Genetic and morphological variation in the *Viola suavis* group in the western Balkan Peninsula

Marek Slovák, Jaromír Kučera, Judita Zozomová-Lihová, Iva Hodálová, Roman Letz, Pavol Mereďa Jr.

Institute of Botany, Slovak Academy of Sciences, Bratislava, Slovak Republic; e-mail: pavol.mereda@savba.sk

Kornati archipelago, Croatia. Habitats of *Viola suavis* subsp. *adriatica*.

I. Introduction

Viola suavis group

- **infrageneric classification:** *Viola* sect. *Viola* subsect. *Viola* – a Eurasian group of ca 25 species; both tetraploids (2n = 20) and octoploids (2n = 40)
- **diagnostic characters:** short and stout stolons, long-fimbriate stipules, bracteoles inserted below the middle of peduncle, calycine appendages appressed to the peduncle, 2n = 40
- **distribution:** Mediterranean Basin and the Middle East, introduced to C & N Europe
- **taxonomically critical species/species complex:** numerous (infra)specific taxa described; at the species level e.g.: 1819 – *V. suavis*, 1849 – *V. sepincola*, 1857 – *V. beraudii*, 1872 – *V. austriaca*, 1872 – *V. cyanea*, 1884 – *V. adriatica*, 1889 – *V. segobricensis*, 1918 – *V. pontica*, 1929 – *V. catalonica*

Results of our previous studies (Mereďa et al. 2008)

- **4 genetically and morphologically differentiated types** within *V. suavis* s.l. in Europe: 1) blue-flowered type from central and south-eastern Europe (C&SE Europe hereafter) (=*V. suavis* s. str.), 2) white-flowered type from C&SE Europe, 3) blue-flowered type from Spain, 4) white-flowered type from Spain (syn. *V. catalonica*)

Viola suavis group in the western Balkan Peninsula

- **one taxon from the *V. suavis* complex recognised on the Adriatic sea-coast:** *V. adriatica* – described in 1884 by Freyn – treated either as a separate species, subspecies [*V. suavis* subsp. *adriatica* (Freyn) Haesler] or not accepted as distinct from *V. suavis*; **Fig. 2, 3**

Research questions

- **?** What is the phylogenetic position and relationships of W Balkan populations to the other European taxa from *Viola* subsect. *Viola*?
- **?** Are western Balkan populations of *V. suavis* s.l. genetically, morphologically and karyologically differentiated from the populations of *V. suavis* s.l. occurring in C&SE Europe and those from NE Spain?
- **?** Are populations from W Balkan genetically, karyologically and morphologically homogenous, or posses some variation? Is the variation geographically structured?
- **?** Do our data support the existence of *V. adriatica* as a separate taxonomic entity?

II. Sampling & methods
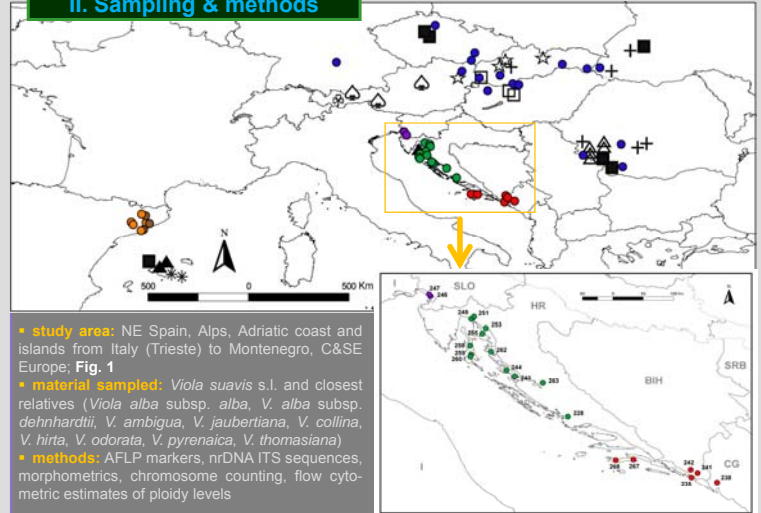
- **study area:** NE Spain, Alps, Adriatic coast and islands from Italy (Trieste) to Montenegro, C&SE Europe; **Fig. 1**
- **material sampled:** *Viola suavis* s.l. and closest relatives (*Viola alba* subsp. *alba*, *V. alba* subsp. *dehnhardtii*, *V. ambigua*, *V. jaubertiana*, *V. collina*, *V. hirta*, *V. odorata*, *V. pyrenaica*, *V. thomasiana*)
- **methods:** AFLP markers, nrDNA ITS sequences, morphometrics, chromosome counting, flow cytometric estimates of ploidy levels

Fig. 1. Map of sample sites of the studied populations of *Viola* subsect. *Viola*: *V. alba* subsp. *alba* (white triangle), *V. alba* subsp. *dehnhardtii* (black triangle), *V. ambigua* (white square), *V. jaubertiana* (asterisk), *V. collina* (star), *V. hirta* (plus), *V. odorata* (black square), *V. pyrenaica* (spade), *V. thomasiana* (trefoil), *V. suavis* s.l. (circle) – blue-flowered populations from Spain (brown circle); white-flowered from Spain (orange circle), blue-flowered from C&SE Europe (dark blue circles), white-flowered from C&SE Europe (light blue circles), populations from Trieste (violet circle), from NW Croatia (green circle) and from S Dalmatia (red circle).

III. Results

1) Ploidy level

- all populations of *V. suavis* s.l. are octoploid (2n = 40)

2) nrDNA ITS data

- dataset of 70 sequences and 612 aligned nucleotide positions; 49 variable sites
- strict consensus tree of the most parsimonious trees revealed **5 well supported clades,** placed in polytomy: 1) *V. alba* subsp. *alba*, 2) *V. alba* subsp. *dehnhardtii*, *V. odorata*, *V. jaubertiana* and *V. collina*, 3) *V. pyrenaica* and *V. suavis* s.l., 4) *V. ambigua*, 5) *V. hirta* and *V. thomasiana*; **Fig. 4**
- all **accessions of *V. suavis* s.l. are monophyletic**, most closely related to and apparently dirived from *V. pyrenaica*; **Fig. 4**
- populations of *V. suavis* s.l. from S Croatia, SW Herzegovina and W Montenegro (S Dalmatia hereafter) form a distinct subclade

3) AFLP data

- dataset of 192 individuals and 184 AFLP markers
- *V. pyrenaica* is clearly separated from all accessions of *V. suavis* s.l. (fig. not shown)
- pronounced **genetic structure in *V. suavis* s.l.** [neighbour-joining (NJ), Bayesian, and neighbor-net clustering, PCoA]; **Figs. 5, 6, 7**
- Bayesian clustering detects **2 and 4 genetic groups** (corroborated also by NJ, PCoA and neighbor-net analyses). At K = 2, one cluster is formed by the populations from S Dalmatia and the other by the remaining population of *V. suavis* s.l. At K = 4, the following groupings are distinguished: 1) S Dalmatia, 2) NW Croatia, 3) C&SE Europe (both blue- and white-flowered morphotypes), 4) NE Spain (also both blue- and white-flowered morphotypes); **Fig. 6**
- populations from S Dalmatia are genetically conspicuously differentiated from all other populations of *V. suavis* s.l.
- two populations from the vicinity of **Trieste** clustered with the populations from C&SE Europe; **Figs. 5, 6, 7**
- populations from W Balkan are **genetically much more variable** than those from C&SE Europe and NE Spain; **Fig. 5**

4) Morphometrics

- 513 individuals from 52 populations; 21 morphological characters
- **no apparent groupings** resolved in principal component analysis (PCA; fig. not shown)
- canonical discriminant analyses **confirmed morphological differentiation** between the 2 genetic clusters (K = 2; fig. not shown) as well as among the 4 clusters (K = 4; **Fig. 8**) resolved by Bayesian clustering

Fig. 2. *Viola suavis* subsp. *adriatica* at the type locality at Bakar (NW Croatia).

Fig. 3. Habitat of *Viola suavis* subsp. *adriatica* on the island of Rab (NW Croatia).
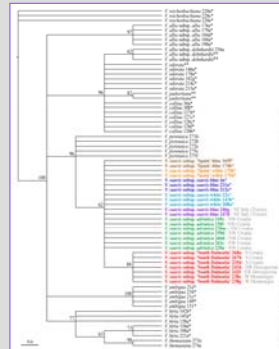

Fig. 4. Strict consensus tree (maximum parsimony) based on nrDNA ITS sequence data. Geographic origin of the samples and the colour morphotypes of *Viola suavis* s.l. are indicated. Ploidy levels for each taxon are shown along the vertical bars. Accessions with asterisks are taken from Mereďa et al. (2008), those with two asterisks indicate GenBank accessions.
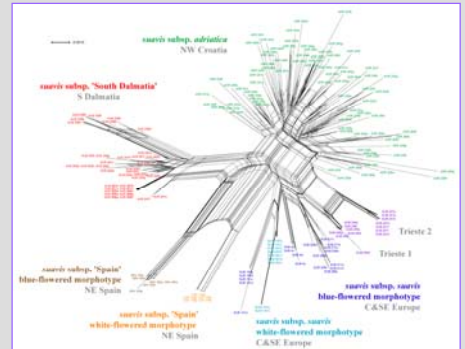

Fig. 5. Neighbor-net diagram based on AFLP data of 192 individuals of *Viola suavis* s.l.

Fig. 6. Genetic structuring in *Viola suavis* s.l. (173 individuals from 28 populations) as resolved by Bayesian clustering of AFLP phenotypes at K = 2 and K = 4. Each individual is represented by a vertical bar, coloured proportionally according to the cluster assignment (red – *V. suavis* subsp. 'South Dalmatia', green – *V. suavis* subsp. *adriatica*, blue – *V. suavis* subsp. *suavis*, brown – *V. suavis* subsp. 'Spain').
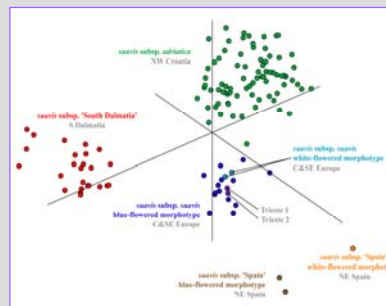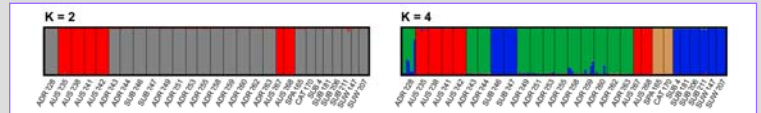

Fig. 7. Principal coordinate analysis based on AFLP data of 192 individuals of *Viola suavis* s.l.
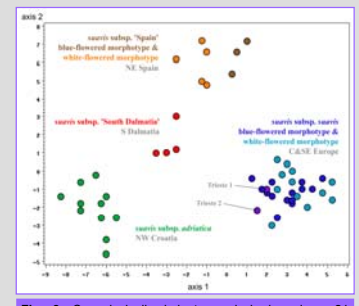

Fig. 8. Canonical discriminate analysis based on 21 morphological characters of 52 populations of *V. suavis* s.l. The four groups were defined on the basis of AFLP data.

IV. Conclusions

Genetic and morphological data support the recognition of **4 taxa within *Viola suavis* at subspecies level:**
1) *V. suavis* subsp. *suavis* (C&SE Europe, C&E Balkan) – with two color morphotypes: typical blue-flowered and white-flowered (varietal rank adequate?)
2) *V. suavis* subsp. *adriatica* (NW Croatia)
3) *V. suavis* subsp. 'South Dalmatia' (S Dalmatia)
4) *V. suavis* subsp. 'Spain' (Iberian Peninsula, SE France?) – with two color morphotypes: blue-flowered and white-flowered (syn. *V. catalonica*; varietal rank adequate?)