

# The genus *Crocus* (*Iridaceae*) in Greece: some noteworthy floristic records and karyotypes

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**Abstract.** Since its first record in 1846, *Crocus reticulatus* is rediscovered in northern Greece. *C. biflorus* subsp. *alexandri*, a rare species in Greece, is confirmed from east Macedonia. The populations of *C. pallasii* subsp. *pallasii* are not confined to the East Aegean Islands only but extend to Macedonia, Thessaly and Sterea Ellas of the Greek mainland as well, southwards to Mt. Imittos. Descriptions of these three taxa are provided and their apparent relationships discussed. Furthermore, photographs of metaphase plates, karyotypes and idiograms of all three taxa are reported: *Crocus reticulatus* has  $2n = 12$ , *C. biflorus* subsp. *alexandri*  $2n = 8$  and *C. pallasii* subsp. *pallasii* either  $2n = 14$  or  $2n = 16$ .

**Key words:** chorology, *Crocus*, chromosome numbers, floristics, Greece, idiograms, new records.

## Introduction

The genus *Crocus* L. (*Iridaceae*) comprises c. 140 taxa (species and subspecies) in its whole distribution area, including the cultivated species *C. sativus* L. (Mathew 1982, 1983, 1988, 2000a, 2000b; Rukčāns 2010). The genus is confined to the Old World and extends longitudinally from the Iberian Peninsula to west China and latitudinally from Poland and the Caucasus region to the northern parts of the Arabian Peninsula (Mathew 1982; Rukčāns 2010).

*Crocus* exhibits a pronounced infraspecific variation with respect to morphology and chromosome numbers, particularly in the eastern parts of its distribution (Balkan Peninsula and Anatolia). According to Maw (1886) and Feinbrun (1958) the genus appears to have its origin in this latter area. Opposite theories argue that the origin of *Crocus* should be placed in northern Africa, particularly Cyrenaica where *C. bou-*

*losii* Greuter is found (Greuter 1968), and the Iberian Peninsula where some primitive species (*C. carpeta-nus* Boiss. & Reuter, *C. nevadensis* Amo) occur. These species exhibit a semi-terete cross section of leaf and share habitat similarities with the related genus *Romulea* (Goldblatt 1971; Mathew & Brighton 1975). According to recent phylogenetic studies (Petersen & al. 2008; Seberg & Petersen 2009), the most probable place of origin for *Crocus* is North Africa and the Iberian Peninsula, although only a small number of taxa are indigenous in these areas.

On Greek territory, 34 *Crocus* taxa are found (Mathew 1982, 1983, 1988, 2000a, 2000b; Papanicolaou & Zacharof 1980; Phitos & Kamari 1983; Rukčāns 2010, Karamplianis & al. 2011), including *C. sativus*. This number accounts for about 25% of all *Crocus* taxa known so far. An important proportion, 14 species or subspecies, should be considered as Greek endemics. *Crocus boryi* J. Gay and *C. hadriaticus* Herbert

subsp. *hadriaticus*, often considered as Greek endemics, are shared also with Albania (e.g. Shuka 2008) and should be considered regional or Balkan endemics. Both have been collected by us a several scores or several hundred of meters away of the Greek-Albanian border, on Greek territory, and their population obviously continues further north within Albania.

This report deals with 3 *Crocus* taxa inadequately known in Greece, i.e. *Crocus reticulatus* Steven ex Adam, *C. biflorus* Mill subsp. *alexandri* (Nicić ex Velen.) B. Mathew and *C. pallasii* Goldb. subsp. *pallasii*.

## Material and methods

All investigations and descriptions are based on Greek plant material, either dry (see Appendix), deposited at the Herbarium of National and Kapodistrian University of Athens (ATHU; Thiers, continuously updated), or alive. The terms Nomos and Eparchia used in the Appendix refer to administrative divisions of Greece.

Living material is being kept in cultivation at a small experimental garden in the Faculty of Biology, Athens University. Although cultivation is generally successful, taxa of high mountains do not flower regularly at the low altitude where the collection is kept, and slowly deteriorate. The classification and nomenclature mostly follows Mathew (1982). Description of capsule and seed is based on collected samples with additional information by Skourtis & Thanos (2012).

The karyological study was carried out in the Department of Ecology & Systematics, Athens University, and is based on material collected from natural populations (see Table 1 and Appendix). Root tips were pretreated in an aqueous solution of 8-hydroxyquinoline (0.003 % w/v) for 4–4½ h at room temperature, or less often in a saturated aqueous solution of  $\alpha$ -bromonaphthalene for 24 h at 4°C. The material was then fixed in Carnoy solution (3:1 v/v absolute ethanol: glacial acetic acid) for at least 24 hrs at 4°C and stored in a solution of 70 % ethanol at -20°C. For chromosome preparations the root tips were hydrolyzed in 1N HCl for 11 min at 60°C, stained with Feulgen's stain for 3–4 hours and macerated in 45 % (v/v) glacial acetic acid. Metaphases were observed using a Zeiss Axio Imager A1 microscope and captured with an AxioCam MR3. Selected photos were measured using Image J v. 1.44p (Rasband, continuously updated). Chromosome terminology mostly follows Levan

& al. (1964). Karyotype asymmetry is calculated using Stebbins's asymmetry index (SA; Stebbins 1971), total form percentage index (TF%; Huziwara 1962), ratio of long arm per total length of chromosome set (Ask%; Arano 1963), and mean length of short arms per mean length of long arms  $\times$  100 index (Syi%; Greilhuber & Speta 1976). These indexes are among the most accurate in describing karyotype asymmetry, according to Zuo & Yuan (2011).

Provenance of material, karyotype formulas and asymmetry indexes are summarized in Table 1.

## Results and discussion

An investigation of Falakron Mountain (east Macedonia, north-eastern Greece) in March 2009 by Spyros Tsiftsis resulted in the discovery of two interesting *Crocus* taxa. The same mountain was visited again by S. Tsiftsis and Theophanis Karamplianis on March 30, 2010 and both alive and pressed plant specimens were collected. The taxa were determined as *Crocus reticulatus* and *C. biflorus* subsp. *alexandri* and were seen growing in separate populations or occasionally mixed together in the same locality. *Crocus reticulatus* was rediscovered in Greece, after its first record by Grisebach (1846) on Mt. Chortiatis (in pratis m. Korthiat). Its occurrence on this latter mountain, however, was not reconfirmed in a thorough investigation by Karagiannakidou & Raus (1996). The subpopulations on Mt. Falakron consist of numerous individuals inhabiting at least three different localities.

The second taxon, *Crocus biflorus* subsp. *alexandri*, is rare and scattered in Greece. It has earlier been reported from Mt Falakron by Petersen & al. (2008), while Rukčāns (2010) mentions it from central Thrace without providing any further details. Its possible occurrence on Mt Athos (Agion Oros) is further discussed below.

*Crocus pallasii* subsp. *pallasii* was so far known in Greece from the phytogeographical region of the East Aegean Islands (EAe, Mathew 1982; Christodoulakis 1986; Panitsa 1997; Bazos 2005). Mathew (1999) also gave North Greece as a distribution area of the subspecies, but without referring to any precise localities. This subspecies has now been found in several places of the Greek mainland during field work conducted between 2008 and 2012. Its southernmost known locality is on Mt Imittos, close to Athens. Fur-

ther collections were made close to the city of Ellassona in Thessaly (Central Greece, autumn 2008) and on Mt Vertiskos (central Macedonia, see Appendix). Two more collections come from Mt Menikion (autumn of 2009 and 2010) and a hill east of Drama (east Macedonia, November 2012).

***Crocus reticulatus* Steven ex Adam, Beitr. Naturk. (Weber & Mohr) I: 45. (Fig. 1)**

**Description:** Corm subglobose to ovoid, flattened at the base, 15–20 mm in diameter, covered by coarsely reticulate-fibrous tunics with fibres up to 0.5 mm wide, splitting into a coriaceous disc with radial fibres at base and forming a neck with flaccid and strong fibres upwards. Cataphylls 3–4, papery, white, occasionally green at the apex. Leaves 4–5, synanthous, reaching perianth throat at flowering time, 5 cm × (0.5)–1(–1.6) mm and up to 20 cm long at maturity, glabrous, sparsely papillose at the margins and along the convex keel beneath, with a white stripe above up to half the lamina width, and two channels alongside keel below, each with two prominent ridges. Prophyll absent. Bract and bracteole membranous, white,

equal to subequal in length, the bracteole narrower than bract. Flowers 1–2; tepals unequal, 17–38 × 4–13 mm, acute to obtuse, inner ones broader than outer, white or pale-lilac, outer ones white, lilac, silvery or buff, with 3 wide stripes and 2–3 secondary longitudinal violet veins at outer surface, throat white or often pale-yellow, glabrous to slightly papillose. Filaments 5–7 mm, white, glabrous; anthers yellow, 10–12(–15) mm long, about twice the length of filaments. Style somewhat exceeding stamens, branched at around the middle of anthers into 3 yellow to deep orange branches, expanded and papillose at apex, 14–20(–25) mm long. Capsule 15–18 × 7–10(–14) mm, ellipsoid, with three chambers each 7–8 mm wide. Seeds brownish, occasionally pale-yellow (immature?) 3.0–3.5 × 1.5–2.5 mm with a prominent raphe beginning about halfway the underside and ending at the apex of the caruncle. Caruncle not so distinct and flattened, reaching 0.5 × 1 mm, paler than the rest of seed, chalaza yellowish, rough, with ridges lacking any pattern, testa brownish, somewhat smooth and papillose.

**Distribution and ecology:** *Crocus reticulatus* is distributed from Northeast Italy, Croatia and Slovenia



**Fig. 1.** Flowering plants of *Crocus reticulatus* (a); a group of plants in their habitat (b); details of a flowering individual (c) and the structure of the outer corm tunics (d). (Photographs by S. Tsiftsis).



(Randelović & al. 1990; Röpert 2000 onwards), Serbia and Bosnia & Herzegovina (Randelović & al. 1990) eastwards to Hungary (Randelović & al. 2007), Crimea, the Caucasus area (Mathew 1982; Randelović & al. 1990; Röpert 2000 onwards) and Turkey (Mathew 1984). In the neighbourhood of Greece, it has been found in F. Y. R. Macedonia (Randelović & al. 1990; Randelović & al. 2007), Bulgaria (Randelović & al. 1990; Randelović & al. 2007; Vladimirov 2007; Fägäras & al. 2010; Assyov & Petrova 2012) and Romania (Randelović & al. 1990; Fägäras & al. 2010).

Three subpopulations of *Crocus reticulatus* have been seen on Mt Falakron (Fig. 4), between 600 m and 1470 m a.s.l. (see Appendix). Their preferred habitat is the clearings in *Quercus*, *Fagus* and *Pinus* forest, with *Juniperus communis* subsp. *nana*, *J. oxycedrus*, *Pulsatilla halleri* subsp. *rhodopaea*, *Galanthus elwesii* subsp. *elwesii*, *Crocus biflorus* subsp. *alexandri*, and *Primula vulgaris*. Plants were also found growing at the margins of melting snow patches in subalpine grasslands, together with *Crocus flavus*, *C. olivieri* subsp. *olivieri*, *Sesleria* sp., *Rosa* sp., and *Scilla bifolia* s.l. The geological substrate of these localities consists mostly of marbles and the soil is fertile due to the high content of rhizomull humus. The plants flower from mid-February to mid-May, depending on how mild or harsh the weather is.

The population on Mt Falakron consists of at least 3000 individuals and is not under any immediate threat at present. It should be stressed however, that massive collection, particularly for horticultural use, should be strongly discouraged.

**Taxonomic relationships:** *Crocus hittiticus* Baytop & B. Mathew, with a very narrow distribution in the Cilician Taurus of South Turkey, is the closest relative of *C. reticulatus*. The former species is often considered a subspecies of *C. reticulatus* under combination *C. reticulatus* subsp. *hittiticus* (Baytop & B. Mathew) B. Mathew, according to Mathew (1982), but the differences between the two taxa are significant. *C. reticulatus* has very coarsely-reticulate corm tunics, with fibres reaching a width of c. 0.5 mm, the leaves have two channels alongside a keel underneath, each with two prominent ridges, the anthers are yellow before dehiscence and the outer tepals are narrower than the inner ones, a rare character for *Crocus*. In contrast, the corm tunics of *C. hittiticus* have finer fibres of interwoven texture, the underside of leaves has a keel but no ridges

along lateral channels (Kandemir 2010), the anthers are maroon to blackish, with yellow pollen, and the outer tepals vary from narrower to broader, as compared to the inner ones. Furthermore, the two taxa do not appear in close phylogenetic positions (Petersen et al. 2008; Seberg & Petersen 2009). Nevertheless, the members of the *Reticulati* series (where *C. reticulatus* and *C. hittiticus* belong), *Speciosi* and *Biflori*, are intermixed in the known phylogenetic trees, forming a moderately supported clade that indicates close and complex relationships. On the basis of the above data, we prefer to regard the two taxa as distinct at species level until their affinities are fully elucidated.

On grounds of tunic structure, *Crocus reticulatus* shares the characteristic wide fibres with *C. cancellatus* Herbert subsp. *mazziaricus* (Herbert) B. Mathew of the same series.

***Crocus biflorus* Miller subsp. *alexandri* (Ničić ex Velen.) B. Mathew, *Crocus*: 85 (1982) (Fig. 2)**

**Description:** Corm flattened-globose, 7–15 mm in diameter, tunics membranous or coriaceous, forming entire or toothed rings at base, with an indistinct corm neck of triangular teeth mostly not exceeding 5 mm in length. Cataphylls 3–5, papery, usually yellowish or brownish, often distinctly speckled brownish-red. Leaves 3–6, synanthous, up to 1.5 mm wide, with a white stripe less than a quarter of lamina's width and without ribs on the underside. Prophyll absent. Bract and bracteole present, subequal to unequal. Perianth segments 20–35 × 7–17 mm, obtuse or rounded but often subacute, white inside, the exterior surface of inner segments stained violet-blue at base, the exterior of outer segments entirely stained violet-blue, occasionally densely feathered, except for the narrow white margin. Throat of perianth lacking yellow colouration, glabrous. Filaments 3–7 mm long, white, glabrous or seldom finely papillose; anthers 8–14 mm long, yellow. Style divided into three yellow to reddish-orange slender branches, in some plants somewhat expanded at apex. Capsule ellipsoid, 10–15 mm long, carried on a short pedicel just above ground level at maturity. Seeds subglobose and flattened, 2.5–3.0 × 1.5–2.0 mm, reddish-brown with an indistinct raphe. Raphe somehow brighter than seed colour, beginning at the middle of the underside and running through seed surface to the apex of the caruncle, where strongly attached. Caruncle prominent, 0.5–1.0 mm long, up



**Fig. 2.** Flowering plants of *Crocus biflorus* subsp. *alexandri* (a); lateral view of flower (b) and detail of corm (c). (Photographs by S. Tsiftsis).

to 1.0 mm wide. Texture with smooth testa and sparse papillae on the raphe.

**Distribution and ecology.** *Crocus biflorus* subsp. *alexandri* is distributed in Bulgaria (Assyov & Petrova 2012), Serbia and F. Y. R. Macedonia (Randelović & al. 1990). A specimen from Mt Athos (Agion Oros) collected on February 6<sup>th</sup> 1914 by Hartmann (B, photo!) and seen by Bornmüller in 1914 and B. Mathew in 1973 may be the first record of this subspecies in Greece. However, any recent confirmation from the same area would be much welcome, since identification of old dry specimens belonging to the critical *C. biflorus* group is not always a straightforward task.

Recent Greek records of the same subspecies are provided by Petersen & al. (2008) and Rukčāns (2010) from Mt Falakron and Thrace, respectively, without further details. All records appear on the map of Fig. 4.

On Mt Falakron, a large and continuous population of *Crocus biflorus* subsp. *alexandri* has been observed, distributed along the southern slopes of the mountain and particularly above the village of Pyrgi, at c. 700–1500 m. In some localities it grows sympatrically with other *Crocus* species. The plants are found either in openings amongst scattered *Fagus* and *Pinus* trees where the soil is particularly rich in organic matter, or in overgrazed grasslands and pseudomaquis formations consisting mainly of scattered *Quercus coccifera* individuals. However, the most common habitat for *C. biflorus* subsp. *alexandri* is the clearings in *Fagus* and *Pinus* forests and the subalpine grasslands, where

it flowers near melting snow patches. This last habitat is often shared with *C. reticulatus*, *C. flavus* and *C. olivieri* subsp. *olivieri*. The geological substrate consists of marble or limestone. Habitat details are much like those of *C. reticulatus* reported before. The subspecies flowers from the end of February to the beginning of April or possibly later, depending on the melting of snow cover.

*Crocus biflorus* subsp. *alexandri* is not particularly rare on Mt Falakron. According to a preliminary estimation, more than 5000 mature individuals are growing *in situ*. The population is distant from any settlement or anthropogenic activities and does not seem to be under any direct threat at present. It is not negatively affected by overgrazing either. However, its narrow distribution in Greece, compared to other species of the genus, may call for regular monitoring in the future.

**Taxonomic comments.** *Crocus biflorus* subsp. *alexandri* is easily distinguished from its Greek allies. It differs from the typical subspecies, *C. biflorus* subsp. *biflorus*, in the characteristic flower coloration of outer tepals: they are stained with violet in a ‘feathery’ way, leaving only a small white margin untouched, a unique feature among the Greek *Crocus* species. In subsp. *biflorus*, the tepals are more or less similar to the other three Greek subspecies, i.e. subsp. *melantherus* (Boiss. & Orph.) B. Mathew, subsp. *stridii* (Pap. & Zach.) B. Mathew and subsp. *nubigena* (Herbert) B. Mathew, with yellow throat and three broad longitudinal stripes on the exte-



rior of the outer tepals that branch into secondary striations towards the tepal margin. The last three subspecies also have in common the blackish-maroon anthers (occasionally yellow in subsp. *stridii*), best observed before dehiscence. In addition to the above, *C. biflorus* subsp. *melantherus* is an autumn-flowered plant distributed in South Greece. *C. biflorus* subsp. *nubigena* has two pairs of ribs on the underside of leaf (Kandemir 2011) and is found in the East Aegean Islands (Raus 1983; Christodoulakis 1986, 1996; Bazos 2005). *C. biflorus* subsp. *stridii* has papillose to sparsely pubescent throat and filaments, and ciliate (occasionally only sparse) leaves with a distinctly broader white stripe that reaches 2/3 of leaf width (Papanicolaou & Zacharof 1980). It occurs on Mt Chortiatis, east of Thessaloniki and the area around Xanthi (Mathew 1995).

Perhaps the closest relative of *Crocus biflorus* subsp. *alexandri* is *C. biflorus* subsp. *weldenii* (Hoppe & Furnr.) B. Mathew, which differs in having ribs or ridges on the underside of leaf. This latter subspecies is known from the Balkans, i.e. north Albania (Rakaj 2009), Croatia (Ževrnja & Vladović 2005), Montenegro, F. Y. R. Macedonia, Serbia, Bosnia & Herzegovina, Slovenia, and also Northeastern Italy (Randelović & al. 1990). It has not been recorded in Greece so far.

The group of *Crocus biflorus* is by far the most diverse and complex in the genus and still needs careful investigations based on field work, dry specimens and cultivated material coupled with experimental approaches. Its members have been treated in vari-

ous ways by relevant botanists, particularly in regional Floras. Their patterns of variation and distribution need to be further studied.

***Crocus pallasii* Goldb. subsp. *pallasii*, Mem. Soc. Nat. Moscou 5: 157 (1817) (Fig. 3)**

**Description.** Corm flattened-globose, 13–20(–30) mm in diameter, tunics finely reticulate-fibrous, extending at the apex into a neck up to 60(–100) mm long. Cataphylls 3–5, white. Leaves (5–)7–17, synanthous, occasionally hysteranthous but always developing immediately after flowers wither, 0.5–1.5 mm wide, grey-green, glabrous or scabrid to papillose along the margins and the keel beneath, with a white stripe up to 1/3 of lamina width. Flowers 1–6, fragrant, pale pinkish-lilac to deep lilac-blue or purplish-blue, usually darker-veined; throat white or lilac, more or less pubescent. Prophyll present. Bract and bractole unequal, membranous, white, tapering at the apex to long, rather flaccid tips. Perianth tube 40–70(–100) mm long, white, lilac or purplish; segments (19–)25–50 mm long, (5–)8–16 mm wide, elliptic, oblanceolate or obovate, acute or subacute, the inner segments often slightly smaller than the outer. Filaments 3–8 mm long, white, glabrous to sparsely papillose-pubescent; anthers 9–21 mm long, yellow. Style divided into three red or occasionally orange branches, point of division varying but usually found above the lower part of anthers, each branch 3–15 mm long. Capsule ellip-



**Fig. 3.** Flowering plants of *Crocus pallasii* subsp. *pallasii* (a, b) and lateral view of flower (c). (Photographs by I. Sylignakis and Th. Karamplianis).

soid, 15–25 × 7–10 mm at maturity, carried at or just above ground level. Seeds deep reddish to crimson, subglobose to globose, 3.0–3.5(–4) × 2.5–3.0 mm. Raphe usually a small ridge, wing-like, triangular, distinct on the side of chalaza, indistinct on the opposite side. Caruncle small and irregularly formed, less than 0.5 mm long and wide. Texture of seed smooth, covered by velvet-like testa and a mat of long papillae.

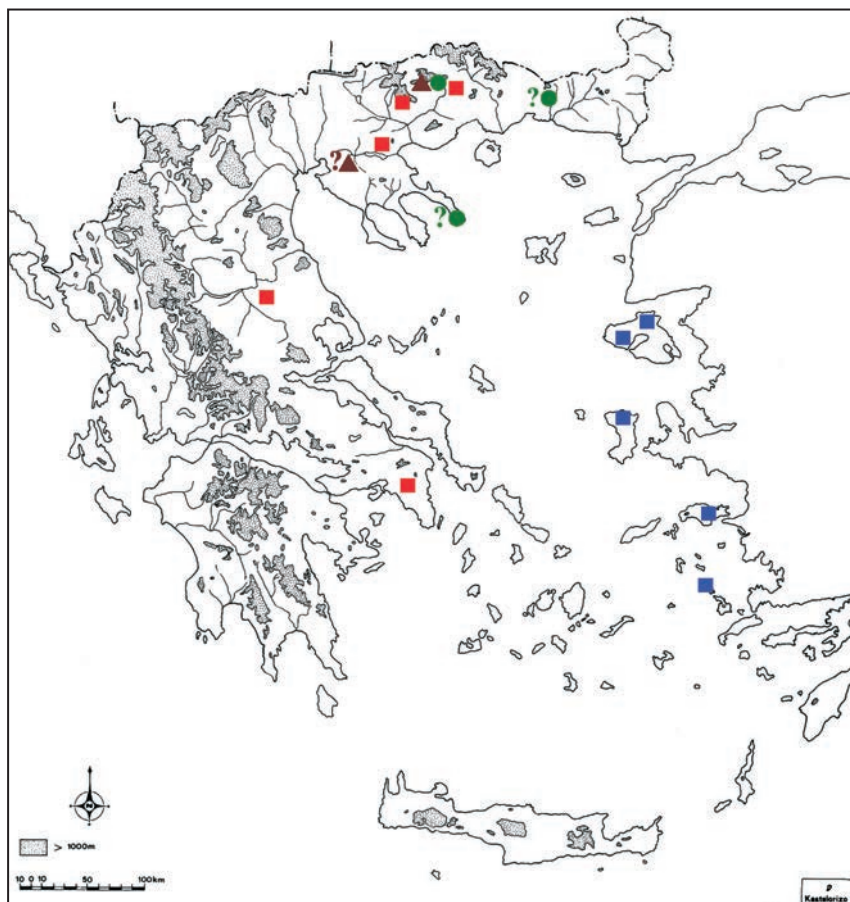
**Distribution and ecology.** *Crocus pallasii* subsp. *pallasii* is the most widespread member of the *Crocus* series and extends from the F. Y. R. Macedonia (Nikolić & al. 2010), Serbia (Randelović & al. 1990), Bulgaria (Făgăraș & al. 2010; Assyov & Petrova 2012) and Romania (Făgăraș & al. 2010) to the Crimea (Seregin 2008) and Anatolia (Turkey), southwards to Lebanon, Syria, Israel and South Jordan (Feinbrun & Shmida 1977; Feinbrun-Dothan 1986; Mathew 1999). In Greece, *C. pallasii* subsp. *pallasii* was known from the East Aegean Islands (Mathew 1982, 1983, 1984, 1988, 2000a; Christodoulakis 1986; Panitsa 1997; Snogerup & al. 2001; Bazos 2005; Bazos & Yannitsaros 2005) and from an unspecified record in

North Greece (Mathew 1999). We confirm the existence of this subspecies in the Greek mainland on the basis of material collected from southeast Sterea El-las to east Macedonia (see Appendix and Fig. 4).

*Crocus pallasii* subsp. *pallasii* grows in a variety of habitats but mostly prefers calcareous grassland with low vegetation, often amongst scattered *Quercus coccifera* shrubs. It is also found in pine or oak woodland openings, usually in shady places with fertile soil. It is not a threatened subspecies in Greece, having been recorded from several localities with a good number of individuals. As in the previous cases, however, an extensive collection of plants from the wild should be strongly discouraged.

**Taxonomic comments.** *Crocus pallasii* subsp. *pallasii* is a rather variable subspecies from a morphological point of view. It is distinguished from its allies by the total style length which is up to half the length of perianth segments, the concolorous (but often striated) perianth that lacks any yellow band and the long corm neck that reaches 60 mm or even 100 mm in Greek plants, depending on the age of the corm. Most collections from

the mainland have extended necks, usually 50–100 mm long, and their leaves are usually absent during flowering. Their perianth segments bear darker veins and measure 25–40 mm long and up to 14 mm wide, longer and broader than the plants from the East Aegean Islands. With their long necks, the Greek plants also resemble subsp. *haussknechtii* (Boiss. & Reut. ex Maw) B. Mathew, distributed in Northeast Iraq, West Iran and Jordan. The leaves of the southernmost mainland population on Mt Imittos are synanthous or begin to appear at the flowering time, unlike the populations of Central and North Greece and in common with the plants from the Aegean region.



**Fig. 4.** Distribution map of *Crocus* taxa in Greece: *Crocus biflorus* subsp. *alexandri* (green circles); *C. pallasii* subsp. *pallasii*, known localities (blue squares) and new localities (red squares); *C. reticulatus* (brown triangles).

Mathew (2000a) commented on a *Crocus* population of Samos Island, between Lazaros and Karvounis summits of Mt Ambelos, and its similarity to *C. mathewii* Kerndorff & Pasche, a species of Anatolia (Kerndorff & Pasche 1994). We had the opportunity to investigate this population in November 2009. The Ambelos plants mostly have pale lilac segments and some of them (but not all!) present the characteristic violet throat of *C. mathewii*. However, on the basis of morphological and karyological evidence, we do not hesitate to assign the Samos population to *C. pallasii* subsp. *pallasii*. The plants of Ambelos have well-developed leaves at flowering time, measuring 0.7 mm to 1.0 mm wide, slightly narrower than *C. mathewii* (1–2 mm; Mathew 2000a). Their filaments are also longer (5–8 mm vs. 3–4 mm long in *C. mathewii*) and the corm tunics are evenly and finely fibrous-reticulated along their whole length, whereas the tunics of *C. mathewii* appear to have slender fibres arranged more or less in a parallel way at the lower part of corm. These morphological differences between *C. pallasii* and *C. mathewii* are by no means pronounced and a clearer evaluation of the whole morphological variation exhibited by the two taxa may be necessary in the future. Karyological work revealed that the Samos population has a chromosome number of  $2n = 14$ , which is different from  $2n = 16$  (Mathew 1999) and  $2n = 70$  (Mathew 2000a, Özhatay 2002) reported for *C. mathewii*.

Together with *Crocus asumaniae* Mathew, *C. pallasii* subsp. *pallasii* is apparently the most divergent member

of the *Crocus* series, a group that also comprises the cultivated *C. sativus* (Grilli-Caiola & al. 2004). Recent phylogenetic studies (Petersen & al. 2008; Seberg & Petersen 2009) confirm its distance from the economically important saffron crocus. Some of the important characters that discriminate taxa within the *Crocus* series are the relative style length, as compared to the length of the perianth segments, the ratio of style length to style branches, the perianth colour, the presence or lack of indumentum on throat and filaments and the presence or absence of a neck at corm apex.

## Karyology

### *Crocus reticulatus*

Earlier works on the chromosomes of this species report  $2n = 10$  (Mathew 1982),  $2n = 12$  (Brighton & al. 1973; Susnik & Lovka 1973; Baytop & al. 1975),  $2n = 12 + 0 - 2B$  (Mathew 1982, Lovka 1995),  $2n = 12 + 3B$ ,  $12 + 5B$  (Brighton & al. 1973),  $2n = 14$  (Popova 1972, as *Crocus variegatus* Hoppe & Hornsch.; Mathew 1982; Randelović & al. 2007), or  $2n = 16$  (Kuzmanov 1993, as *C. variegatus*). The morphology of the karyotype is seldom presented. We studied material from two localities of Mt Falakron (Table 1) and both showed a diploid complement with  $2n = 12$  and a karyotype formula of  $2n = 2x = 2m + 10 sm$  (Fig. 5). No supernumerary chromosomes were found. The arm length ratio of the chromosome pairs varies from 1.13 to 2.95. The karyotype is classified as 3A, according to karyo-

**Table 1.** Chromosome data of the Greek *Crocus* species. Abbreviations: L/S = longest per shortest chromosome ratio; SA = Stebbins's asymmetry index (Stebbins 1971); TF% = total form percentage index (Huziwaru 1962); Ask% = ratio of long arm per total length of chromosome set (Arano 1963); Syi% = mean length of short arms per mean length of long arms  $\times 100$  (Greilhuber & Speta 1976). A. Kar. = Andreas Karamplianis, Const. = Theophanis Constantinidis, Gorl. = Vassilis Gorlitsas, Tsif. = Spyros Tsiftsis, Th. Kar. = Theophanis Karamplianis.

Taxon	Voucher / Origin	Location coordinates	Alt. (m)	Chromosome number (2n)	Karyotype formula	L/S	SA	TF %	Ask %	Syi %
<i>C. biflorus</i> subsp. <i>alexandri</i>	Th. Kar. & Tsif. 1841 / Mt. Falakron	41°17.517'N 24°00.600'N	1210	8	6 sm + 2 st	2.17-3.29	3B	23.50-25.41	74.59 -76.50	30.73-34.07
<i>C. pallasii</i> subsp. <i>pallasii</i>	Th. Kar. & A. Kar. 2050 / Mt. Imittos	37°57.465'N 23°49.055'N	900-920	14	6 m + 2 m-SAT + 6 sm	3.88-4.10	2B-2C	38.13-38.28	61.72-61.87	61.63-62.03
<i>C. pallasii</i> subsp. <i>pallasii</i>	Th. Kar. & Const. 1808 / Samos Island	37°45.520'N 26°50.331'E	890-900	14	10 m + 4 sm	2.75-2.91	2B	36.07-37.83	62.17-63.03	56.45-60.86
<i>C. pallasii</i> subsp. <i>pallasii</i>	Th. Kar. & Gorl. 1895 / Mt. Vertiskos	40°46.620'N 23°15.857'N	575	16	2 m-SAT + 8 sm + 2 sm-SAT + 2 st + 2 st-SAT	3.12-3.52	2B-3B	22.85-34.39	65.61-77.15	29.41-41.80
<i>C. reticulatus</i>	Th. Kar. & Tsif. 1839 / Mt. Falakron	41°17.600'N 24°02.467'N	1470	12	2 m + 10 sm	1.83-1.88	3A	31.12-32.79	67.21-68.88	45.21-48.78
<i>C. reticulatus</i>	Th. Kar. & Tsif. 1843 / Mt. Falakron	41°17.517'N 24°00.600'N	1210	12	2 m + 10 sm	1.77-1.94	3A	31.85-31.97	68.03-68.15	46.75-46.98



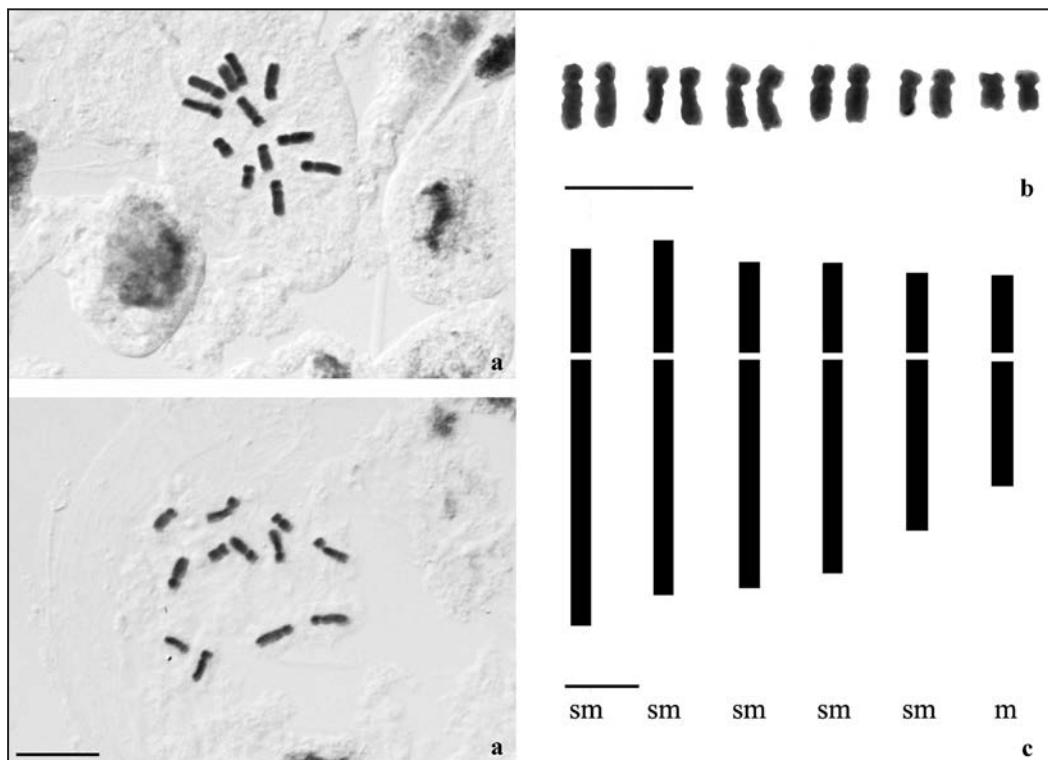


Fig. 5. Chromosome metaphase plates of *Crocus reticulatus* with  $2n = 12$  (a), scale bar = 10  $\mu\text{m}$ ; karyotype (b), scale bar = 10  $\mu\text{m}$  and haploid idiogram (c), scale bar = 1  $\mu\text{m}$ .

type asymmetry index (SA; Stebbins 1971). The longest per shortest chromosome ratio (L/S) ranges from 1.77 to 1.94. The total form percentage (TF%; Huzi-wara 1962) varies from 31.12 to 32.79, the ratio of long arm per total length of chromosome set (Ask%; Arano 1963) ranges from 67.21 to 68.88 and the proportion of mean length of short arms per mean length of long arms (Syi%; Greilhuber & Speta 1976) varies from 45.21 to 48.78 (Table 1).

The two subpopulations exhibit a very similar chromosome complement.

#### ***Crocus biflorus* subsp. *alexandri***

The chromosome numbers of  $2n = 8$  (Mathew 1982) and  $2n = 11$  (Brighton & al. 1973) have been reported for this subspecies; the latter was considered an aneu-ploid karyotype from material of horticultural origin ('Alexandri'). Our plants from Mt Falakron showed a diploid complement with a rather asymmetrical kar-yotype and a formula of  $2n = 2x = 6 \text{ sm} + 2 \text{ st} = 8$  (Table 1), lacking any supernumerary chromosomes (Fig. 6). The relative length ratio of chromosome arms varies from 1.92 to 3.72. The largest chromosome pair is acrocentric (st), followed by three submetacentric (sm) chromosome pairs, and the karyotype asymme-try is classified as 3B (SA; Stebbins 1971). The longest per shortest chromosome ratio (L/S) ranges from 2.17

to 3.29, the total form percentage (TF%) ranges from 23.50 to 25.41, the ratio of long arm per total length of chromosome set (Ask%) ranges from 74.59 to 76.50, and the symmetry index (Syi%) ranges from 30.73 to 34.07, as presented in Table 1.

#### ***Crocus pallasii* subsp. *pallasii***

The chromosome numbers of  $2n = 14$  (Brighton & al. 1973; Brighton 1977; Randelovic & al. 2007; Candan & al. 2009) and  $2n = 16$  (Šopova 1972) have been re-ported for this subspecies. Both numbers were found in Greek material but in different populations.

The plants from Macedonia (Table 1) have a com-plement of  $2n = 16$  chromosomes (Fig. 4 a), classi-fied as  $2n = 2x = 2 \text{ m-SAT} + 8 \text{ sm} + 2 \text{ sm-SAT} + 2 \text{ st} + 2 \text{ st-SAT}$ . Three pairs of satellites can be observed on short chromosome arms and one more satellite on the long arm of the longest chromosome (Fig. 7). The arm length ratio varies from 1.30 to 4.18. The largest chromosome pair is metacentric (m), with satellites on both arms. The second in size chromosome pair is acrocentric (st), with satellites on the short arms. The remaining five chromosome pairs are submeta-centric (sm), the largest one with satellites in the short arms. The last in size chromosome pair is acrocen-tric (st). The karyotype asymmetry (SA) is 2B or 3B and the ratio of the longest per shortest chromosome

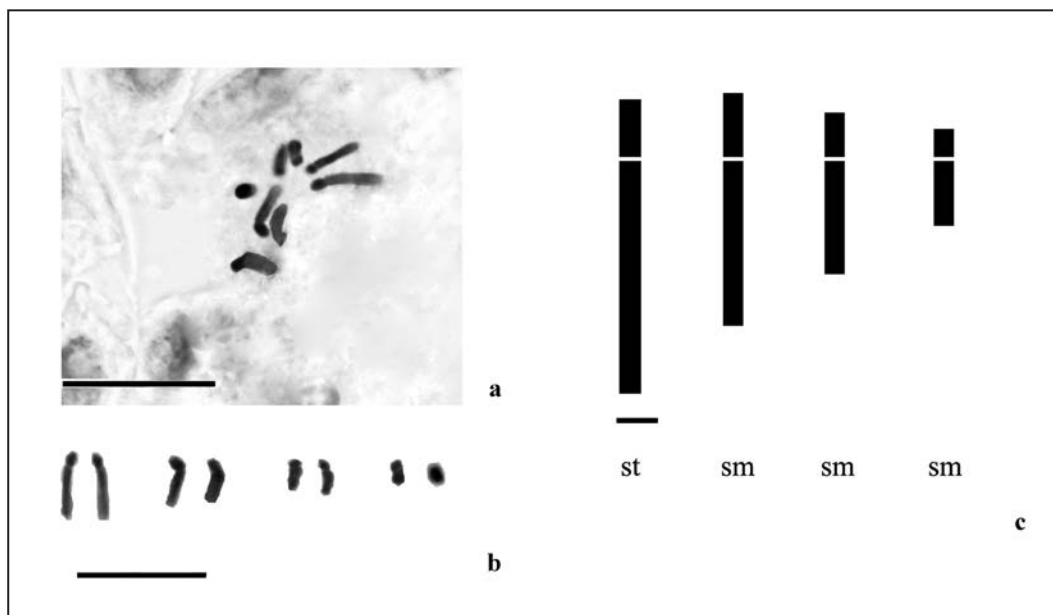


Fig. 6. Chromosome metaphase plate of *Crocus biflorus* subsp. *alexandri* with  $2n = 8$  (a), scale bar = 10  $\mu\text{m}$ ; karyotype (b), scale bar = 10  $\mu\text{m}$  and haploid idiogram (c), scale bar = 3  $\mu\text{m}$ .

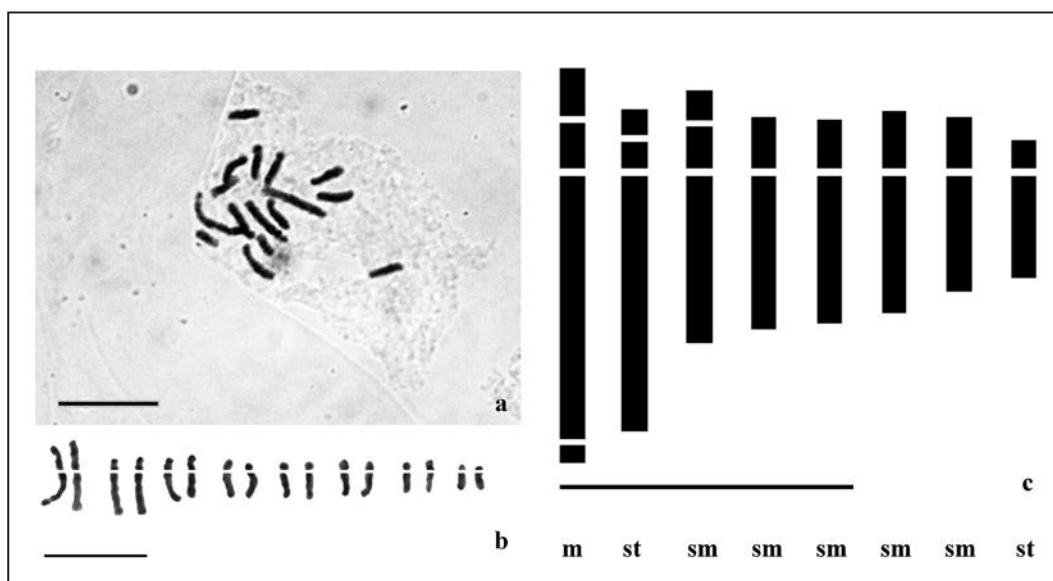


Fig. 7. Chromosome metaphase plate of *Crocus pallasii* subsp. *pallasii* from Macedonia with  $2n = 16$  (a), scale bar = 10  $\mu\text{m}$ ; karyotype (b), scale bar = 10  $\mu\text{m}$  and haploid idiogram (c), scale bar = 5  $\mu\text{m}$ .

(L/S) varies from 3.12 to 3.52. The total form percentage (TF%; Huziwara 1962) varies from 22.85 to 34.39, the ratio of long arm per total length of chromosome set (Ask %) from 65.61 to 77.15 and the proportion of mean length of short arms per mean length of long arms (Syi %) from 29.41 to 41.80.

In contrast, material from the East Aegean Island of Samos afforded  $2n = 14$  (Fig. 8), with a more symmetrical and quite different karyotype, as compared to that of Macedonia. The karyotype formula is  $2n = 10 m + 4 sm$ , without supernumerary chromosomes. Relative length ratio of chromosome arms varies from 1.44 to 2.37. The largest chromosome pair is submetacentric (sm), followed by five metacentric (m) chro-

somes forming a karyotype asymmetry of 2B (SA; Stebbins 1971). The longest per shortest chromosome ratio (L/S) varies from 2.75 to 2.91. Total form percentage (TF %) varies from 36.07 to 37.83, the ratio of long arm per total length of chromosome set (Ask %) ranges from 62.17 to 63.03 and the symmetry index (Syi %) varies from 56.45 to 60.86.

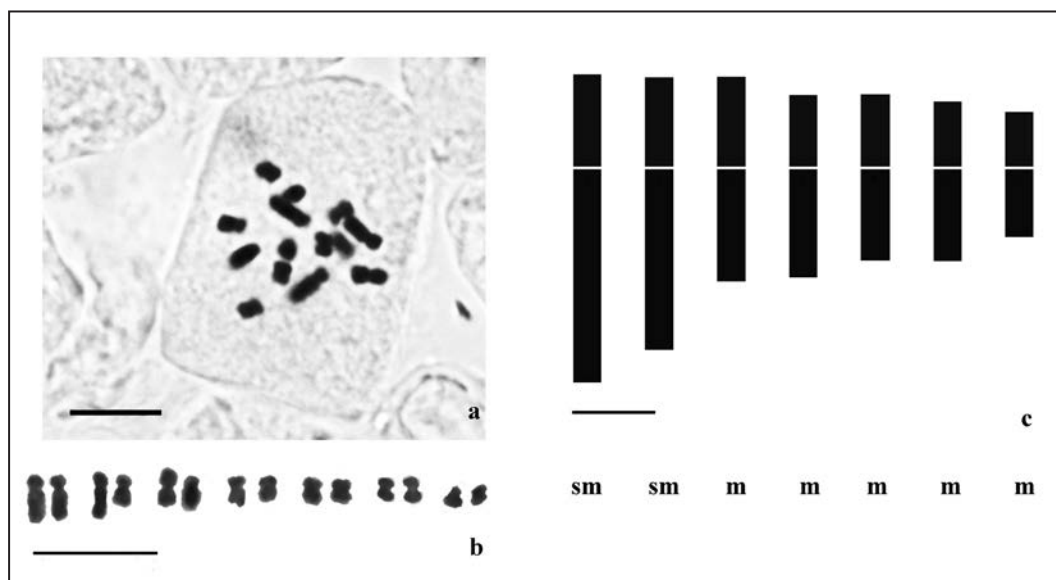
The population of *Crocus pallasii* subsp. *pallasii* from Mt Imittos (Fig. 9) has the same chromosome number with that of Samos. Its chromosomes are classified as  $2n = 2x = 6 m + 2 m\text{-SAT} + 6 sm = 14$ . The relative length ratio of chromosome arms varies between 1.05 and 2.94 and the karyotype asymmetry (SA) is 2B or 2C. TF % is between 38.13 and 38.28, Ask % be-

tween 61.72 and 61.87, and Syi% between 61.63 and 62.03 (see Table 1).

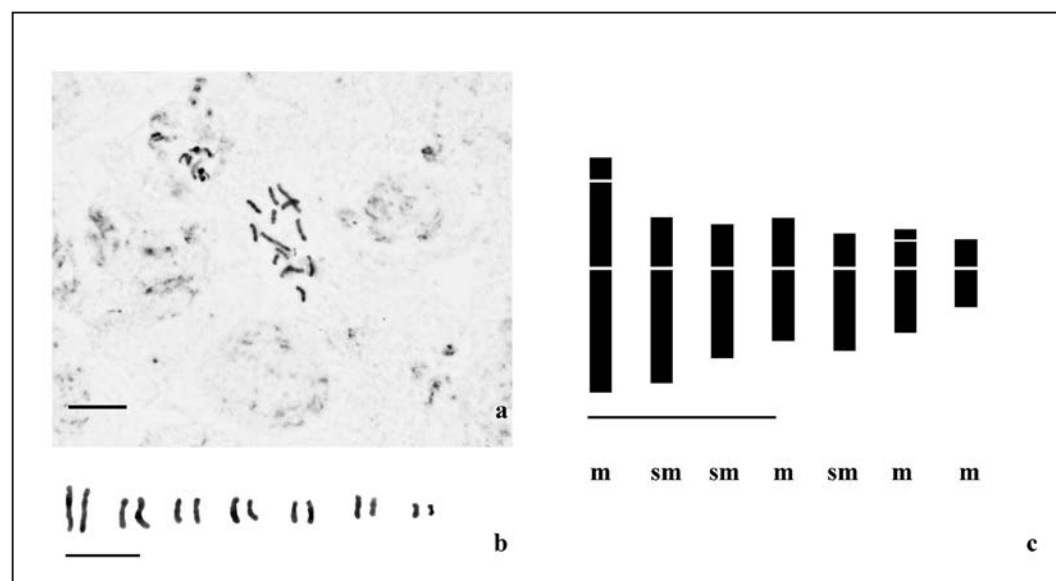
The differences in chromosome number and karyotype morphology between Greek populations of *Crocus pallasii* subsp. *pallasii* are noteworthy. The population of Macedonia has  $2n = 16$ , thus confirming a report by Šopova (1972) from the Central Balkan area. It also exhibits a pronounced intra- and interchromosomal asymmetry (2B or 3B class in Stebbins classification, high AsK value, low TF and Syi indices), as compared to the  $2n = 14$  populations of Mt Imittos and Samos island. The  $2n = 16$  karyotype cannot be explained by simple chromosomal fission of a  $2n = 14$  complement, because it also presents important chromatin reorganization which may be the result of shifts in centromere

position, additions or deletions and/or various translocations. The existence of different cytotypes within *C. pallasii* subsp. *pallasii* may be useful in circumscribing groups within its natural distribution and offers cyto-geographic markers that would shed light on the evolutionary history of the species.

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**Fig. 8.** Chromosome metaphase plate of *Crocus pallasii* subsp. *pallasii* from Samos Island with  $2n = 14$  (a), scale bar = 10  $\mu\text{m}$ ; karyotype (b), scale bar = 10  $\mu\text{m}$  and haploid idiogram (c), scale bar = 1  $\mu\text{m}$ .



**Fig. 9.** Chromosome metaphase plate of *Crocus pallasii* subsp. *pallasii* from Mt. Imittos (Sterea Ellas) with  $2n = 14$  (a), scale bar = 10  $\mu\text{m}$ ; karyotype (b), scale bar = 10  $\mu\text{m}$  and haploid idiogram (c), scale bar = 5  $\mu\text{m}$ .



## Appendix

Plant material seen and populations used for cytological investigation (in **bold**).

*Crocus biflorus* subsp. *alexandri*: Nomos Dramas, Eparchia Dramas, Mt. Falakron, subalpine meadows at the margins of *Pinus* forest, fertile substrate, marble, with *Crocus chrysanthus*, *Crocus reticulatus* and *Crocus biflorus* subsp. *alexandri*, 1470 m, 41°17.600' N, 24°02.467' N, 30.03.2010, *Th. Karamplianis* & S. Tsiftsis 1840; **ibid., at the margins and the openings of *Fagus* and *Pinus* forest with *Pulsatilla halleri* subsp. *rhodopaea*, fertile substrate, marble, 1210 m, Lat. 41°17.517' N, Long. 24°00.600' N, *Th. Karamplianis* & S. Tsiftsis 1841.**

*Crocus pallasii* subsp. *pallasii*: Nomos Dramas, Eparchia Dramas, Korilovos hill, c. 1 km W of Kallifitos village, clearings in *Pinus brutia* reforestations and lowland grasslands with sparse *Quercus coccifera* shrubs, limestone and alluvial deposits, c. 150–200 m, 41°10.630' N, 24°11.890' N, 17.11.2012, S. Tsiftis (*obs.* & *photo*); *ibid.*, Mt Menikion, c. 1.3 km NW of Kali Vrisi village, grasslands with sparse *Quercus coccifera* shrubs, limestone, c. 300–350 m, 41°09.031' N, 23°53.477' N, 10.11.2011 S. Tsiftis (*obs.* & *photo*). Nomos Larissis, Eparchia Elassonos, c. 3.3 km NNW of Elassona, along the road to Servia, margins of *Pinus* woodland, close to the road, shady places with fertile soil, c. 400–420 m, 39°54' N, 22°10' N, 29.10.2007, *Th. Constantinidis* & *Th. Karamplianis* 11619. Nomos Samou, Eparchia Samou, Mt Ambelos, Samos Island, c. 2.3 km NNE of Vourliotes village, c. 400–500 m NNE of Moni Vronta Monastery, small meadow in clearings of *Spartium junceum*, *Cistus* sp. and deciduous shrubs, limestone, 500–510 m, 37°46.250' N, 26°51.333' N, 20.11.2009, *Th. Karamplianis* & *Th. Constantinidis* 1807; **ibid., c. 7.5 km S of Vourliotes village and c. 0.8 km E of Kioulafides settlement, W of Lazaros summit, small meadows and margins of forest road in a burnt area with remnants of *Pinus nigra* and *Pteridium aquilinum*, *Crataegus azorolus* and *Dittrichia viscosa*, limestone, c. 890–900 m, 37°45.520' N, 26°50.331' N, 20.11.2009, *Th. Karamplianis* & *Th. Constantinidis* 1808; *ibid.*, c. 1.5 km NW of Kioulafides settlement, close to the road turn towards Tsouka, the eastern parts of Ambelos summit, clearings of *Pinus nigra* forest, small openings with *Pteridium aquilinum*, *Cistus* sp. and *Phlomis* sp., mostly schist, with some limestone,**

920–930 m, 37°46.109' N, 26°49.271' N, 20.11.2009, *Th. Karamplianis* & *Th. Constantinidis* 1809; *ibid.*, c. 3.6 km S of Pirgos village, on the way to Spatharei village, at roadsides in an earlier burnt area covered with *Quercus pubescens*, *Rubus* sp., *Cistus creticus*, *Pyrus spinosa* and grasses, schist, 580–590 m, 37°41.542' N, 26°47.800' N, 21.11.2009, *Th. Karamplianis* & *Th. Constantinidis* 1820. Nomos Thessalonikis, Eparchia Langada, Mt Vertiskos, c. 1 km before Vertiskos town, meadows by the road, probably in deforested places of *Quercus* forest, perhaps psammitic substrate, 680–700 m, 40°50.743' N, 23°12.647' N, 02.11.2010, *Th. Karamplianis* & V. Gorlitsas 1839; *ibid.*, c. 2 km before Vertiskos town, meadows by the road in a recreation place, probably deforested places of *Quercus* forest and perhaps psammitic substrate, 825 m, 40°52.707' N, 23°13.412' N, 02.11.2010. *Th. Karamplianis* & V. Gorlitsas 1894; **ibid., c. 2 km after the junction to Areti village, on the road towards the town of Sochos, meadows by the road, semiruderal places, probably deforested places of *Quercus* forest and perhaps psammitic substrate, 575 m, 40°46.620' N, 23°15.857' N, 02.11.2010, *Th. Karamplianis* & V. Gorlitsas 1895.** Nomos Attikis, Eparchia Attikis, Mt Imittos, c. 0.5 km before the first cluster of communication antennas at the ridge of the mountain, fallow and waste places by the road, limestone, 900–920 m, 37°57.465' N, 23°49.055' N, 22.11.2012, *Th. Karamplianis* & A. Karamplianis 2050.

*Crocus reticulatus*: Nomos Dramas, Eparchia Dramas, Mt Falakron, 2.8 km NE of Xiropotamos village, mid-altitude grasslands with *Quercus coccifera*, marble, 600–800 m, 41°12.802' N, 24°07.381' N, 26.02.2009, S. Tsiftsis (*obs.* & *photo*); *ibid.*, Mt Falakron, subalpine meadows at the margins of *Pinus* forest, fertile substrate, marble, with *Crocus chrysanthus*, *Crocus reticulatus* subsp. *reticulatus* and *Crocus biflorus* subsp. *alexandri*, 1470 m, 41°17.600' N, 24°02.467' N, 30.03.2010, *Th. Karamplianis* & S. Tsiftsis 1839; *ibid.*, at the margins and the clearings of *Fagus* and *Pinus* forest with *Pulsatilla halleri* subsp. *rhodopaea*, fertile substrate, marble, 1210 m, 41°17.517' N, 24°00.600' N, 30.03.2010, *Th. Karamplianis* & S. Tsiftsis 1843.

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