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POLLEN MICROMORPHOLOGY OF 12 TAXA OF *DELPHINIUM* IN TÜRKİYE AND THEIR TAXONOMIC IMPORTANCE

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ABSTRACT

Pollen micromorphology of 12 taxa belonging to genus *Delphinium* L. (Ranunculaceae) species was investigated by light microscopy and scanning electron microscopy. Pollen slides were prepared using the Wodehouse technique. Measurements were based on 30 or more pollen grains per specimen. For scanning electron microscopy studies, dried pollen grains were transferred to aluminium stubs and coated with gold for 3 min in a sputter-coater. The pollen grains of *Delphinium* taxa are radially symmetric and isopolar. The pollen grains of the taxa are prolate–spheroidal, subprolate with the polar axes 24.51–36.24 µm and the equatorial diameter 22.13–30.43 µm. The pollen grains are tricolpate rarely tetracolpate (*D. thirkeanum*). Colpi were usually long and narrow (Clg: 18.46–28.73 µm; Clt: 5.17–9.31 µm) and granulate. Pollen grains show microechinate, microechinate-perforate, microechinate-verrucate. Exine is subtectate and has a thickness of 0.99–1.58 µm. Ectexine is thicker than endexine. Intine has a thickness of 0.39–0.69 µm. As result of this study, some pollen characteristic as pollen shape, aperture type, and ornamentations are shown to vary within the genus. In particular, pollen surface ornamentation has systematic value for distinguishing taxa from one another. Cluster and principal component analyses supported the separation of taxa into three types. Type 1 and Type 2 differed mainly in pollen size and aperture dimensions, while *D. saccatum* (Type 3) was characterized by thicker exine and intine layers. Taxa formerly classified under *Consolida* clustered together as Type 2, indicating consistent morphological differentiation within the genus *Delphinium*. These statistical results support the taxonomic value of quantitative pollen traits.

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1. INTRODUCTION

The genus *Consolida* (DC.) S.F. Gray and *Delphinium* L. were revised from the Ranunculaceae family by Davis [1] and by Ilarslan [2], respectively. The genus *Consolida* is mostly distributed in the Irano-Turanian and Mediterranean phytogeographical regions, with 56 species in the world and 32 taxa belonging to the genus *Consolida* in Flora of Türkiye [1]. Among those in Türkiye, a total of 14 taxa, 13 species and 1 subspecies, are endemically distributed; the endemism rate of taxa belonging to this genus is 43.75% [1]. The genus *Delpinium* is also mostly distributed in the Irano-Turanian and Mediterranean phytogeographical regions, with 300 species in the world and 31 taxa belonging to the genus *Delphinium* in Flora of Türkiye [1]. In Türkiye, 19 of 31 *Delphinium* taxa are endemic [3]. The endemism rate is 61%.

Ranunculaceae is a eurypalynous family with representatives of several classes whose pollen grains are mostly tricolpate, pantocolpate, or pantoporate (and colporate only in *Paeonia* L.) with exine ornamentation showing a variety of forms, including echinate and reticulate [4,5]. Clarke et al., [6] divided the pollens of the taxa in the genus *Adonis* L. (Ranunculaceae) into 2 types: A. annua type and A. vernalis type. They stated that the most obvious difference in the pollen grains of the taxa in this genus was due to their equatorial appearance. Aytuğ et al., [7] included the pollen morphology of *Ranunculus constantinopolitanus* Urv. They stated that the pollen type of this species is periporate, the number of pores varies between 8-12, and the pollen shape is spheroid, large, and has an irregular granulation. Lee and Blackmore [8] investigated the palinomorphological taxa of the genera *Hydratis* L., *Paeonia* L. and *Trollius* L. from the same family. *Trollius* genus are 2, 3, and 5-colpus and their ornamentation is usually striate. Kurşat et al. [9] conducted palynological studies on 30 different *Ranunculus* L. taxa using light microscopy (LM) and scanning electron microscopy (SEM) methods.

There have been a limited number of studies on *Consolida* [6,10,11]. Recently, Doğanay [12] examined 31 taxa of *Consolida* only by light microscopy and pollen grains tricolpate rarely syncolpate, tetracolpate, prolate-sphaeroidal, subprolate, prolate. Bursali and Doğan, [13] examined the pollen grains of 21 taxa belonging to *Delphinium* using a light microscope. Simon and Blanche [14] studied the pollen morphology of four different species of *Consolida*, namely *C. ajacis* (L.) Schur, *C. orientalis* (Gay) Schrod., *C. pubescens* (DC.) Soo and *C. mauritanica* (Coss.) Muoz, distributed in the Iberian Peninsula, using both light and scanning electron microscopy.

DuPasquier et al. reclasified *Aconitella* Spach., *Consolida* L., and *Delphinium* under one genus, *Delphinium* (c. 300 species), according to the latest molecular phylogenetic studies [15]. Since only 58 species were under the *Aconitella* and *Consolida*, the reclassification resulted in fewer name changes compared to alternative schemes.

In this article, our objective is to determine the morphological characteristics of pollen in the genus.

2. MATERIALS AND METHODS

2.1. Plant materials

In this article, taxa belonging to the genus *Delphinium* were collected from Türkiye (Elazığ, Niğde, and Bitlis provinces). Each taxon was examined as a single specimen. This study used plant materials from the Bitlis Eren University Herbarium (BEUH). The plant material, both fresh and dried, was collected in Türkiye and studied using light and scanning electron microscope.

Specimens investigated: *D. cornutum* Hossain & P.H.Davis: Elazığ, Ağılcık Village and its surroundings, garden inner areas and slopes.01.09.2013, 1300-1450 m. Endemic. BEUH 1025. *D. glandulosum* Boiss. & Huet in Boiss. Elazığ: Upper part of the Baskil marble plant, 26/06/2002, 1320-1450 m. BEUH 1028. *D. oliveriana* DC.: Elazığ, Kamışlık Mountain, lower sides of Topaluşağı Village, slopes, 750-1130 m 04.07.2002, BEUH 1026 *D. hispanicum* Costa: Elazığ: Bolucuk Hamlet and its surroundings, garden interiors,1580 m. 11/06/2002, BEUH 1027. *D. consolida* L. subsp. *paniculatum* (Host) Busch: Niğde, Niğde Omer Halisdemir University Campus, BEUH 1027. *D. saccatum* Huth: Elazığ, Baskil, Doğancık Village, Bolucuk Hamlet and northern slopes 1600 m. 27/07/2021, BEUH 1024. *D. thirkeanum* Boiss. Niğde, Niğde Omer Halisdemir University Campus, Endemic, BEUH1023. *D. dasystachyon* Boiss. & Balansa: Bitlis, North Slopes of Mount Kambos, Steppe, 26.07.2013, 1800-1950, BEUH 1030. *D. dolichostachyum* Chowdhuri & P.H.Davis: Bitlis, Tatvan, north slopes of Obuzlar Village, 27.07.2021, 1850m, Endemic, BEUH1031. *D. peregrinum* L.: Elazığ, Baskil, Bolucuk Hamlet and its surroundings, garden interiors, 1580 m. 10/07/2002, BEUH1029. *D. vanense* Rech.f.: Van, northern slopes of Erek Mountain, southern slopes of Değirmen Village, BEUH 1032. *D. venulosum* Boiss.: Niğde, Niğde Ömer Halisdemir University Campus, BEUH 1022.

2.2. Palynological Analysis

In the examination by light microscopy, pollen samples were prepared using the method of Wodehouse [16] and examined on an Olympus BX-21LED microscope. In total, we examined ten qualitative and quantitative pollen morphological variables. The variables observed or measured were polar axis (P), equatorial diameter (E), pollen shape (P/E), colpus length (Clg), colpus width (Clt), exine thickness (Ex), mesocolpium (Mes), apocolpium (Ap), intine thickness (In), and ornamentation (Orn). Measurements were performed based on 30 pollens for each morphological character, and the mean value and standard deviation of these measurements were given in Table 2. Light micrographs of pollen grains were acquired using an Olympus dp25 digital camera connected to an Olympus BX-31 microscope equipped with a 100 × objective lens.

During the examination performed with a scanning electron microscope, pollen grains selected from dry samples were placed on aluminium stubs with the help of double-sided adhesive tape and coated with gold using a vacuum. Pollen pictures were taken with a ZEISS Supra 55 Scanning Electron Microscope at the SEM Laboratory of the Central Research Laboratory (MERLAB), Van Yuzuncuyil University. Descriptive terminology follows Clarke et al. [6]; Halbritter et al. [17].

2.3. Statistical Analysis

The statistical analyses were performed on polar axis, equatorial diameter, colpus length, colpus width, exine, intine, apocolpium, and mesocolpium data of 12 taxa by using an online application called SourceTable (app.sourcetable.com)[18]. Cluster analysis was performed by using UPGMA (Unweighted Pair Group Method with Arithmetic Mean) with Gower similarity [19, 20, 21]. In order to further visualize the diversity of specimens, PCA (Principal Component Analysis) was performed by using the variance-covariance matrix [22].

3. RESULT

Pollen morphological features of *Delphinium* is presented in Tables 1-2 and Figures 1-2-3. Pollen grains shed as monads, radially symmetrical, isopolar, prolate-spheroidal, and subprolate with a polar axis of 24.51–36.24 µm and an equatorial diameter of 22.13–30.43 µm (Table 1). The polar axis (P) is 36.24 (30.60-41.82) µm and the equatorial axis (E) is 30.43 (19.38–37.74) µm. *D. peregrinum* (subprolate) and other taxa are prolate-spheroidal. The smallest pollen grains were found in *D. dolichostachyum* with P: 24.51 ± 2.13 µm and E: 22.13 ± 1.24 µm. The largest pollen grains were found in *D. thirkeanum* with P: 36.24 ± 2.53 µm and *D. cornutum* with E: 30.43 ± 2.04 µm. The variation in pollen size for all species was shown in Figures 1 and 2. The colpus varies from short to long, but is generally long, with distinct borders and blunt or sharp ends. The outline of all pollen grains is circular in polar view (Table 1; Figures 1-2-3).

Based on SEM and LM observations, two pollen classes were observed in *Delphinium*: tricolpate and tetracolpate (*D. thirkeanum*), colpus is long (18.46–28.73 µm) and narrow or broad (5.27–9.31 µm); margins distinct, regular, and ends acute. Aperture membrane ornamented (Table 1-2; Figures 1-2-3). The exine ranges from 0.99 to 1.58 µm thick in *D. saccatum*. The intine is 0.39–0.58 µm thick in *D. saccatum*. Exine sculpturing is microechinate in LM. Based on SEM microphoto, the number of microechina per 25 μm^2 is 16-108, the number of perforations per 25 μm^2 is 16–43, the microechina length is 0.12–0.52 µm, and the microechina width is 0.18-0.71 µm. The distance between microechinae is 0.38–2.30 µm. Exine sculpturing is microechinate, microechinate-perforate, microechinate-verrucate (Table 1- 2; Figures 1-2).

Table 1. *Micromorphological features of pollen grains of the studied Delphinium taxa (µm) (mean ± standard deviation, minimum-maximum).*

Taxa	(P/E) Polen shape	(P) Polar axis	(E) Equatorial diameter	(Clg) Colpus length	Clt (Colpus width)	(Ex) Exine	(In) Intine	(Ap) Apocolpium	(Mes) Mesocolpium
D. thirkeanum	Subprolate	36.24±2.53 (32.64 – 41.82)	28.33±3.06 (23.46 – 37.74)	28.36±2.96 (23.46 – 36.72)	7.34±1.42 (5.10 – 10.20)	1.43±0.31	0.57±0.25	7.46±1.17 (5.10 – 9.18)	30.04±3.75 (25.50 – 37.74)
D. saccatum	Subprolate	32.98±2.15 (29.58 – 36.72)	26.08±0.96 (24.48 – 28.56)	27.54±1.49 (25.50 – 29.58)	7.12±1.33 (5.10 – 9.18)	1.58±0.18	0.69±0.19	5.17±0.67 (4.08 – 6.12)	27.71±1.46 (25.50 – 30.60)
D. hispanicum	Subprolate	35.90±2.76 (31.62 – 41.82)	28.39±3.83 (22.44 – 35.70)	28.73±2.98 (24.48 – 35.70)	6.05±1.29 (4.08 – 9.18)	1.20±0.20	0.44±0.11	6.43±1.30 (5.10 – 9.18)	28.31±3.31 (22.44 – 33.66)
D. consolida subsp. *paniculatum*	Subprolate	33.79±1.78 (30.60 – 37.74)	26.35±1.68 (23.46 – 30.60)	26.39±1.94 (22.44 – 30.60)	5.64±0.90 (4.08 – 8.16)	1.28±0.29	0.58±0.27	7.09±1.19 (5.10 – 9.18)	25.70±1.32 (23.46 – 28.56)
D. glandulosum	Subprolate	34.30±2.48 (30.60 – 38.76)	25.84±2.71 (21.42 – 30.60)	28.22±2.91 (23.46 – 35.70)	5.54±1.19 (4.08 – 8.16)	1.15±0.18	0.42±0.17	5.66±0.98 (4.08 – 7.14)	27.90±2.11 (23.46 – 31.62)
D. cornutum	Prolate-spheroidal	32.61±1.90 (30.60 – 36.72)	30.43±2.04 (25.50 – 34.68)	27.37±2.05 (22.44 – 30.60)	6.63±1.08 (5.10 – 8.16)	1.17±0.19	0.45±0.17	5.97±1.18 (4.08 – 8.16)	33.10±1.67 (30.60 – 35.70)
D. oliveriana	Subprolate	30.70±1.73 (27.54 – 34.68)	24.41±1.96 (19.38 – 28.56)	24.07±2.11 (20.40 – 28.56)	5.17±0.83 (4.08 – 7.17)	1.12±0.17	0.39±0.14	4.77±0.95 (3.06 – 7.14)	25.40±1.89 (20.40 – 28.56)
D. peregrinum	Subprolate	27.10±1.07 (25.50 – 29.58)	23.08±1.10 (20.40 – 25.50)	21.45±1.52 (18.36 – 24.48)	5.27±0.75 (4.08 – 7.14)	1.15±0.18	0.40±0.14	6.07±1.36 (3.06 – 8.16)	22.75±1.95 (19.38 – 26.52)
D. venulosum	Prolate-spheroidal	26.38±1.27 (24.48 – 28.56)	25.40±1.61 (21.42 – 27.54)	20.84±1.59 (17.34 – 23.46)	8.08±1.05 (6.12 – 9.18)	1.31±0.18	0.53±0.16	6.38±1.28 (4.60 – 9.18)	25.91±1.13 (22.44 – 27.54)
D. dasystachum	Prolate-spheroidal	24.72±1.46 (22.44 – 27.54)	22.72±1.34 (20.40 – 24.48)	20.91±2.93 (17.34 – 27.54)	8.18±0.77 (7.14 – 9.18)	0.99±0.16	0.48±0.15	4.13±0.65 (3.06 – 5.10)	24.04±1.28 (22.44 – 26.52)
D. dolichostachyum	Prolate-spheroidal	24.51±2.13 (20.40 – 28.56)	22.13±1.24 (19.38 – 24.48)	18.46±1.67 (15.30 – 21.42)	6.15±0.86 (4.08 – 8.16)	1.22±0.20	0.50±0.18	4.90±0.83 (4.08 – 6.60)	23.05±1.22 (21.42 – 24.48)
D. *vananse*	Prolate-spheroidal	24.96±1.04 (23.46 – 26.52)	24.49±1.20 (22.44 – 26.52)	20.23±1.18 (18.36 – 22.44)	9.31±0.85 (8.16 – 11.22)	1.03±0.13	0.49±0.16	5.30±0.83 (4.08 – 6.60)	25.67±1.26 (23.46 – 27.54)

Table 2. *Micromorphological features of the studied Delphinium taxa based on SEM microphotographs (µm).*

Taxa	Average number of microechinae in 25 µm^2 (min-max)	Average number of perforations in 25 µm^2 (min-max)	Microechinae length (Sh)	Microechinae width (St)	Distance between microechina	Ornamentation
D. thirkeanum	83 (80-85)	27 (24-29)	0.12	0.21	0.38	Microechinate-perforate
D. saccatum	108 (105-110)	43 (40-45)	0.17	0.28	0.50	Microechinate-perforate
D. hispanicum	28 (25-30)	--	0.19	0.26	0.97	Microechinate-verrucate
D. consolida subsp. *paniculatum*	43 (40-45)	--	0.25	0.42	0.98	Microechinate
D. glandulosum	27 (25-28)	--	0.23	0.32	0.55	Microechinate
D. cornutum	28 (25-30)	18 (15-20)	0.23	0.38	1.60	Microechinate-perforate
D. oliveriana	58 (55-60)	28 (25-30)	0.20	0.18	0.86	Microechinate-perforate
D. peregrinum	16 (14-18)	--	0.38	0.52	1.01	Microechinate
D. venulosum	19 (17-21)	--	0.52	0.71	1.07	Microechinate
D. dasystachum	17 (15-18)	16 (15-17)	0.23	0.41	2.30	Microechinate-perforate
D. dolichostachyum	19 (17-20)	21 (20-22)	0.31	0.44	0.75	Microechinate-perforate
D. vananse	33 (30-35)	--	0.13	0.30	0.92	Microechinate

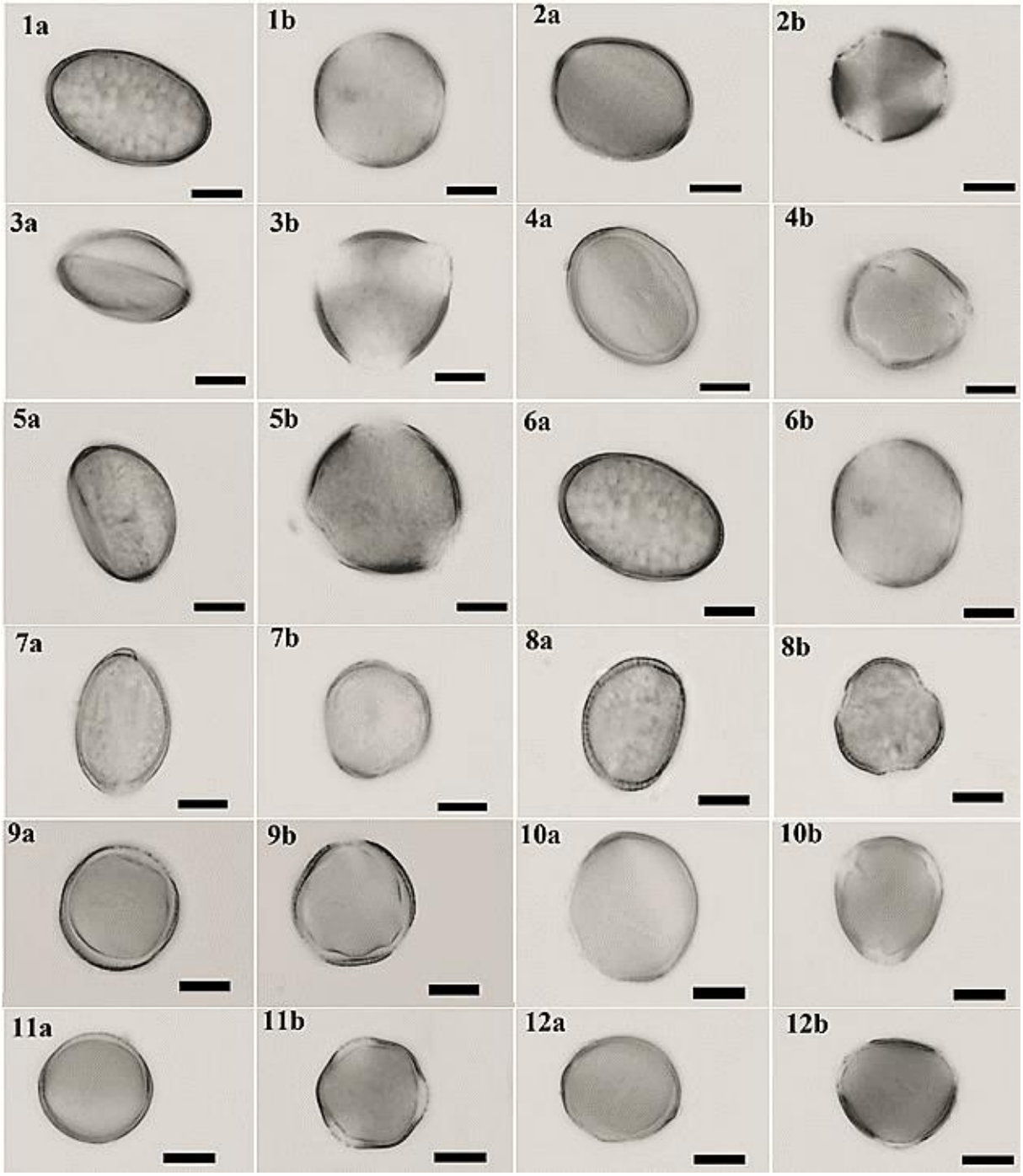


Figure 1. *LM micrograph of Delphinium. Equatorial and polar views:* ***1a-b****: D. thirkeanum,* ***2a-b:*** *D. saccatum,* ***3a-b:*** *D. hispanicum,* ***4a-b:*** *D. consolida* subsp. *paniculatum,* ***5a-b:*** *D. glandulosum,* ***6a-b****: D. cornutum,* ***7a-b:*** *D. oliveriana,* ***8a-b:*** *D. peregrinum,* ***9a-b:*** *D. venulosum,* ***10a-b:*** *D. dasystachum,* ***11a-b:*** *D. dolichostachyum,* ***12a-b****: D. vanense (Scale bar: 10* ***µm****).*

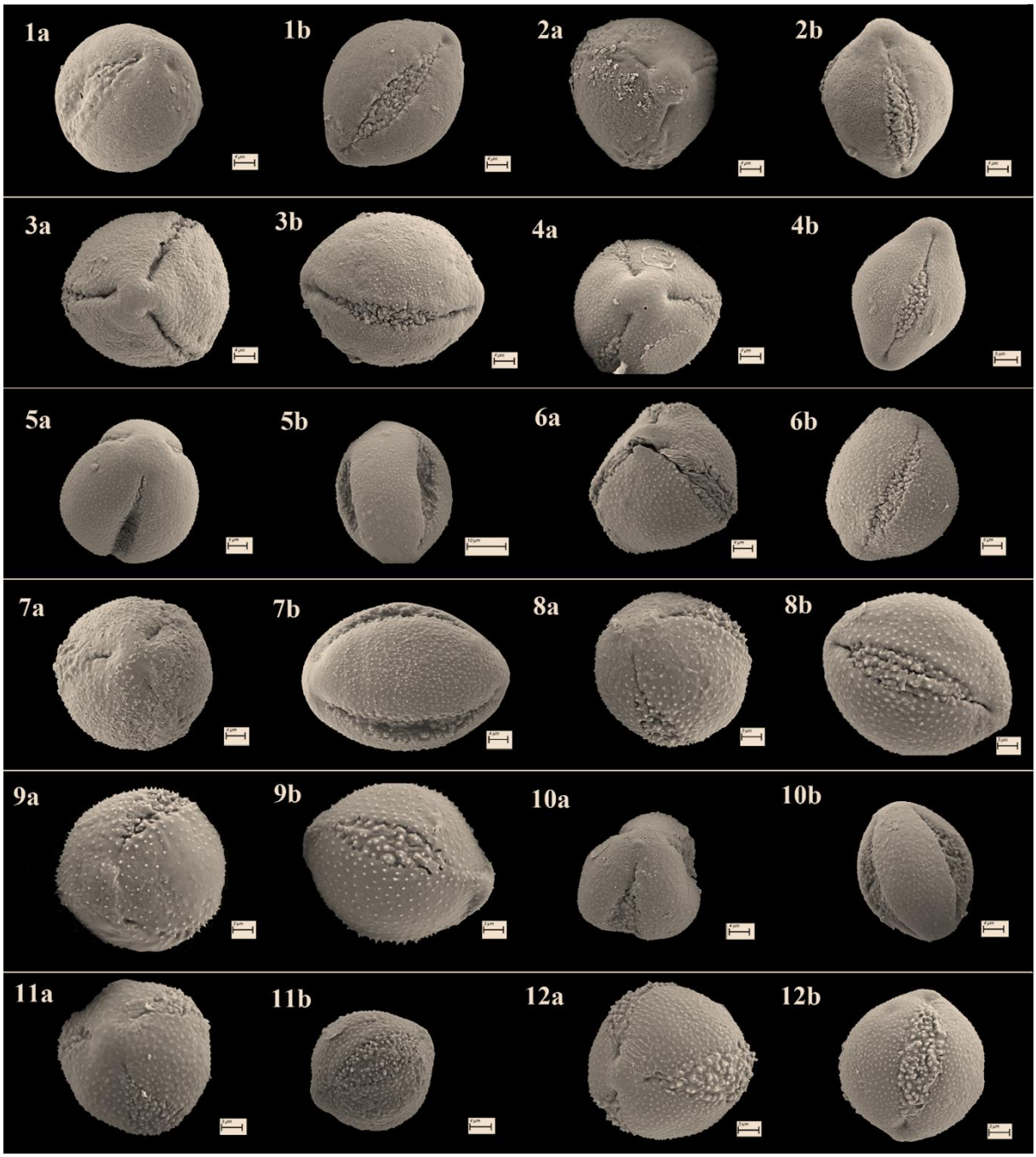


Figure 2. *SEM micrograph of Delphinium. Polar and equatorial view:* ***1a-b****: D. thirkeanum,* ***2a-b:*** *D. saccatum,* ***3a-b:*** *D. hispanicum,* ***4a-b:*** *D. consolida subsp. paniculatum,* ***5a-b:*** *D. glandulosum,* ***6a-b****: D. cornutum,* ***7a-b:*** *D. oliveriana,* ***8a-b:*** *D. peregrinum,* ***9a-b:*** *D. venulosum,* ***10a-b:*** *D. dasystachum,* ***11a-b:*** *D. dolichostachyum,* ***12a-b****: D. vanense.*

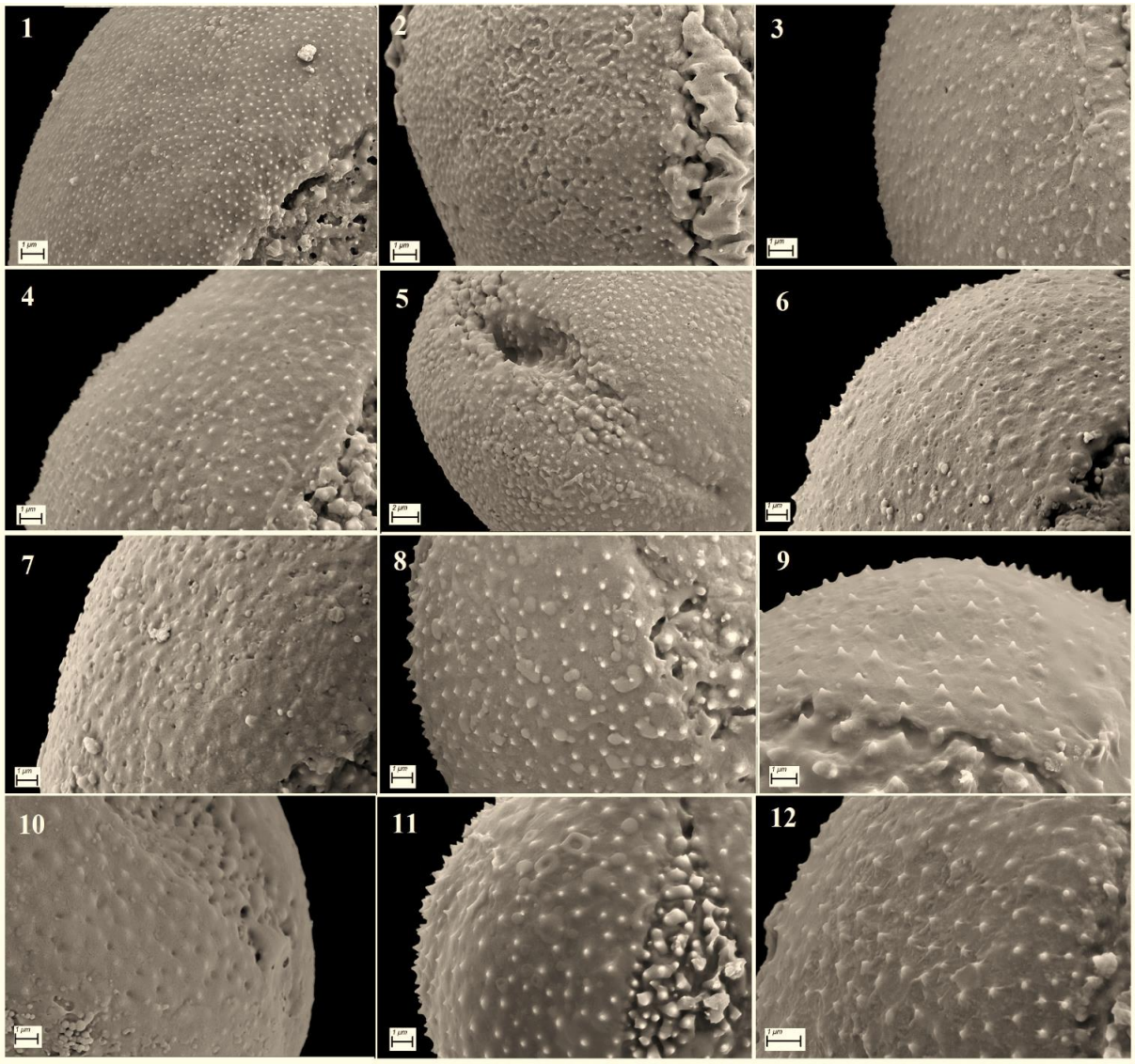


Figure 3. *SEM micrograph of Delphinium Exine sculpture:* ***1:*** *D. thirkeanum,* ***2:*** *D. saccatum,* ***3:*** *D. hispanicum,* ***4:*** *D. consolida subsp. paniculatum,* ***5:*** *D. glandulosum,* ***6****: D. cornutum,* ***7:*** *D. oliveriana,* ***8:*** *D. peregrinum,* ***9:*** *D. venulosum,* ***10:*** *D. dasystachum,* ***11:*** *D. dolichostachyum,* ***12:*** *D. vanense.*

UPGMA-based cluster analysis with Gower similarity coefficiesent devided the 12 taxa into 3 types according to pollen morphology (Figure 4, Table 3): Type 1 includes *D. oliveriana*, *D. peregrinum*, *D. venulosum*, *D. dasystachum D. dolichostachyum*, and *D. vananse*; Type 2 includes *D. thirkeanum*, *D. saccatum*, *D. hispanicum*, *D. consolida* subsp. *paniculatum*, *D. glandulosum*, and *D. cornutum*; and Type 3 includes only *D. saccatum*.

Table 3 shows PCA results, including eigenvalues, percentages, cumulative percentages, PCA scores of all taxa, and PCA variable loading of each parameter for the two principal components with the highest variable explanation (53.9% in PC1 and 21.2% in PC2). The results were visualized in Figure 5. PCA variable loading rates divided the parameters of pollen morphology into four groups: Size variables (polar axis, equatorial diameter, colpus length, mesocolpium), colpus (colpus width), wall structure (exine, intine), and other (apocolpium).

Table 3. *Pollen morphological parameters analysed by principal component analysis (PCA).*

			PC 1	**PC 2**
Eigenvalues			4.71	1.85
Percentage			53.9%	21.2%
Cumulative Percentage			53.9%	75.1%
PCA variable loadings				
Group	**Variable**	**Abbreviation**	**PC 1**	**PC 2**
Size variable	Polar axis	P	0.450	-0.172
Size variable	Equatorial diameter	E	0.437	-0.084
Size variable	Colpus length	Clg	0.446	-0.162
Colpus	Colpus width	Clt	-0.102	0.508
Wall structure	Exine	Ex	0.302	0.455
Wall structure	Intine	In	0.169	0.684
Other	Apocolpium	Ap	0.340	0.025
Size variable	Mesocolpium	Mes	0.402	-0.058
PCA Scores				
Type	**Taxon**		**PC 1**	**PC 2**
Type 2	*D. thirkeanum*		3.187	0.952
Type 3	*D. saccatum*		1.729	2.514
Type 2	*D. hispanicum*		1.990	-1.299
Type 2	*D. consolida* subsp. *paniculatum*		1.454	0.248
Type 2	*D. glandulosum*		0.864	-1.650
Type 2	*D. cornutum*		2.279	-1.061
Type 1	*D. oliveriana*		-1.044	-1.728
Type 1	*D. peregrinum*		-1.814	-1.124
Type 1	*D. venulosum*		-0.639	1.458
Type 1	*D. dasystachum*		-3.095	0.297
Type 1	*D. dolichostachyum*		-2.739	0.487
Type 1	*D. vananse*		-2.171	0.907

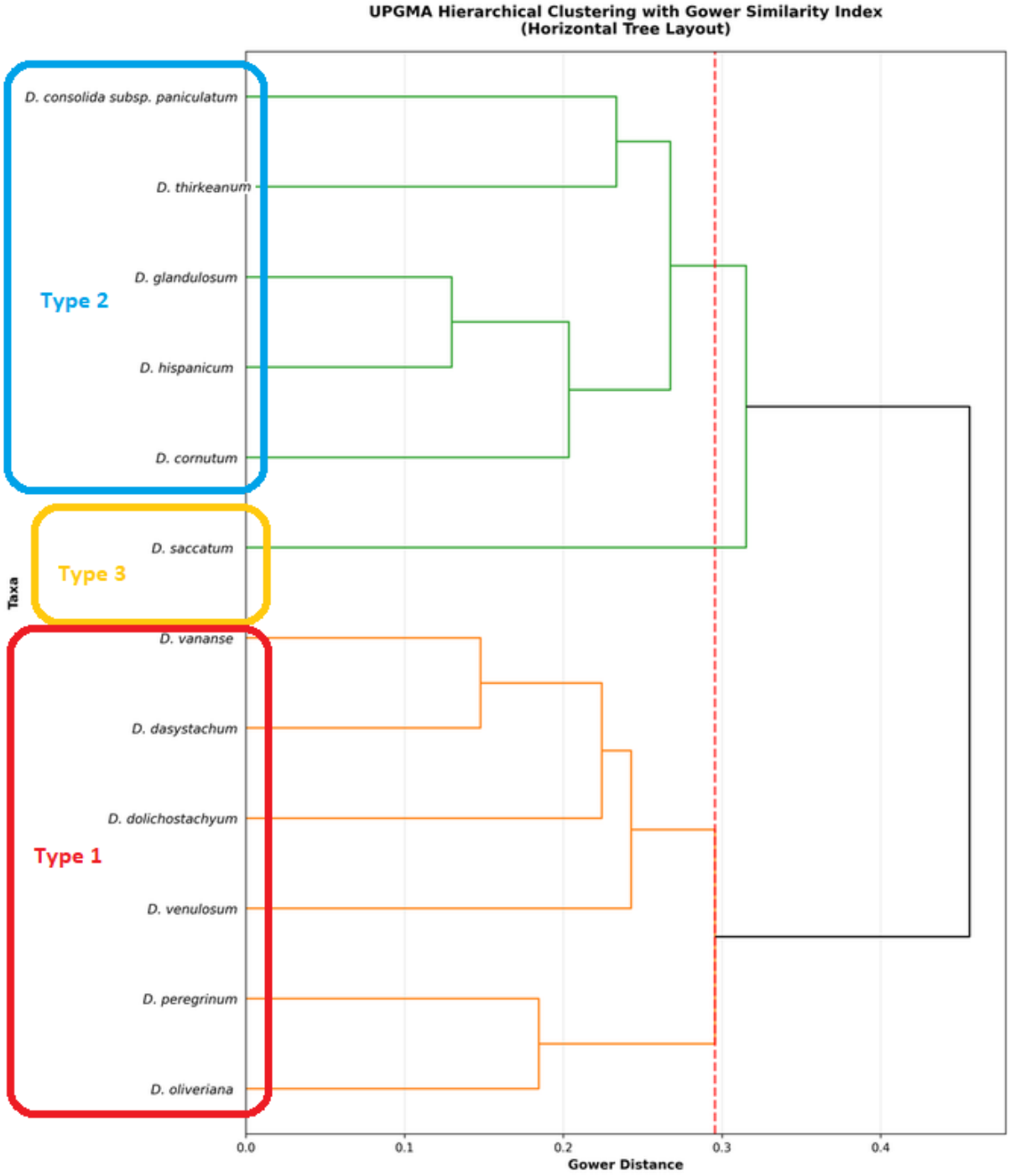


Figure 4. *Dendrogram obtained from the analysis of pollen morphological characters of the studied taxa and their taxonomic relationships.*

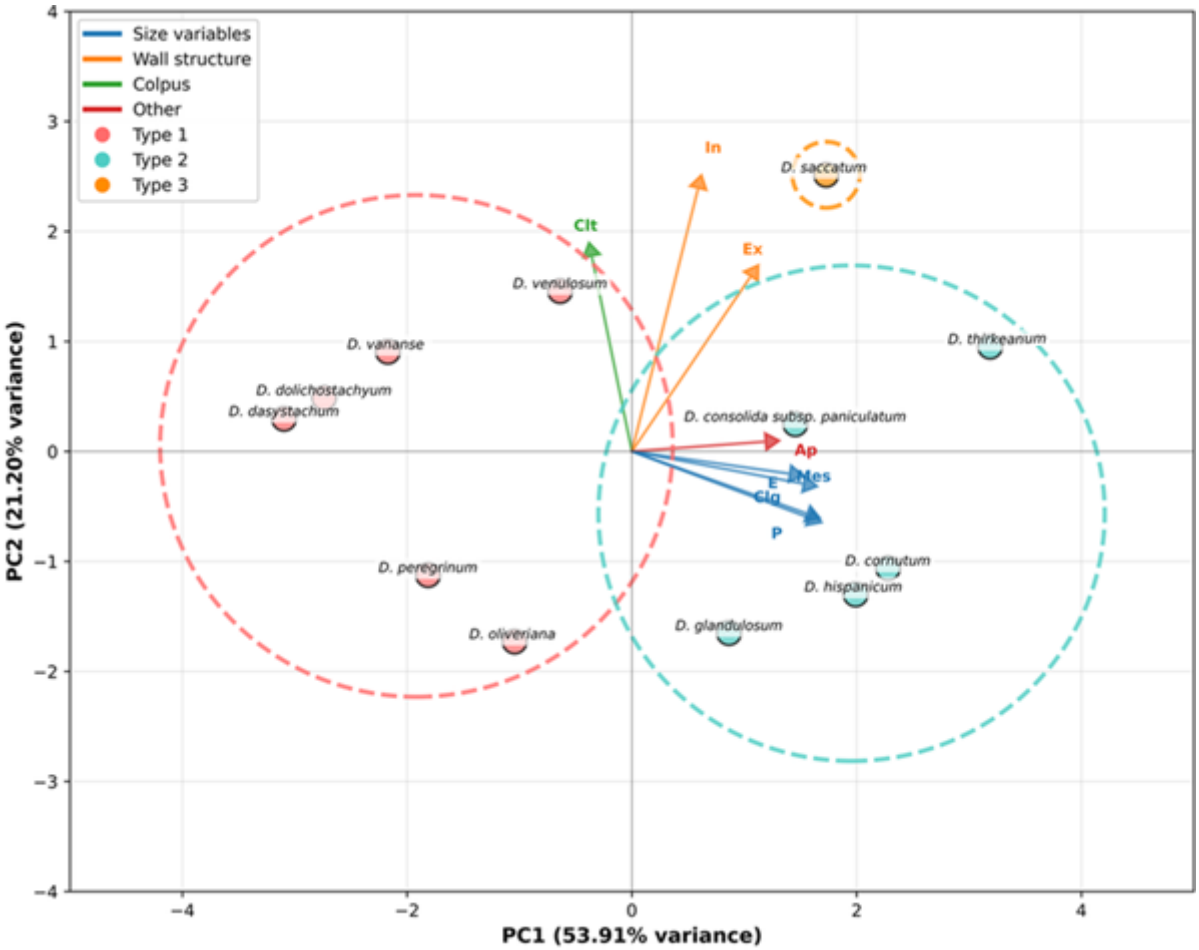


Figure 5. *Principal component analysis biplot with UPGMA types in circle.*

4. DISCUSSION

Ranunculaceae is a eurypalynous family. Pollen grains are (2)-3-(4) colpate, tricolporoidate, pantocolpate, pantoporate, non-aperturate; pollen shape ranges from oblate to spheroidal; ornamentations show a variety of echinate and reticulate [5,12,13]. The pollen grains of different taxa in this family have periporate and microechinate tectum structure [23], syncolpate, tricolpate, and 5-colpate aperture [9], periporate aperture [4,7], striate ornamentation [9], scabrate ornamentation [24], and microechinate ornamentation [12].

Perveen and Qaiser [25] revealed that the family Ranunculaceae had four pollen shape classes: three different aperture types (3-colpate, pantocolpate, and pantoporate oblate-spheroidal), prolate-spheroidal, subprolate, and prolate. The most common pollen shape is subprolate (12 species belonging to the genera *Aconitum*, *Delphinium*, *Clematis*, *Adonis*, and *Ranunculus*), while prolate (*Aquilegia*) is the rarest. The type of tectum is rather uniform, i.e. most species have a microechinate tectum. However, *Ranunculus muricatus* L. has a crustose-verrucate tectum, while *Trollius acaulis* Lindley is the only exception with a coarsely striate tectum with a granulated colpus surface. In the present study, *Delphinum* has tricolpate (except for 3-5% tetracolpate observed in *D. thirkeanum*) and prolate-spheroidal, subprolate pollen grains with microechinate, microreechinate-perforate, and microechinate-verrucate sculpturing (*D. hispanicum*).

Clark et al., [6] identified Consolida ambigua as one of the pollen types in the Ranunculaceae family, which includes *Delphinium* species with 3-zonocolpate, weak microechinate ornamentations. The dominant ornamentation was found to be microechinate in the 12 *Delphinium* taxa examined in this study.

Doğanay [12] studied the morphology of pollen grains of 31 taxa of *Consolida* and reported that pollen grains were tricolpate (rarely syncolpate and tetracolpate), prolate spheroidal, subprolate or prolate, and microechinate ornamentation. However, our study showed that they had tricolpate (except 3-5% tetracolpate observed in *D. thirkeanum*) and prolate-spheroidal, subprolate pollen grains with a microechinate, microreechinate-perforate microechinate-verrucate (*D. hispanicum*), sculpturing.

Faegri & Iversen [29] and Moore & Webb [30] found that the Iranian species of *Consolida* were close to the Adonis annua type due to the narrowness of the colpus. The absence of broad poles in the Adonis annua type is a character seen in the *Caltha palustris* type, *Adonis* L., *Caltha* L., and *Consolida* genera. Therefore, Pakvaran [31] found that all Iranian species of *Consolida* did not occur in a single type (Consolida ambigua type), but could be divided into two groups. The first group consists of broad colpi with thickened exine and coarse-grained membranes at the poles (Consolida ambigua type). The second group has narrow colpus with wide poles. According to SEM images, different exine ornamentations were observed; crusted, weakly or strongly microechinate forms were found in various species; perforated exine was found in *C. oligantha* Schrődinger, *C. persica* (Boiss.) Grossh, *C. regalis* Gray, *C. trigonelloides* (Boiss.) Munz and *C. oliveriana* (DC.) Schrődinger. However, our study showed that they had tricolpate (except 3-5% tetracolpate observed in *D. thirkeanum*) and prolate-spheroidal, subprolate pollen grains with a microechinate, microreechinate-perforate microechinate-verrucate (*D. hispanicum*), sculpturing.

Hasani et al. [32] found that pollen shape and morphology were diagnostic features to distinguish species and were necessary to draw important conclusions about the proximity and remoteness of various taxa, however, they were not sufficient to resolve taxonomic issues within the genus. The results of Pakvaran [31], Jabbour & Renner [26], and Jabbour & Renner [27] confirmed that *Aconitella* Spach. was placed in *Consolida*, as previously proposed, due to *Aconitella* species placement in two pollen groups. Our results comply with those studies (Table 1-2; Figures 1-2).

Hasanbarani and Sharifnia [28] examined the pollen of 27 species of *Delphinium* by SEM in their study. Pollen shapes were grouped as spheroidal, prolate-spheroidal, and subprolate-perprolate. Their surface ornamentation was determined to be scabrate, microperforate-scabrate, and scabrate-reticulate. With their study, they claimed that closely related species did not have very similar morphological features with micromorphological examination of *Delphinium* pollen grains. According to our results, the pollen grains have tricolpate (except 3-5% tetracolpate observed in *D. thirkeanum*) and prolate-spheroidal, subprolate pollen grains with a microechinate, microreechinate-perforate microechinate-verrucate (*D. hispanicum*) sculpturing.

Hasanbarani et al. [33], in pollen studies on the species of *Delphinium* in Iran, reported that *D. venulosum* and *D. peregrinum* had different pollen shapes but formed a clade in molecular studies. In coherence, our results clusters them into Type 1 while having different pollen shapes (Table 1,3; Figures 4,5).

Using light microscopy, Bursalı and Doğan [13] examined the pollen grains of 21 taxa within the genus *Delphinium* L. (Ranunculaceae). They identified that the pollen of these taxa was tricolporate. The pollen shapes included oblate-spheroidal, prolate-spheroidal, subprolate, or prolate, and the exine ornamentation was microechinate. Our findings confirm these results (Table 1-2; Figures 1-2).

Davis [1] reported that most of the taxa belonging to *Delphinium* were probably hybrids. In the light of palynological studies, more than half of the pollen grains expected to be abnormal when they are hybrids. According to [34], the diameters of hybrid pollen grains may vary considerably.

Bursalı and Doğan [13] explained in their study that the diameters of pollen grains of 21 *Delphinium* taxa were not very different and pollen grains showed homogeneity. According to their results, they revealed that the pollen grains of *Delphinium* taxa showed stenopalynous characteristics, which is reconfirmed this our results (Table 1- 2; Figures 1-2).

Recent molecular phylogenetic studies have determined that *Consolida* belongs to *Delphinium* [15,33]. Likewise, our study revealed that the pollen characteristics of taxa previously belonging to *Consolida* are similar to those of *Delphinium* taxa. In cluster analysis of this study, the taxa formerly classified under *Consolida* clustered as Type 2; this grouping corresponds to the annual *Consolida* clade now treated as *Delphinium* subg. *Consolida* [15, 26].

Among the groupings according to the PCA variable loading rate, the separation of colpus length and width into different groups may seem strange at first glance. On second thought, it would be expected since colpus length (Clg) has virtually the same loading vector as the vector of polar axis (P) (Figure 5). Therefore, the colpus length groups with size variables, while colpus width is independent of size parameters. Figure 5 shows that Type 1 and Type 2 are separated mainly by size variables and apocolpium, while *D. saccatum* is separated by wall structure.

5. CONCLUSIONS

This study provides a comparative palynological evaluation of 12 *Delphinium* taxa from Türkiye based on light and scanning electron microscopy. The examined taxa exhibit a largely uniform pollen morphology, particularly with respect to aperture configuration and general exine structure, supporting a stenopalynous pattern within the genus. Exine ornamentation is predominantly microechinate, with additional microechinate-perforate and microechinate-verrucate patterns observed in some taxa, indicating limited but potentially informative micromorphological variation. The close similarity of pollen characters between taxa formerly assigned to *Consolida* and those traditionally placed in *Delphinium* is congruent with recent molecular phylogenetic evidence. Nevertheless, further combined molecular and palynological studies with broad taxa coverage will be useful to further solidify the renewed status of the *Delphinium* genus.

Statistical analyses (UPGMA and PCA) confirmed three distinct pollen types among the studied taxa. The clustering pattern corresponded closely with their taxonomic affinities, particularly separating former *Consolida* species. Quantitative pollen characters such as size, colpus dimensions, and exine thickness proved valuable in distinguishing closely related *Delphinium* taxa.

Conflict of Interest

There is no conflict of interest between the authors.

Authors Contributions (CRediT)

Birol BAŞER: Conceptualization, methodology, investigation, resources, writing - original draft, writing - review & editing, visualization, supervision.

Süleyman AKSU: Investigation, writing - original draft, visualization.

Murat KÜRŞAT: Resources, investigation.

Ozan Kadir BEKMEZCİ: Formal analysis, writing-review & editing, and visualization.

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Statement of Research and Publication Ethics

The authors declare that this study complies with research and publication ethics. This study does not require an ethics committee approval as it does not involve any human or animal subjects, surveys, or clinical data.

Artificial Intelligence (AI) Contribution Statement

This manuscript was entirely written, edited, analyzed, and prepared without the assistance of artificial intelligence (AI). All content, including text, data analysis, and figures, was solely generated by the authors as described in Material and Methods.

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