Botanica SERBICA 38 (1): (2014) 43-56

Original Scientific Paper

Is it possible to identify *Colchicum neapolitanum* s.l. and *C. autumnale* s.l. in vegetative stage? Biometry and flow cytometry approaches

Alain Fridlender[1*], Fatima Pustahija[2], Marija Edita Šolić[3], Sabaheta Abadžić[4], Mickael Bourge[5], Nicolas Pech[1], Sonja Siljak-Yakovlev[6], Spencer C. Brown[5]

1 Faculté des Sciences, Université de Provence – case 75, 3 place Victor Hugo, F-13331 Marseille Cedex 3, France

2 Faculty of Forestry, University of Sarajevo, Zagrebačka 20, 71000 Sarajevo, Bosnia and Herzegovina

3 Institute Mountain and Sea, Franjevački put 1, 21300 Makarska, Croatia

4 National Museum of Bosnia and Herzegovina, Zmaja od Bosne 3, 71000 Sarajevo, Bosnia and Herzegovina

5 Institut des Sciences du Végétal, CNRS UPR2355, IFR87, et Imagif Biologie cellulaire, bâtiment 23, Centre de Recherche de Gif, 91198 Gif-sur-Yvette, France

6 CNRS, Laboratoire Ecologie, Systématique, Evolution, UMR 8079, Université Paris-Sud, Bât. 360, 91405 Orsay, France

ABSTRACT: It is difficult to distinguish hysteranthous *Colchicum* species growing in Dalmatia and Bosnia and Herzegovina, even the two most common taxa (*C. autumnale* and *C. neapolitanum* subsp. *visianii*). There are no clear vegetative characteristics suitable for good identification of these species. The nuclear DNA values, however, reveal differences between these two species and also among other taxa. Genome size was studied for the first time in: *C. hungaricum* Janka (2C = 5.38 ± 0.3 pg); two Dalmatian endemics *C. neapolitanum* (Ten.) Ten. subsp. *kochii* (Parl.) Fridl. (2C = 10.9 ± 0.3 pg) and *C. neapolitanum* (Ten.) Ten. subsp. *visianii* (Parl.) Fridl. (2C = 10.41 ± 0.32 pg) and for Italian *C. neapolitanum* (Ten.) Ten. subsp. *neapolitanum* (2C = 10.0 ± 0.1 pg) endemic to Basilicata – Campania. Genome size of *C. autumnale* L. from Bosnia and Herzegovina (2C = 6.17 ± 0.31 pg) does not differ from other west European populations. Based on this important character and also on morphological studies, we propose taxonomic revision of some taxa. As genome sizes of the two generally confused species *C. neapolitanum* subsp. *visianii* and *C. autumnale* are very different, flow cytometry is useful for identification, conservation inventories and management concerning these two taxa.

Key words: Balkan flora, biometry, *Colchicum*, flow cytometry, genome size

Received: 15 December 2013 Revision accepted 18 January 2014

UDK 582.57

INTRODUCTION

Colchicum L. (incl. *Bulbocodium* L., *Merendera* Ram.) is a Mediterraneo-Irano-turanian genus which comprises about 100 species (Persson 2007). Taxonomic delimitations remain very difficult, even after various recent taxonomic revisions addressing the far-Eastern distribution (Persson 1992), Middle East (Feinbrun 1958, 1986), Turkey and Balkans (Brickell 1984, Persson, 1988-2008), Europe (Brickell 1980), Italy (D'Amato 1955, 1957) and western

*correspondence: alain.fridlender@univ-amu.fr

Mediterranean territories (Fernandez & França 1977; Camarda 1978, 1979, 1990; Lentini & Raimondo 1984; Fridlender 1999a-d; Fridlender & Pignal 2013).

It is quite easy to recognize the synanthous *Colchicum* species in the countries surrounding the Adriatic. *Colchicum cupanii* Guss. blooms in autumn and grows in western Adriatic whereas *Colchicum hungaricum* Janka (= *C. montanum* auct. = *C. bertolonii* Stev. *in Flora Dalmatica* I:156-157, 1842) is an eastern Adriatic species flowering basically in early spring.

On the contrary, hysteranthous species remain very difficult to identify: flowers are observed during a short period in autumn whereas leaves (undeveloped at anthesis) grow in the following spring.

In the past, different taxa were identified or described as endemics in Dalmatia, such as: *C. bivonae* auct. in Mts Velebit and Biokovo (Visiani 1842); *C. visianii* Parl. in Dalmatia and *C. kochii* Parl. in Istria (Parlatore 1860, Stefanoff 1926, Hayek 1932-1933, Pignatti 1982); *C. jankae* Freyn (1877) in Croatia near Salona (Split). In ex-Yugoslavia the two threatened *C. macedonicum* Košanin and *Bulbocodium versicolor* (Ker-Gawler) Spengel (≡ *Colchicum versicolor* Ker Gawl., 1821) do not belong to the Adriatic elements as they are the rare relict plants of high mountains.

In *Flora Europaea*, Brickell (1980) recognizes five species in ex-Yugoslavia: *C. turcicum* Janka, *C. arenarium* Waldst. & Kit., *C. autumnale* L. *C. neapolitanum* (Ten.) Ten. and *C. bivonae* Guss. without, of course, more precision for Illyrian territories (especially for Bosnia and Herzegovina, and Croatia). Western populations of *C. turcicum* are in fact restricted to Greece and Bulgaria, whereas *C. arenarium* seems to be a rare central European species from Hungary which is not present near the Adriatic sea: *Hungaria in pratis arenosis ad Budapest* ("in sandy meadows near Budapest": Stefanoff 1926). Remnant of ice age flora, it is a rare species in northern Serbia: a steppe relic (Stevanović *et al*. 1999). *Colchicum autumnale* is considered as a well-known species present especially in temperate areas (moist soils) of Europe, whereas *C. bivonae* is strictly Mediterranean (south Italy, Sicily, Greece, etc.). Morphological delimitation of *C. neapolitanum* appears to be very controversial: it has been considered as a common species from Western Balkans to Portugal. On the contrary, this species does not exist in Morocco, Iberian Peninsula, France, Corsica and Sardinia where it has been confused with *C. multiflorum* Brot. (Fridlender 1999a, b; Fridlender & Pignal 2013). In the recent revision of Balkan *Colchicum*, Persson (1999) mentioned the following species in Bosnia and Herzegovina and Croatia: *C. haynadii* Heuff., *C. bivonae* and *C. autumnale* common in temperate European climates. According to this author, *C. bivonae* seems to be very rare with one or two occurrences (near Rača) in Croatia, whereas many earlier mentions should probably be attributed to the *C. haynaldii* group.

In *Flora Hrvatske*, Domac (1973, 1994) recognized four species for Croatia: *C. hungaricum, C. autumnale, C. kochii* Parl. and *C. visianii*. In *Flora Croatica index,* Nikolić *et al.* (2000) accepted six species: *C. hungaricum, C. arenarium, C. autumnale, C. neapolitanum, C. visianii* and *C. kochii,* but in the supplement published just one year later — *Notulae ad Indicem Florae Croaticae* (Hršak 2001) — the change is quite important: *C. hungaricum, C. arenarium, C. autumnale, C. neapolitanum, C. bivonae* and *C. haynaldii*!

Clearly, excepting *C. hungaricum*, colchicums observed in Adriatic (from south Italy to southern Croatia to Istria) remain very polymorphic. As demonstrated by these cited contradictory taxonomical analyses, they have proven to be difficult to name and recognize! In this context, identification of these taxa remains impossible for a majority of botanists, ecologists and naturalists! To name these plants as *C. neapolitanum* in Italy and *C. haynaldii* in ex-Yugoslavia (Persson 1999) is not satisfactory. But nor is it acceptable to retain all the names created during the last 200 years.

According to botanical inventories, floras and identification keys, it does not seem difficult to distinguish *C. autumnale* from other Balkan colchicums. However, during a spring trip in southern Croatia and Bosnia and Herzegovina, one of us (AF) immediately saw that it was not so obvious for non-specialist colleagues. Thus, in this work, we explore data on vegetative characters. Our aim was to find some vegetative characters suitable for distinguishing the colchicums. We focused on the two putatively most common hysteranthous species from South Dalmatia - Bosnia and Herzegovina (*C. autumnale* s.l. and *C. visianii* Parl.) for which a new taxonomic approach is proposed.

MATERIAL AND METHODS

Plants and biometrical analysis: Plants (172 specimens from 10 stations) were collected during spring 2011 in Bosnia and Herzegovina (BiH) and South Croatia (Hr) (Appendix 1, Fig. 1). On each individual, all the leaves, corms and fruits of fresh plants were measured according to our previously described methodology (Fridlender 1999a-b). Those measures allowed us to define 17 variables: length, width and their ratio for corms and leaves (first one, second one, third…); fruit and cataphylls' length; numbers of leaves and fruits (fertile and aborted). Only mature individuals, namely floriferous with fertile or aborted fruits, were taken into consideration (Figs. 3 and 5).

Table 1: Proposed taxonomy for *C. neapolitanum* s.l. and *C. autumnale* s.l. around the Adriatic.

***C. neapolitanum* (Ten.) Ten.,** Fl. Neapo Prodr. App. 5:11 (1826). - Type: Unclear but lot of collected plant from Regno de Napoli in Tenore's and Gussone's herbaria (I. Camarda & A. Santangelo NAP curator, *in. lit*. 1998): Monte della Stella F. Giordano coll.; Campania, "In Pratis montanis mediae regni regionis, prope Neapolim ad Camaldoli; et ad montem divi Angeli Stabiarum (Faito)…"; "Nasce nelle praterie montuose della regione media del Regno: presso Napoli cresce in copia ai Camaldoli, ed al Piano di Faito al Monte S. Angelo di Castellammare". - Icon. Fl. Nap. Tav. 138.

C. neapolitanum* (Ten.) Ten. subsp. *neapolitanum It remains unclear if *C. neapolitanum* subsp. *neapolitanum* is endemic to Southern Italy (Campania and Basilicata) due to numerous confusions with *C. multiflorum* Brot., *C. neapolitanum* subsp. *kochii* and maybe *C. bivonae* that also grow in the North part of the Peninsula. In the same way, a certain resemblance also exists with some Italian populations of *C. lusitanum* Brot. whose genome size is also similar (Fridlender *et al*. 2002). But both *C. lusitanum* and *C. bivonae* have bigger flowers and generally more or less deeply tessellated petals. In ex Regno di Napoli we may recognize, at least, other subspecies from Lucania:

***C. neapolitanum* (Ten.) Ten. subsp. *gracile* (K. Perss.)** Fridl.**, comb. stat. nov.** ≡ *C. gracile* K. Perss., Bot. Jahrb. Syst. 127(3): 284 (2008). - Type: Italy, Basilicata, Potenza: SE of Lauria between Pestiere and motorway, 650 m, 28-9-1990.
= *C. tenorii* Parl. Fl. Ital. 3:176 [1860 (1858-1860)]

***C. neapolitanum* (Ten.) Ten. subsp. *kochii* (Parl.)** Fridl.**, stat. nov.** ≡ *C. kochii* Parl., Flora Italiana Vol. III: 188 (1860 [1858-1860]) ≡ *C. neapolitanum* (Ten.) Ten. var. *kochii* (Parl.) Fiori, *in* Malpighia 8:156 (1894) - Type: Croatia Nella parti occidentali d'Istria presso Pola ed altrove. Fiorisce in Settembre e Ottobre. - Lecto.: *in* FI: Croatia, Circa Pola et alibi in Istria australis, Tommasini *s.n.* (FI). This subspecies grows in North East Italy, Slovenia and Croatia (southern limit in Zadar – Krka area) near the sea shore at low altitude (0-200 m, up to 400 m). Flowers are small, 1 or 2 per corm, pink and slightly or not tessellated. It is also a smaller plant than *C. neapolitanum* subsp. *visianii* at vegetative stage (3-4 narrow leaves more or less prostrated on the ground versus 5-7 erect leaves in *C. neapolitnum* subsp. *visianii*. Visiani).

***C. neapolitanum* (Ten.) Ten. subsp. *visianii* (Parl.)** Fridl.**, comb. stat. nov.** ≡ *C. visianii* Parl., Flora Italiana. Vol. III: 175 (1860 [1858-1860]). - Type: "Hab. In pratis et herbidis montis Vellebith et Biokovo nec non circa Dernis, Verlicca, Salona, Ragusa. Floret Septembri, Octobri.». The name of *C. visianii* is based on plants from Dalmatia previously identified as *C. bivonae* Guss. by Visiani in *Flora Dalmatica* I: 156 [1842]). - Lecto.: "In Biokovo" (Herb. Visiani – PAD), see K. Persson (1999). - Icon. Fig. 4 and 5.
= *C. bivonae* auct. p.p.Visiani *in* Flora Dalmatica 1:156 (1842) = *C. jankae* Freyn. p.p. Österr. Bot. Z. 27: 361 (1877). - Type: [Dalmatiae in pratis ad mare adriaticum prope Salonam 9 X 1875.]
This subspecies grows in Dalmatia, above all at an altitude from 300 to 1400 m (some populations at sea level in Herzegovina & South Croatia), in Adriatic–Mediterranean climate, sometimes in dry rocky areas but also in some meadows (polje). It is very polymorphic in all its organs with numerous broad and long leaves. Flowers are numerous (3-6 per corm), sometimes distinctly tessellated, but generally obscurely tessellated (or not at all) even in the same corm and of course in a population. When cultivated, leaves may be smaller and even quite similar to *C. multiflorum* Brot.! It is not surprising that this colchicum has been confused with some other species, e. g. *C. bivonae* and *C. autumnale*.

***C. neapolitanum* (Ten.) Ten. subsp. *haynaldii* (Heuffel) Fridl., stat. nov.**
≡ *C. haynaldii* Heuffel, *Enum. Plant. in Banatu Temesiensi sponte crescentium…* Verh. Zool. Bot. Ges. Wien 8:213 (1858) ≡ *C. neapolitanum* (Ten.) Ten. var. *haynaldii* (Heuffel) Ascherson & Graebner, Syn. Mitteleur. Fl. 3:26 (1905). Type: Romania "Banatus, in Danubis tractus rupestribus calcareis, floret Sept.» (BP). As *C. haynaldii* Heuff. seems to be quite related to *C. neapolitanum s.l.* and, as it is a posterior name, we actually consider it as a subspecies of *C. neapolitanum* complex.

***C. autumnale* L.** Species Plantarum, Tomus I: 341 (1753). - Type: « Habitat in Europae australioris succulentis. »- Icon. Fig. 2 and 3 for Bosnian populations. *Colchicum autumnale* is an easily recognizable colchicum in Western Europe, however in its South-Eastern distribution range it is not well know and well delimited (Persson 1988, 1991; Jung *et al.* 2011). In Balkan, *C. autumnale* is clearly represented by various taxa: two of these (see text) might be present in southernmost Adriatic territories:

***Colchicum autumnale* L. subsp. *graecum* (K. Persson) Fridl. comb. stat. nov.** ≡ *C. graecum* K. Persson, Wildenowia 18:34 (1988). - Type: Messinia Kalamata, Taygetos, Xerovouna, 5 km E of Alagonia 1700-1840 m, J. Persson 4035 GB. Greece, from Pindhos to Peloponnisos, 1300-2400 m, limestone, ophiolithic, schistose substrates.

***Colchicum autumnale* L. subsp. *confusum* (K. Persson) Fridl., comb. stat. nov.** ≡ *C. confusum* K. Persson, Plant Systematics and evolution 217:60 (1999). - Type: Greece, Thessalia: Trikala, 2 km from Pertouli to Elati, large moist meadows, in grass, deep soil, 1150 m, 20-9-1989, GB.

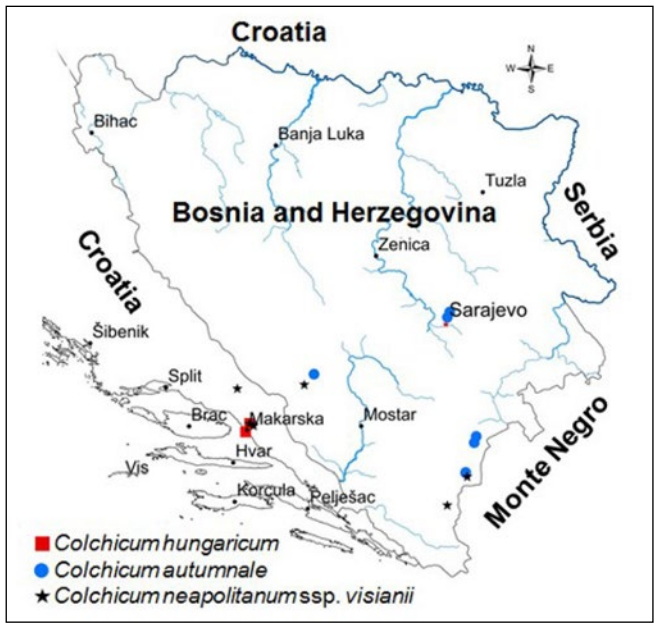

Fig 1. Geographic origin of populations collected for biometry. Plants were collected at vegetative stage in spring 2011 in Bosnia and Herzegovina (BiH) and South Croatia (Hr).

In Mt. Biokovo *C. neapolitanum* subsp. *visianii* and *C. hungaricum* grow together: leaves are mixed in the same meadows. As it is not always easy for a non-botanist to recognize both species in such a context, we have also included some biometric data for *C. hungaricum* leaves from Mt. Biokovo (Table 2).

In order to identify characters able to distinguish stations (and species), we performed a linear discriminate analysis on the 17 variables, where groups are defined by the 10 populations. Missing data (i.e. length of grazed leaves) were initially imputed using the ACP-GEM algorithm (Josse *et al.* 2009) implemented in the missMDA package of R (R core Team 2013).

Genome size analysis: The total nuclear DNA amount was assessed by flow cytometry according to Marie & Brown (1993) for 128 colchicum individuals. *Pisum sativum* cv. Long Express (2C = 8.37 pg) was used as an internal standard. Leaves of the internal standard and *Colchicum* were chopped using a razor blade in a plastic Petri dish with 600 µl of Galbraith nucleus-isolation buffer (Galbraith *et al.* 1983) containing 0.1% (w/v) Triton X–100, supplemented with 10 mM sodium metabisulphite, 1% (w/v) polyvinylpyrrolidone 10,000 and RNAse (2.5 U/ml). The suspension was filtered through 50 µm nylon mesh. The nuclei were stained with 75 µg/ml propidium iodide, a specific DNA fluorochrome intercalating dye, and kept 15 min at 4°C. DNA content of 5,000–10,000 stained nuclei was determined for each sample using a cytometer (CyFlow SL3, Partec, Munster, Germany). The total 2C DNA value was calculated using the linear relationship between the fluorescent signals from stained nuclei of the *Colchicum* species and the internal standard. The mean value was calculated from measurements of samples comprising 4 to 16 individuals according to populations (Tab. 3).

RESULTS AND DISCUSSION

Biometry. The clear differences among species were demonstrated by comparison of mean values of vegetative organ measurements (Table 2). Synanthous species *C. hungaricum* with two narrow and short leaves was, as expected, the more isolated. Discriminant analysis clearly separates it from the other samples (lower leaf width, higher ratio length/width). In contrast, others populations did not show sharp differences but a gradient from *C. autumnale* to *C. neapolitanum* subsp. *visianii* (Fig. 6).

Colchicum autumnale has the biggest leaves, but *C. neapolitanum* subsp. *visianii* leaves are more numerous (n = 4.7 leaves per corm, versus n = 3.6). In general, length of *C. autumnale* leaves is reducing after the fourth leaf (fifth in *C. neapolitanum* subsp. *visianii*), and the last one becomes more or less filiform, especially in *C. autumnale*. However, considering the most used identification morphometric criteria (leaves' number and width) adopted since the 19th century (Fiori 1894), overlapping of data between these two species is clearly significant (Table 2): 45% of *C. autumnale* individuals and 48% of *C. neapolitanum* subsp. *visianii* ones have four leaves (but 7% and 28% have five leaves respectively). Based on the criterion of broadest leaves, 1/3 of individuals from each species do not differ: 30% of *C. neapolitanum* subsp. *visianii* individuals have their broader leaves between]25-35] mm (36% in *C. autumnale*)!

It is far more difficult to separate both species if taking into account just some individuals. Namely, when comparing 5-10 individuals (i.e. different populations, Figs. 3 and 5), differences due to environmental factors (wetness, sunshine, soil richness…) and individual variability predominate in vegetative organs. Means of "Number of leaves" and "Width Broadest Leaf" (Table 2) were different between those two species but overlapping is too important (Fig. 6) for easy and reliable field identification except for some typical *C. autumnale* populations (i.e. from northern Bosnia).

In addition, some flowers (Fig. 2 and 4) do not allow unambiguous identification of these Adriatic geophytes (*C. autumnale, C. bivonae, C. lusitanum,* and *C. neapolitanum*). Consequently, to determine with certainty these species, a non-specialist needs to observe lots of individuals (with numerous fresh flowers in autumn and leaves in spring) from the same population. But it must be

Fig 2. *C. autumnale*. Leaves A - BiH, Čemerno (Sutjeska), 1280-1300 m; B - BiH, Crkva Gospe Snježne - Blidinje Jezero, 1325 m. Flowers C, D - BiH, Sutjeska.

taken into account that field work does not generally allow so many plant measurements!

Genome size. As previously noted for genus *Colchicum* (Fridlender *et al*. 2002), the genome size values were stable for each taxa. The 2C values for *C. autumnale* (2C = 6.17 ± 0.31 pg) from Bosnia and Herzegovina and north Adriatic (Istria: 2C = 6.5 ± 0.2 pg) are similar to previously published genome size data from French and Italian populations (Fridlender *et al*. 2002). Three *C. neapolitanum* subspecies do not have any genome size differences: south west Adriatic *C. neapolitanum* subsp. *neapolitanum*, north Ardiatic *C. neapolitanum* subsp. *kochii* and south-east Adriatic *C. neapolitanum* subsp. *visianii* presented respectively 2C = 10 pg, 10.9 pg and 10.41 pg (Tab. 3).

Genome size value appears to be a good character to easily separate *C. autumnale* and all *C. neapolitanum* subspecies. Next, it is a valuable element for reconsidering some previous identifications, i.e. the genome sizes

Table 2: Comparison of *Colchicum autumnale* L., *C. hungaricum* Janka and *C. neapolitanum* subsp. *visianii* Fridl. from southern Dalmatia and Bosnia and Herzegovina.
Top panel: Average dimensions, standard deviation (SD) and extreme values (range) of different vegetative organs in mature individuals. **Middle panel:** Frequency (%) of individuals according to width of broadest leaf (per 5 mm class). Here was taken into account the largest leaf from each corm (not the average of width of the different leaves of a corm). **Bottom panel:** Frequency (%) of individuals according to number of leaves per corm.
n = number of plants studied; ***a*** = for each plant all the organs could not be measured (i.e. damaged, grazed leaves). In particular, corm measurements were made in fewer individuals for each population.

	C. hungaricum	***C. autumnale***	***C. neapolitanum* subsp. *visianii***
	n = 14 [a] X ± SD [range]	n = 56 [a] X ± SD [range]	n = 79 [a] X ± SD [range]
Corm length (mm) [a]	24.1 ± 3.4 [18-28]	46.7 ± 11.5 [26-68]	37.6 ± 10.5 [25-83]
Corm diameter (mm) [a]	13.2 ± 2.9 [11-20]	33 ± 8.9 [20-48]	28.9 ± 6.3 [18-43]
Stipe length (mm)	52.9 ± 14 [35-85]	175 ± 60.9 [91-300]	85.9 ± 30.5 [36-194]
Number of leaves	2 ± 0 [2-2]	3.6 ± 0.7 [3-6]	4.7 ± 1 [3-8]
Width broadest leaf (mm)	6.2 ± 1.7 [4-10]	41 ± 12.4 [24-76]	24.6 ± 7 [11-43]
Leaf 1 length (mm)	122.5 ± 9.5	273.7 ± 51.8 [193-418]	224 ± 54.3 [127-340]
Leaf 2 length (mm)	123.5 ±3.1	300.7 ± 69.6 [192-492]	235.6 ± 59.8 [137-378]
Leaf 3 length (mm)	-	305.2 ± 78.2 [180-503]	221.7 ± 55.1 [140-365]
Mean length of the first 5 leaves (mm)	122.9 ± 13.6 [98-146]	298 ± 71.1	204.7 ± 10.6
Length fruits (mm)	12.6 ±4	33 ± 9.6	29.7 ± 7
Broadest leaf 0-5 mm (%)	29	-	-
Broadest leaf 5-10 mm (%)	71	-	-
Broadest leaf 10-15 mm (%)	-	-	6
Broadest leaf 15-20 mm (%)	-	-	27
Broadest leaf 20-25 mm (%)	-	2	29
Broadest leaf 25-30 mm (%)	-	20	24
Broadest leaf 30-35 mm (%)	-	16	6
Broadest leaf 35-40 mm (%)	-	18	4
Broadest leaf 40-45 mm (%)	-	18	4
Broadest leaf 45-50 mm (%)	-	9	-
Broadest leaf 50-55 mm (%)	-	7	-
Broadest leaf 55-60 mm (%)	-	3	-
Broadest leaf 60-65 mm (%)	-	-	-
Broadest leaf 65-70 mm (%)	-	2	-
Broadest leaf 70-75 mm (%)	-	3	-
Broadest leaf 75-80 mm (%)	-	2	-
Corms with 1 leaf (%)	-	-	-
Corms with 2 leaves (%)	100	-	-
Corms with 3 leaves (%)	-	46	5
Corms with 4 leaves (%)	-	45	48
Corms with 5 leaves (%)	-	7	28
Corms with 6 leaves (%)	-	2	14
Corms with 7 leaves (%)	-	-	4
Corms with 8 leaves (%)	-	-	1

Table 3: Genome size. 2C DNA in pg ± standard deviation for *Colchicum autumnale, C. hungaricum* and *C. neapolitanum* subsp. *visianii* from Southern Dalmatia and Bosnia Herzegovina. The two others *C. neapolitanum* subspecies (subsp. *neapolitanum* & subsp. *kochii*) from classical/type localities were also measured for comparison.
n = number of plants measured; ***a*** - first known genome size for the taxa; ***b*** - vouchers see Appendix 1; ***c*** – first genome size for this taxa in this country: Ga – France; It – Italy; Hr – Croatia; BiH – Bosnia and Herzegovina.

Species / population	2C DNA ± SD, in pg
***C. hungaricum* Janka**	
Hr, Makarska, 5 m [b]	5.41 ± 0.1 (n = 4) [a]
Hr, Mt. Biokovo, 1075 m [b]	5.37 ± 0.3 (n = 4) [a]
Mean *C. hungaricum* from Dalmatia	5.38 ± 0.3 (n = 8) [a]
***C. autumnale* L.**	
Ga, It: Various populations from France and Italy	5.89 ± 0,1 (Fridlender *et al*. 2002)
Hr, Istria, Lovranska (Rijeka) 680 m [b]	6.5 ± 0.2 (n = 8) [c]
BiH, Čemerno, 1280-1300 m [b]	5.77 ± 0.15 (n = 5) [c]
BiH, Crkva Gospe Snježne - Blidinje Jezero, 1325 m [b]	6.29 ± 0.22 (n = 10) [c]
BiH, Buk Bijela - Tjentište, 495 m [b]	6.12 ± 0.12 (n = 7) [c]
BiH, Mrkovići - Bukovik (Sarajevo), 880 m [b]	6.41 ± 0.21 (n = 9) [c]
BiH, Mrkovići - Bukovik (Sarajevo), 1160 m [b]	6.43 ± 0.39 (n = 5) [c]
BiH, Avtovac (Gacko), 975m [b]	5.89 ± 0.11 (n = 8) [c]
Mean *C. autumnale* from Bosnia and Herzegovina	6.17 ± 0.31 (n = 44) [c]
C. neapolitanum* (Ten.) Ten. subsp. *neapolitanum Italy, Napoli, Monte Faito (*Locus classicus*) [b]	10 ± 0.1 (n = 9) [a]
***C. neapolitanum* (Ten.) Ten. subsp. *kochii* (Parl.) Fridl.** Hr, Istria, Mean of 3 populations near Pula (*Locus classicus*) [b]	10.9 ± 0.3 (n = 20) [a]
***C. neapolitanum* (Ten.) Ten. subsp. *visianii* (Parl.) Fridl.**	
BiH, Bijela Rudina, 660 m [b]	10.82 ± 0.39 (n = 6) [a]
BiH, Rakitno - Donji Poklečani, 865 m [b]	10.20 ± 0.16 (n = 5) [a]
Hr, Biokovo, 1075 m (*Locus classicus*) [b]	10.55 ±0.31 (n = 16) [a]
Hr, Biokovo, 1250- 1300 m (*Locus classicus*) [b]	10.27 ± 0.25 (n = 10) [a]
Croatia, Dinaric Alps between Split and Makarska	10.1 ± 0.2 (n = 2) [a]
Mean *C. neapolitanum* subsp. *visianii* from south Dalmatia and Bosnia and Herzegovina	10.41 ± 0.32 (n = 39) [a]
Hr, Biokovo	9.56 ± 0.34 (Siljak-Yakovlev *et al*. 2010) *sub.* erroneous name of *C. autumnale* L.
BiH , Rudo (serpentine soil)	10.44 ± 0.23 (Pustahija *et al*. 2013) *sub.* erroneous name of *C. autumnale* L.

published from Biokovo (Croatia) and Rudo (Bosnia) by Siljak-Yakovlev *et al*. (2010) and Pustahija *et al*.(2013), do not correspond to that of *C. autumnale*. As all the colchicum flowers observed up to now in Mt. Biokovo (10 populations from 400 to 1400 m in dry and moist soils; A. Fridlender & M.E. Šolić *s.n*., June-2009 to Oct.-2013 *in* Herbier privé Fridlender) belong to C. *neapolitanum* subsp. *visianii*, we deduce that *C. autumnale* was misidentified (Radić 1976; Persson 1999) and is probably absent from this mountain. In Biokovo and Rudo, *C. neapolitanum* subsp. *visianii* was erroneously named *C. autumnale* (Tab. 3).

High morphological polymorphism despite genome size constancy: taxonomic implications. The similar genome size of all analysed subspecies of *C. neapolitanum* may support our taxonomical hypothesis. Yet Persson (1999), a well-known *Colchicum* specialist, had a clearly

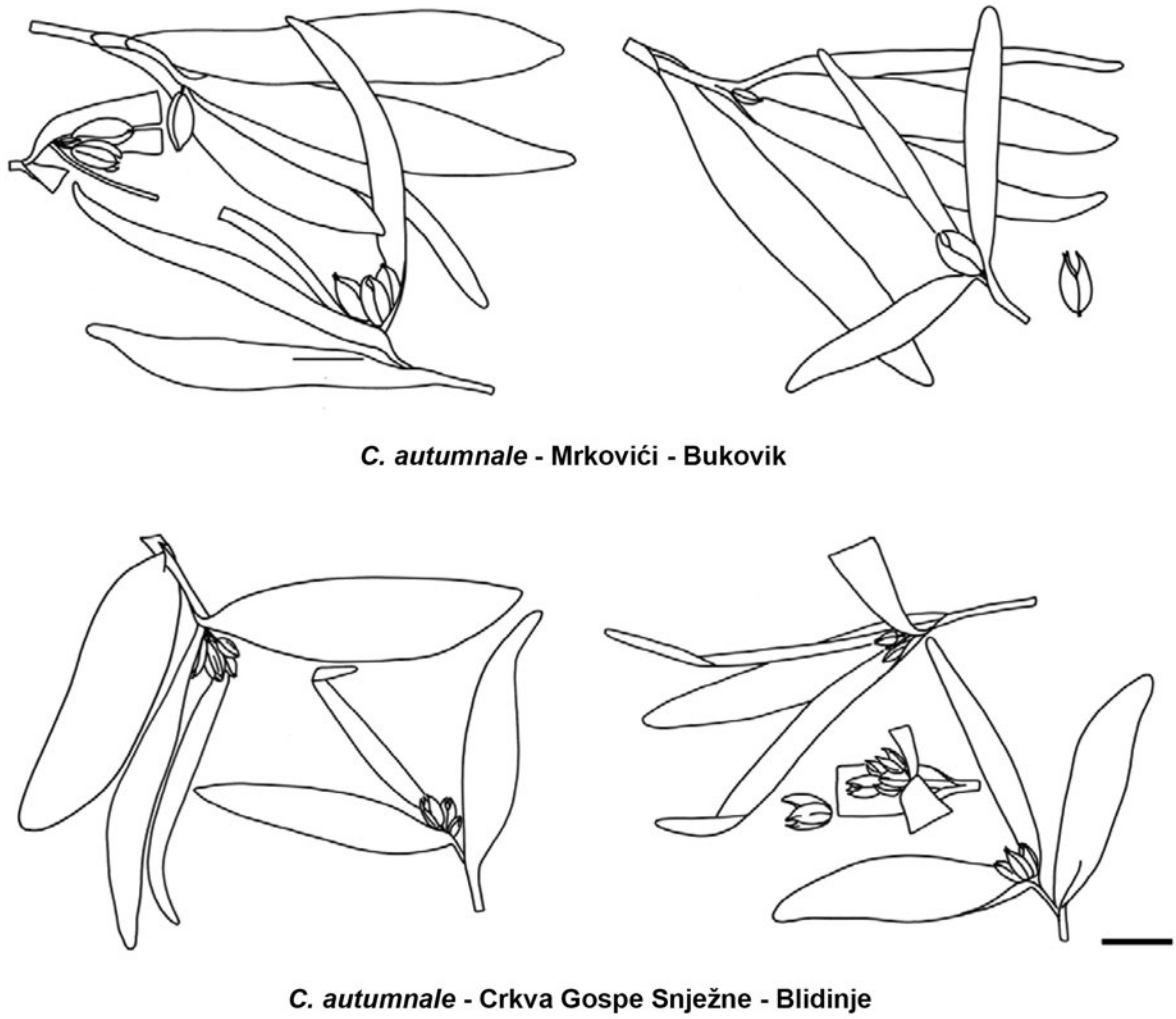

Fig 3. *C. autumnale*. Vegetative silhouette from various fructiferous colchicums from two Bosnia and Herzegovinian populations. Bar scale = 5 cm

different point of view when refuting close relationship between *C. neapolitanum* (Iberian Peninsula, France and Italy) and *C. haynaldii* (Balkans up to Italian border). But we have already demonstrated the mis-identification of *C. neapolitanum* in France and Spain (confused with *C. multiflorum,* Fridlender & Pignal 2013). More investigations are needed to clarify relationships between *C. neapolitanum* subsp. *visianii* from Herzegovina and *C. haynaldii* from Romania (Banatus) and Greece. The well known apparently gradual plant size variation of *C. neapolitanum* s.l. from North Italy to Monte Negro must be investigated: does it correspond to different taxa (i.e subspecies) or ecotypes (i.e. *C. neapolitanum* subsp. *kochii* grows in dry and rocky places in Istria seashore or Pag Island, whereas *C. neapolitanum* subsp. *visianii* from Mt. Biokovo grows in mountainous humid and rich soil)?

According to published chromosome numbers from Italy and Balkans (Goaverts & Persson 2008; Persson 1999, 2008, 2009), *C. neapolitanum* s.l. (here accepted – including *C. haynaldii*) may belong to an octo-decaploid complex with probably many aneuploid populations: 2n = ca 72 sub. *C. tenorei* Parl. nom. utique rej. prop.; 2n = 74 in Macedonia sub. *C. autumnale*; 2n = 76 in Romania sub. *C. haynaldii*; 2n = 80-82 in South Italy sub. *C. gracile* K. Perss.; 2n = 90 in ex-Yugoslavia sub. *C. visianii*; 2n = 90-92 in Italy sub. *C. neapolitanum*; 2n = 96 in ex-Yugoslavia and Greece sub. *C. haynaldii*. Such polyploidy and aneuploidy should explain the observed polymorphism. Unfortunately, we do not have the *C. haynaldii s.s.* genome size. Nevertheless, such variable chromosome numbers are not readily detected by flow cytometry: another point to explore! Moreover, are these different numbers due to chromosome length

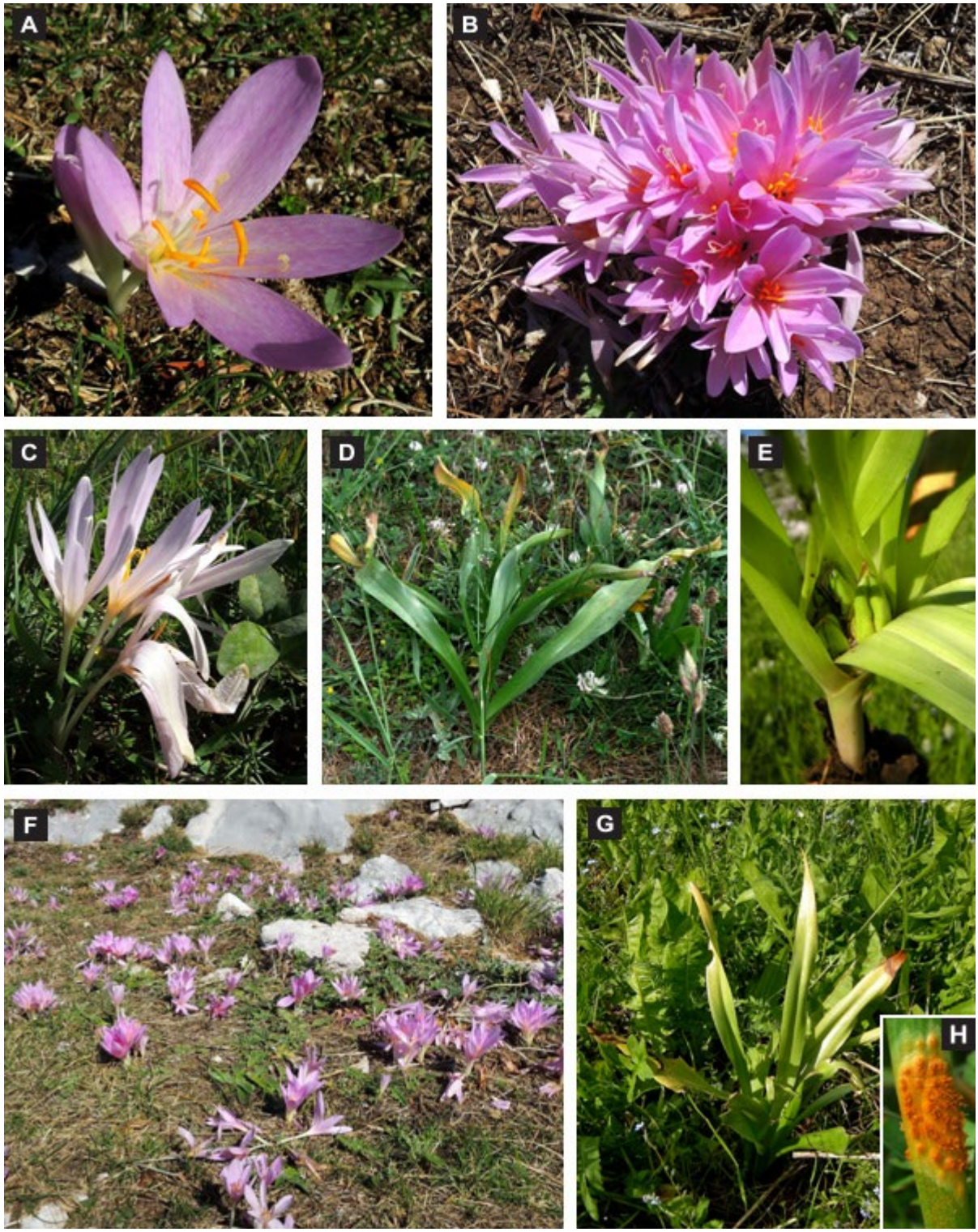

Fig 4. ***C. neapolitanum* subsp. *visianii***. **A** - Tessellated flower. **B** - Non-tessellated big pink flowers resembling *C. autumnale* from one individual after several years of vegetative corm multiplication and synaptospermous seedling. **C** - Three-flor isolated corm with pale pink non-tessellated flower. **D** - Aborted individuals with 4 leaves. **E** - Fertile individual with 4 fruits and 5 broader leaves, notice absence of aerial stipe. **F** - Blooming: each tuft is issued from one maternal individual (cf. B). **G** - Erect leaves. **H** - *Uromyces colchici* on leaves. **A** to **G**: Hr, Biokovo type locality. **H**: BiH, Rakitno - Donji Poklečani, 865 m.

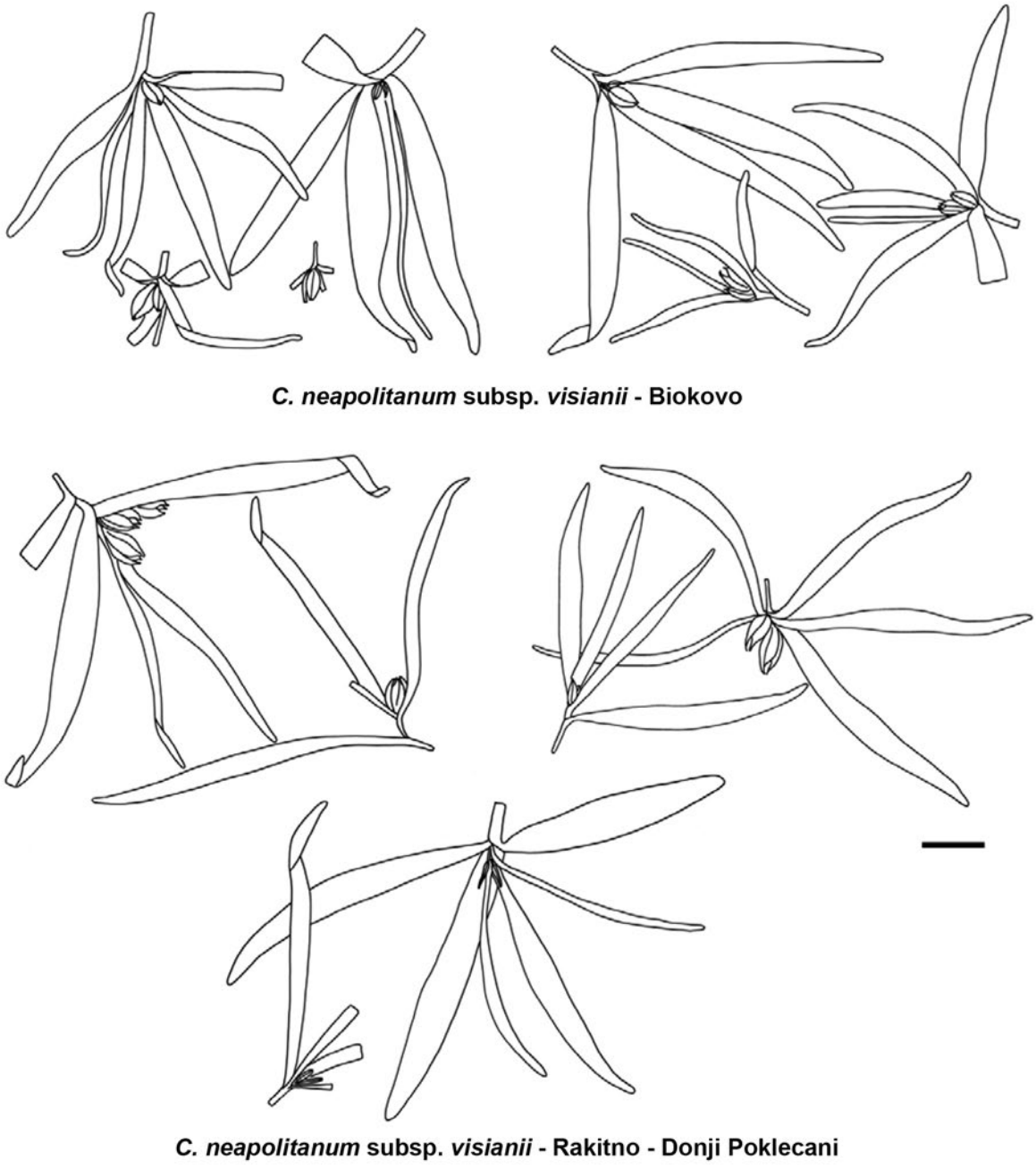

Fig 5. ***C. neapolitanum*** **subsp.** ***visianii***. Vegetative silhouette from various fructiferous colchicums from Herzegovinian populations (some individuals have aborted fruits) and from Biokovo type locality (Hr). Bar scale = 5 cm

variation and/or difficulties to assess exact chromosome numbers in *Colchicum*? The 2n = ca 140-144 chromosomal counts for *C. neapolitanum* (D'Amato 1955, 1957) are probably due to confusion with *C. multiflorum*.

Up to now, chromosome numbers were considered as an important element in *Colchicum* systematics though often difficult to determine. We should also take into account genome size to support new nomenclatural regrouping exposed in Table 1: *C. neapolitanum* s.l. characterised by a genome size of 2C = ca 10.5 pg, and *C. autumnale* s.l. with 2C = ca 6 pg. In our future studies, all investigated populations of Balkan colchicums should be characterised by their chromosome number and genome size.

In South Dalmatia and Bosnia and Herzegovina, *C. neapolitanum* subsp. *visianii* is quite common. *C. autumnale* subsp. *autumnale* grows abundantly in North Bosnia. But on limestone or serpentine soils, in particular in Herzegovina and southern Croatia, many *C. autumnale*

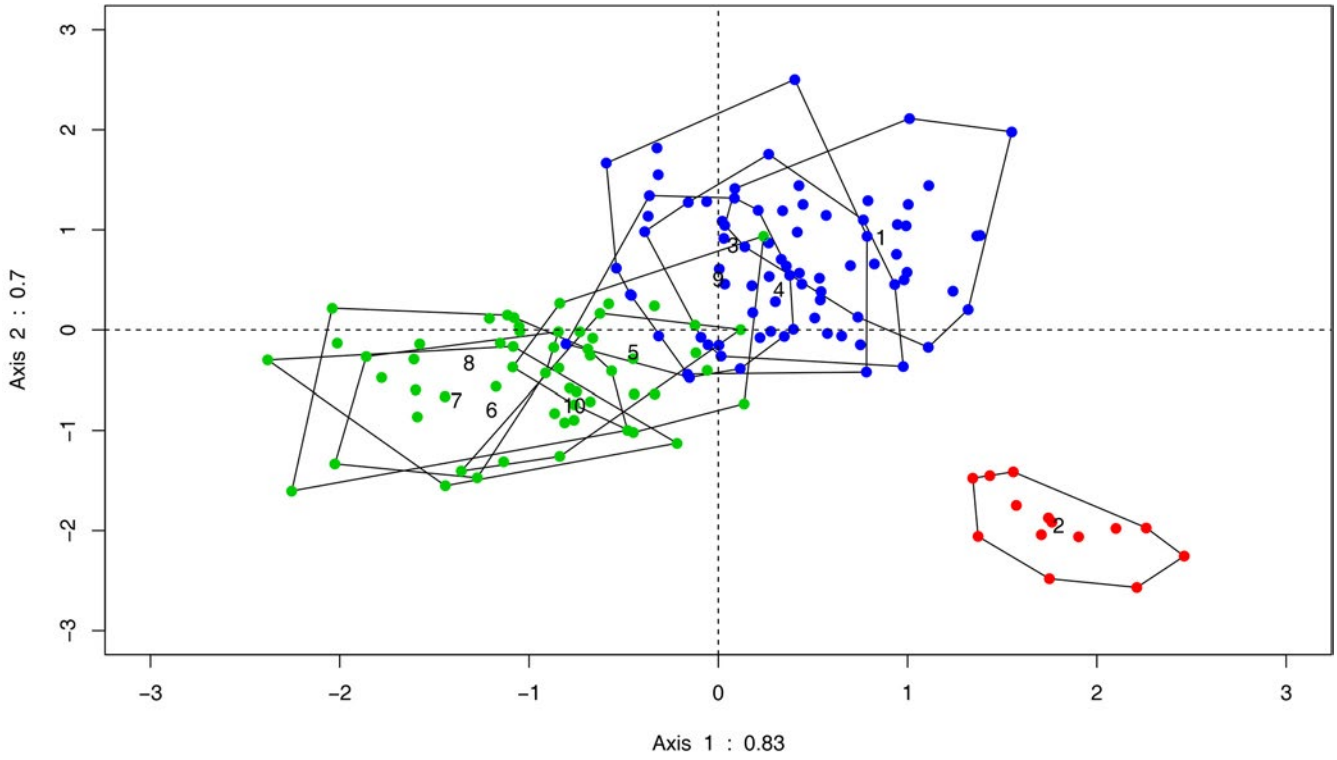

Fig 6. Representation of specimens in the first plane of the discriminant analysis. Axis 1: ratio leaf length/width. Axis 2: width of broadest leaf (Tab. 2). ***C. hungaricum*** in red: 2 - Hr, Mt. Biokovo 1075 m. ***C. neapolitanum*** subsp. ***visianii*** in black: 1 - BiH, Bijela Rudina 660 m; 3 - BiH, Rakitno - Donji Poklečani 865 m; 4 - Hr, Biokovo 1075 m; 5 - Hr, Biokovo 1250-1300 m. ***C. autumnale*** in blue: 6 - BiH, Crkva Gospe Snježne - Blidinje Jezero 1325 m; 7 - BiH, Buk Bijela - Tjentište, 495 m; 8 - BiH, Mrkovići - Bukovik (Sarajevo) 880 m; 9 - BiH, Mrkovići - Bukovik (Sarajevo) 1160 m; 10 - BiH, Čemerno 1280-1300 m.

flowers possess characters intermediate between *C. autumnale* and *C. neapolitanum* subsp. *visianii*. Plants of different Dalmatian populations also present disconcerting affinities with *C. bivonae* s.l. (i.e. anther colour) and even with the two Greek endemic taxa *C. autumnale* subsp. *graecum* and *C. autumnale* subsp. *confusum* (Tab. 1). Do these southern Dalmatian *C. autumnale* populations belong to a new *C. autumnale* subspecies or to one of those Greek mountainous subspecies? We need more field work to elucidate the question as we completely agree with Persson (1999) "…specimen in the floral state [...] are mostly immediately recognizable by an experienced botanist, [but] differences between the species are rather difficult to describe in exact terms and numbers". It could be added that some well visible differences in the wild (like flower colour, tessellated petals) soften on photographic images and of course cannot be detected on herbarium specimens. Ecological preferences are also just indicative and far from being good criteria for both species: generally *C. autumnale* s. l. grows in damp mountain, moist/meadow areas and *Fagus* woodland (Jung *et al.* 2011), whereas *C. neapolitanum* s. l. is usually observed in dryer, stony places but also grows in moist places at altitude on littoral Dinaric Alps (Mt Biokovo).

CONCLUSION

As biometry and punctual field observations are usually insufficient to recognize *Colchicum* taxa, especially in vegetative stage, we suggest the use of cytometry for 2C values as a complement for floristic purpose. This technique (easier than chromosome counting in colchicums, and appropriate for broader sampling) will surely contribute to a better knowledge of ecology, biogeography and conservation of studied *Colchicum* taxa.

Acknowledgments — We thank Fabrice Paranque and Eldin Selimović for help with the english version and preparation of the map.

REFERENCES

Brickell CD. 1980. *Colchicum*. *In* Flora Europaea. Tutin T. G. *et al.* (eds), Cambridge University Press **5**: 21-25.

Brickell CD. 1984. *Colchicum*. *In* Flora of Turkey and the East Aegean Islands. Davis P.H. (eds), Edinburgh University Press.

Camarda I. 1978. *Colchicum gonarei* species nova. *In* Le piante endemiche della Sardegna 21-23, Bolletino della Società Sarda di Scienze Naturali 17: 227-242.

Camarda I. 1979. Actuelles connaissances du genre *Colchicum* en Sardaigne. Webbia 34(1): 481-485.

Camarda I. 1990. Le piante endemiche della Sardegna: 198 *Colchicum corsicum* Baker (1879), Bolletino della Società Sarda di Scienze Naturali **27**: 283-287.

D'Amato F. 1955. Revisione citosistematica del genere *Colchicum* L., I: *C. autumnale* L., *C. lusitanum* Brot. e *C. neapolitanum* Ten. Caryologia **7**: 292-349.

D'Amato F. 1957. Revisione citosistematica del genere *Colchicum* L., III: *C. alpinum* Lam. & DC., *C. cupanii* Guss., *C. bivonae* Guss. e chiave analitica per la determinazione delle specie di *Colchicum* della flora italiana. Caryologia **10**: 111-151.

Domac R. 1973. **Mala Flora Hrvatske i susjednih područja**. Školska knjiga, Zagreb.

Domac R. 1994. **Flora Hrvatske, priručnik za određivanje bilja**, pp. 503, Školska Knjiga – Zagreb.

Feinbrun N. 1958. Chromosome numbers and evolution in the genus *Colchicum*. Evolution **12**: 173-188

Feinbrun N. 1986. **Flora Palestina** Part **4** Israel Academy of sciences and humanities, Jerusalem.

Fernandes A. & Franca F. 1977. Le genre *Colchicum* L. au Portugal, Bol. Soc. Brot., Sér. 2, **51**: 5-36.

Fiori A. 1894. I generi *Tulipa* e *Colchicum* e specie che li rappresentamo nella Flora italiana. Malpighia **VIII**: 131-158.

Fridlender A. 1999a. Sur l'identité des colchiques (*Colchicum* L, *Liliaceae*) à feuilles hystéranthées du Maroc: I. *Colchicum fharii* sp. nov. Bulletin mensuel de la Société Linnéenne de Lyon **68**: 251-278.

Fridlender A. 1999b. Originalités biologiques et systématiques des espèces rares. Quelques exemples choisis dans la flore Tyrrhénienne. Thèse, MNHN, Paris, 500p.

Fridlender A. 1999c. Une nouvelle espèce corse de colchique : *C. arenasii* sp. nov. (*Liliaceae*). Acta Botanica Gallica **146**(2): 157-167.

Fridlender A. 1999d. Description d'une espèce nouvelle de colchique (*Colchicum*, *Liliaceae*) en Sardaigne : *Colchicum actupii* Fridlender. Bulletin mensuel de la Société Linnéenne de Lyon **68**: 193-200.

Fridlender A, Brown S, Verlaque R, Crosnier MT & Pech N. 2002. Cytometric determination of genome size in *Colchicum* species (Liliales, Colchicaceae) of the western Mediterranean area. Plant Cell Rep. **21**: 347-352.

Fridlender A & Pignal M. 2013. Les colchiques de Provence : état de conservation et nouveautés taxonomiques. Nature de Provence ***2***: 19-35.

Galbraith DW, Harkins KR, Maddox JM, Ayres NM, Sharma DP & Firoozabady E. 1983. Rapid flow cytometric analysis of the cell cycle in intact plant tissues. Science **220**: 1049-1051.

Goaverts R & Persson K. 2008. (1834-1835) Proposals to reject the names *Colchicum tenorei* and *Colchicum todaroi* (Colchicaceae). Taxon **57**: 995-996.

Hayek A. 1932-1933. **Prodromus Florae peninsulae Balcanicae**. **3**. Band *Monocotyledoneae*. Dahkem Berlin, 1933.

Hršak V. 2001. Notulae ad indicem Florae Croaticae, 3. *Natura Croatica* Vol. **10**(1): 67-72.

Josse J, Husson F, & Pages J. 2009. Gestion des données manquantes en Analyses en Composantes Principales. Journal de la Société Française de Statistiques **150**: 28-51.

Jung LS, Winter S, Eckstein RL, Kriechbaum M, Karrer G, Welk E, Elsässer M, Donath T W & Otte A. 2011. *Colchicum autumnale* L. *In* Biological Flora of Central Europe. Perspect. Plant Ecol. Evol. Syst. **13**: 227-244.

Lentini F & Raimondo FM. 1984. *Contribution à la connaissance du genre* Colchicum *L. en Sicile.* Webbia 38: 745-755.

Marie D & Brown S. 1993. A cytometric exercise in plant DNA histograms, with 2C values for 70 species. Biol. Cell **78**: 41-51.

Nikolić T. (ed.). 2000. Flora croatica index Florae Croaticae, Pars 3, Natura Croatica **9**, suppl. 1, Hrvatski prirodslovni muzej, Zagreb.

Parlatore F. 1860[1858-1860]. – Colchicaceae *In* Flora Italiana, ossia descrizione delle piante che nascono salvatiche o si sono insalvatichite in Italia e nelle isole ad essa adiacenti. Tip. Le Monnier, Firenze. Vol. **III**: 171-196.

Persson K. 1988. New species of *Colchicum* (Colchicaceae) from the Greek mountains. Materials for the Mountain Flora of Greece, 30. Willdenowia **18**: 29-46.

Persson K. 1991. *Colchicum* L. *In* Strid A & Tan K (eds), Mountain Flora of Greece, Edinburgh University Press, **2**: 650-662.

Persson K. 1992. Liliaceae subfam. Wurmbaeoideae Buxbaum. *In* Rechinge K.H. (ed) Flora Iranica N°**170**: p. 1-40 tt 1-14. Graz – Austria.

Persson K. 1999. New and revised species of *Colchicum* (*Colchicaceae*) from the Balkan Peninsula. Plant Syst. Evol. **217**: 55-80.

Persson K. 2007. Nomenclatural synopsis of the genus *Colchicum* (Colchicaceae), with some new species and combinations. Bot. Jahrb. Syst. **127**(2): 165-242.

Persson K. 2008. A new species of *Colchicum* (Colchiccaceae) from southern Italy. Bot. Jahrb. Syst. **127**: 283-288.

Persson K. 2009. *Colchiccaceae*. *In* IAPT/IOBP chromosome data 7, Marhold K. & Breitwieser I. (ed). Taxon **58**(1):181-183.

Pignatti S. 1982. *Colchicum* L. In *Flora d'Italia*. Edagricola (éd.), Bologna, **3**: 350-352.

Pustahija F, Brown SC, Bogunic F, Basic N, Muratovic E, Ollier S, Hidalgo O, Bourge M, Stevanović V & Siljak-Yakovlev S. 2013. Small genomes dominate in plants growing on serpentine soils in West Balkans, an exhaustive study of 8 habitats covering 308 taxa. Plant and Soil **373**: 427-453 DOI 10.1007/s11104-013-1794-x.

R Core Team. 2013. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-projest.org/.

Radić J. 1976. Bilje Biokova.: Institut Planina i more – Malakološki muzej, Makarska.

Siljak- Yakovlev S, Pustahija F, Solic EM, Bogunic F, Muratovic E, Basic N, Catrice O & Brown SC. 2010. Towards a genome size and chromosome number database of Balkan flora: C-values in 343 taxa with novel values for 242. Adv. Sci. Lett. **3**: 190-213.

Stefanoff B. 1926. Monographiya na roda *Colchicum* L. Sbornik Balg Akademii Nauk Sofiya Kniga **XXII**-9: 3-102.

Stevanović V, Tatić B, Janković M, Diklić N, Jovanović B, Vasić O, Jovanović S, Niketić M, Butorac B & Boza P. 1999. **Crvena knjiga Flore Srbije**, 1 Extinct and critically endangered taxa. Beograd.

Visiani RD. 1842. **Flora Dalmatica sive enumeratio stirpium vascularium quas hactenus in Dalmatia lectas et sibi observatas**. Vo I, Lipsiae, apud Friedericum Hosmeister.

REZIME

Je li moguće identificirati *Colchicum neapolitanum* s.l. i *C. autumnale* s.l. u vegetativnom stanju? Pristup biometrijom i protočnom citometrijom

Alain Fridlender, Fatima Pustahija, Marija Edita Šolić, Sabaheta Abadžić, Mickael Bourge, Nicolas Pech, Sonja Šiljak-Yakovlev, Spencer C. Brown

Teško je razlikovati *Colchicum* vrste koje rastu u Dalmaciji i Bosni i Hercegovini, pa čak i dva najzastupljenija taksona (*C. autumnale* i *C. neapolitanum* subsp. *visianii*). Ne postoje jasni vegetativni karakteri koji su pogodni za dobru identifikaciju tih vrsta. Količina nuklearne DNK, međutim, ukazuje na razlike između ove dvije vrste kao i između drugih taksona. Veličina genoma je određena po prvi put kod: *C. hungaricum* Janka (2C = 5.38 ± 0.3 pg); dvije dalmatinske endemične podvrste *C. neapolitanum* (Ten.) Ten. subsp. *kochii* (Parl.) Fridl. (2C = 10.9 ± 0.3 pg) i *C. neapolitanum* (Ten.) Ten. subsp. *visianii* (Parl.) Fridl. (2C = 10.41 ± 0.32 pg); te kod talijanske podvrste *C. neapolitanum* (Ten.) Ten. subsp. *neapolitanum* (2C = 10.0 ± 0.1 pg), endema područja Basilicata - Campania. Veličina genoma *C. autumnale* L. iz Bosne i Hercegovine (2C = 6.17 ± 0.31 pg) ne razlikuje se od drugih zapadnoevropskih populacija. Oslanjajući se na taj važni karakter, kao i na morfološke studije, predlažemo taksonomsku reviziju nekih taksona. Obzirom na razliku u veličini genoma između ova dva morfološki vrlo slična taksona (*C. neapolitanum* subsp. *visianii* i *C. autumnale*), protočna citometrija se pokazala veoma korisnom za njihovu identifikaciju.

Obzirom da su biometrija i sporadična promatranja na terenu nedovoljna za prepoznavanje taksona iz roda *Colchicum*, naročito u njihovom vegetativnom stanju, predlažemo korištenje protočne citometrije za određivanje 2C vrijednosti (veličine genoma) kao dodatnu metodu za bržu i lakšu identifikaciju navedenih taksona. Ova tehnika (lakša nego određivanje hromosomskog broja u rodu *Colchicum*, i prikladna za veća uzorkovanja) zasigurno će pridonijeti boljem poznavanju ekologije, biogeografije i očuvanja studiranih *Colchicum* taksona.

Ključne riječi: biometrija, *Colchicum*, flora Balkana, protočna citometrija, veličina genoma

Appendix 1: Plants collected by A. Fridlender for genome size analysis and deposited in *Herbier privé Fridlender (s.n.)* & CLF!

***C. autumnale* L. - Bosna i Hercegovina** : Buk Bijela entre Foča et Tjentište, Talus de bord de route, 9-6-2011, 495 m ; Čemerno, (nord de Gacko vers le croisement pour Borač) vastes prairies à *Narcissus poeticus* L., *Asphodelus* cf. *albus* Miller, *Chamaespartium sagittale* (L.) P. Gibbs, *Scorzonera purpurea* (L) subsp. *rosea* (Nyman), *Helleborus* cf. *odorus* Waldst. & Kit.…, 9-6-2011, 1280-1300 m ; Avtovac (Gacko) vaste polje en pâturage ovin et bovin (prairie inondable à *Scilla* sp., *N. poeticus, etc.*), 9-6-2011, 975 m ; prairie enfrichée à *Botrychium* en lisière de hêtraie, montagne au sud de Crkva Gospe Snježne au-dessus du Blidinje Jezero, 12-6-2011, 1 325 m; Mrkovići (massif de Bukovik) Colchiques ça et là en bordure de pinèdes recolonisées par les *Fagus*, abondance de *Neottia*, 14-6-2011, 1 160m ; Mrkovići (massif de Bukovik) prairie de fauche, 14-6-2011, 880m. - **Hrvatska** : Istria, Lovranska Draga le long du sentier qui monte au Vojak, herbages abandonnés entre les châtaigneraies, 2-10-2011, 680 m.

***C. neapolitanum* (Ten.) Ten. subsp. *visianii* Fridlender - Bosna i Hercegovina** : Bijela Rudina prairies de fauche ceintes de murets de pierres, juste au nord du village avec *Dictamnus albus* L., 9-6-2011, 660 m ; plaine de Rakitno vers Donji Poklečani, polje avec *N. poeticus, Dianthus sanguineus* Vis. à corolles rouge vif, feuilles parasitées par *Uromyces colchici*, 12-6-2011, 865m. - **Hrvatska** : Massif de Biokovo route qui monte à Sveti Jure, piano (calcaire) à *C. hungaricum* et *C. visianii*, 10-6-2011, 1075 m ; Massif de Biokovo, route qui monte à Sveti Jure en contrebas du croisement pour Vošac, jardinets et prairies de fauche, marges et fonds de dolines (avec *Saponaria bellidifolia* Sm.), 10-6-2011, 1250- 1300 m ; contrefort des alpes Dinariques, à 200 m de Kekezi à 6 km de Cista Provo, prairies et champs en friches séparés par des murets en ruine (haies à *Acer monspessulanum* L.), 12-6-2011, 405 m.

***C. hungaricum* Janka - Hrvatska**, Massif calcaire de Biokovo route qui monte à Sveti Jure, piano très riche à *C. hungaricum* et *C. visianii* en mélange, 10-6-2011, 1075 m ; au pied du Massif de Biokovo, bord de mer à proximité du phare de Makarska, pelouses a *Romulea bulbocodium* (L.) S. & M. et *C. hungaricum* (feuilles desséchées depuis longtemps !), 11-6-2011, 3 à 10 m.

***C. neapolitanum* (Ten.) Ten. subsp. *neapolitanum* - Italia,** Campania, Penisola Sorrentina, Monti Lattara, châtaigneraies du Monte Faito entre 1000 et 1100m (Gragnano - Napoli), mai 1997 avec de vastes peuplements de *Cyclamen neapolitanum* Tenore.

***C. neapolitanum* (Ten.) Ten. subsp. *kochii* Fridlender – Hrvatska**, presqu'île d'Istria, garrigues et pelouses littorales de Baderna, Poreč et Rovinj, septembre 2011.