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Pollen morphology of Scaligeria DC. (Apiaceae) in Turkey

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Abstract: In this study, the pollen morphology of all *Scaligeria* DC. species distributed in Turkey were studied with light and scanning electron microscopy for the first time. The aim is to contribute to knowledge of the variation among seven *Scaligeria* species, which have taxonomic problems, based on pollen morphological characteristics by population analysis. The pollen is radially symmetrical, isopolar, and tricolporate in all examined species. Pollen grains are prolate and subprolate with the polar axes ranging from 18.4 to 37 μ m and the equatorial axes from 9.8 to 30 μ m. Their polar shapes are triangular, triangular to subcircular, circular to subcircular, or triangular to subtriangular. Several types of exine ornamentations have been observed on pollen by scanning electron microscopy. Numerical analysis showed that pore structure, the ratio of P/E, and ornamentation in the polar and equatorial views are most valuable variables for separating the *Scaligeria* species. All *S. lazica* taxa were grouped together and *S. capillifolia* and *S. tripartita* were the external taxa due to cluster and PCA analysis.

Key words: Cluster analysis, pollen morphology, principle component analysis, Scaligeria, Turkey

1. Introduction

The genus Scaligeria DC. (Apiaceae) comprises species that are rich in essential oils that have antibacterial and antifungal properties (Baser et al., 1995; Tabanca et al., 2007; Navaei and Mirza, 2009; Rowshan and Tarakemeh, 2013; Baldemir et al., 2016, 2018). The taxonomy of the genus Scaligeria has been significantly altered in the last few decades (Hand et al., 2012). In some early systematic studies of Apiaceae, it was associated with other genera including Elaeosticta Fenzl, Bunium L., and Carum L. (Nasir, 1972). The cotyledon number of Apiaceae has been used for classificatory purposes to some extent. There are at least nine genera with monocotyledonous species, and most of these are tuberous geophytes (Haccius, 1952). However, all dicotyledonous Scaligeria species were recently placed within Elaeosticta such that Scaligeria proper includes only monocotyledonous species (Degtjareva et al., 2013). A classification for Middle Asian geophilic Umbelliferae-Apioideae genera was presented by Degtjareva et al. (2013), where they accommodated 81 taxa in 8 genera based on morphology, embryology, and internal transcribed spacer (ITS) and external transcribed spacer (ETS) sequence data. Different types of life forms, cotyledon numbers, the presence/absence of petioles of basal leaflets, shape of mericarp ribs, the width of mericarp commissures, and the number of secretory ducts in mericarp furrows were also observed. ITS and psbA-trnH analyses indicated that the species of eastern *Bunium, Elaeosticta, Galagania, Gongylotaxis, Hyalolaena, Mogoltavia*, and *Oedibasis* are closely related and form a strongly supported clade. However, *Scaligeria* is not grouped with these genera (Degtjareva et al., 2009, 2013; Hand et al., 2012). This result is in agreement with the treatment suggested by Kljuykov et al. (1976) and Pimenov (1983). Meanwhile, the taxonomic issues of *Scaligeria* still remain unresolved and studies continue.

Scaligeria is widespread, with its presence ranging from Turkey, Cyprus, the southwestern Balkan Peninsula, and Greece eastwards to Syria, Lebanon, Israel, and Jordan with 25 species (Kyriakopoulos et al., 2014). The genus is represented in Turkey by seven species: the endemic *S. capillifolia* Post. in Adana located in the south of Turkey; *S. lazica* Boiss., endemic to the Eastern Black Sea region; and *S. glaucescens* (DC.) Boiss., *S. hermonis* Post, *S. meifolia* (Fenzl) Boiss., *S. napiformis* (Willd.) Grande, and *S. tripartita* (Kalen.) Tamamsch. with a wide geographical distribution (Stevens, 1972; Güner et al., 2012). *S. lazica* and *S. capillifolia* have been evaluated in the vulnerable

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category within the International Union for Conservation of Nature's Red List Categories (Ekim et al., 2000).

The morphological features of pollen from various taxa of the family Apiaceae have been used for elaboration of pollen keys (Erdtman et al., 1969; Faegri and Iversen, 1975; Punt, 1984; Perveen and Qaiser, 2006) and descriptions have been given in regional pollen floras (Aytuğ et al., 1971; Kuprianova and Alyoshina, 1972; Punt, 1984; Perveen and Qaiser, 2006; Güner et al., 2011; Mungan et al., 2011) by several authors. Cerceau-Larrival (1962) and Roland-Heydacker and Cerceau-Larrival (1978) divided the pollen of Apiaceae into five distinct types based on P/E ratio, outline of the inner and outer contour, and exine thickness. Van Zeist et al. (1977) also described nine pollen types from Iran: Anisoscidium-type, Bunium-type, Bupleurum-type, Eryngium-type, Ferula-type, Malabailatype, Pimpinella-type, Sium erectum-type, and Thurgeniatype. The pollen morphology of 105 Apiaceae species was examined by light microscopy (LM) and scanning electron microscopy (SEM) and divided into 50 types by Punt (1984). The pollen morphology of 50 species representing 27 genera of the family Apiaceae from Pakistan was examined by Perveen and Qaiser (2006), and on the basis of exine pattern, three distinct pollen types were recognized: Bupleurum gilessii-type, Pleurospermum hookeri-type, and Trachyspermum ammi-type, which includes Scaligeria aitchisonii Wolff.

The taxonomic issues of *Scaligeria* still remain unresolved and work continues. This study includes a detailed morphological analysis of pollen from all species of *Scaligeria* that are distributed in Turkey. To date, only one study has been conducted on micromorphological aspects of pollen from this genus (Perveen and Qaiser, 2006). Pollen grains were investigated using LM and SEM, and inter- and intrageneric relationships based on these data were discussed. This study aims to contribute to the systematics of the Turkish *Scaligeria*.

2. Materials and methods

2.1. Plant material

The material used for this study was collected during 2014 and 2015 from different periods of the year and from various locations in Turkey. The voucher specimens were deposited in the herbarium of the Faculty of Science of Erciyes University, Kayseri, Turkey (ERCH). Plant localities, collection dates, and herbarium numbers are shown in Table 1.

2.2. Palynological and morphological analysis

Pollen slides were prepared using the Wodehouse (1935) technique. Pollen grains were placed in glycerin jelly, stained with safranin, and studied under a light microscope. Photographs were taken with a Leica DM 3000 digital photomicrograph system (Germany). Measurements were based on 30 or more pollen grains per specimen. Pollen morphological characteristics were similar between the localities for *S. meifolia* (two localities), *S. napiformis* (three localities), and *S. tripartita* (two localities). Therefore, the mean values of the measurements of all taxa were given, except for *S. lazica*. Both in LM and SEM analysis pollen microphotographs were given due to this arrangement. For SEM studies, dried pollen grains were transferred onto aluminum stubs and coated with gold for 4 min in

Species	Collection site	Collection date	Herbarium number
S. capillifolia	Osmaniye: South of Yarpuz, Yağlıpınar, 1400 m	July 2014	ERCH 5108
S. glaucescens	Van: Between Gürpınar and Hoşap, around Zemek dam	July 2015	ERCH 5110
S. hermonis	Konya: Between Seydişehir and Gölyüzü village, Tavuktepe, 1200 m	July 2015	ERCH 5111
S. lazica	(A) Rize: Çayeli, Kaptanpaşa, over the village of Çataldere	August 2014	ERCH 5114
	(B) Trabzon: Köprübaşı, Beşköy, Büyükdoğanlı village, 800 m	July 2014	ERCH 5102
	(C) Trabzon: Altındere, Sümela monastery surroundings	July 2014	ERCH 5103
S. meifolia	Siirt: Pervari, between Tandırköy and Göl village gateway, among oaks	June 2014	ERCH 5115
	Mardin: Bakırkırı, Şeyh Şaran tomb surroundings, among oaks, 1020 m	June 2014	ERCH 5107
S. napiformis	Antalya: Akseki, Çukur village, Istarlas, 1050 m	May 2014	ERCH 5104
	Muğla: Köyceğiz, Ekincik village, Sandallıtepe, 480 m	May 2014	ERCH 5105
	Isparta: Eğirdir, upper parts of Gökdere, Kasnak forest, 1700 m	July 2014	ERCH 5116
S. tripartita	Rize: Çamlıhemşin, between Çat and Orta village, 1750 m	July 2014	ERCH 5109
	Aksaray: Hasan Mountain, above Pırpırlıseki, 1560 m	August 2014	ERCH 5117

Table 1. Locality information of Scaligeria samples used in this study.

a sputter-coater. Morphological observations were made with a LEO 440 Polaron SC7620 Sputter Coater Scanning Electron Microscope at the SEM laboratory of the Technology Research and Application Center (TAUM) of Erciyes University, Kayseri.

The pollen terminology was adopted from Faegri and Iversen (1975), Punt (1984), and Punt et al. (2007), and the shape classification followed that of Erdtman (1969), based on the P/E ratios in Table 2.

2.3. Numerical analysis

The Simpson and Roe graphical test (Van der Pluym and Hideux, 1997) was used for statistical calculations. Using the pollen morphological characteristics of the species, coefficients of correlation were determined, and they were grouped using the clustering analysis method (UPGMA, similarity, standardized variable). Nine palynological characters were selected to distinguish the 9 populations (operational taxonomic units: 7 species and 2 extra different populations for S. lazica) of Scaligeria. Every morphological character state and their values or scales are given in Table 3. The clustering analysis was based on Gower's (1971) general coefficient similarity (Sneath and Sokal, 1973), which can be used directly with a mixture of character types (binary, qualitative, and quantitative characters). The unweighted pair group method using arithmetic averages (UPGMA) was selected because it is the most commonly used method (Mohammadi and Prasanna, 2003) and it has advantages over other methods in accurate reflection of the similarity matrix as measured by the cophenetic correlation coefficient of Sokal and Rohlf (1962), symmetrical hierarchical structure [the "structural value" concept of McNeill (1979)], and congruence with classification derived by

traditional methods (Ward, 1993). All computations for cluster analysis were made using MVSP 3.1 software. In addition, principal component analysis (PCA) was used to ordinate the variables and identify the valuable pollen morphological characters used in taxonomy. The variance/covariance matrix was obtained from the average of the palynological data and the coordinates in a biplot graph were based on Euclidean distance. The results are shown in a two-dimensional graph with the first and second principal components. Untransformed, centered, and standardized data were used to create a covariance matrix, and 3 eigenvectors were extracted. The values of the eigenvectors and the total cumulative variance are represented in Table 2. With the help of Statistica StatSoft, v. 12 (Dell Software, Tulsa, OK, USA), exploratory data analysis was performed.

3. Results

3.1. Pollen morphology

The main palynological features of the *Scaligeria* species are examined and summarized in Table 3 and Figures 1–5.

3.2 Size, symmetry, and shape

The pollen grains of *Scaligeria* are radially symmetrical and isopolar. The pollen grains are prolate and subprolate (*S. capillifolia*) with polar axes ranging from 18.4 to 37 μ m and equatorial axes ranging from 9.8 to 30 μ m (Figures 1A and 1B), sides convex to subparallel, concave or concave to subparallel in equatorial view. The pollen sides of *S. meifolia* are 98% convex and 2% convex to subparallel, *S. napiformis* 98% concave to subparallel and 2% concave. The dimensions are smaller in *S. tripartita* and larger in *S. capillifolia* (Table 3; Figures 2–5).

Table 2. Summary statistics for nine characters examined in 9 populations (cases) of Scaligeria.

	P/E	Polar view (Amb)*	Sides	Exine thickness	Orn. polar view**	Pore structure	Equa. shape***	Clg	Pore shape
S. capillifolia	1.32	0	0	2.13	0	0	0	14.0	0
S. glaucescens	1.76	1	0	1.25	1	1	1	14.0	0
S. hermonis	1.71	2	1	2.5	0	1	1	16.8	1
S. lazica (A)	1.55	1	2	1.6	0	1	1	15.75	0
S. lazica (B)	1.55	1	2	1.6	2	1	1	15.75	0
S. lazica (C)	1.55	1	2	1.6	3	1	1	15.75	0
S. meifolia	1.65	0	3	1.1	4	2	1	17.8	0
S. napiformis	1.62	0	2	2.25	0	3	1	12.5	0
S. tripartita	1.88	0	1	1.7	2	4	1	10.5	1

*Pollen shape in polar view (Amb).

**Ornamentation of polar view.

***Pollen shape in equatorial view.

Species	Polar ;	axes (P	(um) (,	Equator	rial axes	(E) (μm)	P/E	Pollen shape		Sides	Exine thickness (µm)	Intine thickness (μm)
	Min	Max	Mean	Min	Max	Mean		Equatorial view	Polar view (Amb)			
S. capillifolia	33.10	37.0	35.20	23.30	30.0	26.70	1.32	Subprolate	Triangular	Convex to subparallel	1.75-2.50	0.30-0.50
S. glaucescens	19.0	25.0	22.0	10.0	15.0	12.50	1.76	Prolate	Triangular to subcircular	Convex to subparallel	1.0-1.50	0.30-0.50
S. hermonis	20.10	26.0	21.70	10.0	15.40	12.70	1.71	Prolate	Circular to subcircular	Concave	2.0-3.0	0.50-0.90
S. lazica (A)												
S. lazica (B)	18.50	25.70	22.30	12.30	16.50	14.40	1.55	Prolate	Triangular to subtriangular	Concave to subparallel	1.50-1.70	0.50-1.0
S. lazica (C)									0			
S. meifolia	19.50	23.30	20.90	11.30	13.80	12.60	1.65	Prolate	Triangular	98% convex, 2% convex to subparallel	0.90-1.30	0.50
S. napiformis	23.10	27.0	25.10	13.80	17.20	15.50	1.62	Prolate	Triangular	98% concave to subparallel, 2% concave	2.0–2.50	0.50
S. tripartita	18.40	23.0	20.70	9.80	12.20	11.0	1.88	Prolate	Triangular	Concave	1.50-2.0	0.75

Table 3. Pollen morphological measurements of Scaligeria species

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Species	Ornamentation		Aperture type	Colpi (µm)		Pore			
	Polar view	Equatorial view		Clg	Clt	Plg	Plt	Structure	Shape
S. capillifolia	Striate	Striate-rugulate	Tricolporate	13.0-15.0	0.50-0.90	2.0-2.50	4.0-4.50	Not to slightly protruding thick endexine	Lalongate
S. glaucescens	Striate-rugulate	Striate-rugulate	Tricolporate	13.0-15.0	0.80-1.20	2.0-3.0	2.50-3.5	Slightly protruding thickened endexine, arching ectexine	Lalongate
S. hermonis	Striate	Striate	Tricolporate	15.0-18.70	1.0-2.0	0.90-1.50	3.30-5.0	Slightly protruding thickened endexine, arching ectexine	Rectangular elliptical
S. lazica (A)	Striate	Striate-rugulate							
S. lazica (B)	Rugulate	Verrucate	Tricolporate	14.0-17.50	1.20-2.0	3.0-3.50	3.50-4.0	Slightly protruding thickened endexine, arching ectexine	Lalongate
S. lazica (C)	Rugulate-perforate	Rugulate							
S. meifolia	Striate reticulate	Striate reticulate	Tricolporate	16.0-19.70	1.0-2.0	1.20-1.70	1.50-3.0	Usually recessed, thick endexine	Lalongate
S. napiformis	Striate	Rugulate	Tricolporate	12.0-13.0	0.50 - 1.0	2.0-2.50	1.50-2.0	Slightly protruding thickened endexine, slightly arching ectexine	Lalongate
S. tripartita	Rugulate	Rugulate	Tricolporate	13.0-15.0	0.50-0.75	3.0-3.50	3.50-4.0	Protruding thickened endexine, arching ectexine	Rectangular elliptical

Clg: Colpus length; Clt: colpus width; Plg: pore length; Plt: pore width.

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Table 3. (Continued).



Figure 1. Simpson and Roe test for the *Scaligeria* species: A- polar axes (P), B- equatorial axes (E), and C-exine ornamentation variability of the species.

3.3. Apertures

All species examined are tricolporate. The colpus is short to rather long (9–19.7 μ m), narrow (0.5–2 μ m) and slit-like.

Margins are distinct, wavy and ends are distinct, acute. The highest values were observed in *S. hermonis* and *S. meifolia*. *S. tripartita* has the smallest measures of colpus.



Figure 2. Pollen morphology of *Scaligeria* by LM. 1–3- *S. capillifolia*, 3a: fastigium, 3b: exine structure. 4–6- *S. glaucescens*. 7–9- *S. hermonis*, 9: equatorial view with fastigium. 10–12- *S. lazica* (A). 13–15- *S. lazica* (B), 15: equatorial view with fastigium (scale bar = 10 μ m).



Figure 3. Pollen morphology of *Scaligeria* by LM. 1–3- *S. lazica* (C), 3: equatorial view with exine structure. 4–6- *S. meifolia*, 6: equatorial view with fastigium. 7–9- *S. napiformis*, 8a: fastigium, 8b: exine structure. 10–12- *S. tripartita*, 12a: fastigium, 12b: exine structure (scale bar = 10 µm).



Figure 4. Pollen morphology of *Scaligeria* by SEM. 1–3- *S. capillifolia*. 4–6- *S. glaucescens*. 7–9- *S. hermonis*. 10–12- *S. lazica* (A). 13–15- *S. lazica* (B). Scale bars: 2 μm (1, 4, 7, 10, 13); 10 μm (2, 5, 8, 11, 14); 1 μm (3, 6, 9, 12, 15).

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Figure 5. Pollen morphology of *Scaligeria* by scanning electron microscope. 1–3- *S. lazica* (C). 4–6- *S. meifolia.* 7–9- *S. napiformis.* 10–12- *S. tripartita.* Scale bars: 2 μ m (1, 4, 7, 10); 10 μ m (2, 5, 8, 11); 1 μ m (3, 6, 9, 12).

The colpus membrane is more or less granulate (Table 3; Figures 2–5). The pore is lalongate or rectangular-elliptical (*S. hermonis* and *S. napiformis*) in outline, often with straight margins. Pore area structure protruding, ectexine arching away from thickened endexine (*S. tripartita*); slightly protruding, ectexine arching away from thickened endexine; not to slightly protruding, thickened endexine (*S. capillifolia*) or usually recessed, thick endexine (*S. meifolia*).

3.4. Exine and intine

The exine is subtectate and $0.9-3 \ \mu m$ in thickness in the equatorial area. Nexine thinner than sexine at poles or about equally thick, even slightly thicker than sexine at equator. The columellar hypertrophy appears between the subpolar and equatorial position. Intine thickness ranges between 0.3 and 1 µm. A fastigium was observed in all of the species (Figures 2 and 3), whereas a costa was indistinct or very narrow, not forming a band in all species. By SEM, several types of ornamentations were observed on pollen surfaces: striate in polar view and striaterugulate in equatorial view (S. capillifolia, S. hermonis, S. lazica (A)), striate-rugulate in polar and equatorial views (S. glaucescens), striate-reticulate in polar and equatorial views (S. meifolia), striate in polar view and rugulate in equatorial view (S. napiformis), rugulate in polar and equatorial views (S. tripartita), rugulate-perforate in polar and rugulate in equatorial view (S. lazica (C)), rugulate in polar view and verrucate in equatorial view (S. lazica (B)) (Figures 1C and 2-5; Table 3).

3.5. Numerical analysis of the palynological character states

A dendrogram resulting from the cluster analysis based on nine palynological variables of 9 populations of *Scaligeria*

is presented in Figure 6. The dendrogram constructed by UPGMA revealed three main groups with 51.6% similarity; the first one comprises *S. tripartita*, the second one comprises *S. capillifolia*, and the third one, which is the largest, consists of the other five species. From the dendrogram, it is evident that *S. tripartita* and *S. capillifolia* are quite different from the other species and separated first. In the third group, *S. lazica* (B) and (C) and are quite similar at a level of 97.2% and *S. lazica* (A) is closely related to these with 93.1% similarity. *S. glaucescens* is quite similar at a level of 79.3% to populations of *S. lazica*.

PCA analysis was performed to determine which variables are important in explaining the total variation among the 9 cases examined. The PCA explained 81.461% of the variation with the first three axes (Figure 7; Table 4). The first principal component explains 34.004% of the total variation in the examined species. The pore structure, the ratio of P/E, and the ornamentation in equatorial view and polar view are the most significant variables in the first principal component because they have the highest relative variation rate (Table 4). Colpus length, pore shape, exine thickness, and sides have the strongest influence on the species in the second principal component; this explains 26.863% of the total variation. The third principal component explains 20.594% of the total variation, mainly through variables such as ornamentation in polar view and equatorial view as well as colpus length. The first three components that underlined the three primary valuable variables for separating the species of Scaligeria examined in this study are pore structure, the ratio of P/E, and equatorial shape. The ornamentation of polar view, colpus length, and exine thickness were secondary important characters. Additionally, 3D surface plot analysis was



Figure 6. Dendrogram showing similarity distance of Scaligeria species. Asterisk marks endemic species.



Figure 7. Scatter plots of the projections of variables and species (cases) along PC1 and PC2 (A, B).

performed. These plots are useful in regression analysis for viewing the relationships among variables. Multiple regression analysis states that this surface is perfectly flat. Hence, the surface plot lets us visually determine if multiple regression is appropriate between the variables (Figures 7 and 8).

Variables	PC 1	PC 2	PC 3
P/E	0.845	0.334	-0.279
Polar view amb	-0.194	-0.014	-0.953
Sides	0.437	-0.664	-0.050
Exine thickness	-0.410	0.670	-0.237
Orn. polar view	0.667	-0.475	0.214
Pore structure	0.847	0.358	0.275
Equa. shape	0.758	-0.202	-0.501
Clg	-0.254	-0.739	-0.520
Pore shape	0.379	0.686	-0.404
Eigenvalues % Total variance	3.060 34.004	2.417 26.863	1.853 20.594
Cumulative eigenvalue	3.060	5.478	7.331
Cumulative percentage	34.004	60.867	81.461

Table 4. PCA variable loadings for the first 3 components.

4. Discussion

The pollen morphology of the Turkish Scaligeria species shows taxonomically significant features where the main differences were found at the species level. The pollen grains of the genus are homogeneous in size and shape. On the basis of the classification of Cerceau-Larrival (1962, 1971) and Roland-Heydacker and Cerceau-Larrival (1978), all pollen grains in this study were consistently oval type with a P/E ratio of 1.5-2 on the outside of S. capillifolia (subcircular, type 2, P/E: 1-1.5). The diversification in the family Apiaceae of the phyletic series was subrhomboidal > subcircular > oval > subrectangular > equatorially constricted (Gruas-Cavagnetto and Cerceau-Larrival, 1978), where a suboval or rectangular shape is a more advanced feature (De Leonardis et al., 2008). Punt (1984) divided the family into 50 pollen types based on the two outer contour shapes (outer contour of mesocolpium side straight or convex and concave or slightly concave). The outer contours of S. capillifolia, S. glaucescens, and S. meifolia are convex or straight while those of S. hermonis, S. lazica, S. napiformes, and S. tripartita are concave or slightly concave according to Punt's classification. Cerceau (1959, 1962) and Punt (1984) observed that the polar view of Apiaceae was important for the differentiation of the pollen types. In this study, we found that the genus Scaligeria had three types of polar shapes: triangular, triangular to subtriangular, and circular to subcircular.

The common aperture type of Apiaceae pollen grains is 3-colporate (rarely 4-colporate porate) and colpi with costae (Perveen and Qaiser, 2006; Yousefzadi, 2006; Pehlivan et al., 2009, Güner et al., 2011). *Scaligeria* pollen grains only have tricolporate aperture. It was suggested that

the outline of the endoaperture is most important for the identification of Apiaceae (Cerceau-Larrival, 1962; Punt, 1984; Hebda, 1985). Ligusticum L. has predominantly dumbbell-, X-, or H-shaped to occasionally rectangular, usually lalongate pores (Hebda, 1985), whereas Scaligeria shows lalongate or rectangular-elliptical aperture type pores. Remarkable changes were found in the degree of ectexinal arching over the pore area and shape of the endoaperture and exoaperture (Cerceau-Larrival, 1971; Hebda, 1985). In most taxa of this genus, the ectexine arches either slightly out or not at all from the colpus. The ectexine of Scaligeria arches markedly, creating exopores that are irregular and difficult to distinguish. The fastigium, which is a cavity in a colporate grain, is either not visible or is a small and indistinct feature for Apiaceae (Reitsma, 1970; Punt, 1984). However, we observed a distinctly small fastigium in all the species (Figures 2-5). Bupleurum falcatum L. and Meum athamanticum Jacq. pollen types of Punt's classification usually have a distinct and small fastigium. The costae of Apiaceae taxa are always most common, present all around the equator, and are broad and band-like (Punt, 1984; Perveen and Qaiser, 2006; Güner et al., 2011). The costae of Scaligeria pollen are restricted to the pores, and the nexine at the mesocolpium side is no thicker than the shoulders or the poles like in Torilis nodosa L. Gaertner and Hydrocotyle vulgaris L.

Pollen ornamentation is one of the most significant characteristics that can be used to separate taxa (Pınar et al., 2009; Mačukanović-Jocić et al., 2017; Zhang et al., 2017). We observed that *Scaligeria* was striate, striate-rugulate, striate-reticulate, rugulate, and rugulate-perforate in polar view and striate-rugulate, striate-reticulate, rugulate, and

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Figure 8. The 3D surface plot analysis shows the interaction of the first important three variables (A) and the secondary important three variables (B).

verrucate in equatorial view in exine ornamentation. We also found that endemic *S. lazica* is striate in polar view and striate-rugulate in equatorial view (A), regulate-perforate in polar and regulate in equatorial view (C), and rugulate in polar view and verrucate in equatorial view (B). This situation reflects the variation between the species. According to the cluster and population structure and genetic diversity analysis of *S. lazica* collected from different locations of Turkey, all accessions were grouped into four clusters based on the collection sites (Baldemir et al., 2017). Because of the striate rugulate ornamentation, size, and shape, *S. capillifolia*, *S. glaucescens*, *S. hermonis*, and *S. lazica* (A) conformed to the *Bupleurum gilesii* type

of Perveen and Qaiser (2006). However, ornamentation is of little value as a discriminating factor for the identification of pollen grains in Umbelliferae (Punt, 1984). Three ornamentation types were determined in that study: irregularly rugulate, irregularly striate, or cerebroid. However, Punt (1984) did not examine any species of *Scaligeria*. In another study, Güner et al. (2011) observed the following ornamentation types of *Seseli* L.: rugulate in the equatorial area, psilate at the poles; striate-reticulate at equator, rugulate at poles; rugulate at equator, striate at poles; and rugulate-granulate at equator, striate at poles.

The results from cluster analysis show that the examined members of *Scaligeria* that fall into three main

groups coincide with pollen sizes (Figure 6). According to UPGMA analysis based on pollen morphological data, each species was distinctly separated from each other.

Pollen morphological characteristics such as pore structure, the ratio of P/E, and ornamentation at the polar and equatorial view are the most valuable variables for separating the *Scaligeria* species. The results of UPGMA clustering and scatter plot projections for species are quite common. This compatibility was observed in the study by Umdale et al. (2017). In the PCA scatter plot, all *S. lazica* species were grouped together, the same as in the dendrogram. *S. tripartita* and *S. capilifolia* were found as the external taxa separating from the other taxa at first in the dendrogram and the plot (Figures 6–8).

In conclusion, analysis of pollen grains of seven *Scaligeria* species in Turkey by LM and SEM revealed that the palynological characteristics (e.g., pore structure,

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equatorial shape, ornamentation of exine at polar view, sides of pollen, and exine sculpturing) are reliable criteria for explaining the relationships between these species. Pollen properties such as pore structure and P/E ratio ensure that the majority of the *Scaligeria* species are easily separated from each other and confirm the pollen heterogeneity. In further studies we predict that the systematic problems of the genus *Scaligeria* will be solved by supporting anatomical, morphological, and molecular studies.

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