# *Crocus adamii* Gay (Liliiflorae, Iridaceae) and some of its relatives in Iran

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**Abstract:** *Crocus adamii* Gay and its relatives belong to one of the largest and complex groups of associated species in section *Nudiscapus* in the genus *Crocus*. The group is known to have members in the Anatolian Diagonal (Turkey) and in the Caucasus. New findings support the suggestion that it is also widely distributed in Iran. Our findings shed some light on this situation and revealed new species which are described here.

**Zusammenfassung:** *Crocus adamii* Gay und seine Verwandten gehören zu einer der größten und kompliziertesten Gruppen zusammengehörender Arten in Sektion *Nudiscapus* in der Gattung *Crocus*. Neuere Ergebnisse unterstützen die Vermutung, dass die „adamii-Gruppe" auch im Iran weit verbreitet ist. Unsere Ergebnisse bringen einige neue Erkenntnisse in diesem Zusammenhang, inklusive neuer Arten, die hier beschrieben werden.

**Key words:** *Crocus adamii* species group, Iran.

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## INTRODUCTION

Results of molecular analyses in the genus *Crocus* show a rather complex situation in the relationship of species belonging to *Crocus adamii* Gay. The distribution of this species-group has a large extension all along the Turkish Anatolian Diagonal, from there north-eastwards into the Caucasus range, eastwards into Armenia and Azerbaijan and south-eastwards into Iran where they can be found southwards along the Zagros mountains at least down to Sanandaj and eastwards along different mountain stocks belonging to the Elburz mountains as far as Almeh. The entire group turned out to be one of the genetically most distinct and largest in the genus and will be defined later as series *Adami*.

## PLANT MATERIAL

For molecular analysis we investigated two to five individuals per population. For morphological analyses a minimum set of characters was measured or determined in the field of 24 to 49 individuals (Tab. 1). In some cases for different parameters also dried material from type locality was used. Voucher specimens of all analyzed individuals were deposited at the herbaria of the IPK Gatersleben (GAT). The distribution of the investigated material is shown in Fig. 1. The map was generated using a 1 arc-minute global relief model of Earth's surface integrating land topography (ETOPO1; Amante and Eakins 2009), and the raster package (Hijmans and van Etten 2012) in R v.3.2 (R Develop-

**Table 1:** Investigated Iranian *Crocus* populations belonging to the "adamii-group".

| Population identity | Taxon | Investigated specimens | Geographical distribution in Iran |
|---|---|---|---|
| HKEP1638 | *C.* cf. *reinhardii* | 49 | Semnan, north of Damghan, 2090 m |
| HKEP1555 | *C.* cf. *reinhardii* | 41 | Zanjan, pass east northeast of Zanjan, 2100 m |
| HKEP1636 | *C. iranicus* type | 33 | Kordestan, near Saqqez, 1560 m |
| HKEP1548 | *C. sanandajensis* sp. nova | 35 | Kordestan, east of Sanandaj, 2100 m |
| HKEP1637 | *C. zagrosensis* sp. nova | 44 | Kordestan, Divandarreh, 2070 m |
| HKEP1628 | *C. zanjanensis* sp. nova | 38 | Zanjan, Qedar, 2160 m |
| HKEP1629 | *C.* cf. hybrid | 44 | Azarbayjan-E-Sharoi, S of Ahar, 1850 m |
| HKEP1630 | undefined, no molecular analysis possible | 24 | Azarbayjan-E-Sharoi, S of Varzaghan, 1850 m |
| HKEP1631 | *C.* cf. *roopiae* | 41 | Azarbayjan-E-Gharel, Orumiyeh to Silvana, 1470 m |

MENT CORE TEAM 2008). Important morphological (continuous parameters) values, results of statistical calculations, as well as phenotypical characters of the investigated populations are compiled for differentiation and comparison in Tab. 2.

## MOLECULAR METHODS AND RESULTS

To infer the affiliation of the Iranian crocuses also other taxa of the *C. adamii* group were included. Five other species of section *Nudiscapus* and five species of section *Crocus* were molecularly investigated as outgroups. For each of the internal transcribed spacer region (ITS) the nuclear ribosomal DNA (rDNA) was investigated. DNA extraction, PCR and sequencing were done according to HARPKE et al. (2014). All sequences newly obtained in this project were deposited in the EMBL nucleotide database under accession numbers (for all others see HARPKE et al. 2013; 2016).

### Sequence alignments and phylogenetic analyses

Sequences for all loci were manually aligned. If sequences were identical within the same population, only one sequence per population was included in the phylogenetic analysis. Bayesian inference (BI) was conducted in MrBayes 3.2 (RONQUIST et al. 2012) using the GTR + Γ + I model. In BI two times four chains were run for two million generations each, sampling a tree every 100 generations. Converging log-likelihoods, potential scale reduction factors for each parameter and inspection of

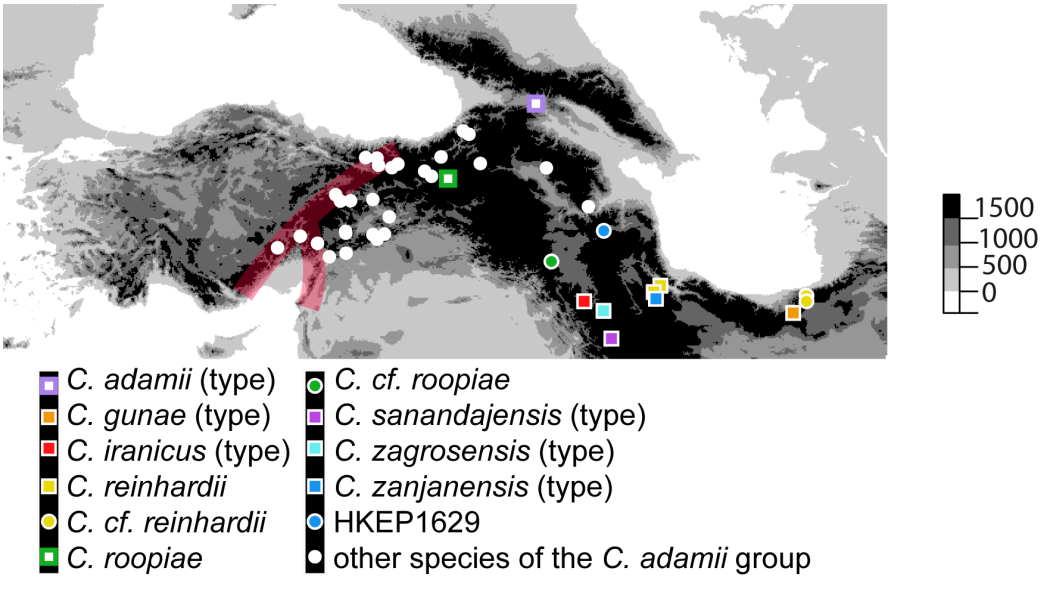


**Fig. 1**: Map of geographical distribution of the investigated population of *C. adamii* group. Elevation levels are indicated in different shades of grey. The Anatolian Diagonal is indicated in red.

**Table 2:** Continuous parameters and phenotypic characteristics of some Iranian *Crocus* populations belonging to the "*C. adamii*-Komplex"

| Parameter group | annulate corm tunic | | | | | | | colour of sheathing leaves | segm prop. | true leaves | | | | | | stamen and styles | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| population identity | outer | inner | splits (mm) | sub-splits | neck-length (mm) | teeth on rings (length, mm) | cata-phylls | bract/ bracteoles | | leaf-no. mean[3] | col leaves | white stripe[1] | leaf-diam. (mm) | leaf-ribs no.[2] | length fila (mm)[3] | length anth (mm)[3] | length style-bran. (mm)[3] | col fila | col anth | col conn | col style | style acc. stam. |
| HKEP1638 | c | c | 2-5 | ab | 5-7 | ab to ma <<0.5 | silv tbr | silv | 3.0 | 3-5.4-7 | g gr | 1/5-1/4 | 2-3 | (2)3(5) | 4-5.1-7 | 8.5-11.7-14 | 5.9 | dy | dy | dy | or | 80el |
| HKEP1555 | c | so | none or >5 | ab | 5-10 | f-dist <<0.5 | silv, ty-tgr | silv | 2.7 | 3-5.5-7 | g gr | 1/3 to >1/3 | 1.5-2 | (1)2(3) | 4-5.5-7 | 7-10.4-13 | 5.2 | dy-or | dy | dy | or | 100el |
| HKEP1636 | c | c | none or >5 | ab | 10-13 | ab to ma dist <1 | silv | silv | - | 3-4.5-7 | g gr | <1/3-1/3 | 1.5-2.5 | (3)4(5) | - | - | - | - | - | - | - | - |
| HKEP1548 | c | so | >5 | ab | 8-10 | ab to f <<0.5 | silv, tbr | silv | 2.7 | 3-4-7 | dgr | <1/3-1/3 | 2-4 | (3)4(5) | 4-5.9-7 | 9-11.5-17 | 6.6 | y-dy | dy | c | or | 91se |
| HKEP1637 | c | so | >5 | ab | 9-13 | ab | silv | silv, tbr | 2.5 | 3-4-6 | g | 1/5-1/3 | 1.5-2 | (4)5 | 4-5.3-6.5 | 7.5-11-15.3 | 6.2 | dy | dy | c-ly | or | 86el |
| HKEP1628 | c/m | c/m | 2-5 | pr (r) | 8-10 | ab | silv,ty/br | silv, long | 2.8 | 2-3.7-5 | g | 1/5-1/3 | 1.5-3 | (2)3(5) | 4-5.9-9.5 | 10-12.5-17 | 6.0 | c-ly | dy | c-ly | dy | 97le |
| HKEP1629 | c | so | 2-5 | r | 6-10 | ab | silv ty | silv | 3.0 | - | - | - | - | - | 2.5-4.2-6 | 7-10.8-13 | 5.2 | dy | dy | c-ly | or | 91el |
| HKEP1630 | c | so | 2-5 | pr (occ) | 5-8 | ab | silv | silv | 3.2 | - | - | - | - | - | 3-4-5 | 8.9-10.8-13.5 | 6.7 | dy | dy | c-ly | dy-or | 78el |
| HKEP1631 | c/m | c/m | >5 | ab | 5-10 | ab | silv/bry | silv | 2.6 | - | - | - | - | - | 5.5-7.4-9 | 8-11.7-14 | 6.0 | c-ly | dy | ly-dy | dy-or | 90le |

Explanatory notes:
corm tunic: c = coriaceous, m = membranous, so = softer, ab = absent, pr =present, r = rare, ma = many, f = few, dist = distant, up = on upper rings; <smaller than, <<much smaller than;
color of sheathing leaves at flowering time: silv = silvery (skinny); br = brownish, tips yellow = ty, tips greenish = tgr, tips brownish = tbr
segmprop.: segment proportion (= average length of outer segments/average width of outer segments) as a rule: 2-2.5 bowl-shaped flowers, 2.5-3.5 "normal" flowers, 3.5-5 rather starry star-like flowers;
true leaves: col = colour, gr = green, g = grey, d = dark, l = light, (sub) = subulate;
stamen and styles: fila = filaments, anth = anthers, conn = connectives, col = colour; colours of filaments: c = colourless, y = yellow, or = orange, d = dark, l = light; colour of anthers: y = yellow, b = black, g = grey; colours of connectives: c = colourless, ly = light yellow; colours of styles: y-or = yellow to orange, or = orange, orr = orange-red;
style acc. stam = length of style according to stamen (numbers are % of investigated specimens of a population, le = longer to equal, se = shorter to equal.

[1]in dimension of the leaf-diameter
[2]on both sides of the groves
[3]average of investigated specimens

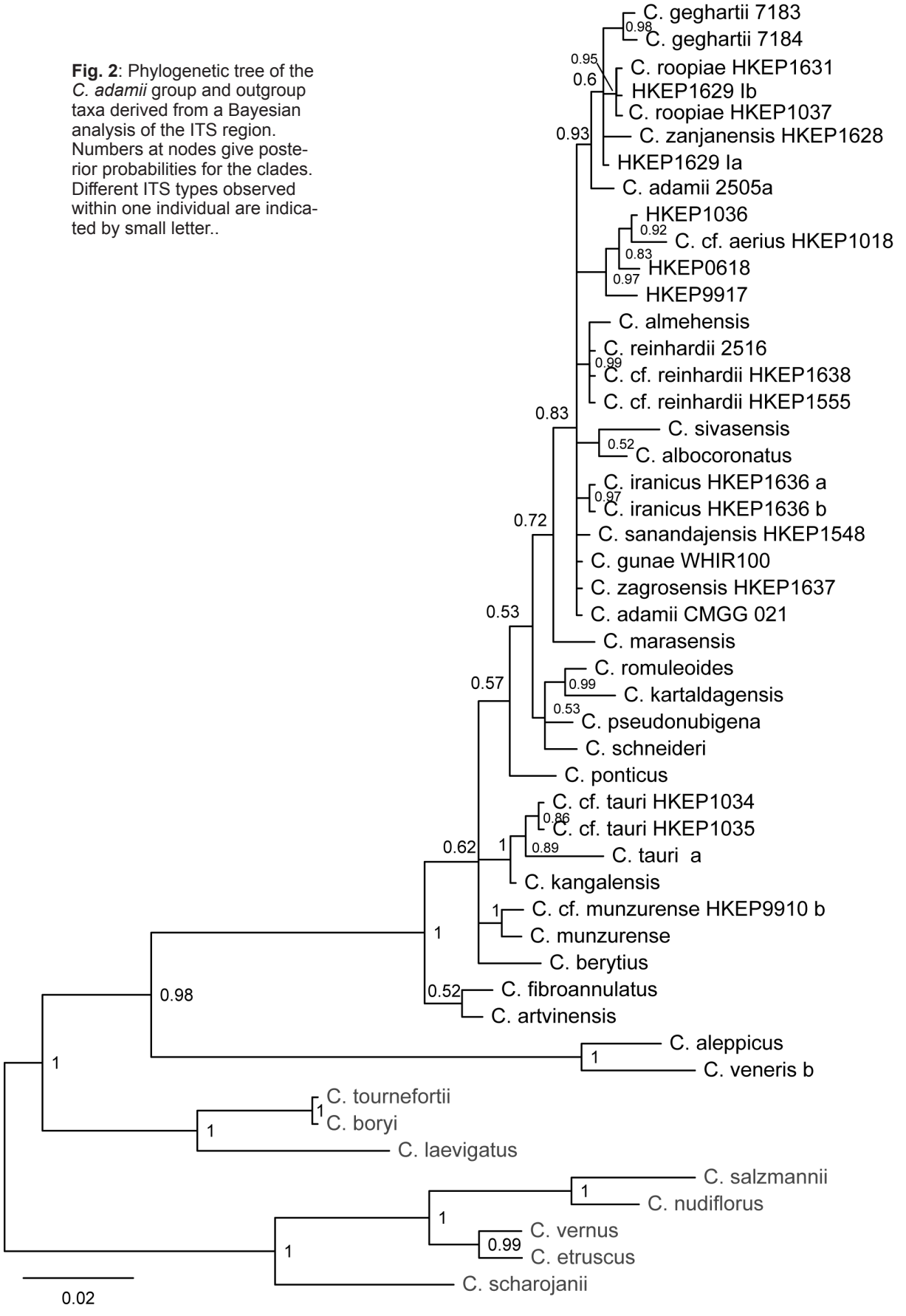


**Fig. 2**: Phylogenetic tree of the *C. adamii* group and outgroup taxa derived from a Bayesian analysis of the ITS region. Numbers at nodes give posterior probabilities for the clades. Different ITS types observed within one individual are indicated by small letter..

tabulated model parameters in MrBayes suggested that stationarity had been reached in all analyses. The first 25% of trees of each run were discarded as burn-in. Three independent runs of BI analysis were performed to confirm that separate analyses converged on the same result. In each of these analyses, the same topology and similar posterior probabilities (pp) of nodal support were found.

## PHYLOGENY

Although the evolutionary linage of the "*Crocus adamii* group" is one of the oldest in section *Nudiscapus* (HARPKE et al., 2013), most species originated within the last few Mya. The Iranian crocuses cluster together with *C. adamii* (Georgia), *C. alboconoratus* (KERND.) KERND. ET AL. (Anatolian Diagonal, Turkey), *C. geghartii* SOSN. (Armenia), *C. roopiae* WORONOW (Palandöken mountains, eastern Turkey). It is probably a recently evolved group with rapid radiation, which is reflected (*i*) by the low degree of differences in the used phylogenetic marker (nrITS region), and (*ii*) the comparatively large distribution area.

As a result of low genetic variability branch supports and resolution are low in the phylogenetic tree, too (Fig. 2). However, although the molecular data do not reflect the clear morphological differentiation it also supports that some of the investigated Iranian populations clearly represent new species.

In *Crocus* genetic distances correlate often with geographical distances. It is therefore surprising to find *C. gunae* RUKŠĀNS to be molecular identical to *C. zagrosensis*, growing 650 km away from each other. Also, *C. reinhardii* (north-east of Zanjan), identical to *C. cf. reinhardii* (population HKEP1555, some 10 km apart from the location north-east of Zanjan) is geographically far apart (450 km) from the molecular also identical population HKEP1638 (north of Damghan). In both cases not only distance, but also different mountain stocks are separating these populations. Here more sampling and additional markers and the application of population genetic methods can provide a better understanding of this finding.

Another point which yet needs to be investigated more intensely concerns the population HKEP1629. The presence of two different ITS types (HKEP1629a and b in Fig. 2) indicates that it might represent a hybrid probably involving *C. zanjanensis*. To evaluate the status of the HKEP1629 further and extended molecular and karyological analyses are necessary. We, therefore, refrain from describing it as a new species at present.

## DESCRIPTION OF NEW SPECIES

### 1. *Crocus sanandajensis* KERNDORFF & PASCHE, species nova

Holotypus: Iran, Province Kordestan, Zagros mountains, east of Sanandaj, 2100 m, 13.4.2015, HKEP 1548 (GAT, Gatersleben).

Corm subglobose, flattened at base, 15-20 mm in diameter; outer tunics coriaceous, inner tunics somewhat softer, neck conspicuous, 8-10 mm long, bristly; tunics split into broad segments of >5mm, sub-splits absent; rings well developed, predominantly whole-edged or slightly saw-toothed, very rarely with tiny teeth of <<0,5 mm. Cataphylls 3-4, white at flowering time but often with brownish tips. Leaves 3-**4**-7 (n = 31), slightly lanceolate, dark green, 2-4 mm in diameter at broadest position, glabrous, white stripe small, 1/5 to <1/3 of leaf-diameter, 3-4(5) ribs underneath of each side of the blade. Leaves often reach the flowers at anthesis. Throat is deep yellow without hair. Perianth tube white or light blue, near the apex striped or spotted brownish, bluish or violet. Outer segments between 25 and 35 mm but usually 30.2 mm long (n = 35), between 7.5 and 14.7 mm but usually 11.2 mm wide (n=35). Inner segments between 22.8 and 34.6 mm but usually 28.7 mm long, between 7 and 14 mm but usually 10 mm wide (n = 35). Inside all segments are evenly white with the striping of the outside sometimes shining through on outside. Outside of outer segments finely striped or feathered bluish or slightly violet, more intense at base. Outside of inner segments plain whitish-blue, towards perianth tube more or less brownish or bluish-violet spotted. Prophyll absent. Bract and bracteole present, silvery, not conspicuous. Length of filaments 4-**5.9**-7 mm (n = 35), yellow to dark-yellow, no hairs. Anthers 9-**11.5**-17 mm long (n = 35), always yellow. Connective colourless or yellowish. Pollen yellow. Style divided into 3 branches stiffly upright and closely hold together, broadly trumpet-shaped at apex mostly orange sometimes deep yellow, branches 4-**6.6**-12 mm long (n = 35). Style length according to stamen is 91% shorter to equal, and 9% longer (n = 35). Capsule and seed not seen. Chromosome number unknown.

Remarkable of *C. sanandajensis* are the more or less uniformly white and large flowers with long segments of around 3 cm on average, relatively broad lanceolate leaves with a very narrow white stripe, many ribs in both grooves of the leaves underneath and long anthers which can reach up to 17 mm. The outside of the outer segments has a fine bluish feathering or faint striping but is never speckled (see also parameter compilation in Tab. 2). *Crocus sanandajensis* is a close relative to *Crocus reinhardii* (HKEP 1555, Fritsch 2516) and *C. almehensis*.

Distribution and habitat: *C. sanandajensis* is until now only known from the type locality east of Sanandaj, Province Kordestan, Iran. The plant grows in rocky and grassy areas without trees, together with *Astragalus, Hypericum, Anemone, Iris, Fritillaria, Alysssum, Gagea, Muscari, Scilla, Allium*.

### 2. *Crocus zanjanensis* KERNDORFF & PASCHE, species nova

Holotypus: Iran, Province Zanjan, Qedar, 2160 m, 7.3.2016, HKEP 1628 (GAT, Gatersleben).

Corm subglobose, somewhat flattened at base, 13-20 mm in diameter, outer and inner tunics coriaceous to membranous, neck conspicuous, bristly, 8-10 mm long; tunics split into segments of 2-5 mm, sub-splits rarely present; rings well developed but mostly rather narrow, all smooth-edged. Cataphylls 3-4, silvery at flowering time often with yellowish or brownish tips. Leaves 2-**3.7**-5 (n = 21), green, poorly developed at anthesis mostly much shorter than flowers 1.5-3 mm in diameter, glabrous, apex obtuse, white stripe small 1/5 to maximal 1/3 of leaf-diameter; variable no. of ribs underneath (2)3(5) on both sides of the keel. Throat without hair, yellow. Perianth tube white

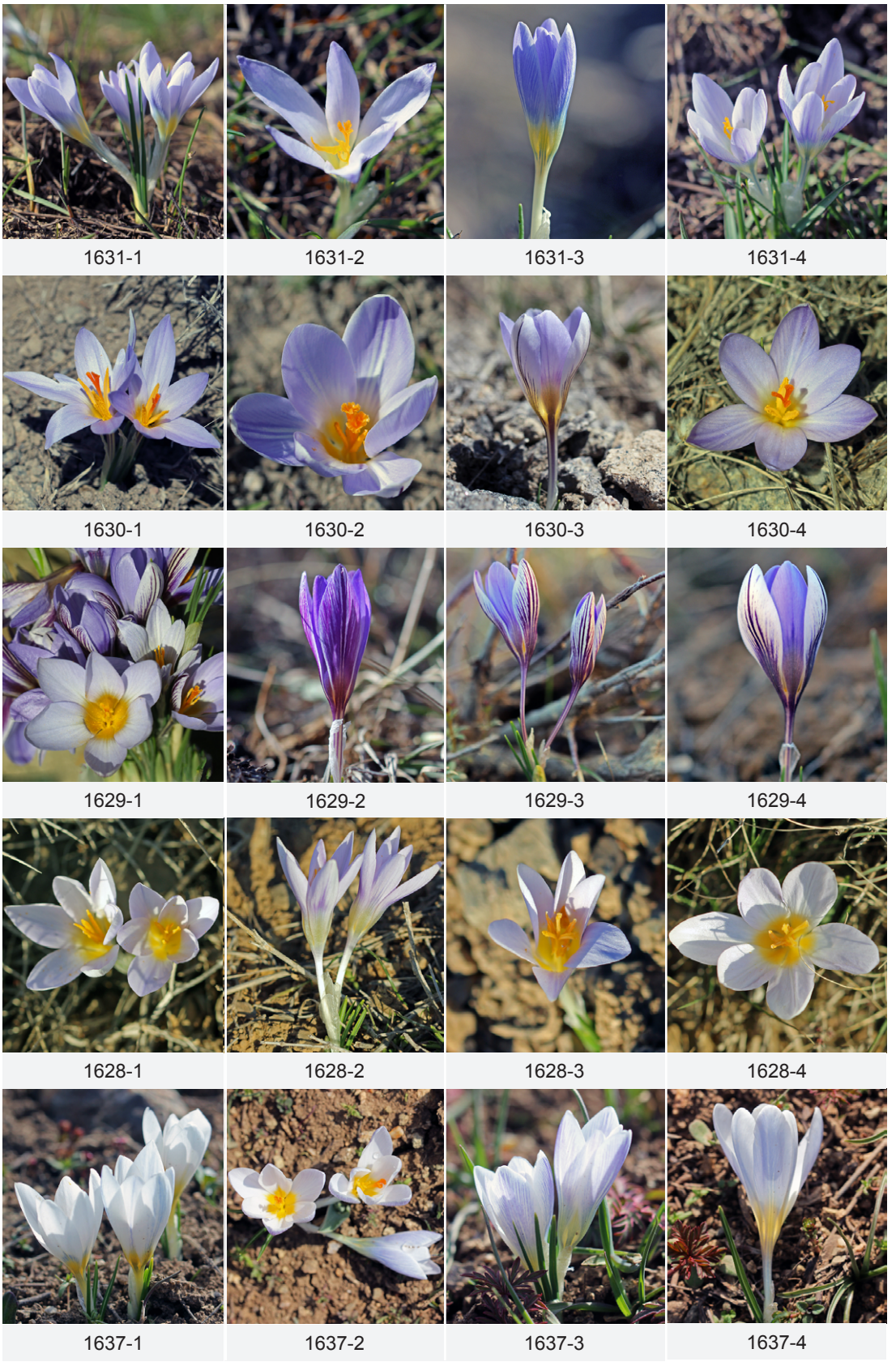


**Fig. 3**: Selection of four photographs of each investigated population to document their variability.

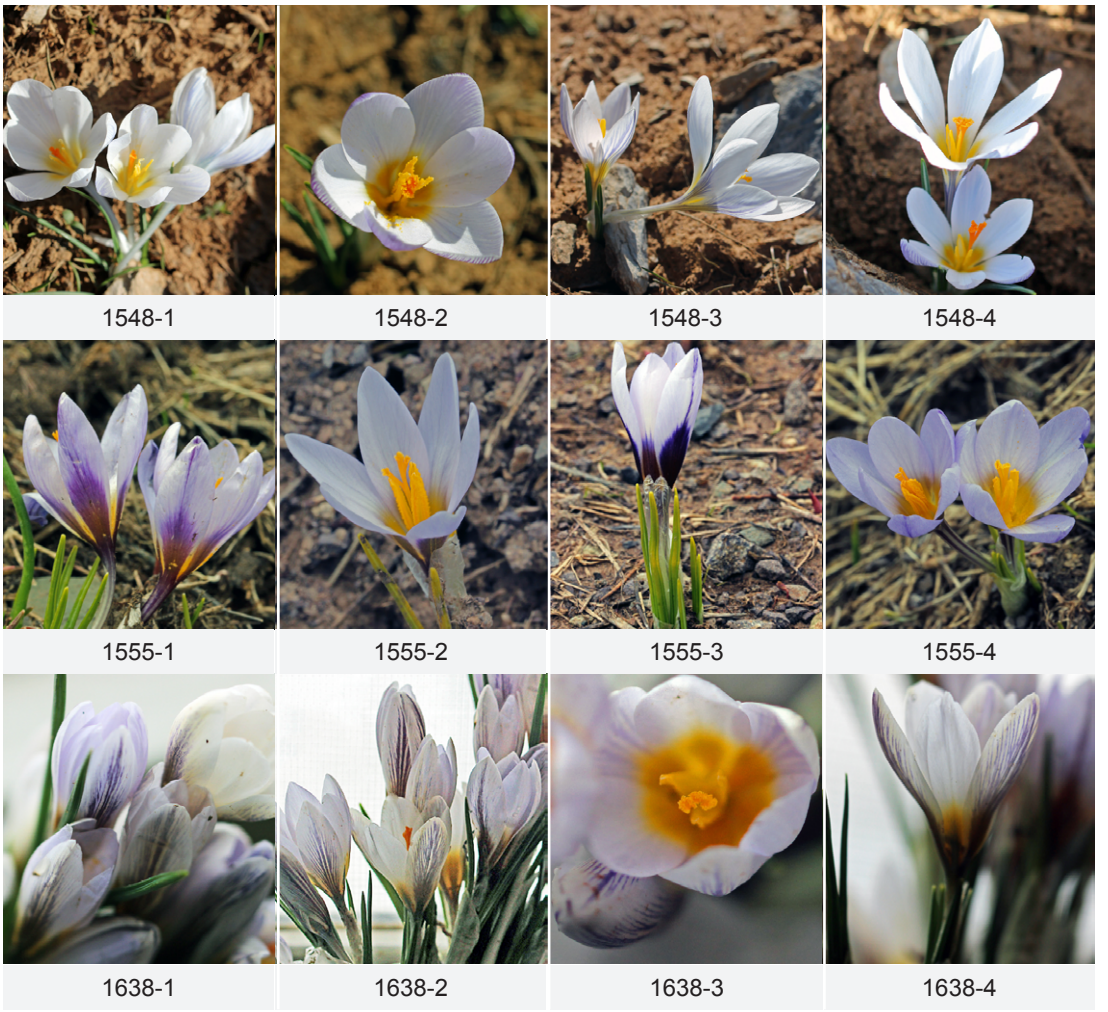


**Fig. 3** (continued): Selection of four photographs of each investigated population to document their variability.

but near the apex rarely with small bluish or violet spots. Outer segments between 23.3 and 33 mm but usually 27.9 mm long (n = 33), between 7.7 and 12 mm but usually 9.9 mm wide (n = 33). Inner segments between 21.6 and 32 mm but usually 26.4 mm long, between 7 and 12.5 mm but usually 9,6 mm wide (n = 33). Outsides and insides of all segments are light bluish lilac. The outside of the outer and inner segments is in general not much different and often without any markings. The outer ones have sometimes bluish-violet spots near the base which are extended into faint radial stripes up to half of the segments. Prophyll absent. Bract and bracteole present, silvery, conspicuous. Length of filaments 4-**5.9**-9.5 mm (n = 36), colourless to light-yellow, no hair. Anthers 10-**12.5**-17 mm long (n = 36) and narrow, deep yellow. Connective is colourless to yellowish, pollen yellow. Style deep yellow divided into 3 branches, not extended or trumpet-shaped at the upper end, branches 3.5-**6**-9.5 mm long (n = 36) Style length according to stamen is (n = 36). Capsule and seeds not seen. Chromosome number unknown.

*Crocus zanjanensis* is characterised by its medium-sized flowers having a soft bluish-lilac ground colour without conspicuous markings. Remarkable are the long narrow anthers with an average length of 12.5 mm. Its near relatives are HKEP 0616 from Güllü dağları and *C. roopiae* (HKEP1037) from Palandöken dağları, both in eastern Turkey (KERNDORFF et al., 2013), HKEP1629 (see Tab. 1 and 2 of this paper), *C. geghartii*, *C. adamii* of collection CMGG021 and the plants defined as type of *C. adamii* by RUKSANS (2015).

Distribution and habitat: *C. zanjanensis* is until now only known from the type locality in the mountains south of Zanjan, Zanjan Province, Iran. The plant grows in rocky and grassy areas without trees on limestone among *Iris hymenospatha, I. reticulata, Astragalus, Colchicum, Muscari.*

### *3. Crocus zagrosensis* KERNDORFF & PASCHE, species nova

Holotypus: Iran, Province Kordestan, Zagros mountains, near Divandarreh, 2100 m, 12.3.2016, HKEP 1637 (GAT, Gatersleben).

Corm subglobose slightly flattened at base, about 14 to 20 mm in diameter, tunics coriaceous, the inner ones softer, neck conspicuous, 9-13 mm long, very bristly; tunic splits into broad segments >5 mm, sub-splits absent; rings well developed more or less smooth-edged. Cataphylls 3-4, white even at tips. Leaves 3-**4**-6 green, 1.5-2 mm in diameter at flowering time, glabrous, white stripe small 1/5 to 1/3 of leaf-diameter, 5 rarely 4 ribs underneath of each side of the keel. Leaves poorly developed at anthesis shorter than flowers. Throat deep yellow without hairs. Perianth tube white, near the apex sometimes coloured or striped bluish.

Outer segments between 21.4 and 31.2 mm but usually 26.4 mm long (n = 33), between 6.5 and 13 mm but usually 10.5 mm wide (n = 33). Inner segments between 18.6 and 30 mm but usually 24.7 mm long, between 7 and 13.2 mm but usually 10 mm wide (n = 36). Inside all segments are white. The same is observable for the outside of the inner segments. Outside of outer segments is finely striped, feathered or speckled light blue or bluish-lilac or rarely without markings. Both, outer and inner segments may have bluish zones near the apex of the perianth tube. Prophyll absent. Bract and bracteole present, silvery, sometimes with light brownish tips. Length of filaments 4-**5.3**-6.5 mm (n = 36), yellow to orange-yellow, no hairs. Anthers 7.5-**11**-15.3 mm long (n = 36), yellow, with rounded tips. Connective colourless to yellowish, pollen yellow. Style orange divided into 3 branches hold stiffly upright and closely together, expanded and fringed at the upper end, branches 4-**6.2**-10.4 mm long (n = 36). Style length according to stamen is 84% equal (56%) and shorter (28%), and 16% longer (n = 36). Capsule and seeds not seen. Chromosome number unknown.

*Crocus zagrosensis* is characterised e. g. by white flowers with faint light blue stripes, feathers or speckles on the outside of the outer segments. Remarkable are the stiffly upright style-branches which are expanded and fringed at the apex, and the unusually high number of ribs in both sides of the grooves of the leaves. The closest relatives of *C. zagrosensis* is *C. reinhardii* (HKEP 1548, HKEP 1638 ≡ HKEP 1555 ≡ *C. reinhardii* 2516 (Dr. Fritsch) and *C. almehensis*.

Distribution and habitat: *C. zagrosensis* is a plant of alpine meadows. It is until now only known from the type locality in the Zagros Mountains around Divandarreh, Kordestan Province, Iran. The plant grows in rocky and grassy areas (short alpine turf) among *Astragalus*, *Verbascum*, *Allium*, *Colchicum*, *Corydalis*, *Ranunculus*, thistles.

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