 2n = 16 in root tips (Fig. 5). The basic chromosome number of was found as x = 8.

Arabis kaynakiae is an endemic distributed in a limited area, and is only known from two populations in Mersin/Gülnar in southern Turkey (Daşkın 2013). In this study, we found a new population consisted of less than 50 individuals in Karaman/Ermenek (Fig. 6).

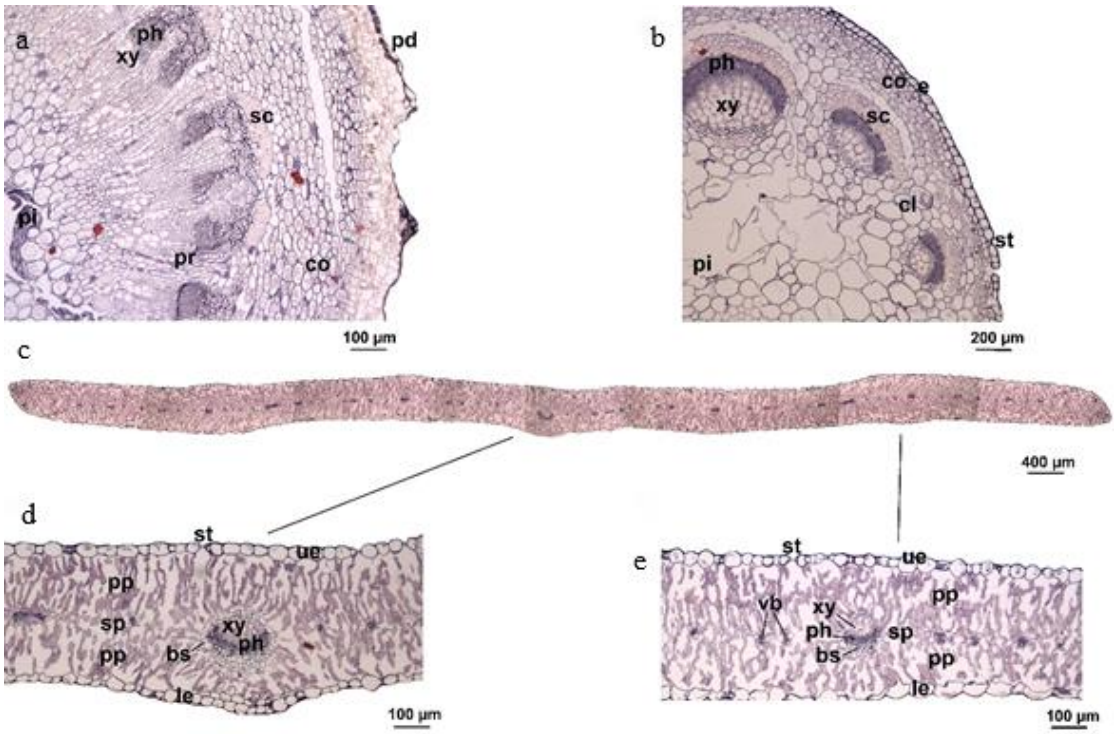

Fig. 4. The anatomical cross-sections of *A. kaynakiae*; **a:** root, **b:** stem, **c-e:** leaf (pd: periderm, co: cortex, ph: phloem, xy: xylem, pi: pith region, pr: pith ray, e: epidermis, cl: chlorenchyma, ue: upper epidermis, le: lower epidermis, pp: palisade parenchyma, sp: spongy parenchyma, vb: vascular bundle, sc: sclerenchyma, st: stoma, bs: bundle sheath).

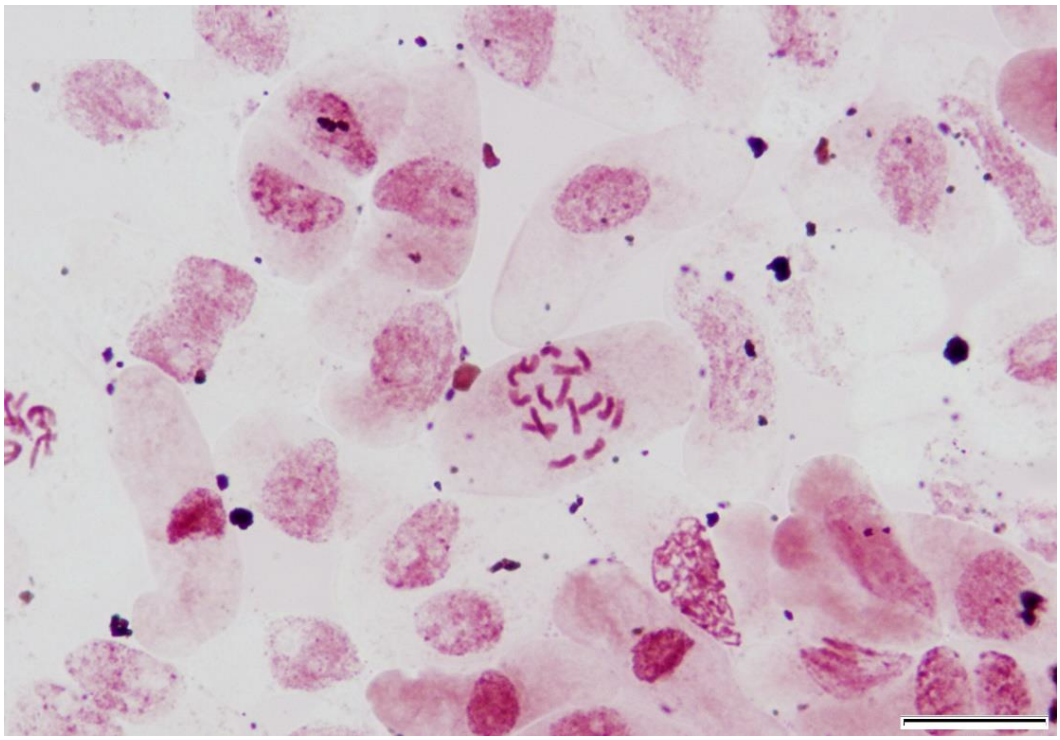

Fig. 5. The chromosomes of *A. kaynakiae* in a metaphase plate (2n=16, scale bar=10 µm).

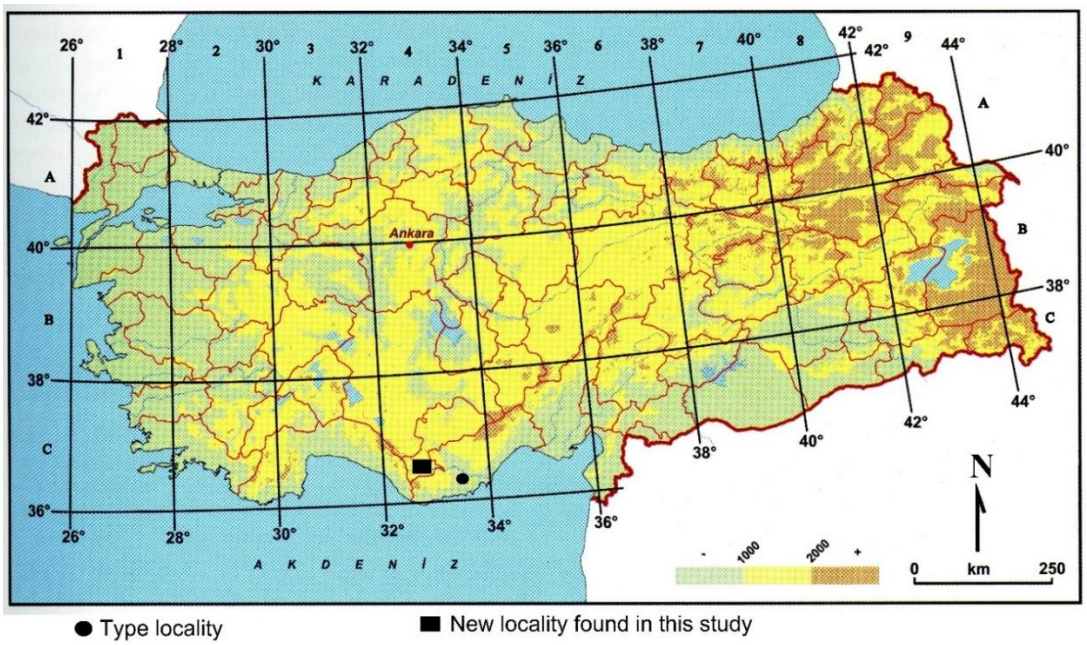

Fig. 6. The distribution map of *A. kaynakiae.*

Discussion

The results of the present study expanded the former morphological description of some parts (flower, seed, leaves, fruits) of *Arabis kaynakiae* and provided the first data on anatomy, palynology, micromorphology, and karyology of the species.

Seed coat structure is considered as an important character in systematic studies of Brassicaceae. It is mostly used to establish taxonomic and evolutionary relationships (Khalik & Maesen 2002, Tantawy *et al.* 2004, Kaya *et al.* 2011, Bona 2013, Karaismailoğlu 2016, 2019a, Karaismailoğlu & Erol 2018). Seed micromorphology studies conducted so far on *Arabis* were based on 6 taxa in Mutlu (2002) and 2 taxa in Khalik & Maesen (2002). Our study is the first report on seed micromorphology of *A. kaynakiae*. The surface ornamentation of the species is recorded as papillate and

tuberculate, although the previously reported types of other species of the genus are reticulate and alveolate. This unique character easily distinguishes *A. kaynakiae* from other studied species. Also, this type is rarely observed in other genera of the family (Murley 1951, Koul *et al.* 2000, Zeng *et al.* 2004, Moazzeni *et al.* 2007).

The pollen features may present useful information to better understand the benefits of pollen studies in discrimination of closely related taxa. *Arabis kaynakiae* pollen has not been studied so far. The pollen shape was defined in this study as subprolate, which is one of the most common types in the family, and is consistent with the results of Khalik & Maesn Van Der (2002), Mutlu & Erik (2012), and Karaismailoğlu (2017, 2019b), who studied pollen grains of different genera in Brassicaceae. The pollen surface ornamentation type is coarse reticulate, similar to several other previously studied Brassicaceae taxa (İnceoğlu & Karamustafa 1975, Anchev & Deneva 1997, Bıçakcı & Güleryüz 1988, Mutlu & Erik 2012, Karaismailoğlu 2017, 2019b, Şirin & Karaismailoğlu 2020).

Mutlu & Erik (2012) studied palynomorphological features of 22 *Arabis* taxa from Turkey. They have described three different pollen morphotypes as Alpina (Polar axes equal or longer than 25 µm, equatorial axes longer than 23 µm, abm axes longer than 23 µm), Nova (Polar axes 18–23 µm, equatorial axes shorter than 23 µm, exine thickness longer than 1.80 µm) and Hirsuta (Polar axes shorter than 18 µm, equatorial axes shorter than 23 µm, exine thickness shorter than 1.80 µm) based on polar axes and exine thickness. Accordingly, *A. kaynakiae* is of Nova type, which has polar axes between 18 and 23 µm, and exine thickness longer than 1.80 µm.

The taxonomic application of anatomical characters is useful for discrimination of closely related taxa within Brassicaceae (Metcalfe & Chalk 1957). The anatomical features of root, stem, and leaf of *A. kaynakiae* were presented here for the first time. The roots have a multi-layered periderm in outermost part, with large pith rays arranged together with xylem components and sclerenchymatic structures. The cortex cells are below an epidermis layer (5-45 µm) in the stem and seem similar to the descriptions of some *Alyssum* L. and *Aubrieta* Adans. species (Orcan & Binzet 2003), and *Pachypragma macrophyllum* (Karaismailoğlu 2019a). The leaves are of the equifacial type. The palisade parenchyma in the mesophyll covers more space than the spongy parenchyma, which widely happens within the Brassicaceae family (Orcan & Binzet 2003, Cansaran *et al.* 2007, Karaismailoğlu 2016, 2019a).

Chromosome number in Brassicaceae is one of the most important characters in the evaluation of systematic relationships in the family (Karaismailoğlu 2018). The genus *Arabis* is represented by 118 species worldwide and chromosome numbers of 59 of these have been studied so far (Warwick & Al-Shehbaz 2006). Chromosome numbers of 14 *Arabis* species from Turkey have previously been reported (Mutlu 2002). The chromosome number of *A. kaynakiae* was found as $2n = 16$ ($\times=8$). The basic chromosome number differs from those of other studied *Arabis* taxa (6 in *A. glabra* (L.) Bernh., 9 in *A. drabiformis* Boiss. and 11 in *A. laxa* L.) (Mutlu 2002).

According to IUCN (2010), the extent of occurrence and the area of occupancy of *A. kaynakiae* are less than 100 km^2 and 10 km^2, respectively. The known populations and their localities are destroyed by road construction activities (Mutlu & Erik 2015). According to the criteria of geographic scale dimension (criteria B) in IUCN classes (Mutlu & Erik 2015), *A. kaynakiae* is assessed as CR; B1ab (i,v), B2ab (i,v). Although we found a new population consisted of less than 50 individuals in Karaman/Ermenek, this species is currently known from 3 localities occupying less than 100 km^2 (Fig. 6). According to IUCN 2010 criteria [B1ab (i,v), B2ab (i,v)], the CR status is still recommended.

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