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Morphological, Anatomical, Palynological and Cytological Investigation on *Silene urvillei* Schott. (Caryophyllaceae)

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Abstract

Silene urvillei Schott. (Caryophyllaceae) which is endemic to West Anatolia and Aegean Islands was investigated morphologically, anatomically, palynologically and cytologically. The plant is perennial and the leaves are spiny therefore it is different from other Silene L. species. Stem and leaves are covered with glandular hairs, the stem has multi-layered collenchyma. Petals are whitish, anthophore is hairy. Seeds are reniform, suture outlines of testa cells are lobed and sinuate. Pollens are tectate, spheroidal, microechinate and microperforate, 15-24 pores. Chromosome number was founded 2n= 24.

Key Words: Anatomy, cytology, endemic, morphology, palinology, Silene urvillei.

INTRODUCTION

Silene is one of the largest genera of flowering plants in the world, consisting of ca. 700 species, many of which are distributed in the Mediterrenean region [1]. One of the important genera of this family is Silene in Turkey is Silene. Since 1967, 14 new species and three subspecies have been described from Turkey. Thus, the total number of Turkish Silene taxa is c.160 and the endemism percentage of Silene in the Flora of Turkey is approximately 48% [2–9].

Chowdhuri made a revision of *Silene* taxa in the world [10], and Melzheimer conducted biosystematical studies of *Silene* taxa available in the Balkans [11]. Both Chowdhuri and Melzheimer used seed testa cells as diagnostic characters. Ghazanfar, in his palynogical investigation of 44 taxa represented in the section *Pinifoliae* Chowd. demonstrated that the sexine sculpture of *Silene caryophylloides* (Poiret) Otth subsp. *subulata* (Boiss.) Coode & Cullen was semireticulate [12]. Yıldız and Çırpıcı studied seed morphology of 19 *Silene* species from Turkey (Northwest Anatolia) [13]. The other studies known in the seed morphology of the *Caryopyllaceae* members include those of Chowdhuri [10], Melzheimer [11] and Yıldız and Çırpıcı [14]. Yıldız, showed 37 *Silene* taxa from Turkey using LM and SEM, and demonstrated the precense of various type of exine such as tectate, semitectate; spinulose (microechinate), microperforate and semireticulate [15–17]. Morphological description of *S. urvillei* presented on Flora of Turkey and East Aegen Islands and Flora of Hellenica however, no other work on anatomical, palynological and cytological is available [2,18].

S. urvillei is an endemic species, restricted to Eagean Islands and West Anatolia. Conservation status is vulnerable (VU) according to the most recent IUCN categorization [19]. The purpose of this study is to investigate anatomical, palynological and cytological properties of *S. urvillei*, in addition to the morphological features.

MATERIALS AND METHODS

Plant samples were collected from natural populations in the Sipil (Manisa) mountain between May 2004- July 2004. A portion of the specimens were placed into 70% alcohol to be used in anotomical studies. Seeds collected from the plant specimens containing mature fruits were deposited into envelopes in the field to be used in seed examinations. Samples were collected from the following location at different times:

[B1] Manisa: Turgutalp village above, rocky area, Sipil Mountain, 1200m.

Taxonomical identification of the plants was done according to Flora of Turkey and Flora Hellenica [2,18]. Owing to their significance in the determination of *Silene* species, diagnostics such as plant lenght, arrangement of the basal and cauline leaves, inflororescence, sepal, petal and fruit (capsula) were used in the morphological study. Seed morphology of the plant was assessed according to Prentice [20] and Stearn [21] with the help of microphotographs obtained from SEM images. For preparing a cross section of plants stem and leaf were used in anatomical analysis. All sections were taken manually and not stained. Hairs on stem and leaf surface and stomata on the leaf surface were investigated.

For palynological studies, the pollen grains were acetolysed according the method of Erdtman [22]. Different morphological characters of pollen parts such as pollen diameter, pore diameter, distance between two pores and exine thickness were measured by means of light microscopy. About 50 pollens were used to obtain these measurements. The descriptive terminology of Punt et al. and Moore et al. [23, 24] are used in this paper. For SEM investigations, seeds and pollens were directly mounted on stubs using double-sided adhesive tape. Samples were coated with gold/palladium in POLARON SC 7620 ion-sputter and then observed by standard techniques using a LEO 440 scanning electron microscope.

The seeds were germinated on filter paper placed inside petri dishes. Root tips which have a length of 0.5 mm–1 cm was removed and pretreated with 0,5% colchicine for 1–5 hour the aceto-orcein squash method [25] was applied to the root tips. Olympus CX21FS1 microscope with D plan 1.00–1.25 160/0.17 oil immersion objective and NFKx3.3 LD 125 lens and photomicrographs were taken by Pentax Optio S camera were used for anatomical, palynological and cytological studies.

RESULTS

Description (Figure 1–3): Caespitose, perennials. Stems erect, scabrid upper glabrous, 15–30 cm. BASAL LEAVES triquetrous, rigid, 25–30 x 1 mm, sparsely puberulent, very spiny. Inflorescence raceme-like, many flowered. Pedicel 1–40 mm. Calyx 10–12,5 mm, glabrous, strongly 10-nerved (Fig.1.a). Petals whitish, deeply bipartite, lips 4–5 mm, upper margin densely long-cilliate, lipbase no ligule but small bulge. Anthophore 4–6 mm, puberulous. Immature capsule oblong-ovoid, c. 5–6 mm (Fig. 1.b). Seeds reniform. Fl. 5–7.

***Habitat*:** Macchie, limestone rocks, cliffs and walls, in Southeast and Southwest slopes and frequently in sunny, sometimes in North, up 1250 m.

Distribution: Muğla, Manisa, İzmir (Turkey), Lesvos, Chios, Samos and Kos islands (Greece).

Figure 1. *S. urvillei* **a.** Flower and **b.** Capsule

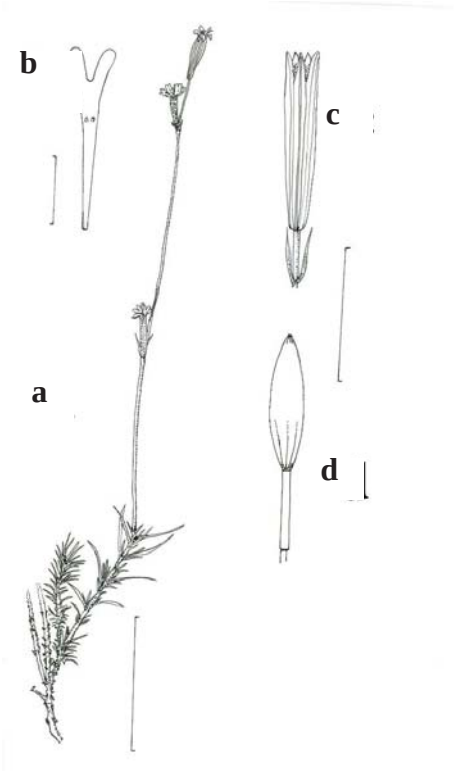


Figure 2. a. General appearance (— : 5 cm), b. Petal, c. Calyx, d. Capsule (b, c, d; — : 1 cm).

Leaf Surface Morphology

Upper and lower epidermis cells of leaf are covered with thin cuticle. Upper cuticle is thicker than the lower one. Glandular hairs and stomata are present on both surfaces of leaves (Figure 3).

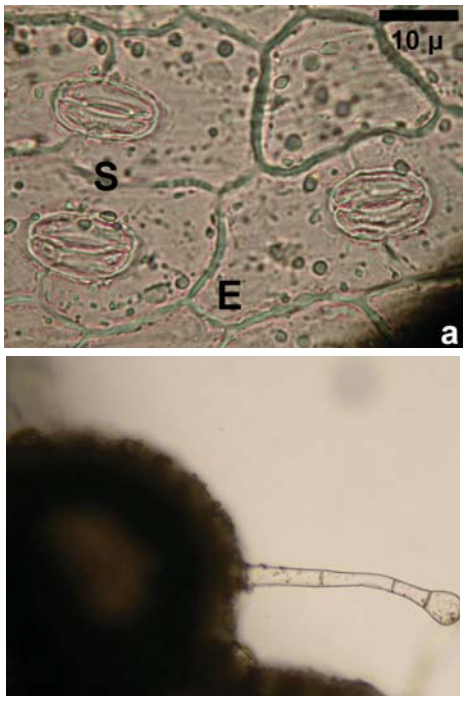


Figure 3. a. Apperance of upper leaf surface S: stomata E: epiderma **b.** Glandular hair (x 1000).

ANATOMICAL PROPERTIES

Stem

Epidermis is single layered. Shape of this cell is hexagonal or ovoidal. There are multicellular capitate glandular hairs on epidermis. Hypoderma is 2–3 layered. Following cortex cells are 4–6 layered sclerenchymas with very thick cellwall and than, it has 10 – 12 layered parenchyma with oval cells and vascular bundle (xylem and phloem tissues). Cambium is not distinguishable. The pith is large and consists of parenchymatic cells (Figure 4).

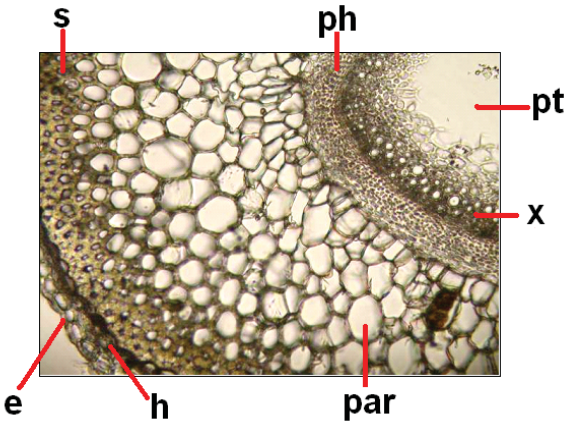
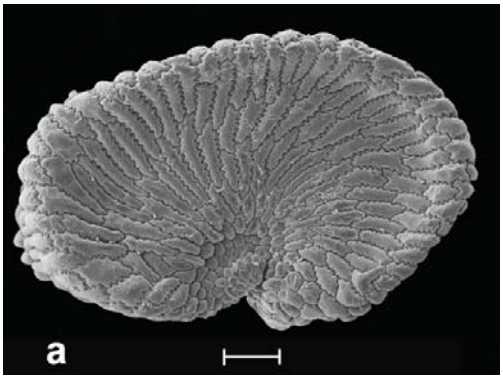


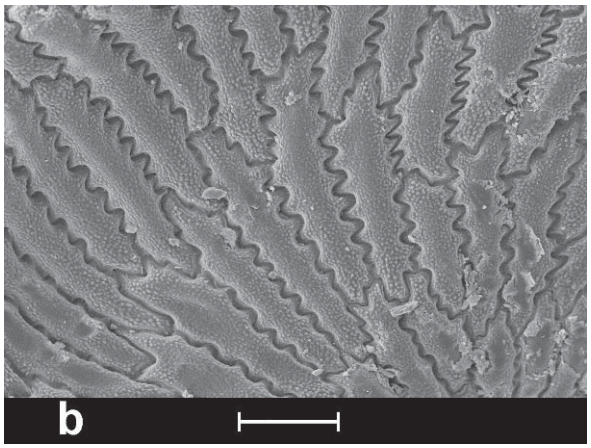
Figure 4. Cross-section of stem of *S. urvillei* e: epidermis, h: hypoderma, s: sclerenchyma, par: parenchyma, ph: phloem, x: xylem, pt: pith (x 40).

Seed properties (Table 1, Figure 5)

Table 1. Seed properties of *S. urvillei*

Seed characteristics	
Seed lenght x width (mm)	1.6 – 1.8 x 0.8 – 1.1
Lenght / width ratio	1.57
Testa cell lenght x width (µm)	40 – 75 x 160 – 260
Teeth of testa cell lenght x width (µm)	9 – 12 x 15 – 19
Seed type	Reniform
Colour	Light brownish or greyish brown
Surface type	Concave, sometimes flat
Seed back	Concave and flat
Hylar zone type shape	Recessed
Seed surface granulation	Medium
Suture outline	Lobate or sinuous
Number of suture point per plate	13 – 24
Seed tubercle shape	Obtuse
Seed tubercle tip colour	Greyish or light brown





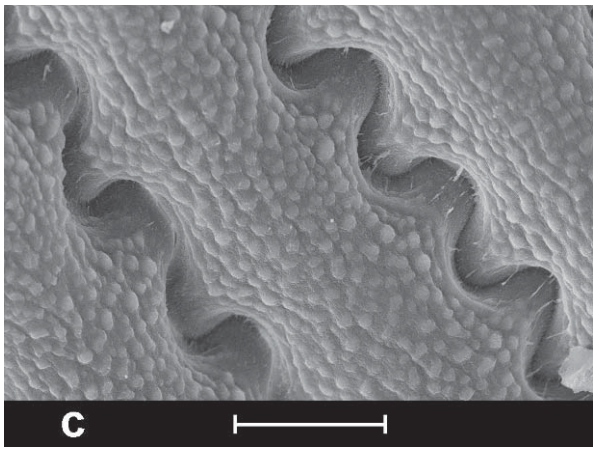
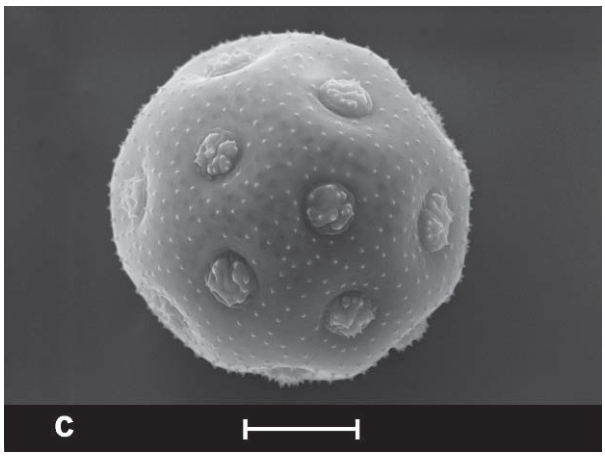


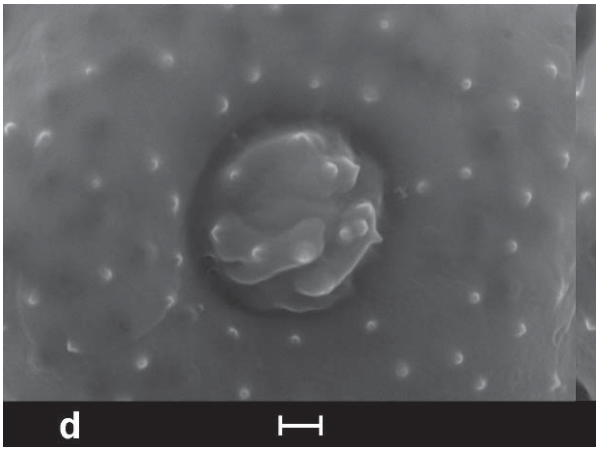
Figure 5. SEM seed photographs of S. urvillei. a. General appearance, b. Testa cells c. Suture outline and surface granulations. (Scale a: 200 µm; b: 100 µm; c: 30 µm)

Palynological properties (Table 2, Figure 6)

Table 2. Pollen properties of *S. urvillei*.

Pollen characteristics	
Diameter of pollen (µm)	32.07 – 36.18
Diameter of pore (µm)	5.15 – 6.21
Distance between two pores (µm)	5.9 – 10.7
Number of granules on pore	15-19
Spinule length x base width (µm)	0.383–0.411x0.446–0. 477
Diameter of microperforate (µm)	0.2 – 0.45
Number of pore per polen	15 – 24
Polen type	Spheroidal
Ornemantation	Microechinate
Polen structure	Tectate
Structure of pore aperture	Columella is protuberant





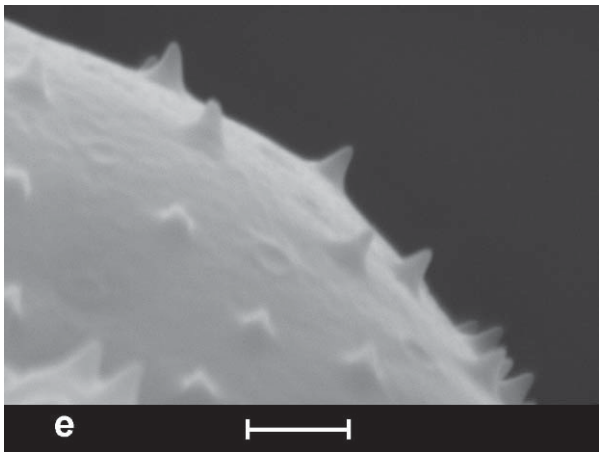


Figure 6. Light (a,b) and SEM (c, d, e) microphotographs of pollen morphology of *S. urvillei*. a. Structure, b. Surface ornamentation, c. General appearance, d. Pore, e. Microechinates (Scale a,b,c: 10 µm; d,e: 1 µm).

Cytological properties

The cromosome numbers of *S. urvillei* was determined to be 2n= 24 (Figure 7). The chromosome lenghts are about 1,54 – 2,63 µm. No differences in the chromosome numbers or any abnormalities in the chromosomes were observed in chromosome investigations carried out on different plant specimens of *S. urvillei*.

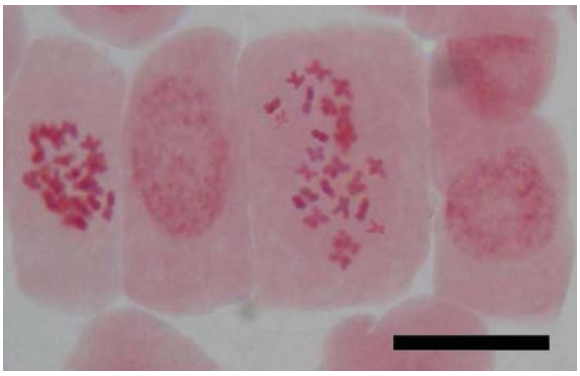


Figure 7. Mitotic metaphase chromosomes in root tip cells of *S. urvillei* (Scale 10 µm).

DISCUSSION

In this study the morphological, stem anatomical, palynological and karyological features of *Silene urvillei* that is endemic to West Anatolia (Eagean) and East Aegean Islands. In the results presented here, the morphological properties of *S.urvillei* showed many similarities compared to the Flora of Turkey [2].

In our anatomical study, it was determined that the species have a typical dicotyledones stem. However, the cambium is not distinguishable and the cortex of the stem is rather broad in cross section. The sclerenchyma tissue is thick and surrouns all around of the stem (Figure 3). *S. urvillei* grows on rocky, windy and boisterous habitats. *S. urvillei* is adapted to its region thanks to scleranchyma especially.

The seeds as a whole bear numerous morphological characters which can be used for taxonomic purposes. The ornamentation features of the seed surface and its relief are different from one species to another, even by high magnifications (X500). This fact has been noted as an important taxonomical criterion. Davis and Heywood emphasized the use of seed characters as these are reliable and constant within taxa [26]. The advanced families usually have complex seed types. Melzheimer is distinguished and revisioned for *S. fabaria* (L.) Sm. subsp. *thebana, S.ionica* Hal. and *S. fabaria* (L.) Sm. subsp. *fabaria* taxa according to seed morphology [27]. Melzheimer also used seed surface micromorphological characters of *Silene* in Flora of Iran [28].

Yıldız and Çırpıcı worked on seed morphology of the *Silene* species distributed in Turkey, using a stereo and scanning electron microscope [13]. In their study, seed structures were termed reniform, ovate; seed tubercles low round, and long cylindrical. In the other study on *Caryophyllaceae* species of North Anatolia, Yıldız stated the seed types of the *Silene* species [*S.montbretiana* Boiss., *S.caryophylloides* subsp.*subulata* (Boiss.) Coode&Cullen, *S.conica* L.] as reniform, tubercles as obtuse, seed surface as flat, concave and convex, testa surface cell walls (suture outline) as sinuous and sharp toothed [29]. *S.caryophylloides* subsp. subulata and *S.urvilleii* are in the same section. The seed morphology of these species are compared on table 3. The back side of seed, surface granulation and number of suture point per plate (testa cells) are very different properties from each other especially.

Table 3. Comparation of seed morphology of *S. urvillei* and *S. caryophylloides* subsp. *subulata*.

Seed	***S. urvillei***	***S. caryophylloides*** subsp. ***subulata*** Yıldız (2002)
Surface type	Concave or flat	Concave
Back type	Concave and flat	Winged flat
Hylar zone type shape	Recessed	Recessed
Surface granulation	Medium	Course and medium
Number of suture point per plate	13 – 24	6–10
Suture outline	Lobate or sinuous	Sinuous
Tubercle shape	Obtuse	Obtuse

Palynological studies of some *Silene* taxa revealed that pollens are generally spheroidal, periporate, tectate, microechinate-microperforate and semireticulate [12,15–16,30]. *S. urvilleii* was investigated had similar results (Table 2). In *Silene caryophylloides* subsp. *subulata* collected from Turkey by Ghazanfar, diameter of pollen 35–44 µm; diameter of pore 4–5 µm; distance between two pori 4–5 µm; number of pore 45–49; sculpture of ektexine semireticulate are recognized [12]. Average of pollen dimeter of same species mentioned are 44.57±2.71 µm, pore diameter 5.93±0.80 µm, distance between two pores 5.29±1.06 µm, number of pore 34–40, ornamentation of exine (sexine) semireticulate are recognized [15]. However, in the present study, pollen diameter and distance among pori are comparatively smaller, also the number of pore is less in number (Table 4).

Imperforate exine and fewer number of pori are generally accepted as primitive [31–33]. The pore number of *S. caryophylloides* subsp. *subulata* is more than *S. urvilleii* (Table 4). *S. caryophylloides* subsp. *subulata* regarding evolution are more advanced than *S. urvilleii*. The phylogenetic order of Flora of Turkey seems to be true according to this result [2].

In the biosystematic revision by Melzheimer on *Silene* taxa distributed in the Balkans, he also determined the chromosome number of all the species which he studied [11]. They had 2n=24 for all of the studied taxa, which agrees with the data obtained in our study. In a karyological investigation carried out on 19 *Silene* species distributed in Turkey [34] that based on x=12. The chromosome numbers of the 14 species were 2n=24 (2x) and 4 species were 2n=48 (4x). The chromosome number of *S.urvilleii* was 2n=24 in Greece [18]. In the karyological investigation we carried out, the chromosome number and the basic chromosome number of *S. urvilleii* was also determined as 2n=24 (x=12).

Table 4. Comparation of polen measurement of *S. urvillei* and *S. caryophylloides* subsp. *subulata*.

Pollen	*S.urvilleii* (min.-max.) µm	*S. caryophylloides* subsp. *subulata* (min.-max.) µm (Ghazanfar, 1984)
Diameter of pollen	32.07 – 36.18	41.14 – 47.57
Diameter of pore	5.15 – 6.21	4.56 – 6.73
Distance between two pores	5.9 – 10.7	4.29 – 6.24
Number of granules on pore	15 – 19	8 – 10
Number of pore per polen	15 – 24	34 – 40

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