

A phylogeny of the genus *Crocus* (Iridaceae) based on sequence data from five plastid regions

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For the first time a phylogenetic analysis of the genus *Crocus* is presented. The phylogeny includes all but two of the currently 88 recognized species of the genus. The analysis is based on a total of 222 phylogenetically informative characters derived from nucleotide sequence data from three protein-coding (*ndhF*, *accD*, *rpoC1*) and two non-coding (*trnH-psbA*, *rpl36-rps8*) plastid regions. The phylogenetic hypothesis is in conflict with the primary classification of Mathew into subgenera and sections, most notably by placing the highly morphologically deviant *C. banaticus* (subgenus *Crociris*) not as sister group to subgenus *Crocus* but imbedded within it. The grouping of the taxa into series is better supported, though not entirely. Of the fifteen series recognized by Mathew, eight are confirmed as being monophyletic, monophyly of one more remains a possibility, and monophyly of a further two series is only violated by one taxon. Not unexpectedly, the two most species-rich groups, series *Reticulati* and series *Biflori* encompassing almost 1/3 of all species, are clearly non-monophyletic, but the present data do not satisfactorily solve their phylogenetic relationships.

KEYWORDS: *accD*, *Crocus*, *ndhF*, phylogeny, *rpl36-rps8*, *rpoC1*, *trnH-psbA*

INTRODUCTION

The genus *Crocus* L. consists of 88 small, corm-bearing, perennial species. In the most recent revision of the genus, Mathew (1982) recognized 81¹ species, but 7 species have been described since (see Table 1). The genus is distributed in Central and Southern Europe, North Africa, and from Southwest Asia to western China. The majority of species are restricted to Turkey and the Balkans.

Based on morphology, Mathew (1982) divided the genus into two subgenera, subgenus *Crocus* with extrorse anthers, and subgenus *Crociris* with introrse anthers. The latter subgenus is monotypic and includes only *C. banaticus*. Subgenus *Crocus* was further subdivided into two sections (section *Crocus* and section *Nudiscapus*) including six and nine series, respectively. These sections were defined by presence or absence of a prophyll, and some important characters for the delimitation of the series include corm tunic structure, leaf structure, presence/absence of a bracteole, division of the style, anther colour, and flowering time.

A single species, the triploid *C. sativus*, is commercially grown for the production of saffron—the world's most expensive spice. Only the stigmas are harvested manually and used for the production of saffron. Numer-

ous species have attracted attention as garden plants and the rarer species as collector's items. Considering the commercial interest in the genus, surprisingly few studies have tried to elucidate its phylogeny. A few studies based on distance analyses of AFLP or RAPD data, have investigated the relationships within series *Crocus*, to which *C. sativus* belongs (Grilli Caiola & al., 2004; Zubor & al., 2004). Further studies covering the entire genus have used data such as composition of floral flavonoids (Nørbæk & al., 2002) and occurrence of repetitive DNA sequences detected through Southern hybridization (Frello & Heslop-Harrison, 2000; Frello & al., 2004). However, none of these studies have employed any kind of phylogenetic analysis, and their contribution to the understanding of *Crocus* phylogeny is very limited.

According to the recent classification of Iridaceae subfamily Crocoideae by Goldblatt & al. (2006), *Crocus* is included in the tribe Croceae. The three genera *Crocus*, *Romulea* Maratti, and *Syringodea* D. Don together constitute subtribe Romuleinae, characterized morphologically by woody corm tunics, flowers solitary on the branches of the flowering stems, and often divided style branches (Goldblatt, 1990). However, Goldblatt (1990, 1991) interpreted the corm tunic character for *Crocus* incorrectly—the tunics of *Crocus* are fibrous or more or less membranous—mostly not woody (Mathew, 1982).

According to the most recent phylogenetic analysis of subfamily Crocoideae including representatives from

1 Mathew (1982) numbered only 80 species. He suggested that *C. sativus* is a selection of *C. cartwrightianus* without formally synonymizing it.

Table 1. Classification of *Crocus* following Mathew (1982) including seven species described later (*) and one nomen novum (). For these a reference is included.**

Subgenus <i>Crocus</i>
Section <i>Crocus</i>
Series <i>Verni</i> (VERN)
<i>C. vernus</i> , <i>C. tommasinianus</i> , <i>C. etruscus</i> ,
<i>C. kosaninii</i> , <i>C. baytopiorum</i>
Series <i>Scardici</i> (SCAR)
<i>C. scardicus</i> , <i>C. pelistericus</i>
Series <i>Versicolores</i> (VERS)
<i>C. versicolor</i> , <i>C. malyi</i> , <i>C. imperati</i> , <i>C. minimus</i> , <i>C.</i>
<i>corsicus</i> , <i>C. cambessedesii</i>
Series <i>Longiflori</i> (LONG)
<i>C. longiflorus</i> , <i>C. nudiflorus</i> , <i>C. serotinus</i> ,
<i>C. niveus</i> , <i>C. goulimyii</i> , <i>C. ligusticus</i> ** (syn.
<i>C. medius</i> hort. non Balbis) (Mariotti, 1988)
Series <i>Kotschyani</i> (KOTS)
<i>C. kotschyanus</i> , <i>C. scharojanii</i> , <i>C. vallicola</i> ,
<i>C. autranii</i> , <i>C. karduchorum</i> , <i>C. gilanicus</i> ,
<i>C. ochroleucus</i>
Series <i>Crocus</i> (CROC)
<i>C. sativus</i> , <i>C. pallasii</i> , <i>C. thomasii</i> , <i>C. cartwright-</i>
<i>ianus</i> , <i>C. moabiticus</i> , <i>C. oreoreticus</i> , <i>C. asuma-</i>
<i>niae</i> , <i>C. hadriaticus</i> , <i>C. mathewii</i> * (Kerndorff &
Pasche, 1994), <i>C. naqabensis</i> * (Al-Eisawi, 2001)
Section <i>Nudiscapus</i>
Series <i>Reticulati</i> (RETI)
<i>C. reticulatus</i> , <i>C. veluchensis</i> , <i>C. cvjicii</i> ,
<i>C. dalmaticus</i> , <i>C. sieberi</i> , <i>C. robertianus</i> ,
<i>C. cancellatus</i> , <i>C. hermonicus</i> , <i>C. abantensis</i> ,
<i>C. angustifolius</i> , <i>C. ancyrensis</i> , <i>C. gargaricus</i> ,
<i>C. sieheanus</i> , <i>C. rujanensis</i> * (Randjelović & al.,
1990)
Series <i>Biflori</i> (BIFL)
<i>C. biflorus</i> , <i>C. chrysanthus</i> , <i>C. almehensis</i> ,
<i>C. danfordiae</i> , <i>C. pestalozzae</i> , <i>C. aeriis</i> ,
<i>C. cyprius</i> , <i>C. hartmannianus</i> , <i>C. adanensis</i> , <i>C.</i>
<i>leichtlinii</i> , <i>C. caspius</i> , <i>C. kerndorffiorum</i> * (Pasche,
1993), <i>C. wattiorum</i> * (Mathew, 1995; 2000), <i>C.</i>
<i>nerimaniae</i> * (Yüzbaşıoğlu & Varol, 2004)
Series <i>Orientalis</i> (ORIE)
<i>C. korolkowii</i> , <i>C. michelsonii</i> , <i>C. alatavicus</i>
Series <i>Flavi</i> (FLAV)
<i>C. flavus</i> , <i>C. antalyensis</i> , <i>C. olivieri</i> , <i>C. candidus</i> ,
<i>C. vitellinus</i> , <i>C. graveolens</i> , <i>C. hyemalis</i> ,
<i>C. paschei</i> * (Kerndorff, 1993)
Series <i>Aleppici</i> (ALEP)
<i>C. aleppicus</i> , <i>C. veneris</i> , <i>C. boulosii</i>
Series <i>Carpetani</i> (CARP)
<i>C. carpetanus</i> , <i>C. nevadensis</i>
Series <i>Intertexti</i> (INTE)
<i>C. fleischeri</i>
Series <i>Speciosi</i> (SPEC)
<i>C. speciosus</i> , <i>C. pulchellus</i>
Series <i>Laevigati</i> (LAEV)
<i>C. laevigatus</i> , <i>C. boryi</i> , <i>C. tournefortii</i>
Subgenus <i>Crociris</i> (CROCI)
<i>C. banaticus</i>
<i>Incertae cedis</i>
<i>C. boissieri</i>

The abbreviations for supraspecific taxa are also used in Fig. 1.

all but one of the currently recognized genera and DNA sequence data from multiple plastid regions the sister group to *Crocus* is the South African genus *Syringodea* (Goldblatt & al., 2006). *Romulea* is placed as the sister group to these two genera. Previous phylogenetic analyses of Iridaceae including *Crocus*, *Syringodea*, and *Romulea*, but otherwise based on fewer representatives of Crocoideae and fewer molecular loci, did not clearly clarify the relationship between the three genera (Souza-Chies & al., 1997; Reeves & al., 2001). Manning & al. (2007) mention that *Afrocrocus* Goldblatt & Manning (Goldblatt & Manning, in press) is the sister to *Crocus*. The genus *Afrocrocus* is monotypic and based on *Syringodea unifolia* Goldblatt (J. Manning, pers. comm.), and according to the phylogenetic tree available at P. Goldblatt's homepage (<http://www.mobot.org/MOBOT/Research/curators/pdf/fig1a.pdf>), *Syringodea* and *Afrocrocus* are sister taxa and jointly sisters to *Crocus*.

In our efforts to produce a phylogenetic hypothesis for the genus *Crocus*, we have chosen here to focus on DNA sequence data from the plastid genome. We acknowledge that plastid data on their own are unlikely to provide the full evolutionary story, especially in a genus where speciation has very likely involved hybridization events. Incompatibility seems to be less pronounced among species belonging to the same series (Chichiricò, 1996) where naturally occurring hybrids are known (Pasche & Kerndorff, 1999). However, the phylogenetic hypothesis constructed by the present plastid data may serve as a guideline for further studies using nuclear sequence data. The five plastid regions included in the present study include three protein coding genes (*ndhF*, *accD*, *rpoC1*) and two intergenic regions (*trnH-psbA*, *rpl36-rps8*). These regions were chosen for their potential relative high level of variation. Except for *ndhF*, all regions have been considered as candidates for barcoding in plants (Kress & al., 2005; Chase & al., 2007).

MATERIALS AND METHODS

Taxon sampling. — Taxon sampling was intended to be as extensive as possible. Mathew (1982) recognized 81 species of *Crocus*, and the present taxon sampling includes all but one of these: *C. boissieri* Maw, known only from a herbarium specimen in Geneva (G-BOIS). Mathew (1982) recognized *Crocus medius* Balbis, but this is now considered a synonym of *C. nudiflorus* whereas specimens in the trade and botanical collections under the name *C. medius* should be referred to *C. ligusticus*. Of the 50 subspecies recognized by Mathew (1982) 48 are included here. Since 1982, seven new species and ten new subspecies have been described. Of these new taxa *C. naqabensis* Al-Eisawi & Kiswani and eight of the subspecies are not

included here. A few taxa were represented by more than one specimen, and the total number of included accessions of *Crocus* is 131. Two species of *Romulea*, and one species each of *Syringodea*, *Babiana*, and *Tigridia* were included as outgroups. *Babiana* is a phylogenetically more distant member of tribe Croceae (Goldblatt & al., 2006) and *Tigridia*, a member of Iridaceae subfamily Iridoideae (Goldblatt, 2000), was used to root the trees. For verification of some unexpected phylogenetic relationships extra sequences for one of the genes were obtained mainly from herbarium specimens. These sequences were not included in the phylogenetic analyses, but the specimens and the genes sequenced are listed in the Appendix. Voucher information and GenBank accession numbers may be found in the Appendix.

Molecular methods. — DNA extractions were performed using the DNeasy Plant Mini Kit (QIAGEN Ltd., Crawley, West Sussex) after tissue disruption in a FastPrep FP-120 bead mill (Qbiogene, Carlsbad, California). PCR amplifications followed standard procedures except for the addition of 0.1 mg/ml BSA to most reactions. For PCR amplification and sequencing of the five plastid regions the following primers were used: *ndhF1318F* and *ndhF2110R* (Olmstead & Sweere, 1994), *accD1F* and *accD3R* (<http://www.kew.org/barcoding/protocols.html>), *rpoC1F* and *rpoC4R* (<http://www.kew.org/barcoding/protocols.html>), *rpl36F* and *rps8R* (Kress & al., 2005), and *psbAF* and *trnH2* (Sang & al., 1997; Tate & Simpson, 2003). Direct sequencing of purified PCR products was performed using BIGDYE 1.1 (Applied Biosystems, Wellesley, Massachusetts, U.S.A.) and purified sequencing products were run on an AB3130xl automated sequencer (Applied Biosystems). Sequence editing was done using Sequencher versions 4.5 to 4.7 (Gene Codes Corporation, Ann Arbor, Michigan, U.S.A.). Alignments were done manually and the matrix is available at Tree-Base (acc no. M3519, S1912).

Phylogenetic analysis. — All phylogenetic analyses were performed using PAUP*, version 4.0b8 (Swofford, 2001). This version was preferred to the more recent version 4.0b10, as the latter under some circumstances outputs erroneous tree lengths and an excessive number of tree islands. Uninformative characters were excluded from the analyses, and informative characters were equally weighted and treated as unordered. Gaps were treated as ambiguous data (?). Analyses were performed using both the default branch collapsing rule (collapse if maximum length is zero) and *amb-* (collapse if minimum length is zero). The latter option was used for facilitating comparison of results from phylogenetic analyses using PAUP* with results from analyses using WinClada (Nixon, 2002). Under the default branch collapsing rule and simple sequence addition the number of equally parsimonious trees was very high (hitting the limit of 637,000 defined by memory allocation) and

analyses without an upper limit for the number of saved trees could not be run to completion. Thus, we also used a two step approach first running 1,000 random addition sequences saving no more than 25 trees per replicate. The trees saved in this analysis were used as starting trees for a new analysis with a maximum number of trees saved set to 100,000. In order to assess the phylogenetic performance of each of the five data partitions, we calculated the number of steps, consistency index (CI), and retention index (RI) of each partition on the total evidence trees.

Phylogenetic analyses performed using WinClada, version 1.00.08 (Nixon, 2002), spawning the matrix to NONA version 2.0 (Goloboff, 1993) were executed using heuristic search options *hold10000*, *mult*100*, *max**, *hold/10*, and the default branch collapsing rule, *amb-*. To assess branch support 1,000 jackknife replicates collecting no more than 1,000 trees per replicate were performed using PAUP* emulating Jac with 36% deletion. In the following the terms used to describe various levels of support follow Chase & al. (2000) using the categories “strong” ($\geq 85\%$), “moderate” (75%–84%), “weak” (50%–74%), and “lacking” ($< 50\%$)

RESULTS

The amplified fragments of *ndhF* have a length of 763 bp in all but one specimen (*C. kotschyanus* subsp. *suworowianus*), which has a 6 bp insertion. Numbers of variable sites and phylogenetically informative sites for this and other data partitions are listed in Table 2. The fragments of the two other protein coding genes have no length variation. The two non-coding regions both show length variation. The *rpl36-rps8* region varied in length from 489–531 bp and was aligned to a total length of 554 bp. The *trnH-psbA* region varied in length from 594–635 bp and was aligned to a total length of 698 bp. Within the region (positions 101–213 in the matrix) an inverted repeat able to form a more or less perfect hairpin structure caused sequencing problems as well as alignment problems. In most taxa the inverted repeat has a length of 51 or 53 bp, but numerous indels caused the alignment of the region to be more than twice as long. Perfection of the hairpin structure and sequencing problems were clearly correlated. As the alignment of this region was ambiguous, two phylogenetic analyses were performed: one including and one excluding the region. Though some inserts in the *rpl36-rps8* and *trnH-psbA* regions are easily interpretable as shorter duplications the complexity caused by overlapping length mutations makes coding of the gaps inserted in the matrix highly problematic.

Using the default branch collapsing rule in PAUP* without a defined upper limit for the number of saved trees combined analysis of all data (except the ambigu-

ously aligned *trnH-psbA* region) resulted in the 637,000 equally parsimonious trees (length = 488, CI = 0.57, RI = 0.89), which maximally could be saved. The second two step analysis restricting numbers of saved trees resulted in 100,000 trees of the same length. Under collapsing rule amb- only 8,315 or 8,304 most parsimonious trees were found allowing analyses to be run to completion. The different numbers of trees were recovered from analyses using 100 random addition sequences and simple addition sequence, respectively. The WinClada analysis recovered 8,344 trees. Strict consensus trees calculated from each of the above analyses were identical (Fig. 1). The individual data partitions behaved very uniformly as judged by their CI and RI values (Table 2). Combined analysis of all data (including the ambiguously aligned region) also resulted in more than 637,000 equally parsimonious trees (length = 578, CI = 0.51, RI = 0.86). The strict consensus tree (not shown) differs from the above, but mainly with respect to non-supported and weakly supported branches.

The genus *Crocus* is not monophyletic in the present data analysis. However, one possible resolution of the trichotomy involving *Romulea* + *Syringodea*, two species of *Crocus* as sisters, and the remaining species of *Crocus* is a monophyletic *Crocus*. If monophyly of *Crocus* is accepted, a basal split within the genus divides the genus into two clades: one strongly supported clade (85% jackknife) including *C. carpetanus* and *C. nevadensis* (series *Carpetani*), another weakly supported clade (56% jackknife) including all remaining species. The latter clade is basally split into two groups: a strongly supported group (95% jackknife) including the species of series *Orientalis* plus *C. caspius* (series *Biflori*) and a large, weakly supported clade (60% jackknife) including all species not already mentioned. This large clade has a basal trichotomy, with one weakly supported clade (73% jackknife) corresponding to section *Crocus* except for the inclusion of a group of species from section *Nudiscapus* series *Reticulati*. The

other well-supported clades (93% and 94% jackknife) include species of section *Nudiscapus*. Further subdivision of the clades will be commented upon below.

DISCUSSION

In the below discussion information about morphology, cytology, and distribution of taxa is from Mathew (1982) unless otherwise cited. The present analysis (Fig. 1) provides moderate support for monophyly of subtribe Romuleinae (76% jackknife), but in contrast to the relationships suggested by Goldblatt & al. (2006), *Romulea* and *Syringodea* are sister taxa. However, this relationship is weakly supported (55% jackknife) and considering the limited taxon sampling in tribe Croceae and of the two genera *Romulea* and *Syringodea*, this should not be given too much weight. The analysis does not support monophyly of *Crocus*, but neither is monophyly contradicted. Based on the morphological distinction of *Crocus* (structure of corm tunics and leaves) compared to *Romulea* and *Syringodea*, we assume that *Crocus* is monophyletic. The resolution within *Crocus* does not follow the primary classification of Mathew (1982) into subgenera and sections but the grouping into series is better supported, although not entirely (Fig. 1). However, for ease of understanding we will structure the following discussion according to Mathew's classification rather than the tree.

As indicated above, the subgeneric classification by Mathew (1982) is not supported: *C. banaticus*, the sole member of subgenus *Crociris*, is embedded within a clade including all species of section *Crocus* plus members of series *Reticulati* (Fig. 1). *C. banaticus* is placed in a clade with *C. malyi*, but the clade is weakly supported (51% jackknife). The presence in *C. banaticus* of a prophyll supports its position within section *Crocus*, but its unique morphological traits such as introrse anthers, inner perianth seg-

Table 2. Alignment length, numbers of variable and phylogenetically informative sites, tree length, CI, and RI for different data partitions.

	Aligned length	Variable sites	Informative sites	Tree length	CI	RI
All data	2,963	487	233	578	0.51	0.86
All data ^a	2,850	465	222	488	0.57	0.89
<i>ndhF</i>	769	204	102	249–251	0.52–0.53	0.88
<i>rpoC1</i>	575	54	24	45–46	0.57–0.58	0.84–0.85
<i>accD</i>	367	58	23	57	0.54	0.90
<i>rpl36-rps8</i>	554	76	41	80–81	0.61	0.91
<i>trnH-psbA</i> ^a	585	73	32	55–56	0.70–0.71	0.89

^aExcluding the inverted repeat region of *trnH-psbA* (positions 101–213).

Tree lengths, CI, and RI values for each partition (excluding uninformative characters) are calculated from all equally parsimonious trees derived from combined analysis of all data except the inverted repeat region of *trnH-psbA*, thus values may vary within a partition.

ments being much shorter than the outer, and style splitting into numerous, slender, lilac branches, are not paralleled by a similar accumulation of changes at the molecular level. The deviant morphological characters of *C. banaticus* are most likely autapomorphies, e.g., extrorse anthers are not just the plesiomorphic condition in *Crocus*, but characteristic of the entire Iridaceae (Dahlgren & al., 1985).

The division of subgenus *Crocus* into two sections is not supported by the present phylogenetic hypothesis either. The species of section *Crocus* are placed in a clade together with *C. banaticus* and a group of species usually included in series *Reticulati* of section *Nudiscapus* (Fig. 1). However, the basally unresolved structure of the clade allows for a resolution that would make section *Crocus* (+*C. banaticus*) monophyletic with presence of a prophyll a synapomorphy of the clade (though the prophyll then appears secondarily lost in *C. asumaniae*; however, this character requires checking in a wider range of individuals). Any possible resolution of unresolved clades in the tree makes section *Nudiscapus* non-monophyletic.

Within section *Crocus* series *Scardici* is strongly supported as monophyletic (99% jackknife), but its position in a clade including members of series *Longiflori* and series *Versicolores* is only weakly supported (52% jackknife) (Fig. 1). Series *Scardici* is also morphologically well-defined primarily by unique absence of a pale stripe on the upper surface of the leaves (Fig. 1).

Series *Kotschyani* is also strongly supported (94% jackknife) as monophyletic (Fig. 1). Within the group, two clades are weakly to moderately supported: one including *C. kotschyanus*, *C. karduchorum*, and *C. ochroleucus* (62% jackknife), the other including *C. vallicola*, *C. scharojanii*, *C. autranii*, and *C. gilanicus* (80% jackknife). In the former group *C. ochroleucus* is strongly supported as the sister to *C. kotschyanus* subsp. *kotschyanus* (95% jackknife) and *C. karduchorum* is placed in a strongly supported clade with *C. kotschyanus* subsp. *cappadocicus* and *C. kotschyanus* subsp. *hakkariensis* (86% jackknife). *C. ochroleucus* and *C. kotschyanus* subsp. *kotschyanus* share an upright corm, whereas *C. karduchorum* and all other subspecies of *C. kotschyanus* have corms lying on the side. It is noteworthy that in the latter clade *C. vallicola*, *C. scharojanii*, *C. gilanicus*, and *C. autranii* have chromosome numbers of $2n = 8, 8, 24, 32$ ($x = 8$) respectively (Brighton & al., 1973), while in the former clade *C. karduchorum*, *C. ochroleucus*, and *C. kotschyanus* have $2n = 10$ and *C. kotschyanus* subsp. *suworowianus* $2n = 20$ ($x = 10$) (however, there is a record of $2n = 8$ for *C. kotschyanus*, which requires checking). The results of the present study support the recognition of series *Kotschyani* as a morphologically and geographically discrete group.

Series *Crocus* also forms a strongly supported monophyletic group (96% jackknife) (Fig. 1). The species of series *Crocus* form three clades: one moderately supported

clade (82% jackknife) includes *C. thomasi*, *C. hadriaticus*, *C. cartwrightianus*, *C. oreoreticus* and *C. sativus*, a second strongly supported clade (87% jackknife) includes *C. asumaniae* and *C. mathewii*, and a third strongly supported clade (87% jackknife) includes *C. pallasii* and *C. moabiticus* (Fig. 1). The close relationship between *C. asumaniae* and *C. mathewii* is supported by morphological similarity (Kerndorff & Pasche, 1994). *C. sativus* is a sterile triploid only known from cultivation. It is usually thought to be a hybrid involving *C. cartwrightianus* (e.g., Grilli Caiola & al., 2004; Zubor & al., 2004). *C. sativus* is sister to one of samples of *C. cartwrightianus* included in our study (Fig. 1).

Most species of series *Versicolores* form a moderately supported monophyletic group (82% jackknife), but *C. malyi* represented by two specimens is not included in the clade (Fig. 1). *C. malyi* is placed as the sister to *C. banaticus*, but a relationship that is weakly supported (51% jackknife). Within *Versicolores* the species form two strongly supported clades: one including *C. versicolor* and *C. cambessedesii* (95% jackknife), the other including *C. minimus*, *C. corsicus*, and *C. imperati* (93% jackknife) (Fig. 1).

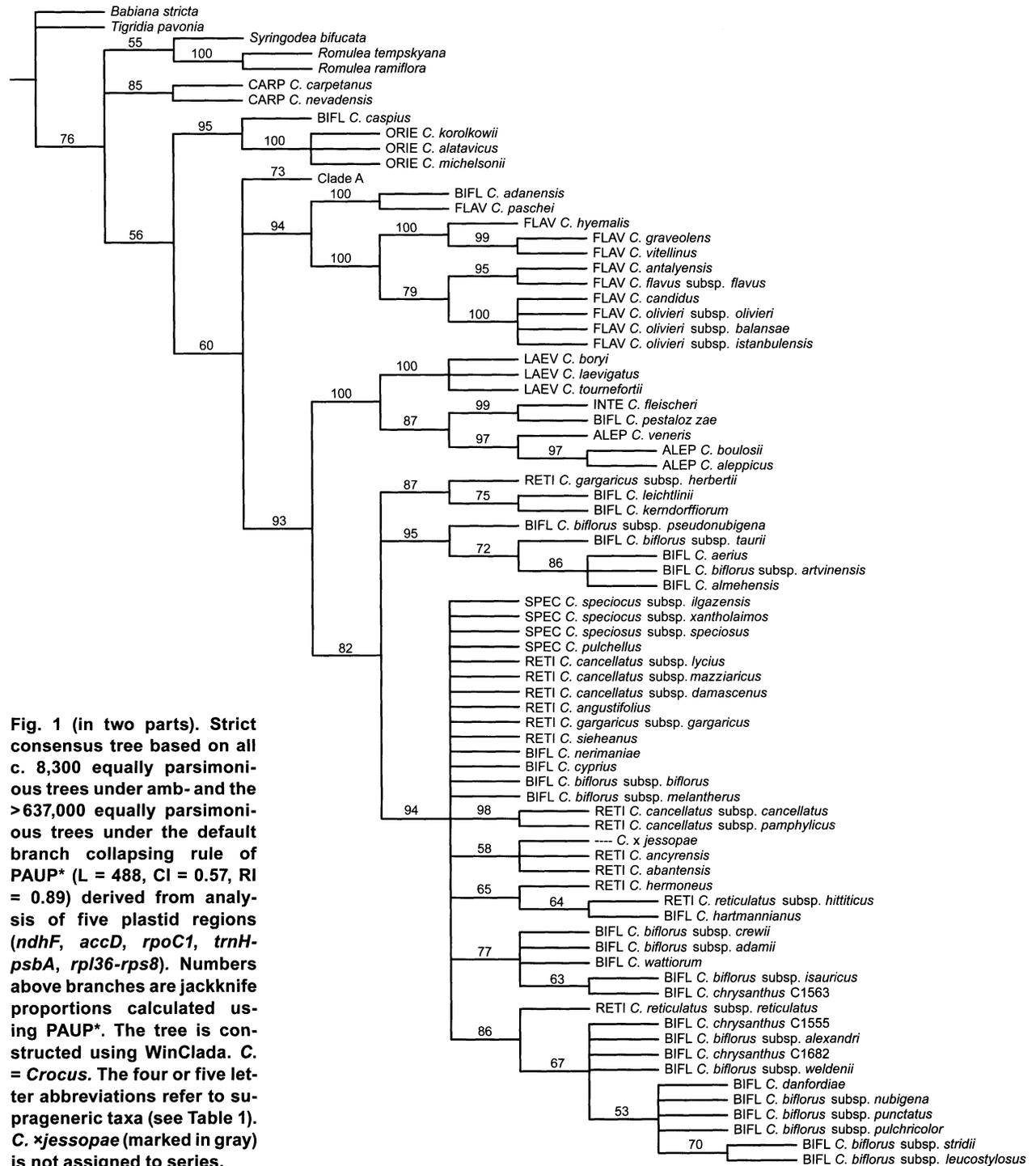
Most species of series *Verni* form a strongly supported monophyletic group (96% jackknife), however *C. longiflorus* (series *Longiflori*) is included in the group and *C. baytopiorum* is strongly supported (99% jackknife) as sister to series *Crocus* (Fig. 1). Mathew (1984, 2002) previously expressed doubt about inclusion of *C. baytopiorum* in series *Verni*, suggesting that the species might best be placed in a series of its own. Unless included in series *Crocus*, the present phylogenetic analysis supports this view. The inclusion of the autumn-flowering *C. longiflorus* within the same clade as the species of series *Verni* is unexpected, but setting aside the differences in flowering time—all species of series *Verni* are spring-flowering—*C. longiflorus* does in fact share several of the morphological characters of series *Verni*. The identity of the included specimen of *C. longiflorus* has been verified by sequencing the *accD* gene from two herbarium specimens. All three *C. longiflorus* sequences are identical and different from the *accD* sequences from all other species of *Crocus*. Within series *Verni*, the two subspecies of *C. vernus* are placed in each of two strongly supported clades (Fig. 1). *C. vernus* subsp. *vernus* is grouped together with *C. kosaninii* and *C. tommasinianus*, whereas *C. vernus* subsp. *albiflorus* is grouped with *C. etruscus* (99% and 88% jackknife, respectively). In view of the wide distribution and variability of *C. vernus* s.l. (Spain, eastwards to Poland and southwards to Sicily) it is essential that further studies incorporate a range of material from different areas.

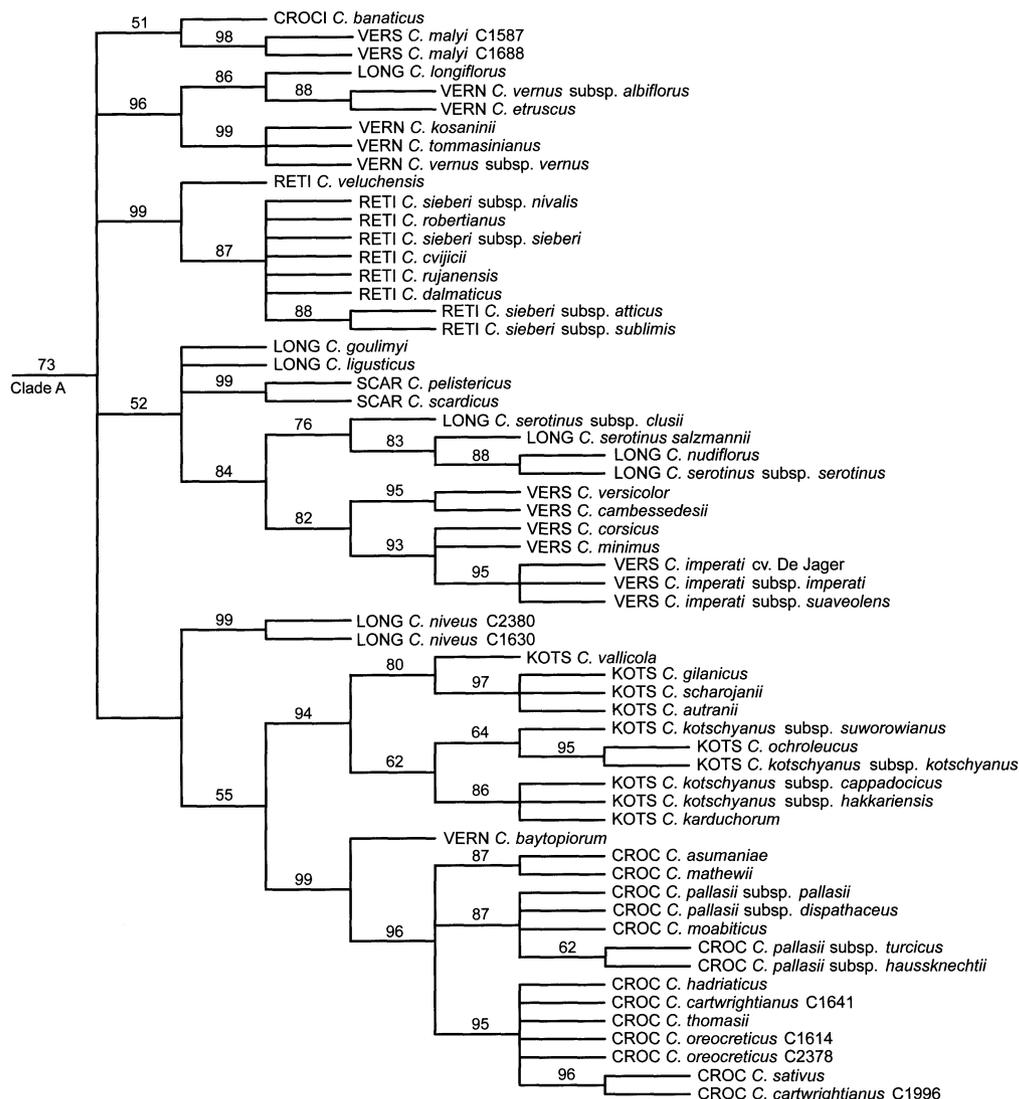
Series *Longiflori* is shown to be polyphyletic by the present analysis. As mentioned above *C. longiflorus* is included in the *Verni* clade, *C. niveus* is the sister to a

clade including series *Kotschyani*, series *Crocus* and *C. baytopiorum*, and at best *C. goulimyi* and *C. ligusticus* (*C. medius* hort., non Balbis) may be the sister group to a moderately supported clade (84% jackknife) including series *Versicolores* and the two remaining species of series *Longiflori*, *C. nudiflorus* and *C. serotinus*, as their moderately supported sister group (76% jackknife)

(Fig. 1). These results are not entirely surprising as series *Longiflori* is morphologically a rather loose assemblage in need of reevaluation.

Within the non-monophyletic section *Nudiscapus*, series *Carpetani*, consisting of only two species, *C. carpetanus* and *C. nevadensis*, is strongly supported (85% jackknife) as monophyletic and depending on the resolution of





the trichotomy in which the clade is placed, it may be the sister group to the remaining species of *Crocus* (Fig. 1). The abaxial leaf structure of *C. carpetanus* differs from that of all other species of *Crocus* by lack of a keel and by presence of several minor grooves. The typical *Crocus* leaf has a flattened keel between two deep grooves. The leaf of *C. nevadensis* has a less developed keel than other species of *Crocus* and in this respect may be considered structurally intermediate between that of *C. carpetanus* and other more conventionally-leaved *Crocus* species. The leaf structure of *C. carpetanus* bears some resemblance to the bifacial leaf of species of *Syringodea* (Manning & al., 2002), but meaningful comparison is difficult due to uncertainty in interpretation of anatomical structures (Rudall & Mathew, 1990). It has even been suggested that the entire leaf of *C. carpetanus* should be interpreted as a leaf sheath, thus not being homologous to the leaves of other *Crocus* species (Arber, 1921).

Series *Orientalis* is also strongly supported as monophyletic (100% jackknife), but the clade consisting of only three species is unresolved with *C. caspius* (series *Biflori*) as strongly supported (95% jackknife) sister group (Fig. 1). This group is sister to all other species of *Crocus* except the two species of series *Carpetani*. *Crocus caspius* occurs in the same area as the species of series *Orientalis*, but being autumn-flowering in contrast to the spring-flowering species of *Orientalis* makes hybridization an unlikely explanation for the unsuspected relationship. We have verified the position of *C. caspius* by sequencing the *ndhF* gene from a herbarium specimen. Both sequences are identical and different from all other *Crocus ndhF* sequences. Morphologically, *C. caspius* is not a typical member of series *Biflori* so its position is not too surprising. *Crocus caspius* and the species of *Orientalis* share the unusual behaviour of ripening their capsules at ground level, thus supporting a close relationship. Thus, both on

morphological and geographical grounds it could equally be regarded as related to species of series *Orietales*.

Series *Laevigati* is strongly supported as monophyletic (100% jackknife) (Fig. 1). The relationship between its three species is unresolved, which may not be surprising in view of their close morphological resemblance. It would be desirable to include in a further study material from Crete where all three species occur and where specimens of *C. laevigatus* are slightly different from those of mainland Greece.

Series *Aleppici* is also strongly supported as monophyletic (97% jackknife) and within the clade *C. boulosii* and *C. aleppicus* are strongly supported as sisters (97% jackknife) (Fig. 1). These two species are from North Africa (Libya) and the Middle East (Lebanon, Syria, Jordan, Israel) respectively, whereas the third species, *C. veneris*, is from Cyprus.

Series *Aleppici* is strongly supported (87% jackknife) as the sister group to another strongly supported clade (99% jackknife) consisting of the monotypic series *Intertexti* (including only *C. fleischeri*) and *C. pestalozzae* from series *Biflori* (Fig. 1). The relationships of *C. fleischeri* have previously been uncertain, though Mathew (1982) noted a similarity between *C. fleischeri* and *C. boulosii* in their unusual corm colour (yellow). The apparent relationship, indicated here, of *C. fleischeri* with the species of series *Aleppici* and series *Laevigati*, is interesting. In classifications based on morphology alone, great weight has been placed on the unique hence most likely autapomorphic structure of the corm tunic of *C. fleischeri* (Maw, 1886; Mathew, 1982). However, excluding this character, the species has much in common with the species of series *Laevigati* and series *Aleppici*. The disparate flowering times of *C. fleischeri* (vernal) and the other species under discussion is probably not of great significance as the three species of series *Aleppici* vary in flowering time from late autumn through to early in the new year, and there are autumn-, winter-, and early spring-flowering populations of *C. laevigatus*. The most surprising member of the *Aleppici-Intertexti-Laevigati* group is definitely *C. pestalozzae*, with its tunic basally splitting into rings, a characteristic for series *Biflori*. Whereas series *Aleppici*, *Laevigati*, and *Intertexti* can be characterized by the presence of styles divided into six or usually more branches, *C. pestalozzae* has styles divided into only three branches. It should be noted that although morphologically similar to the species of series *Biflori*, cytologically and ecologically *C. pestalozzae* is one of the most distinct members of the series. It occurs in north-western Turkey on acid soils near Istanbul, has a high chromosome number of $2n = 28$ and does not appear to hybridise with *C. biflorus* ($2n = 8$ in this region) (Brighton & al., 1973) which occurs in the same area, although in a different habitat. We have verified the identity of the voucher specimen of *C. pestaloz-*

zae and the sequence has been confirmed by sequencing the *ndhF* gene in another accession. The two sequences from a white- and a blue-flowered variant respectively, are identical and different from all other *Crocus ndhF* sequences.

Another strongly supported clade (94% jackknife) includes all species of series *Flavi* plus *C. adanensis* (series *Biflori*) (Fig. 1). The latter species is strongly supported (100% jackknife) as the sister to *C. paschei*, and these two species form the sister group to the other species of series *Flavi*. *Crocus adanensis* lacks or develops only very weakly the characteristic rings formed by the splitting corm tunic of series *Biflori*, thus, it may have been misplaced in that series. We have verified the placement of *C. adanensis* by sequencing the *ndhF* gene in another accession of the species. The two sequences are identical to each other and to the *ndhF* sequence of *C. paschei*. The membranous tunic of *C. adanensis* splitting into parallel strips or fibres fits well into series *Flavi*, and a sister group relationship to *C. paschei* is supported by lilac-blue flower colour and styles divided into only three branches. *Crocus antalyensis* shares the lilac-blue colour, but based on similarities in the tunic structure—presence of a “neck” build up of persistent remains of old cataphylls—Mathew (1982) suggested that *C. antalyensis* was the closest relative of *C. flavus*, a relationship strongly supported (95% jackknife) by the present data (Fig. 1). A close relationship between *C. candidus*, the only white-flowering member of the series, and *C. olivieri* was also suggested by Mathew (1982) and is likewise strongly supported here (100% jackknife) (Fig. 1). A sister group relationship of these two groups is moderately supported (79% jackknife). The three species *C. hyemalis*, *C. graveolens*, and *C. vitellinus* form another strongly supported group (100% jackknife), with the two latter species being strongly supported as sisters (99% jackknife) (Fig. 1). The close relationship between *C. graveolens* and *C. vitellinus* was also found by Mathew (1982), whereas he had problems placing *C. hyemalis*. The three species have overlapping distribution areas in southern Turkey, Syria, Lebanon, and Israel, whereas other members of series *Flavi* mainly occur further to the north.

Members of the three series *Speciosi*, *Reticulati*, and *Biflori* are unsurprisingly mixed in a moderately supported clade (82% jackknife) (Fig. 1). The *Reticulati* and *Biflori* groups present some of the most challenging taxonomic problems within the genus. Apart from corm tunic characteristics—membranous/annulate versus reticulate—many of the species of these two series are very similar in a suite of morphological features. In a study of the structure of calcium oxalate crystals in the corm tunics, Wolter (1990) found that occurrence of prismatic crystals of a particular type was confined to species of these three series. Several species within series *Biflori*

and *Reticulati*, however, possess needle shaped crystals or a combination of both types. Though occurrence of the prismatic crystals is also confined to the clade found here (i.e., this crystal type is not found in any of the taxa of two sections placed outside the clade), there is no correlation between the occurrence of the two crystal types within the clade.

Within the above clade, the two autumn-flowering species *C. speciosus* and *C. pulchellus* of series *Speciosi* are both placed in a large, strongly supported (94% jackknife), but very unresolved clade also including species of series *Reticulati* and series *Biflori* (Fig. 1). Thus, monophyly of series *Speciosi* cannot be confirmed, but the unresolved position of the accessions of the two species, does not contradict monophyly of the group.

The suggested relationships between species of series *Biflori* and series *Reticulati* are considerably more complicated, and a few groups having strong support include species from both series (Fig. 1). *Crocus reticulatus* subsp. *reticulatus* (series *Reticulati*) is grouped with *C. danfordiae*, some accessions of *C. chrysanthus*, and some subspecific taxa of *C. biflorus* (series *Biflori*) (86% jackknife). For the reasons stated above, this is not surprising in view of the morphological similarity between the members of *Biflori* and *Reticulati*. However, more unexpected is the grouping of *C. gargaricus* subsp. *herbertii* (series *Reticulati*) with *C. leichtlinii* and *C. kerndorffiorum* (series *Biflori*) (87% jackknife) as there are only slight morphological features to distinguish this from subsp. *gargaricus*. In addition, none of the species represented by more than one accession (*C. chrysanthus*, *C. biflorus*, *C. reticulatus*, *C. cancellatus*, *C. gargaricus*) come out as monophyletic though monophyly of *C. cancellatus* is not contradicted. It seems very likely that the taxa within series *Biflori* have weak sterility barriers and at least some of them are known to hybridise readily (e.g., *C. biflorus*, *C. chrysanthus*) giving rise to many horticulturally important cultivars (Brighton & al., 1980; Jacobsen & al., 1997). Also, it seems possible that certain species have arisen through natural hybridization, for example *C. sieheanus* (series *Reticulati*) which has characteristics that could be interpreted as morphologically intermediate between *C. chrysanthus* (series *Biflori*) and *C. ancyrensis* (series *Reticulati*) (pers. obs.). Further detailed studies are therefore desirable, particularly including nuclear data, but the present study suggests that there is a case for the merger of and amendment of series *Biflori* and series *Reticulati*.

A few groupings suggested by the present analysis may be supported by other types of data and shall be commented upon here. Inclusion of *C. × jessopae* in a weakly supported group (58% jackknife) with *C. abantensis* or *C. ancyrensis* (Fig. 1) may suggest that formation of this hybrid of unknown origin could have involved either of these species as the female parent. However, the sugges-

tion of Mathew (1982) that *C. reticulatus* could be one of the parental species is not contradicted. The moderately supported (75% jackknife) sister group relationship of *C. kerndorffiorum* and *C. leichtlinii* (Fig. 1) is further supported by their pronounced morphological similarity (Pasche, 1993). The strongly supported (95% jackknife) clade including *C. aereus*, *C. biflorus* subsp. *tauri*, *C. biflorus* subsp. *pseudonubigena*, *C. biflorus* subsp. *artvinensis* and *C. almehensis* (series *Biflori*) (Fig. 1) may be supported by their chromosome numbers (from: Brighton & al., 1973): *C. aereus* ($2n = 22$) *C. biflorus* subsp. *tauri* ($2n = 20, 22$), *C. biflorus* subsp. *pseudonubigena* ($2n = 18, 20$), *C. almehensis* ($2n = 20$), *C. biflorus* subsp. *artvinensis* (unknown). Except for *C. almehensis*, which is endemic to north eastern Iran, the remaining members of the clade are geographically confined to eastern Turkey. In this area, the only other member of series *Biflori* included in the present analysis is the very widespread *C. biflorus* subsp. *adamii* (the accession included here is from Serbia). It seems difficult to find morphological characters in support for the above group.

In addition to the pronounced confusion of series *Reticulati*, *Biflori*, and *Speciosi* comes the position of a group of species traditionally belonging to series *Reticulati* as part of or as the sister group to Section *Crocus* (see above). The group of species includes *C. sieberi*, *C. robertianus*, *C. cvjicii*, *C. rujanensis*, *C. dalmaticus*, and *C. veluchensis* (Fig. 1). This group of species ($2n = 18, 20, 22, 24, 26$) may possibly be cytologically distinct from the remaining species ($2n = 8, 10, 12, 14, 16, 18, 30$), though accessions with $2n = 18$ are known from both *C. veluchensis*, *C. cvjicii*, and *C. cancellatus* subsp. *mazziaricus* (data from Brighton & al., 1973; Brighton, 1977). In Mathew (1982) these species—except *C. rujanensis*—are generally referred to as more closely related to each other than any other species of the group, but it is difficult to find morphological characters supporting the group.

As indicated above, the present phylogenetic hypothesis is based on plastid sequences only, thus in case of hybridization the tree will only reflect the relationship of the plastid donor, typically the female parent. Accordingly, we will at this stage not formally propose any taxonomic changes, but await the addition of nucleotide sequence data from the nuclear genome. Despite the fact that many inconsistencies between the classification of *Crocus* suggested by Mathew (1982) and the present phylogenetic hypothesis have been pointed out above, the main assignment of species to the sections and series of Mathew (1982) is actually supported. Nevertheless, future re-classification is likely to involve all infrageneric levels: subgenera, sections, and series.

Though the phylogeny has clarified some relationships between supraspecific taxa, more data are clearly needed for fully resolving the phylogenetic tree. With ex-

ception of the little understood groups in series *Biflori*, *Reticulati*, and *Speciosi*, the high level of resolution of the present tree opposes the notions of “explosive” speciation or evolution by Frello and co-workers (Frello & Heslop-Harrison, 2000: 907; Frello & al., 2004: 87), who failed to find a correlation between the distribution of repetitive DNA sequences and the classification of Mathew (1982). Though an improved correlation exists between the distribution pattern of the sequence (pCvKB8) shown in Table 1 of Frello & al. (2004: 83–84) and the current phylogenetic hypothesis, we disagree with the view that repetitive DNA sequences are appropriate for tracking speciation (Frello & Heslop-Harrison, 2000; Frello & al., 2004). Homology assessment involving repetitive DNA sequences is notoriously difficult even at the nucleotide sequence level, and methods of detection based on probe hybridization never provided more than rough estimates of similarity. Despite the difficulties encountered in obtaining nucleotide sequence data from single copy nuclear genes, in particular from polyploid species or species which by other evolutionary mechanisms have gained extra gene copies, we consider this kind of data the most appropriate for future studies in *Crocus* phylogenetics.

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LITERATURE CITED

- Al-Eisawi, D. 2001. Two new species of Iridaceae, *Crocus naqabensis* and *Romulea petraea*, from Jordan. *Arab Gulf J. Sci. Res.* 19: 167–169.
- Arber, A. 1921. The leaf structure of the Iridaceae, considered in relation to the phyllode theory. *Ann. Bot. (Oxford)* 35: 310–336.
- Brighton, C.A. 1977. Cytological problems in the genus *Crocus* (Iridaceae) II. *Crocus cancellatus* aggregate. *Kew Bull.* 32: 33–45.
- Brighton, C.A., Mathew, B. & Marchant, C.J. 1973. Chromosome counts in the genus *Crocus* (Iridaceae). *Kew Bull.* 28: 451–464.
- Brighton, C.A., Scarlett, C.J. & Mathew, B. 1980. Cytological studies and origins of some *Crocus* cultivars. Pp. 139–160 in: Brickell, C.D., Cutler, D.F. & Gregory, M. (eds.), *Petaloid Monocotyledons. Horticultural and Botanical Research*. Academic Press, London.
- Chase, M.W., Cowan, R.S., Hollingsworth, P., van den Berg, C., Madriñán, S., Petersen, G., Seberg, O., Cameron, K.M., Kress, J.W., Hedderson, T.A.J., Conrad, F., Salazar, G., Richardson, J.E., Hollingsworth, M., Jørgensen, T., Kelly, L. & Wilkinson, M. 2007. A proposal for a standardized protocol to barcode all land plants. *Taxon* 56: 295–299.
- Chase, M.W., Soltis, P.S., Ruddall, P.J., Fay, M.F., Hahn, W.H., Sullivan, S., Joseph, J., Molvray, M., Kores, P.J., Givnish, T.J., Sytsma, K.J. & Pires, J.C. 2000. Higher-level systematics of the monocotyledons: an assessment of current knowledge and a new classification. Pp. 3–16 in: Wilson, K.L. & Morrison, D.A. (eds.), *Monocots: Systematics and Evolution*. CSIRO, Collingwood.
- Chichiriccò, G. 1996. Intra- and interspecific reproductive barriers in *Crocus* (Iridaceae). *Pl. Syst. Evol.* 201: 83–92.
- Dahlgren, R.M.T., Clifford, H.T. & Yeo, P.F. 1985. *The Families of the Monocotyledons*. Springer-Verlag, Berlin.
- Frello, S. & Heslop-Harrison, J.S. 2000. Repetitive DNA sequences in *Crocus vernus* Hill (Iridaceae): the genomic organization and distribution of dispersed elements in the genus *Crocus* and its allies. *Genome* 43: 902–909.
- Frello, S., Ørgaard, M., Jacobsen, N. & Heslop-Harrison, J.S. 2004. The genomic organization and evolutionary distribution of a tandemly repeated DNA sequence in the genus *Crocus* (Iridaceae). *Hereditas* 141: 81–88.
- Goldblatt, P. 1990. Phylogeny and classification of Iridaceae. *Ann. Missouri Bot. Gard.* 77: 607–627.
- Goldblatt, P. 1991. An overview of the systematics, phylogeny and biology of the African Iridaceae. *Contr. Bolus Herb.* 13: 1–74.
- Goldblatt, P. 2000 [2001]. Phylogeny and classification of the Iridaceae and the relationships of *Iris*. *Ann. Bot. (Rome)*, n.s., 58: 13–28.
- Goldblatt, P., Davies, T.J., Manning, J.C., van der Bank, M. & Savolainen, V. 2006. Phylogeny of Iridaceae subfamily Crocoideae based on a combined multigene plastid DNA analysis. *Aliso* 22: 399–411.
- Goldblatt, P. & Manning, J. In press. *The Natural History of the Iridaceae*. Timber Press, Cape Town.
- Goloboff, P.A. 1993. *Nona*, version 2.0. Published by the author, www.zmuc.dk/public/phylogeny/Nona-PeeWee/.
- Grilli Caiola, M., Caputto, P. & Zanier, R. 2004. RAPD analysis in *Crocus sativus* L. accessions and related *Crocus* species. *Biol. Pl.* 48: 375–380.
- Jacobsen, N., van Scheepen, J. & Ørgaard, M. 1997. The *Crocus chrysanthus-biflorus* cultivars. *New Plantsman* 4: 6–38.
- Kerndorff, H. 1993. Two new taxa in Turkish *Crocus* (Iridaceae). *Herbertia* 49: 76–86.
- Kerndorff, H. & Pasche, E. 1994. *Crocus mathewii* a new autumn flowering crocus from Turkey. *New Plantsman* 1: 102–106.
- Kress, W.J., Wurdach, K.J., Zimmer, E.A., Weigt, L.A. & Janzen, D.H. 2005. Use of DNA barcodes to identify flowering plants. *Proc. Natl. Acad. Sci. U.S.A.* 102: 8369–8374.
- Manning, J., Forest, F. & Vinnersten, A. 2007. The genus

- Colchicum* L. redefined to include *Androcymbium* Willd. based on molecular evidence. *Taxon* 56: 872–882.
- Manning, J., Goldblatt, P. & Snijman, D. 2002. *The Color Encyclopedia of Cape Bulbs*. Timber Press, Portland & Cambridge.
- Mariotti, M.G. 1988. *Crocus ligusticus* n. sp., a well known species. *Candollea* 43: 667–680.
- Mathew, B. 1982. *The Crocus. A Revision of the Genus Crocus (Iridaceae)*. Timber Press, Portland.
- Mathew, B. 1984. Rare and little-known crocuses in cultivation. *Kew Mag.* 1: 68–74.
- Mathew, B. 1995. An interesting new autumn-flowering *Crocus* from Turkey. *New Plantsman* 2: 183–183.
- Mathew, B. 2000 [2001]. 4. *Crocus* L. Pp. 271–274 in: Güner, A., Özhatay, N., Ekim, T. & Başer, K.H.C. (eds.), *Flora of Turkey and the East Aegean Islands*, vol. 11. Edinburgh Univ. Press, Edinburgh.
- Mathew, B. 2002. *Crocus* up-date. *Plantsman* 1: 44–56.
- Maw, G. 1886. *A Monograph of the Genus Crocus*. Dulau & Co., London.
- Nixon, K.C. 2002. *Winclada*, version 1.00.08. Published by the author, Ithaca, New York, www.cladistics.com.
- Nørnbæk, R., Brandt, K., Nielsen, J.K., Ørgaard, M. & Jacobsen, N. 2002. Flower pigment composition of *Crocus* species and cultivars used for a chemotaxonomic investigation. *Biochem. Syst. Ecol.* 30: 763–791.
- Olmstead, R.G. & Sweere, J.A. 1994. Combining data in phylogenetic systematics: an empirical approach using three molecular data sets in the Solanaceae. *Syst. Biol.* 43: 467–481.
- Pasche, E. 1993. A new *Crocus* (Iridaceae) from Turkey. *Herbertia* 49: 67–75.
- Pasche, E. & Kerndorff, H. 1999. A new natural hybrid in the genus *Crocus* (Iridaceae). *New Plantsman* 6: 43–45.
- Randjelović, N., Hill, A.D., Stamenković, V. & Randjelović, V. 1990. A new species of *Crocus* from Yugoslavia. *Kew Mag.* 7: 182–186.
- Reeves, G., Chase, M.W., Goldblatt, P., Rudall, P., Fay, M.F., Cox, A.V., Lejeune, B. & Souza-Chies, T. 2001. Molecular systematics of Iridaceae: evidence from four plastid DNA regions. *Amer. J. Bot.* 88: 2074–2087.
- Rudall, P. & Mathew, B. 1990. Leaf anatomy in *Crocus* (Iridaceae). *Kew Bull.* 45: 535–544.
- Sang, T., Crawford, D.J. & Stuessy, T.F. 1997. Chloroplast DNA phylogeny, reticulate evolution, and biogeography of *Paeonia* (Paeoniaceae). *Amer. J. Bot.* 84: 1120–1136.
- Souza-Chies, T.T., Bittar, G., Nadot, S., Carter, L., Besin, E. & Lejeune, B. 1997. Phylogenetic analysis of Iridaceae with parsimony and distance methods using the plastid gene *rps4*. *Pl. Syst. Evol.* 204: 109–123.
- Swofford, D.L. 2001. *PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods)*, Version 4.0b8. Sinauer, Sunderland
- Tate, J.A. & Simpson, B.B. 2003. Paraphyly of *Tarasa* (Malvaceae) and diverse origins of the polyploidy species. *Syst. Bot.* 28: 723–737.
- Wolter, M. 1990. Calciumoxalat-Kristalle in den Knollen-Hüllen von *Crocus* L. (Iridaceae) und ihre systematische Bedeutung. *Bot. Jahrb. Syst.* 112: 99–114.
- Yüzbasıoğlu, S. & Varol, Ö. 2004. A new autumn-flowering *Crocus* from SW Turkey. *Plantsman* 3: 104–106.
- Zubor, Á., Surányi, G., Györi, Z., Borbély, G. & Prokisch, J. 2004. Molecular biological approach of the systematics of *Crocus sativus* L. and its allies. *Acta Hort.* 650: 85–93.

Appendix. Voucher information and GenBank accession numbers of taxa sampled for the genus *Crocus* and outgroup representatives. Order of GenBank accession numbers: *ndhF*, *rpoC1*, *accD*, *trnH-psbA*, *rpl36-rps8*. * denotes specimens for which only one gene has been sequenced for verification purposes. – denotes lacking sequence. Nomenclature and authorities follow Mathew (1982) unless otherwise stated in the text.

C. abantensis T. Baytop & B. Mathew, Turkey, *V. Vasak* 315, C2328 (C), EU110504, EU110642, EU110366, EU110232, EU110814; *C. adanensis* T. Baytop & B. Mathew, Turkey, *O. Sønderhousen* 1027, C1582 (C), EU110405, EU110543, EU110268, EU110133, EU110715; **C. adanensis* T. Baytop & B. Mathew, Turkey, *O. Sønderhousen* 1024A, C1669 (C), EU110509, –, –, –, –; *C. aeriens* Herb., Turkey, *O. Sønderhousen* 0547⁴, C1875 (C), EU110453, EU110591, EU110316, EU110181, EU110763; *C. alatavicus* Semenow & Regel, Cult., GBG⁴, C1876 (C), EU110454, EU110592, EU110317, EU110182, EU110764; *C. aleppicus* Baker, Jordan, Edgewood Gardens S9914², C1923, EU110496, EU110634, EU110358, EU110224, EU110806; *C. almehensis* C.D. Brickell & B. Mathew, Cult., GBG⁴, C1914 (C), EU110487, EU110625, EU110350, EU110215, EU110797; *C. ancyrensis* (Herb.) Maw, Turkey, *M. Kammerlander & al. (KPPZ)* 90-254⁴, C1899 (C), EU110473, EU110611, EU110336, EU110201, EU110783; *C. angustifolius* Weston, Cult., CBG¹, C1689 (C), EU110446, EU110584, EU110309, EU110174, EU110756; *C. antalyensis* B. Mathew, Turkey, *O. Sønderhousen* 0482, C1550 (C), EU110388, EU110526, EU110251, EU110116, EU110698; *C. asumaniae* B. Mathew & T. Baytop, Cult., CBG¹, C1619 (C), EU110425, EU110563, EU110288, EU110153, EU110735; *C. autranii* Albov, Cult., GBG⁴, C1919 (C), EU110492, EU110630, EU110355, EU110220, EU110802; *C. banaticus* J. Gay, Cult., CBG¹, C1821 (C), EU110447, EU110585, EU110310, EU110175, EU110757; *C. baytopiorum* B. Mathew, Turkey, *O. Sønderhousen* 0976, C1549 (C), EU110387, EU110525, EU110250, EU110115, EU110697; *C. biflorus* Mill. subsp. *adamii* (J. Gay) B. Mathew, Yugoslavia, *S & Z* 88-103⁴, C1879 (C), EU110457, EU110595, EU110320, EU110185, EU110767; *C. biflorus* Mill. subsp. *alexandri* (Nčić ex Velen.) B. Mathew, Greece, *N. Jacobsen* G98-76, C1561 (C), EU110395, EU110533, EU110258, EU110123, EU110705; *C. biflorus* Mill. subsp. *artvinensis* (Philippov) B. Mathew, Turkey, *H. Kerndorff & E. Pasche* 9359⁴, C1877 (C), EU110455, EU110593, EU110318, EU110183, EU110765; *C. biflorus* Mill. subsp. *biflorus*, Turkey, *O. Sønderhousen* s.n., C1547 (C), EU110386, EU110524, EU110249, EU110114, EU110696; *C. biflorus* Mill. subsp. *crewii* (Hook. f.) B. Mathew, Turkey, *O. Sønderhousen* 1323, C1552 (C), EU110389, EU110527, EU110252, EU110117, EU110699; *C. biflorus* Mill. subsp. *isauricus* (Siehe ex Bowles) B. Mathew, Turkey, *G. Petersen & al.* 90-80a, C1556 (C), EU110392, EU110530, EU110255, EU110120, EU110702; *C. biflorus* Mill. subsp. *leucostylosus* Kernd. & Pasche, Turkey, *H. Kerndorff & E. Pasche* 0214⁴, C1892 (C), EU110467, EU110605, EU110330, EU110195, EU110777; *C. biflorus* Mill. subsp. *melantherus* (Boiss. & Orph.) B. Mathew, Greece, *A. Strid* 25302, C1613 (C), EU110422, EU110560, EU110285, EU110150, EU110732; *C. biflorus* Mill. subsp. *nubigena* (Herb.) B. Mathew, Greece, *H. Nielsen* HNL GK 37, C1546 (C), EU110385, EU110523, EU110248, EU110113, EU110695; *C. biflorus*

Appendix. Continued.

Mill. subsp. *pseudonubigena* B. Mathew, Turkey, *M. Kammerlander & al. (KPPZ) 90-131*⁴, *C1878* (C), EU110456, EU110594, EU110319, EU110184, EU110766; *C. biflorus* Mill. subsp. *pulchricolor* (Herb.) B. Mathew, Turkey, *O. Sønderhausen 0489, C1594* (C), EU110412, EU110550, EU110275, EU110140, EU110722; *C. biflorus* Mill. subsp. *punctatus* B. Mathew, Greece, *G. Petersen & al. 90-80b, C1583* (C), EU110406, EU110544, EU110269, EU110134, EU110716; *C. biflorus* Mill. subsp. *stridii* (Papan. & Zacharof) B. Mathew, Greece, *H. Nielsen HNL7058, C1574* (C), EU110401, EU110539, EU110264, EU110129, EU110711; *C. biflorus* Mill. subsp. *taurii* (Maw) B. Mathew, Turkey, *CBG*¹, *C1545* (C), EU110384, EU110522, EU110247, EU110112, EU110694; *C. biflorus* Mill. subsp. *weldenii* (Hoppe & Fümrohr) B. Mathew, Slovenia, *M. Ørngaard & K. Kristiansen 95-110*⁴, *C1900* (C), EU110474, EU110612, EU110337, EU110202, EU110784; *C. boryi* J. Gay, Greece, *A. Strid 25323, C1626* (C), EU110428, EU110566, EU110291, EU110156, EU110738; *C. boulosii* Greuter, Libya, *Koenen & Sarnetzkii s.n., C1913* (C), EU110486, EU110624, EU110349, EU110214, EU110796; *C. cambessedesii* J. Gay, Spain, *M. Sørensen s.n., C1627* (C), EU110429, EU110567, EU110292, EU110157, EU110739; *C. cancellatus* Herb. subsp. *cancellatus*, Turkey, *O. Sønderhausen 1060, C1633* (C), EU110433, EU110571, EU110296, EU110161, EU110743; *C. cancellatus* Herb. subsp. *damascenus* (Herb.) B. Mathew, Turkey, *O. Sønderhausen 1140, C1646* (C), EU110437, EU110575, EU110300, EU110165, EU110747; *C. cancellatus* Herb. subsp. *lycius* B. Mathew, Turkey, *O. Sønderhausen 1301, C1609* (C), EU110420, EU110558, EU110283, EU110148, EU110730; *C. cancellatus* Herb. subsp. *mazziaricus* (Herb.) B. Mathew, Greece, *A. Strid 25301, C1617* (C), EU110424, EU110562, EU110287, EU110152, EU110734; *C. cancellatus* Herb. subsp. *pamphylicus* B. Mathew, Turkey, *J. Persson 87-7, C1639* (C), EU110435, EU110573, EU110298, EU110163, EU110745; *C. candidus* Clarke, Turkey, *O. Sønderhausen 1200, C1554* (C), EU110390, EU110528, EU110253, EU110118, EU110700; *C. carpetanus* Boiss. & Reut., Portugal, *CBG*¹, *C1586* (C), EU110407, EU110545, EU110270, EU110135, EU110717; *C. cartwrightianus* Herb., Greece, *A. Strid s.n., C1641* (C), EU110436, EU110574, EU110299, EU110164, EU110746; *C. cartwrightianus* Herb., Cult., *CBG*¹, *C1996* (C), EU110502, EU110640, EU110364, EU110230, EU110812; *C. caspius* Fisch. & C.A. Mey. ex Hohen, *O. Sønderhausen*⁴, *C1911* (C), EU110484, EU110622, EU110347, EU110212, EU110794; **C. caspius* Fisch. & C.A. Mey. ex Hohen, Unknown, *P1992-5263, C2359* (C), EU110511, -, -, -, *C. chrysanthus* (Herb.) Herb., Greece, *K. Papanicolaou 903, C1555* (C), EU110391, EU110529, EU110254, EU110119, EU110701; *C. chrysanthus* (Herb.) Herb., Turkey, *O. Sønderhausen 0463, C1563* (C), EU110397, EU110535, EU110260, EU110125, EU110707; *C. chrysanthus* (Herb.) Herb., Greece, *S. Diemar & O. Seberg OSA242, C1682* (C), EU110443, EU110581, EU110306, EU110171, EU110753; *C. corsicus* Vanucc. ex Maw, France, *G. Petersen 91-10, C368* (C), EU110378, EU110516, EU110241, EU110106, EU110688; *C. cvijicii* Košanin, Greece, *J. & J. Archibald 343.600*⁴, *C1901* (C), EU110475, EU110613, EU110338, EU110203, EU110785; *C. cyprius* Boiss. & Kotschy, Cyprus, *G. Petersen & J. Petersen 04-18, C1503* (C), EU110381, EU110519, EU110244, EU110109, EU110691; *C. dalmaticus* Vis., Yugoslavia, *CEH 536/537*⁴, *C1912* (C), EU110485, EU110623, EU110348, EU110213, EU110795; *C. danfordiae* Maw, Turkey, *O. Sønderhausen 0569, C1543* (C), EU110382, EU110520, EU110245, EU110110, EU110692; *C. etruscus* Parl., Cult., *CBG*¹, *C1997* (C), EU110503, EU110641, EU110365, EU110231, EU110813; *C. flavus* Weston subsp. *flavus*, Cult., *CBG*¹, *C1832* (C), EU110452, EU110590, EU110315, EU110180, EU110762; *C. fleischeri* J. Gay, Cult., *CBG*¹, *C1559* (C), EU110394, EU110532, EU110257, EU110122, EU110704; *C. gargaricus* Herb. subsp. *gargaricus*, Cult., *CBG*¹, *C1830* (C), EU110451, EU110589, EU110314, EU110179, EU110761; *C. gargaricus* Herb. subsp. *herbertii* B. Mathew, Turkey, *GBG*⁴, *C1880* (C), EU110458, EU110596, EU110321, EU110186, EU110768; *C. gilanicus* B. Mathew, Cult., *GBG*⁴, *C1896* (C), EU110470, EU110608, EU110333, EU110198, EU110780; *C. goulimyi* Turrill, Greece, *O. Sønderhausen 0515, C1647* (C), EU110438, EU110576, EU110301, EU110166, EU110748; *C. graveolens* Boiss. & Reut. ex Boiss., Turkey, *O. Sønderhausen 1290, C1576* (C), EU110402, EU110540, EU110265, EU110130, EU110712; *C. hadriaticus* Herb., Greece, *A. Strid 25317, C1611* (C), EU110421, EU110559, EU110284, EU110149, EU110731; *C. hartmannianus* Holmboe, Cyprus, *Lady Loch 160, C2344* (K), EU110506, EU110644, EU110368, EU110234, EU110816; *C. hermoneus* Kotschy ex Maw, Israel, *N. Feinbrun s.n., C1889* (C), EU110465, EU110603, EU110603, EU110328, EU110193, EU110775; *C. hyemalis* Boiss. & Blanche, Israel, Edgewood Gardens², *C1926*, EU110498, EU110636, EU110360, EU110226, EU110808; *C. imperati* Ten. cv. De Jager, Cult., *CBG*¹, *C1825* (C), EU110449, EU110587, EU110312, EU110177, EU110759; *C. imperati* Ten. subsp. *imperati*, Italy, *EM 1066*⁴, *C2355* (C), EU110507, EU110644, EU110369, EU110235, EU110817; *C. imperati* Ten. subsp. *suaveolens* (Bertol.) B. Mathew, Italy, *M. Salmon 962*⁴, *C2356* (C), EU110508, EU110645, EU110370, EU110236, EU110818; *C. × jessopae* Bowles, Cult., *CBG*¹, *C1588* (C), EU110409, EU110547, EU110272, EU110137, EU110719; *C. karduchorum* Kotschy ex Maw, Turkey, *O. Sønderhausen 1108, C1658* (C), EU110440, EU110578, EU110303, EU110168, EU110750; *C. kerndorffiorum* Pasche, Turkey, Edgewood Gardens *HKEP 9010*², *C1922*, EU110495, EU110633, EU110357, EU110223, EU110805; *C. korolkowii* Regel ex Maw, Cult., *CBG*¹, *C1565* (C), EU110398, EU110536, EU110261, EU110126, EU110708; *C. kosaninii* Pulević, Cult., *GBG*⁴, *C1910* (C), EU110483, EU110621, EU110346, EU110211, EU110793; *C. kotschyanus* K. Koch subsp. *cappadocicus* B. Mathew, Turkey, *O. Sønderhausen 1142, C1600* (C), EU110415, EU110553, EU110278, EU110143, EU110725; *C. kotschyanus* K. Koch subsp. *hakkariensis* B. Mathew, Turkey, *O. Sønderhausen 0816, C1605* (C), EU110418, EU110556, EU110281, EU110146, EU110728; *C. kotschyanus* K. Koch subsp. *kotschyanus*, Turkey, *J. Persson 88-37*⁴, *C1897* (C), EU110471, EU110609, EU110334, EU110199, EU110781; *C. kotschyanus* K. Koch subsp. *suworowianus* (K. Koch) B. Mathew, Turkey, *G. Petersen & M. Ørngaard 90-92, C551* (C), EU110379, EU110517, EU110242, EU110107, EU110689; *C. laevigatus* Bory & Chaub., Greece, *O. Sønderhausen 0968, C1656* (C), EU110439, EU110577, EU110302, EU110167, EU110749; *C. leichtlinii* (Dewar) Bowles, Turkey, *M. Kammerlander & al. (KPPZ) 90-182*⁴, *C1915* (C), EU110488, EU110626, EU110351, EU110216, EU110798; *C. ligusticus* M.G. Mariotti, Italy, *J. & K. Persson 99-29*⁴, *C1884* (no herbarium voucher), EU110461, EU110599, EU110324, EU110189, EU110771; *C. longiflorus* Raf., Italy, *O. Sønderhausen 1089, C1636* (C), EU110434, EU110572, EU110297, EU110162, EU110744; **C. longiflorus* Raf., Italy, *O. Sønderhausen 1089, C2360* (C), -, -, EU110371, -, -, **C. longiflorus* Raf., Unknown *1248-7, C2361* (C), -, -, EU110372, -, -, *C. malyi* Vis., Cult., *CBG*¹, *C1587* (C), EU110408, EU110546, EU110271, EU110136, EU110718; *C. malyi* Vis., Cult., *CBG*¹, *C1688* (C), EU110445, EU110583, EU110308, EU110173, EU110755; *C. mathewii* Kernd. & Pasche, Turkey, *H. Nielsen s.n., C1632* (C), EU110432, EU110570, EU110295, EU110160, EU110742; *C. michelsonii* B. Fedtsch., Turkmenistan, *GBG*³, *C1885* (C), EU110462, EU110600, EU110325, EU110190, EU110772; *C. minimus* DC., Italy, *G. Petersen 91-15, C1580* (C), EU110404, EU110542, EU110267, EU110132, EU110714; *C. moabiticus* Bornm. & Dinsm. ex Bornm., Cult., Edgewood Gardens², *C1927*, EU110499, EU110637, EU110361, EU110227, EU110809; *C. nerimaniae*

Appendix. Continued.

Yüzü, Turkey, *H. Kerndorff & E. Pasche 0327⁴*, *C1891* (C), EU110466, EU110604, EU110329, EU110194, EU110776; *C. nevadensis* Amo, Spain, *H. Zetterlund 2000-002²*, *C1907* (C), EU110481, EU110619, EU110344, EU110209, EU110791; *C. niveus* Bowles, Greece, *K. Tan 10760*, *C1630* (C), EU110430, EU110568, EU110293, EU110158, EU110740; *C. niveus* Bowles, Greece, *O. Sønderhausen 1077*, *C2380* (C), EU257497, EU257488, EU257485, EU257491, EU257494; *C. nudiflorus* Sm., Cult., CBG¹, *C1598* (C), EU110413, EU110551, EU110276, EU110141, EU110723; *C. ochroleucus* Boiss. & Gaill., Cult., CBG¹, *C1827* (C), EU110450, EU110588, EU110313, EU110178, EU110760; *C. olivieri* J. Gay subsp. *balansae* (J. Gay ex Bak.) B. Mathew, Turkey, *O. Sønderhausen 1212*, *C1592* (C), EU110411, EU110549, EU110274, EU110139, EU110721; *C. olivieri* J. Gay subsp. *istanbulensis* B. Mathew, Cult., GBG⁴, *C1909* (C), EU110482, EU110620, EU110345, EU110210, EU110792; *C. olivieri* J. Gay subsp. *olivieri*, Turkey, *O. Sønderhausen 1180*, *C1589* (C), EU110410, EU110548, EU110273, EU110138, EU110720; *C. oreoreticus* B.L. Burtt, Greece, *O. Sønderhausen 0916*, *C1614* (C), EU257498, EU257489, EU257486, EU257492, EU257495; *C. oreoreticus* B.L. Burtt, Cult., CBG¹, *C2378* (C), EU257499, EU257490, EU257487, EU257493, EU257496; *C. pallasii* Goldb. subsp. *dispathaceus* (Bowles) B. Mathew, Turkey, *J. Persson 88-50⁴*, *C1893* (C), EU110468, EU110606, EU110331, EU110196, EU110778; *C. pallasii* Goldb. subsp. *hausknechtii* (Boiss. & Reut. ex Maw) B. Mathew, Iran, *P. Furse 1032*, *C2342* (K), EU110505, EU110643, EU110367, EU110233, EU110815; *C. pallasii* Goldb. subsp. *pallasii*, Turkey, *O. Sønderhausen 0850A*, *C1631* (C), EU110431, EU110569, EU110294, EU110159, EU110741; *C. pallasii* Goldb. subsp. *turcicus* B. Mathew, Turkey, *M. Kammerlander & al. (KPPZ) 90-184⁴*, *C1894* (C), EU110469, EU110607, EU110332, EU110197, EU110779; *C. paschei* Kernd., Turkey, *H. Kerndorff & E. Pasche 9034⁴*, *C1916* (C), EU110489, EU110627, EU110352, EU110217, EU110799; *C. pelistericus* Pulević, Greece, *H. Zetterlund, A. Eriksson & A. Strid 51678-B*, *C1918* (C), EU110491, EU110629, EU110354, EU110219, EU110801; *C. pestalozzae* Boiss. (white form), Cult., CBG¹, *C1685* (C), EU110444, EU110582, EU110307, EU110172, EU110754; **C. pestalozzae* Boiss. (blue form), Cult., *C1934* (no herbarium voucher), EU110510, —, —, —; *C. pulchellus* Herb., Turkey, *O. Sønderhausen 1208*, *C1601* (C), EU110416, EU110554, EU110279, EU110144, EU110726; *C. reticulatus* Steven ex Adams subsp. *hittiticus* (T. Baytop & B. Mathew) B. Mathew, Turkey, CBG¹, *C1544* (C), EU110383, EU110521, EU110246, EU110111, EU110693; *C. reticulatus* Steven ex Adams subsp. *reticulatus*, Cult., GBG⁴, *C1882* (C), EU110459, EU110597, EU110322, EU110187, EU110769; *C. robertianus* C.D. Brickell, Greece, *O. Sønderhausen 1190*, *C1599* (C), EU110414, EU110552, EU110277, EU110142, EU110724; *C. rujanensis* Randjel. & D.A. Hill., Serbia, *U. Strindberg & H. Zetterlund 88-081⁴*, *C1906* (C), EU110480, EU110618, EU110343, EU110208, EU110790; *C. sativus* L., Cult., CBG¹, *C1606* (C), EU110419, EU110557, EU110282, EU110147, EU110729; *C. scardicus* Košanin, Macedonia, GBG⁴, *C1937* (C), EU110501, EU110639, EU110363, EU110229, EU110811; *C. scharojanii* Rupr., Turkey, *J. & J. Archibald 8196*, *C1917* (C), EU110490, EU110628, EU110353, EU110218, EU110800; *C. serotinus* Salisb. subsp. *clusii* (J. Gay) B. Mathew, Portugal, *H. Christiansen 5344*, *C1621* (C), EU110426, EU110564, EU110289, EU110154, EU110736; *C. serotinus* Salisb. subsp. *salzmannii* (J. Gay) B. Mathew, Portugal, *H. Christiansen 3069*, *C1622* (C), EU110427, EU110565, EU110290, EU110155, EU110737; *C. serotinus* Salisb. subsp. *serotinus*, Spain, *M. Lidén 1721⁴*, *C1898* (C), EU110472, EU110610, EU110335, EU110200, EU110782; *C. sieberi* J. Gay subsp. *atticus* (Boiss. & Orph.) B. Mathew, Greece, *P. Hartvig 278-01*, *C1571* (C), EU110399, EU110537, EU110262, EU110127, EU110709; *C. sieberi* J. Gay subsp. *nivalis* (Bory & Chaub.) B. Mathew, Greece, *O. Sønderhausen 1020*, *C1562* (C), EU110396, EU110534, EU110259, EU110124, EU110706; *C. sieberi* J. Gay subsp. *sieberi*, Greece, *J. Petersen 1989*, *C1680* (C), EU110442, EU110580, EU110305, EU110170, EU110752; *C. sieberi* J. Gay subsp. *sublimis* (Herb.) B. Mathew, Greece, *O. Sønderhausen 0920⁴*, *C1904* (C), EU110478, EU110616, EU110341, EU110206, EU110788; *C. sieheanus* Barr ex B.L. Burtt, Cult., GBG⁴, *C1883* (C), EU110460, EU110598, EU110323, EU110188, EU110770; *C. speciosus* M. Bieb. subsp. *ilgazensis* B. Mathew, Cult., GBG⁴, *C1902* (C), EU110476, EU110614, EU110339, EU110204, EU110786; *C. speciosus* M. Bieb. subsp. *xantholaimos* B. Mathew, Turkey, *A.M.D. Hoog & E. Pasche 8324⁴*, *C1903* (C), EU110477, EU110615, EU110340, EU110205, EU110787; *C. speciosus* M. Bieb. subsp. *speciosus*, Cult., CBG¹, *C1602* (C), EU110417, EU110555, EU110280, EU110145, EU110727; *C. thomasii* Ten., Yugoslavia, *B. Mathew 7589*, *C1886* (C), EU110463, EU110601, EU110326, EU110191, EU110773; *C. tommasianus* Herb., Cult., CBG¹, *C1822* (C), EU110448, EU110586, EU110311, EU110176, EU110758; *C. tournefortii* J. Gay, Greece, *M. Salmon 1093*, *C1887* (C), EU110464, EU110602, EU110327, EU110192, EU110774; *C. vallicola* Herb., Turkey, *O. Sønderhausen 723*, *C1920* (C), EU110493, EU110631, EU110356, EU110221, EU110803; *C. veluchensis* Herb., Cult., CBG¹, *C1578* (C), EU110403, EU110541, EU110266, EU110131, EU110713; *C. veneris* Tapp. ex Poech, Cyprus, Edgewood Gardens *PB181²*, *C1925*, EU110497, EU110635, EU110359, EU110225, EU110807; *C. vernus* Hill subsp. *albiflorus* (Kit. ex Schult.) Asch. & Graebn., Cult., CBG¹, *C1573* (C), EU110400, EU110538, EU110263, EU110128, EU110710; *C. vernus* Hill subsp. *vernus*, Slovakia, GBG⁴, *C1905* (C), EU110479, EU110617, EU110342, EU110207, EU110789; *C. versicolor* Ker Gawl., France, *O. Sønderhausen 1271*, *C1558* (C), EU110393, EU110531, EU110256, EU110121, EU110703; *C. vitellinus* Wahlenb., Turkey, *O. Sønderhausen 1283c*, *C1664* (C), EU110441, EU110579, EU110304, EU110169, EU110751; *C. wattiorum* (B. Mathew) B. Mathew, Cult., Edgewood Gardens *HKEP 9548²*, *C1928*, EU110500, EU110638, EU110362, EU110228, EU110810.

Outgroup taxa

Babiana stricta (Aiton) Ker Gawl., South Africa, CBG¹, *C0695* (C), EU110375, EU110512, EU110237, EU110102, EU110684; *Romulea ramiflora* Ten., Portugal, CBG¹, *C1527* (C), EU110377, EU110515, EU110240, EU110104, EU110687; *Romulea temp-skyana* Freyn, Cyprus, *G. Petersen & J. Petersen C1512* (C), EU110376, EU110514, EU110239, EU110105, EU110686; *Syringodea bifucata* M.P. de Vos, South Africa, *Davidson 3180³*, *C2346* (J), EU110380, EU110518, EU110243, EU110108, EU110690; *Tigridia pavonia* (L. f.) DC, Cult., CBG¹, *C2345* (C), AY225087, EU110513, EU110238, EU110103, EU110685.

1 DNA from plants cultivated in Copenhagen Botanic Garden. If preceded by a country name, the plants are of wild origin; if preceded by "Cult.", the origin is unknown.

2 DNA extracted from dried leaves received from Edgewood Gardens, Pennsylvania. No herbarium vouchers available.

3 DNA from SANBI.

4 DNA from plants cultivated in Göteborg Botanical Garden, GBG. If preceded by a country name, the plants are of wild origin; if preceded by "Cult.", the origin is unknown.