

Supplementary material

Title: Species delimitation in the Ponto-Caucasian *Viola sieheana* complex, based on evidence from allozymes, morphology, ploidy levels, and crossing experiments

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Supplementary material A. *Viola* materials examined using allozymes, flow-cytometry and chromosome counts. Single asterisks indicate localities with more than one species present. Counts on *V. caspia* were performed on artificial interpopulation crosses, for the whitish-coloured morph 234×236 (**) and for the blue-flowered morph 221×226 (***). All collections were made by the authors, except where indicated: (KB) K. Blaxland; (MD) M. Dinç; (MR) M. Ronikier; (WK) W. Klettring.

Accession number	Locality	Allozymes <i>N</i>	Flowcytometry <i>N</i>	Counts <i>N</i>
<i>V. caspia</i> (whitish-flowered morph)				
237	Azerbaijan, İsmayilli: 1 k m S of Təzəkənd, 800 m	4	1	.
234	Azerbaijan, İsmayilli: nr. Qalaçıq, 500 m	4	2	**1
238	Azerbaijan, İsmayilli: nr. Təzəkənd, 800 m	2	.	.
235	Azerbaijan, Şamaxı: Ağsu River valley, W of Şamaxı, 700 m	2	1	.
232	Azerbaijan, Şəki: Hill btw. Şəki & Baş Zəyzit, 1600 m	2	2	.
231-1	Azerbaijan, Şəki: Bottom of hill btw. Şəki & Baş Zəyzit	1	1	.
<i>V. caspia</i> (blue-flowered morph)				
221	Azerbaijan, Quba: Btw. Quba & Qəçrəs, 800 m	5	5	***1
222	Azerbaijan, Quba: Qəçrəs, 1000 m	4	3	.
226	Azerbaijan, Qəbələ: Vəndəm, along Qaraçay River creek, 800 m	4	4	.
231-2	Azerbaijan, Şəki: Top of hill btw. Şəki & Baş Zəyzit	1	1	.
<i>V. caspia</i> (introgressants between whitish- and blue-flowered morph)				
236	Azerbaijan, Qusar: Çıləgir, 800 m	5	3	.
<i>V. dirphys</i>				
352	Greece, Evvia: Mt Dirphys, above the the refuge, 1170-1200 m	6	.	.
*357	Greece, Evvia: Mt Dirphys, along the dirtroad S of the pass of the Steni-Stropones road, 1040 m	11	1	.
353	Greece, Evvia: Mt Dirphys, btw. the refuge and the summit, 1140 m	8	.	.
351	Greece, Evvia: Mt Dirphys, close to the refuge, 1160 m	3	1	.
<i>V. oligyrtia</i>				
336	Greece, Argolida: Mt Oligyrtos, 1 km NW of the pass, along dirtroad, 1240 m	11	1	.
337	Greece, Argolida: Mt Oligyrtos, 900 m NW of the pass, along dirtroad, 1275 m	4	.	.
<i>V. reichenbachiana</i>				
322	Greece, Arkadia (Párnon): 1.5 km E of Agios Petros, 800 m	7	.	.
*320	Greece, Arkadia (Párnon): Agios Petros, 850 m	3	.	.

333	Greece, Arkadía: btw. Limbovisi & Eláti, 1370 m	1	.	.
356	Greece, Evvia: btw. Metohio & Kymi, 540 m	3	.	.
*355	Greece, Evvia: Highest point on the dirtroad btw. Stropones & Metohio, 700 m	2	.	.
*357	Greece, Evvia: Mt Dirphys, along the dirtroad S of the pass of the Steni-Stropones road, 1040 m	4	.	.
478	Greece, Ioánnina: nr. St. Paraskevi Monastery, btw. Aristi & Papigo (MR)	1	.	.
*350	Greece, Kardítsa: btw. Neraída & Klisto, 1170 m	2	.	.
*348	Greece, Kardítsa: btw. Sarandoporo & Neraída, 710 m	7	.	.
*343	Greece, Kardítsa: Hill above Kastania, 900 m	4	.	.
*346	Greece, Kardítsa: N side of Mt Itamos, btw. Karoplesi & Rahoúla, 1040 m	3	.	.
*344	Greece, Kardítsa: Roadside btw. Mucha & Lake Tavropoú weir, 890 m	3	.	.
*326	Greece, Messinía (Taÿgetos): 3 km S of Langada Pass along dirtroad to Pigádia, 1460 m	7	.	.
*327	Greece, Messinía (Taÿgetos): 3.5 km S of Langada Pass along dirtroad to Pigádia, 1460 m	2	.	.
456	Turkey, A7 Trabzon: Zumela nr. Trabzon (WK)	1	.	.

V. reichenbachiana* × *sieheana

*320	Greece, Arkadía (Párnon): Agios Petros, 850 m	4	.	.
*344	Greece, Kardítsa: Roadside btw. Mucha & Lake Tavropoú weir, 890 m	3	.	.
*326	Greece, Messinía (Taÿgetos): 3 km S of Langada Pass along dirtroad to Pigádia, 1460 m	3	2	.

V. riviniana

341	Greece, Achaía: Mt Chelmos above the ski centre, 2100 m	4	.	.
430	Greece, Ioánnina: Mt Smolikas (KB)	1	.	.
338	Greece, Korinthía: Mt. Kyllini, NW slope along dirtroad btw. Trikala & Agios Nikolaos above Goúra, 1550 m	6	.	.

V. sieheana

461	Cyprus, Lemesos: Troödos Massif, btw. Platres & the Caledonia Falls	16	14	.
462	Cyprus, Lemesos: Troödos Massif, btw. the Presidential Cottage & the Kyros river	4	4	.
464	Cyprus, Lemesos: Troödos Massif, btw. Troöditissa Monastery & Trikoukia E of Prodomos	3	3	.
470	Cyprus, Lemesos: Troödos Massif, c 1 km S of Mesopotamos, above Saïttas	1	1	.
471	Cyprus, Lemesos: Troödos Massif, nr. Kouris creek btw. Mesapotamos & Platres	4	4	1
469	Cyprus, Lemesos: Troödos Massif, Saïttas, along Mesapotamos creek	4	4	.
463	Cyprus, Lemesos: Troödos Massif, Troöditissa picnic area, E of the Monastery	5	4	.
475	Cyprus, Nicosía: 2 km E of TM-474	3	3	.
473	Cyprus, Nicosía: Above Sarándi, btw. Polystypos & Spília	6	5	.
474	Cyprus, Nicosía: nr. B9 S of Kakopetria	4	3	.
476	Cyprus, Nicosía: Pafos forest, 1 km E of Cedar Valley	4	4	1
465	Cyprus, Nicosía: Troödos Massif, above Prodomos among the road to Troödos	2	2	.

466	Cyprus, Nicosía: Troödos Massif, Mt. Olympos, 100 m W of the Ski Centre btw. Prodromos & Troödos	2	1	.
468	Cyprus, Nicosía: Troödos Massif, nr. Restaurant Platania above Pedoulas, btw. Kakopetria & Prodromos	5	5	.
467	Cyprus, Nicosía: Troödos Massif, Pedoulas	3	3	.
334	Greece, Arkadía (Mainalon): 500 m E of Alonistena, 1200 m	6	6	.
335	Greece, Arkadía (Mainalon): Btw. Kardaras & N. Kardaras, 990 m	6	6	1
*320	Greece, Arkadía (Párnon): Agios Petros, 850 m	6	4	.
*355	Greece, Evvia: Highest point on the dirtroad btw. Stropones & Metohio, 700 m	3	3	.
354	Greece, Evvia: Mt Dirphys, nr. the refuge on the road to Steni, 1080 m	1	1	.
345	Greece, Kardítsa: 1 km from Kastania on the road to Mucha, 835 m	3	3	.
349	Greece, Kardítsa: btw. Neraída & Klisto, 1030 m	3	3	.
*350	Greece, Kardítsa: btw. Neraída & Klisto, 1170 m	4	4	.
*348	Greece, Kardítsa: btw. Sarandoporo & Neraída, 710 m	1	1	.
*343	Greece, Kardítsa: Hill above Kastania, 900 m	3	3	.
*346	Greece, Kardítsa: N side of Mt Itamos, btw. Karoplesi & Rahoúla, 1040 m	8	8	.
*344	Greece, Kardítsa: Roadside btw. Mucha & Lake Tavropoú weir, 890 m	8	8	.
*326	Greece, Messinía (Taÿgetos): 3 km S of Langada Pass along dirtroad to Pigádia, 1460 m	5	4	.
328	Greece, Messinía (Taÿgetos): 3.2 km S of Langada Pass along dirtroad to Pigádia, 1460 m	6	6	.
*327	Greece, Messinía (Taÿgetos): 3.5 km S of Langada Pass along dirtroad to Pigádia, 1460 m	4	4	.
457	Turkey, A8 Artvin: Artvin, 1450 m	2	1	.
482	Turkey, C3 Konya: Beyşehir, Kuruçuoğlu, 1500 m (MD)	3	.	.
483	Turkey, C5 Kayseri: Yahyalı, Aladağ National Park, around Kapuzbaşı village, 800 m (MD)	10	1	.
sum:		296	154	5

Supplementary material B. Allozyme band frequencies within the investigated taxa of *Viola*: *V. caspia* split down to the blue and whitish colour morphs and one population (TM-236) of introgressants between the two morphs, *V. dirphya*, *V. oligyrtia*, *V. reichenbachiana*, *V. riviniana*, *V. sieheana* s. str., and the sterile F₁ hybrid *V. reichenbachiana* × *sieheana*. Ploidy levels, average number of allozyme bands (*excluding IDH), and average band frequency (*excluding IDH) are shown.

Taxon	2n=	N=	Average number of bands*	Average band frequency*	AAT					ACO						GPI				
					a	b	c	d	e	a	b	c	d	e	f	a	b	c	d	e
<i>caspia</i> whitish	40	13	18.15	0.92	1	.	1	1	.	0.62	1	.	1	0.77	0.85	.	.	.	1	.
<i>caspia</i> whitish × bluish	40	5	18.20	0.88	1	.	1	1	.	0.80	0.80	.	1	0.80	0.80	.	.	0.40	1	.
<i>caspia</i> blue	40	15	15.20	0.91	1	.	.	1	.	.	1	.	1	.	.	.	.	1	0.93	.
<i>dirphya</i>	40	28	18.86	0.94	.	1	.	1	.	.	0.86	1	1	.	.	.	.	1	.	.
<i>oligyrtia</i>	40	15	19.00	1.00	.	1	1	1	.	.	1	1	1	1	.	.	.	1	1	.
<i>reichenbachiana</i>	20	50	13.44	0.67	0.48	.	0.54	0.98	.	.	.	1	1	.	.	0.02	.	0.02	0.20	0.56
<i>riviniana</i>	40	11	16.91	0.85	0.91	.	1	1	.	.	.	1	1	.	.	.	.	0.91	0.09	0.91
<i>sieheana</i>	60	148	19.98	0.74	.	.	1	1	0.36	.	0.98	0.99	1	0.21	.	.	0.01	1	0.02	0.07
<i>reichenbachiana</i> × <i>sieheana</i>	40	10	22.20	0.82	0.70	.	1	1	0.70	.	1	1	1	0.50	.	.	.	1	0.30	0.30

				IDH			MDH					PGM										SDH		
<i>f</i>	<i>g</i>	<i>h</i>	<i>i</i>	<i>a</i>	<i>b</i>	<i>c</i>	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>e</i>	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>e</i>	<i>f</i>	<i>g</i>	<i>h</i>	<i>i</i>	<i>j</i>	<i>k</i>	<i>a</i>	<i>b</i>
.	1	1		1	1	.	1	1	1	.	.	.	1	.	.	.	0.69	0.23	1	1	.	.	1	.
.	0.80	0.80	0.20	1	.	1	1	1	1	.	.	.	1	.	.	.	0.80	.	1	1	.	.	1	.
.	0.67	.	1	1	.	1	1	1	1	.	.	.	1	.	.	.	1	0.13	0.47	1	.	.	1	.
1	.	.	1	—	—	—	1	1	1	.	1	.	1	1	.	1	.	1	1	1	.	.	1	.
.	1	.	1	—	—	—	1	1	1	.	.	1	1	.	.	.	.	1	.	1	.	.	1	.
0.20	1	.	.	—	—	—	1	1	1	.	.	0.62	0.82	.	.	.	.	1	.	1	.	.	0.54	0.46
.	1	.	.	—	—	—	1	1	1	.	.	0.09	1	.	.	0.09	0.91	1	.	1	.	1	1	.
0.67	0.86	.	0.86	—	—	—	1	1	1	0.98	.	.	1	0.52	0.44	.	1	1	.	0.99	0.01	0.99	1	.
0.40	1	.	1	—	—	—	1	1	1	1	.	0.40	1	0.60	.	.	1	1	.	1	.	1	1	0.30