

Anatomical and Ecological Study on Turkish Endemic *Viola kizildaghensis* M. Dinç and Ş. Yıldırım

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Abstract: In this study, anatomical and ecological properties of endemic *Viola kizildaghensis* M.Dinç and Ş. Yıldırım were determined. It was introduced to the scientific world from Kızıldağ National Park (Isparta-Şarkikaraağaç) in 2002 and is only known from there. For the anatomical studies, cross-sections of roots, leaves, petioles, stipules, peduncles and petals and surface sections of the leaves of *Viola kizildaghensis* were investigated. For ecological investigations, the chemical properties of the soil were analysed and the accompanying species were given. Also, conservation status of the species was proposed according to our observation on its population size and the distribution area.

Key words: *Viola kizildaghensis* · anatomy · ecology

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. The *Viola* L. is the largest genus of the family and has about 400 species in the world [1]. There are 33 natural *Viola* taxa belonging to 29 species and four subspecies in Turkey [2-11]. The species without stolons of the section *Viola* L. subsection *Viola* L. are, traditionally considered as series *Eflagellatae* [12, 13]. Series *Eflagellatae*, including *V. kizildaghensis*, consists of 11 species are all relictual with narrow endemic or disjunct distributions and native to the high montane and alpine regions of Central Europe, North Africa and the Middle East [8, 14-18].

The genus *Viola* has commercial significance. *Viola odorata*, included in section *Viola* like *V. kizildaghensis*, is grown for essential oils used in manufacture of perfumes, flavorings, toiletries and the very sweet, violet-colored liqueur *parfait amour*. The flowers of many species are preserved in sugar as crystallized violets used largely for decoration. Also, many species of *Viola* are grown as ornamentals [1]. Morphological and anatomical aspects of some species of subsect. *Viola* L. and

Rostratae Kuppfer were previously investigated [19, 20]. Here, we report on the anatomical and ecological features of local Turkish endemic *V. kizildaghensis* for the first time.

MATERIALS AND METHODS

Plant and soil samples of *Viola kizildaghensis* were collected from its type locality, Kızıldağ National Park. The specimens were dried according to standard herbarium techniques and stored at the Selçuk University Herbarium (KNYA). The collecting locality of the species as follows;

B3 isparta: Şarkikaraağaç, Kızıldağ National Park, stony slopes, 1400 m, 15.04.2002, M. Dinç 1160 and Y. Bağcı.

For anatomical analysis, some plant samples were fixed in 70% alcohol. Anatomical investigations were performed on the cross-sections of the root, petiole, peduncle, petal, stipule and leaves and the surface sections of leaves. The cross and surface sections were painted with basic fuchsin and covered with glycerin-gelatin [21]. Photographs of them were taken with a Olympus BX-50 microscope. The stomatal index and stomatal index rate were calculated as described by Meidner and Mansfield [22]. Soil sample was taken from 0-20 cm. The analysis of soil sample was done according

to organic matter Smith-Weldon method [23]; CaCO_3 Scheibler calcimeter method [24]; phosphorus Olsen method [25]; K, Ca, Mg N NH_4OAc method [23] and Cu, Fe, Mn, Zn at DTPA extract ICP-AES with apparatus [26].

RESULTS

Anatomical properties

Root: Periderm is multilayered on the outer surface of the root. Fellem elements are brown. Cortex is present under the periderm. It is parenchymatous and consists of 15-20 layered flattened quadrangular cells. Cambium can be distinguished in some parts of the border of cortex and central cylinder. It is 2-3 layered. The xylem comprises trachea and tracheids. Pith rays are 1-2 layered. In the pith region there is primary xylem tissue (Fig. 1).

Leaf: Leaves are covered by a thick cuticular layer on both surfaces. The upper and lower epidermis consists of a single row of ovoid, squarish and rectangular cells. There are some mucilaginous cells which are larger than the epidermis cells in upper and lower epidermis. Also, there are simple and one-celled hairs on both epidermis. Leaf is bifacial and amphistomatic, with anisocytic stomata. The number of stomata is 103 ± 4 on the upper epidermis and 88 ± 4 on the lower epidermis of the leaf. The stomata index is 35.4 for the upper epidermis and 35.2 for the lower epidermis. The stomata index rate is 1.005 (Table 1). There is a large vascular bundle on the median region of the leaf. Parenchymatic bundle sheath surrounds the vascular bundle. The collenchyma is present under both epidermis. Lower collenchyma tissue is 3-4 layered and the upper one is only 1-layered. Vascular bundles are collateral. There are two layers of palisade parenchyma cells below the upper epidermis. Also, 3-4 layers of spongy parenchyma cells are observed. There are druse crystals in some spongy parenchyma cells (Fig. 2-4).

Stipule: Stipules are covered by a thin cuticular layer on both surfaces. Epidermis consists of single layered ovoid cells on both surfaces. Mucilaginous cells are present among the epidermis cells. Stipule is unifacial. Mesophyll is not differentiated and consists of 3-6 layered spherical parenchymatic cells. Parenchymatic cells have intercellular space and lack chloroplast. Vascular bundle is near the upper epidermis. It is much reduced and surrounded by a parenchymatic bundle sheath (Fig. 5).

Petiole: Petiole is covered by ovoidal epidermal cells. There are simple and one-celled hairs on the epidermis.

Table 1: Some features on leaf epidermis of *Viola kizildaghensis*

	Upper surface	Lower surface
Number of stoma	103 ± 4	88 ± 4
Number of epidermis cells	188 ± 5	162 ± 5
Stoma length	$22-26 \mu$	$25-29 \mu$
Stoma width	$18-20 \mu$	$19-21 \mu$
Stoma index	35.4	35.2
Stoma index rate	1.005	

Table 2: Soil chemical analysis of *Viola kizildaghensis*

Analysis	Unit	Result
pH (1:2.5, soil:water)	---	8.10
CaCO_3 (lime)	%	2.25
Organic matter	%	6.04
NaCl (1:5, soil:water)	mS cm^{-1}	0.70
Phosphorus (P_2O_5)	mg kg^{-1}	6.10
Potassium (K)	mg kg^{-1}	529.00
Calcium (Ca)	mg kg^{-1}	3579.00
Magnesium (Mg)	mg kg^{-1}	481.00
Sodium (Na)	mg kg^{-1}	300.00
Copper (Cu)	mg kg^{-1}	0.54
Iron (Fe)	mg kg^{-1}	32.80
Zinc (Zn)	mg kg^{-1}	1.96
Mangan (Mn)	mg kg^{-1}	54.37

The collenchyma is present under the epidermis. Collenchyma consists of 4-5 layered cells at the corners of the petiole, whereas it is 1-2 layered at the other parts. Parenchymatous cortex is present under the collenchyma. Cortex cells are spherical, 15-20 layered with intercellular space. There is a large crescent shaped vascular bundle on the median region of the petiole. Also, a same shaped lateral small vascular bundle is present at each corner of the petiole. All three vascular bundles are collateral and the each one is covered by a parenchymatic bundle sheath. The xylem turns towards the upper side of the petiole and the phloem turns towards the lower side of the petiole (Fig. 6).

Peduncle: Peduncle is covered by ovoidal epidermal cells. There are simple and one-celled hairs on epidermis. The collenchyma consisting of 1-2 layered cells is present under the epidermis. There is parenchymatous cortex below the collenchyma. Cortex cells are spherical, 8-10 layered and with intercellular space. Endodermis is distinguished under the cortex. Endodermis consists of 1-layered hexagonal and ovoidal cells encircling vascular bundles. There are 4 vascular bundles in the median region of the peduncle. Vascular bundles are collateral and situated on circular axle. The xylem turns towards inside and the phloem turns towards outside. Pith consists of 12-15 layered spherical parenchymatic cells (Fig. 7).

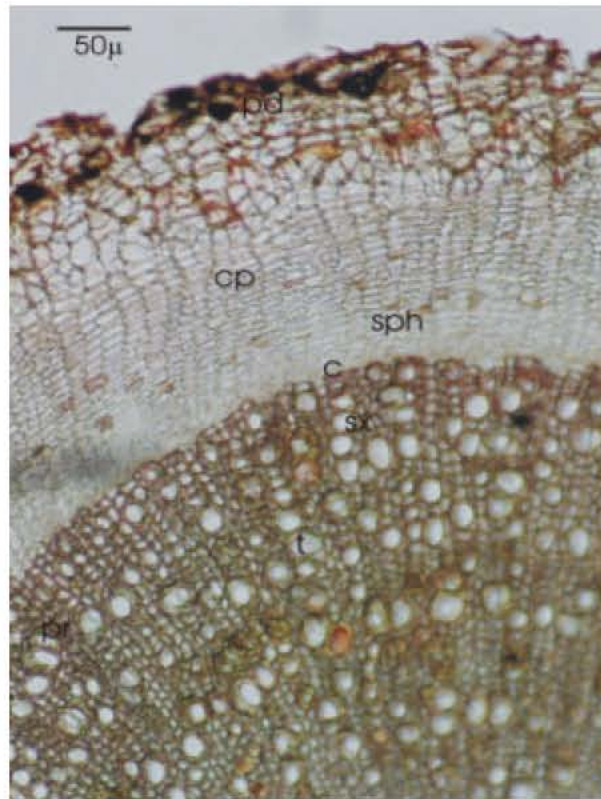


Fig. 1: Cross-section of root. pd) Peridermis, cp) Cortex parenchyma, sph) Secondary phloem, c) Cambium, sx) Secondary xylem, t) Trachea, pr) Pith ray



Fig. 2: Cross-section of leaf. h) Hair, cu) Cuticle, ue) Upper epidermis, mc) Mucilaginous cell, pp) Palisade parenchyma, sp) Spongy parenchyma, v) Vascular bundle, co) Collenchyma, le) Lower epidermis

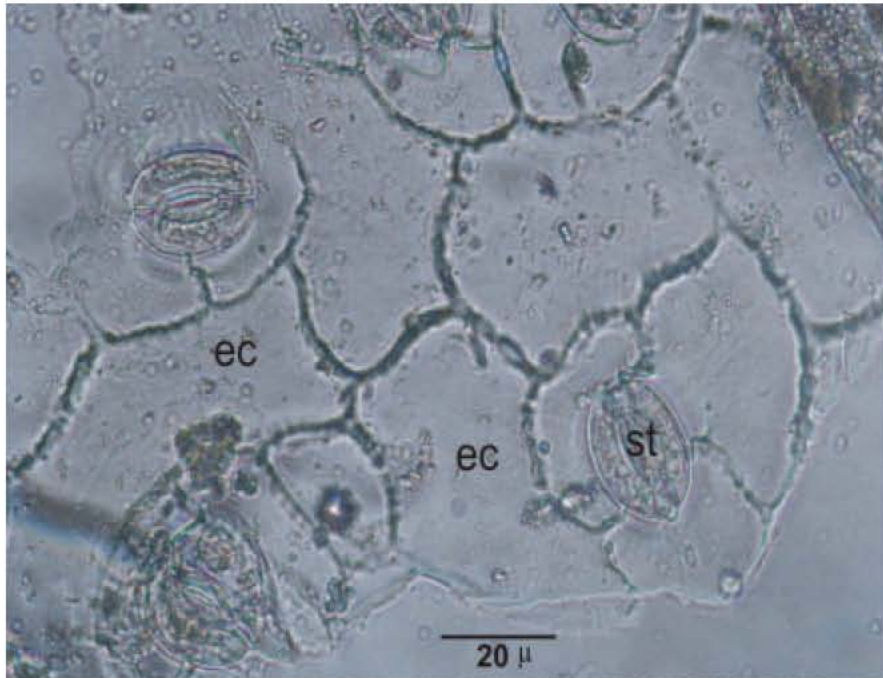


Fig. 3: Surface section of the upper side of leaf. st) Stoma, ec) Epidermis cell

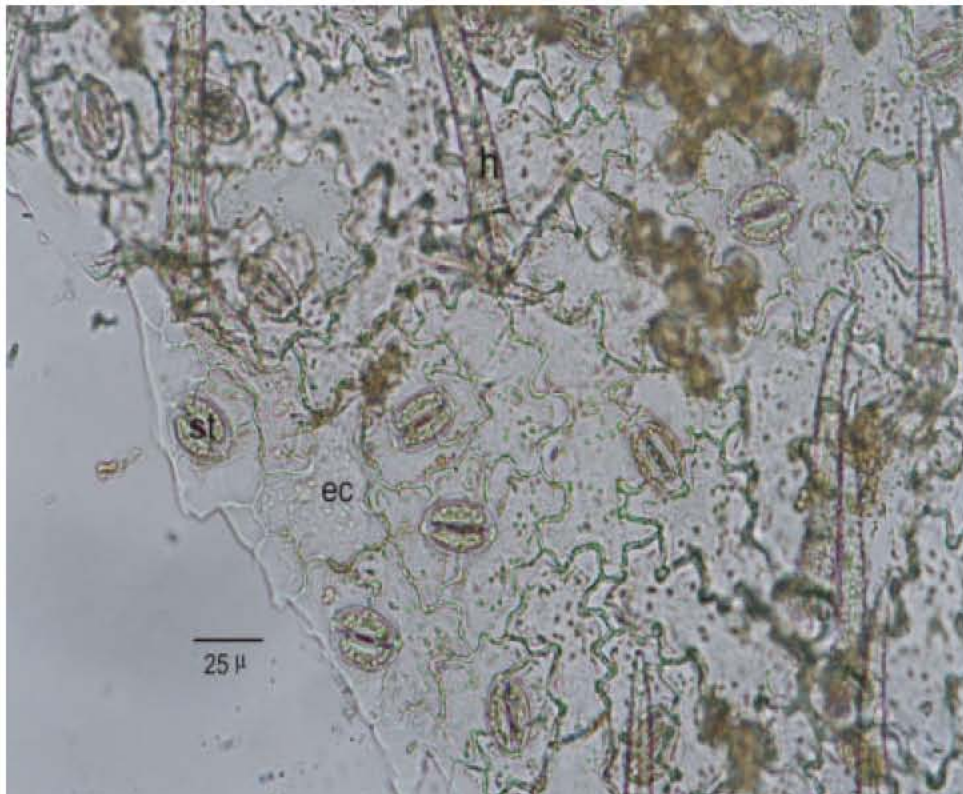


Fig. 4: Surface section of the lower side of leaf. st) Stoma, ec) Epidermis cell, h) Hair



Fig. 5: Cross-section of stipule. ue) Upper epidermis, mc) Mucilaginous cell, p) Parenchyma, v) Vascular bundle, le) Lower epidermis

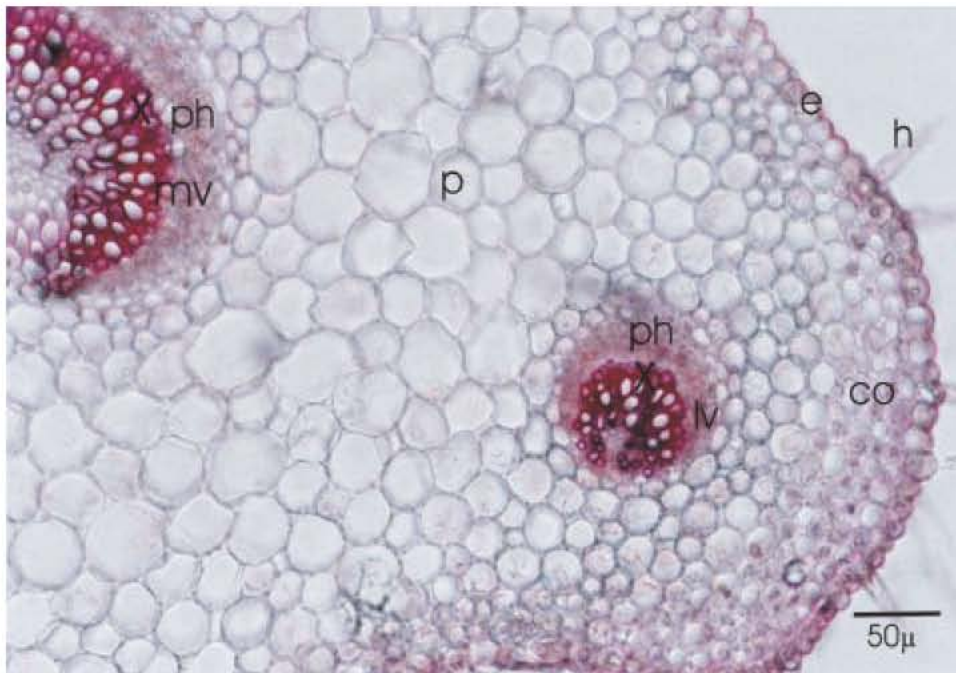


Fig. 6: Cross-section of petiole. h) Hair, e) Epidermis, co) Collenchyma, p) Parenchyma, mv) Median vascular bundle, lv) Lateral vascular bundle, ph) Phloem, x) Xylem

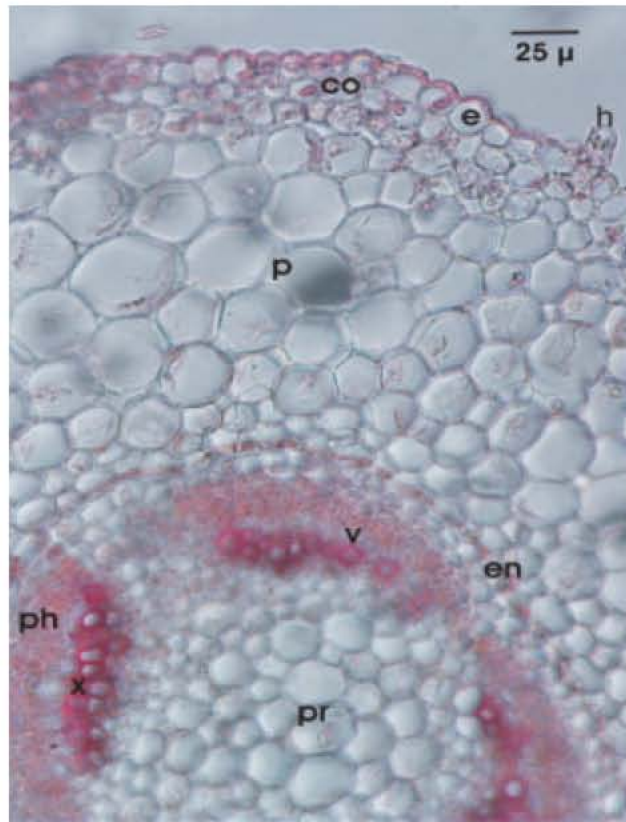


Fig. 7: Cross-section of peduncle. h) Hair, e) Epidermis, co) Collenchyma, p) Parenchyma, en) Endodermis, v) Vascular bundle, ph) Phloem, x) Xylem, pr) Pith region

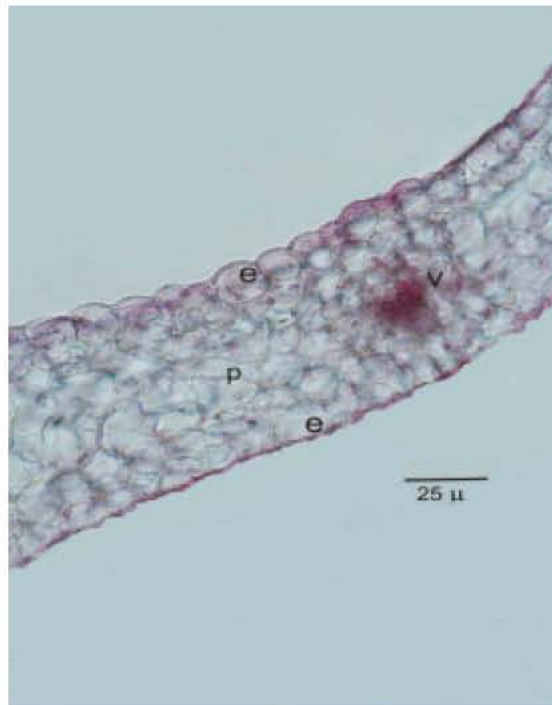


Fig. 8: Cross-section of petal. e) Epidermis, p) Parenchyma, v) Vascular bundle

Petal: Petals are covered by a thin cuticular layer on both surfaces. Epidermis consists of single layered ovoidal and angular cells on both surfaces and lack papilla. Mesophyll consists of 5-6 layered spherical parenchymatic cells. Parenchymatic cells have intercellular space. Vascular bundle is much reduced and occur in the middle region of petal in the cross-section (Fig. 8).

Ecological properties: *V. kizildaghensis* grows on stony slopes in the clearings of *Cedrus libani* forest at an elevation of 1350-1600 m. In this study, chemical features of the soil on which the species naturally grow were investigated. The soil sample was found to be rich of organic matters, to have medium pH values (alchalic), sufficient levels of P₂O₅, K, Mg, Na, Cu, Fe, Zn and Mn. Also, the soil was very poor in CaCO₃ and NaCl according to the results of the analyses (Table 2).

V. kizildaghensis is found with *Erysimum crassipes* Fisch and Mey., *Alliaria petiolata* (M.Bieb.) Cavara and Grande, *Helianthemum canum* (L.) Baumg., *Saponaria kotschyi* Boiss., *Bromus tomentellus* Boiss., *Fritillaria whittallii* Baker and *Muscari neglectum* Guss. according to our observation. The range of *V. kizildaghensis* is restricted to only one locality. The population include approximately 100 individuals and the area on which it grows occupies less than 10 km². Therefore, we suggest that *Viola kizildaghensis* should be placed under the IUCN category Critically Endangered (CR) [27].

DISCUSSION

General anatomical characteristics of *Viola* species were pointed out that they have generally dorsiventral leaves with gland-tipped teeth, transition stoma form between *Cruciferous* and *Rubiaceous*, simple and one-celled trichomes, epidermis often with mucillaceous, aggregate calcium oxalate crystals [28]. Paracytic stoma is also known as *Rubiaceous* type and anisocytic stoma also as *Cruciferous* type [29]. Our results basically agree with the characteristics pointed out by Metcalfe and Chalk [28]. But, the stomata of *V. kizildaghensis* are all anisocytic.

The *Viola* species in Turkey are divided into two sections as *Viola* L. and *Melanium* Gingins and sect. *Viola* is divided into two subsections as *Viola* and *Rostratae* according to Becker's [30] sectional classification. Akarsu [19] stated that *Viola odorata*, included in sect. *Viola* subsect. *Viola*, has amphistomatic leaves with one layered palisad parenchyma and the petal epidermis without papilla. Similarly, *V. kizildaghensis*, the other species of sect. *Viola* subsect. *Viola*, has

amphistomatic leaves and the petal epidermis lacking papilla according to our results. But, *V. kizildaghensis* differs in that its palisad parenchyma consists of two layered cells in cross section. It was also reported that although the petals lack papillae on epidermis in some species of sect. *Viola* subsect. *Viola*, they have papillate epidermis in some species of sect. *Viola* subsect. *Rostratae* [20]. Consequently, it can be said that papillate epidermis on the petals is an important anatomical character for the sectional classification of the genus *Viola*.

The stipule shape is one of the most important morphological characters in classification of *Viola* species [2]. It is not similar to the leaf in sect. *Viola*. However, it is green in subsect. *Rostratae* and not green in subsect. *Viola*. The results derived from the anatomical study on *V. kizildaghensis* exhibited that the stipule mesophyll is not differentiated and consists of 3-6 layered spherical parenchymatic cells without chloroplast. Namely, stipule anatomy is convenient with its morphological shape.

V. kizildaghensis grows on stony places in the clearings of *Cedrus libani* forest in where the soil were alchalic, without salt and lime and had much organic and inorganic matters according to our results. According to the floristic study previously carried out in the area, the average annual precipitation is 453.4 mm, the precipitation type is W.Sp.A.S, the most arid and hottest months are June and July with a mean temperature of 28.4°C, the mean temperature for the year is 11.2°C and the climate is Mediterranean semi-dry [31].

The conservation status of *V. kizildaghensis* should be graded as Critically Endangered according to our observation. The same assessment was made before [31].

V. kizildaghensis is found with *Erysimum crassipes* Fisch and Mey., *Briza humilis* M.Bieb., *Alliaria petiolata* (M.Bieb.) Cavara and Grande, *Helianthemum canum* (L.) Baumg., *Saponaria kotschyi* Boiss., *Bromus tomentellus* Boiss., *Pilosella hoppeana* (Sch.Bip.) Schultz and F.W.Schultz subsp. *cilicica* (NP) P.D.Sell and C.West, *Pilosella piloselloides* (Vill.) Sojak. subsp. *megalomatrix* (Naeg and Peter) P.D.Sell and C.West, *Fritillaria whittallii* Baker, *Silene cappadocica* Boiss and Heldr, *Silene spergulifolia* (Desf.) M. Bieb and *Muscari neglectum* Guss.

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