



**Some notes on *Galanthus cilicicus* and *Galanthus peshmenii* (Amaryllidaceae)**

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**Abstract**

*Galanthus cilicicus* Baker and *Galanthus peshmenii* A.P. Davis & C.D. Brickell are taxonomically problematic species. In this study, they were morphologically examined in detail and their chromosomes were counted. Moreover, descriptions, illustrations and distribution areas are given; in addition to the SEM observations of seed coats and pollen grains.

**Key words:** Amaryllidaceae, *Galanthus*, Morphology, Palynology, Taxonomy

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***Galanthus cilicicus* and *Galanthus peshmenii* (Amaryllidaceae) hakkında bazı notlar**

**Özet**

*Galanthus cilicicus* Baker ve *Galanthus peshmenii* A.P.Davis & C.D.Brickell taksonomik açıdan problemliler türlerdir. Bu çalışmada bu türlerin morfolojik yapıları detaylı olarak incelenmiş ve kromozomları sayılmıştır. Ayrıca, tohum yüzeyi ve polen tanelerinin SEM görüntülerine ek olarak, betimleri, şekilleri ve yayılış alanları verilmiştir.

**Anahtar kelimeler:** Amaryllidaceae, *Galanthus*, Morfoloji, Palinoloji, Taksonomi

**1. Introduction**

*Galanthus* L. (kardelen) is a member of the family Amaryllidaceae which has c. 60 genera and 850 species throughout the world. The family is occurs mainly in the tropics and subtropics, although there are several representatives in temperate areas in Europe and a few in Asia (Heywood et al., 2007). The genus is confined to Europe, Asia Minor, and the Near East (Davis, 1999).

There are 19 *Galanthus* species (22 taxa) in the world; 12 of them and one natural hybrid are distributed in Turkey, a center of species diversity. Among the 14 taxa in Turkey, five of them and one hybrid grow solely in Anatolia. These are: *G. plicatus* M.Bieb. subsp. *byzantinus* (Baker) D.A.Webb (İstanbul kardeleni), *G. cilicicus* Baker (İçel kardeleni), *G. elwesii* Hook.f. var. *monostictus* P.D.Sell (kardelen), *G. koenianus* Lobin, C.D.Brickell & A.P.Davis (garipçe), *G. trojanus* A.P.Davis & N.Özhatay (Truva kardeleni) and *G. x valentinei* Beck nothosubsp. *subplicatus* (N.Zeybek) A.P.Davis (melez kardelen), (Brickell, 1984; Davis et al., 1988; Davis, 2000; Davis, 2001; Davis and Özhatay, 2001; Davis et al., 2001; Demir, 2010).

In the present study, *G. peshmenii* (bey kardeleni) and *G. cilicicus* (İçel kardeleni) were comprehensively investigated in terms of morphology and taxonomy. These are considered to be closely related. *G. cilicicus*, initially introduced to the world of science by Baker (1897), was transferred as subspecies to *G. nivalis* L. as *G. nivalis* subsp. *cilicicus* (Baker) Gott.-Tann. by Gottlieb-Tannenhain (1904). In 1999, the independent status of *G. cilicicus* was

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accepted by A.P. Davis who prepared the monograph of the genus. According to Zonneveld et al. (2004), *G. cilicicus* is clearly different from *G. nivalis* with its DNA, which confirms that it is not a subspecies of the latter as suggested by Stern (1956) and later discounted by Davis (1999). *G. peshmenii* samples were taken from various places at different times and named as *G. cilicicus*, *G. nivalis* subsp. *cilicicus* and *G. reginae-olgae* Orph. However, these samples were introduced to the world of science as *G. peshmenii* (Davis and Brickell, 1994).

The present study was to determine morpho- and cyto-taxonomic similarities and differences between *G. cilicicus* and *G. peshmenii*. Key characters used to distinguish species in *Galanthus* are always easy to determine satisfactorily on the living material but are not easy to determine on herbarium specimens. Arrangement of leaves in the bud known as vernation is a good character that can be observed in the early stage of the flowering. However, it is very difficult to observe this feature in dry specimens and those in the late stage of flowering. Another important character is the leaf colour, which was impossible to detect with the herbarium materials. The other important character is the type of coloring in the inner perianth segment, which can easily be observed in the herbarium samples.

The species in this study are clearly different from the other species of the genus in their flowering time. *G. peshmenii* which flowers in November and December is found within the borders of Antalya province, and it extends to the border of Kaş peninsula in the west and cannot be found towards the east of the city center. *G. cilicicus* flowers in December and January and its distribution is more limited than *G. peshmenii*; it is found only within the borders of Mersin province. The other taxon which flowers in late autumn-early winter period is *G. elwesii* var. *monostictus*. In the light of data obtained so far now, its distribution area was concluded to be in Antalya-Mersin provinces. This variety flowers in November-April depending on the altitude. Since *G. elwesii* var. *monostictus* has supervolute vernation, it clearly differs from *G. peshmenii* and *G. cilicicus* which have applanate vernation. In these three taxa, only apical coloration can be seen in the inner perianth segment.

## 2. Materials and methods

Plant specimens were collected during the field-works between the years 2002-2007. All plant specimens used in the present study were dried according to the standard herbarium techniques and deposited in the herbaria of ISTE. Morphological features of *G. cilicicus* and *G. peshmenii* were described based on field-works, observations and measurements of herbarium specimens.

The seed morphology of both species was examined using SEM (Scanning electron microscopy) techniques. For this, the seeds were covered with gold on stubs. The micro-photographs were taken with a Zeiss LEO 1430 Scanning Electron Microscope.

The pollen morphology of *G. cilicicus* and *G. peshmenii* was examined by light microscopy (LM) and SEM. For the LM, the pollen grains were first treated with 96% alcohol to remove the oily substances; subsequently, they were embedded in glycerin-jelly and stained with basic fuchsin (Wodehouse, 1935). The following parameters were measured: the polar axis (P), the equatorial axis (E), as well as the exine and the intine thickness. The measured pollen diameters were based on 50 samples. To examine the exine sculpture in detail, scanning electron microscopy (SEM) was also used. For SEM study, pollens were first treated with 70% alcohol, and then dried before mounting on stubs with gold. The micro-photographs were taken with a Zeiss LEO-1430 Scanning Electron Microscope. Pollen shapes and ornamentation were classified according to Punt et al. (1994).

Cytological investigations were limited to mitotic studies using a root-tip squash technique. The root tips were placed in  $\alpha$ -monobromonaphthalene and kept for 24 hours at +4°C. Afterwards, they were fixed in a 3:1 (v/v) absolute alcohol: glacial acetic acid mixture and stored in 70% (v/v) alcohol at +4°C. The root tips were hydrolyzed in 1 N HCl for 10 minutes at +60°C and stained with Schiff reagent. The squashed preparations of root tips were made with 45% (v/v) aceto-orcin on permanent slides. Chromosomes at the metaphase stage of the mitosis were counted and photographed (Brighton et al., 1973; Zeybek and Sauer, 1995).

Red list categories of taxa were revised according to IUCN Red List Categories (IUCN, 2001).

## 3. Results

### 1.1. Morphological study

*Galanthus cilicicus* Baker in Gard. Chron. ser. 3, 21: 214 (1897). (Figure 1).

Type: Cilicia [Cilician Taurus], 560 m, 1896, Siehe (holotype K)- cf. A.P. Davis, 1999)

Bulb  $\pm$ ovoid, (1.4-)1.6-2.1  $\times$  (1-)1.2-1.5(-1.8) cm. Sheath (2-)4-6.5(-8.8)  $\times$  0.4-0.6 cm. Vernation applanate. Leaves linear, at flowering (3.5-)5.7-9.5(-20.5)  $\times$  (0.4-)0.5-0.7(-0.8) cm, after flowering developing to 16.5-25(-43)  $\times$  0.5-0.8 cm, midrib conspicuous; margins flat; apex acute to acute-obtuse, flat to very slightly hooded; upper and lower surfaces  $\pm$  the same color, glaucous or rarely glaucescent, matt, upper surface with or without a faint grayish median stripe. Scape (7-)8.5-18.6(-25) cm long, glaucous. Spathe always longer than pedicel, 2.2-3.6(-4.6) cm long. Pedicel (1.1)1.5-2.2(-2.5) cm long. Outer perianth segments,  $\pm$ elliptic-obovate, (1.7-)2-3(-3.3)  $\times$  0.75-0.95(-1.3) cm, slightly unguiculate, inner perianth segments  $\pm$ narrowly obovate-obtriangular, (0.9-)1-1.3(-1.5)  $\times$  0.5-0.7 cm, emarginate,

narrow to broad,  $\pm\Delta$  to  $\cap$  shaped, or  $\pm$ heart-shaped green mark, usually covering  $(1/3-1/2-2/3)$  of segment; inner face of each segment with a faint green mark covering  $2/3-3/3$  of segment. Anthers tapering to a long point, 5-6.6 mm long. Filaments 1.5-2 mm long. Style 6.5-8(-10) mm long. Stigma acapitate or capitate. Capsule globose-ellipsoid, 1.2-1.8  $\times$  1-1.4 cm. Seeds broadly ovate to rounded, 2.5-4  $\times$  2.2-4 mm, pale brown; seed surface rugose.

Examined specimens: C5 Mersin: Mersin-Arslanköy road, Yeniköy, SW side of Çaltık hill, rocky areas, *Quercus coccifera* beneath, 892 m, 28.12.2002, S.Yüzbaşıoğlu, ISTE 93271. *ibid.*, 13.01.2007, S.Yüzbaşıoğlu ISTE 93274. *ibid.*, 08.01.2009, S. Yüzbaşıoğlu, ISTE 93275. Mersin: Doruklu, Dalakdere, rocky areas, *Quercus coccifera* beneath, 440 m, 13.01.2007, S. Yüzbaşıoğlu, ISTE 93272. Mersin: Mersin-Arslanköy road, Kayrakkeşli, rocky areas, *Quercus coccifera* beneath, 620 m, 08.01.2009, S.Yüzbaşıoğlu, ISTE 93276; *ibid.*, 11.03.2009, S.Yüzbaşıoğlu, ISTE 93277. Kagiraki, 560 m, 1896, Siehe, (K 000464080!). Mostly restricted to limestone.

Conservation status: CR [B1 ab(i, ii, iii, v) + 2ab (i, ii, iii, v)]

Zeybek & Sauer (1995) reported that *G. nivalis* subsp. *cilicicus* also grew in two other localities, namely Çanakkale and Mersin. However, as a result of the field-works for the present study, the sample obtained from Bayramiç (Çanakkale) was proved as *G. trojanus* and published by A.P. Davis and N. Özhatay (2001).

Baker (1897) stated that the living specimen used in the description of *G. cilicicus* was sent to him by the nurseryman T.S.Ware at January 7, 1897. Dried samples were collected by W.Siehe from the Cilician Taurus at 560 m in 1896 were reported in the same publication. In the studies carried out by Stern (1956) and Brickell (1984), the T.S.Ware plant was said to be in the Kew herbarium and suggested as the type specimen of *G. cilicicus*. However, no T.S.Ware specimen was found by the first author while working for his Phd thesis in the Kew herbarium. In the monographic study of A.P.Davis (1999), the Siehe gathering (1896) was regarded as the holotype.

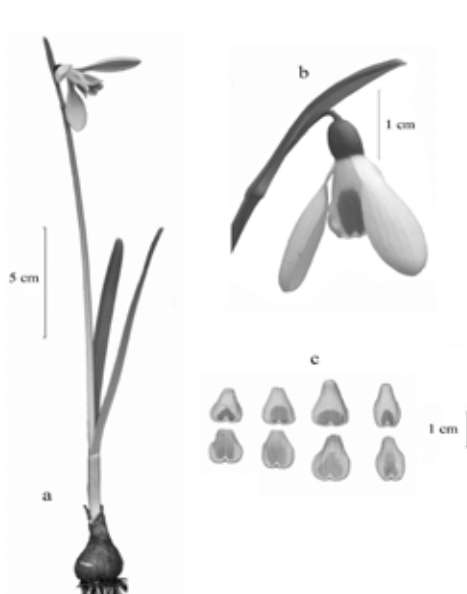


Figure 1: *Galanthus cilicicus*. a) general view b) spathe and flower c) inner perianth segments (outer surfaces on the upper row and inner surfaces on the lower row).

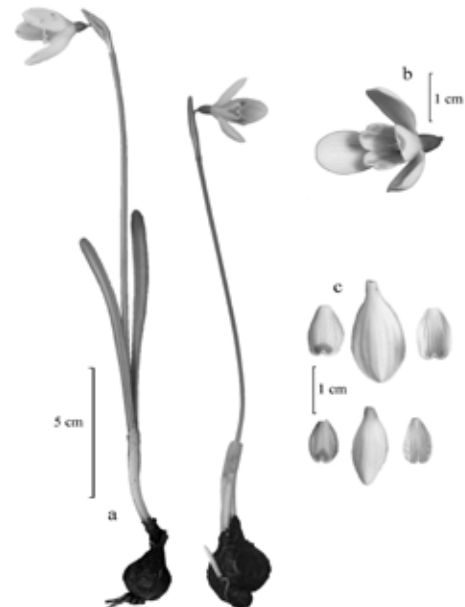


Figure 2: *Galanthus peshmenii*. a) general view b) flower c) inner and outer perianth segments (from left to right: inner perianth segments outer surfaces, outer perianth segments, inner perianth segments inner surfaces)

***Galanthus peshmenii*** A.P. Davis & C.D. Brickell in *New Plantsman* 1: 14, fig. 1 (1994). (Figure 2).

Type: Turkey, [C3 Antalya] Kemer, Kesmeboğazi-Gedelma Köyü arasi, c. 300 m, 3.11.1978, Peşmen, Yıldız & Güneş 4125 (holotype HUB!; isotype E photo!).

Bulb  $\pm$  globose-ovoid, 1.5-2.4  $\times$  (0.8-)1.2-2 cm. Sheath (1-)2.5-7(-9.5)  $\times$  (0.3-)0.4-0.65 cm. Vernation applanate. Leaves linear, at flowering absent or much shorter than the scape, (0-)1-5.5(-9)  $\times$  0.2-0.4(-0.6) cm, after flowering developing to 15-27(-33)  $\times$  0.4-0.6 cm, midrib conspicuous; margins flat or slightly rolled under near the base; apex acute to acute-obtuse, flat; upper and lower surfaces slightly different in colour or  $\pm$  the same, upper surface glaucescent to almost glaucous, usually with a faint grayish median strip, lower surface glaucescent to  $\pm$  glaucous, matt. Scape 7-13(-20) cm long, glaucescent. Spathe always longer than pedicel, (1.5-)2.1-2.5(-2.9) cm long. Pedicel (1-)1.5-1.7(-2.1) cm long. Outer perianth segments narrowly obovate, elliptic-broadly elliptic, (1.1-)1.5-1.8(-2.1)  $\times$  0.4-0.5 cm, slightly unguiculate, inner perianth segments  $\pm$  obovate, 0.8-1.1(-1.3)  $\times$  0.45-0.7(-0.85) cm, emarginate,  $\pm\cap$  to  $\Delta$ , heart-shaped green mark or two small green spots (either side of the sinus) usually covering  $1/3$ (- $1/2$ ) of segment; inner face of each segment with a faint green mark covering  $2/3-3/3$  of segment. Anthers tapering to a long point, 4-4.7 mm long.

Filaments 0.7-1 mm long. Style 6-8 mm long. Stigma acapitate or capitate. Capsule globose-ellipsoid,  $0.9-1.2 \times 0.8-1.2$  cm. Seeds broadly ovate to rounded,  $2.5-4 \times 2.2-3.5$  mm, pale brown; seed surface rugose.

Examined specimens: C3 Antalya: Geyikbayırı, light *Pinus brutia* forest and *Quercus coccifera* beneath, 600 m, 09.11.2006, S. Yüzbaşıoğlu, ISTE 93267. Antalya: Finike, Sahilkent Municipality, Alakır dam way, inside of valley, 50 m, 13.11.2006, S. Yüzbaşıoğlu, ISTE 93268. Antalya: Kaş, Peninsula entrance, 5 m, 01.12.2007, S. Yüzbaşıoğlu, ISTE 93269. Antalya: Kemer, Kesmeboğazi, *Pinus brutia* forest, 600 m, 29.03.2009, S. Yüzbaşıoğlu, ISTE 93270. Not: The species is also in Greece-island of Kastellorhizo [Megisti] (Davis, 1999).

Conservation status: EN [B1 ab (i, ii, iii, v) + 2ab (i, ii, iii, v)]

During the revision of the genus *Galanthus* L. in Turkey, numerous field trips have been made by the first author. Living specimens were collected from all around Turkey. Collected bulbs were planted for observation Nezahat Gökyiğit and Alfred Heilbronn Botanic Gardens in İstanbul, Turkey. Based on these studies *G. cilicicus* is known only in a few localities of Mersin province. The field-works demonstrated that *G. cilicicus* is the rarest *Galanthus* species and endangered in the wild. The natural distributions of *G. cilicicus* and *G. peshmenii* do not overlap (Figure 3). Between Antalya and Mersin, there is no locality found that these two species grow together. Morphological differences between *G. cilicicus* and *G. peshmenii* are given in Table 1.

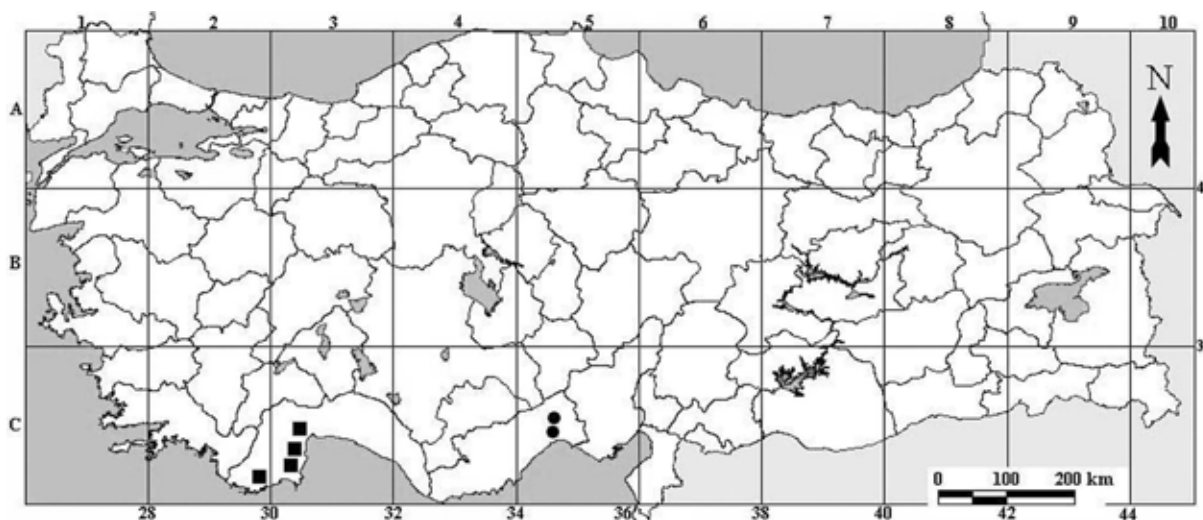


Figure 3. Distribution areas of *G. cilicicus* (●) and *G. peshmenii* (■) in Turkey

Table 1. Comparison of some diagnostic morphological and palynological characters of *G. cilicicus* and *G. peshmenii*

		<i>G. peshmenii</i>	<i>G. cilicicus</i>
Morphological characters	Flowering period	October-December	December-February
	Leaves	absent or (0-)1-5.5 cm $\times$ 0.2-0.4 cm at start of flowering	(3.5-)5.7-9.5 cm $\times$ 0.5-0.7 cm at start of flowering
	Leaf colour	Adaxial surfaces glaucescent with a faint underlying median stripe, abaxial surface glaucous	Leaves surfaces $\pm$ same colour, glaucous, adaxial surfaces usually without a faint underlying median stripe
	Inner perianth segments	Apex and margins flat	Apex usually flared and margins wavy
	Inner segment markings on the outer surface	$\pm$ $\cap$ to $\Lambda$ , heart-shaped or two small green spots either side of the sinus, usually covering (-1/2)1/3 of segment	$\pm$ $\cap$ to $\Lambda$ or heart shaped, usually covering (1/3-)1/2-2/3 of segment
	Outer perianth segments	(1.1-)1.5-1.8(-2.1) $\times$ 0.4-0.5 cm	(1.7-)2-3(-3.3) $\times$ 0.75-0.95 (-1.3) cm
Palynological characters	Polar axis (P)	26.16 $\pm$ 0.68 $\mu$ m	26.32 $\pm$ 0.64 $\mu$ m
	Equatorial axis (E)	18.94 $\pm$ 0.78 $\mu$ m	19.71 $\pm$ 0.53 $\mu$ m
	P/E	1.38	1.33
	Shape	prolate	prolate
	Aperture	monosulcate	monosulcate
	Ornamentation	micro-rugulate	micro-rugulate
	Exine	0.77-1.28 $\mu$ m	0.76-1.13 $\mu$ m
Intine	0.5-0.75 $\mu$ m	0.5-0.75 $\mu$ m	

Seed morphologies of *G. cilicicus* and *G. peshmenii* were examined. Seed size, colour and seed surface of these species were found similar. The seed shapes of *G. cilicicus* and *G. peshmenii* were broadly ovate to rounded; the colours were pale brown with size ranging from 2.5-4 mm in length and 2.2-4 mm in width; the seed surfaces were rugose. The details of the seed shape and seed surface are given in Figure 4.

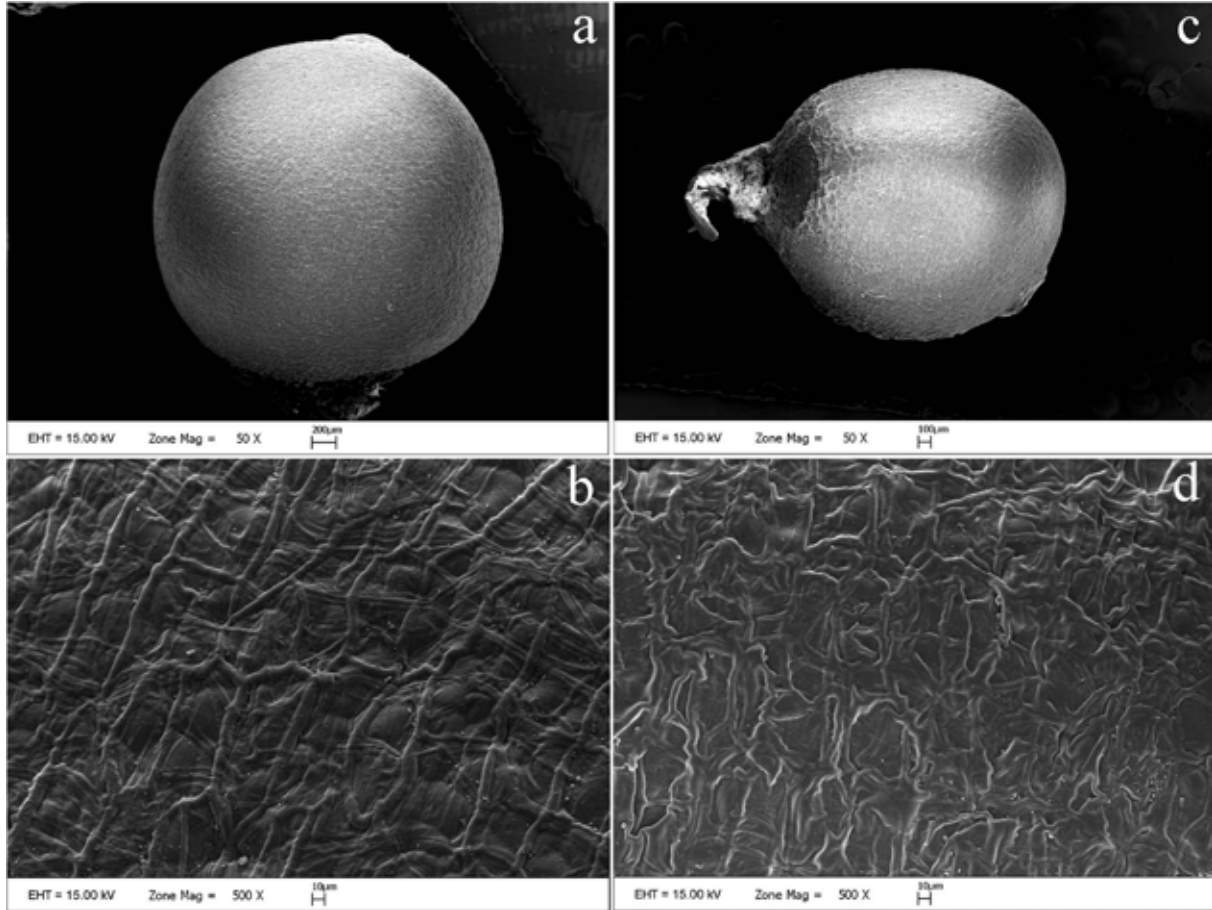


Figure 4. SEM micro-photographs of seeds. (a, b) *G. cilicicus*; (c, d) *G. peshmenii*

### 1.2. Palynological study

The main palynological features of *G. cilicicus* and *G. peshmenii* are summarized in the Table. It was established from the LM (Figure 5) and SEM (Figure 6) investigations that, the pollen grains are monad, monosulcate and heteropolar; they are medium in size (26-50  $\mu\text{m}$ ). The pollen shapes (based on P/E ratio) are prolate and elliptical in polar view. These results are similar to those of earlier studies (Dönmez & Işık 2008; Şahin et al., 1997).

The pollen grains of *G. cilicicus* were prolate, polar axis was 25.63-(26.32)-27.68  $\mu\text{m}$ , and equatorial axis was 18.45-(19.71)-21.03  $\mu\text{m}$ . Ornamentation was micro-rugulate; exine was 0.76-1.13  $\mu\text{m}$ ; and intine was 0.5-0.75  $\mu\text{m}$ . The pollen grains of *G. peshmenii* were prolate, polar axis was 25.63-(26.16)-27.68  $\mu\text{m}$ , and equatorial axis was 18.45-(18.94)-20.5  $\mu\text{m}$ . Ornamentation was micro-rugulate; exine 0.77-1.28  $\mu\text{m}$ , and intine 0.5-0.75  $\mu\text{m}$ . The results showed that the pollen grains of *G. cilicicus* and *G. peshmenii* were morphologically similar.

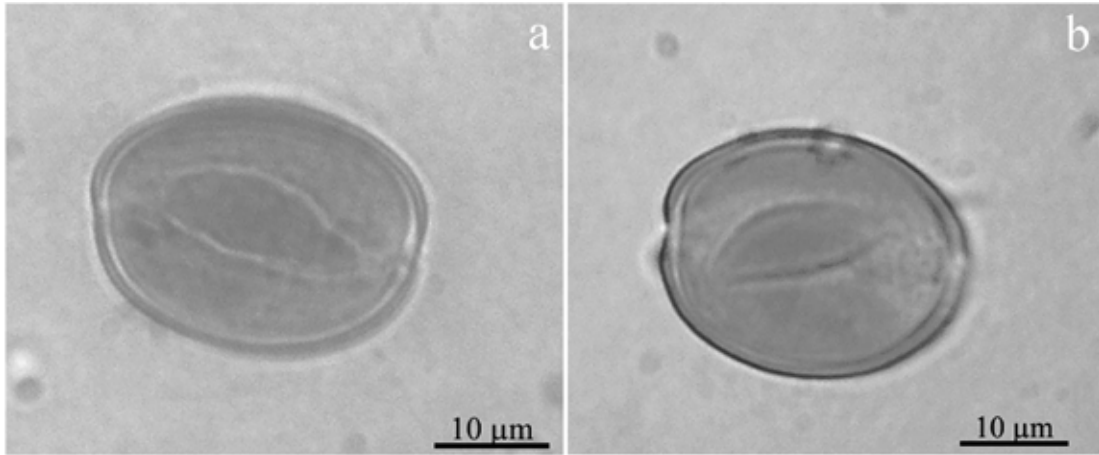


Figure 5: LM photographs of the pollen grains. **a)** *G. cilicicus*; **b)** *G. peshmenii*

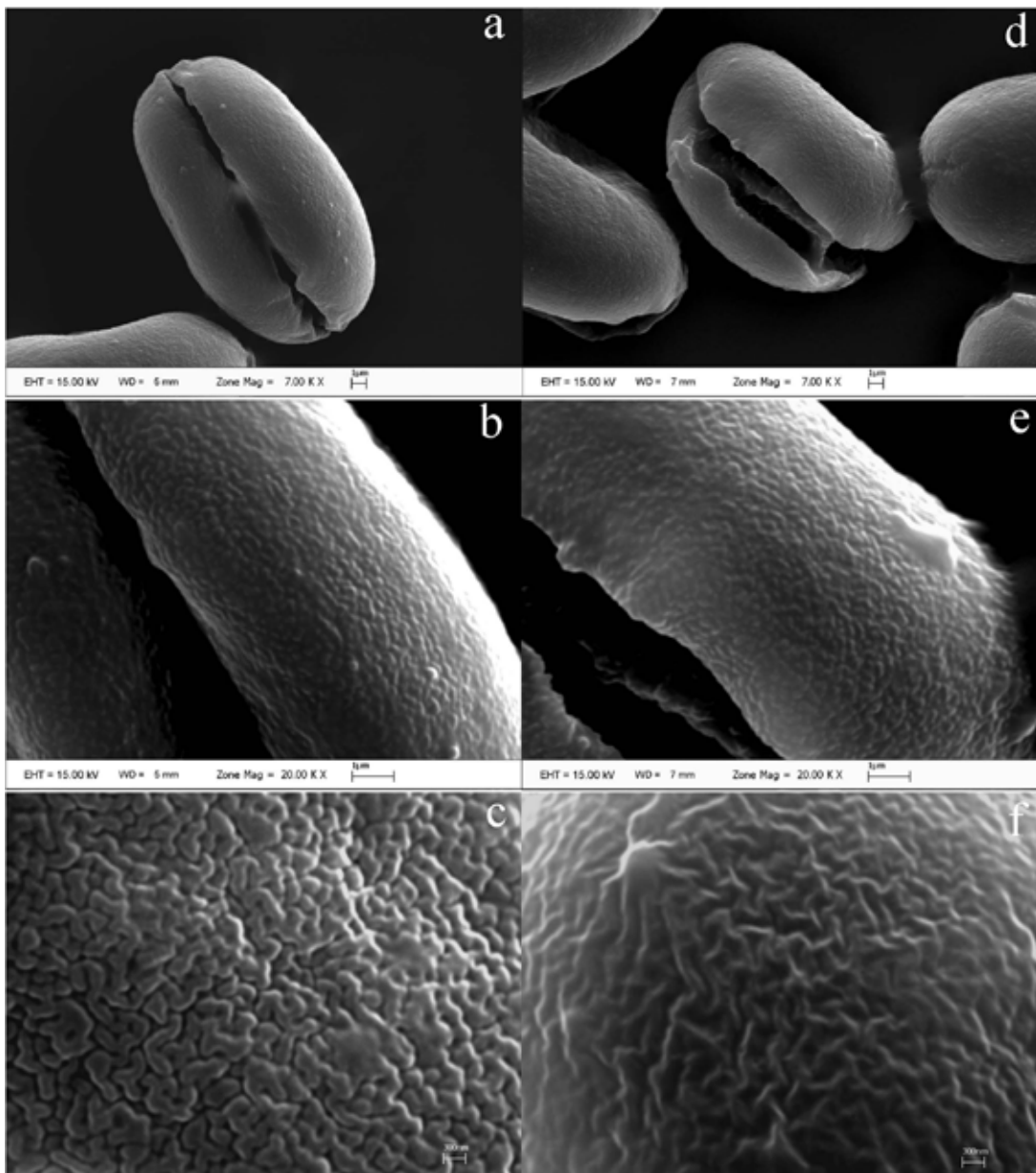


Figure 6: SEM micro-photographs of the pollen and pollen surfaces. **a, b, c)** *G. cilicicus*; **d, e, f)** *G. peshmenii*

### 1.3. Chromosome counts

All species of *Galanthus* counted (with exception of the polyploid clones) have the same basic chromosome number,  $2n = 2x = 24$  (Sveshnikova, 1965). There are different studies in literature reporting the chromosome number of *G. peshmenii*. (i.e. Özhatay, 2002).

According to our chromosomal studies, chromosome number of *G. peshmenii* was counted as  $2n = 24$  and this result is with suitable previous reports for concerning species. But, *G. cilicicus* had to triploid ( $2n = 3x = 36$ ) chromosome numbers (Figure 7). This chromosome number for *G. cilicicus* may be used as diagnostic characters. Therefore, this ploidy level own to the species can be used taxonomically to separate it from closely related species *G. peshmenii*.

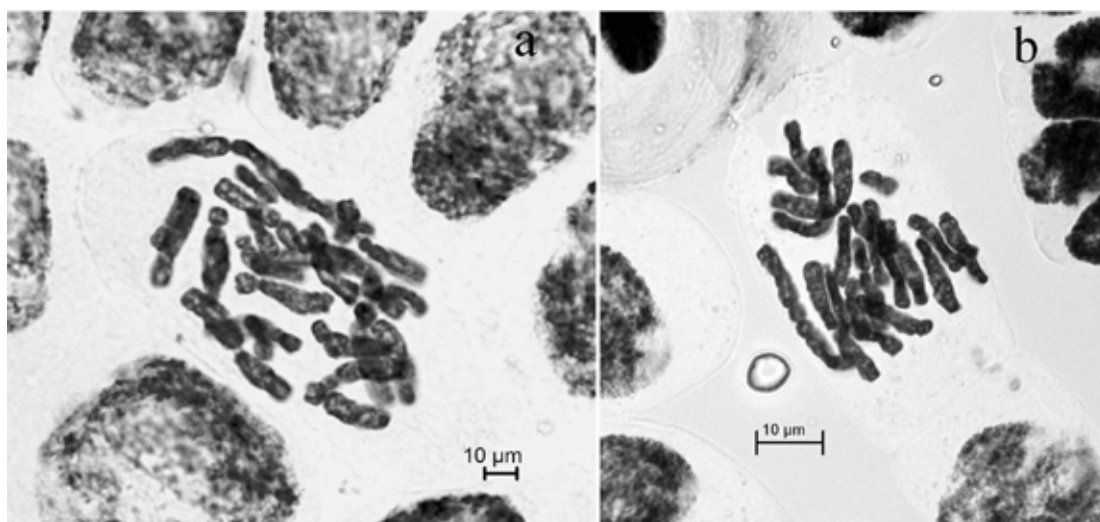


Figure 7: Mitotic metaphase chromosomes of a) *G. cilicicus*  $2n = 36$ ; b) *G. peshmenii*  $2n = 24$ .

## 4. Conclusions

*G. cilicicus* and *G. peshmenii* are closely related to each other and share applanate vernation, linear leaves and a single green mark on each inner perianth segment. *G. cilicicus* is an autumn to winter-flowering plant. The leaves of *G. cilicicus* are several centimeters longer and several millimeters wider than *G. peshmenii* at flowering time. *G. peshmenii* is an autumn-flowering plant. The flowers are produced before the leaves emerge from the soil, or when the leaves are only 1-5.5 cm long. *G. cilicicus* is taller than *G. peshmenii* and has larger leaves and flowers. In *G. cilicicus* apex of the inner perianth segments are often flared with wavy margins, whereas flared inner perianth segments and wavy margins were not observed in *G. peshmenii* during field-studies. The leaves of *G. peshmenii* usually have a faint stripe on upper surfaces, but this faint stripe is very rare in *G. cilicicus*.

In our palynological studies, pollen grains of both species were shown to be prolate shaped, and the size and the ornamentalations of the grains were similar. The sample named as *G. cilicicus* [H. Sümbül 2229 (HUB)] by Dönmez and Işık (2008) was considered to be *G. elwesii* var. *monostictus*. Based on these results, pollen structures of these three taxa were found morphologically quite similar to each other's.

In addition, seeds of the species were quite similar in terms of size, shape and surface.

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