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Crocus cobbii Kerndorff, Pasche & Harpke species nova (Liliiflorae, Iridaceae) and its relatives

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Abstract: In this article we present the new species *Crocus cobbii* from the northwestern part of the Iberian Peninsula. The definition of *Crocus cobbii* is based on molecular and detailed morphological findings, including the molecular examination and morphological revision of its four relatives, *C. nudiflorus*, *C. clusii*, *C. salzmannii*, and *C. serotinus*. An epitypfication for *C. serotinus* was done and a lectotype for *C. nudiflorus* designated. In addition, our molecular data assign the origin of the English *C. nudiflorus*, from which the plant was originally described, to the eastern Pyrenees.

Zusammenfassung: In diesem Artikel stellen wir die neue Art *Crocus cobbii* aus dem nordwestlichen Teil der Iberischen Halbinsel vor. Die Definition von *Crocus cobbii* basiert auf molekularen und detaillierten morphologischen Ergebnissen, einschließlich der molekularen Untersuchung und morphologischen Überarbeitung seiner vier Verwandten, *C. nudiflorus*, *C. clusii*, *C. salzmannii* und *C. serotinus*. Zusätzlich ordnen unsere molekularen Daten den Ursprung des englischen *C. nudiflorus*, von dem die Pflanze ursprünglich beschrieben wurde, den östlichen Pyrenäen zu.

Key words: New species *Crocus cobbii*, Iberian autumn crocuses, molecular analyses, lectotypification of *C. nudiflorus*, improved detailed morphology.

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1 INTRODUCTION

The new species belongs to the Iberian autumnal crocuses. Up to now it was hidden among its relatives most probably because all of them have little variation in their overall appearance especially concerning their pinkish, bluish-lilac or violet flowers and comparable colour-markings. In the course of our work on Iberian crocuses it was accidently detected by evaluating the results of molecular analyses.

In herbarium specimens the decay of the flower-colours of these crocuses is rapid and the original colour changes after 5-10 years into colourless or brown tobacco-like conditions, which makes an already difficult determination often impossible. This is one reason why many crocus collections of the past stored in Iberian herbaria have uncertain, false or no determination labels. Another reason is a lack of information or insufficient knowledge of the collectors about the genus. From the results of molecular analyses we know that it is important to look for minor but fairly constant differences between species, which were subjectively treated in the past as forms, variants, and subspecies. Taxonomically relevant morphological/phenotypic details were often not known or not recognised as relevant and therefore neglected.

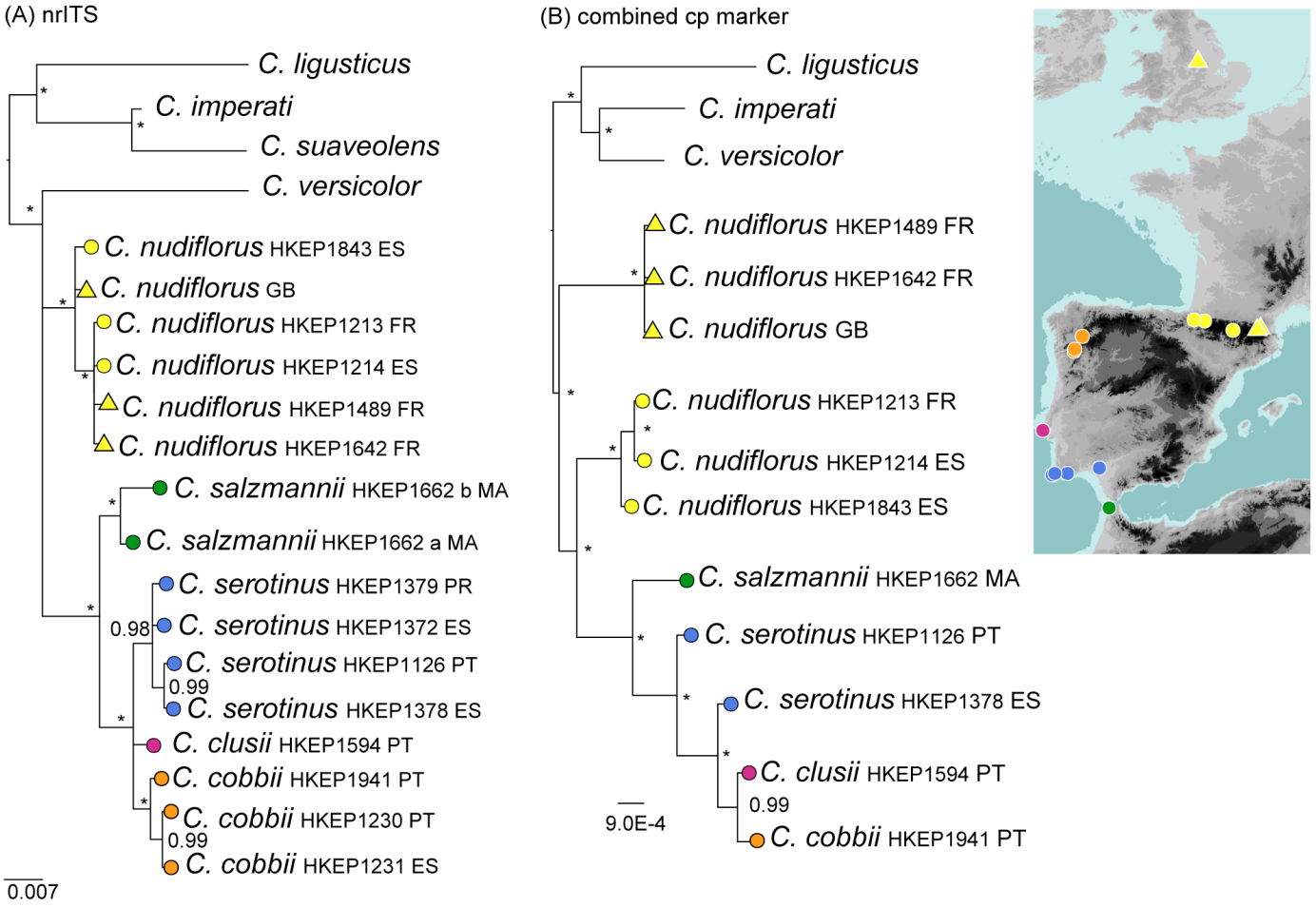


Fig. 1: Phylogenetic trees derived from Bayasian analysis based on the nrITS data set (A) and the combined chloroplast marker set (cp) of the concatenated matK– trnQ, trnL–trnF plus ycf1 region (B). Sample origins are plotted on the map, their positions are indicated in the trees (purple dots = *C. clusii*, organge dots = *C. cobbii*, yellow triangles and dots = *C. nudiflorus*, green dots = *C. salzmannii*, blue dots = *C. serotinus*). Posterior probabilities (pp) are given at the nodes of the tree, pp of 1 are indicated with an asterisk.

The correlation between molecular results and morphological parameters of a species is evident. In cases where it is possible to define a species by molecular results it is also possible to find its morphological peculiarities but not vice versa. Several recent species descriptions in *Crocus* were made basically of reduced morphological description from a few plants cultivated in a pot ("ex horto"). No molecular analyses were done before the description with the result that later conducted molecular analyses of several of those "species" proofed their identity with already known species. This means the "new species" turns out in the best case as a variation of a local population and must go to synonymy. Results of molecular investigations of the Iberian autumn crocuses also give evidence that formerly not known, overlooked or wrongly estimated characteristics can be essential prerequisites for their right determination.

In the last review of the genus *Crocus,* MATHEW (1982) obviously also encountered some of these problems. However, it was wise of him not to make the existent puzzling situation worse. He separated the taxa mainly by their obvious differences of corm tunics, number of leaves and their development at anthesis, size of segments, and length of perianth tubes. This treatment resulted in just four separable autumn-flowering Iberian crocuses, namely *C. nudiflorus* and *C. serotinus* divided by him into the three subspecies *serotinus*, *salzmannii,* and *clusii*. According to our molecular results the subspecies of *C. serotinus* cannot maintain their present taxonomical status and must receive back their species level which will be explained in detail in the revision of the genus.

In this paper we introduce the new species *Crocus cobbii* from the north-western part of the Iberian Peninsula and compare it to its known relatives. It is named to honour Ray Cobb (*1922) who, besides many other botanical activities, was 29 years holder of the NCCPG National Crocus Collection of the UK from shortly after its foundation in 1978 to 2007. Also, we are very thankful to him because he helped us significantly to get plant material of *C. nudiflorus* from its type-locality in Nottinghamshire for a more detailed investigation of this crocus.

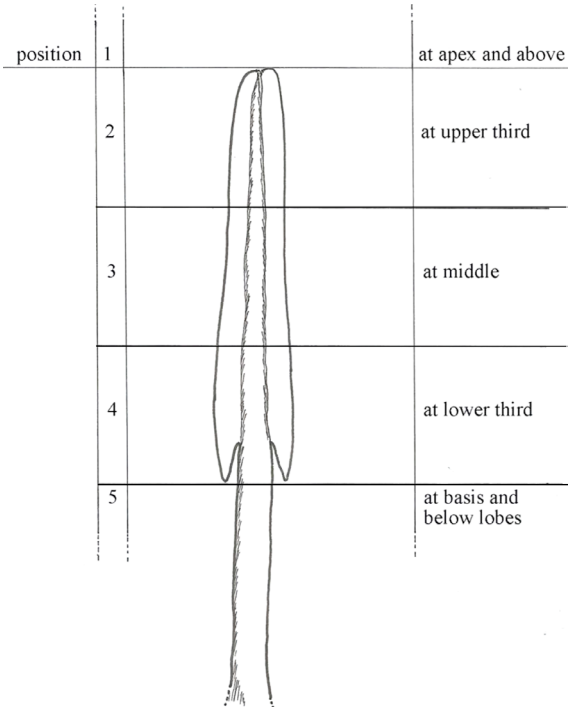


Fig. 2: Standardised positions of the first style division in relation to the anther-length.

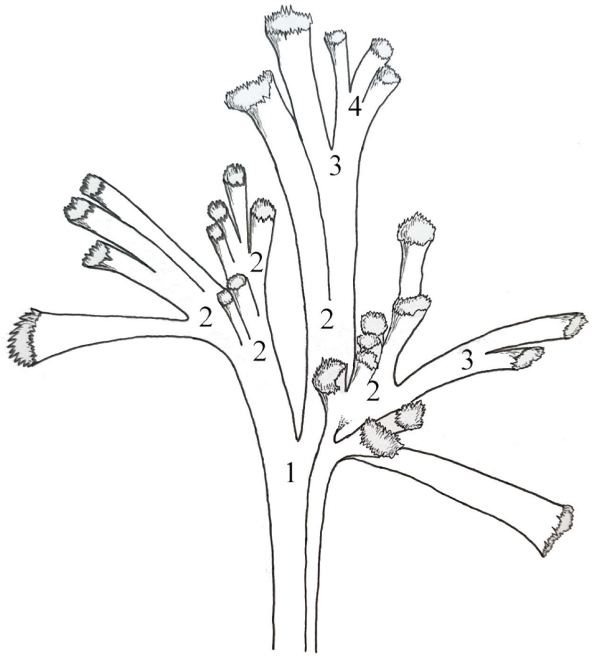


Fig. 3: Example of a style with four irregular style-divisions and different branch-lengths.

The definition of *Crocus cobbii* is based on molecular and detailed morphological results. Also, its other four relatives were molecularly investigated and are revised morphologically in the same way as recommended by KERNDORFF et al. (2015, 2016b). This is necessary because several species and subspecies descriptions of the past are incomplete in this sense or obviously arose from subsuming different taxa, which in some cases gave distorted results of several morphological parameters.

2 MATERIAL & METHODS

2.1 Provenance of investigated species

Provenance of investigated species (tab. 1) was illustrated using R (R CORE TEAM 2020) and the raster package HIJMANS and VAN ETTEN (2012). Our sampling covered the whole distribution range of autumn flowering crocuses of the Iberian Peninsula from Morocco to France (fig. 1).

2.2 Molecular analysis, phylogenetic analysis, and genome sizes

Molecular analysis – we included 15 individuals from different populations representing 5 taxa (three *C. cobbii*, one *C. clusii*, six *C. nudiflorus*, one *C. salzmannii*, four *C. serotinus*) and four outgroup taxa (*C. imperati*, *C. ligusticus*, *C. suaveolens*, and *C. versicolour*) for internal transcribed spacer (ITS) region of the nuclear ribosomal DNA (nrDNA) and eleven individuals (one *C. cobbii*, one *C. clusii*, six *C. nudiflorus*, one *C. salzmannii*, and two *C. serotinus*) and three outgroup taxa (*C. imperati*, *C. ligusticus*, and *C. versicolour*) for the chloroplast markers. Herbarium vouchers are deposited at the herbarium of the IPK Gatersleben (GAT).

Extraction of genomic DNA and amplification of the nuclear rDNA ITS and intergenic spacer between the *trn*L and *trn*F(GAA) genes were conducted according to HARPKE et al. (2013, 2014) and JAKOB & BLATTNER (2006), respectivly. Two further chloroplast markers were amplified with 1 U Phusion High-Fidelity DNA Polymerase (Thermo Scientific) in the supplied Phusion GC Buffer, 200 µM of each dNTP, 0.5 µM of each primer, and about 20 ng of total DNA in 50 µl reaction volume in a GeneAmp PCR System 9700 (Perkin-Elmer). For the plastid region comprising the *trn*K intron, *rp*S16 and *trn*Q and their intergenic spacers, four primers were used (matkf combined with rpS16in1_r, rpS16in1_f combined with trnQr; see tab. 2) and the *ycf*1 gene.

Forward and reverse sequences were manually checked, edited where necessary, and combined in consensus sequences for each locus and individual. All newly obtained sequences were submitted to the EMBL nucleotide database. Sequence accession numbers for the studied individuals are accessible via the ID PRJEB43897. Sequences were manually aligned. The sequences of chloroplast loci were concatenated resulting in one dataset.

Table 1: Provenance of molecularly analyzed crocuses

Species	HKEP coll. ID	Provenance	Collection date
C. clusii	1594	Portugal, Estremadura, Serra de Sintra, 380 m	24.10.15
C. cobbii	1230	Portugal, Trás-os-Montes e Alto Douro, Montalegre, 1040 m	11.10.12
	1231	Spain, Castilla y Leon, Seoane 850 m	12.10.12
	1941	Portugal, Trás-os-Montes e Alto Douro, Padornelos, 1100 m	24.09.19
C. nudiflorus	1213	France, Pyrénées Atlantiques, Col de la Pierre St. Martin, 1300 m	30.03.12
	1214	Spain, Catalunya, Vielha, 1500 m	13.04.12
	1489	France, Pyrénées Orientales, Col de Jau, 1500 m	24.04.14
	1642	France, Pyrénées Orientales, Esposolla, 1700 m	29.04.16
	1843	Spain, Pamplona, Puerto Ibaneta, 1050m	03.05.18
	-	Great Britain, Nottinghamshire, type area, ex Ray Cobb	10.19
C. salzmannii	1662	Morocco, Tangier, 50 m	22.11.16
C. serotinus	1378	Portugal, Algarve, Odeceixe, 80 m	06.11.13
	1126	Portugal, Algarve, Vilamoura, 20 m	06.11.11
	1379	Portugal, Algarve, Sagres, 80 m,	16.11.13
	1372	Spain, Andalucia, Hinojos, 90 m	03.11.13

Phylogenetic analysis – The nrITS and the chloroplast data set were subjected to Bayesian phylogenetic inference (BI) with MrBayes 3.2.6 (Ronquist et al. 2012). For BI 2 times 4 chains were run for 1 million generations under the GTR+Gamma+I model of sequence evolution for the ITS data set and 1.5 million generations for the chloroplast marker data set, sampling a tree every 1000 generations. Converging log-likelihoods, potential scale reduction factors for each parameter and inspection of tabulated model parameters in MrBayes suggested that stationarity had been reached. The first 25% of trees of each run were discarded as burn-in. Two independent runs of BI analysis were performed to confirm that separate analyses converged on the same result. In each of the 2 analyses the same topology and similar posterior probabilities (pp) of nodal support resulted.

Genome sizes – genome sizes of silica dried leaf material of two to three individuals were measured in a flow cytometer (Meudt et al. 2015), with *Pisum sativum* L. as standard (2C = 9.09 pg; Doležel et al. 2007). If possible, the genome sizes were connected to already published chromosome counts (Brighton et al. 1973). Chromosomes were counted according to Miljković et al. (2016).

2.3 Application of additional morphological parameters for a better distinction of species

Position of style-division in relation to anther-length – At present a rarely used style-parameter in crocus morphology and species differentiation is at which position of the anther the style begins to branch out. We include this parameter in our investigations of Iberian autumnal crocuses because we noticed significant differences in the species investigated. This parameter is, like many others in *Crocus*, rather variable and can only be studied meaningfully having enough material available. For reliable comparison and integration in statistical procedures it is necessary to standardise this parameter what is being done here for the first time.

As can be seen in fig. 2, we divided the position of the first style division into five areas related to the length of the anther. Area one starts from the apex of the anther and extends to beyond of it with no limit. Area two is the upper third of the anther to the apex, area three the middle of the anther, area four the lower third of the anther, and area five the bottom of the anther lobe and below of it.

Kind of style-division – Another useful style-parameter for Iberian autumnal-flowering crocuses is, regardless of the branch out position, the kind of division. In all species of the genus the style divides primarily into three branches although it might be difficult in cases with further divisions and plenty of resulting branches to recognise this without special techniques or tools. The first division of the style into three branches is the most typical in the genus and in many species the only one. Furthermore, we found out that the branching of the three branches has not necessarily to take place exactly in the same vertical position. Especially with species having several hierarchy levels of branching it can take place somewhat offset.

Length of style according to stamens – A widely used style parameter in *Crocus* is the length of the entire style according to the stamens. It is generally differentiated into three cases, (1) styles (with branches) are longer than the stamens, (2) they are equal to the top of the stamens and, (3) they are shorter than stamens.

Length of anther lobes – In the present investigation, we tested another morphological parameter for general taxonomical purposes. It is the length of the lobes of the anthers at their basis which can vary in species to a certain extend as we found out in the investigations of Serbian crocuses (Harpke et al. 2017).

Table 2: Primers and analyzed regions.

Region	Primer name	Primer sequence (5'-3')	Amplicon size in bp	PCR annealing temperature
ITS	ITSA [1]	GGA AGG AGA AGT CGT AAC AAG G	~750	54 °C
	ITSB [1]	CTT TTC CTC CGC TTA TTG ATA TG		
*trn*L^{UAA}–*trn*F^{GAA}	Cp07 [2]	GGA AAT GGG GAT ATG GCG	~720	54 °C
	trnFr [2]	AAA ATC GTG AGG GTT CAA GTC		
*mat*K–*trn*Q^{UUG}	matKf [3]	CAT TTC CAC TTG AAC CAT AAG CAG G	~1600	64 °C*
	rpS16in1_r	GAATCGTTGCAATTGATGTTCG		
	rpS16in1_f	CGAAGGTCTCTTCCTTCTCTTCGG	~1600	68 °C*
	trnQr [3]	GGT CCC GTT ACT CGG AGG TTC G		
*ycf*1	ycf1bF	TCCGATGAAAGTCTGATTGTTGTG	~900	65 °C*
	ycf1bR	ATACATGCTCAATTGATGGAAAAC		

[1]BLATTNER (1999); [2]JAKOB & BLATTNER (2006); [3]HARPKE et al. (2014)
* amplified with Phusion Taq

Calcium-oxalate crystals in the corm tunics – Calcium-oxalate crystals in plants are known for some time and comprehensively dealt with in FRANCESCHI (1980). In Crocus, they were discovered for the first time by WOLTER (1990) who found them rather hidden in the corm tunics of almost all taxa known at that time, using special treatment techniques to make them observable under polarized light. We used the same treatment as WOLTER (1990) for the preparation of the corm tunics, a short soaking with bleach, rinsing with water and fixation in glycerol. They were visualized with a Dino-Lite Edge Digital Microscope using polarized light and photo-stacking technique in cases where necessary. In these cases, scale bars cannot be shown, but enlargements have the same order of magnitude as the pictures with scale bars. The Ca-oxalate monohydrate crystals have a high birefringence index of nγ-nα = 0.16, which is near the one of calcite (0.172), means they can create strong interference colours, dependent on species. The form, size, localisation, and frequency of the crystals in the tunics can be different and are usable as an additional distinguishing parameter for crocuses.

Shape of transversal cross-sections of leaves – RUDALL & MATHEW (1990) studied crocus leaf anatomy in the context of other leaf anatomical studies on its subfamily *Ixioidae* and other *Iridaceae*. Taxonomically useful findings of them were that similar leaf shapes often correspond to nearly allied species e.g., belonging to a common series. This can be confirmed for Iberian autumn crocuses, but it is also possible to see several differences in the leaf shape between them.

The leaves of the crocuses investigated were kept in 70% Ethanol, cut with a microtome to thin slices of 75 µm and coloured with "Astrablau" and Chrysoidine. It is important to mention that after cutting the samples must be transferred into water again and washed thoroughly to receive back their natural form, as many species may spirally roll up their leaves especially when they were kept in acidic preservatives. In such cases and without this pre-treatment the following characterization of leaves is not possible.

To systematize our leaf cross-section results, the following definitions are made based on the bilateral symmetry of the crocus leaf. The only axis of symmetry runs vertically through the middle of the "white stripe" (keel). The shape of the cross-section is divided into two main parts, the "base" of the leaf and the "arms" of the leaf. The shape of the basis of the investigated crocuses is divided into three categories, planar, concave, and convex. The arms of the leaves are either with or without "ribs" underneath. The end of the arm can point in different directions. It is characterized as "open", when it has an angle of significantly more than 0 degree or "half-open" when it has an angle of around 0 degree or "closed" when it has an angle of significantly smaller than 0 degree compared to the symmetry-axis of the leaf.

Macroscopic and microscopic seed parameters – To get the most representative seeds of a species for photographing we selected three typical ones in good condition out of about 20-30 individuals to show their size, shape, colour, caruncle, and raphe. For photographing, the seeds were mounted on a Petri-plate filled with a thin layer of dried Agar-Agar to avoid rolling of the seeds while moving under the microscope. For a good "round-view" of seeds they were slightly pressed down with a flexible steel tweezers into different positions on the Agar-Agar layer. The photographs were made at the Stuttgart State Museum (Germany) with a Zeiss light microscope with an EDOF camera and software of the same firm (KERNDORFF et al. 2016a).

For the characterization of the microscopic sculptures of the testa, scanning electron microscopy (SEM) was carried out. The three selected seeds were mounted on a graphite sample holder, coated with gold and photographed and examined in a Hitachi S4100 SEM (Hisco Europe, Ratingen, Germany) at 5 kV acceleration-voltage.

Table 3: Genome size measuring using *Pisum sativum* as standard.

Species	collection ID	CV % standard	CV % sample	Genome size 2C in pg
C. cobbii	HKEP1230	2.52	4.23	3.57
		2.46	4.72	3.43
	HKEP1231	2.51	3.64	3.45
	HKEP1941	3.16	4.5	3.5
C. clusii	HKEP1594	2.38	5.18	9.23
		2.67	2.73	8.28
C. nudiflorus	GB	3.51	4.3	7.09
		2.26	3.03	6.91
	HKEP1214	2.57	3.81	6.96
	HKEP1843	2.52	4.23	7.47
		2.99	4.24	7.61
C. salzmannii	HKEP1662	2.36	3.67	6.34
		2.04	2.18	6.12
C. serotinus	HKEP1379	1.85	4.73	4.45
	HKEP1378	2.45	3.03	3.48

3. RESULTS & DISCUSSION

3.1. Phylogenetic relationships

Sequences of nrITS region had a length between 629 bp (*C. cobbii*) and 647 bp (*C. salzmannii*) resulting in an alignment of 675 bp. The concatenated chloroplast markers resulted in an alignment of 4927 bp with sequences ranging in length from 4639 (*C. ligusticus*) to 4833 bp (*C. cobbii*).

The five species of the *C. serotinus* group were revealed in two major clades in the nrITS phylogeny (fig. 1, (A)). One clade comprising *C. nudiflorus* and a second clade comprising *C. clusii*, *C. cobbii*, *C. salzmannii* and *C. serotinus*, where *Crocus cobbii* is placed in a strong supported polytomic clade as sister to *C. clusii* and *C. serotinus* (fig. 1, (A)).

The two major clades are grouping together with *C. versicolour* in a polytomy.

The chloroplast marker phylogeny could provide a better resolution with the five species of the *C. serotinus* group forming a full supported monophyletic group (pp 1; fig. 1, (B)). *Crocus nudiflorus* from the Eastern Pyrenees and the type from England (fig. 1) are separated from the other species as well as from the other locations of *C. nudiflorus* from the Central and Western Pyrenees. Therefore, the plants growing today in England probably originate from the Eastern Pyrenees.

Crocus cobbii was placed together with *C. clusii* (fig. 1, (B)) in a strongly supported clade (pp 0.99) with *Crocus serotinus* as sister clade. Both data sets show that *C. cobbii* is molecularly clearly separated from its four related species.

Generally, the resolution of the chloroplast data set was better than that of the nrITS data set. In some cases, the topology of both phylogenetic trees is incongruent, which could be caused by hybridization or incomplete lineage sorting. In case of incomplete lineage sorting the Eastern Pyrenean *C. nudiflorus* could have kept an ancient chloroplast type. Considering the chromosome count of $2n = 48$ (Brigthon et al. 1973) as well as its genome size (2C of 6.9 to7.6 pg) being about twice of that of closely related species, *C. nudiflorus* is tetraploid. It might be possible that it originated via a hybridization event involving extinct or still undiscovered parental species.

Crocus nudiflorus is not the only polyploid *Crocus* in this investigation. Genome sizes suggest that *C. clusii* (2C of 8.28 to 9.23 pg) and *C. salzmanii* (2C of 6.1 to 6.3 pg) are also polyploids since their genome size is twice as high as the genome size the other (tab. 3). For *C. salzmannii* chromosome counts of $2n = 22$ and $2n = 44$ were published (Brigthon et al. 1973). Considering the high genome size, the material of the type-region most likely possesses a karyotype having $2n = 44$ chromosomes. Chromosome counts for *C. clusii* ranged at highest between $2n = 22$ and $2n = 24$. Counting for material included in our analysis is currently in progress. *Crocus serotinus* from Sagres was counted with 24 chromosomes by Brigthon et al. (1973) and confirmed by our own analyses of individuals of the HKEP1379 population.

Additional nuclear markers, genome sizes, chromosome numbers and additional sampling is necessary to clearly identify past allopolyploidisation events and involved parental species.

3.2. Morphological results

Position of style-division in relation to anther-length

The results we received for the style-division in relation to anther-length (fig. 2) of the five species that need to be characterised more precisely are presented in tab. 4.

Table 4: Position of the first style division of Iberian autum crocuses in relation to their anther-length

Position of style-division	HKEP 1214 *C. nudiflorus* (n = 45)	HKEP1379 *C. serotinus* (n = 24)	HKEP1662 *C. salzmannii* (n = 19)	HKEP1594 *C. clusii* (n = 28)	HKEP1230 *C. cobbii* (n = 67)
1	29 (64.4%)	4 (16.7%)	0	2 (7.1%)	22 (32.8%)
2	15 (33.3%)	7 (29.2%)	3 (15.8%)	8 (28.6%)	34 (50.7%)
3	1 (2.2%)	7 (29.2%)	10 (52.6%)	16 (57.1%)	10 (14.9%)
4	0	5 (20.8%)	6 (31.6%)	2 (7.1%)	1 (1.6%)
5	0	1 (4.2%)	0	0	0

Table 5: Length of style according to stamens

	HKEP1214	HKEP1379	HKEP1594	HKEP1662	HKEP1230
styles shorter (3)	1 (2.2%)	2 (8.3%)	1 (3.6%)	4 (21.1%)	3 (4.4%)
styles equal (2)	2 (4.4%)	2 (8.3%)	7 (25%	8 (42.1%)	7 (10.3%)
styles longer (1)	42 (93.3%)	20 (83.3%)	20 (71.4%)	7 (36.8%)	58 (85.3)
crocuses investigated	45	24	28	19	68

It can be recognised that only *C. serotinus* has a spread of style division position all over the defined areas 1-5 with the majority dividing in the middle and upper third of anthers (58.4%). Compared to this, *C. nudiflorus* shows quite different results in having mostly style-divisions in areas one and two (97.7%) compared to 2.2% in areas three, four, and five. Only *C. salzmannii* shows style divisions in the three areas of the anther-length (areas 2, 3, and 4) and never above (1) or below of it (5). *C. clusii* has comparable style-divisions to *C. salzmannii*, which is mainly at the centre of the anthers in areas two and three (85.7%) and less in areas one, four, and five (14.2%). Finally, *C. cobbii* has an obvious tendency for style-divisions in the middle and upper areas of the anthers and above (areas 2, 3, and 1) with 98.4% compared to only 1.6% in areas four and five.

The results show clear differences between the species which leads to the conclusion that this morphological peculiarity can also be helpful as an additional parameter for species differentiation in other series of the genus in cases where they are not easy to separate morphologically.

Kind of style-division

In case of the Iberian autumn crocuses the basic branching of the style is followed by two to three further divisions, resulting with a maximum of four. These are normally arranged hierarchically, or, in some cases, rather chaotic as shown in fig. 3

In the latter case it is not easy to systematize the styles to find out differences between them as there can be around 50 branches present with a high number of unequal lengths and forms especially in the higher hierarchy-levels (fig. 3). Most of the styles investigated had four hierarchical divisions although the fourth one is often rare and poorly developed. The style-ends can also be different and may be trumpet shaped or only slightly or not expanded at the apex (fig. 3). Level two and three of the divisions are in most cases evenly developed, and their branches +/- of the same length. Branches of the first division are generally the longest. There are also transitional lengths and forms between division two and three and three and four (fig. 3). The mostly jagged fourth division of the style-branches has different lengths too, but they are tiny and too variable to be considered in a systematic assessment. The length of style-branches is often taxon-specific as is known from other species of the genus (e.g., *C. wattiorum*).

Like the length of the branches, their thickness can also vary. Two main forms are observable in Iberian autumn crocuses. One is a continuous broadening towards the apex (*C. nudiflorus*) the other one is a constant thickness up to the apex (*C. serotinus*). In accordance with fig. 4 the following results could be received:

Crocus nudiflorus (HKEP1214) has style-branches of 3-4 hierarchical levels. Branches are in all levels +/- upright and brush-like because the thickness is increasing in all levels with length, ending in a trumpet-like irregularly jagged edge. The total number of branches reaches 30-50.

Crocus serotinus (HKEP1379) has style-branches of 3-4 hierarchical levels. The first and the second level have the longest branches. For the whole length the branches are thin. In less frequent cases they have only a slight thickening at the apex. Branches are not only upright but also bend downwards. The total number of branches is around 30-50.

Crocus clusii (HKEP1594) has style-branches of 2 (rarely 3) hierarchical levels. Branches are in both levels +/- upright, filiform or thickening upwards. Branches of the first division are significantly longer than of level two which are about half as long. Branches of level three (if present) are short. The apex is

Fig. 4: Style-branches of Iberian autumn crocuses.

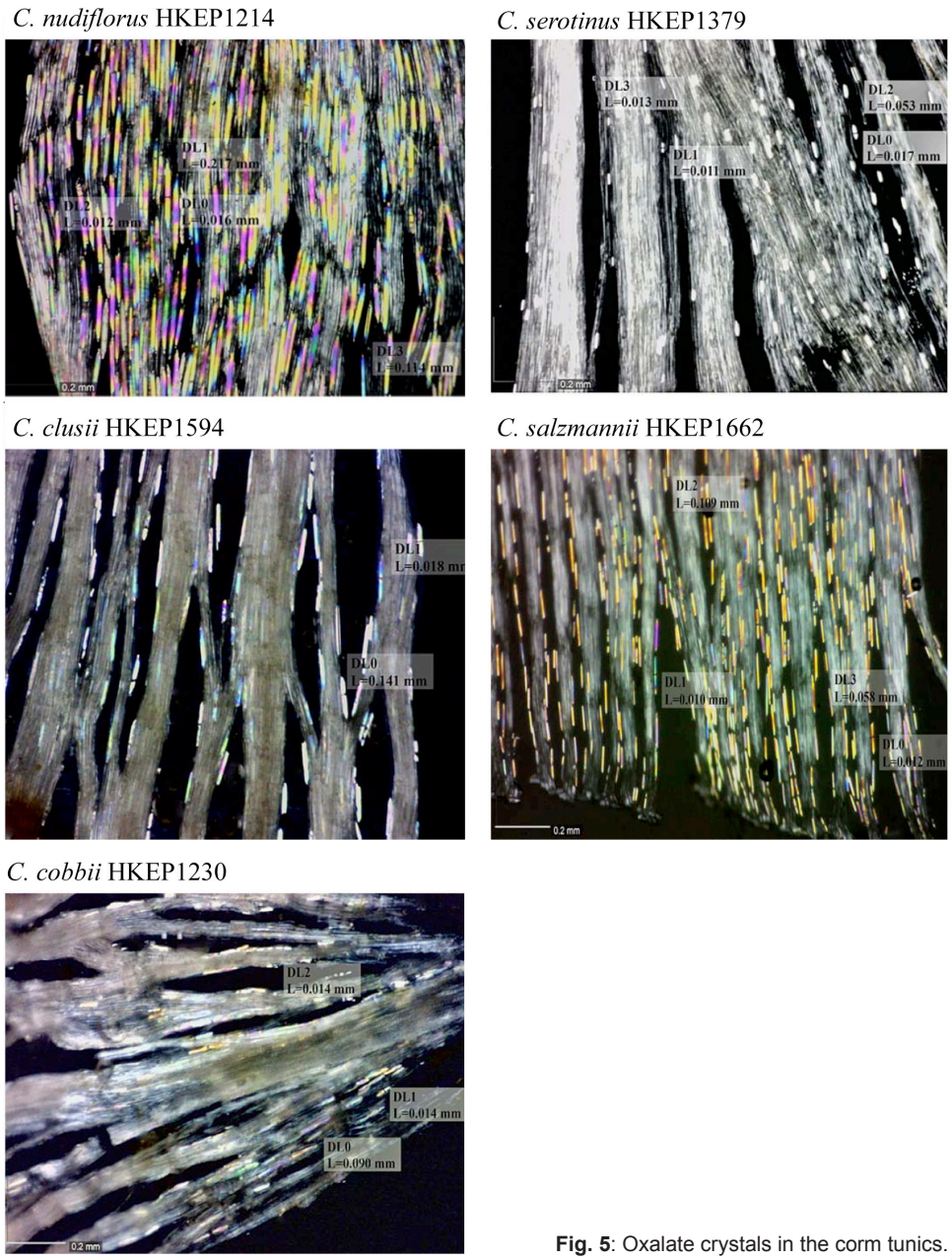


Fig. 5: Oxalate crystals in the corm tunics.

either of the same thickness or a little thicker. The total number of branches is about 10-25.

Crocus salzmannii (HKEP1662) has style-branches of 3-4 hierarchy levels. Branches are in all levels +/- upright. The first level has the longest branches, approximately two times longer than branches of level two and three. Level four is not always present, if so, branches are short. The thickness of all branches is +/- equal even to the apex. Number of all branches is around 15-20.

Crocus cobbii (HKEP1230) has style-branches of 3-4 hierarchy levels with the tendency to spread out in level three and four. The first level has the longest branches, 2-3 times as long as level two and three. Level four is rare and branches rather short. Thickness of all branches is equal or becoming slightly thicker towards the apex. Number of all branches is around 20-40.

Length of style according to stamens

As documented in tab. 5 only *C. salzmannii* has a +/- even distribution of style length in all three categories. In the other four species styles significantly longer than anthers are dominant.

Length of anther lobes – The results we received for the Iberian autumn crocuses are almost the same for each species and of low taxonomic value. For *C. nudiflorus, C. serotinus, C. clusii*, and *C. cobbii*, the average of the investigated specimens is

Fig. 6: Shapes of leaf cross-sections of Iberian autumn crocuses.

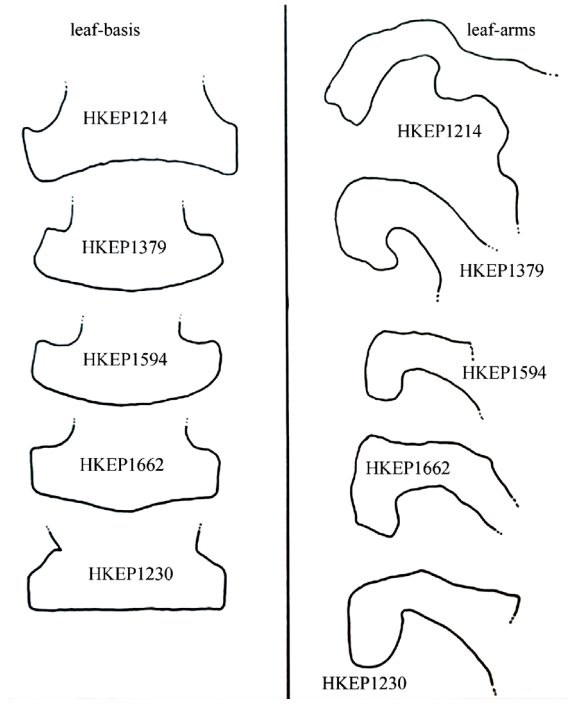


Fig. 7: Leaf-basis and leaf-arms of Iberian autumn crocuses.

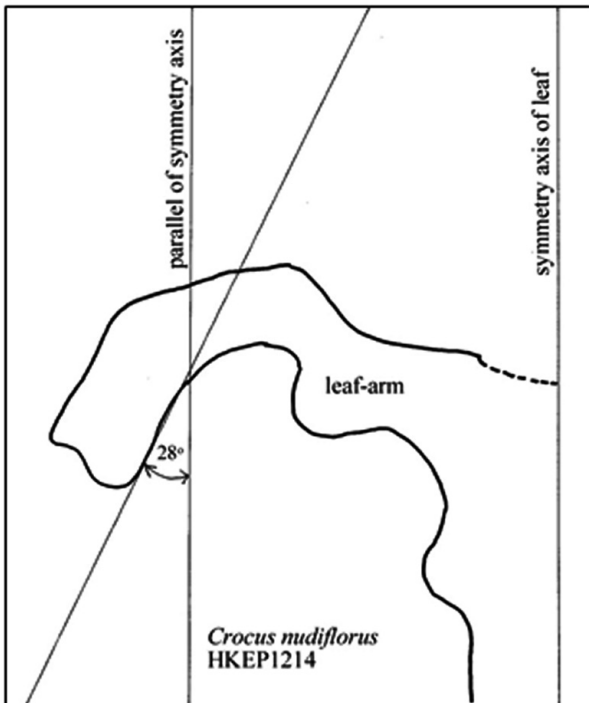


Fig. 8: Calculation of leaf-arm inclination.

between 1.56 mm and 1.63 mm, the median of all four species is 1.5 mm the minimum is 1mm and the maximum 3 mm. The only remarkable difference to these results has *C. salzmannii* which has shorter lobes with an average of 1.05 mm, a median of 1 mm, a minimum of 0.5 mm and a maximum of 1.5 mm. The results clearly shows that this parameter is mainly useful in cases, where the lobes are already recognised in a pre-investigation of a new species either as unusually long or short.

Calcium-oxalate crystals in the corm tunics

In various plant tissues, the crystals can either appear throughout the tissue in each individual cell, or they can be localized in special cells. There are all transitions between these two extremes. In order to obtain a separation between the one and the other extreme, we introduce the term "missing localization" if every cell of the tissue contains a crystal, and of "complete localization" if the calcium oxalate is present in anatomically special cells (FREY, 1924). The results of the investigated Iberian autumnal crocuses are according to fig. 5 as follows:

Crocus nudiflorus (HKEP1214) is the only one of the investigated crocuses which has an almost "missing localization" of the crystals in the tunic as they occur obviously in most of the (dead) cells of the tunic. The needle-like crystals strongly create interference colours and are rather long with a length between 0.114 and 0.217 mm and a width with 0.012-0.016 mm.

Crocus serotinus (HKEP1379) shows a quite different distribution and shape of the crystals compared to *C. nudiflorus*. They occur in a "complete localization" scattered along the surface of the vascular bundles and show no interference colours. They are not "needle-like" and their dimension and frequency also much less than those of *C. nudiflorus*, having a length of only 0.017-0.053 mm and a tiny diameter of 0.011-0.013 mm.

Crocus clusii (HKEP1594) has a similar crystal distribution as *C. serotinus* scattered all along the former vascular bundles but the crystals are much longer (0.137-0.141 mm) and a little bit broader (0.017-0.018 mm). The Interference colours are present but not well developed.

Crocus salzmannii (HKEP1662) has very frequent, long but narrow needles with interference colours mainly in the spectral areas of the yellow-orange wavelengths, well localized along the vascular bundles. Their length is between 0.058 mm and 0.109 mm, their width between 0.010 and 0.012 mm.

Crocus cobbii (HKEP1230) has few, rather haphazardly distributed crystals, occurring in few places of the tunic while others are totally without crystals. The orientation and localization is scattered along the vascular bundles. Interference colours are present but poorly developed. The dimension of the crystals is about 0.09 times 0.014 mm.

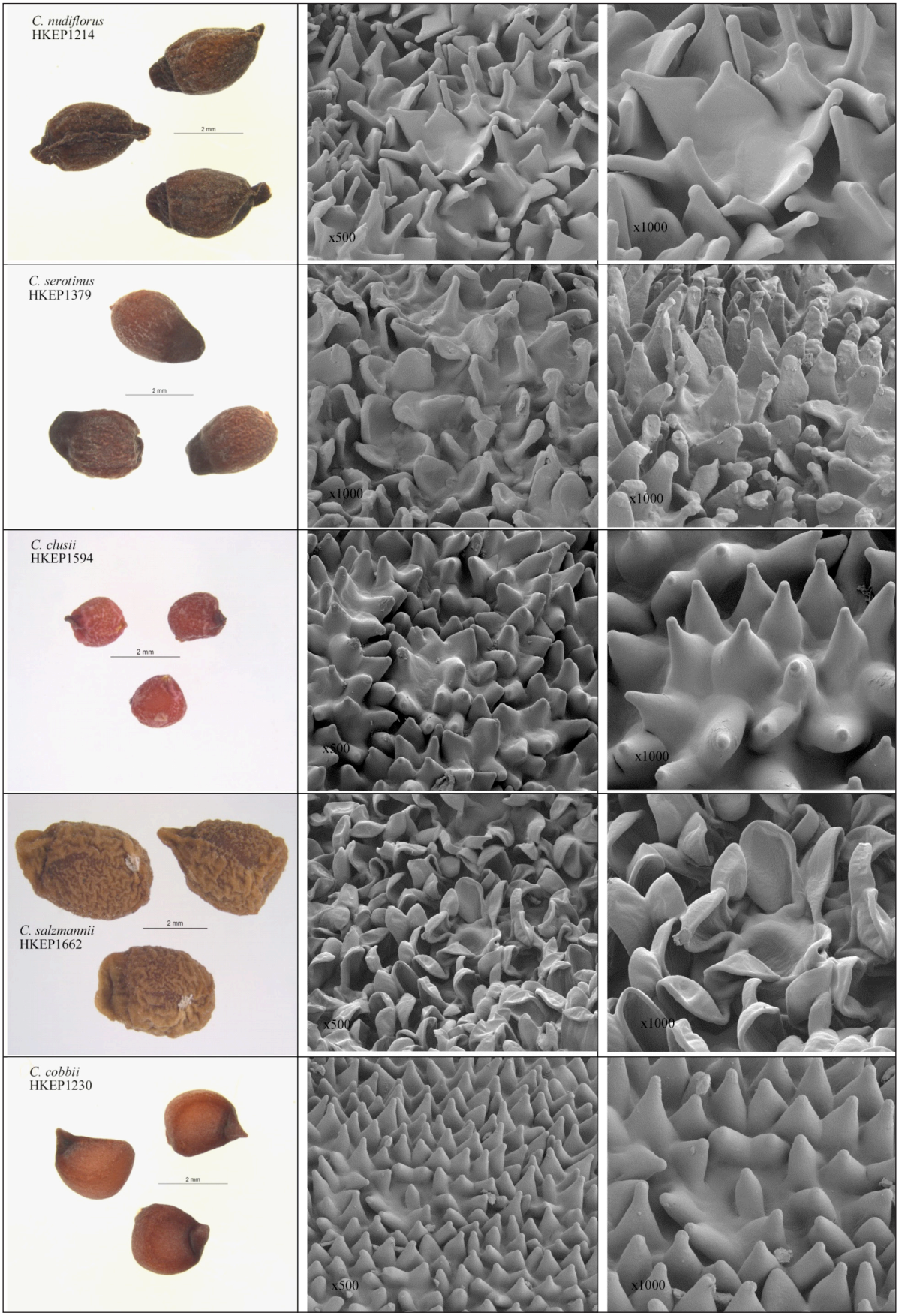


Fig. 9: Macroscopic and microscopic seed properties.

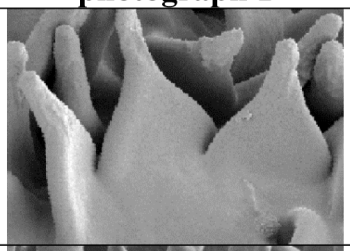
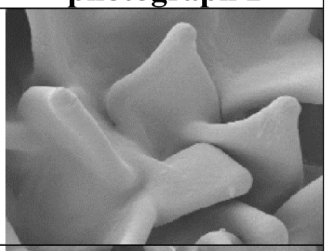
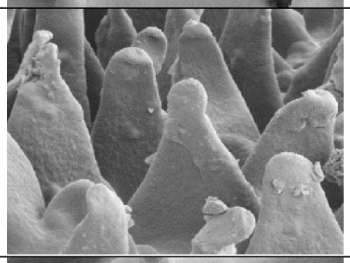
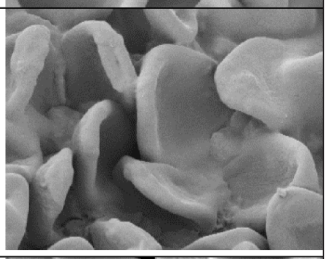
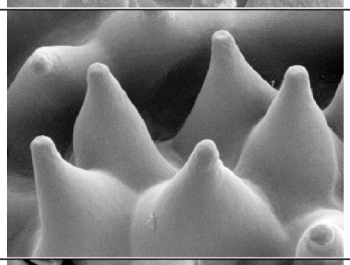
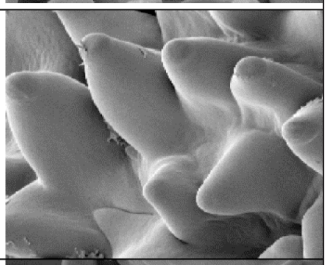
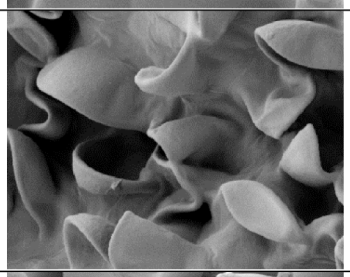
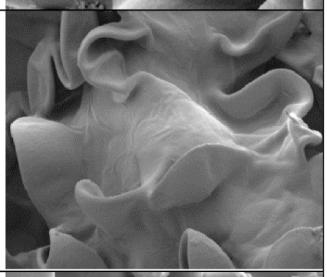
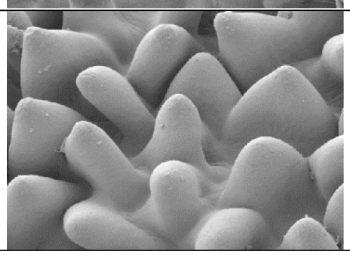
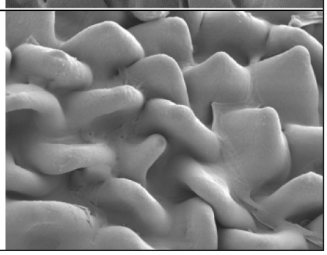
Crocus	description	photograph 1	photograph 2
nudiflorus **HKEP1214**	The sculptures are flat, rather thin with a broad basis, narrowing to the apex pin-like. Apex often thickened like a button (1, 2). The sculptures are connected to one, two or three others (2).		
serotinus **HKEP1379**	Two kinds of sculptures can be found. One similar to *C. nudiflorus* but much narrower triangles rounded at the top (1). The other kind is ear-like and broadly rounded. Sculptures connected to one or two others (2).		
clusii **HKEP1594**	Two kinds of sculptures could be found, both massive not flat (1, 2). One is conical with a wide base strongly narrowing towards the apex (1). The other resembles more thick toes often widened before the button-like apex (2).		
salzmannii **HKEP1662**	The sculptures represent rounded “ears” in perfection hollow hemispherical (1). Ears connected to one or two others, often with some distance in between (2).		
cobbii **HKEP1230**	The seed sculptures of *C. cobbii* can be flat to thick rounded triangles or thick and thumb-like, rarely with a button-like apex (1, 2). They can be single or connected in different ways (2).		

Fig. 10: Description of seed-surface microstructures.

Shape of transversal cross-sections of leaves

Crocus nudiflorus (HKEP1214) has a concave leaf-basis the arm is open with an angle of about +28 degrees and has two ribs on the underneath surface of the arm (fig. 6, fig. 7).

Crocus serotinus (HKEP1379) has a convex leaf-basis the arm is closed with an angle of about -40 degrees (fig. 6, fig. 7).

Crocus clusii (HKEP1594) has a convex leaf-basis the arm is half-open with an angle of about zero degrees (fig. 6, fig. 7).

Crocus salzmannii (HKEP1662) has a convex leaf-basis with a slight central recess. The arm is only a bit closed with an angle of around -12 degrees (fig. 6, fig. 7).

Crocus cobbii (HKEP1230) has a planar leaf-basis and the arm is slightly open with an angle of about +17 degrees (fig. 6, fig. 7).

Macroscopic and microscopic seed parameters

Size, shape, colour, caruncle, and raphe of the five compared seeds are different as can be seen in fig. 9 which is expectable for different species (details in the descriptions).

The micro-surface of the investigated crocus seeds is comparably variable from species to species as is shown in fig. 9 and explained in fig. 10.

Fig. 11: Painting of *C. nudiflorus* of the original description (SOWSBERY & SMITH 1798).

4. Description of species

4.1. *Crocus nudiflorus* SMITH: In: SMITH, Engl. Bot. 7: t. 491 (1798):

Type locality: England, Nottinghamshire, 'between Nottingham Castle and the River Trent'. Type (lectotype, designated here): An illustration of *C. nudiflorus* was provided alongside with its description by SOWERBY in SMITH, Engl. Bot. 7: 491 (1798) (fig. 11)

4.1a *C. nudiflorus* type from Nottinghamshire

Corm depressed globose, 12-15 mm diameter, producing stolons. Outer and inner tunic consistent of parallel fibers, the inner ones connected by membrane. Cataphylls three, membranous, white. Leaves 3-**3.6**-4, hysteranthous, 2.5-3 mm wide with 1(2) ribs underneath of both leaf-arms, green, glabrous, white stripe 1/3 of leaf-diameter. Neck 5-7 mm long sometimes membranous. Flowers are solitary, deep purple, not prominently ve-

Table 6.1: Results for corm and corm tunic of *C. nudiflorus* of different provenance

Crocus	corm	corm tunic
nudiflorus sensu Mathew (1982)	depressed globose, 8-15 mm diameter usually with stolons	papery, interspearsed with strong parallel fibres, the older becoming more or less wholly fibrous
nudiflorus type area Nottinghamshire	sub-globose, 12-15 mm diameter, producing stolons	outer and inner tunic consistent of parallel fibres inner connected by membrane
nudiflorus Pyrenees HKEP1214	sub-globose to globose, 10-15 mm diameter, producing stolons	inner tunics similar but all fibres connected by membrane

ined, throat white or lilac, perianth tube 15-20 cm long, white suffused lilac or purple at the apex. Segments are unequal, elliptic to oblanceolate. Outer ones 44-**51**-60 mm long (n = 16), 12-**16**-20 mm wide. Inner ones 40-**46**-55 mm long (n = 14), 10-**16**-21 mm wide (n = 13). Segment proportion 3.2. Filaments white, 12-**15**-20 mm long, glabrous (n = 36). Anthers yellow to orange 13-**19**-22 mm long (n = 42). Connectives deep yellow. Styles mostly orange, rarely yellow, glabrous. Style-branches of 3-4 hierarchical levels, branches of level one and two upright, upper branches brush-like inclined outwards. The thickness is increasing in all levels with length, ending in a trumpet-like irregularly jagged edge. The total number of branches is around 30-50. Style division position is 53% in area 3, 16% in areas 1, 2, 4 (n = 38). Style-length according to stamen is 62.2% shorter, 29.7 % longer, and 8.1% equal. Capsule and seeds not seen. Chromosome number unknown.

4.1b *C. nudiflorus* by Mathew (1982)

Corm depressed globose, 8-15 mm diameter, usually producing with stolons which are clothed with leaf sheats, and eventually producing separate cormlets; tunics papery, interspersed with strong parallel fibres, the older tunics becoming +/- wholly fibrous. Cataphylls 3-4, membranous. Leaves 3-4, hysteranthous and remaining below ground for a long period after flowering, 2-4 mm wide, green glabrous. Flowers are solitary, deep purple or rarely lilac-purple, not prominently veined, throat white or lilac, glabrous or rarely finely papillose pubescent. Prophyl present. Bract membranous white suffused green at apex; bracteole absent. Perianth tube usually 10-22 cm long, white suffused lilac or purple at the apex; segments 3-6 cm long, 0.9-2 cm wide, equal, elliptic to lanceolate, obtuse. Filaments 0.9-1.2 cm long, white, glabrous or papillose; anthers 1.2-1.9 cm long, yellow. Style much dissected into many slender orange branches. Capsule about 1.3-2 cm long, ellipsoid; seeds brown, c. 3-5 mm long, with a prominent caruncle and inconspicuous raphe. Chromosome number 2n = 48.

4.1c *C. nudiflorus* from Pyrenees (HKEP 1214) new description according to fig. 12 and others; GAT23098, GAT72983

Corm sub-globose to globose, 10-15 mm diameter (fig. 13), producing stolons; outer tunic consistent of parallel fibers, often connected by membrane; inner tunics similar but all fibers connected by membrane; diameter of fibers approximately 0.12-0.23 mm in diameter (fig. 14). *C. nudiflorus* is the only one of the investigated crocuses which has an almost "missing localization" of the Calcium-oxalate crystals in the tunic as they occur obviously in most of the (dead) cells of the tunic (fig. 5). The needle-like crystals strongly create interference colours and are rather long with a length between 0.114 and 0.217 mm and a width of 0.012-0.016 mm. The neck is often rudimentary but can be up to 10 mm. Cataphylls membranous, white or greenish. Leaves 3-**3.7**-4, hysteranthous, 2.5-3.5 mm wide with two ribs underneath of both leaf-arms, dark green, glabrous, white stripe 1/3 to >1/3 of leaf-diameter. The leaf-basis is concave the leaf-arm is open with an angle of about +28 degrees (fig. 6, fig. 7). Throat mostly of the same colour as flowers, if white then only little extended up the segments, glabrous (fig. 12). Perianth tube of different length depending on locality. In mountain pastures around 10 cm in high grass often more than 20 cm; it is white becoming violet towards apex (fig. 12). Segments unequal outer segments 28-**40**-51 mm long, 11-**15**-21 mm wide, inner segments 26-**36**-48 mm long, 10-**14**-18 mm wide, elliptic to obovate. Segment proportion 2.7. The outside and inside colour of all segments is mostly deep purple with very faint markings (fig. 12). Filaments white, glabrous, 7-**10.5**-17.5 mm long, anthers deep yellow to orange 10-**15.2**-22 mm long (n= 45). Connective narrow and deep yellow; pollen yellow. Style orange, yellow, or white. Style-branches of 3-4 hierarchical levels. Branches are in all levels +/- upright and brush-like because the thickness is increasing in all levels with length, ending in a trumpet-like irregularly jagged edge (fig. 4). The total number of branches reaches 30-50. The first style-division in relation to the anther-length occurs mostly in areas one and two (97.7%) compared to 2.2% in areas three, four, and five (tab. 4). Style-length according to stamen is 2.2% shorter, 4.4% equal and 93.3% longer. Capsule 18-25 mm long and 8-10 mm broad, ellipsoid with long attenuate apex. Seeds are ovoid flattened at caruncle, 2.1-2.5 • 1.7-1.9 mm. Testa rough, without structure, very dark brown (Red umber variation), raphe distinct, often winged and broadened at end, caruncle sac-shaped, elaiosome absent, seed end at opposite side of caruncle sac-shaped to peaky (fig. 9). The micro-sculptures are flat, rather thin with a broad basis, narrowing to the apex pin-like. Apex often thickened like a button. The sculptures are connected to one, two or three others (fig. 10). Chromosomes not investigated.

The painting representing the type shows a high agreement with the descriptions for the few parameters recognizable there. These are the parallel fibres of the corm tunic, the stolons,

Table 6.2: Results for cataphylls, bract, and true leaves of *C. nudiflorus* of different provenance

	cataphylls	bract[1]	true leaves							
Crocus	**no. and colour**	**colour**	**range**	**mean**	**median**	**diameter (mm)**	**ribs under-neath**	**white stripe[2]**	**colour**	**hair**
nudiflorus sensu Mathew (1982)	3-4, membranous	membranous, white, suffused green at the apex	3-4, hyst.	n.d.	n.d.	2-4	n.d.	n.d.	green	glab.
nudiflorus from the type area Nottinghamshire	3, membranous, white	menbranous, white, mostly with a split apex	3-4, hyst.	**3.6**	4	2.5-3 (n = 5)	1 (2) on each side	1/3	green	glab.
nudiflorus from the Pyrenees HKEP1214	no. n.d., membranous, white or greenish	membranous, white, with age faintly brown	3-4 hyst. (n = 10)	**3.7**	4	2.5-3.5 (n = 10)	2 on each side	1/3 - >1/3	dark green	glab.

[1]bracteole absent, prophyll present

[2]in dimension of leaf-diameter (approximately 1/3 is recognised as "normal")

n.d. = not determined

Table 6.3: Results for perianth tubes and range of segment sizes of *C. nudiflorus* of different provenance

Crocus	**perianth tube /throat**	**range of segment sizes (mm)**				
	length, colour, hair	**outer length[1]**	**outer width[1]**	**inner length[1]**	**inner width[1]**	**segment proportion[2]**
nudiflorus sensu Mathew (1982)	flowers solitary, deep purple or rarely lilac-purple, not prominently veined, throat white or lilac, glabrous or rarely finely papillose-pubescent; perianth tube 10-22 cm long, white suffused lilac or purple at the apex. Segments equal, elliptic to oblanceolate	30-60	9-20	n.d.	n.d.	n.d.
nudiflorus type area Nottinghamshire	flowers solitary, deep purple, not prominently veined, throat white or lilac, perianth tube 13-20 cm long, white suffused lilac or purple at the apex. Segments unequal, elliptic to oblanceolate.	44-**51**-60 (n = 16)	12-**16**-20 (n = 14)	40-**46**-55 (n = 14)	10-**16**-21 (n = 13)	3.2
nudiflorus HKEP1214 Pyrenees	flowers solitary, deep purple, not prominently veined, throat white or lilac, glabrous or rarely papillose; perianth tube 10-20 cm long, white suffused lilac or purple at the apex; segments unequal, elliptic to oblanceolate	28-**40**-51 (n = 45)	11-**15**-21 (n = 44)	26-**36**-48 (n = 44)	10-**14**-18 (n = 44)	2.7

[1]left side minimum, middle **mean**, right side maximum values (mm)

[2]segment proportion is the average length of outer segments divided by the average width of outer segments; proportions of 2-2.5 create significantly bowl-shaped flowers, values of 2.5-3.5 "normal" flowers, and values of 3.5-5 rather starry star-like flowers.

Table 6.4: Results for stamen and styles of *C. nudiflorus* of different provenance

Crocus	filament length (mm)[1]	anther length (mm)[1]	style-branches	style-division at[2]	style-length according to stamen[3]	colour of filaments	colour of anthers	colour of connec-tives	colour of styles	Papillae on styles
nudiflorus sensu Mathew (1982)	9-12	12-19	much dissected into slender branches	n.d.	n.d.	white, glabrous / papillose	yellow	n.d.	orange	n.d.
nudiflorus type area Nottinghamshire	12-15-20 (n = 36)	13-19-22 (n = 42)	3-4 hierarchical levels, level one and two upright, upper levels brush-like inclined outwards. Thickness increasing in all levels with length, ends trumpet-like with jagged edge. Total number of branches 30-50.	53% area 3 16% area 1, 2, 4 (n = 38)	62.2% shorter, 29.7 % longer, 8.1% equal	white, glabrous	yellow to orange	deep yellow	mostly orange	glabrous
nudiflorus HKEP1214 Pyrenees	7-10.5-17.5 (n = 45)	10-15.2-22 (n = 45)	3-4 hierarchical levels, upright, get thicker in all levels with length, apex trumpet-like, branches 30-50	64% area 1 33% area 2 2.2% area 3 (n = 45)	2.3% shorter 93.3% longer 4.4% equal	white, glabrous	deep yellow to orange	deep yellow	white, yellow, orange	glabrous

[1] average of investigated specimens
[2] position of the first style-division compared to anther-length (table 2)
[3] style-length according to stamen (numbers are % of investigated specimens of a population)

the flower colour and their markings, the bract, the stamen, and the style. However, these few parameters are not sufficient to distinguish *C. nudiflorus* from its related species. An extensive morphological description is required for this.

The purpose of presenting descriptions of three *C. nudiflorus* of different provenances is to show and interpret the morphological and phenotypical differences between them (tab. 6.1, 6.2, 6.3, and 6.4). All along the Pyrenees *C. nudiflorus* populations also have differences. Some of these are due to the fact, that their habitats have different ecological conditions like e.g., high mountain pastures or montane meadows. In the first case flowers are small and perianth tubes short compared to those growing in montane meadows having large flowers and perianth tubes often more than 20 cm long.

For comparison with its relatives, we have chosen a *C. nudiflorus* population from the central Pyrenees (HKEP1214), which is neither alpine nor montane but in between. South and west of the Pyrenees we found other populations showing more differences with yet unsolved reason but corresponding herbarium material was found to be labelled in many cases as *C. nudiflorus* as is also documented in older literature and still many herbaria. To shed more light on this problem we studied comprehensive material of the herbaria in Seville, Jaca, Madrid, and Paris. In many cases, determinations based on Mathew (1982) were found in which the findings were classified either as *C. nudiflorus* or as a subspecies of *C. serotinus* or as a separate species, e.g., *C. serotinus*, *C. salzmannii* or *C. clusii.* Numerous cases we have found to be doubtful. In case of Mathew's description of *C. nudiflorus* the reason for the differences to the description of HKEP1214 might be the inadvertent inclusion of some of those mentioned crocuses that were believed to be *C. nudiflorus*.

In case of the type of *C. nudiflorus* from Nottinghamshire things are more complicate. A classic paper on *C. nudiflorus* in England was originally published by Crump & Sledge, 1950. "This paper first proposed a link between *C. nudiflorus* and the Knights of St. John in the Halifax area. It also suggests, almost in passing, that saffron was used as a textile dye in Britain – and they present historical evidence from Nottinghamshire where *C. nudiflorus* can still be found. I don't really need to remind readers that West Yorkshire and Lancashire were, until recent times, extremely important textile areas - so has too much been made of *C. nudiflorus* as a medicinal plant, and was its most important function as a source of dye for dyeing cloth with?" Cited from a comment by Dave Bishop in the "Friends of Chorlton Meadows" of October, 2009.

The crocuses, for what reason ever, were brought to England several hundred years ago from the Pyrenees probably as a source of saffron cultivated in gardens. For the sake of a good saffron harvest probably large individuals having well developed styles were collected in the Pyrenees and planted in Nottinghamshire and other counties, where they eventually escaped from the culture and overgrown.

The still much larger flowers of the type compared to the plants from the Pyrenees favors this hypothesis. We assume that the genetic and morphological diversity of those is much less than in the population of the Pyrenees where they were once collected. It is probable that only a few certain geno- as well as morphotypes have prevailed because they were better adapted and able to cope with the local conditions (selection). Conside-

Fig. 12: Habitus of *C. nudiflorus*, Vielha, Spain.

C. nudiflorus HKEP1214

C. serotinus HKEP1379

C. clusii HKEP1594

C. salzmannii HKEP1662

C. cobbii HKEP1230

Fig. 13: Corms of Iberian autumnal crocuses.

Fig. 14: Corm tunics of Iberian autumn crocuses. Magnification factor for outer and inner tunics is 35. Fibre measurements made of outer tunics. *Crocus nudiflorus* HKEP1214 is shown in row (a), *C. serotinus* HKEP1379 in row (b), *C. clusii* HKEP1594 in row (d) and *C. cobbii* HKEP1230 in row (e) with the first column showing the outer tunics, the second the inner tunics and the fibres with diamters in the third column

ring that the Pyrenees harbor a much higher genetic diversity and crocuses are often outcrossing species, a crocus grows there in Nottinghamshire (and other counties) today, which is different from the Pyreneen *C. nudiflorus* similar to what is known from *C. sativus* and its wild progenitor *C. cartwrightianus* (NEMATI et al. 2019). To summarize the findings following factors probably play a decisive role:

- Targeted selection when collecting plants at the original location probably on attractiveness for the saffron usage.
- Natural selection at the new locations around Nottingham and other counties adjustment if necessary.
- Effect of the corresponding different growth parameters and location conditions on the morphological characteristics certainly plays a role.

4.2. *Crocus serotinus* SALISB.: Parad. Lond. t. 30 (1806):

Type locality: Portugal, in rocks near the sea

Type (lectotype, designated here): An illustration was published together with the description of the species by SALISBURY, Parad. Lond. t. 30 (1806).

Epitype (in support of the above designated lectotype): Portugal, Sagres, 80m NN, H. Kerndorff, collection ID HKEP1379, 16.11.2013 (GAT 56995!).

The crocus shown in the cited illustration is said to come from rocky coastal areas in the surroundings of Sagres in southern Portugal. Unfortunately, the corm tunic drawn by SOWERSBY in SALISBURY's description is so indistinct that *C. serotinus* cannot be identified with it. Therefore, an epitypification is necessary as this taxon will be the type species of the newly to be defined series *Serotini*. Over the course of several years of searching for it we were able to find this plant in rocky areas near the coast not far from Sagres, means we are able to provide a new and detailed morphological description of *C. serotinus* based on the plants from there (HKEP 1379; GAT 56995!).

New description according to fig. 15 and others

Corm broadly ovoid, somewhat flattened at base, 10-25 mm in diameter (fig. 13), outer and inner tunics fibrous, fibres about 0.126-0.323 mm in diameter and coarsely netted, net-spaces between 2 and 6 mm (fig. 13, fig. 14). The tunic of *C. serotinus* is unique in the genus because the "fibres" are predominantly bands running downwards with the narrower side outwards (!) down to the corm base (fig. 14, top right, right corm). The lower third of the tunic is frequently consistent of those bands which end in a boomerang-like shape pointed to the centre of the basal tunic (fig. 13, fig. 14). The monohydrate Ca-oxalate crystals are short and thin monoclinic "needles", about 0.011-0.013 mm thick and 0.017-0.053 mm long (fig. 6), they occur in a "complete localization" scattered along the surface of the dead vascular bundles and show no interference colours (fig. 5). The neck, if developed, is bristly and up to 10 mm long. The cataphylls are mostly greenish but can be also white in some cases (fig. 15).

Leaves are green, 3-**4.2**-5, 1.5-2 mm in diameter, glabrous to slightly ciliated. Leaf-basis convex, the leaf-arm is closed with an angle of about -40 degrees, no ribs underneath (fig. 7). White stripe is <1/3 to 1/3 of leaf-diameter (fig. 6). Leaves hysterantous but can be developed in rare cases to a small extent. Throat white, rarely with a hint of yellow, no hair. Perianth tube is faintly pinkish-lilac more intense towards the apex. Outer segments between 26 and 44 mm but usually 34 mm long (n = 26), between 8 and 14 mm but usually 10 mm wide (n = 26). Inner segments between 23 and 39 mm but usually 30 mm long, between 6 and 12 mm but usually 10 mm wide (n = 26). The inside and outside of all segments is light to deep pinkish lilac with generally weak or sometimes strongly coloured veins but no speckles or featherings. Prophyll present. Bract greenish-white and rather skinny, bracteole absent. Mathew (1982) mentioned rarely a present but inconspicuous bracteole which could not be observed by us in the investigated specimens. Length of filaments 5-**7.3**-11 mm (n = 26), white to light yellow, no hair. The anthers are deep yellow to orange, rather narrow elongated, slightly peaky at top (fig. 15), 9-**12.5**-18 mm long (n = 26). Connective very narrow, yellow, pollen yellow. Style-branches deep yellow to orange and have 3-4 hierarchical levels. The first and the second level have the longest branches. The entire length of the branches is thin. In less frequent cases they have only a slight thickening at the apex. Branches are not only upright but also bend downwards. The total number of branches is around 30-50 (fig. 17). The position of branching is mainly in area 2 and 3 (tab. 4). Style length according to stamen is in 83.3% longer than stamen (tab. 5). Capsule beige, 12-16 mm long and 7-9 mm broad, ellipsoid, little peaks at apex. Seeds elongated, 1 to 1.5 mm broad, 3-3.5 mm long, very dark brown with a prominent caruncle and an indistinct raphe. Two kinds of micro-sculptures can be found. One is similar of *C. nudiflorus* but with much narrower triangles rounded at the top (fig. 9, fig. 10). The other kind is ear-like and broadly rounded. Sculptures connected to one or two others (2). Chromosome number 2n = 24

Distribution and habitat: At present, based on molecular results, the reliably known localities of *C. serotinus* are confined to open *Pinus pinea* forests, coastal phrygana, and similar scrublands, always on very sandy soils with low humus content (fig. 15) in species-rich biotopes with many genera of bulbous / tuberous species like orchids, *Muscari, Ornithogalum, Iris, Anemone*, and others. It is obviously restricted to south-western parts of the Iberian Atlantic coast on Cenozoic and Mesozoic basins approximately from the Spanish "Parque Nacional de Doñana" to the south-westernmost corner of Europe, the "Cabo de São Vicente" in Portugal and a disjoint locality about 30 km north of this cape in the surroundings of the village Odeceixe. All other in literature given localities for *C. serotinus* are according to our present knowledge doubtful. It should not go unmentioned that the once continuous and extensive *Pinus pinea* forest belt of the South Iberian Atlantic coast has been largely destroyed by mass tourism, except of the Spanish "Parque Nacional de Doñana" which is strictly protected and can only be entered with a guide.

4.3. *Crocus clusii* GAY: In Féruss. Bull. Sc. Nat. XXV, 320

Type locality: Portugal 'Habitat circa Olyssiponem' (Lisbon), Prof. Link (specimen at Kew). Our investigated material (HKEP 1594) comes from the type-area west of Lisbon in the Serra de Sintra. (GAT56916)

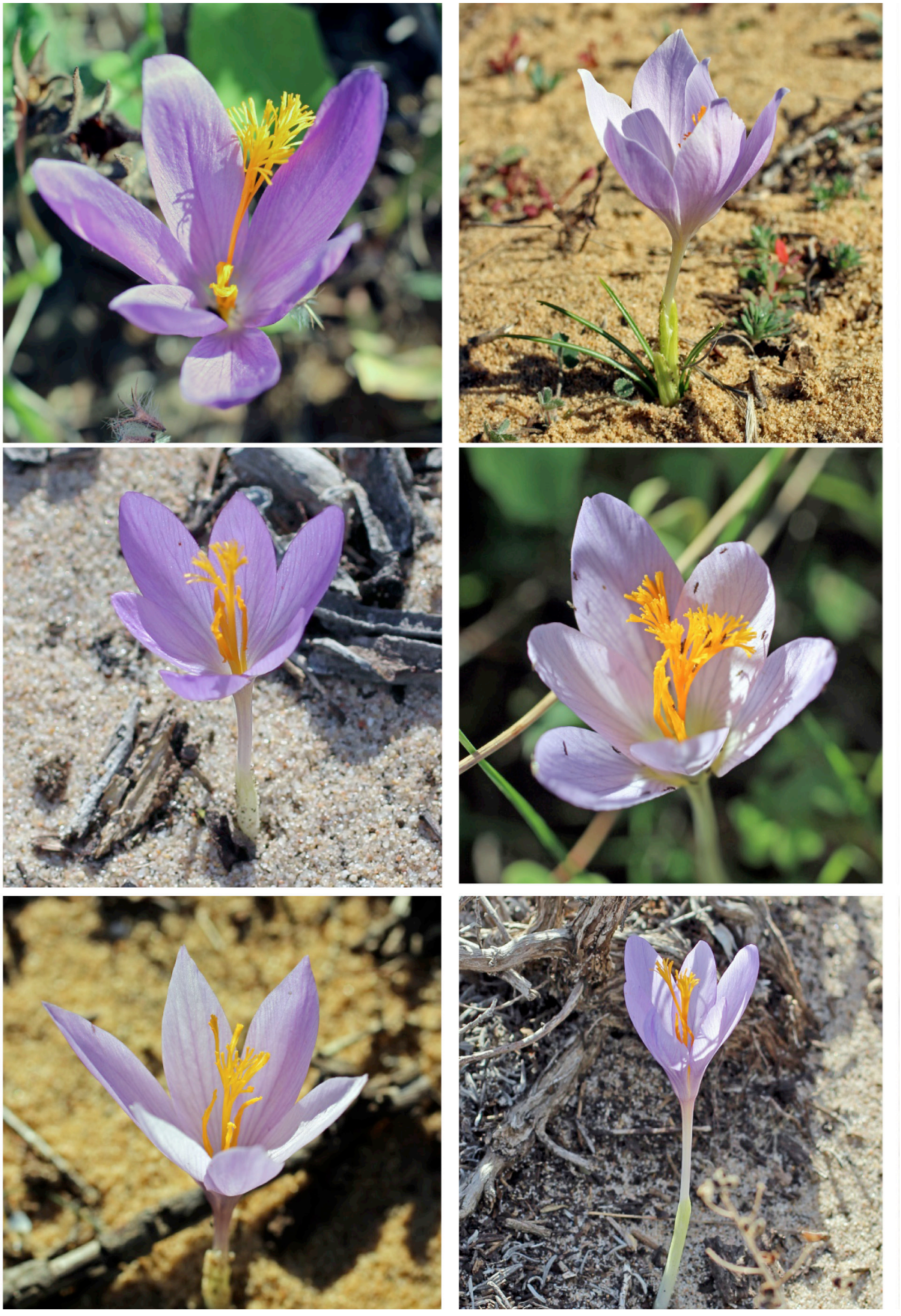


Fig. 15: Habitus of *C. serotinus*, Portugal, Sagres.

Fig. 16: Habitus of *C. clusii*, Portugal, Serra de Sintra (type area).

New description according to fig. 16 and others

Corm sub-globose somewhat flattened at base, 15-18 mm in diameter (fig. 13), outer and inner tunics fibrous, fibers are thin threads about 0.075-0.109 mm in diameter (fig. 13, fig. 14). The fibers of the inner tunics are still connected by membranes (fig. 14 middle). Fibers mostly parallel but towards apex slightly anastomosing, net-spaces around 0.4-2.3 mm long narrow and elongated. The rather frequent Ca-oxalate monohydrate crystals are monocline needles (about 0.018 mm thick and 0.141 mm long and located at the outside of the vascular bundles with the same orientation (fig. 5). The neck is bristly and 5-10 mm long. The cataphylls are greenish (fig. 16). Leaf-number 3-**4.8**-7, green 1-2 mm in diameter glabrous or rarely with scattered tiny hair. Leaf-basis convex leaf-arm half-open with an angle of about zero degrees (fig. 6, fig. 7). White stripe <1/3 to 1/3 of leaf-diameter slightly broader at base, no ribs underneath (fig. 6). Leaves synanthous shorter or of same length as flowers. Throat white, little extended up the segments, bearded. Colour of perianth tube deep bluish violet often extended up to the top of the outer segments. Outer segments between 24 and 44 mm but usually 33 mm long (n = 27), between 6 and 15 mm but usually 11 mm wide (n = 27). Inner segments between 22 and 39 mm but usually 30 mm long, between 6 and 15 mm but usually 11 mm wide (n = 27). The inside and outside of all segments is light to deep bluish lilac with generally weak coloured veins (fig. 16). The outside of the outer segments can have deeper coloured stripes or slight featherings. Prophyll present, bract greenish and rather skinny, bracteole absent.

Length of filaments 5.5-7-10 mm (n = 29), yellow, near anthers darker or brownish, no hair. The anthers are deep yellow to orange (orange red), arrow-shaped mostly rounded at top (fig. 16), 8.5-11.5-16 mm long (n = 29). Connective of the same colour as anthers; pollen yellow. Style orange to orange-red, style-branches divided into 2 (rarely 3) hierarchical levels. Branches are in both levels +/- upright, filiform or thickening upwards. Branches of the first division are significantly longer than of level two which are about half as long. Branches of level three (if present) are short. The apex is either of the same thickness or a little thicker. The total number of branches is about 10-25 (fig. 16).

Style length according to stamen is in 96% longer to equal, in 4% shorter (n = 22). Capsule not seen. Seeds small, subglobose around 1.5 mm in diameter, "English red with flesh tint" (pinkish brown), surface rough to smooth without structure, raphe distinct, caruncle sac-shaped, elaiosome absent, shape of seed end at opposite side of caruncle rounded or peaky (fig. 9). Two kinds of micro-sculptures could be found on the surface, both voluminous not flat (fig. 10). One is conical with a wide base strongly narrowing towards the apex. The other resembles thicker toes often widened before the button-like apex. (fig. 10). Chromosome number 2n = 22.

Distribution and habitat: *Crocus clusii* was originally thought to be widely distributed on the western Iberian Peninsula, from around Lisbon to most of northern Portugal and at a very disjoint locality near Cadiz (Andalusia, Spain). Up to now we only can confirm its distribution near the type-locality in the Serra de Sintra west of Lisbon where it is presently rare because almost all its former natural habitats in this mountain ridge (native cork-oak forests) were destroyed by eucalyptus plantations. Some small relict populations in this area are in severe threatening of extinction. In contrary to the former knowledge the habitats are not on limestone formations but solely between Precambriam-Paleozoic igneous rocks (granite) in pockets of black humus-rich soil.

4.4. *Crocus salzmannii* GAY: In: Féruss. Bull. Sc. Nat. XXV, 320

Type locality: Morocco, Tangier, 'Habitat circa Tingidem', Salzmann (specimen at Kew). Investigated plants originate from the type-area close to Tangier (Morocco), HKEP 1662, GAT56897.

New description according to fig. 17 and others

Corm ovoid flattened at base (fig. 13), 22-26 mm in diameter, outer and inner tunics banded, the inner ones partly connected by membranes (fig. 13, fig. 16). Bands are between 0.09 and 0.2 mm wide throughout parallel, very rarely anastomosing towards apex. Calcium-oxalate monohydrate crystals frequent, thin and comparatively long needles, 0.01-0.012 mm wide and 0.058-0.109 mm long (fig. 5), located at the outside of the vascular bundles with the long side in the same orientation (fig. 5). The neck is about 10 mm long and consists of bands partly connected with membrane (fig. 13). The cataphylls are silvery-white to greenish (fig. 17), edges becoming brownish with age. Leaf-number 4-**7**-8 (n = 16), dark green, linear to slightly subulate, 2-3 mm in diameter, glabrous, white stripe mostly narrow <1/3 up to 1/3 at base, no ribs underneath (fig. 6). Leaf-basis is convex with a slight dent in the middle. The arm is only a bit closed with an angle of around -12 degrees. Leaves hysteranthous, rarely only tips visible at flowering time. Throat is yellowish or deep yellow and bearded. Perianth tube is whitish but becoming brownish or bluish violet at the apex which is often extended somewhat up the segments. Outer segments between 29 and 42 mm but usually 35 mm long (n = 19), between 8 and 16 mm but usually 12 mm wide (n = 19). Inner segments between 27 and 40 mm but usually 32 mm long, between 7 and 14 mm but usually 11 mm wide (n = 19). The inside and outside of all segments is generally light rosy lilac with deeper coloured veins (fig. 17). The outside of the outer segments also can have deeper coloured speckles but never featherings. Prophyll present. Bract bright light green veined darker, bracteole absent.

Length of filaments 5.5-**7.1**-9.5 mm (n = 29), yellow to orange, no hair. The anthers are deep yellow to orange, broadly linear, ending at two rounded lobes at top (fig. 17), 11-14.3-18 mm long (n = 29). Connective of the same colour as anthers, pollen yellow. Style orange to orange red with 3-4 hierarchy levels. Branches are in all levels +/- upright. The first level has the longest branches, approximately two times longer than branches of level two and three. Level four is not always present, if so, branches are short. The thickness of all branches is +/- equal even to the apex. Number of all branches is around 15-20. Style length according to stamen is in 79% longer to equal, in 21% shorter (n = 19). Capsule not seen. Seeds large, ovoid often flattened at base, 2.4-3.4 times 2.0-2.5 mm in diameter, surface coarsely undulate, beige-brown ("Mars Brown variation"), raphe indistinct, caruncle indistinctly sac-like, elaiosome absent, shape of seed end at opposite side of caruncle rounded (fig. 9).

The micro-sculptures represent rounded "ears" in perfection

Fig. 17: Habitus of *C. salzmannii*, Morocco, near Tangier (type area).

Fig. 18: Habitus of *C. cobbii*, Portugal, Montalegre (type).

hollow hemispherical. Ears connected to one or two others, often with some distance in between (fig. 10). Chromosome number 2n = 44.

Distribution and habitat: The distribution of *Crocus salzmannii* is given in the literature from northwest Morocco to Galicia and from some central and southern Spanish mountains. However, according to our molecular results it seems by far not so widely distributed. Therefore, we only included plants from the type-region near Tangier (Morocco) in the current investigation. The crocus grows not far from the city of Tangier in coastal macchie in heavy clay-rich soil above Cretaceous-Miocene units belonging to the Maghrebian flysh basin.

4.5. *Crocus cobbii* KERNDORFF, PASCHE & HARPKE sp. nov.

Type locality: Portugal, Trás-os-Montes e Alto Douro, Montalegre (HKEP 1230, specimen at Gatersleben, GAT56968!).

Description according to fig. 18 and others

Corm depressed globose (fig. 13), 15-20 mm in diameter, outer and inner tunics fibrous, the inner ones partly connected by membranes (figs. s and y). Fibers are +/- round in cross-section, between 0.055 and 0.128 mm wide mostly parallel, very rarely connected. Calcium- oxalate crystals few, rather haphazardly distributed, occurring in few places of the tunic while others are totally without crystals. Orientation and localization like of all the other investigated crocuses scattered along the vascular bundles. Interference colours are present but poorly developed. The dimension of the crystals is about 0.09 times 0.014 mm. (fig. 5), located at the outside of the vascular bundles with the long side in the same orientation (fig. 5). The fibrous neck is about 5-10 mm long (fig. 13). The cataphylls are silvery-white to greenish (fig. 18). Leaves hysteranthous, leaf-number 3-3.6-6 (n = 23), green, linear, 2-3 mm in diameter, glabrous, white stripe mostly narrow 1/4 up to 1/3 at base, no ribs underneath (fig.6); leaf-basis planar and leaf-arm slightly open with an angle of about +17 degrees (fig. 6).

Throat is yellowish and bearded. Perianth tube is whitish or brownish becoming more intense at the apex. Outer segments between 21 and 47 mm but usually 37 mm long (n = 19), between 6 and 16 mm but usually 11 mm wide (n = 19). Inner segments between 21 and 43 mm but usually 33 mm long, between 7 and 17 mm but usually 11 mm wide (n = 19). The inside and outside of all segments is mostly deep lilac-purple predominately with deeper coloured veins especially in the throat (fig. 18). Prophyll present. Bract bright green with dark green veins, bracteole absent.

Length of filaments 6-8.1-12 mm (n = 29), yellow, no hair. The anthers are deep yellow to orange, broadly linear, flattened at apex and notched in the middle (fig. 6), 9-13.3-20 mm long (n = 29). Connective is deep yellow to orange, pollen yellow. Style deep yellow to orange. Style-branches have 3-4 hierarchy levels with the tendency to spread out in level three and four. The first level has the longest branches, 2-3 times as long as level two and three. Level four is rare branches are short. Thickness of all branches is +/- equal or becoming slightly thicker towards the apex. Number of all branches is around 20-40. Style length according to stamen is in 85.3% longer, in 4.4% shorter, and in 10.3% equal (n = 68). Capsule beige-brown, stretched ovoid, 10-15 mm long and 6-9 mm broad, chambers of opened capsule peaky and often with persistent bracts, a phenomenon which was not recognized before. Seeds rather small, irregular shaped (chestnut-like), 1.7-2.1 times 1.5-1.8 mm in diameter, surface rough without structure, chestnut-brown, raphe indistinct, caruncle conically sac-like, elaiosome absent, shape of seed end at opposite side of caruncle rounded (fig. 9). The micro-sculptures of the seed can be flat to thick rounded triangles or thick and thumb-like, rarely with a button-like apex. They can be single or connected in different ways (fig. 10). Chromosome number unkown.

Distribution and habitat: *Crocus cobbi* is presently only known from two localities, the type-locality near Montalegre, Trás-os-Montes, Portugal, and from near Seoane, Castilla y Leon, Spain. The crocus grows on humus-rich blakkish or dark brown soils above Precambriam-Paleozoic igneous rocks (granite and granite-like), often in meadows and light deciduous forests and clearings.

5 CONCLUDING REMARKS

Crocus cobbii is a new species for science. It differs genetically and morphologically from its related species. In contrast to being clearly separated by molecular markers, characterizing the morphological differences required thorough investigation of the crocuses with the inclusion of new parameter. Particularly useful for the examined crocuses are the type of style- division, the position of the first style-division by length of the stamens, size, shape, and location of the calcium oxalate crystals in the corm tunic, the shape of the cross-section of the leaves and all seed parameters. With the results, a more detailed description of the morphology for all five closely related species from their type regions is performed. All five investigated species were placed in series *Longiflori* (MATHEW 1982). However, this series does not represent a legitimate taxonomic unit since its type species was included into series *Verni* (HARPKE et al. 2015). The detailed circumscription and molecular investigation of *C. serotinus* and its allies are an important first step towards a newly defining their affiliation within the genus. Further comprehensive morphological, karyological and molecular analyses are already under way.

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