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Taxonomic and anatomical notes on *Viola* sect. *Viola* (Violaceae) in Iran

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Summary: Comparative anatomy of 7 taxa of *Viola* sect. *Viola* distributed in N and NW of Iran was studied. The investigated species are *V. alba*, *V. odorata*, *V. sintenisii* (subsect. *Viola*), *V. caspia*, *V. reichenbachiana* and *V. rupestris* (subsect. *Rostratae*). The following characters were determined to be taxonomically informative: presence or absence of phloem fibers in lateral stem cross-sections, the higher diameter of the pith region in taxa of subsect. *Rostratae* relative to taxa of subsect. *Viola*, the number of collenchyma layers in abaxial and adaxial surfaces and the variation of midrib outlines of the leaf lamina. *Viola rupestris* differs from other studied species in not having a protruding midrib in cross section. Our results show that comparative anatomy of lateral stems and leaves may provide additional characters to be considered in taxonomic studies within the sect. *Viola*.

Keywords: *Viola*, anatomy, taxonomy, Iran

The genus *Viola* L. comprises about 600 mostly herbaceous species and is distributed throughout most temperate regions of the world (Ballard et al. 1999; Yockteng et al. 2003). This genus is divided into 16 sections worldwide (Marcussen et al. accepted). Sect. *Viola*, one of the largest infrageneric groups of violets in Europe, is subdivided into subsect. *Viola* and subsect. *Rostratae* (Kupffer) W. Becker (Marcussen et al. 2012). The lateral stems of subsect. *Viola* and subsect. *Rostratae*, stolons and aerial stems, respectively, are believed to be homologous structures.

According to Yousefi et al. (2012), six species of sect. *Viola* are present in Iran, i.e. *V. alba* Bess. (as subsp. *alba*), *V. odorata* L., *V. sintenisii* W. Becker, *V. caspia* (Rupr.) Freyn, *V. reichenbachiana* Jord. ex Bor. and *V. rupestris* F. W. Schmidt. The taxonomy of three of the species has traditionally been confused in Iran (Khatamsaz 1991). *Viola alba* subsp. *alba* and *V. sintenisii* have been treated collectively as ill-defined *V. alba* subsp. *sintenisii* (W. Becker) W. Becker or *V. suavis* M. Bieb. *Viola caspia* has been misidentified as *V. sieheana* W. Becker in the Flora Iranica area (Schmidt 1992). On the basis of evidence from allozymes, morphology, ploidy levels and cross-compatibility, Marcussen & Borgen (2011) suggested that the Ponto-Caucasian plants belong to a separate species, *V. caspia*. Furthermore, the main distribution of *V. sieheana* s. str. is in the eastern Balkans, Turkey, Cyprus and in Lebanon, whereas *V. caspia* is restricted to Crimea, northeastern Turkey, Caucasus and the Caspian coast (Marcussen & Borgen 2011). *Viola caspia* can be further divided into two subspecies: *V. caspia* subsp. *caspia*, *V. caspia* subsp. *sylvestrioides* Marcussen, based on the corolla colour. The former taxon has whitish corollas while the corolla colour is lavender blue in the latter one and the two taxa differ considerably in their allozyme multilocus profiles. *Viola alba* occurs from the Caucasus, westwards to Central Europe and northern Spain (Marcussen 2003). *Viola odorata* is morphologically rather uniform and is commonly distributed in most parts of Europe and adjacent parts of Asia and North Africa (Marcussen 2006). *Viola sintenisii* is native to the Caucasus and western Asia (Marcussen et al. 2005) and is distributed on the Caspian coast from Azerbaijan to Turkmenistan. *Viola reichenbachiana* occurs from central Europe and is scattered eastwards to the Himalayas (eFloras

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Table 1. Collection data for *Viola* sect. *Viola* species examined for anatomy.

Species	Subsect.	Collection data
V. caspia (Rupr.) Freyn subsp. *caspia*	*Rostratae*	Iran: Guilan, Lahijan, Sheitan kuh, 300–400 m, March 2007, Yousefi, 13682
V. caspia subsp. *sylvestrioides* Marcussen		Iran: Ardebil, Almas road, 1700–1800 m, April 2010, Vafi & Yousefi,13753
V. reichenbachiana Jord. ex Bor.		Iran: Guilan, Asalem to Khalkhal road, 1500–1600 m, May 2010, Vafi & Yousefi, 13720
V. rupestris F. W. Schmidt		Iran: Ardebil, Almas road, 1700–1800 m, April 2009, Yousefi & Asaadi, 13672
V. alba Bess. subsp. *alba*	*Viola*	Iran: Guilan, Lahijan, 200–250 m, April 2011, Vafi & Saeidi, 13264
V. odorata L.		Iran: Ardebil, Almas road, 1700–1800 m, March 2008, Yousefi & Saeidi, 13665
V. sintenisii W. Becker		Iran: Guilan, Lahijan, Sheitan kuh, 300–400 m, February 2010, Vafi & Yousefi, 13675

2011). *Viola rupestris* is a Eurasiatic species with a wide distribution, ranging from Sakhalin in the Russian Far East to southern, central and northernmost Europe, although it is also present in the sub- to low-alpine belts in the mountains and the subarctic zone in Siberia and Caucasus (Nordal & Jonsell 1998).

There are several anatomical studies covering certain geographical areas of the genus *Viola* (Metcalf & Chalk 1950; Rubin & Paolillo 1978; Bağci et al. 2008; Dinç 2009). Morphometric studies of the European species have also been carried out (Marcussen & Borgen 2000; Marcussen et al. 2001; Hodálová et al. 2008). Recently, anatomical studies of root, stem, petiole and peduncle in *Viola* distributed in northern Iran have been published by Yousefi et al. (2012).

This paper provides a detailed description of anatomical features of lateral stems and leaves in taxa of sect. *Viola* growing in Iran and evaluates the systematic significance of such characteristics.

Materials and methods

Plants were collected from natural habitats of northern Iran and each specimen was given a voucher and a collector's number. The specimens were dried according to standard herbarium methods and are kept at Guilan University Herbarium. The collection data for the examined specimens are given in Table 1. Collected live materials were fixed in 70% alcohol for 3 days. Transverse sections of lateral stems and leaves were made manually using commercial razor blades. The cross sections were stained with methylene blue and Congo red and covered with glycerin gelatin (Vardar 1987). Photographs were taken with an Olympus BH-2 microscope under 100× and 400× magnifications.

All measurements and observations were made three or four times from several sections taken from at least two selected specimens. For each taxon three to four replicates from several cross-sections of the selected specimens were measured. Midrib outline was classified using current terminology described in Croat & Bunting (1979).

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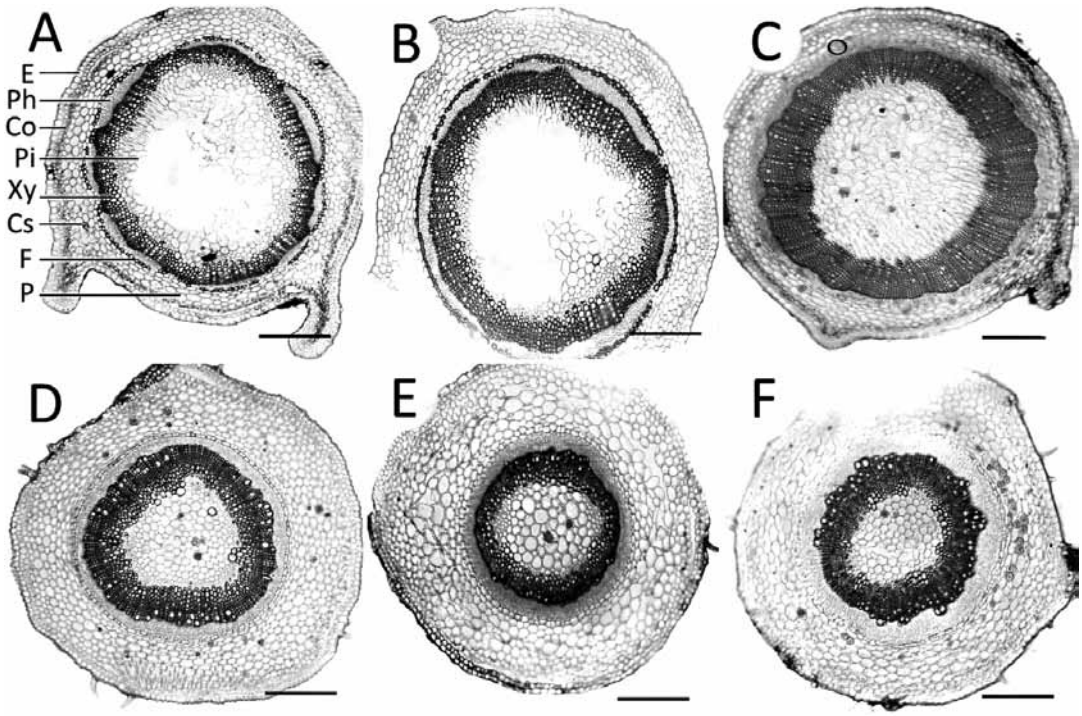

Figure 1. Cross-sections of lateral stems in *Viola* sect. *Viola*: A – *V. caspia* subsp. *caspia*; B – *V. caspia* subsp. *sylvestrioides*; C – *V. reichenbachiana*; D – *V. alba* subsp. *alba*; E – *V. odorata*; F – *V. sintenisii*.
Abbreviations: E = epidermis, Ph = phloem, Co = collenchyma, Pi = pith region, Xy = xylem, Cs = crystal storage, F = fiber, P = parenchyma. Scale bars = 0.5 mm.

Results

Cross-sections of lateral stems (Fig. 1 A–F; Table 2). In taxa of subsect. *Rostratae*, cross-sections of lateral stems are circular to semi-circular in shape with two wings, while in subsect. *Viola* they are circular with or without one wing. The epidermis in taxa of subsect. *Viola* consists of simple and unicellular trichomes and 1–2 internal layers of collenchyma. Inside the collenchymatic cell layer is a parenchymatous zone with circular cells and intercellular spaces. The presence of phloem fibres in *V. caspia* is evident. The pith region consists of spherical parenchymatous cells which store calcium oxalate crystals in taxa of both subsections. The diameter of the pith region in taxa of subsect. *Rostratae* are larger than those of subsect. *Viola*.

Leaf cross-sections (Fig. 2 A–G; Table 3). The midrib outline of the studied taxa varies along the leaf lamina. This variation is different on the adaxial and abaxial surfaces. In cross-sections, the median veins of all species are convex, except *V. rupestris* which is flat. The external surface is covered by a layer of quadrangular to ovoidal epidermal cells, which is cutinized and possesses mucilagous cells on both surfaces. The size of epidermal cells varies between species and between the adaxial and abaxial surfaces of the same species. Simple and unicellular trichomes are present on the epidermis with more density on the upper surface. The collenchyma, consisting of two to four cell layers, is located between the epidermis and the parenchymatous cortex around the vascular bundle. The number of the collenchyma differs in the upper and lower surface of leaves. All species are characterised by the presence of crystal cells. Unicellular trichomes on the adaxial side of leaves are restricted only to the midrib region of *V. alba* subsp. *alba* and *V. odorata*. The

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Table 2. Lateral stem cross-sections of *Viola* species examined.
Abbreviations: PD = pith diameter, LSD = lateral stem cross-section diameter, FP = Presence of fibre in phloem, b/a = the ratio of lateral stem cross-section diameter to pith diameter.

Species	Subsect.	PD (a) µm	LSD (b) µm	b/a	FP	Shape
V. caspia subsp. *caspia*	*Rostratae*	(366) 388±22 (401)	(1020) 1067±48 (1113)	2.75	+	Circular to semi-circular with 2 wings
V. caspia subsp. *sylvestrioides*		(395) 430±35 (467)	(685) 722±37 (760)	1.8	+	Circular to semi-circular with 2 wings
V. reichenbachiana		(705) 748±40 (786)	(1516) 1563±42 (1604)	2.09	+	Circular to semi-circular with 2 wings
V. alba subsp. *alba*	*Viola*	(198) 216±17 (237)	(713) 749±28 (779)	3.46	–	Circular with 1 wing
V. odorata		(353) 379±24 (304)	(977) 1018±62 (1080)	2.69	–	Circular without wing
V. sintenisii		(196) 211±13 (226)	(895) 940±55 (995)	4.46	–	Circular without wing

mesophyll comprises one to three layers of palisade parenchyma and loosely arranged spongy parenchyma. The veins shape ranges from semi-orbicular (Fig. 2 A, C) to narrow elliptical (Fig. 2 E, F) and oblong (Fig. 2 B, D, G).

Discussion

Our anatomical results indicate that characters such as the number of wings in lateral stem cross-sections (0–1 in subsect. *Viola* vs. 2 in subsect. *Rostratae*) and the diameter of the pith region are useful for the delimitation of species within sect. *Viola*. Within *V. caspia*, the ratio of lateral stem cross-section diameter to pith diameter is significantly greater ($p<0.05$) in subsp. *caspia* (ratio: 2.75) than in subsp. *sylvestrioides* (ratio: 1.8). Vegetative characteristics, such as leaf lamina outline in cross-section, have already been applied to the diagnosis of plant species (Metcalf & Chalk

Table 3. Leaf cross-sections of *Viola* species examined.
Abbreviations: CB = Number of collenchyma in abaxial surface; CD = Number of collenchyma in adaxial surface.

Species	Subsect.	CB	CD	Median veins shape
V. caspia subsp. *caspia*	*Rostratae*	2	1	Convex
V. caspia subsp. *sylvestrioides*		2	1	Convex
V. reichenbachiana		3	1	Convex
V. rupestris		3	1	Flat
V. alba subsp. *alba*	*Viola*	3	2	Convex
V. odorata		3	1	Convex
V. sintenisii		2	1	Convex

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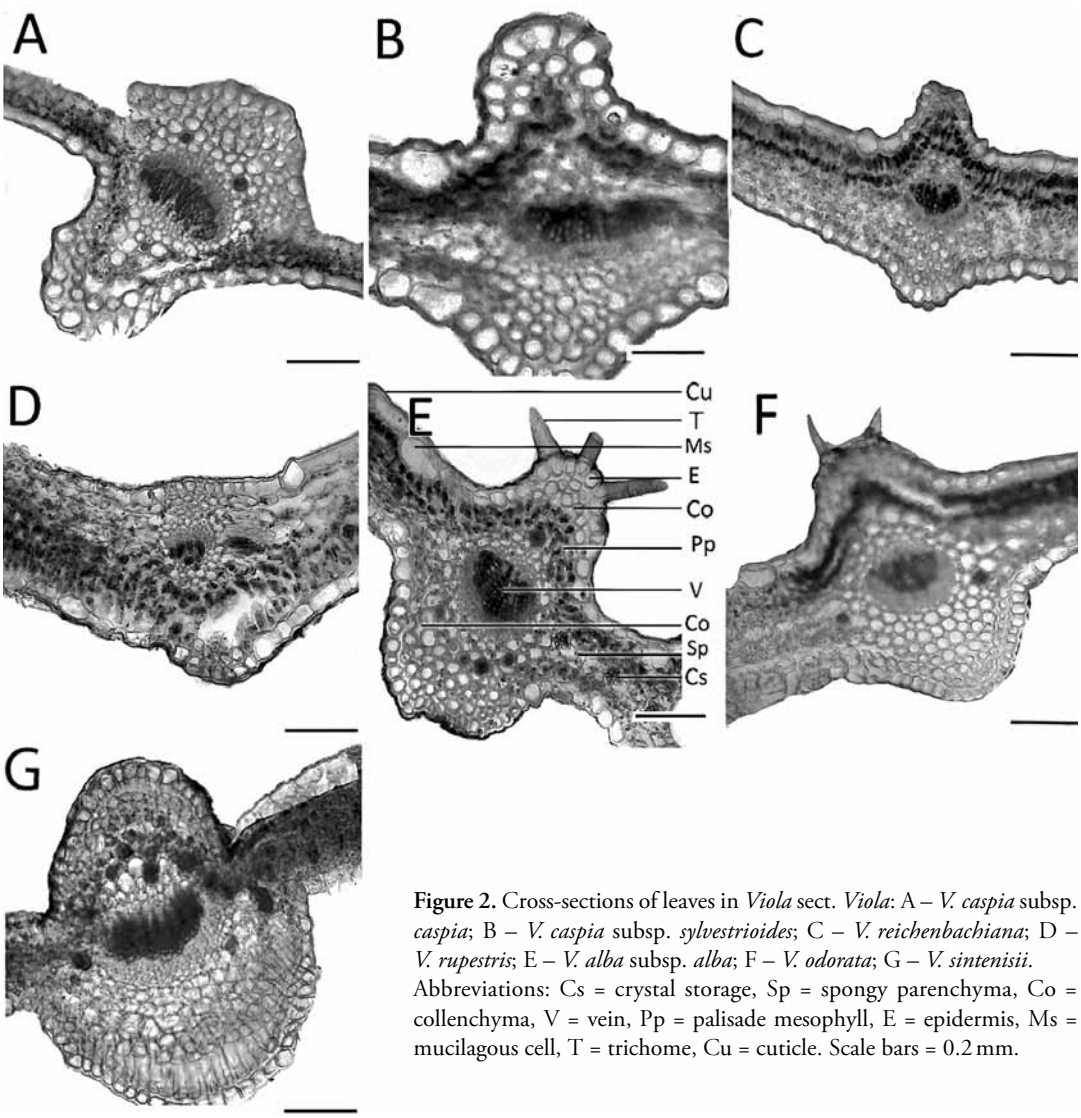

Figure 2. Cross-sections of leaves in *Viola* sect. *Viola*: A – *V. caspia* subsp. *caspia*; B – *V. caspia* subsp. *sylvestrioides*; C – *V. reichenbachiana*; D – *V. rupestris*; E – *V. alba* subsp. *alba*; F – *V. odorata*; G – *V. sintenisii*. Abbreviations: Cs = crystal storage, Sp = spongy parenchyma, Co = collenchyma, V = vein, Pp = palisade mesophyll, E = epidermis, Ms = mucilagous cell, T = trichome, Cu = cuticle. Scale bars = 0.2 mm.

1950; Leo et al. 1997; Sajo et al. 1995). The midrib outline of the leaf lamina in taxa of sect. *Viola* is highly variable and could be useful for the taxonomy of the genus.

Within subsect. *Viola*, *V. alba* subsp. *alba* and *V. sintenisii* are morphologically very similar and also closely related but clearly distinguishable based on allozyme data (Marcussen et al. 2005; Malécot et al. 2007). The two species further differ in details of root anatomy, where a pith region is present in *V. sintenisii* but absent in *V. alba* subsp. *alba* root cross-sections (Yousefi et al. 2012); and also in pollen exine, which is perforate-subpsilate and perforate-granulate in *V. alba* subsp. *alba* but perforate-granulate in *V. sintenisii* (Saeidi Mehrvarz et al. accepted). This study on leaf cross-sections shows that *V. alba* subsp. *alba* has three collenchyma layers in its abaxial surface and two collenchyma layers in its adaxial surface; whereas *V. sintenisii* has two collenchyma layers in abaxial surface and one collenchyma layer in the adaxial surface (Fig. 2 E, G). In addition, the abaxial midrib surface of *V. alba* subsp. *alba* is concave, but it is more prominent in *V. sintenisii*.

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Our study shows that *V. caspia* is morphologically the most variable species of sect. *Viola* in Iran. Among the analysed *Viola* species, the adaxial surface of the midrib is flat only in *V. rupestris* (subsect. *Rostratae*). *Viola caspia*, which has two collenchyma layers in the abaxial surface and one layer in the adaxial surface, can be distinguished from *V. rupestris* and *V. reichenbachiana*, both of which have three collenchyma layers in the abaxial surface and one collenchyma layer in the adaxial surface. Yousefi et al. (2012) have reported that the number of vascular bundles in the stem cross-section is a useful character for separating *Viola* species; they concluded that *V. rupestris* with seven vascular bundles can be distinguished from *V. caspia* and *V. reichenbachiana* with eleven and with eight vascular bundles, respectively. Saeidi Mehrvarz et al. (accepted) reported that the pollen grains of *V. caspia* are prolate-spheroidal in equatorial view and with granulate exine, while *V. rupestris* and *V. reichenbachiana* have oblate-spheroidal pollen grains with a perforate-granulate exine. Despite high morphological similarity between *V. caspia* subsp. *caspia* and *V. caspia* subsp. *sylvestrioides*, they can be differentiated by anatomical characters. The abaxial midrib surface of *V. caspia* subsp. *caspia* is U-shaped, whereas it is concave in *V. caspia* subsp. *sylvestrioides*. The results reveal that molecular studies are essential for solving the problems of relationships and taxonomy of taxa of this section.

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