

Crocus pallidus (Iridaceae)

Subjects: **Plant Sciences**

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The genus *Crocus* L., with a center of diversity on the Balkan Peninsula and Asia Minor, constitutes 235 species. For a long time, the Balkan endemic species *Crocus pallidus* has been unconfirmed and neglected for the flora of Bulgaria. It has remained an uncertain species from the Balkans, often listed as a synonym of *C. weldenii*. The morphological resemblance to the albinistic forms of *C. chrysanthus* has led to incorrect identification in the past, resulting in uncertainty regarding the distribution of this species in Bulgaria.

Iridaceae

Bulgaria

ITS region

Crocus pallidus

Crocus thracicus

Nudiscapus

anatomy

morphology

1. Introduction

The genus *Crocus* L., with a center of diversity on the Balkan Peninsula and Asia Minor ^[1], constitutes 235 species ^[2]. The number of species has increased due to the descriptions of the new species from this area in the last few years ^{[3][4]}. However, a taxonomic revision of the genus *Crocus* in Bulgarian flora has not been conducted in the last 50 years. After the last taxonomic treatment in Bulgaria, the genus included nine species ^[5]. *Crocus pallidus* Kitan. & Drenk. was reported as a new species, based on the revised specimens of *C. chrysanthus* Herb. var. *albidus* Maw, collected from the region of North Macedonia ^[6]. Later, this species persisted in the research of Kitanov et al. ^[7] and was included in the list of species for the flora of Dobrudja ^[8]. For more than 35 years, this species has been neglected in the Bulgarian floristic literature and identification keys ^[9]. However, *C. pallidus* was mentioned in a review for the Balkan endemics in Bulgaria ^[10]. On the basis of the publication of Kitanov et al. ^[7], the fourth edition of “Conspectus of the Bulgarian Vascular Flora” ^[11] included *C. pallidus*, with a distribution range along the Black Sea Coast (Northern) and Northeast Bulgaria. Kitanov and other authors collected the available deposited materials from Bulgarian sites during 1965–1972. The limited studies so far have caused a lack of morphological and anatomical characteristics for *C. pallidus* in the referent taxonomic sources. Various authors have perceived white-flowered crocuses in the Balkans with different taxonomic statuses. Pulević ^[12] considered that the specimens described by Kitanov & Drenkovsky represent *C. chrysanthus* var. *citrinus* Velen. *Crocus pallidus* was neglected by Mathew ^[13] and noted as a heterotypic synonym of *C. weldenii* Hoppe & Fühnrohr. This has probably been the reason for *C. pallidus* being listed as a synonym of *C. weldenii* in the system of WCSP ^[14].

Crocus weldenii is a taxon with controversial taxonomic status. It has been perceived as a subspecies of *C. biflorus* s.l. ^[13]. This taxon is Balkan-endemic and Illyrian–Adriatic-endemic, distributed from Trieste to the border of

Albania, with general distribution in Slovenia, Croatia, Herzegovina, Montenegro, Serbia, and North Macedonia [15]. The northwestern border of the species' range reaches the vicinity of Trieste in NE Italy [16][17][18].

According to Randelović et al. [19], both species are found in Serbia. *Crocus weldenii* inhabits the hilly and southern regions, while *C. pallidus* is a species from the mountainous and more northern areas. The taxonomical status of *C. pallidus* remains very unclear [2].

All members of the genus *Crocus* have shown exclusively high diversity in their chromosome numbers [20]. The chromosome number of *C. biflorus* sensu Mathew (1982) varies between $2n = 8$ and $2n = 36$ [21]. The changes in the chromosome number in the related species play an important role in the speciation [22][23]. This could be an important feature if the close related taxa are different according to taxonomic numbers. Nevertheless, the representatives of the group discussed here have different morphology and the same chromosome number. The cytological models in *Crocus* are complex, with extensive dysploidy, and not satisfactorily explained [13].

The white-flowering crocuses on the Balkan Peninsula and Adriatic coast [19] have not been covered by the recent molecular and phylogenetic studies [24][25]. Furthermore, no annotated ITS sequences of *C. pallidus* were found in the NCBI GenBank Nucleotide database [26].

In the protologue of the older taxonomic literature, the morphology of *C. pallidus* has been laconic and incompletely reflected [5][17], which also leads to uncertainty of its status. A species named *C. thracicus* with a similar but more detailed morphology was described in northwest Turkey [27].

The information about the species in the Bulgarian floristic literature is based on sporadic reports, without precise localities and current diagnostic data. The various assumptions about the taxon have led to confusion and neglect in the Bulgarian literature. Moreover, the contradictory taxonomic treatments of white-flowered Crocuses from the Balkans need further study of a more thorough sample.

2. Description Based on Bulgarian Materials

A spring synanthous geophyte is shown in **Figure 1** with the following features: corm oval-suborbicular, 13.5–25 mm in diameter, with membranous-coriaceous tunic, yellowish-brown, with separating basal rings; Basal rings 1.2–2.5 mm thick (**Figure 1G**) with unclear, slightly visible irregular dentation; plant height 100–210 mm; leaves 4–6(–7), 0.8–2.3 mm wide; flowers 2–3(–4); perigone segments niveous-white or outer ones with sprayed pale-blue coloration (**Figure 1A,C**); rounded to slightly acuminate; 19–33 × 6–11 mm long and 15–26 × 5–12 mm wide; perigone tube 6–10 cm long, white or bluish–lilac. (**Figure 2**); throat glabrous, white; filaments 3.8–10 mm; anthers 8–12(16) mm long with blackish basal lobes, or exceptionally with blackish stripes on the internal side, along the thecas (**Figure 1B**), on both sides of the white connective; stigma orange-reddish, trilobate (stylodia 3–5 mm), usually shorter than or equal to anther length; Capsule ellipsoid 15–21 × 4.3–7.4 mm (**Figure 1D**); seeds oval, dark brown 2.3–3.4 × 1.5–2.1 mm.



Figure 1. Morphological features of *Crocus pallidus* (specimen SOA 062791, grid 1 mm): (A) whole plant; (B) anthers and stigma; (C) flower section; (D) mature capsules; (E) seeds; (F) corm; (G) basal rings and bottom of the corm.



Figure 2. Variation in the color of the outer perigone segments of *Crocus pallidus*—white and speckled violet (population with voucher 062791).

A comparison with the known morphological features of the closely related species is provided in **Table 1**.

Table 1. A comparison of morphological features of *Crocus pallidus* (current data), *C. weldenii* [13][17], and *C. thracicus* [27].

	<i>C. pallidus</i>	<i>C. weldenii</i>	<i>C thracicus</i>
Corm	13.5–25 mm	13–19.7 mm	10–12 mm
Plant height	(8)10–20 cm	8–17 cm	7.35–12 mm
Teeth of the basal rings	Irregular	Regular/Irregular	Irregular
Count of leaves	3–5	3–5	3–4(–5)
Leaf width	0.84–2.3 mm	max 1 mm	0.75–1.3 mm
Ribs on the abaxial leaf surface	Missing or vaguely visible	1–2, clearly visible	Missing
Color of the perigone tube	White, rarely violet	White, often violet	White, rarely violet near the apex
Color of outer perigone segments	White or sprayed in violet	White	White or sprayed in violet
Size of perigone segments (out/in)	19–33 × 6–11/15–26 × 5–12 mm	19–37 × 4.8–12.8	17–24 × 6–9/14.6–23 × 5.7–8.7 mm

	<i>C. pallidus</i>	<i>C. weldenii</i>	<i>C thracicus</i>
Perigone throat	Glabrous, white	Yellow	Glabrous, white
Filaments	3.8–13.7 mm	8–16 mm	10–13.5 mm
Anthers	8–16 mm, yellow, with blackish basal lobes, or entirely black edge	yellow, without blackish lobes	7.3–11.8 mm, yellow, with blackish basal lobes.
Capsule	Ellipsoid; 15–21 mm long; 4.3–7.4 mm wide.		Ellipsoid, about 14 mm long
Seeds	2.2–3.4 × 1.5–2.1 mm, with convex caruncle		2.5 mm in diameter, with convex caruncle
Distribution	Bulgaria (Eastern parts), North Macedonia, Serbia.	Italy, Albania, Serbia.	Turkey in Europe (Thrakia)
Elevation	30–190 m	100–750 m	45–170 m
Flowering period	February–March		February–March

Sea coast represents the eastern border (**Figure 3**).

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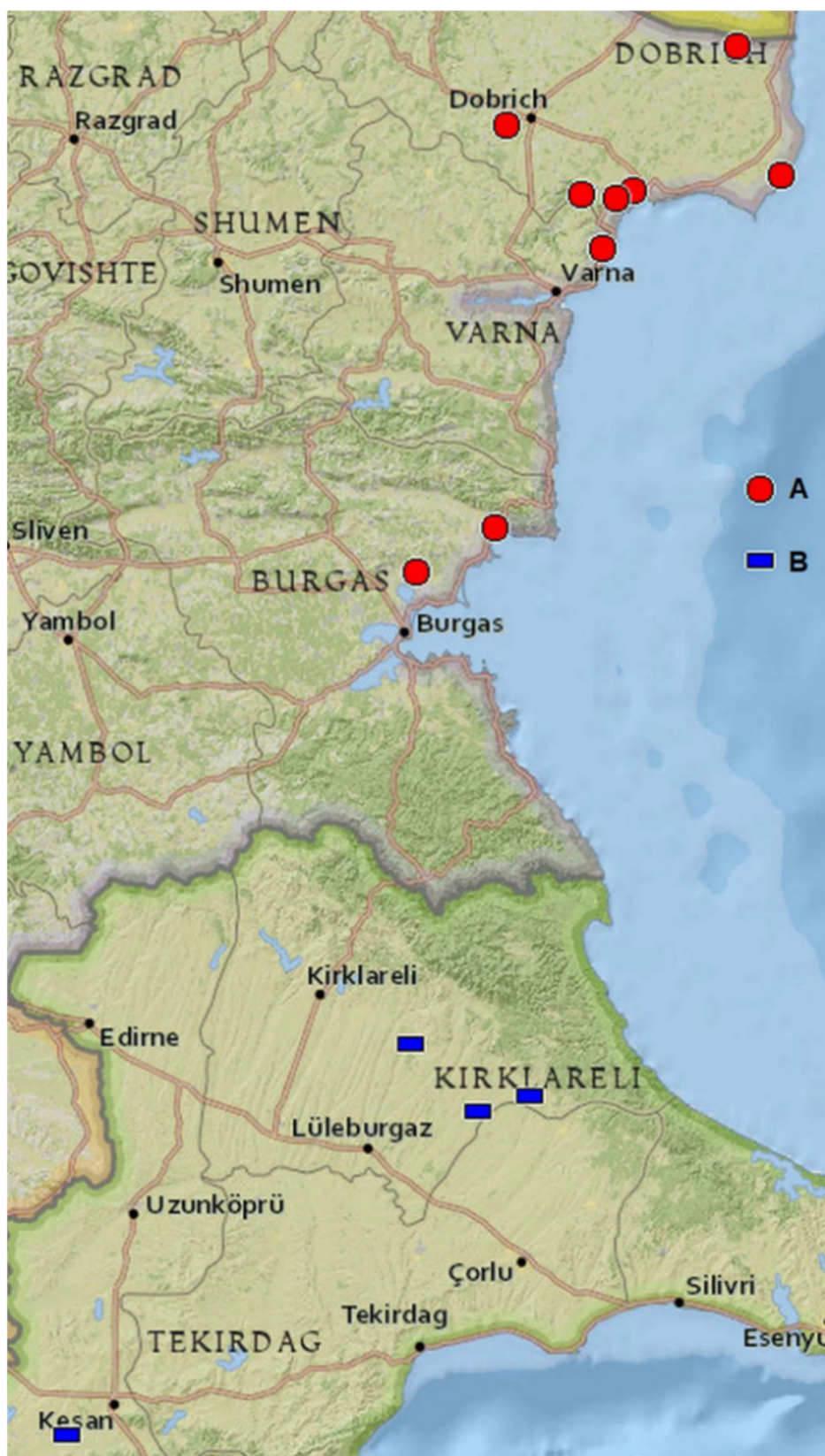


Figure 3. Localities of *Crocus pallidus* (A) in Bulgaria and *C. thracicus* (B) in Turkey. Map created at GPSVisualizer.com (accessed on 16 March 2021). Leaflet | NGS maps from ESRI/ArcGIS.

4. Phenology and Habitats

The flowering period lasts from the end of January to the end of March. The population of *C. pallidus* near the village of Kamen Bryag (SOA 062791) inhabits the open grass communities in the margin of an oak grove, accompanied by individuals of *Paliurus spina-christi* Mill., *Ficaria verna* Huds., *Crocus chrysanthus* (Herb.) Herb., *Geranium mole* L., *Fragaria vesca* L., *Viola* sp. div., *Trifolium* sp. div., *Poaceae* sp. div., and tufts of mosses. The whole habitat is surrounded by agricultural land. Approximately 150–200 individuals represent the population. A dense sympatric population of *C. chrysanthus* was found inside the grove. On the margin of the grove, populations of both species are mixed, whereas hybrids may occur (**Figure 4**). The locality is under anthropogenic pressure due to agricultural work, the main road laying on the western border of the population, and proximity to Kamen Bryag.

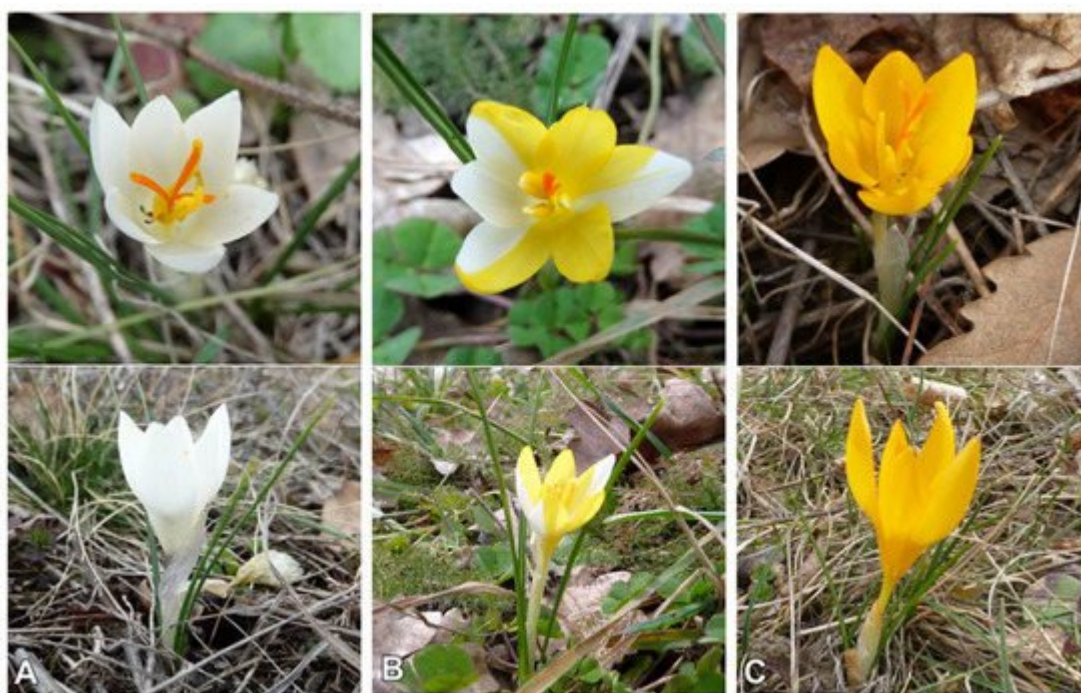


Figure 4. *Crocus pallidus* (A), *C. chrysanthus* (C), and their hybrid (B) in the population with voucher 062791.

The population in Pomorie Narrow (SOA 062791) exists in sparse oak forest with the same accompanying species and *Anemone blanda* Schott & Kotchy. The few observed individuals of *C. chrysanthus* were flowering earlier than *C. pallidus* flowering time. The anthropogenic pressure is also significant (the habitat is an illegal landfill for household waste).

Under high anthropogenic pressure is the locus classicus of *C. p. f. bulgaricus* Kitan. & Drenk. It is an oak grove, subjected to unregulated logging and pollution, situated on the main road near the city of Dobrich (SO 21305).

The population near the Rudnik suburb of Burgas (SOA 063064) is under relatively low anthropogenic pressure. It is located in an open habitat, protected by the shrubs of *Paliurus*, and accompanied by *C. chrysanthus*.

5. Leaf Anatomy

The anatomical features of *C. pallidus* from the evaluated populations are presented in **Table 2**.

Table 2. Anatomical parameters of the examined populations of *Crocus pallidus*. Values are given as a range (minimum–maximum), mean ± standard deviation.

Population *	062791 Kamen Bryag	062797 Pomorie Narrow	063064 Roudnik
Section width, μm	1701–2224 1927.6 ± 133	955–1722 1210.9 ± 193	910–2017 1432.6 ± 327.9
Section height, μm	709–807 761.1 ± 39.8	356–664 481.8 ± 89.2	480–633 562.8 ± 54.8
Arm width, μm	657–973 830.1 ± 96.3	385–696 547.2 ± 91.1	455–1071 703.4 ± 146.6
White stripe to leaf width ratio, %	15.1–21 17.4 ± 2	12.7–28.8 20 ± 4.2	14.9–19.3 16.64 ± 1.5
Vascular bundles, count	23–25 23.8 ± 1	13–15 13.8 ± 1	15–17 15.5 ± 0.3
Vascular bundles, height, μm	25.9–177 81.7 ± 45.3	27.5–187 95.6 ± 45.8	33.2–190 104.6 ± 50.1
Vascular bundles, width, μm	17.9–103 59.2 ± 24.6	22.9–141 73.2 ± 36.5	23.9–127 74 ± 29.8
Vascular tissues in the bundles, %	22.2–50.7 37.3 ± 8.2	7–61 19.9 ± 9.3	4.1–30.8 19.4 ± 7.7
Palisade tissue: thickness, μm	13.7–77 44 ± 14.5	38–80 56.8 ± 8.8	25.1–75 57.9 ± 11.2
Spongy tissue-thickness, μm	25–91 48 ± 16.6	43–104 61.6 ± 13.8	33.8–65 46.3 ± 9.7
White stripe, μm	287–407 341 ± 49.2	150–295 241.1 ± 44.5	247–305 276.9 ± 22.8
Adaxial epidermal cell: length, μm	173–290 236 ± 47.3	98–431 237.2 ± 61.7	155–473 286.2 ± 66.8
Adaxial epidermal cell: height, μm	15–23.8 18.5 ± 1.96	11–22.6 17.83 ± 2.56	6.7–21 15.3 ± 3
Adaxial epidermal cell: width, μm	10.4–23.1 17.5 ± 2.93	11.5–20.8 16.45 ± 2.4	9.8–23.5 16.8 ± 3

Population *	062791 Kamen Bryag	062797 Pomorie Narrow	063064 Roudnik	
Palisade cell: height, μm	13.8–36.4 26.4 ± 4.25	21.3–40.7 32.1 ± 6.49	17.2–41.3 30.3 ± 5.3	
Palisade cell: width, μm	8.8–21.2 13.9 ± 2.47	9.6–18.6 13.53 ± 2.46	8.9–17.5 12.7 ± 2	
Spongy cell: height, μm	14.4–29.6 21.4 ± 3.14	19.1–25.1 22.36 ± 2.07	13.9–28.6 20.7 ± 4.1	or square
Spongy cell: width, μm	8.3–23.1 14.7 ± 3.39	13.7–17.9 15.74 ± 1.57	7.4–21.5 14.2 ± 2.6	
Abaxial epidermal cell: height, μm	11.2–24.3 19.6 ± 2.92	15–28 18.2 ± 3.33	7.7–18.1 13.5 ± 2.7	hite stripe he bigger

vascular bundles (Figure 5).

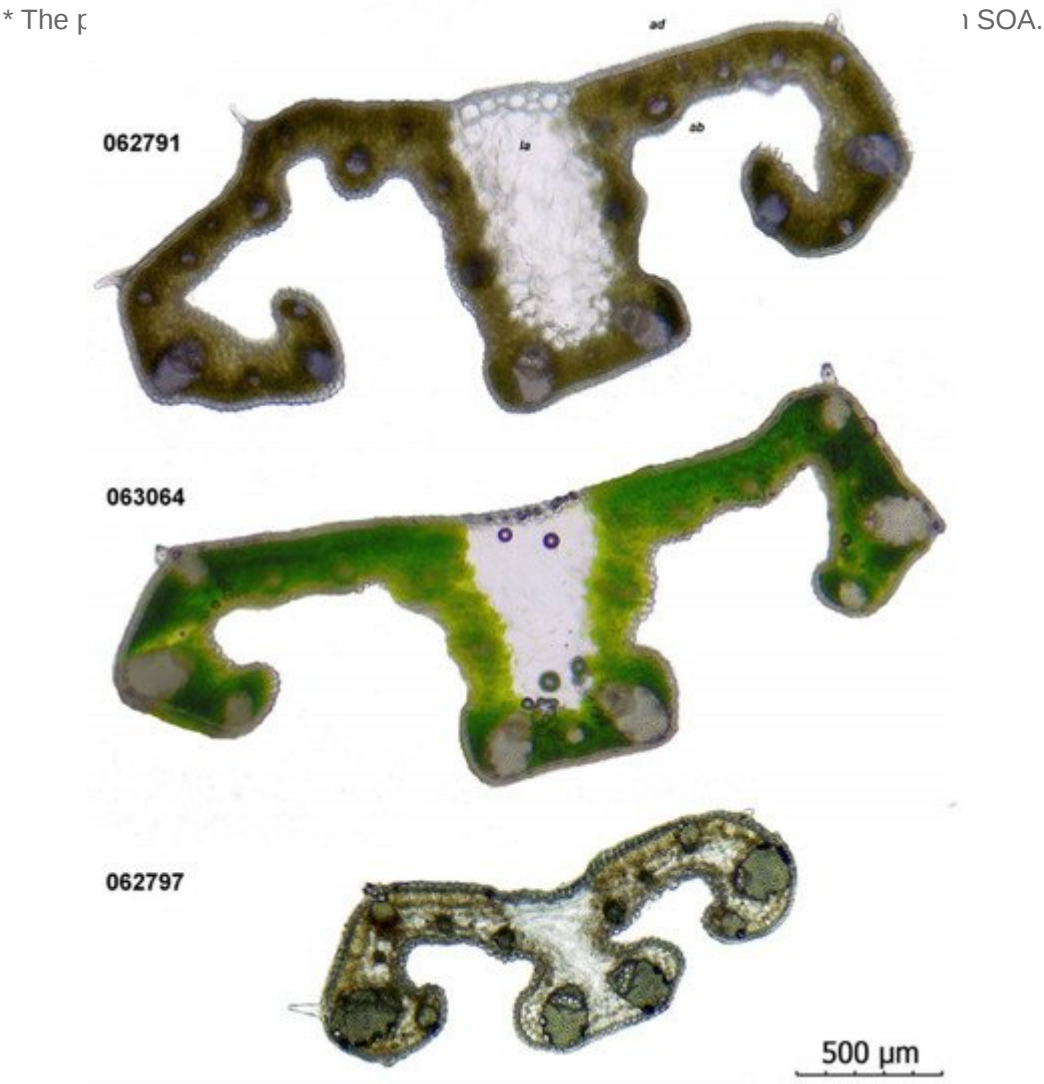


Figure 5. *Crocus pallidus* leaf cross-sections (4×). Abbreviations: ad—adaxial side, ab—abaxial side, la—lacuna area. Voucher numbers are shown near the cross-sections.

The arms' length and their curving degree vary in the investigated populations. The ribs are present on the abaxial side of the arms (**Figure 6B,E**). Papillae are located in the apical regions of the arms and along the ribs (**Figure 6A**).

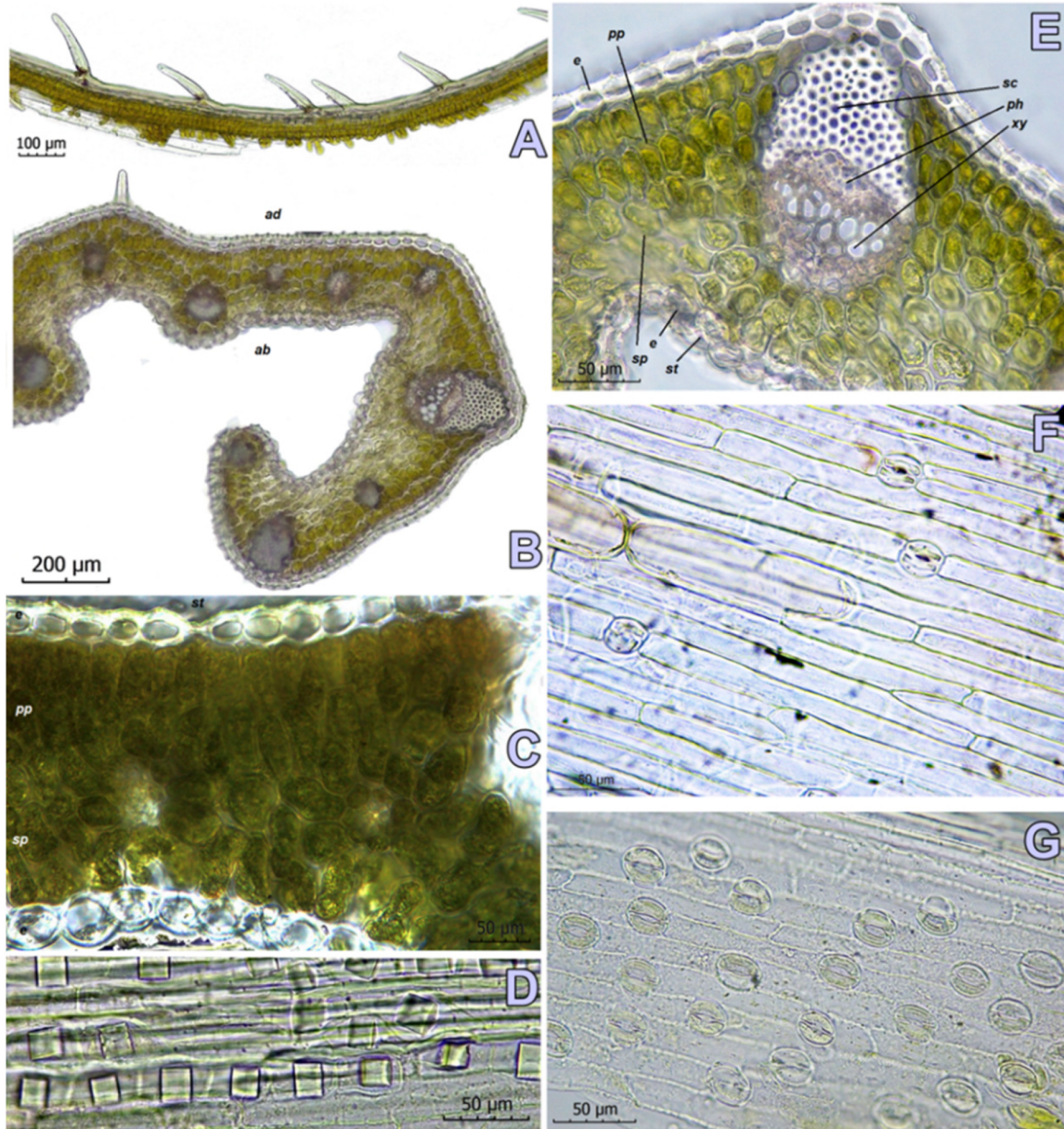


Figure 6. Leaf anatomy of *Crocus pallidus*. (A) Papillae on the ribs—adaxial surface; (B) section of the shoulder (SOA 062791); (C) detail of the leaf with visible adaxial stomata (SOA 062797); (D) crystals; (E) subterminal vascular bundle in the shoulder; (F) adaxial epidermis with stomata; (G) abaxial epidermis with stomata (SOA 062791). Abbreviations: ad—adaxial side, ab—abaxial side, la—lacuna area, e—epidermis, pp—palisade parenchyma, sp—spongy parenchyma, sc—sclerenchyma cap, ph—phloem, xy—xylem.

The adaxial epidermal cells are rectangular, with a thicker cuticle. The abaxial epidermal cells are elliptic in shape, while the cuticle layer is thinner. The stomata are located on the abaxial side of the leaves, (**Figure 6E,G**) in the

zones of the arms and keel. The stomatal type is anomocytic. Single stomata are also sporadically present on the abaxial epidermis (**Figure 6C,F**).

The parenchyma in the central zone of the keel is represented by big rectangular cells with thin walls and without chloroplasts, forming a lacuna zone, visible like a white stripe along the leaf blade. The assimilating mesophyll in the zone of the arms consists of palisade and spongy layers. The palisade cells are oblong, organized in two rows, without any space between them. The spongy cells form zones of 2–4 rows near the abaxial epidermis, between the ribs. They have an elliptic to irregular shape, with slightly visible intercellular spaces between them (**Figure 6C,E**). Crystalline formations as oxalate sand or crystals with an approximately cubic shape are present in all three studied populations of *C. pallidus* (**Figure 6D**).

The vascular bundles are collateral, located in a row along the arms and the keel zone. The vascular tissues take 7–40(–61)% while the remaining bundles (about 2/3) consist of sclerenchymatous “caps”. The subterminal bundles in the folding area of the arms are bigger than the other (81–187 μm $\times$ 56–141 μm). The terminal bundles in the keel are the biggest in that area (89–190 μm $\times$ 70–136 μm). The number and size of the other bundles vary depending on the width of the section. The population from Kamen Bryag (SOA 062791) shows a bigger number of vascular bundles. Despite the quantitative variation in their number, in all of the sections, the biggest vascular bundle is subterminal, followed by 1–3 narrower terminal bundles in the shoulder.

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