Turkish Journal of Bioscience and Collections
Volume 8, Number 2, 2024
E-ISSN: 2601-4292

RESEARCH ARTICLE

Comparative Seed Morphology of Eight *Allium* L. Species from Sections *Codonoprasum* and *Scorodon*

Özge Vatandaşlar[1], Mine Koçyiğit[2]

Abstract

Objective: The *Allium* L. genus is one of the largest species of sectoral importance due to its consumption as food and its medical importance. For this reason, different characters are used to solve taxonomic problems and clearly identify species. One of these characteristics is the micromorphological characteristics of the seed.

Materials and Methods: Light microscopy was used to examine the size and maturity of the seeds, and their micromorphology was studied using an FEI Quanta 450 FEG-EDS scanning electron microscope (SEM) at Istanbul University, Faculty of Science Department of Physics Nano- and Optoelectronics Research Laboratories. Twelve characteristics were determined on the seed surfaces of the examined eight *Allium* species. Cluster analysis was performed on these characteristics using Jaccard similarity analysis with PAST 1.81.

Results: Seed surfaces of eight *Allium* species (4 species from the sect. *Codonoprasum* and 4 species from the sect. *Scorodon* were examined. *Allium phrygium* Boiss., *A. balansae* Boiss., *A. sivasicum* N. Özhatay & Kollmann, *A. djilgense* Boiss. ex Regel, and *A. tauricola* Boiss. are endemics for Türkiye. The epidermal cells of the testa were either regularly or irregularly polygonal. The arrangement of the cells was generally compact, primarily consisting of cellular reticulate tissue. The anticlinal walls of the testa epidermal cells were either straight or curved, while the periclinal walls varied from flat or concave to various extents. Additionally, the epidermal cells exhibited verrucae and granulose features. The micromorphological characteristics of the seeds are summarized in a list. According to seed characteristics, two main branches formed in the similarity tree. In particular, the species in the Sect. *Scorodon* are grouped together.

Conclusion: Micromorphological traits, such as surface texture, cell shape, and seed testa patterns, can provide valuable information for identifying species and understanding taxonomic and evolutionary relationships. In the context of *Allium*, these traits can be particularly useful because they often exhibit significant variation among species and are stable and characteristic enough to serve as reliable taxonomic markers. By analyzing these micromorphological features, it is possible to gain insight into the evolutionary history and relationships of different taxa within the species.

Keywords: *Allium*, endemic, Cluster analysis, SEM, testa

[1]İstanbul University, Institute of Graduate Studies in Health Sciences, İstanbul, Türkiye

[2]İstanbul University, Faculty of Pharmacy, Department of Pharmaceutical Botany, İstanbul, Türkiye

ORCID: Ö.V. 0000-0003-1604-3905; M.K. 0000-0002-7831-0151

Received: 01.08.2024
Revision Requested: 07.08.2024
Last Revision Received: 09.08.2024
Accepted: 10.08.2024
Published Online: 21.08.2024

Correspondence: Mine Koçyiğit
mkocyigit@istanbul.edu.tr

Citation: Vatandaşlar, Ö., & Koçyiğit, M. (2024). Comparative Seed Morphology of Eight *Allium* L. Species from Sections *Codonoprasum* and *Scorodon*. *Turkish Journal of Bioscience and Collections*, *8*(2), 41–48.
https://doi.org/10.26650/tjbc.1525986

Introduction

One of the important plants known and cultivated since ancient times is *Allium* L. In particular, epidemic diseases such as cholera and plague in the Middle Ages, II. During World War II, garlic was used for the treatment of wounded people (Baytop, 1999). In addition to being used as a vegetable and spice, garlic is used for medicinal purposes in Türkiye and around the world (Ekşi *et al.,* 2020). In Türkiye, the leaves and bulbs of *Allium* species, both naturally grown and cultivated, are used by the public for various purposes. *Allium* species are used in the treatment of hemorrhoids around Ankara, Bartın and Gönen (Gürhan & Ezer, 2004; Tuzlacı & Aymaz, 2001), in the treatment of hemorrhoids, earache, and poisoning in Kırklareli (Kültür, 2007), and as an expectorant, cough suppressant, and blood pressure reducer in Çankırı (Ezer & Avcı, 2004), in the Middle and Western Black Sea region, for boil ripening, diabetes, and rheumatism pain, around Kırşehir, as an expectorant, blood pressure reducer, for reducing intoxication after alcohol consumption, around Trabzon, for wound treatment, earache, hair loss (Yazıcıoğlu & Alpınar, 1993), and wounds and boils. They are used to treat worms around Muğla, as food around Bodrum, for inflamed wounds around Malatya (Yeşil & Akalın, 2009), and for inflamed wounds and stomach ache around Kayseri (Özkan Gençler & Koyuncu, 2005). It is used for the treatment of boils in Portugal (Novais *et al.,* 2004), in veterinary medicine, insect bites, and as a diuretic in Italy (Guarrera *et al.*, 2005), and in Morocco for the treatment of diabetes, heart diseases, hypertension, skin diseases, scorpion, and snake bites (Jouad *et al.,* 2001).

The taxonomically difficult *Allium* is the largest genus of petaloid monocots, comprising approximately 1200 taxa (Govaerts *et al.*, 2024). In Türkiye, the genus is represented by 225 taxa in 15 sections. In recent years, seed characteristics have also been frequently used to solve taxonomic problems.

In studies based on the morphological characteristics of *Allium* seeds, differences in size and shape were determined in the epidermal cells of the testa. In particular, testa characteristics have been revealed in 17 sections of the *Allium* genus (Friesen *et al*., 2006; Kruse, 1994). Additionally, the seed surface of 20 *Allium* taxa from Iran have been studied (Neshati & Fritsch, 2009). In a study conducted in Canada, the genus was revised according to seed characteristics (Choi & Cota-Sanchez, 2010). Seed surface of 24 *Allium* species in Korea and northeastern China were examined, and it was concluded that there were differences (Choi & Oh, 2011). In Poland, the seeds of eight *Allium* species were examined and different species were identified (Bednorz *et al*., 2011). Studies have shown that seed morphology contributes to *Allium* taxonomy in Central Asia (Baasanmunkh *et al.,* 2020; Shukherdorj *et al.,* 2021).

In Turkey, 62 *Allium* seeds were examined (Celep *et al*., 2012). Seed surface examinations have also been included in studies describing new species in recent years. It is stated in all studies that the identification of micromorphological seed characters is useful for evaluating the taxonomic relationships among *Allium* taxa.

In this study, the seed surfaces of eight *Allium* species belonging to two very similar sections, *Codonoprasum* and *Scorodon*, were examined. Five of the species examined were endemic. We evaluated whether there was any similarity between the sections in terms of seed surfaces.

Materials and Methods

Allium samples in the Herbarium of the Faculty of Pharmacy, Istanbul University (ISTE) were examined. The seeds of each species were examined under a stereomicroscope to ensure that only normal-sized and mature seeds were used. Herbarium numbers and locality information are given in Table 1. Light microscopy was used to examine the

Table 1. Localities and herbarium numbers of the examined *Allium* samples.

ISTE no	Scientific name (Endemism)	Localities	Altitudes (m)	Collected Date
87671	*A. phrygium* Boiss. **(E)**	Isparta, Barla Village	967	20.07.2008
87585	*A. pseudoflavum* Vved.	Kars, Ani Ruins	1260	6.07.2007
92251	*A. flavum* L.	Kırklareli, Demirköy, Yıldız Mountains, and Mahya Hill	1031	4.07.2009
87725	*A. staticiforme* Sm.	Balıkesir, Between Bandırma and Biga, the Denizkent dunes	0	20.06.2009
97291	*A. balansae* Boiss. **(E)**	Nevşehir, Avanos Kayseri road	992	7.07.2012
40183	*A. sivasicum* N. Özhatay & Kollmann **(E)**	Sivas, 20 km from Sivas to Hafik		15.06.1978
87807	*A. djimilense* Boiss. ex Regel **(E)**	Trabzon, From Soğanlı Pass to Uzungöl	2245	19.08.2009
87674	*A. tauricola* Boiss. **(E)**	Kars, Boğatepe Province	2300	20.07.2008

size and maturity of the seeds, and their micromorphology was studied using an FEI Quanta 450 FEG-EDS scanning electron microscope (SEM) at Istanbul University. All measurements were performed using the image analysis systems KAMERAM and the Canon A 640 camera. Twelve characteristics were determined based on the seed surfaces of the examined *Allium* species (Table 2), and the codas used to evaluate the 12 identified characteristics are listed in Table 3. Cluster analysis was performed on these characteristics using Jaccard similarity analysis with PAST 1.81 (Hammer *et al*., 2001).

Results and Discussion

The seed surfaces of eight *Allium* species, four species from the Sect. *Codonoprasum* and four species from Sect. *Scorodon* were examined (Figs. 1, 2). The micromorphological characteristics of the seeds are summarized in Table 4. The testa epidermal cells were regular or irregular polygonal. The cellular arrangement was tight with cellular reticulate tissue. The anticline walls of seed testa epidermal cells were straight or straight to arched, the periclinal walls ranged from flat or concave to

Table 2. Data Matrix of *Allium* Seed Characteristics, Characteristics -states and their codings.

Cs	Characteristics	Characteristics-states and their codings	
C1	Seed shape	semicircle = 0	drop-shaped-semicircle = 1
C2	Epidermal cell shape	≤4 = 0	≥ 4 = 1
C3	Relief of intercellular space (cell boundary)	Reticulate tissue = 0	Not reticulate tissue = 1
C4	Periclinal wall (PW)	Flat = 0	Concave = 1
C5	Fine relief of the PW	Many small domes with dispersed verrucae = 0	Others = 1
C6	Diameter of verrucae on PW	Small to large = 0	Others = 1
C7	Number of verruca on PW	≥ 15 = 0	Others = 1
C8	Epidermal cell area (µm2)	≥ 750 µm2 = 0	≤ 750 µm2 = 1
C9	Intercellular space length (µm)	≥ 6 µm = 0	≤ 6 µm = 1
C10	Seed length (mm)	≥ 3 µm = 0	≤ 3 µm = 1
C11	Seed width (mm)	≥ 1.50 µm = 0	≤ 1.50 µm = 1
C12	Seed area (µm2)	≥ 5 µm = 0	≤ 5 µm = 1

Table 3. Seed Macro-Micromorphological data matrix for *Allium* based on scoring of Characteristics, Characteristics -states from Table 2.

Cs	*A. phrygium*	*A. pseudoflavum*	*A. flavum*	*A. staticiforme*	*A. balansae*	*A. sivasicum*	*A. djimilense*	*A. tauricola*
C1	0	0	0	0	1	1	1	1
C2	1	1	1	1	1	1	0	0
C3	1	0	0	0	1	1	1	1
C4	0	0	1	1	0	0	0	1
C5	0	0	1	1	0	1	1	0
C6	1	0	0	1	0	0	0	1
C7	0	0	1	1	1	1	1	0
C8	1	1	1	1	0	0	0	0
C9	0	1	1	0	1	1	0	1
C10	1	0	0	1	1	0	1	1
C11	1	0	0	0	1	0	1	1
C12	1	0	0	0	1	1	1	1

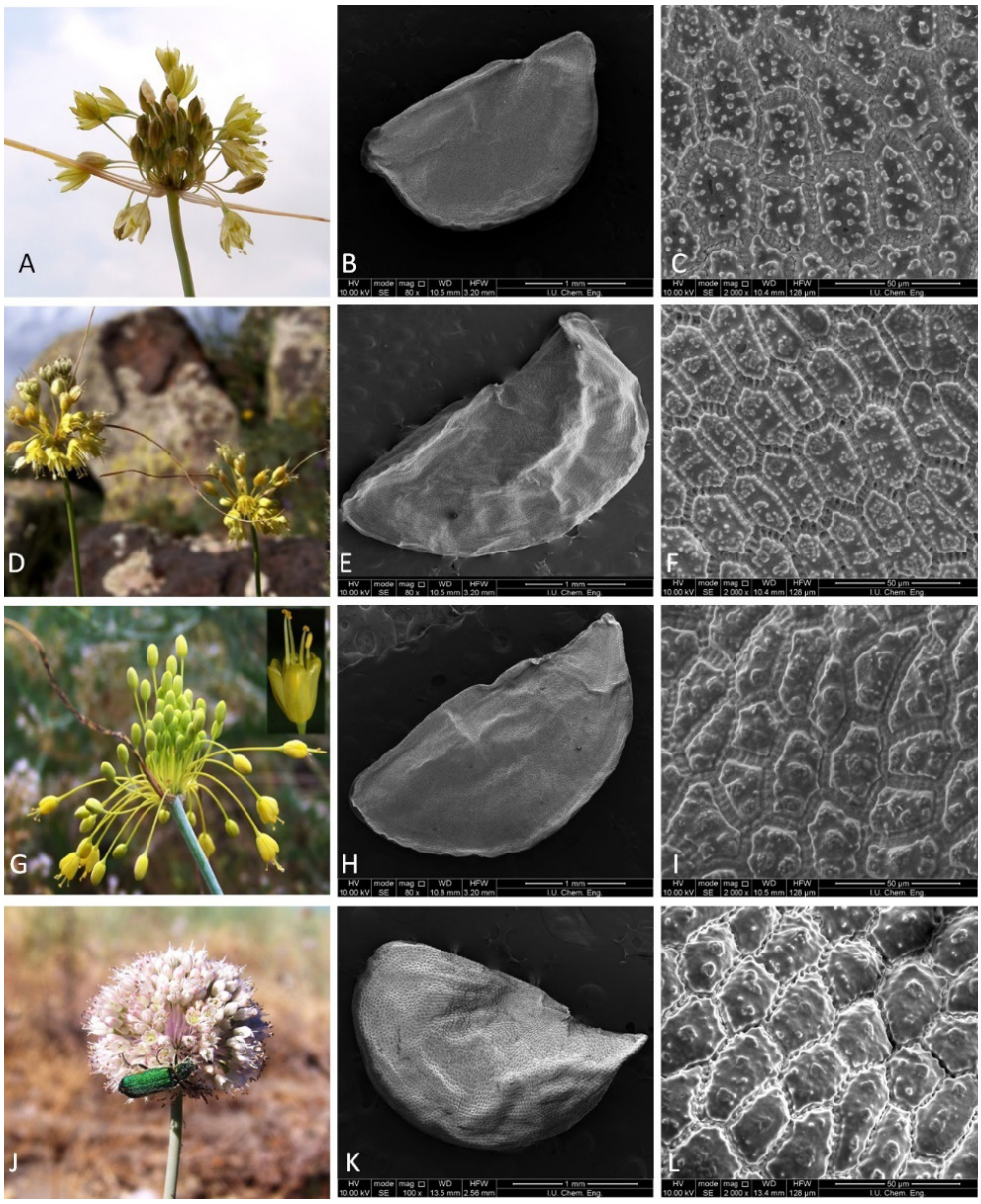

Figure 1. The examined *Allium* species from sect. *Codonoprasum: A. phrygium* (A, B, C), *A. pseudoflavum* (D, E, F), *A. flavum* (G, H, I), *A. staticiforme* (J, K, L).

various degrees, and there were verrucae and granulose on the epidermal cells. In *A. balansae*, no granules were observed in the periclinal walls. *Allium sivasicum* rarely exhibits an indistinct granular structure. The seed shapes of *Allium* species from the Sect. *Codonoprasum* were semicircular, the species from the Sect. *Scorodon* had slightly narrowed in the area where the seed attached to the placenta, and therefore, it was observed as a drop. While *A. sivasicum* has the longest seed length (3.85 mm), *A. phrygium* has the shortest seed length (2.53 mm). The widest seed was *A. staticiforme* (1.80 mm), while the narrowest seed was *A. tauricola* (1.25 mm).

In the Jaccard similarity analysis based on these data, the phenetic correlation value was calculated as 0.839. According to seed characteristics, two main branches formed in the similarity tree. In particular, the species in the Sect. *Scorodon* are grouped together. Although *A. phrygium* is described in Section *Codonoprasum* remains outside the main branch and is essentially located within its own section (Fig. 3).

Macro- and micro-morphological features have proven valuable for defining taxa (Choi & Oh, 2011; Lin & Tan, 2015; Namin *et al.,* 2009). In *Allium*, the micromorphological characteristics of the seed testa are particularly important for describing new taxa (Koçyiğit *et al.,* 2024). Our findings indicate that stable and distinct seed

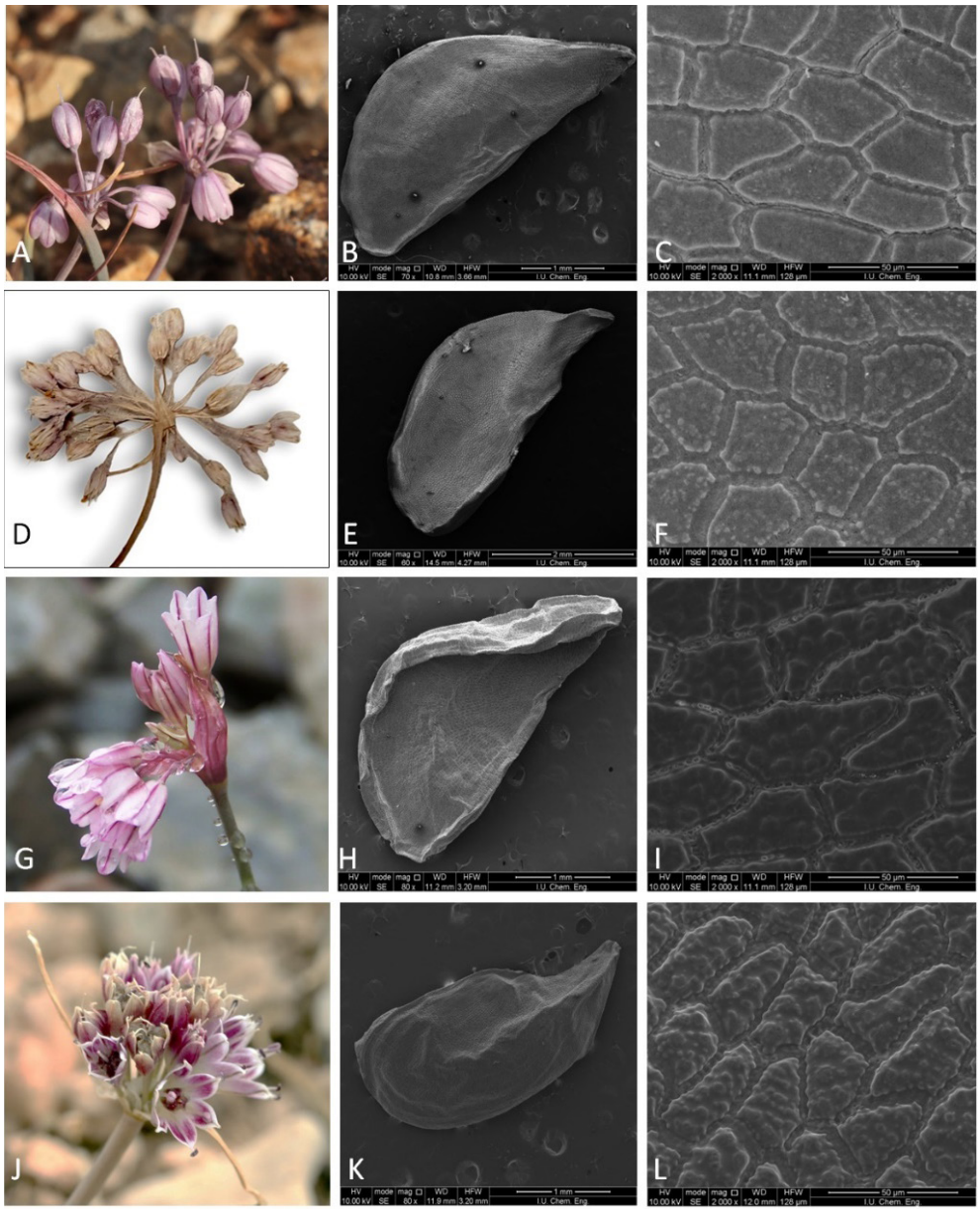

Figure 2. The examined *Allium* species from sect. *Scorodon: A. balansae* (A, B, C), *A. sivasicum* (D, E, F), *A. djimilense* (G, H, I), *A. tauricola* (J, K, L).

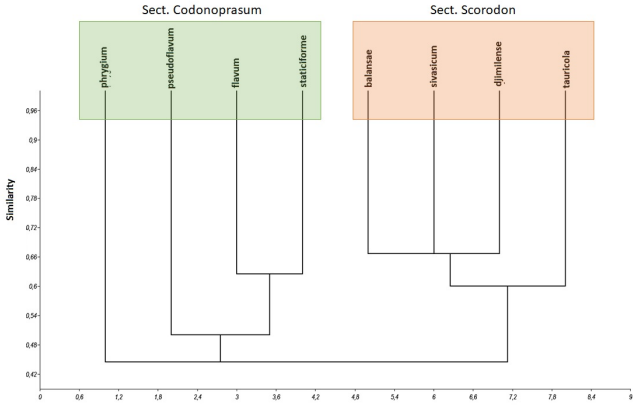

Figure 3. Cluster analysis of the considered *Allium* species, based on the Seed Macro-Micromorphological data matrix presented in Table 3.

micromorphological traits can effectively identify species and reflect taxonomic relationships and evolutionary stages. Thus, features such as testa curvature, anticlinal wall relief, and the microsculpture of outer periclinal walls are important for taxonomy in *Allium*. We propose that the cellular arrangement, presence, and nature of reticulated tissue, periclinal wall outline, and degree of verrucation are important for distinguishing between *Allium* subdivisions. Additionally, the characteristics of anticlinal walls, along with their size and density, are important for understanding species relationships. Therefore, seed coat micromorphological traits can be instrumental in species identification and taxonomy within *Allium*.

Table 4. Micromorphological characteristics of seed testa of eight species of *Allium* from Sections *Codonoprasum* and *Scorodon*.

	A. phrygium	*A. pseudoflavum*	*A. flavum*	*A. staticiforme*	*A. balansae*	*A. sivasicum*	*A. djimilense*	*A. tauricola*
Seed shape	Semicircle	Semicircle	Semicircle	Semicircle	Drop-shaped-semicircle	Drop-shaped-semicircle	Drop-shaped-semicircle	Drop-shaped-semicircle
Epidermal cell shape	Variably polygonal (4-5)	Variably polygonal (5-6)	Variably polygonal (4-5)	Variably polygonal (4-5)	Variably polygonal (4-5)	Variably polygonal (4-5)	Variably polygonal (3-5)	Variably polygonal (3-5)
Epidermal cell arrangement	Side-by-side	Side-by-side	Side-by-side	Side-by-side	Side-by-side	Side-by-side	Side-by-side	Side-by-side
Anticlinal wall	Straight to arched	Straight to arched	Straight to arched	Straight to arched	Straight to arched	Straight to arched	Straight to arched	Straight to arched
Relief of intercellular space (cell boundary)	Scabrate	Reticulate tissue with a broad mesh of connecting threads	Reticulate tissue with a broad mesh of connecting threads	Reticulate tissue with a broad mesh of connecting threads	Slightly scabrate	Slightly scabrate	Scabrate	Scabrate
Periclinal wall (PW)	Flat	Flat	Concave	Concave	Flat	Flat	Flat	Concave
Fine relief of the PW	Many small domes with dispersed verrucae	Many small domes with dispersed verrucae	One large central dome with central and marginal verrucae	One large central dome with central and marginal verrucae	Rare small dispersed verrucae	Many small dispersed verrucae	Dispersed same size verrucae without domes	Many small domes with dispersed verrucae
Diameter of verrucae on PW	Small to medium	Small to large	Small to large	Small to medium	Almost absent	Very small	Small	Small to large
Number of verrucae on PW	≥ 15	≥ 15	≤15	≤15	0	≤15	≤15	≥ 15
Epidermal cell area (μm^2)	66.63±13.4	741.22±11.2	724.68±17.3	659.82±10.62	893.79±15.8	1088.99±17.04	1392.90±14.3	765.03±11.41
Intercellular space length (μm)	8.27±3.4	5.33±6.7	6.26±6.5	5.12±4.7	6.35±5.4	7.4±3.8	5.12±5.1	6.18±2.4
Seed length (mm)	2.53±3.12	3.11±4.11	3.22±3.17	2.91±5.13	2.98±4.13	3.85±3.72	2.94±4.22	2.62±3.16
Seed width (mm)	1.47±2.52	1.72±3.32	1.69±4.72	1.80±4.62	1.39±3.86	1.67±4.22	1.41±3.42	1.25±4.72
Seed area (μm^2)	4.43±3.18	6.40±3.31	5.87±3.62	5.92±3.72	4.15±3.21	4.83±2.32	4.20±2.83	3.66±3.11

Peer-review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study – Ö.V.; Data Acquisition- Ö.V.; Data Analysis/ Interpretation- M.K.; Drafting Manuscript- Ö.V.; Critical Revision of Manuscript- M.K.; Final Approval and Accountability- M.K.; Technical or Material Support – Ö.V.; Supervision – M.K.

Acknowledgements: We thank the curators and staff members of the following herbaria: ISTE and NGBB.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: This project is financially supported by the Scientific Investigation Project Coordinator of Istanbul University (Project no: 39135)

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