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Anatomical, palynological, morphological, karyological, and ecological investigations on *Gypsophila davisii*

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Gypsophila davisii üzerinde anatomik, palinolojik, morfolojik, karyolojik ve ekolojik araştırmalar

Abstract: *Gypsophila davisii* Barkoudah, an endemic species from Muğla (Turkey), was investigated for the first time as a whole in terms anatomy, morphology, ecology, karyology, and palynology. No druse crystals were observed in stems and roots of examined specimens in contrast to the known. Seeds are pyriform with shiny, smooth grooves on the surface. The pollen grains are polyporate, spheroidal, granulate-microechinate-microperforate. *G. davisii* has 30 somatic chromosomes (2n=30). The samples are compared morphologically with the description given in Flora of Turkey.

Key words: Anatomy, chromosome number, ecology, *Gypsophila davisii*, morphology, palynology.

Özet: Muğla'ya endemik bir tür olan *Gypsophila davisii* Barkoudah, anatomik, morfolojik, ekolojik ve palinolojik açıdan bir bütün olarak ilk kez incelenmiştir. Bilinenin aksine incelenen örneklerin kök ve gövdelerinde hiçbir druz kristaline rastlanmamıştır. Tohumlar armut şeklinde olup yüzeyinde parlak düz tüberküller vardır. Polen taneleri, çok porlu, küremsi, granül-mikroekinat-mikroperforattır. *G. davisii*'nin 30 somatik kromozomu vardır (2n = 30). Örnekler morfolojik açıdan Türkiye Florası'ndaki betim ile karşılaştırılmıştır.

Anahtar Kelimeler: Anatomi, kromozom sayısı, ekoloji, *Gypsophila davisii*, morfoloji, palinoloji.

1. Introduction

The genus name *Gypsophila* Barkoudah was derived with the combination of the Greek terms "gypsos=gypsiferous soil" and "philos=like, prefer", since the members of this genus prefers gypsiferous soils or limestones (Korkmaz et al., 2012). Seventy four *Gypsophila* species, 43 of which are endemic, currently exist in Turkey (Barkoudah, 1962; Huber-Morath, 1967; Davis et al., 1988; Ataşlar, 2000; Ekim, 2012; Armağan et al., 2017). The existence of all the three subgenera (*Gypsophila*, *Pseudosaponaria* and *Macrorhizaea*) of *Gypsophila* increases the importance of this genus in terms of Turkey. Among the 126 species of the subgenus *Gypsophila*, 75 species have distribution in the region containing Turkey, North Iraq, North Iran, Caucasus and Black Sea region (Barkoudah, 1962). The pollens of *Gypsophila* taxa are spheroid and polyporate, and generally have granulate-microechinate-microperforate ornamentations (Ataşlar et al., 2009). Members of *Gypsophila* contains excess amount of calcium oxalate crystals in the structure of their roots and stems (Ataşlar and Ocak, 2017). Although diploid number of the chromosomes (2n=34) is a typical representative of this genus, the differences at a ploidy level has been found between the *Gypsophila* species (Vettori et al., 2015). *Gypsophila* species generally prefer of gyps and erosive areas. They are widespread in arid and semiarid steppe areas and also distributed on dry and calcareous rocks, serpentine rocks, and stony–sandy lands (Korkmaz and Özçelik, 2013).

The aims of this study is to report of pollen morphological, anatomical, karyological and ecological characteristics of *G. davisii* for the first time and thus to provide a contribution to the taxonomy of the genus *Gypsophila*.

2. Materials and Method

The *Gypsophila davisii* specimens which are collected during PhD thesis and the herbarium specimens previously collected within the boundaries of Turkey constitute the materials of the study. The initial samples were collected from Muğla province (Figure 1). During field works ecological and morphological characteristics of the populations were recorded, photographed in their natural habitat and some of them were collected to prepare herbarium specimens and some of them keep in 70 % ethanol. For further ecological studies, soil samples were also collected from a deep of 20 cm. Morphological, studies was carried out in the herbarium of VANF, HUB, GAZI, K, and E (herbaria acronyms according to Thiers 2016+), while anatomical, ecological, palynological, and karyological studies were conducted in the laboratories of Yüzüncü Yıl University.

2.1. Morphological studies

Morphological findings were obtained from the field, herbaria and laboratories works. Macromorphological and some micromorphological measurements were performed from the herbarium samples. High resolution photographs of the specimens kept in different virtual herbaria were also investigated. The terminology of Stearn (1992) and Bittrich (1993) was adopted to describe the seed coat.

2.2. Anatomical studies

Anatomical findings were obtained by thin sections from the roots, stems and leaves of the samples kept in %70

ethanol. These sections were stained with Fast Green and Safranin dyes. Metcalfe and Chalk (1957) were followed for the anatomical terminology.

2.3. Ecological studies

Soil samples which were transported to the laboratory in cloth bags were used for further ecological studies. They were dried and passed through from 2 mm sieves. Then, the physical and chemical (texture, pH, salinity, CaCO3, N, P, K, Ca, Mg, Fe, Cu, Zn, Mn and Na) analysis of them were carried out by using standard techniques, in the laboratories of Agricultural Analysis Laboratory of Eğirdir Fruit Research Station.

2.4. Palynological studies

Pollen grains were investigated using both light and scanning electron microscopes (SEM). Wodehouse (1935) technique was followed for light microscopy preparations. Pollen determining terminology is in accordance with Hesse et al. (2009). Terminology for pollen morphology proposed by Hesse et al. (1966) and Ataşlar et al. (2009) was considered.

2.5. Seed surface studies

Seed surface investigations were carried out using a Leica EZ4 Stereo Microscope. Shape, colour, length, width, surface structure, and length/width ratio of the seeds were determined. For this purpose, more than 20 seeds were measured and photographed. A millimetric ruler was used to measure the length and the width of the seeds, and the smallest and the biggest average data were provided.

Figure 1. The distribution map of *Gypsophila davisii*. ▲ In Flora of Turkey, ● in this study.

2.6. Karyological studies

Mature seeds of the collected *G. davisii* samples were germinated in petri dishes and root tips of these plantlets were used for karyological investigations. The seeds were germinated within one week at room temperature. Though methods used to obtain chromosomes vary more or less according to the researchers and the species, the common first step for all the methods was fixation, hydrolysis and staining (Darlington and La Cour, 1976; Elçi, 1994, Atasagun et al, 2016).

Root tips were pretreated with 1% α-monobromonaphthalene at 4°C for 16-17 h. Root tips were fixed with Farmer fixative (3:1 ethyl alcohol: glacial acetic acid) for 24 h at 4°C. The material was hydrolyzed with 1N HCl for 13-14 minutes at room temperature after the alcohol-extracted root tips have been washed with distilled water several times. The chromosomes were stained with 2% acetic orcein and mounted in 45% acetic acid. Permanent slides were prepared using Canada balsam. Photographs were taken using a Leica EZ4 Stereo Microscope.

3. Results

Gypsophila davisii Barkoudah, Wentia 9: 62 (1962), (Figure 2, 3, and 6).

Type: Turkey-Muğla: Gökçeova near to Sandras mountain, 1700 m, 23.07.1947, Davis 13516a (holo. E, iso. K)

Perennial, caespitose, glabrous, minutely scabrous. Stem numerous, flowering stems 4-16 cm. Basal leaves rigid, linear, acute, subspiny, ciliate below, distinctly three veins, keeled in the middle vein, scarious papillous at margins, 7-10 × 0.8-1.2 mm, numerous. Stem leaves similar to basal leaves, 2-8 × 0.3-0.8 mm, 3-4 pairs. Inflorescence minutely scabrous somewhere, dichasial (from 2/3 of the body), 3-7 flowered. Bracts leafy, lanceolate-ovate, green, finely ciliate at the base, 1.2-2.3 × 0.4-0.5 mm. Pedicels 3-13 mm. Calyx campanulate-turbinate, 2.5-3.5 × 2-3 mm. Calyx teeth ovate, subobtuse, ciliate, 0.9-1.3 × 0.9-1.1 mm. Petals pink with darker veins, linear-cuneate, obtuse, 4.2-5 × 1-1.6 mm. Style 1.6-2.3 mm. Ovarium 1.3-2.0 × 1.0-1.3 mm. Capsules 3.8-4.6

× 2.5-2.6 mm. Number of ovules 8-11. Seeds pyriform, flat tubercles, dark brown to black, shiny, 0.98-1.17 × 1.08-1.39 mm.

Flowering-fruiting: July
Habitat: Subalpine meadows
Altitude: 1750-1770 m

Figure 2. Herbarium sample of *Gypsophila davisii* (VANF162322!).

Conservation status: Its habitat is located in the Sandras Mountain which is one of important Nature Areas in Turkey. This endemic species has a distributing area of about 10 km^2 just in Muğla. The picnickers, the creation of recreational areas and grazing threat its habitat. Based on the criteria B1a, B1b(iii), B2b(iii, v), we propose to assess *G. davisii* as Critically Endangered (CR) (IUCN 2014).

Specimens examined: Muğla: Around Gökçeova lake, on organic matter-rich (wet) soil deposited on the serpentine bedrock, 37°03'34.3"N 28°48'17.5"E, 1770 m, 10.07.2009, VANF162322 (Figure 2); subalpine meadows, 37°03'39.4"N 28°48'22.4"E, 1752 m, 18.07.2017, Armağan 7708 (Figure 3).

Anatomic properties:

Stem: Secondary enlargement was not observed. A thin cuticle layer exists as an outermost layer and an epidermal layer, which is composed of oval cells, takes place just under this cuticle. Intercellular space doesn't exist, but a single celled scabrid which was formed by the differentiation of epidermis, takes place. Cortex starts just under the epidermis and much more coloured sclerenchyma cells take place between them. Phloem cells lined up beside the cortex. Just after phloem, much larger xylem cells exist. Thee pith takes place at the innermost region where many large cells with hyaline appearance are seen. No druse crystals were observed in the stems of examined specimens (Figure 4c).

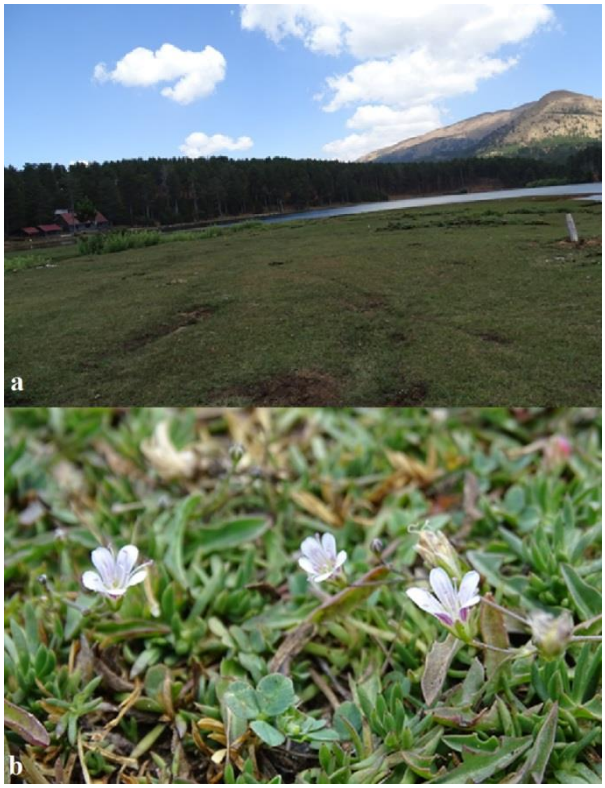

Figure 3. The habitat (Gökçeova lake and Sandras mountain) (a) and habit (b) of *Gypsophila davisii.*

Caudex and root; at the outermost layer, 6-7 layered cork exists as the protective tissue. Cortex takes place just under the cork layer. An endodermis layer which is composed of smaller cells compared to cortex cells exists at the innermost layer of the cortex. At the central cylinder which starts just under the endodermis, vascular bundles take place. No druse crystals were observed in the roots of examined specimens (Figure 4a, 4b).

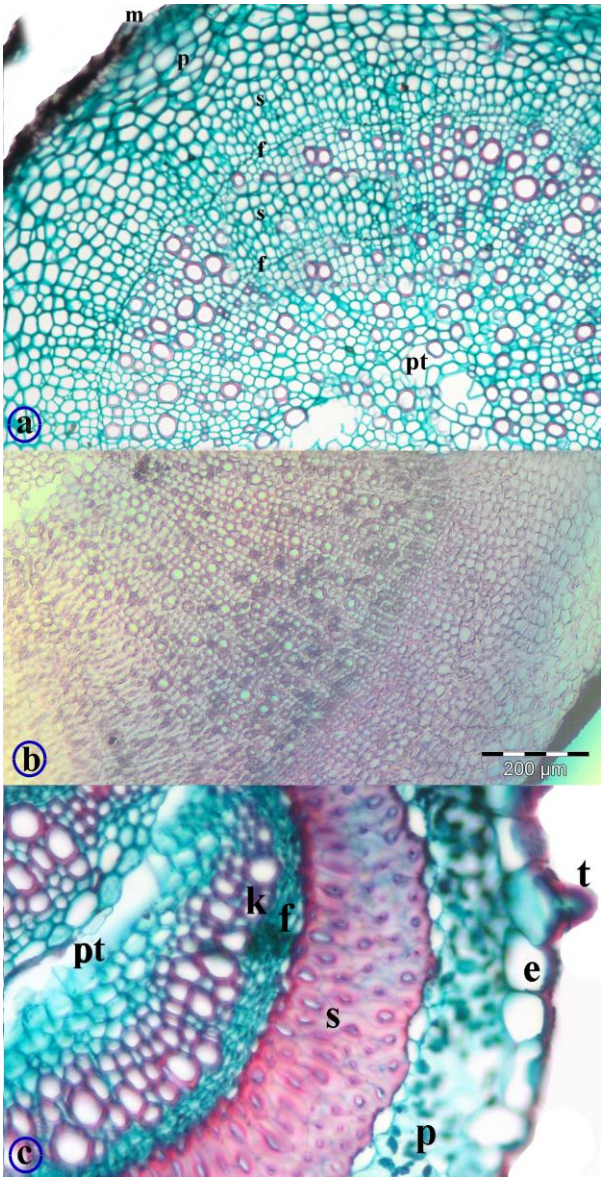

Figure 4. The root (a), caudex (b) and stem (c) anatomy of *Gypsophila davisii* (m. cortex, p. parenchyma, s. sclerenchyma, f. phloem, k. xylem, pt. pith, t. scabrid).

Leaf; an epidermis which is composed of ovate-to rectangular and single layered cell, forms the outermost layer. Mesophyll is composed of 9-13 layered cells which start from upper epidermis and extends till lower epidermis. There isn't a parenchymatic differentiation in mesophyll. Other than chloroplasts, druse crystals are seen seldomly. Venation is parallel and the central vein is larger compared to others (Figure 5a).

Through the sections taken from the surface of the leaves, amaryllis type stoma, the form belonging to the family Caryophyllaceae was observed. Neighbouring cells have a varying shape from ovoid to rectangular with more or less smooth cell walls (Figure 5b)

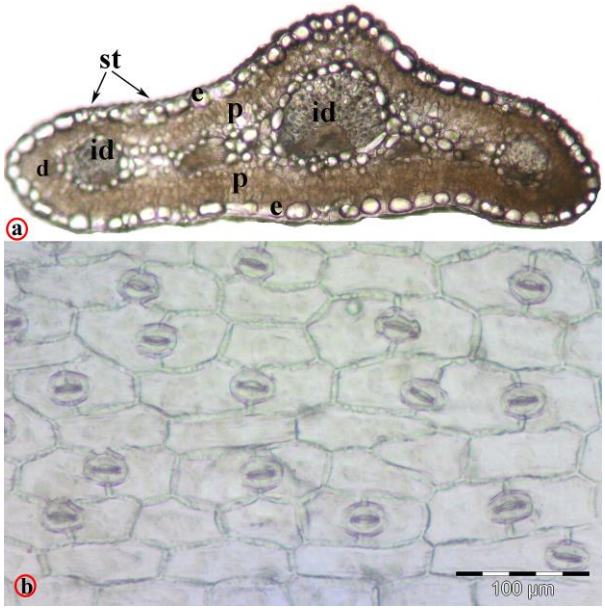

Figure 5. The leaf (a) and leaf surface (b) anatomy of *Gypsophila davisii* (e. epidermis, st. stoma, d. druse crystal, id. vascular bandle, p. palisade parenchyma).

Seed Morphology:

The seeds are pyriform, mature seeds dark brown to black, 0.98-1.17×1.08-1.39 mm. Unripe seeds red. Seed surface with flat tubercles (Figure 6).

Pollen Morphology:

The pollen grains are polyporate, spheroidal, granulate-microechinate-microperforate; the equatorial diameters are 25.87561-28.397911 µm (Figure 7).

Ecological properties:

Gypsophila davisii, prefers soils with medium texture; neutral; unsalted; low in terms of lime, phosphorus, potassium, calcium, sodium and zinc; medium in terms of magnesium and cupper; rich in terms of iron and organic matter (Table 1). Though it doesn't have a side preference, it grows on wet soils accumulated on serpentine bedrock.

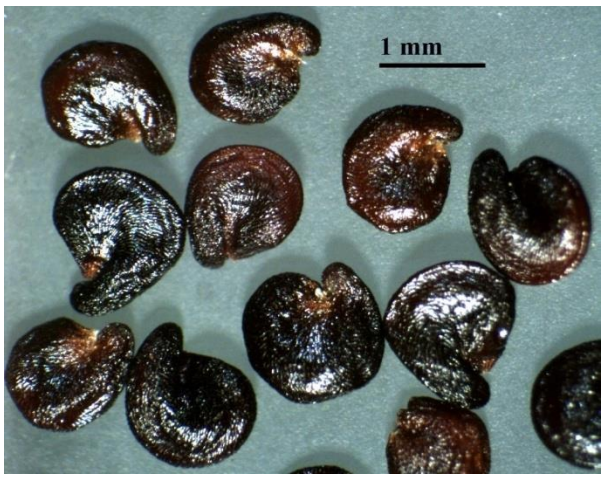

Figure 6. The seeds of *Gypsophila davisii*.

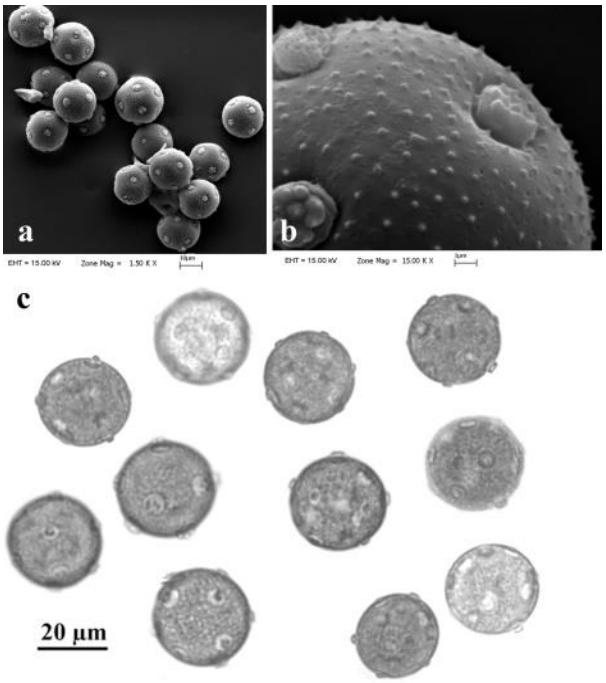

Figure 7. The pollen grains of *Gypsophila davisii* (a & b. SEM, c. LM).

It was determined to have a natural distribution around Köyceğiz (Muğla) district. It is generally distributed on red-brown Mediterranean soils, accumulated on mesosoic peridatit bedrock. Annual precipitations, summer time precipitations, precipitation regimes, annual average temperatures, maximum mean temperature of the hottest months, minimum average temperatures of the coldest months and the climate types of Köyceğiz, Dalaman and Muğla are given in Table 2 in accordance with Emberger (Akman, 2011). There have also been sedimentary or volcanic rocks around the region.

Karyological properties:

The chromosome numbers of *G. davisii* an endemic species in Turkey were examined. The results of our studies showed that the chromosome number of *G. davisii* was 2n = 30. The basic chromosome number of species was determined as x = 15 (Figure 8).

4. Discussions

The other species (*G. repens* L., *G. nana* Bory et Chaub., *G. spergulifolia* Grisebach, and *G. achaia* Bornm.) of the section *Gypsophila* have distributions in Europe. *G. davisii* is known from a few localities only within the boundaries of Muğla province in Turkey. It prefers wet meadows as habitat. The major difference of this species from the others is the smooth, bright and pyriform structure of the seeds. As a result of this study, the description of the species in Flora of Turkey was expanded by adding the missing properties (Table 2).

Table 1. Soil properties of *Gypsophila davisii*

Satur.	EC	pH	Lime	Org. Subs.	P	K	Ca	Mg	Na	Fe	Cu	Mn	Zn
50	125	6.66	2.2	4.9	11	74.8	858	590	14	63.6	0.7	24.5	1.2

Table 2. Climatic data, precipitation regimes and climate type of Köyceğiz, Dalaman and Muğla (Anonymous 1).

Locality	Annual rainfall (mm)	Summer rainfall (mm)	Precipitation Regime	Average temperature (°C)	Max. av. temp. of the hottest month (°C)	Min. av. temp. of the coldest month (°C)	Climate type
Köyceğiz	1122.1	23.8	WinterAutumnSpringSummer	18.3	35.0	4.7	Humid sub-temperate Mediterranean
Dalaman	1080.9	6.2	WinterAutumnSpringSummer	18.0	33.7	5.1	Humid sub-temperate Mediterranean
Muğla	1209.2	38.4	WinterSpringAutumnSummer	15.0	33.4	1.6	Humid sub-cool Mediterranean

Figure 8. The metaphase chromosomes of *Gypsophila davisii* (2n=30).

None of the determined samples have a view preference. *G. davisii* prefers to grow at mountain meadows where excess amount of water exist. Peridotites belonging to mesozoic exist in the habitats of *G. davisii* and it lives on the soils formed by the metamorphosis of these rocks.

Druse crystals don't exist in the pith, pith branches and cortex of *G. davisii*. They were not observed in preparations from the cross section of the stem either. Although druses are present in woody species of the Caryophyllaceae family (Carlquist 1995), it was not found in stem and caudex of *G. davisii*. It may not have produced the druse crystals as *G. davisii*'s habitat is humid meadow. The epidermis of the stem has prominent protrusions. It is diacytic in terms of the number of cells surrounding the stoma.

Anatomy of root, stem and leaves well fit with the descriptions of Metcalfe & Chalk (1957), Barkoudah

(1962) and Ataşlar & Ocak (2017). Leaves of *G. davisii* are isolateral and have 2 or rarely 3 neighbouring cells. Stoma neighbouring cells rectangular underside while generally ovate to square shaped at upper side. Stomal cells are abundant at upper side of the leaves.

The chromosome number ranges between x=6 and 34 in the genus *Gypsophila*. The chromosome number of *G. perfoliata* L. was x=17, 18, 24, and 34 (Rice et al. 2014). It could be suggested that there is polyploidy in some species of *Gypsophila* based on these amounts. The chromosome number of *G. davisii* an endemic species in Turkey was examined. The results of our studies showed that the chromosome number of *G. davisii* was 2n = 30. The basic chromosome number of species was determined as x = 15 such as *G. cerastoides* D.Don (Sharma, 1970).

Table 3. Morphological comparison of our samples and the species description given in Flora of Turkey for *Gypsophila davisii*.

	Flora of Turkey	**Our samples**
Habitat	Subalpine meadows, 1700 m	Subalpine meadows, 1700-1800 m
Stem	Caespitose, 5-10 cm	Caespitose, 4-16 cm, minutely scabrous
Basal leaves	Linear, ciliate at the base, acute, subspiny, 5-10×0.6-1 mm, numerous	Linear, scarious papillous at the margins, acute, subspiny, distinctly three veins, keeled in the middle vein on the back, ciliate at the base, 7-10×0.8-1.2 mm, numerous
Stem leaves	-	2-8 × 0.3-0.8 mm, 3–4 pairs, linear, acute, scarious papillous at the margins, ciliate at the base
Inflorescence	-	Minutely scabrous somewhere, dichasial, branched 2/3 of stem
Flowers	2-7	3-7
Pedicel (mm)	5-13	3-13
Petals	Linear-cuneate, obtuse-retuse, pink with darker veins, 4-6 mm	Linear-cuneate, obtuse, pink with darker veins, 4.2-5×1.0-1.6 mm
Calyx	Campanulate-turbinate, 3-3.5 mm	Campanulate-turbinate, 2.5-3.5×2-3 mm
Calyx teeth	Ovate, subobtuse, ciliate	Ovate, subobtuse, ciliate, 0.9-1.3×0.9-1.1 mm
Bracts	Lanceolate-triangular, green, ciliate at the margins	Leafy, lanceolate-ovate, green, ciliate at the base, 1.2-2.3×0.4-0.5 mm
Style length	-	1.6-2.3 mm
Ovarium	-	1.3-2.0×1.0-1.3 mm
Capsules	-	3.8-4.6×2.5-2.6 mm
Ovules	-	8-11
Seeds	-	Pyriform, flat tubercles, dark brown-black, shiny, 0.98-1.17×1.08-1.39 mm

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