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Cytological status of Viola kitaibeliana (Section Melanium, Violaceae) in Europe

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Viola kitaibeliana Schultes in Roemer & Schultes (1819: 383) was described for "*Pannonia*" as a small pansy with "*caule erecto simplicissimo, foliis grosse crenatis, inferioribus subrotundis glabris, superioribus obovatis, stipulis similibus subintegerrimis, basive subpinnatis, petalo cornuto calycem glabrum aequante, reliquis brevioribus. Arvensi proxima, hac praecocior multo, (Aprili florens!) tenerior, et notis indicatis satis distincta. Flores 2, vel unicus, raro tres, oblique erecti in pedunculis folia excedentibus.*" (*Typus:* M0112803; Fig. 1). Thereafter, many other names have been assigned to similar pansies described outside the Pannonian region: e.g. *V. tricolor* Linneus (1753: 935) var. *nana* De Candolle (1824: 304) and *V. nemausensis* Jordan (1846: 18) in France, *V. tricolor* subsp. *minima* Gaudin (1828: 210) in Switzerland, *V. tricolor* var. *trimestris* De Candolle (1824: 304), *V. tricolor* var. *henriquesii* Willk. ex Coutinho (1892: 36) and *V. tricolor* var. *machadeana* Coutinho (1892: 36) in the Iberian Peninsula. None of these taxa have been reported in *Flora Europaea* by Valentine *et al.* (1968) and later they have been included in *V. kitaibeliana* or placed in synonymy with it in the main floras (e.g. Muñoz Garmendia *et al.* 1993). According to Valentine *et al.* (1968) and to the updates of Magrini & Scoppola (2015b), in Europe *V. kitaibeliana* is widespread from Southwest to East, extending to Ukraine (Fig. 2).

The taxonomy of the *V. kitaibeliana* aggregate is very complicated because of the ambiguity of some morphological characteristics, the frequency of polyploidy, dysploidy, and hybridisation events within the section *Melanium* Gingins de la Sarraz (1823: 23) (Marcussen *et al.* 2015), and the conflicting cytotaxonomic treatments. During the last two centuries, seven cytotypes have been reported for plants named *V. kitaibeliana*, 2n = 14, 16, 18, 24, 36, 40 and 48 (see Table 1 and Fig. 2) (e.g. Clausen 1931, Valentine *et al.* 1968, Hess *et al.* 1970, Randall 2004). Thus, *V. kitaibeliana* has been thoroughly investigated to assess if it should be considered an aggregate of mere cytotypes or it should be split in several narrow species differing in chromosome number, ecology or distribution.

The main cytotype given by authors to this species through its distribution area is 2n = 16. This number has been counted in plants from Hungary, Austria, and Switzerland (Erben 1985), Slovakia (Uhrlkova & Majovsky 1978), Turingia, Germany (Hand & Gregor 2011), Republic of Macedonia (Schmidt 1964), Central Italy (Scoppola *et al.* 2014), Southern France (Verlaque & Espeut 2007), and Northern Spain (FCO!, Fernández Casado 1984).

Clausen (1931: 230) first reported several chromosome numbers for *V. kitaibeliana* writing "*The species [....] comprised* a number of chromosomal different types, namely with n = 7, 8, 12, 18 and 24 and possibly more numbers." citing the results of a previous study (Clausen 1927). On the other hand, in such paper it was written: "*I have several times received seeds* from the Tiflis Botanical Garden under the name of V. kitaibeliana. One of the consignments consisted of a mixture of V. arvensis with n = 17 and a V. kitaibeliana type with n = 18. Another batch was more uniform in appearance; it was made up exclusively of typical V. kitaibeliana, which I presumed had also n = 18, though the type was of rather slighter build than the first. A crossing with arvensis was made before I had an opportunity of subjecting the new type to cytological investigation. In the F_1 of this crossing, however, there were about 10 univalents, not, as I had expected, only one. Cytological investigation of the root-tips from the crossed kitaibeliana type showed, to my surprise, that this had 2n = 14." (Clausen 1927: 691). These evidences suggest that the chromosome numbers counted only by Clausen (14 and 36) should not be attributed to *V. kitaibeliana*: 2n = 36 has been counted from stout plants grown from a mixture of pansy seeds of Tbilisi Botanical Gardens (Georgia) (Clausen 1927) and 2n = 14 from a hybrid obtained by Clausen himself (1927) and, afterwards, wrongly referred to *V. kitaibeliana* (Clausen 1931).

Among the others cytotypes, two have been assigned exclusively to other species: 2n = 24 was counted for some Greek populations (Van Loon 1980, Livaniou-Tiniakou 1983, Franzen & Gustavsson 1983) later assigned to *Viola phitosiana* Erben (1985: 396); 2n = 40 was counted in populations of the Galician-Portuguese sector of the Iberian Peninsula (MA!, Aldasoro & Laínz 1992) recently assigned to *V. henriquesii* (Willk. ex Cout.) W. Becker (1906: 190) by Magrini & Scoppola (2015a).