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Comparative morphological and palynological study on poorly known *Viola sandrasea* and its closest relative *V. kizildaghensis*

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Abstract: The relationships between the closely related Turkish endemic species, *Viola sandrasea* Melchior and *V. kizildaghensis* M. Dinç & Ş. Yıldırımlı, are discussed in the present study. *Viola sandrasea* has been known only from a few gatherings and insufficient description since 1939. Morphological and palynological properties of it are determined in detail and compared to its closest relative *V. kizildaghensis* in this study. The special habitats these species occupy, their typical associates and some other ecological features are also briefly discussed. The obtained data suggests that the morphological differences between them are sometimes subtle, but the petal colour, the leaf wrinkles and the pattern of indument are distinct. The pollen characteristics clearly appear to have taxonomic value. The pollen exine ornemantion of *V. sandrasea* characterized by the rugulate clearly differs from that of *V. kizildaghensis* characterized by psilate-perforate. Moreover, the two species are seasonally and geographically isolated from each other.

Key words: *Viola kizildaghensis*; *V. sandrasea*; taxonomy; morphology; palynology

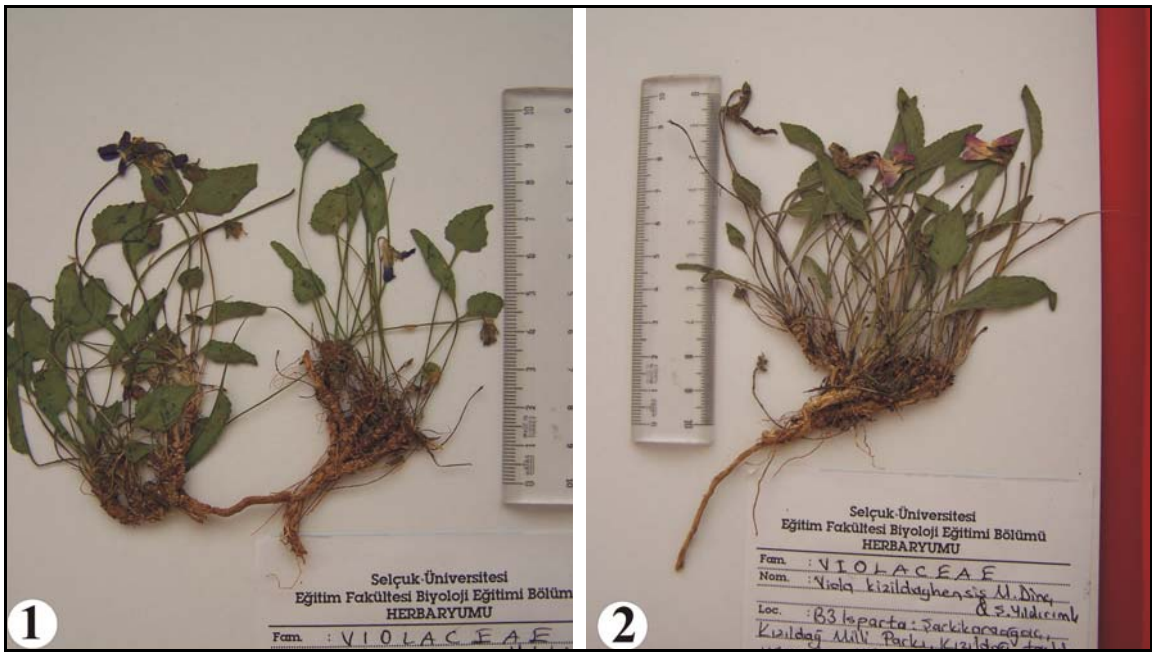
Introduction

The *Violaceae* is a medium-sized family of perennial or rarely annual herbs or shrubs, including the violets or pansies. The family is cosmopolitan, but more typical of the temperate regions and tends to be restricted to higher mountainous areas. It contains about 900 species belonging to 22 genera (Heywood 1993). The *Viola* L. is the largest genus of the family and has about 525–600 species in the world (Ballard 1999). There are 33 naturally growing *Viola* taxa belonging to 29 species and four subspecies in Turkey (Coode & Cullen 1965; Davis et al. 1988; Yıldırımlı 1994, 2000; Dinç et al. 2001; Dinç & Yıldırımlı 2002; Yıldırımlı & Dinç 2002; Dinç et al. 2003; Blaxland 2004).

V. sandrasea and *V. kizildaghensis* belong to the critical Eurasian subsection *Viola*, which includes 25 species (Yıldırımlı 1994; Marcussen & Borgen 2000; Marcussen et al. 2001; Dinç & Yıldırımlı 2002; Dinç et al. 2003; Marcussen et al. 2005). Their delimitations have been topics of discussion among some European authors for two centuries due to the variability and hybridization of these (Marcussen & Borgen, 2000; Marcussen et al. 2005). The taxa belonging to subsection *Viola* typically differ too in relatively few or inconspicuous characters, such as stipule shape and fimbrication, presence of stolons, bracteole insertion on peduncles, flower fragrance, spur and petal colour. The species in subsection *Viola* are all perennials and, produce two types of flowers; showy, chasmogamous flowers in early spring, and inconspicuous cleistogamous ones during the rest of the growth season. Members of the subsection are also characterised by the production of pedicels directly from the rhizome, sphaeric and inexplosive capsules, with a basic chromosome number of $x = 10$ (Okamoto et al. 1993).

Subsection *Viola* L. has traditionally been divided into two series, *Flagellatae* and *Eflagellatae*, based on whether stolons are present or not (Melchior 1939; Okamoto et al. 1993). The current subdivision into two series probably does not reflect true phylogenetic relationships within the group. Hovewer, alpine species of *Eflagellatae* series constitute *Lignosae* W.Becker group (Marcussen & Borgen 2000). The *Lignosae* species are readily distinguished from the other taxa in the subsection *Viola* by lower stature, somewhat woody and branched rhizome, absence of stolons, and the often erect fruiting peduncles (Becker 1925). The seven species included in this group; *Viola chelmae* Boiss. & Heldr., *V. libanotica* Boiss., *V. isaurica* Contandr. & Quézel, *V. yildirimlii* M. Dinç et al., *V. bocquetiana* Yıldırımlı, *V. sandrasea* and *V. kizildaghensis* are all relictual with narrow endemics or disjuncts and native to the montane and alpine regions of South Europe, North Africa, and the Middle East (Melchior 1939; Contandriopoulos & Quézel 1976; Yıldırımlı 1994; Marcussen & Nordal 1998; Dinç & Yıldırımlı 2002; Dinç et. al. 2003).

V. sandrasea, introduced to science from Muğla province of southwest Anatolia in 1939, is only known from a few gatherings and an insufficient description (Melchior 1939). Moreover, it was graded as "possibly extinct" by some authors (Marcussen & Borgen 2000). *V. kizildaghensis*, the closest relative often mistaken as *V. sandrasea*, was described as a new species from Is-



Figs 1–2. Herbarium speciemens of the two species. 1 – *V. sandrasea* (M.Dinç 2713), 2 – *V. kizildaghensis* (M. Dinç 964 & Ş. Yıldırımlı, isotype).

parta province of South Anatolia. There are some similar characteristics such as leaf shape and lower stature in these two Turkish endemic *Viola* species (Dinç & Yıldırımlı 2002).

The aim of this paper is to present a detailed morphological and palynological description of *V. sandrasea* and to discuss taxonomic identities and differences with its closest relative *V. kizildaghensis*. The obtained ecological data will be also included in the discussion since the taxonomy of subsection *Viola* including the two species is critical.

Material and methods

Flowering and fruiting samples of the two species were collected from their type localities. Some samples of *V. kizildaghensis* and *V. sandrasea* were fixed in 70% alcohol. A total 50 samples belonging to these two taxa were used to conduct the morphological studies of the present study. One sample for each species is stored at the Selçuk University Herbarium (KNYA). The collecting localities of the species as follows;

V. kizildaghensis B3 Isparta: Şarkikaraağaç, Kızıldağ National Park, stony slopes, 1400 m a.s.l., 15.04.2002, M. Dinç 962 & Ş. Yıldırımlı.

V. sandrasea C2 Muğla: Köyceğiz, Sandras Dağı, near spring water, rocky places, 1800 m a.s.l., 11.06.2006, M.Dinç 2713.

The surface sections of the leaves were studied under a light microscope. The leaves taken from the specimen of the two species were also placed on prepared stubs and their surface photographs were taken with SEM. The sizes of stomata were measured on thirty stomata on both surfaces. The values of stomatal size and parameters were given as mean ± standart deviation (minimum–maximum). The stomatal index was calculated as described by Meidner & Mansfield (1968).

Palynological investigations were made by both light and scanning electron microscope. For light microscope studies, the pollen slides were prepared according to Wodehouse technique (1935). Pollen grains were dissected from herbarium specimens and placed on a clean microscope slides. Glycerin-gelatin with basic fuchsine was placed on pollens and allowed to melt and mixed by a clean pin to get scattered pollen grains. Measurements and morphological observations were made with an Olympus BX-50 microscope. Measurements of polar axis, equatorial diameter, colpus length, exine thickness and intine thickness were taken of 30 pollen grains per species under the light microscope (×400). Thus, the P/E quotient is determined. The palynological data was given as mean ± standart deviation (minimum–maximum) and presented in Table 2. In addition, pollen grains were placed on prepared stubs and the photographs were taken with SEM to determine exine ornamentation. The pollen terminology used in the present study follows Faegri & Iversen (1989) and Punt et al. (1994).

Results

The studies on the specimens collected from Sandras Dağı in Muğla province lead to the conclusion that they are identical with that described by Melchior as *Viola sandrasea* Melchior.

Viola sandrasea Melchior in Feddes Rep. 46: 39 (1939). Type: [Turkey C2] prov. Muğla, distr. Köyceğis: Sandras Dağ sub lapidibus ad nivem deliquescentem cacuminis Cicekli Baba, in solo serpentinico, 10 vii 1938, *O. Schwarz* 108.

Description

Acaulous perennial herb to 8 cm high, lacking stolons. Rootstock woody and more or less branched, clothed with withered leaves and petioles. Leaves triangular-

Table 1. A comparison of *Viola sandrasea* and *V. kizildaghensis*.

	Viola sandrasea	*V. kizildaghensis*
Indumentum	glabrous or rarely hairy, green	densely pubescent, grayish
Stipules	ovate-lanceolate to linear with stalked glandular fimbria generally longer than half wide of stipule above, shorter below, sometimes purplish tinged	ovate-lanceolate to linear-lanceolate, with mostly stalkless or shortly stalked glandular fimbria throughout, sometimes pinkish tinged
Leaves	triangular-ovate to lanceolate, glabrous or rarely pubescent only at veins and margins, minutely wrinkled on lower surfaces, with shallowly cordate to cuneate bases	lanceolate, rarely ovate when young, densely pubescent and wrinkled on both surfaces, with truncate to cuneate bases
Peduncles	glabrous with bracteoles at about the middle, sometimes purplish tinged at base	densely retrorsely pubescent with bracteoles at about the middle, sometimes pinkish tinged at base
Corolla	deep violet with a white throat, lateral petals beardless inside	purplish-pink with a white throat, lateral petals beardless inside
Sepals	oblong to oblong-lanceolate, glabrous, lateral ones with 1–2 stalkless rough gland at base	narrowly oblong, generally ciliate, with densely pubescent appendages, lateral ones with 1–4 stalkless rough gland at base
Capsules	glabrous	densely pubescent
Flowering	May–June	March–early April
Fruiting	June–July	late April

Table 2. Some features on leaf surfaces of *Viola sandrasea* and *V. kizildaghensis*.

	V. sandrasea		*V. kizildaghensis*	
	Upper surface	Lower surface	Upper surface	Lower surface
Stoma (1 mm^2)	98 ± 5 (90–105)	90 ± 4 (80–97)	106 ± 6 (94–115)	92 ± 4 (85–98)
Epidermis cells (1 mm^2)	194 ± 8 (180–212)	190 ± 8 (175–200)	190 ± 5 (180–198)	172 ± 5 (165–180)
Stoma lenght (μm)	26.2 ± 1.2 (24–28)	26.6 ± 1.0 (24–28)	24.4 ± 1.3 (22–26)	27 ± 1.5 (25–29)
Stoma width (μm)	21.1 ± 0.9 (20–23)	20.7 ± 0.8 (19–23)	19.6 ± 1.0 (18–22)	20.2 ± 0.4 (19–21)
Stoma index	33.6 ± 1.8 (29.8–36.8)	32.1 ± 1.6 (28.6–35.7)	35.8 ± 1.5 (32.2–38.9)	34.8 ± 1.3 (32.1–37.3)

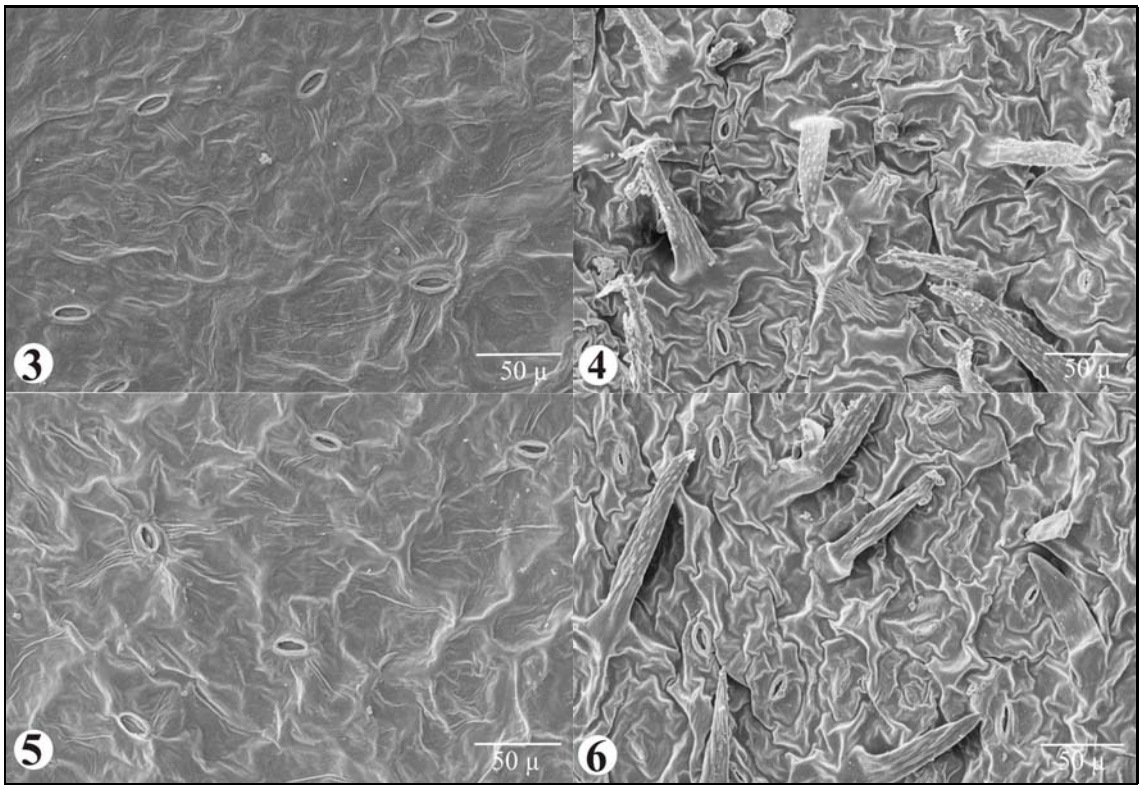
ovate to lanceolate, blade 5–50 × 5–20 mm, shallowly cordate to cuneate at the base, glabrous, rarely hairy especially at the veins and margins, crenate-serrate, petioles 15–80 mm, narrowly winged, glabrous. Stipules adnate at base of petioles, ovate-lanceolate to linear, 5–15 × 1–2.5 mm wide, membraneous, with 3–8 fimbriae on each side, sometimes purplish tinged. Fimbria glandular tipped, generally longer than half width of stipul above, shorter below. Peduncles (of chasmogamous flowers) quadrangular in section, 35–90 mm long, glabrous; bracteoles 2, borne at about the middle of the peduncle, 3–5 mm, glandular-fimbriate throughout or at the lower half, sometimes purplish tinged; peduncles of cleistogamous flowers much shorter. Sepals oblong to oblong-lanceolate, 3–5 × 1.1–2.1 mm; lateral ones with 1–2 rough stalkless gland at base; appendages 0.5–1 mm, glabrous. Flowers not fragrant, 7–14 mm in diameter, violet with a white throat. Lower petals 11–14 mm; lateral petals hairless inside, 9–11 mm; upper petals 9–11 mm; spur violet or whitish, 2–3 mm, curved upwards. Style 1.5–2 mm, sigmoid at the base, apex curved, beaked. Capsules borne on stalks which are procumbent at maturity, globose or obovoid, compressed at apex, 6–9 mm in diameter, glabrous. Seeds 2.5–3 × 1.2–1.5 mm with distinctive elaisomes.

The photographs of *V. sandrasea* and *V. kizildaghensis* are presented (Figs 1, 2). Morphological comparison of the two taxa are given in detail (Table 1) based on the investigation of the specimens from their type localities. *Viola sandrasea* and *V. kizildaghensis* differed in several morphological traits such as leaf shape, floral pigmentation and patterns of indument. Whereas, *V. sandrasea* is completely glabrous, or rarely hairy especially on leaf veins and margins; *V. kizildaghensis* is densely pubescent throughout. The leaves of *V. sandrasea* are deeply green, triangular-ovate to lanceolate, having shallowly cordate to cuneate bases; whereas, those of *V. kizildaghensis* are greyish green, lanceolate, and have cuneate bases. Finally, the flowers of *V. sandrasea* are violet, whereas those of *V. kizildaghensis* are purplish-pink.

The leaves epidermal structures on the upper and lower surfaces of the two species are similar on account of stomatal distributions, types and sizes. The leaves are amphistomatic, and the stomata are of anisocytic in both species. However, the surface wrinkles are fairly denser in *V. kizildaghensis* compared to *V. sandrasea*. The leaf surface of *V. kizildaghensis* has 90 ± 10 hairs per mm^2 , *V. sandrasea* on the other hand lack hairs or is sparsely haired at veins and margins (Figs 3–6, Table 2).

Pollen characteristics

In *V. sandrasea*, pollen small, polar axis 24–30 μm, equatorial axis 26–32 μm, P/E 0.80–1.08; shape in equatorial view suboblate to spheroidal, in polar view



Figs 3–6. Scanning electron photomicrographs of leaf surfaces. 3, 4 – Upper surfaces, 5, 6 – Lower surfaces. *V. sandrasea* (3, 5.), *V. kizildaghensis* (4, 6).

Table 3. Pollen morphological characters for *V. sandrasea* and *V. kizildaghensis*.

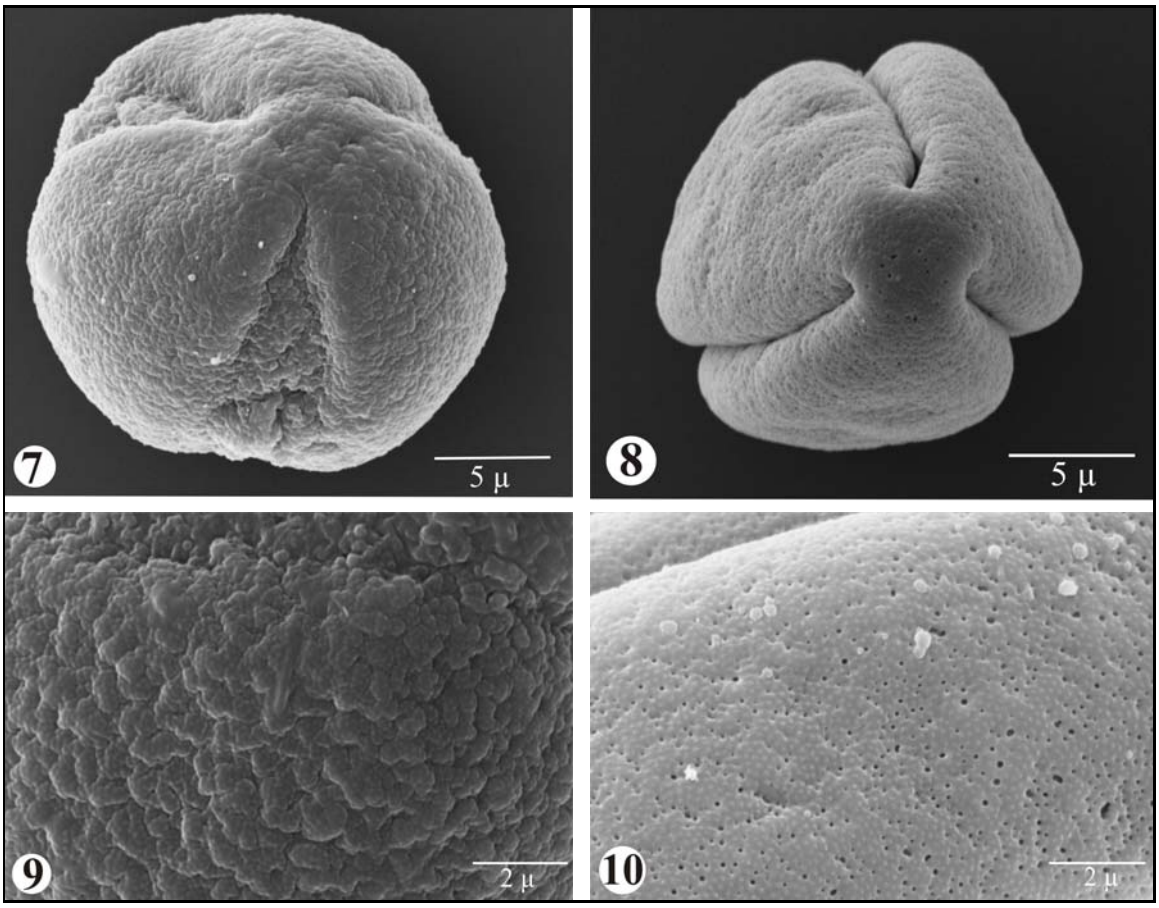
	V. sandrasea	V. kizildaghensis
Polar axis (μm)	26.56 ± 1.11 (24–30)	24.4 ± 0.96 (22–26)
Equatorial axis (μm)	30.21 ± 1.16 (26–32)	27.26 ± 0.88 (26–30)
P/E ratio	0.92 ± 0.5 (0.80–1.08)	0.90 ± 0.5 (0.78–1.00)
The shape equatorial view	suboblate to spheroidal	suboblate to spheroidal
Colpus length	20.28 ± 0.78 (18–21)	19.44 ± 0.50 (18–20)
Exine thickness (μm)	2.12 ± 0.5 (1.90–2.20)	1.94 ± 0.4 (1.80–2.00)
Intine thickness (μm)	0.58 ± 0.06 (0.50–0.65)	0.53 ± 0.05 (0.45–0.60)
Ornamentation	rugulate	psilate-perforate
The shape polar view	circular to subtriangular	circular to subtriangular
Aperture type	3-colporate	3-colporate

circular to subtriangular. Apertures 3, colporate with 18–21 μm long ectocolpi; colpus ends acute; the exine ornamentation rugulate. Exine 1.90–2.20 μm thick, intine 0.50–0.65 μm in mesocolpium.

In *V. kizildaghensis*, pollen small, polar axis 22–26 μm, equatorial axis 26–30 μm, P/E 0.78–1.00; shape in equatorial view suboblate to spheroidal, in polar view circular to subtriangular. Apertures 3, colporate with 18–20 μm long ectocolpi; colpus ends acute; the exine ornamentation psilate-perforate. Exine 1.80–2.00 μm thick, intine 0.45–0.60 μm in mesocolpium (Figs 7–10, Table 3).

Discussion

The Sandras Mountains on which *V. sandrasea* grows are an interesting and isolated area ascending from sea level to approximately 2500 m. The area is one of the main centres of endemism in Turkey (Davis 1971) and a shelter 76 Anatolian endemics. Out of these, 11 taxa, like *Viola sandrasea,* originate from this area (Özhatay 1986). Together with *Viola sandrasea*, the species such as *Scorzonera sandrasica* Hartvig & Strid, *Genista sandrasica* Hartwig & Strid, *Senecio sandrasicus* P.H. Davis, *Allium sandrasicum* Kollmann, *Ferulago sandrasica* Peşmen & Quézel, *Lamium sandrasicum* P.H. Davis, *Teucrium sandrasicum* O. Schwarz took their names fom the area, and are highly local endemics. On the contrary, *V. kizildaghensis* grows in Kızıldağ National Park located at a distance about 350 km from Sandras Mountains. *V. sandrasea* is thus geographically isolated from its closest relative. *V. sandrasea* is also seasonally isolated from *V. kizildaghensis*. Whereas, *V. sandrasea* blooms in May to June and fruits in June to July, *V. kizildaghensis* blooms in March to early April and fruits in the end of April.



Figs 7–10. Scanning electron photomicrographs of pollen grains. 7, 8 – Polar view of pollen grains, 9, 10 – Exine surfaces in detail. *V. sandrasea* (7, 9), *V. kizildaghensis* (8, 10).

The *Lignosae* group, including these two species as well, is ecologically composed of glacial refugee species and restricted to alpine regions near melting snow. *V. kizildaghensis* grows at a lower altitude compared to *V. sandrasea*. Therefore, the generative development period of *V. kizildaghensis* is faster and shorter compared to *V. sandrasea*.

V. sandrasea grows on stony places in the clearings of *Pinus nigra* J.F. Arnold subsp. *nigra* var. *caramanica* (Loudon) Rehder at an elevation of over 1800 m, with *Ornithogalum nivale* Boiss., *O. alpigenum* Stapf, *Primula vulgaris* Huds. subsp. v*ulgaris*, *P. auriculata* Lam., *Centaurea ensiformis* P.H. Davis., *Acinos troodi* (Post) Leblebici subsp. *vardaranus* Leblebici. *V. kizildaghensis* on the other hand grows in the clearings of *Cedrus libani* A.Rich. var. *libani* at an elevation of 1300–1600 m, with *Acantholimon caesareum* Boiss. & Balansa, *Ornithogalum narbonense* L., *O. ulophyllum* Hand.-Mazz., *Erysimum crassipes* Fisch. & C.A.Mey., *Alliaria petiolata* (M.Bieb.) Cavara & Grande, *Helianthemum canum* (L.) Baumg., *Saponaria kotschyi* Boiss., *Bromus tomentellus* Boiss., *Fritillaria whittallii* Baker and *Muscari neglectum* Guss.

Viola sandrasea has been only known from a few gatherings since 1939. In the earlier description, the stipules were given to be long-fimbriate and eglandular, the leaves up to 2.5 cm with subcordate to truncate bases (Melchior 1939; Coode & Cullen 1965). The present study showed that the stipules are long and short-fimbriate, and the fimbriae are glandular-tipped, and the leaves are up to 5 cm with shallowly cordate to cuneate bases.

The leaf shapes of the subsection *Viola* species are mostly overtopping. These are ovate to orbicular with cordate to cuneate bases. Those of *V. sandrasea* and *V. kizildaghensis* are partly overtopping as well, but are clearly different from their relatives. Lanceolate shaped leaves are only seen in the two species in subsection *Viola* (Becker 1910; Yuzepchuk 1949; Valentine et al. 1968; Marcussen & Nordal 1998). However, the leaves are fairly variable, triangular-ovate to lanceolate with shallowly cordate to cuneate bases in *V. sandrasea*. They are less variable, lanceolate or rarely ovate when young with truncate to cuneate in *V. kizildaghensis*.

Pigmentation has been known to be a highly variable feature below the species level, and floral albinism is found in the species of subsection *Viola* (Becker 1910; Marcussen & Nordal 1998). According to the field observation, corolla colour is invariable in the two species. All individuals have purplish-pink petals in the population of *V. kizildaghensis*, whereas those of *V. sandrasea* are deeply violet. The lateral petals are more or less bearded in the other species of subsection *Viola* (Becker 1910; Marcussen & Nordal 1998), but certainly not in all specimens of these two species studied in the present study.

Contandriopoulos & Quézel (1976) described *Viola sandrasea* subsp. *cilicica* as a new taxon. However, it was suggested that this was the same as *V. alba* ssp. *dehnhardtii* rather than a new, very disjunct subspecies of *V. sandrasea* (Davis et al. 1988). Indeed, this taxon neither could be collected from the type locality, nor seen in Turkish herbaria. Moreover, *V. alba* ssp. *dehnhardtii* was seen to grow in the putative type locality of *Viola sandrasea* subsp. *cilicica*. This situation might support to a great extent the argument of Davis et al. (1988). *Viola kizildaghensis* was given as the closest relative of Lebanese endemic *V. libanotica* Boiss. (Dinç & Yıldırımlı 2002). But, the present study suggests that *V. kizildaghensis* and *V. sandrasea* are the closest relatives by their leaf shapes and lateral sepals with rough glands at base, and with these features isolated from the other species of subsection *Viola*.

Although pollen morphology supports the segregation of some genera, it is often regarded as unsufficient criteria for separating the species (Moore et al. 1991). Moore et al. (1991) classified the pollen morphologies of some species of subsection *Viola* such as *Viola palustris*, *V. hirta*, *V. odorata* and some species of subsection *Rostratae* such as *V. riviniana*, *V. canina* under the group called *Viola palustris* type. In this group, the polen grains of subsection *Rostratae* species are trizonocolpate and tetrazonocolporate, but those of subsection *Viola* species are only trizonocolpate. In addition, polen grain is completely psilate, colpi is very long, and apocolpium is correspondingly small in *Viola palustris* type (Moore et al. 1991). The pollen grains completely exhibit the features of *V. palustris* type in *V. kizildaghensis*. But, the exine ornamentation clearly differs from *V. palustris* type in *V. sandrasea* (Figs 7, 9).

However the pollens of *V. sandrasea* are slightly larger than those of *V. kizildaghensis*. The pollen grains of the two species show similar characteristics in relation to pollen dimensions, the shapes and the aperture types (Table 3); but, the exine ornamentation clearly appear to have taxonomic value. Rugulate ornamentation is observed in *V. sandrasea*, but psilate-perforate ornamentation in *V. kizildaghensis* (Figs 9, 10). The present palynological data points out that the exine ornamentation may be used for interspecific classification of subsection *Viola* which is taxonomically critical.

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