

Taxonomy of *Campanula tomentosa* Lam. and *C. vardariana* Bocquet from Turkey

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Abstract: The taxonomical status of *Campanula tomentosa* Lam. and *C. vardariana* Bocquet, belonging to the section *Quinqueloculares* (Boiss.) Phitos, which have important taxonomical problems, was reviewed in this study. Furthermore, morphological, anatomical leaf surface, and palynological features of these 2 species were examined in detail. Habit, shape of leaves, calyx, corolla, stamen, pistil, and seed micromorphological features as morphological characters; and pollen diameter, pore diameter, spinule length, and number, ornamentation, etc. as palynological characters, were examined and discussed. An identification key for the species is presented according to the results obtained. Following this extensive study, the mentioned problems of *C. tomentosa* and *C. vardariana* in the flora of Turkey were solved, and it was verified that they were 2 different species.

Key words: Anatomy, *Campanula*, *Quinqueloculares*, taxonomy, Turkey

Türkiye’de yayılış gösteren *Campanula tomentosa* Lam. and *C. vardariana* Bocquet’nın taksonomisi

Özet: Önemli taksonomik problemleri bulunan *Quinqueloculares* (Boiss.) Phitos seksiyonundan *C. tomentosa* Lam. ile *C. vardariana* Bocquet türleri tekrar gözden geçirilmiştir. Bu iki tür morfolojik, yaprak yüzeyi anatomisi ve palinolojik özellikleri bakımından detaylı bir şekilde incelenmiştir. Bitkilerin morfolojik karakterleri olarak genel yapısı, yaprakların şekli, kaliks, korolla, stamen, pistil ve mikromorfolojik tohum özellikleri ve polen çap, por çap, spinül ölçüsü ve sayısı, ornemantasyon gibi palinolojik karakterleri incelenmiş ve tartışılmıştır. Bu çalışmalardan elde edilen verilere göre yeniden yaptığımız tür tayin anahtarları verilmiştir. Bu kapsamlı çalışmadan sonra, *C. tomentosa* ve *C. vardariana*’nın Türkiye Florası’nda belirtilen taksonomik sorunları çözülmüş ve farklı türler olduğu doğrulanmıştır.

Anahtar sözcükler: Anatomi, *Campanula*, *Quinqueloculares*, taksonomi, Türkiye

Introduction

The East Mediterranean region, which includes Turkey, is considered the diversification region of

Campanula L. according to Contandriopoulos (1984). *Campanula* is among the genera containing the highest endemic species, with an endemic ratio of

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more than 50% and is represented by a total of 134 taxa in the flora of Turkey (Damboldt, 1978; Davis et al., 1988; Özhatay et al., 1999; Güner et al., 2000; Özhatay et al., 2006; Özhatay et al., 2009).

Regarding the studies on *Campanula*, it is observed that *Campanula* is divided into 2 sections (based on whether the calyx extension exists or not) in De Candolle's (1830) monograph. Phitos (1965) researched the species belonging to the section *Quinqueloculares* based on morphological, cytological, and geographical data. Eddie et al. (1999), in their paper on polymorphism in species with 5 loculi in the Aegean region, mentioned the study by Phitos, and stated that it is not sufficient for determining phylogenetic relations by itself and therefore should be supported with molecular studies.

General information regarding the Campanulaceae is encountered only in Metcalfe and Chalk's (1983) study. Damboldt (1976) mentioned this absence of information regarding the Campanulaceae. Ocak and Tokur (1996) studied the anatomy of some *Campanula* species from Turkey.

Chapman (1966) and Avetisjan (1967, 1973) were the first researchers to analyse the relation between pollen morphology and taxonomy. Dunbar (1973, 1975a, 1975b, 1981) and Dunbar and Wallentinus (1976) took pictures of every phase regarding the pollen ontogenesis with a scanning electron microscope. They thought that the structure of the pollen surface would solve the phylogenetic and taxonomic problems in their studies.

Problems in the identification of some taxa were encountered during the preparation of the *Flora of Turkey* (Dambolt, 1978) because the distribution of these taxa was not recorded, or the description of species and genera were prepared using populations with insufficient number of specimens. Two taxa among these problematic species are *C. tomentosa* Lam. and *C. vardariana* Bocquet, which are also endemic for Turkey.

Prof. Dr. Phitos described *C. vardariana* in the Turkish flora as a form of *C. tomentosa* with no taxonomic value. Therefore, only a short informative passage about *C. vardariana*, regarding its name and

the date and the name of its first publication, was presented. In the publication in which *C. vardariana* was first presented as a new species (Bocquet, 1968), it was indicated that it could be separated from *C. tomentosa* only by its smaller sized flowers; and it was mentioned that its holotype and isotype are located in the ZT (Zurich) and G (Geneva) Herbaria, respectively. In the interview conducted with the G Herbarium it was stated that Bocquet had not given the isotype specimen to their herbarium. ZT Herbarium, on the other hand, did not answer the request for the type specimen. During the written communication conducted with Dr. Hans Runemark from Lund University, he complained that he also was not able to obtain the type specimen. When the findings of this study were presented to Prof. Dr. Phitos, he confuted his own aforementioned thoughts about the *Flora of Turkey* and affirmed the findings of this study. He stated that his conclusion was based on the literature and had not seen the type specimen at the time of his research. Therefore, the problems between the 2 species were aimed to be overcome by collecting plentiful topotype specimens, to determine whether *C. tomentosa* and *C. vardariana* are similar or distinct species, in this study.

Materials and methods

Specimens of *C. tomentosa* and *C. vardariana* were collected during field studies in the vegetation periods of 2001-2005. Moreover, specimens found in EGE, GAZI, HUB, ISTE, and ISTF herbaria were examined, while the photographs of the specimens contained in B and LD herbaria were taken. Attempts were made to obtain type specimens or type photographs of the species but they could not be provided by the related associations.

A detailed description of *C. vardariana* was created in this study for the first time, while the description of the *C. tomentosa* was broadened based on the findings. The identification keys of the species were prepared both morphologically and palynologically. The threat categories of the species were also evaluated (IUCN, 2001).

Micromorphological measurements were obtained using a millimetric ruler under a stereomicroscope whereas they were obtained using a regular ruler in larger structures. The micromorphological measurements are given comparatively in Table 1. The surface sections taken from the leaves were examined under a light microscope (LM) and fixed with glycerine-gelatine (İnce, 1989). Photographs obtained from the preparations were taken by Olympus brand camera connected to the light microscope with $\times 40$ objective and $\times 10$ ocular. Preparations fixed via Erdtman's method (Erdtman, 1966) for the pollen measurements were used. The examination was performed with a binocular Hund microscope. An apochromatic oil immersion objective ($\times 100$) and ocular ($\times 10$) were used during the examination. The averages and standard deviations of the measurements were calculated according to formulae previously given (Sokal et al., 1969). A minimum of 30 measurements from at least 5 specimens belonging to each taxon were obtained for all the characters.

A scanning electron microscope (SEM) was also used for more detailed examination of the pollens. The pollen was kept in 2.5% glutaraldehyde solution at $+4^\circ\text{C}$ in the refrigerator and then passed through alcohol series of 25%, 50%, 75%, 90%, and 100% solutions. They were placed on metal pollen stubs, with 2-sided adhesive tape, under a binocular microscope. Then the stubs were covered with gold in order for the pollen to be transferred into a conductive state. Microphotographs of the examined pollen were taken using a JEOL JSM 5200 SEM. This study was performed in the Faculty of Dentistry, Ege University. Palynological characters were determined according to Punt et al. (1994). Dried plant specimens, and anatomic and palynological preparations are kept in the Biology Department, Science and Letters Faculty, Celal Bayar University.

Results

General Morphology

Morphological drawings were made of both species (Figures 1, 2); measurements of stem length,

basal leaf length and width, petiole length, calyx, calyx lobe, calyx appendages, corolla length, corolla tube, stamen, and pistil were obtained and they were defined using comparative tables (Table 1).

Surface Morphology of Leaves

C. tomentosa

An amaryllis type stoma was encountered in the tangential section taken from the leaves; thus the species displays amphistomatic leaf features. Stomata, on the other hand, are of anomostic type since they do not have special neighbour cells. The cell walls of the lower epidermis cells are significantly undulate while their upper surface cells have fairly straight cell walls. Moreover, more stomata are contained in the lower surface than in the upper surface (Table 1, Figure 3).

C. vardariana

An amaryllis type stoma was encountered in the both surface tangential sections taken from the upper and lower surface of the leaves; thus the species displays amphistomatic leaf features. The cell walls of the lower epidermis cells are significantly undulate while its upper surface cells have fairly straight cell walls. Furthermore, more stomata are contained in the lower surface than in the upper surface. Stomata, on the other hand, are of anomostic type since they do not have special neighbour cells (Table 1, Figure 3).

Seed Morphology

Seeds display major similarities in both species. Seeds of *C. tomentosa* and *C. vardariana* are ovoid while their surfaces are ribbed. The seeds of *C. tomentosa* are frequently light brown whereas those of *C. vardariana* are generally yellowish-light brown (Table 1, Figure 4) (Stearn, 1996).

Pollen Morphology

Pollen morphologies were examined with LM and SEM in this study, and identification keys of the taxa based on their pollen morphologies were prepared. The species were triporate having Campanula type pollen. The pollen grains were spheroidal for both species. The structure of the exine, on the other hand,



Figure 1. *C. tomentosa*: A. Habit, B. Flower, C. Calyx (scale bars: A: 5 cm, B, C: 1 cm) (Alçitepe 2138).

is tectate. The edges of the pores are frequently obvious and contain small granules. Operculum frequently contains a large columella. Columellae are obvious, granular, and sometimes containing 2 parts. The sizes of spinule may differ among the same species' pollen (İnceoğlu, 1975). Palynological comparisons of the studied species are shown below (Table 1, Figure 5).

Discussion

The dimensions of corolla tube length of *Campanula tomentosa* are shown as 30-35 × c. 30 mm in the identification key for the *Flora of Turkey* (Dambolt, 1978). *C. vardariana*, on the other hand, is not included in the key and is mentioned as "a form of *C. tomentosa* without any taxonomic value" near the

Figure 2. *C. vardariana*: A. Habit, B. Flower (scale bars: A: 5 cm, B: 1 cm) (Alçitepe 2256).Table 1. Comparative characters of *C. tomentosa* and *C. vardariana* (Figures 1-5).

		<i>C. tomentosa</i> M ± S (V)		<i>C. vardariana</i> M ± S (V)	
Stem length	(cm)	50.0 ± 12.22	(30.0-70.0)	28.4 ± 12.31	(14.0-48.0)
Basal leaves	Width (cm)	3.40 ± 1.02	(1.8-5.0)	2.46 ± 0.48	(1.5-3.5)
	Length (cm)	7.65 ± 2.23	(6.5-17.5)	6.97 ± 2.11	(3.5-10.8)
Petiole length	(cm)	11.17 ± 3.31	(5.5-17)	5.9 ± 1.09	(3.7-8.5)
Pedicel length	(mm)	9.7 ± 3.22	(4.0-13.0)	9.8 ± 6.79	(3.0-27.0)
Calyx length	(mm)	22.5 ± 2.98	(16.0-26.0)	14.7 ± 2.05	(12.0-30.0)
Calyx lobe length	(mm)	15.9 ± 2.60	(10.0-21.0)	11.4 ± 1.49	(9.0-16.0)
Calyx appendages length	(mm)	8.35 ± 1.42	(6.0-11.0)	5.87 ± 1.13	(4.0-9.0)
Corolla length	(mm)	33.8 ± 4.5	(26.0-53.0)	22.0 ± 3.06	(17.0-35.0)
Corolla tube length	(mm)	28.5 ± 9.32	(18.0-42.0)	15.18 ± 1.67	(12.0-28.0)
Stamen	Width (mm)	0.96 ± 0.05	(0.9-1.1)	0.89 ± 0.15	(0.6-1.0)
	Length (mm)	14.40 ± 3.32	(8.0-18.0)	6.10 ± 0.83	(5.0-14.0)
Pistil	Width (mm)	2.95 ± 0.36	(2.5-3.5)	0.89 ± 0.15	(0.6-1.0)
	Length (mm)	28.25 ± 10.14	(13.0-37.0)	12.6 ± 2.91	(9.0-23.0)
Cuticle	Thickness (µm)	-	(3-11)	-	(4-5)
Leaf cell width	Upper epidermis (µm)	24 ± 0.74	(16-29)	26 ± 1.35	(19-30)
	Lower epidermis (µm)	14 ± 0.59	(9-19)	17 ± 1.56	(11-22)
Leaf cell length	Upper epidermis (µm)	26 ± 0.73	(21-32)	23 ± 1.74	(19-33)
	Lower epidermis (µm)	20 ± 0.88	(12-26)	27 ± 2.77	(16-35)
Seed	Width (µm)	300 ± 30	(250-350)	280 ± 30	(250-300)
	Length (µm)	450 ± 40	(400-500)	410 ± 80	(300-500)
Pollen	Diameter (µm)	27.3 ± 2.3	(18.9-37.8)	22.3 ± 1.29	(18.9-25.3)
	Pore diameter (µm)	4.41 ± 1.0	(3.1-7.3)	5.85 ± 0.64	(5.2-8.4)
	Exine thickness (µm)	1.12 ± 0.28	(0.5-2.1)	1.00 ± 0.06	(0.7-1.2)
	Spinule length (µm)	-	(0.6-1.2)	-	(0.2-0.5)
	5 µm ² spinule number	-	(3-4)	-	(34-36)

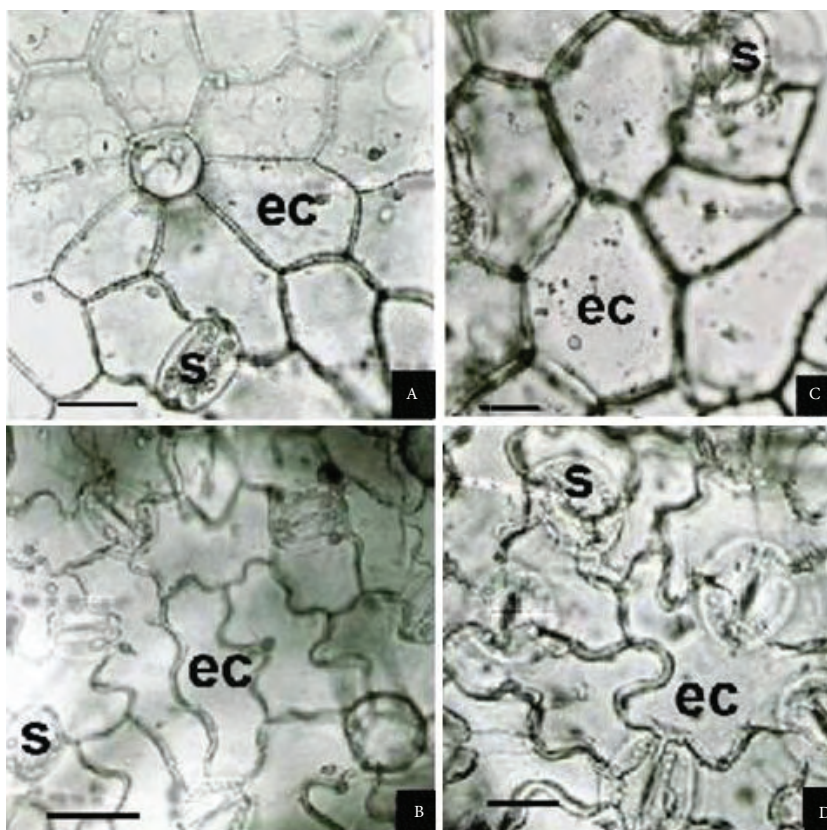


Figure 3. Leaf surface anatomy of *C. tomentosa* and *C. vardariana*. *C. tomentosa*: A. Upper surface, B. Lower surface (Alçitepe 2138); *C. vardariana*: C. Upper surface, D. Lower surface (Alçitepe 2256). ec: epiderma cell, s: stoma (scale bar: 15 μ m).

end of the description for *C. tomentosa*. Furthermore, only one character is given for *C. vardariana*, i.e. having very small sized flower, both in the *Flora of Turkey* and its original description (Bocquet, 1968).

The dimensions of corolla tube length of *C. tomentosa* were determined as 18-42 \times 12-37 mm and in *C. vardariana* as 12-28 \times 8-17 mm in this study.

C. tomentosa, as can be observed from the values, displays significant differences in its values from the values mentioned in the identification key for the *Flora of Turkey*. Thus, an investigation was conducted on numerous specimens, by using different characters, for the aim of revealing the similarities and differences among the 2 species and whether they were really 2 distinct species or members of the same species generated in 2 different locations. According

to the findings of the study, conducted with the above-mentioned aim, *C. tomentosa* has a longer stem, larger lamina of basal leaf and also has higher values for calyx, calyx lobe, calyx appendages, corolla, and corolla tube sizes than *C. vardariana*. The lengths of the pedicels were similar for both species while *C. tomentosa*'s dimensions were found to be longer and wider when the measurements of the stamen and pistil widths were compared. *C. tomentosa* were observed to have slightly larger seeds than *C. vardariana* (Table 1). Significant differences obtained from these findings are given below (Table 2).

No significant differences regarding the general cell morphology were encountered during the examination of the lower and upper surface leaf morphology but the cuticula was found to be thicker

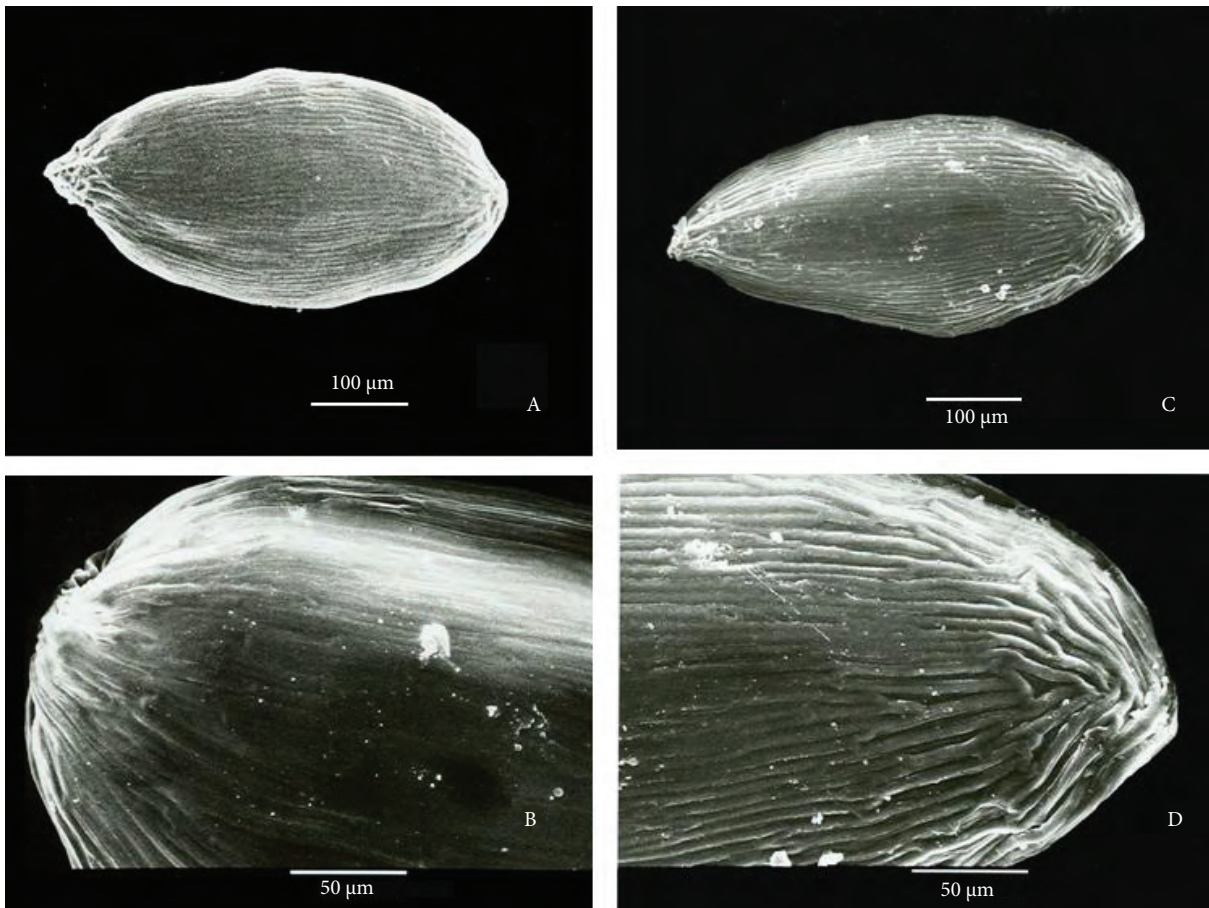


Figure 4. Seed morphology of *C. tomentosa* and *C. vardariana* (SEM). *C. tomentosa*: A. General view, B. Detailed surface view (Alçitepe 2138). *C. vardariana*: C. General view, D. Detailed surface view (Alçitepe 2256).

Table 2. Comparative diagnostic characters of *C. tomentosa* and *C. vardariana*.

	<i>C. tomentosa</i>	<i>C. vardariana</i>
Stem length	to 70 cm	to 48 cm
Basal leaves	6.5-17.5 × 1.8-5 cm	3.5-10.8 × 1.5-3.5 cm
Cauline leaves	ovate to ovate-triangular seldom lyrate, 1.5-7.5 cm length	lyrate or ovate 1.5-3.8 cm length
Corolla	cylindrical-campanulate or urn-shaped,	cylindrical-campanulate,
Corolla tube	26-53 × 18-45 mm 18-42 mm	17-35 × 12-32 mm 12-28 mm

in *C. tomentosa* whereas the lower epidermis was longer for *C. vardariana* (Table 1, Figure 3).

No significant differences were observed regarding the seeds among the 2 species. Seeds in both *C. tomentosa* and *C. vardariana* were ovoid, had ribbed

surfaces, and generally were brownish. However, seeds were smaller in *C. vardariana* (Table 1, Figure 4).

Ornamentation became more significant as the pollen size increased (İnceoğlu 1975). Spinule sizes

were increasing in relation with the increase in the pollen sizes in this study and displayed ornamentation towards distinctive. Pollen diameter, exine thickness, and spinule length were found to be greater for *C. tomentosa* than the pollen of *C. vardariana* whereas the number of spinules located in 5 mm², except pore diameter, was found to be much more for *C. vardariana* than *C. tomentosa* during the palynological studies conducted. *C. tomentosa*, on the other hand, can be easily distinguished from *C. vardariana* since its spinules are longer and fewer (Table 1, Figure 5).

Identification key:

- 1. Upper cauline leaves ovate to ovate-triangular, regularly dentate; corolla 26-53 × 18-45 mm, cylindrical-campanulate or urn-shaped*C. tomentosa*
- 1. Upper cauline lyrate to ovate, irregularly dentate; corolla 17-35 × 12-32 mm, cylindrical-campanulate*C. vardariana*

As can be seen from the identification key, it is possible to distinguish the 2 species from each other based on morphological characters although there are no significant differences in their seed morphology.

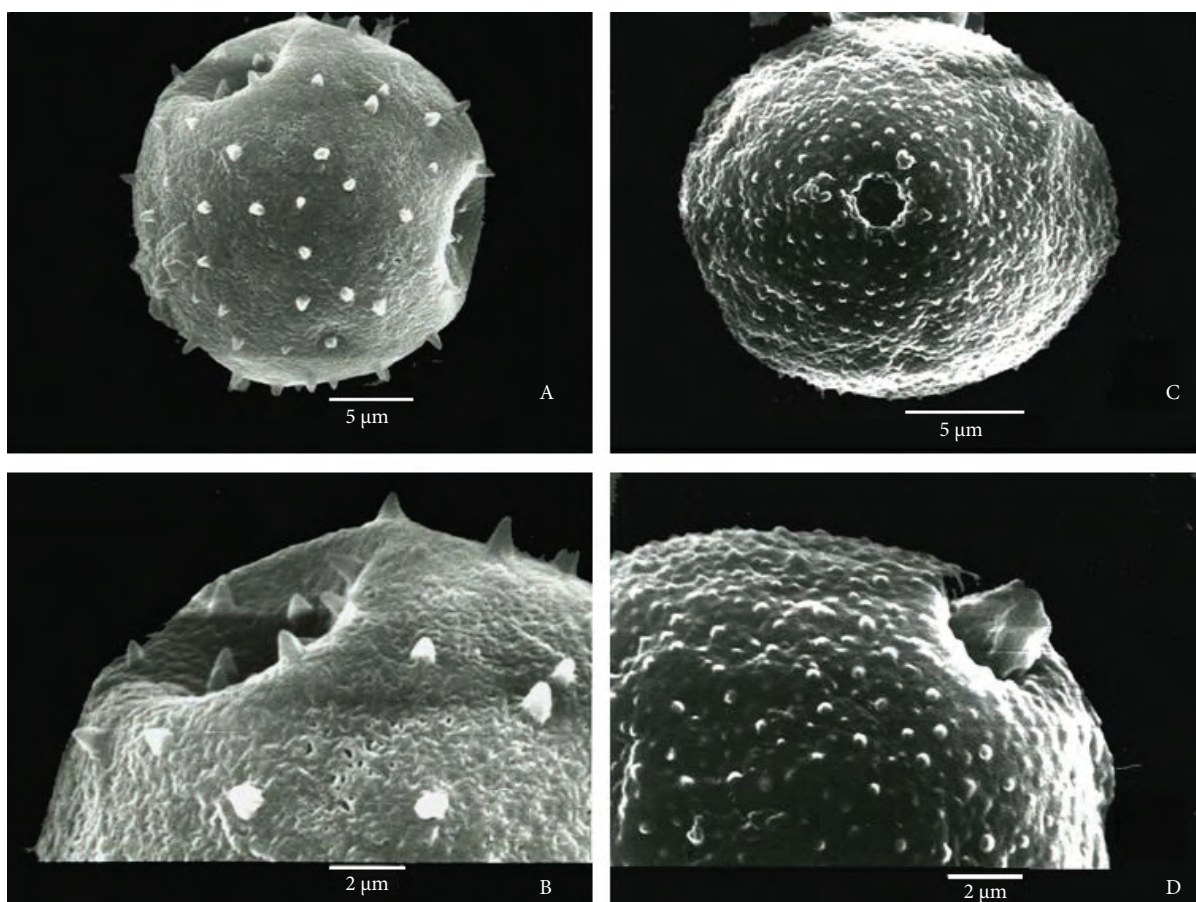


Figure 5. Pollen morphology of *C. tomentosa* and *C. vardariana* (SEM). *C. tomentosa*: A. General view, B. Ornamentation and pore view (Alçitepe 2138). *C. vardariana*: C. General view, D. Ornamentation and pore view (Alçitepe 2256).

The threat category of *C. tomentosa* was determined as VU (vulnerable) while the threat category of *C. vardariana*, which is known as doubtful or insufficient in the *Flora of Turkey*, was found to be CR (critically endangered) as a result of the field investigation of the 2 species (IUCN, 2001).

Investigated Specimens:

C. tomentosa

C1 Aydın: Dilek Peninsula, Samsun Mountain, 5 km westlich Güzelçamlı Canyon, 37°40' n.Br. 27°15' 8.L., Spalten in besonnten kalkfelsen, 100 m, 25.5.1995, *P.Tlein* 0126/2003-3, (B, Photo!); in declivitatibus meridionalibus montis Samsun-dagh. (Mykale), inter, Priene et Sokhia, 1-200 m, 2-3.6.1906, *J.Bornmüller* 9759, 9753 (B, Photo!); Ortaklar to Çamlık, near road, c. 300 m, 12.5.1967, *H.Peşmen*, *G.Oğuz* 16856 (EGE); Kuşadası, Kalamaki brook, c. 200 m, 12.5.1968, *F.Mayer*, *H.Peşmen* 8834 (EGE); Söke Prienen ruins, 25.6.1971, *H.Peşmen* & *B.Yıldız* 8650 (EGE); ibid. c. 200 m, 24.4.1977, *A.Yürül*, *T.Çetindağ*, *A.Yayıntaş* 15138 (EGE) ibid., Limestone cliffs, 50 m, 9.5.1972, *H.Runemark* & *P.Wendelbo* 625 (LD, Photo!); ibid. 16.05.1983, *K.Faber* 126/2003-12 (B Photo!); ibid. 100 m, 4.10.1989, *Th.Raus* 14758 (B, Photo!); Samsun Mountain, Kalamaki, near road, stony places, 50 m, 12.5.1982, *A.Çırpıcı* 35055 (ISTF); Söke, near Cement Plant, rocky areas, 100-130 m, 26.5.2002, *E.Alçitepe* 2114; Aydın-İzmir road, limestone rocks, 50-60 m, 26.5.2002, *E.Alçitepe* 2113; Kuşadası, Dilek peninsula national park, military area,

c. 20-30 m, 3.5.2003, *E.Alçitepe* 2144; İzmir: İzmir ad anoenia, arupta Ephes, 1.6.1906, *J.Bornmüller* 9747 (B, Photo!); ibid. 9751, 9752 (B, Photo!); Mai 1932, *A.Scheibe* 0126/2003 11 (B, Photo!); ibid., 6.1965 *C.Regel* 2442 (EGE); 19.5.1971, *N.Zeybek*, *H.Peşmen*, *Y.Aydar*, *T.Kesercioğlu* 8660 (EGE); ibid., 31.05.1972, *A.Baytop* 22095, (ISTE); ibid., 7.5.2003, *E.Alçitepe* 2138; Selçuk-Meryem Ana road, rocks, 18.05.1967, *F.Öktem* 11114, (ISTE); ibid., 18.5.1969, *N.Zeybek* 8649 (EGE); ibid., 23.4.1966, *C.Regel* 182 (EGE); from Selçuk 1-2 km north, above rocky area, 50-100 m, 20.4.1990, *H.Duman* 4332 (GAZI); C2 Muğla: Marmaris to Datça road, 23.4.1969, *K.Walther* 26336 (HUB).

C. vardariana

C1 Aydın: Aydın prov. 2 km N of Söke, 100 m, limestone cliffs, near the road, 01.05.1983, *H.Runemark* & *A.Carlström* 49312 (LD, Photo!); Aydın: Söke, near Cement Plant 21.05.1976, *Ö.Seçmen* 23922 (EGE); ibid., rocks, 100-130 m, 26.05.2002, *E.Alçitepe* 2114a; ibid., 15.05.2002, *E.Alçitepe* 2256; ibid., 17.05.2004, *E.Alçitepe* 2265.

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