The taxa of *Crocus* ser. *Flavi* (Iridaceae) in Greece: a taxonomic and karyomorphometric study

THEOPHANIS KARAMPLIANIS & THEOPHANIS CONSTANTINIDIS

Abstract

Karamplianis, Th. & Constantinidis Th. 2019: The taxa of *Crocus* ser. *Flavi* (Iridaceae) in Greece: a taxonomic and karyomorphometric study. – Bot. Chron.22: 171-194.

This study focuses on the morphology and karyology of Greek members of *Crocus* ser. *Flavi* and their consequences for taxonomy. The series, in Greece, consists of *Crocus balansae*, *C. olivieri, C. flavus* subsp. *flavus,* and *C. flavus* subsp. *dissectus*. The former two have $2n = 6$, whereas the latter two, $2n = 8$ chromosomes. Their karyotypes are basically similar, but differ in relative length, presence of B-chromosomes, and presumed chromosomal translocations. Gross morphology varies within the group with respect to perigon coloration, style dissection, as well as number and width of leaves. In the case of *C. flavus*, intra-population variation of morphological features supports a conservative taxonomic approach, with *C. flavus* subsp. *atticus* reduced to synonymy under *C. flavus* subsp. *flavus,* because morphology and karyomorphology do not support subspecific distinctness. On the other hand, *C. olivieri*, *C. balansae* and their Turkish endemic ally, *C. istanbulensis,* that exhibit additional differences in corm tunic features, are best regarded as distinct species.

Th. Karamplianis & * Th. Constantinidis, Department of Ecology & Systematics, Faculty of Biology, National & Kapodistrian University of Athens, Panepistimiopolis, GR-157 03 Athens, Greece. E-mails: theo_karamp@biol.uoa.gr; constgr@biol.uoa.gr (* = corresponding author).

Key words: Crocus ser. *Flavi*, karyotype asymmetry, morphology, somatic chromosomes, statistics, taxonomy.

Introduction

The genus *Crocus* L. (Iridaceae) comprises c. 200 taxa (HARPKE & al. 2016), its total distribution area extending from the Iberian Peninsula and Morocco to west China longitudinally, and from Poland and the Caucasus region to the northern parts of the Arabian Peninsula and Cyrenaica, latitudinally (MATHEW 1982, RUKŠĀNS 2010). According to DIMOPOULOS & al. (2013), 29 taxa (species and subspecies) are found in Greece, a number that recent taxonomic amendments and re-circumscriptions (RUKŠĀNS 2013, 2014, HARPKE & al. 2015, TAN & al. 2014) have raised to 35. According to current understanding, 4 taxa of *Crocus* ser. *Flavi* B. Mathew are growing in Greece, viz., *C. olivieri* J. Gay, *C. balansae* J. Gay ex Maw, *C. flavus* Weston subsp. *flavus* and *C. flavus* subsp. *dissectus* Baytop & B. Mathew. The first species has a wide distribution and altitudinal range on both mainland and insular Greece (GAY 1831, HERBERT 1847, ORPHANIDES 1869, BAKER 1873, HOOKER 1873, BOISSIER 1882, HALÁCSY 1896, ZAGANIARIS 1938, PAPANICOLAOU & ZACHAROF 1980, VOLIOTIS 1986, MATHEW 2000, SNOGERUP & al. 2001), and karyological data are plentiful (MATHEW &

BRIGHTON 1972, BRIGHTON & al. 1973, BRIGHTON 1976, MATHEW 1976, 1982, PAPANICOLAOU & ZACHAROF (1980), USLU & al. 2012, YETIŞEN & al. 2013, and YÜZBAŞIOĞLU & al. 2013. The second species is distributed from the East Aegean Islands (Chios and Samos, Greece) to Anatolia (Turkey), and its karyomorphology is reported here for the first time. Both species have a karyotype of $2n = 6$ somatic but no supernumerary B-chromosomes. *C. flavus* subsp. *flavus* is mainly found in the northern parts of the Greek mainland: its northern Greek populations, together with an outlying one in Central Greece, form the southernmost part of its total distribution area (MATHEW & BAYTOP 1976, PAPANICOLAOU & ZACHAROF 1980). It has also been reported from the island of Kerkyra (Corfu) by MAZZIARI (1834), but this report lacks recent confirmation. Its karyology was studied by MATHEW (1976, 1982) and PAPANICOLAOU & ZACHAROF (1980). *C. flavus* subsp. *dissectus* was considered a Turkish endemic until recently, when PAPANICOLAOU & ZACHAROF (1980) reported a population from Mt. Gramos (NW Greece) and found its chromosome number to be $2n = 8$. The southernmost Greek population of *C. flavus* subsp. *flavus* is located in the Tatoi area in Attiki, and was already known to *Heldreich*, who collected it in 1878, followed by *S. Atchley* and *E. A. Bowles* in 1926, *E. A. Bowles* in 1938, *K. Goulimis* in 1956, and *E. Stamatiadou* in 1969 (see HALÁCSY 1904, BOWLES 1924, GOULIMIS 1956 and MATHEW 1982).

Chromosome numbers and karyotype morphology, within *Crocus,* are important to assess species relationship and taxonomy, as pointed by out PERUZZI & ALTINORDU (2014) in their study of *Crocus* ser. *Verni*. Karyological data often correlate well with taxonomy and may support phylogenetic hypotheses (e.g. HARPKE & al. 2015). Karyotype studies may therefore be valuable in tracing evolutionary lineages within the genus. In *Crocus* ser. *Flavi*, the evolutionary advanced taxa show a clear tendency toward more asymmetrical karyotypes (HARPKE & al. 2013). Supernumerary B-chromosomes also may have played a role in genome evolution (HARPKE & al. 2013: 626), underpinning hybridization and phylogenetic trends within the genus.

Materials and methods

The material investigated in this study consists of dried specimens collected in Greece and deposited at the Herbarium of the National and Kapodistrian University of Athens (ATHU), including voucher specimens for our karyological data. Material from the herbaria P, K, WU, B, IPK (see THIERS, continuously updated) was consulted in addition. Living plants collected in the field and cultivated in an experimental garden of School of Biology at National & Kapodistrian University of Athens were used for karyological studies, a topic to be included in the first author's PhD thesis, currently in progress. Their origin, chromosome number and karyomorphometric data are presented in Table 1.

For our morphometric study, quantitative morphological features of 5 taxa were measured, and qualitative features assessed, on 184 individuals from 24 populations distributed in Greece, the Balkans and NW Turkey. Measurements were taken on dried specimens, using a Zeiss Stemi 2000 stereomicroscope, whereas specimen photographs were processed by the Image J v.1.50f software (SCHNEIDER & al. 2012).

For compiling the morphological data matrix we used both quantitative and qualitative characters. The most informative characters in terms of univariate analysis were used (Table 2 and 3). At first, variables of 71 vegetative and floral characters with 2 (binary) to 10 states (multistate characters) and quantitative characters (Table 3) were analysed for the whole genus, in

Table 1. Origin, karyomorphometry and chromosome relative length of *Crocus olivieri* and *Cr. flavus* aggregates according to literature and our own data. For details of the headings see the text (material and methods). The following references were used, particularly for the last column: [1]STEBBINS (1971), [2]PLUMMER & al. (2003), [3]MATHEW & BRIGHTON (1972), [4]BRIGHTON (1976), [5]YÜZBAŞIOĞLU & al.(2013).

***Crocus* taxa**	**Lat.**	**Long.**	**x**	**2n**	**THL**	**CV_{CI}**	**CV_{CL}**	**M_{CA}**	**1-4 A-C**	**B -**	**SAT-**			PLUMMER & al. [2]					**Ref.**
									STEBBINS [1]	**chrom**	**L**	**S**	**int**	**VS**	**S**	**M**	**L**	**VL**	
Cr. antalyensis	s.d.	s.d	4	8	19.94	11.99	26.94	41.22	2A	0	0	0	0	0	0	0.88	0.12	0	3
Cr. balansae	38.04	26.1167	3	6	29.92	15.07	11.03	51.16	4A	0	0	0	2	0	0	1	0	0	new
	s.d.	s.d.	3	6	26.74	6.84	4.04	20.80	4A	0	0	0	2	0	0	1	0	0	4
Cr. candidus	s.d.	s.d	3	6	29.12	9.64	6.29	22.97	4A	0	0	0	2	0	0.33	0.33	0.33	0	4
Cr. flavus* subsp. *flavus	40.0184	21.5146	4	8	29.83	18.92	32.02	42.04	3A	1	0	2	0	0.11	0	0.22	0.67	0	new
	40.0184	21.5146	4	8	34.1	38.18	41.12	45.54	3A	2	0	2	0	0	0.2	0.4	0.4	0	new
	40.0184	21.5146	4	8	29.48	26.74	24.6	40.54	3A	0	0	2	0	0	0	1	0	0	new
	38.1977	23.7846	4	8	34.93	27.68	19.72	42.62	3A	1	0	2	0	0.11	0	0.67	0.22	0	new
	s.d.	s.d	4	8	32.49	6.38	19.53	25.53	3A	0	0	2	0	0	0	1	0	0	4
	s.d.	s.d	4	8	25.13	8.41	23.40	25.95	3A	11	0	2	0	0.58	0	0.42	0	0	4
Cr. flavus* subsp. *dissectus	s.d.	s.d	4	8	28.18	7.00	16.64	29.10	3A	0	0	2	0	0	0	1	0	0	4
Cr. graveolens	s.d.	s.d	3	6	28.09	19.06	24.58	34.76	2A	0	0	0	2	0	0	1	0	0	4
Cr. hyemalis	s.d.	s.d	3	6	33.02	19.45	17.94	28.80	3B	2	0	2	0	0.4	0	0.2	0.2	0.2	4
Cr. istanbulensis	s.d	s.d	3	6	24.68	55.07	7.55	53.76	3A	0	0	0	2	0	0	1	0	0	5
Cr. olivieri	38.4019	26.0200	3	6	24.02	7.66	8.36	57.48	4A	1	0	0	2	0	0	1	0	0	new
	41.2214	23.7703	3	6	27.47	26.67	8.739	59.4	4A	0, 2	0	0	2	0	0	1	0	0	new
	38.5664	26.011	3	6	24.86	12.73	6.86	53.05	4A	1	0	0	2	0	0	1	0	0	new
	37.7727	22.5222	3	6	25.48	10.12	13.27	22.52	4A	0-3	0	0	2	0	0	1	0	0	new
	s.d.	s.d	3	6	22.27	3.49	6.90	21.81	3A	0	0	0	2	0	0	1	0	0	4
	s.d.	s.d	3	6	23.74	8.40	5.93	19.42	3A	2	0	0	2	0.25	0	0.5	0.25	0	4
Cr. vitellinus	s.d.	s.d	4	8	25.30	8.81	23.95	20.14	A	0	0	1	0	0	0	1	0	0	4
	s.d.	s.d	4	8	18.51	8.43	20.37	22.87	A	0	0	0	0	0	0	1	0	0	4

s.d.: without geodata

Table 2. Most informative morphological characters (both qualitative and quantitative), with their loadings as implemented by the Principal Coordinate Analysis.

	Character code	Character abbreviation	Character description	Axis 1	Axis 2	Axis 3	Axis 4
1	**51**	**leaf_l**	Leaf length	1.7725	-28.082	12.966	-21.615
2	**54**	**tub_l**	Perigone tube length	1.1155	-9.0739	18.603	-24.091
3	**49**	**cor_n**	Length of corm cup	1.0871	-0.37562	-1.2609	-13.48
4	**58**	**L_in**	Length of inner perigone segments	0.17725	-2.1809	3.4142	-1.298
5	**55**	**L_out**	Length of outer perigone segments	0.17598	-3.0064	3.665	-0.65309
6	**70**	**Anth_l**	Anthers length	0.16	-0.49034	2.2991	0.58583
7	**1**	**cor_shp**	Corm shape	0.087736	0.004749	-0.003291	0.005106
8	**2**	**cor_tun**	Corm tunic type	1.29E-29	8.50E-30	4.06E-30	-5.09E-30
9	**15**	**bract_col**	Bract coloration	7.73E-30	2.90E-30	1.63E-30	-2.23E-30
10	**28**	**Phar_pub**	Pharynx pubescence	5.04E-30	2.11E-30	1.09E-30	-1.63E-30
11	**22**	**Out_sgm_homo**	Outer perigone segments homogeneity	1.66E-30	7.55E-31	4.75E-31	-5.08E-31
12	**20**	**In_sgm_homo**	Inner perigone segments homogeneity	1.64E-30	3.53E-31	2.07E-31	-3.85E-31
13	**26**	**tub_mot**	Perigone tube motif	3.65E-31	1.27E-31	8.55E-32	-9.30E-32
14	**24**	**sgm_mot**	Perigone segments motif	1.82E-31	6.34E-32	4.28E-32	-4.65E-32
15	**27**	**Phar_col**	Pharynx coloration	-4.39E-31	-2.12E-31	-1.11E-31	1.02E-31
16	**13**	**Cataph_col**	Cataphylls coloration	-1.16E-30	-3.51E-31	-1.80E-31	3.32E-31
17	**43**	**Elaiosome**	Elaiosome	-5.10E-30	-3.17E-30	-1.62E-30	1.99E-30
18	**44**	**Raphe_typ**	Raphe type	-5.10E-30	-3.17E-30	-1.62E-30	1.99E-30
19	**9**	**Leaf_ind**	Leaf indumentum	-7.73E-30	-2.90E-30	-1.63E-30	2.23E-30

Table 3. Dataset of the most informative qualitative morphological characters of *Crocus* ser. *Flavi* (abbreviations as indicated below).

Taxa	**Accession numbers**	**Characters**										
		0	**0**	**0**	**1**	**1**	**2**	**2**	**2**	**2**	**2**	**2**
		1	**2**	**9**	**3**	**5**	**0**	**2**	**4**	**6**	**7**	**8**
Crocus olivieri **J. Gay**	1286_1	1	0	4	3	0	0	1	0	0	1	2
Crocus olivieri **J. Gay**	1286_2	1	0	4	3	0	0	1	0	0	1	2
Crocus olivieri **J. Gay**	1286_3	1	0	4	3	0	0	1	0	0	1	2
Crocus olivieri **J. Gay**	1286_4	1	0	4	3	0	0	1	0	0	1	2
Crocus olivieri **J. Gay**	1760_1	1	0	4	3	0	0	1	0	0	1	2
Crocus olivieri **J. Gay**	1760_2	1	0	4	3	0	0	1	0	0	1	2
Crocus olivieri **J. Gay**	1760_3	1	0	4	3	0	0	1	0	0	1	2
Crocus olivieri **J. Gay**	1760_4	1	0	4	3	0	0	1	0	0	1	2
Crocus olivieri **J. Gay**	1760_5	1	0	4	3	0	0	1	0	0	1	2
Crocus olivieri **J. Gay**	1760_6	1	0	4	3	0	0	1	0	0	1	2
Crocus olivieri **J. Gay**	1760_7	1	0	4	3	0	0	1	0	0	1	2
Crocus olivieri **J. Gay**	1760_8	1	0	4	3	0	0	1	0	0	1	2
Crocus olivieri **J. Gay**	1761_1	1	0	4	3	0	0	1	0	0	1	2
Crocus olivieri **J. Gay**	1761_2	1	0	4	3	0	0	1	0	0	1	2
Crocus olivieri **J. Gay**	1761_3	1	0	4	3	0	0	1	0	0	1	2
Crocus olivieri **J. Gay**	2081_1	1	0	4	3	0	0	1	0	0	1	2
Crocus olivieri **J. Gay**	2081_2	1	0	4	3	0	0	1	0	0	1	2
Crocus olivieri **J. Gay**	2081_3	1	0	4	3	0	0	1	0	0	1	2
Crocus olivieri **J. Gay**	2081_4	1	0	4	3	0	0	1	0	0	1	2
Crocus olivieri **J. Gay**	2081_5	1	0	4	3	0	0	1	0	0	1	2
Crocus olivieri **J. Gay**	2081_6	1	0	4	3	0	0	1	0	0	1	2
Crocus olivieri **J. Gay**	2081_7	1	0	4	3	0	0	1	0	0	1	2
Crocus olivieri **J. Gay**	2081_8	1	0	4	3	0	0	1	0	0	1	2
Crocus olivieri **J. Gay**	2097_1	1	0	4	3	0	0	1	0	0	1	2
Crocus olivieri **J. Gay**	2097_10	1	0	4	3	0	0	1	0	0	1	2
Crocus olivieri **J. Gay**	2097_11	1	0	4	3	0	0	1	0	0	1	2

***Crocus olivieri* J. Gay**	2097_12	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2097_13	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2097_14	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2097_15	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2097_2	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2097_3	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2097_4	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2097_5	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2097_6	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2097_7	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2097_8	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2097_9	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2102_b	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2104_1	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2104_10	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2104_11	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2104_12	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2104_13	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2104_2	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2104_3	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2104_4	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2104_5	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2104_6	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2104_7	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2104_8	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2104_9	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2888_1	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2888_2	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2888_3	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	2888_4	1	0	4	3	0	0	1	0	0	1	2
***Crocus olivieri* J. Gay**	72_16	1	0	4	3	0	0	1	0	0	1	2
Crocus flavus* Weston subsp. *flavus	2113_1	3	4	1	2	3	1	0	0	0	1	0

Crocus flavus **Weston subsp.** ***flavus***	2113_10	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_11	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_12	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_13	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_14	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_15	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_16	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_17	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_18	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_19	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_2	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_20	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_21	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_22	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_23	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_24	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_3	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_4	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_5	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_6	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_7	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_8	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_9	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_cult_1	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_cult_10	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_cult_11	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_cult_12	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_cult_13	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_cult_14	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_cult_15	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_cult_16	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	2113_cult_17	3	4	1	2	3	1	0	0	0	1	0

Crocus flavus* Weston subsp. *flavus	2113_cult_18	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_19	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_2	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_20	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_21	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_22	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_23	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_24	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_25	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_26	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_27	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_28	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_29	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_3	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_30	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_31	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_32	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_33	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_34	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_35	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_36	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_37	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_38	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_39	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_4	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_40	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_5	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_6	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_7	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	72_1	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	72_10	3	4	1	2	3	1	0	0	0	1	0

Crocus flavus* Weston subsp. *flavus	72_11	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	72_12	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	72_13	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	72_14	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	72_15	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_8	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	2113_cult_9	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	72_2	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	72_3	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	72_4	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	72_5	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	72_6	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	72_7	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	72_8	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus* Weston subsp. *flavus	72_9	3	4	1	2	3	1	0	0	0	1	0
***Crocus flavus* Weston subsp. *dissectus* Baytop & Mathew**	2124_1	3	4	1	2	3	1	0	0	0	1	0
***Crocus flavus* Weston subsp. *dissectus* Baytop & Mathew**	2124_2	3	4	1	2	3	1	0	0	0	1	0
***Crocus flavus* Weston subsp. d*issectus* Baytop & Mathew**	2124_3	3	4	1	2	3	1	0	0	0	1	0
***Crocus flavus* Weston subsp. d*issectus* Baytop & Mathew**	2124_4	3	4	1	2	3	1	0	0	0	1	0
***Crocus flavus* Weston subsp. d*issectus* Baytop & Mathew**	23990_1	3	4	1	2	3	1	0	0	0	1	0
***Crocus flavus* Weston subsp. d*issectus* Baytop & Mathew**	23990_2	3	4	1	2	3	1	0	0	0	1	0
***Crocus flavus* Weston subsp. d*issectus* Baytop & Mathew**	23990_3	3	4	1	2	3	1	0	0	0	1	0
***Crocus flavus* Weston subsp. d*issectus* Baytop & Mathew**	23990_4	3	4	1	2	3	1	0	0	0	1	0
***Crocus flavus* Weston subsp. d*issectus* Baytop & Mathew**	23990_5	3	4	1	2	3	1	0	0	0	1	0

Crocus flavus **Weston subsp. d*issectus*** **Baytop & Mathew**	23990_6	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp. d*issectus*** **Baytop & Mathew**	23990_7	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp. d*issectus*** **Baytop & Mathew**	23990_8	3	4	1	2	3	1	0	0	0	1	0
Crocus istabulensis **(B.Mathew) Ruksans**	46030_1	1	6	4	3	0	0	1	0	0	1	2
Crocus istabulensis **(B.Mathew) Ruksans**	46030_2	1	6	4	3	0	0	1	0	0	1	2
Crocus istabulensis **(B.Mathew) Ruksans**	46030_3	1	6	4	3	0	0	1	0	0	1	2
Crocus istabulensis **(B.Mathew) Ruksans**	46030_4	1	6	4	3	0	0	1	0	0	1	2
Crocus istabulensis **(B.Mathew) Ruksans**	46030_5	1	6	4	3	0	0	1	0	0	1	2
Crocus istabulensis **(B.Mathew) Ruksans**	46030_6	2	6	4	3	0	0	1	0	0	1	2
Crocus istabulensis **(B.Mathew) Ruksans**	46030_7	2	6	4	3	0	0	1	0	0	1	2
Crocus istabulensis **(B.Mathew) Ruksans**	46030_8	1	6	4	3	0	0	1	0	0	1	2
Crocus balansae **J. Gay**	B34_1	1	0	4	3	0	0	0	1	2	2	3
Crocus balansae **J. Gay**	B34_2	1	0	4	3	0	0	0	1	2	2	3
Crocus balansae **J. Gay**	B34_3	1	0	4	3	0	0	0	1	2	2	3
Crocus balansae **J. Gay**	B34_4	1	0	4	3	0	0	0	1	2	2	3
Crocus balansae **J. Gay**	B34_5	1	0	4	3	0	0	0	1	2	2	3
Crocus flavus **Weston subsp. *flavus***	P2107918_1	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp. *flavus***	P2107918_2	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp. *flavus***	P2107918_3	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp. *flavus***	P2107918_4	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp. *flavus***	P2107918_5	3	4	1	2	3	1	0	0	0	1	0

Crocus flavus **Weston subsp.** ***flavus***	P2107918_6	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	P2107918_7	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	P2107918_8	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	P2107922_1	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	P2107922_2	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	P2107922_3	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	P2107922_4	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	P2107924_1	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	P2107930_1	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	P2107930_2	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	P2107930_3	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	P2107930_4	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	P2107932_1	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	P2107932_2	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	P2107932_3	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	P2107932_4	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	P2107933_1	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	P2107933_2	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	P2107933_3	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	P2107933_4	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	P2107933_5	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	P2107941_1	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	P2107941_2	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	P2107941_3	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	PA 3014_1	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	PA 3014_2	3	4	1	2	3	1	0	0	0	1	0
Crocus flavus **Weston subsp.** ***flavus***	PA 3014_3	3	4	1	2	3	1	0	0	0	1	0
Crocus balansae **J. Gay**	s.n._1	1	0	4	3	0	0	0	1	2	2	3
Crocus balansae **J. Gay**	s.n._2	1	0	4	3	0	0	0	1	2	2	3
Crocus balansae **J. Gay**	s.n._3	1	0	4	3	0	0	0	1	2	2	3
Crocus flavus **Weston subsp.** ***dissectus*** **Baytop & Mathew**	ZE544	3	4	1	2	3	1	0	0	0	1	0

***Crocus flavus* Weston subsp. *dissectus* Baytop & Mathew**	ZE544_1	3	4	1	2	3	1	0	0	0	1	0
***Crocus flavus* Weston subsp. *dissectus* Baytop & Mathew**	ZE544_2	3	4	1	2	3	1	0	0	0	1	0

	State / **Trait**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1	**corm shape**	flattened-globose	subglobose	ovate	globose	subovate						
2	**corm tunic**	smooth with acute dentate basal appendages	membranous or papery	papery or stiff with basal rings	coarsely – parallel fibrous reaching the cap	membranous with parallel fibres	fine reticulate fibrous	coarsely reticulate fibrous	hard reticulate fibrous	parrallel fibrous lower, reticulate upper	papery with vertical bands	interwoven
9	**leaf indumentum**	glabrous	pubescent at margins	pubescent at upper surface	pubescent underneath	pubescent	other					
13	**cataphyl colour**	white	greenish	brownish	maculate							
15	**bract colour**	white	greenish	brownish	maculate							
20	**inner segments colour homogenity**	no	yes									
22	**outer segments colour homogenity**	no	yes									
24	**segments motif**	lacking	vertical striped	feathered	tinged basally	tinged apically	uncolored margins					
26	**tube motif**	lacking	vertical striped	tinged								
27	**pharynx colour**	white	yellow	violet	deep violet							
28	**pharynx pubescence**	glabrous	pubescent	ciliate	papillose							

order to estimate which characters are most informative for taxon discrimination, especially for closely related taxa. A dissimilarity matrix was calculated using the Gower distance measure in three clustering modes (Single Linkage, Complete Linkage and Unweighted Pair-Group Method with Arithmetic Mean, UPGMA) to render phenograms. We selected, as best suited, those phenograms with Cophenetic Correlation values above 75%, an indication that these phenograms exhibit the most reliable classification (SOKAL 1986). The analysis was carried out via Past version 3.15 (HAMMER & al. 2001) and the derived morphological data matrix was compared with the karyomorphology correlations within the *Crocus flavus* group.

Karyology and karyomorphometric analysis. −Root tips (see Table 1) were pretreated in 1 : 1 solution of cycloheximide (0.006 % w/v) and 8-hydroxyquinoline (0.006 % w/v) for 2–3 h. They were fixed in Carnoy solution (3 : 1 v/v absolute ethanol : glacial acetic acid) for at least 24 h at 4°C and stored in a solution of 70 % ethanol at −20 °C until use. The tips were hydrolysed in 1n HCl for 11 min at 60°C, and stained with Feulgen's stain. After 3-4 h of staining the tips showed a uniform rose appearance, and their chopped tips were macerated in 45% (v/v) glacial acetic acid for the squash procedure. Metaphases were observed using a Zeiss Axio Imager A_1 microscope and documented with an AxioCam MR3. Selected photographs (at least five mitotic plates per population) were measured using KaryoType version 2 (ALTINORDU & al. 2016) software. Chromosome karyotype formula follows the classification of LEVAN & al. (1964). The formation of the karyotype is crucial in taxonomic treatment either for species with different centromere positions or different relative size. The former case might reflects chromosome translocations whereas the latter segmental interchange. Furthermore, the known karyotype formula was compared with their New Relative Length (NRL; PLUMMER & al. 2003) in order to match the homologous chromosomes.

Karyograms were constructed by organizing the chromosomes into groups, ordering them according to their arm length ratio and overall length. The data are presented in relative lengths over physical ones to avoid metaphase biases. The Total Haploid Length (THL), in µm, reported in this study is an estimate of the length of the haploid chromosome complement. Ideograms are based on the mean centromeric index per population and arranged in decreasing size.

Karyotype asymmetry was estimated according to the asymmetry classification of STEBBINS (1971) and analysed according to the most informative indices, as proposed by PERUZZI & ALTINORDU (2014) for *Crocus* ser. *Verni*. Chromosome Length of Chromosome Index (CV_{CI}; CV_{CL}; PASZKO 2006), and the mean centromeric asymmetry (M_{CA}; PERUZZI & ALTINORDU 2014) were calculated.

Furthermore, a multivariate analysis was carried out using Past v. 3.15 (HAMMER & al. 2001, HAMMER 2013). A dataset of 20 OTU's (operational taxonomic units) × 16 variables (8 quantitative and 8 qualitative) was used in order to calculate the average taxonomic distance and construct a dendrogram. The clustering analysis was performed using UPGMA and Gower general Dissimilarity Coefficient, as described by GOWER (1971). The proposed method follows PODANI & SCHMERA (2006). Gower's algorithm is best suited for a mixture of scale types, and is tolerant of missing values.

The results of the karyomorphometric study were presented both in Discriminant Analysis plots and phenograms, after Cluster Analysis.

Results and Discussion

Taxonomic treatment

The results of our study indicate that the members of *Crocus olivieri* aggregate sensu MATHEW (1982) are best treated as distinct species. Hence, we accept specific status for *C. olivieri*, *C. balansae* and the Turkish endemic *C. istanbulensis* (B.

Mathew) Rukšāns (included here so as to consider the aggregate as a whole), because they differ in morphology and show minor karyological differences as well. Conversely, the members of the *C. flavus* group are morphologically variable. The characters used to circumscribe its members are the number of stigmatic branches, the width and length of the leaves, and the shape and cup length of the corm. An evaluation of these and other characters shows that the corm tunics are important for taxonomic delimitation in the group, but leaf dimensions and stigma dissection are of lesser value. This is consistent with the idea of a but recent divergence of taxa in this group (HARPKE & al. 2013).

Crocus flavus subsp. *atticus* Kit Tan & al. was claimed to differ from *C. flavus* subsp. *flavus* in style dissection (TAN & al. 2014: 123): in the former, the style would be divided into 6 distinct branches, whereas in the latter the style is shortly trifid. On a visit to the type locality of *C. flavus* subsp. *atticus,* in mid-February 2016, we found that style division is variable: Almost half of the studied flowers had trifid styles. Also, multifid styles were but apically divided (for up to 2 mm) and deeply frilled. Style division number, in half of the individuals, ranged from 4 to 7, and was not correlated with any other morphological feature. Stigma maturity in the material collected in the Tatoi district was evaluated using their hydrogen peroxide response (MCINNIS & al. 2006). We found that several stigmata were unreceptive and therefore immature. The degree of style dissection in the studied population may indeed be correlated with the maturity stage of the stigmas.

Several *Crocus flavus* plants from Mt. Parnitha near Athens, which is the locus classicus of *C. flavus* subsp. *atticus*, show non-bracteolate flowers, described as characteristic for the latter (TAN & al. 2014). This morphological character was thoroughly studied by CHOOB (2000) for *Crocus* as a whole. It was found that, anatomically, the presence or absence of a bracteole is correlated with the developmental stage of the inflorescence. CHOOB (2000) linked the "anomaly" of lacking bracteoles with the rank of inflorescence axis. He writes (CHOOB 2000:103): "all Crocus species have prophylls at the bases of paracladia"; and "the bract and bracteole in *C. flavus* are prophylls. The flower is on the 3rd (4th) axis". Therefore, the peculiarity of *C. flavus* subsp. *atticus* (and *C. flavus* in general) is due to organ reduction (CHOOB 2000:104): "organ reduction occurs by three processes (a) abortion, (b) ablasty and (c) fusion", and is an ontogenetic developmental process. This may explain why the ratio of bracteolate against non-bracteolate flowers depends on inflorescence development and, hence, collecting date. The type material of *C. flavus* subsp. *atticus* was collected early in its flowering period (8th of February), whereas the material studied by TAN & al. (2014) was gathered later (2nd of March), is in a more advanced developmental stage and, therefore, its secondary axial inflorescence lacks bracteoles.

Crocus flavus Weston, Bot. Univ. 2: 237. 1771, **subsp. *flavus***

= *C. flavus* subsp. *atticus* Kit Tan & al. in Phytol. Balcan. 20: 123. 2014.

Morphometric study. – The UPGMA phenogram constructed on the basis of morphological data shows four major branches (Fig. 1). The first, uppermost branch consists of *Crocus balansae* individuals only; the next branches are more complex, with

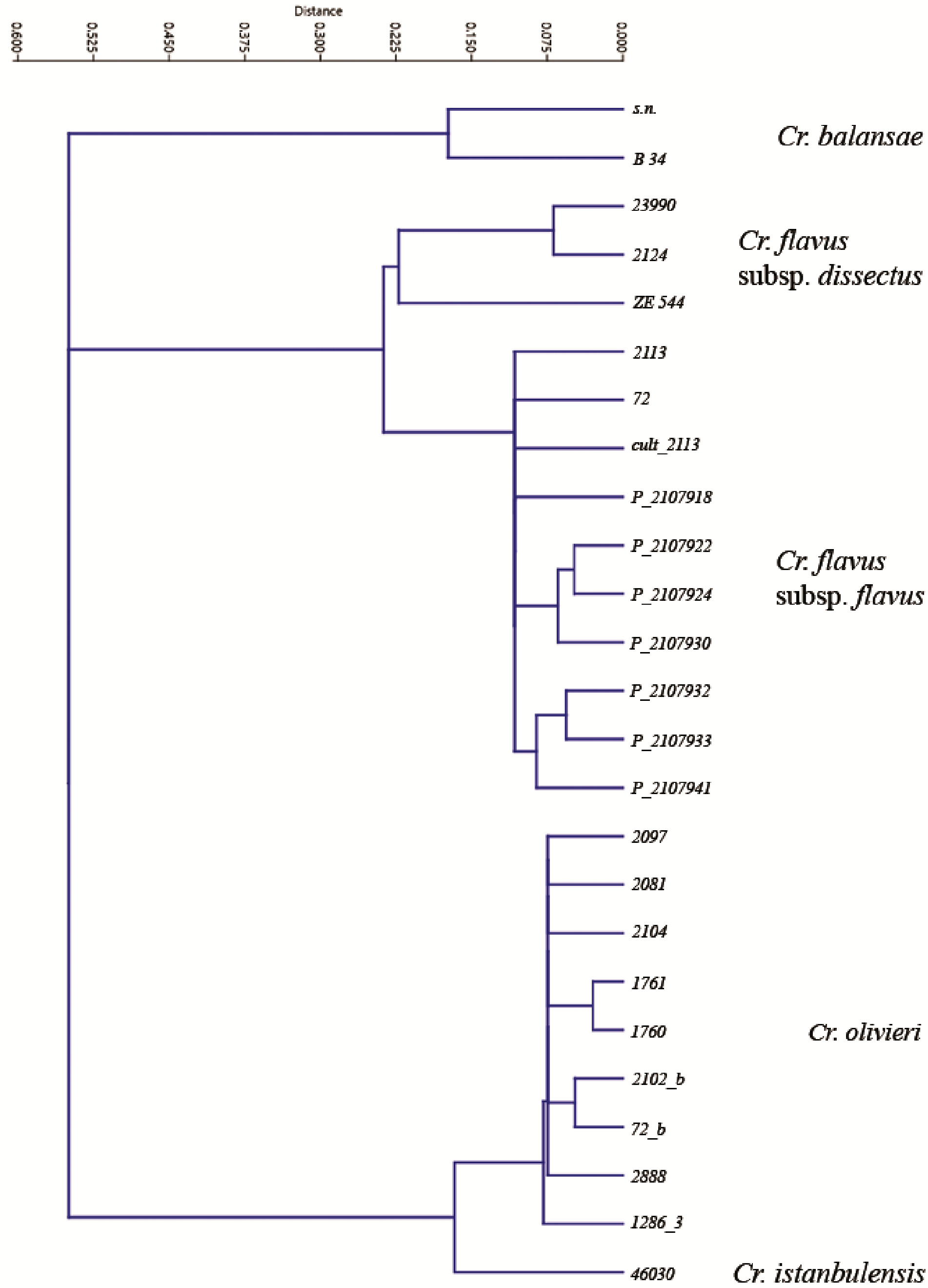

Fig. 1. Dendrogram of 25 *Crocus* ser. *Flavi* populations from Greece and adjacent areas (Balkans and Turkey) using Gower's distance clustering analysis. Cophenetic correlation r = 0.9284.

C. istanbulensis and *C. olivieri* at the bottom and *C. flavus* subsp. *flavus* (incl. subsp. *atticus*) and *C. flavus* subsp. *dissectus* forming another branch at the middle. This phenogram sorts out characters that are major factors in segregating taxa within *C.* ser. *Flavi,* and supports our taxonomic approach. The first two characters, namely, leaf length (measured at flowering time and discriminating between synanthous and hysteranthous species) and perigon tube length account for 79.2 % of variation in the group, followed by leaf width, perigon segment length, corm cup length, anther length, corm shape, and pistil per stamen ratio.

The Principal Coordinate Analysis (PCoA) and Discriminant Analysis (DA) of the morphometric data show that the *Crocus flavus* cluster overlap (Fig. 2a). On the other hand, PCoA and DA of the whole taxonomic group indicate the three taxa of the *C. olivieri* aggregate as distinct (Fig. 2b). As a consequence of the morphometric study, the two members of the *C. flavus* aggregate remain at subspecific rank, whereas members of the *C. olivieri* group are better be treated as distinct species.

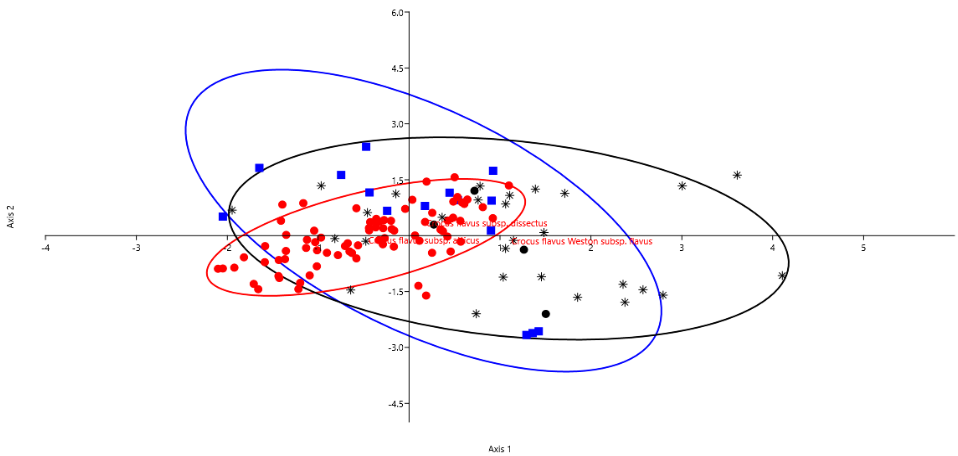

Fig. 2a. Discriminant Analysis of the Greek members of *Crocus flavus* aggregate.

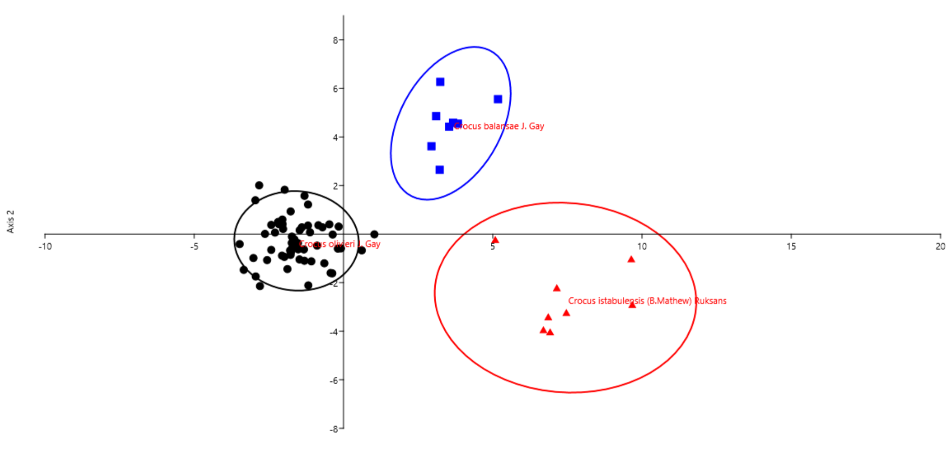

Fig. 2b. Discriminant Analysis of the Greek members of *Crocus olivieri* aggregate.

Karyology, karyomorphometry and chromosome evolution. – The karyology and karyomorphology of the *Crocus flavus* subsp. *flavus* population from Attiki ("subsp. *atticus*") are first reported in this study (Fig. 3g-h). The karyotype consists of eight chromosomes coupled with a B-chromosome (2*n* = 8 = 2m + 2sm + 2sm-SAT + 2st + 0-1B) and is compared with *C. flavus* subsp. *flavus* from a Macedonian population (Grevena, Figs 3e-f). The two populations present a similar, if not identical, karyotype with respect to chromosome number and asymmetry, which does not deviate significantly from the general, stable karyotypic pattern of *C. flavus* subsp. *flavus*.

Crocus flavus subsp. *flavus* shows remarkable morphological variation, hence its large synonymy. Yet, its karyological variability turns out to be the lowest of any *Crocus* taxon. The karyotype, in *C. flavus* subsp. *flavus,* usually varies in the presence and number of supernumerary B-chromosomes. Up to 11 B-chromosomes have been observed in *C. flavus* subsp. *flavus* populations from Turkey (BRIGHTON 1976). The presence of supernumerary B-chromosomes does not seem to correlate with major phylogenetic discontinuities, as HARPKE & al. (2013: 626) have shown in their phylogenetic analysis of the genus. B-chromosomes are present in many other *Crocus* taxa, not included in this study. We may conclude that, in the case of *C. flavus,* morphological variation is not reflected in karyotype differentiation. In terms of cytogeography, plants from the Pindos range as well as those from Mt. Vermion in Macedonia (BRIGHTON 1976) and those from Mt. Parnitha have a similar karyotype and karyotype asymmetry (Table 1). Their karyomorphometric indices are also very similar, if not identical, and form the closest tree branches in the clustering analysis to follow.

We take it that the basic chromosome number in *Crocus* ser. *Flavi* is x = 4, one of the most widespread basic numbers in the whole genus (see FEINBRUN 1958:186), being present in all generic subdivisions; the vast majority of taxa with a different chromosome numbers have derived through polyploidy and dysploidy. HARPKE & al. (2013: 625) postulate that the haploid series in *Crocus* has *n* = 4/5, 8/10 and 16 chromosomes. Their conclusion, that "reconstruction assumes fewer polyploidization events but at deeper nodes of the tree, and a high frequency of chromosome losses towards the tips", was corroborated by means of the CHROMEVOL chromosome evolution software (GLICK & MAYROSE, 2014).

The somatic chromosome number in Greek *Crocus* ser. *Flavi* is either 6 or 8. The clearest difference amongst the taxa is intra-chromosomal asymmetry. The derived species of this series (*Cr. olivieri* aggregate; HARPKE & al. 2013) reported with karyotype dominated by acrocentric (ST, T) chromosomes and it seems more asymmetric than its most primitive allies consisted of M and SM chromosomes (*Cr. flavus* aggregate). Secondly, the inter-chromosomal asymmetry discriminates the taxa taxonomically as L/S index ranges between 1.28 - 1.49 and 1.5 - 1.79 for *Cr. olivieri* and *Cr. flavus* groups, respectively. Additionally, the presence of B-chromosomes in some populations as well as satellites or secondary constricted areas, either intercalary as in *C. olivieri* or on the short arm as in *C. flavus*.

Chromosome number seems to have evolved by descending dysploidy, as the most basal species of the series, *C. paschei* Kernd. has 2*n* = 14 chromosomes (HARPKE & al. 2013), whereas our species, which are most derived, have 2*n* = 6, 8 chromosomes. This indicates that chromosome rearrangements took place, involving possible changes of centromeric position and a satellited chromosome pair with a secondary constriction, and a loss of a chromosome pair as it is depicted in Fig. 4.

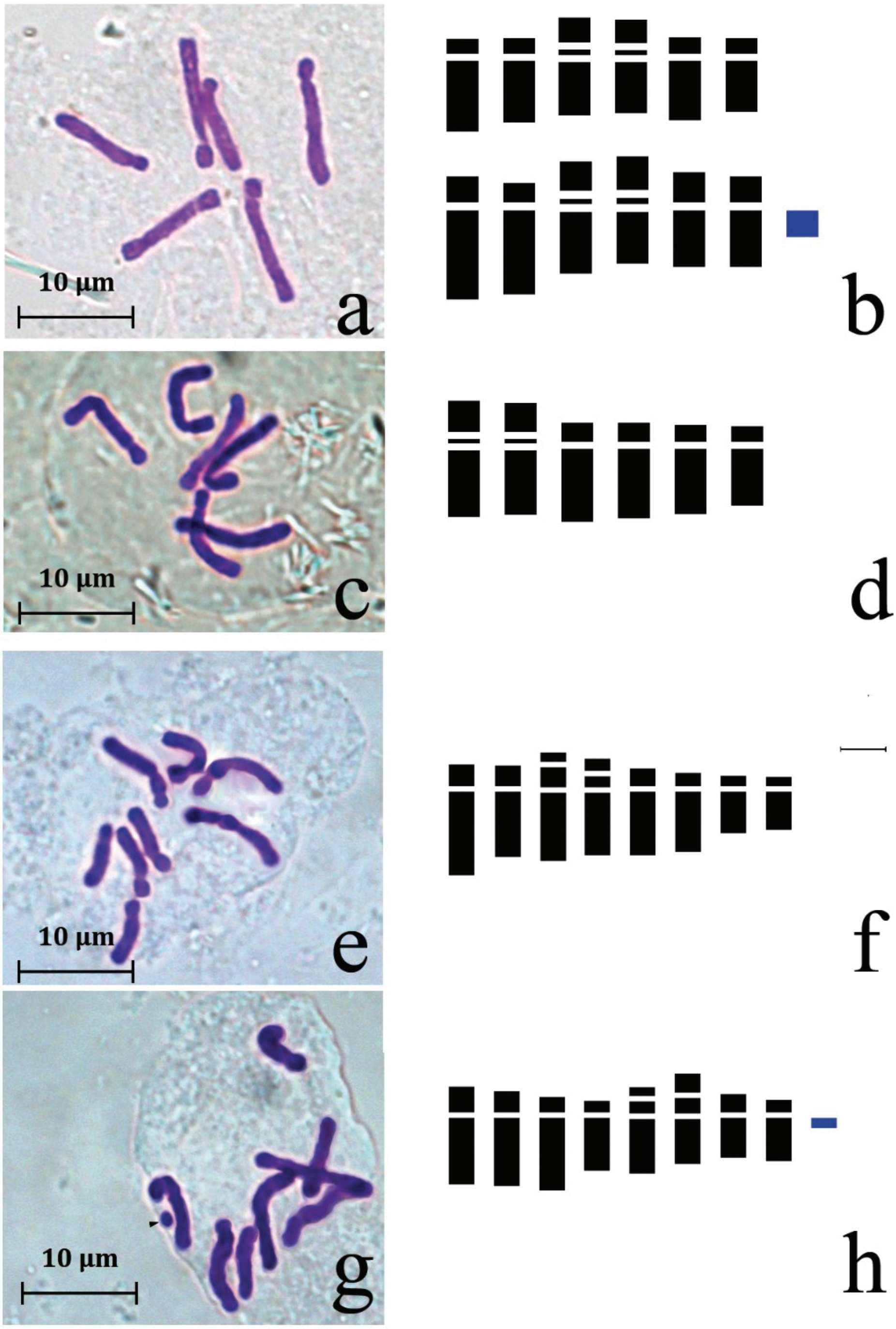

Fig. 3. Karyotypes and their corresponding idiograms of Greek *Crocus* ser. *Flavi:* a, b, *Cr. olivieri*; c, d, *Cr. balansae* and e-h, *Cr. flavus* subsp. *flavus*.

FIG. 4. The average idiograms of *Crocus olivieri* (2*n* = 6; pink idiogram) and *Cr. flavus* (2*n* = 8; black idiogram) superimposed for comparison in the evolutionary scenario of descended dysploidy – Scale bar = 10 µm.

Our findings indicate that THL differs significantly between the *Crocus flavus* and *C. olivieri* aggregates, with 34.969 ± 3.94 µm against 24.24 ± 0.39 µm, respectively. It is significant that the THL decreases from the more ancestral *C. flavus* to the most derived *C. olivieri*, lending support to our evolutionary scenario of descending dysploidy.

In the PCoA analysis of the karyomorphometrical data (Fig. 5), the first two coordinates account for 83.26 % of the total variation. The first axis of the plot discriminates the taxa in accordance with Stebbins class and L/S ratio, followed by the chromosome number, THL, M_{CA} and CV_{CI}. It is clear that chromosome types according to STEBBINS's (1971) classification have major impact, as they describe karyotype asymmetry. Also, the ratio between the longest and the shortest chromosomes of a karyotype (L/S), which is partially an index of inter-chromosomal asymmetry (also included in the Stebbins classification), correlates well with the taxon similarities depicted in a phenogram based on the most informative karyomorphological data (Fig. 6), where *C. olivieri* samples both from the Greek mainland and islands cluster in the same branch. This indicates that the species is distinct, lacking infraspecific differentiation due to topographic isolation barriers. *Crocus flavus* subsp. *flavus* from Attiki and Macedonia have a similar karyotype and belong to the same cluster, which supports their conspecificity.

The CV_{CL} karyotype asymmetry index is the most variable parameter, especially when B-chromosomes are present. As JONES & HOUBEN (2003) propose, the B-chromosomes seem to be "escapees" from the A chromosomes (see also MARTIS & al. 2012), being found in both *Crocus flavus* and *C. olivieri*. Chromosome length measurements suggests that the B-chromosomes are derived from the third longest chromosome pair in the case of *C. olivieri* and from the second longest in the case of *C. flavus*.

The M_{CA} asymmetry index discriminates well between the *Crocus flavus* and *C. olivieri* group of taxa. The index is well correlated with the presumed phylogeny of the

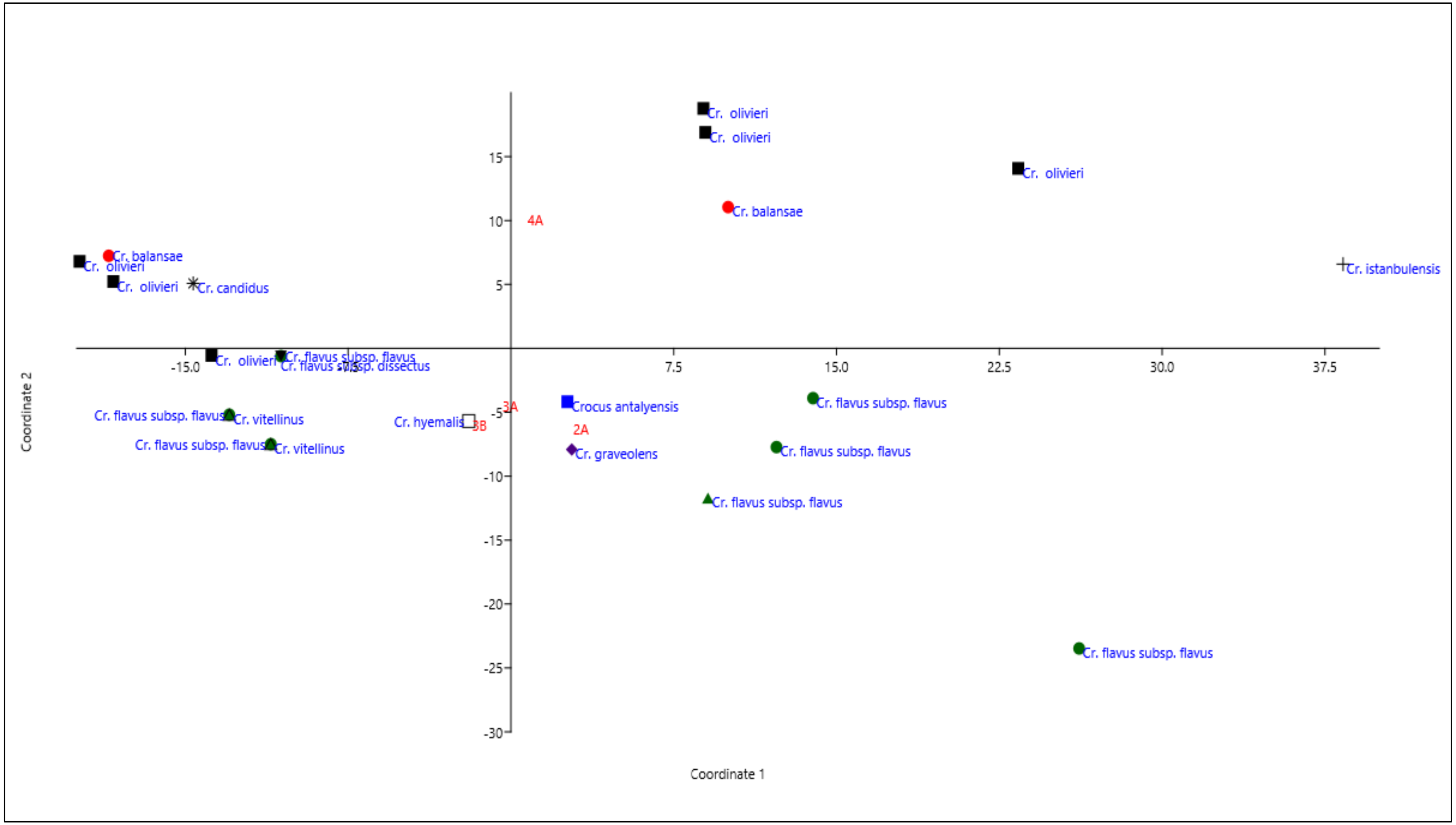

Fig. 5. Principal Coordinate Analysis (PCoA) of the most informative karyomorphometric indices within *Crocus* ser. *Flavi*. Variation correlates well with the Stebbins classification. The *Crocus flavus* subsp. *flavus* population from Attiki is indicated by a green triangle.

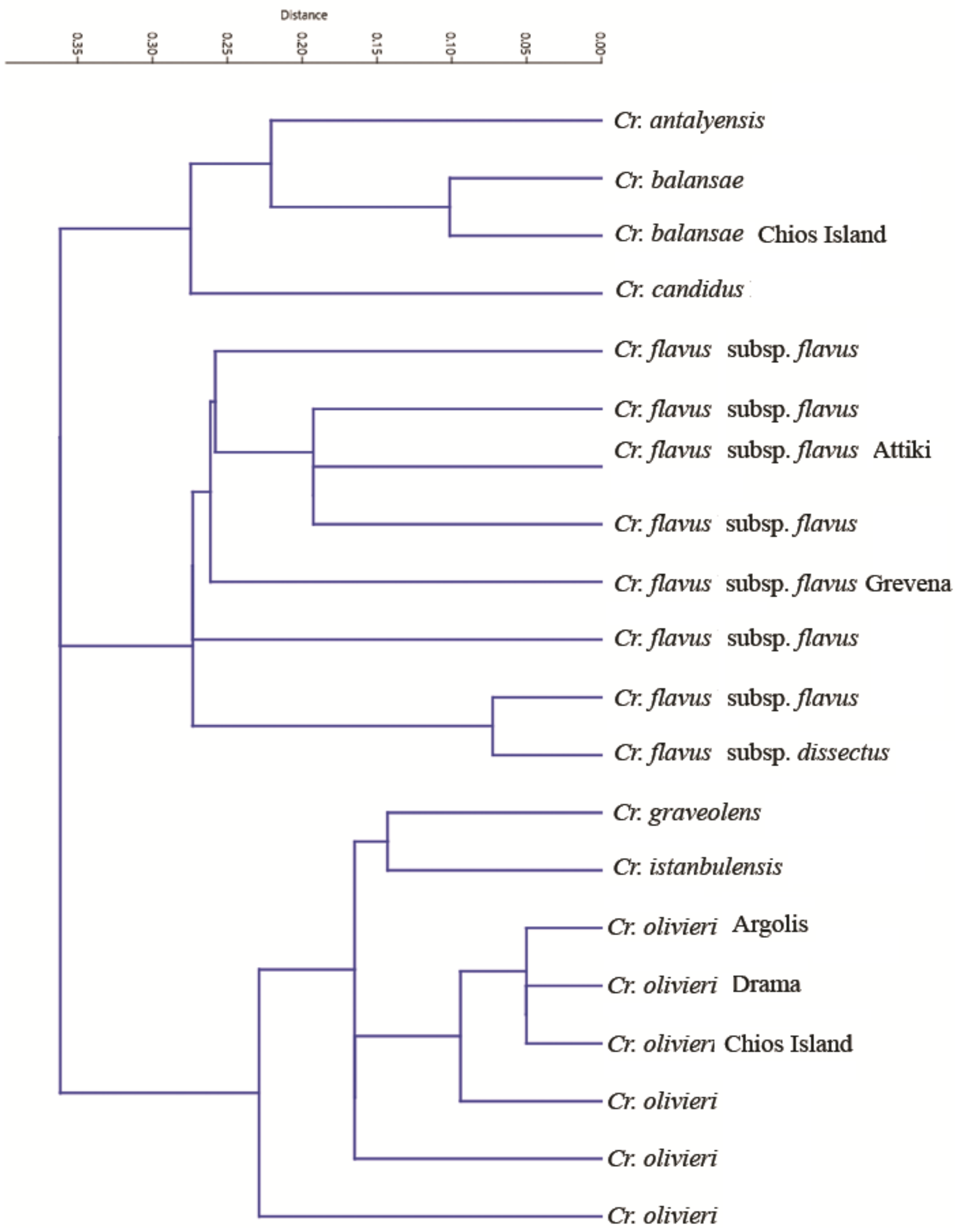

FIG. 6. Dendrogram of the Gower Distance of the karyomorphometric data of the *Crocus* ser. *Flavi*. Cophenetic correlation $r = 0.789$.

group, as its values are higher in the most derived species. M_{CA} also supports a descending dysploidy, from $n = 8$ to $n = 6$, within this group. This hypothesis is also consistent with a scenario of Robertsonian translocations, especially considering that the L/S index decreases from a range of 1.79-1.76 in the more ancestral *C. flavus* to a range of 1.57-1.28 in the derived *C. olivieri*.

Acknowledgements

This paper is dedicated to Dimitrios Phitos, Professor Emeritus of the Biology Department, University of Patras, in appreciation of his many achievements. The authors sincerely thank Assoc. Prof. E. Kapsanaki, Ass. Prof. Z. Gonou, Dr I. Pyrri and Dr A. Sergentani (Department of Ecology & Systematics, Faculty of Biology, National & Kapodistrian University of Athens) for their help and support, in particular for granting access to the Zeiss Axio Imager A_1.

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