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POLLEN MORPHOLOGY OF *POLYGALA* L. (POLYGALACEAE) FROM TURKEY AND ITS TAXONOMIC IMPLICATIONS

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Pollen morphology of *Polygala* taxa from the family Polygalaceae in Turkey is presented in this study. Pollen features of 18 species along with one undescribed species in the section *Polygala* were examined with light and scanning electron microscopy, 11 of which were studied and defined for the first time. Cluster analysis and principal components analysis were conducted to determine informative palynological characters and to discover similarities among the studied taxa. Based on qualitative and quantitative variables in the phenogram, the studied taxa were divided into three major clusters. Multivariate analyses revealed that apocolpium characters, including a psilate apocolpium, the presence of apocolpial lumens with granules and small depressions with psilate or rugulate walls are the most distinct features for discriminating *Polygala* taxa. Intraspecific variations in some pollen characters, such as the exine pattern and aperture membrane features, are reported for several taxa. Pollen morphological data obtained in the present study are compared with those from previous studies for a number of species, and the results are evaluated. In addition, the aperture number and its probable significance in the Turkish *Polygala* are considered for some taxa, with emphasis on their known pollination strategies.

Keywords: cluster analysis, pollen, *Polygala*, principal component analysis, taxonomy

INTRODUCTION

The genus *Polygala* L. (Polygalaceae) includes over 600 species, which are most widely represented in temperate and tropical regions (Paiva, 1998; Castro et al., 2009; Abbott, 2011; Kerrigan, 2012; Pastore et al., 2019), but a recent evaluation accepts the genus *Polygala* with more restricted delimitations (Pastore et al., 2023). It is a highly diverse genus comprising herbs, shrubs, small trees, and rarely climbers (Westerkamp and Weber, 1997; Castro et al., 2008a). It is clear that the genus is non-monophyletic (Pastore et al., 2019), and biogeographically two well supported clades of *Polygala* s. str. are referred to as the New World clade (NWC) and the Old World clade (OWC) (Abbott, 2009). The *Polygala* OWC was divided into 11 sections (Chodat, 1891), and the section *Polygala* includes all Turkish *Polygala* taxa (Dönmez et al., 2015), while the NWC clade of *Polygala* are recently recognized as the genus *Senega* Spach and divided into three subgenera, the typical *Senega* (DC.) J. F. B. Pastore & Agust. Martinez and *Monninopsis* (S. F. Blake) J. F. B. Pastore & J. R. Abbott (Pastore et al., 2023).

The pollen morphology of the genus has received attention and it has been recognized as one of the major taxonomic characters by some authors (e.g., Chodat, 1889; Erdtman, 1952, 1969; Castro et al., 2009; Krachai et al., 2009). Most of previous pollen studies on the genus focused on several taxa, mainly from northern, central and western Europe (Erdtman, 1969; Heubl, 1984; Villanueva and Ramos, 1986; De Leonardis et al., 1989; Ubera and Diez, 1994; Furness and Stafford, 1995; Reille, 1995; Halbritter et al., 2021), the Northern Caucasus (Telitsina et al., 2019), Iran (Sarvi et al., 2022), Karachi (Pakistan) (Perveen et al., 2000), Thailand (Krachai et al., 2009), and South Africa (Paiva and Santos Dias,

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1990). Recent comprehensive pollen morphological investigations into the Polygalaceae family comprise many *Polygala* species from the Far East, northern and southern Africa, Europe, and Americas including their northern subcontinent and Brazil, providing pollen data for phylogenetic analyses (Banks et al., 2008; Castro et al., 2009).

Twenty native and one cultivated *Polygala* taxa from the section *Polygala* have been recorded in Turkey (Cullen, 1965; Baytop, 1971; Peşmen, 1980; Davis et al., 1988; Eren et al., 2008; Dönmez et al., 2015; Dönmez and Uğurlu Aydın, 2018). Recently, a new species, *P. fadimeana* Dönmez and Çeçen, has been described (Çeçen et al., 2023), while another new species has been proposed by Dönmez and Uğurlu Aydın (in preparation). The native species are mostly perennial herbs that grow in various habitats in Turkey, including steppe and calcareous rocky slopes, edges of forests, meadows, and rarely wet sandy shores of shallow lakes, from sea level up to about 2000 meters. The cultivated species *P. myrtifolia* L. is an evergreen shrub adapted to gardens (Paiva, 1998).

The pollen morphology of the *Polygala* species from Turkey, which is located between two continents: Europe and Asia has not been extensively examined. Only brief pollen descriptions for two species, *P. peshmenii* Eren, Parolly, Rauss & Kürschner and *P. turcica* Dönmez & Uğurlu, have been given by Dönmez et al. (2015) so far. Therefore, one of the main aims of the present investigation was to enlighten pollen characters of the Turkish representatives of the genus in detail using light microscopy (LM) and scanning electron microscopy (SEM). It also aimed to provide diagnostic pollen features at the taxon level and contribute to a better understanding of *Polygala* taxonomy.

Six of the known taxa examined herein are endemic to Turkey: *P. azizsancarii* Dönmez, *P. inexpectata* Peşmen & Erik, *P. peshmenii*, *P. pruinosa* Boiss. subsp. *megaptera* Cullen, *P. turcica*, and *P. fadimeana*.

MATERIALS AND METHODS

PLANT MATERIAL

For pollen morphological studies a total of 35 specimens from 18 known species identified by Ali A. Dönmez, and one proposed species, including two subspecies of *P. pruinosa*, were investigated. The specimens were collected in the field and deposited in the herbarium of Hacettepe University (HUB). They are listed in alphabetical order in Table 1.

TABLE 1. Examined material of the species of *Polygala* used in this study. All specimens are from the mainland Turkey, except the two specimens from the island country Northern Cyprus in the south of the Anatolian Peninsula, where stated.

Taxa	Locality	Voucher
Polygala alpestris C.A.Mey	Bayburt	*A.A.Dönmez 20754-Y.Kaya*
P. anatolica Boiss. & Heldr.	Sakarya	*A.A.Dönmez 20856-E.O.Karahan*
	Ardahan	*A.A.Dönmez 20648*
	Antalya	*A.A.Dönmez 20382*
P. azizsancarii Dönmez	Mardin	*A.A.Dönmez 19902*
	Mardin	*A.A.Dönmez 19906*
	Mardin	*A.A.Dönmez 19909*
P. comosa Schkuhr	Antalya	*A.A.Dönmez 20384*
	Sivas	*A.A.Dönmez 20421 et al.*
	Sivas	*A.A.Dönmez 20422 et al.*
P. fadimeana Dönmez & Çeçen	Karaman	*A.A.Dönmez 20946*
P. hohenackeriana Fisch. & C.A.Mey.	Iğdır	*A.A.Dönmez 11415*
P. inexpectata Peşmen & Erik	Karaman	*A.A.Dönmez 20373-Z.Uğurlu*
P. major Jacq.	Artvin	*A.A.Dönmez 20914-S.Yüzbaşıoğlu*

Taxa	Locality	Voucher
P. monspeliaca L.	Tekirdağ	*A.A.Dönmez 6748*
	Muğla	*A.A.Dönmez 20797*
P. myritifolia L.	Antalya	*A.A.Dönmez 20975-E.O.Karahan*
P. papilionacea Boiss.	Iğdır	*A.A.Dönmez 20406-Y.Kaya*
	Bayburt	*A.A.Dönmez 20746*
P. peshmenii Eren, Parolly, Raus & Kürschner	Antalya	*A.A.Dönmez 20798-E.O.Karahan*
P. pruinosa Boiss. subsp. *pruinosa*	Ankara	*A.A.Dönmez 20358*
	Nevşehir	*A.A.Dönmez 21159- Z.Uğurlu*
	Erzincan	*A.A.Dönmez 20418-Y.Kaya*
	Erzincan	*A.A.Dönmez 20818-E.O.Karahan*
	Denizli	*A.A.Dönmez 20301-K.Şenova*
P. pruinosa subsp. *megaptera* Cullen	Niğde	*A.A.Dönmez 20574*
P. supina Schreb.	Bursa	*A.A.Dönmez 19772-Z.Uğurlu*
	Erzurum	*A.A.Dönmez 20722- Y.Kaya*
P. turcica Dönmez & Uğurlu	Kars	*A.A.Dönmez 20390*
	Kars	*A.A.Dönmez 20391-Y.Kaya*
	Kars	*A.A.Dönmez 15242*
P. venulosa Sm.	Aydın	*A.A.Dönmez 20794-E.O.Karahan*
	N. Cyprus	*A.Candaş 001*
	N. Cyprus	*A.Candaş 002*
P. vulgaris L.	Kastamonu	*A.A.Dönmez 19849*
	Erzurum	*A.A.Dönmez 20628*
	Erzurum	*A.A.Dönmez 20743-Y.Kaya*
Polygala species nova	Kırşehir	*A.A.Dönmez 20983*

In many cases more than one specimen for each taxon was examined to try and determine any possible intraspecific variations in different localities, but for some taxa there was no sufficient material, or their distribution areas were too limited to carry out such investigations.

POLLEN MORPHOLOGICAL STUDIES

For LM study, the pollen was first treated with 70% ethyl alcohol on a hot plate to remove oily pollen coat material (pollenkitt). It was then embedded in glycerine-jelly, stained with basic fuchsine (Wodehouse, 1935).

The following parameters were measured using LM: pollen size – polar axis (P) and equatorial diameter (E), ectoaperture size – colpus length and width, apocolpium diameter, width of lumens (forming a reticulate pattern) in the apocolpial area when they were present, exine and intine thickness in the polar area where these layers were clearly defined. Pollen size represents the mean of about 50 pollen grains. SPSS program (version 23) was used for descriptive statistics. Pollen grain sizes with variation values from 20-50 µm were defined as 'medium', while pollen grain sizes with maximum values over 50 µm were defined as 'large', adapted from Hesse et al. (2009). P/E ratios were defined based on the main values. Ten measurements for other parameters were made, and the mean values are presented for them, except the minimum and maximum width values for the apocolpium diameter and of apocolpial lumens. In *P. major* and *P. myrtifolia*, relatively smaller and

larger pollen grains from the same pollen sac were recorded; smaller grains had a thicker exine, while larger grains had a thinner exine. For these species, the mean exine thickness values were given separately.

The endoaperture was endocingulate, continuous around the pollen grain, lying in the equatorial plane; since its dimension was not clearly discernable, it was not measured. The pollen shape, colpi morphological features in equatorial view, the pollen shape and colpi number in polar view, and mesocolpium ramification when present were also recorded.

For SEM study, non-acetolysed pollen grains were treated with 70% ethyl alcohol. They were left for air-drying and mounted on specimen stubs. Then the stubs were coated with gold. The photomicrographs were taken using a Tescan GAIA-3 electron microscope.

The following characters were recorded under SEM: shapes of colpi margins and ends, exine pattern in the mesocolpium and on membranes of apertures, presence of psilate apocolpium without lumens or pore-like small depressions, width of small depressions at apocolpium when they were present, presence or absence of microperforations near or in the mesocolpial area, and of operculum, which were clearly discernable in SEM. The number of apocolpial lumens or depressions varied greatly within a species. Therefore, they were not counted.

Since the LM photographs were not clear, only the clearest SEM photographs representing the main pollen characters were selected for this paper.

The terminology that was used mainly follows that recommended by Punt et al. (2007) and Hesse et al. (2009).

MULTIVARIATE DATA ANALYSES

Principal component analysis (PCA) was performed to evaluate whether the pollen quantitative data allowed the grouping of taxa using Minitab 18. For the PCA analysis, the arithmetic means of seven metric variables were used: polar axis (P), equatorial diameter (E), the ratio of polar axis to equatorial diameter (P/E), colpus length (CL), colpus width (CW), exine thickness (ET), intine thickness (IT). One population of *P. azizsancarii* with insufficient data was excluded from the analyses. The frst two principal components (PCs) with eigenvalues greater than one were represented and the results were presented in a two-dimensional plot of the first and second PCs.

A hierarchical cluster analysis (HCA) based on the Euclidian distance measure using the Gower coefficient (Gower, 1971) was performed to classify the studied taxa. For HCA, five selected qualitative and seven quantitative characters were used (Table 2). The multivariate analyses were performed using PAST version 2.17c (Hammer et al., 2001).

TABLE 2. Pollen character matrix with their character states used in multivariate analyses.

Character	State
*Polar axis (P)	µm
*Equatorial diameter (E)	µm
*P/E ratio	Ratio
*Colpus Length (CL)	µm
*Colpus Width (CW)	µm
*Exine Thickness (ET)	1 µm = 1; 2 µm = 2; 2, 3 µm = 3
*Intine Thickness (IT)	<1 µm = 1; 1 µm = 2
Psilate Apolcolpium (PA)	Absent, present
Apocolpial Lumen (AL)	Absent, granulate
Lumen Wall ornamentation at apocolpium (LW)	Absent, rugulate, psilate
Small Depression at apocolpium (SD)	Absent, granulate
Depression Wall ornamentation (DW)	Absent, psilate, rugulate

*Used only for PCA.

RESULTS

POLLEN MORPHOLOGY

Pollen measurements and the main pollen morphological characteristics of Turkish *Polygala* taxa are presented in Table 3. Details of pollen surface patterns and the presence or absence of operculum and of microperforations near/in the mesocolpial area in SEM are given in Table 4. A general pollen morphological description is given below.

POLLEN MORPHOLOGICAL DESCRIPTION (PLATES 1-7)

Pollen grains of *Polygala* from Turkey are isopolar, predominantly oblate-spheroidal and rarely spheroidal in equatorial view, circular in polar view, medium to large in size; the polar axis (P) ranges from 20 to 57.5 µm, while the equatorial diameter (E) ranges from 20 to 60 µm, and the P/E ratio ranges from 0.90 to 1.08. The aperture system is polyzonocolporate (stephanocolporate) and endocingulate, with 8-21colpi (e.g., Plate 1, Fig. 3; Plate 3, Fig. 5). The colpi length is between 20.13 and 33 µm, while the colpi width is between 1.98 and 8.25 µm; usually long and narrow or broad, usually with regular margins and rounded ends (e.g., Plate 2, Figs. 1-2; Plate 3, Fig. 4). The endocingulate endoaperture is without costae. The mesocolpia are ramified in some taxa and specimens (e.g., Table 3, Plate 1, Fig. 5; Plate 7, Fig. 9). The apocolpium diameter ranges from 7.8 to 37.44 µm. The apocolpial field is with lumina (e.g., Plate 1, Figs. 3-4; Plate 6, Figs. 6, 8) or small pore-like depressions (e.g., Plate 1, Fig. 1; Plate 2, Fig. 7); the lumen size ranges from 1 to 15 µm in LM, while the small depression size ranges from <1 to 4 µm in SEM, or psilate with no lumina or small depressions (e.g., Plate 1, Figs. 5; Plate 2, Fig. 4; Plate 7, Fig. 1). The exine is 2-3 µm thick, while the intine is rather thin, <1-1 µm, at poles. The exine patterns in mesocolpial regions and in walls of lumina/depressions in apocolpial regions when present are diverse in SEM (Table 4); psilate (e.g., Plate 2, Fig. 2; Plate 6, Fig. 7) or finely striate (e.g., Plate 1, Figs. 2, 6) to striate (e.g., Plate 5, Fig. 3), or rugulate (e.g., Plate 2**,** Fig. 5) in the mesocolpium, and granulate in lumens/small depressions (e.g., Plate 1, Figs. 1, 4), and psilate (e.g., Plate 1, Fig. 1; Plate 2, Fig. 3) to rugulate (e.g., Plate 1, Fig. 4; Plate 2, Fig. 7) on their walls. The colpus and pore membranes are psilate (Plate 1, Fig. 3) to psilate-granulate (Plate 6, Fig. 7), granulate (e.g., Plate 1, Fig. 2), or granulate-perforate (e.g., Plate 6, Fig. 2) to perforate (e.g., Plate 2, Fig. 6). In some taxa, the opercula on membranes of both colpi and pores (e.g., Plate 1**,** Fig. 6; Plate 7, Fig. 2) or only on pores (e.g., Plate 3, Fig. 6) are distinguished by SEM (Table 4). In SEM, other structures recorded include microperforations; they are found either near the apocolpial field (e.g., Plate 5, Fig. 5) or both near the apocolpial field and in the mesocolpial region (e.g., Plate 7, Figs. 5-6).

There are some qualitative and quantitative differences between the pollen of Turkish *Polygala* taxa, such as pollen size, colpi number, apocolpium diameter, and pollen surface features (Tables 3-4). However, it is difficult, even impossible, to distinguish most species due to low pollen morphological diversity, and some intraspecific variations. Although differences between some species in the exine patterns in the mesocolpial and apocolpial regions and on the aperture membranes are noticed in SEM (Table 4), these characters do not seem to be useful for further divisions due to overlapping. In addition, the exine pattern would vary in a single species (as in *P. myrtifolia*) (Plate 4, Figs. 2, 4) or the aperture membrane features would vary intraspecifically as in *P. vulgaris*; with opercula (Plate 7, Fig. 2) or without them (Plate 7, Figures 3-4).

MULTIVARIATE DATA ANALYSES

The pollen quantitative data are collected to explore the relationships among the taxa using PCA: The first two dimensions of PCA (Table 5 and Plate 8) express 65% of the total variation. The first axis (PC1) explains 46.6% of the total variation and is associated with the polar axis (P), equatorial diameter (E), the ratio of the polar axis/equatorial diameter (P/E), colpi length (CL), and colpi width (CW). The second axis (PC2) accounts for 18.4% of the variance, based on the exine thickness (ET) and intine thickness (IT).

Based on the pollen metric variables, *P. azizsancarii* is distributed to the extreme negative side of the PC1 axis. This axis also separates *P. pruinosa* subsp. *pruinosa*, *P. vulgaris*, *P. supina*, *P. turcica* and some specimens of *P. venulosa* and *P. comosa*. However, the remaining specimens of *P. venulosa* and *P. comosa* are clustered on the other side of the axis, and the other subspecies of *P. pruinosa* are grouped in the negative side of the PC2 axis.

TABLE 3. Main pollen characteristics of Turkish *Polygala* in LM.

Taxa and specimens	***P* (µm)**			***E* (µm)**			**Pollen size**	***P/E* ratio**	**Shape**	**Colpi n**	**Colpi length (µm)**	**Colpi width (µm)**
	M	SD	V	M	SD	V						
P. alpestris *AAD 20754*	30.5	± 2.76	25–35	33.65	±2.95	27.5–40	me	0.90	os	9–10	22.25	6
P. anatolica *AAD 20856*	35.19	±2.41	27.5–40	38.26	±2.86	27.5–45	me	0.91	os	10–14	22.5	7
AAD 20648	47.5	±2.2	42.5–52.5	49.6	±2.04	45–55	la	0.95	os	12–13	30.5	8.25
AAD 20382	34.05	±2.13	30–37.5	34.65	±3.53	30–40	me	0.98	os	20–21	24.25	5
P. azizsancarii *AAD 19902*	30.14	±2.76	25–35	28.92	±1.53	25–35	me	1.04	sp	16–19	20.13	1.98
AAD 19906	36.22	±2.13	35–37.5	38.77	±2.95	37.5–40	me	0.93	os	16–19	27.04	1.98
AAD 19909	33.03	±2.41	27.5–37.5	35.34	±2.04	27.5–42.5	me	0.93	os	15–17	22.83	1.98
P. comosa *AAD 20384*	40	±2.37	35–45	43.2	±2.81	37.5–47.5	me	0.92	os	10–13	25.25	7.25
AAD 20421	44.9	±4.57	35–57.5	48	±5.2	35–60	la	0.93	os	11–12	28.08	6,86
AAD 20422	38.67	±4.13	27.5–47.5	41.76	±4.22	35–50	me	0.92	os	12–15	28.5	6.75
P. fadimeana *AAD 20946*	28.41	±1.71	25–32.5	29.66	± 1.78	27.5–32.5	me	0.95	os	16–18	20.5	2.5
P. hohaneckeriana *AAD 11415*	30.25	±1.83	25–32.5	32.25	±1.53	27.5–35	me	0.93	os	9–10	23.5	7.25
P. inexpectata *AAD 20373*	28.84	±3.1	25–35	30.3	±3.59	25–37.5	me	0.95	os	9–10	21.3	4.99
P. major *AAD 20914*	36	±5.19	27.5–47.5	36.58	±7.15	25–50	me	0.98	os	11–12	23.4	5.68

TABLE 3. Continued

Taxa and specimens	***P* (µm)**			***E* (µm)**			**Pollen size**	***P/E* ratio**	**Shape**	**Colpi n**	**Colpi length (µm)**	**Colpi width (µm)**
	M	SD	V	M	SD	V						
P. monspeliaca *AAD 6748*	24.5	±2.2	20–27.5	26	±3.03	20–30	me	0.94	os	10–11	21.5	5
AAD 20797	30	±2.52	25–32.5	31.3	±3.28	25–37.5	me	0.95	os	10–11	26.5	5
P. myrtifolia *AAD 20975*	35.49	±4	30–50	33.82	±4.6	30–50	me	1.04	sp	19–20	24.25	2.5
P. papilionacea *AAD 20406-YK*	38.7	±2.4	32.5–42.5	39.59	±3.9	27.5–45	me	0.97	os	9–11	30.25	6.5
AAD 20746	29.08	±2.47	22.5–32.5	30.43	±1.89	27.5–35	me	0.95	os	8–9	22.25	5
P. peshmenii *AAD 20798*	36.75	±4.04	25–42.5	38.05	±6.25	25–45	me	0.96	os	17–19	29.5	4.25
P. pruinosa subsp. *pruinosa* *AAD 20301-KŞ*	32.59	**±**2.28	27.5–37.5	33.18	**±**3.08	25–37.5	me	0.98	os	19–21	24	2.5
AAD 21159	32.25	±3.43	27.5–40	30	±2.9	25–37.5	me	1.08	sp	16–18	22.5	2.75
AAD 20418	31.6	±3.18	25–37.5	32.5	±4.72	22.5–40	me	0.97	os	18–21	22.5	2.5
AAD 20818	29.01	±2.45	25–37.5	28.87	±2.66	25–37.5	me	1	sp	18–20	20.75	2.5
P. pruinosa subsp. *megaptera* *AAD 20574*	29.35	±1.66	25–32.5	30.8	±1.91	25-32.5	me	0.95	os	18–19	21	2.5
P. supina *AAD 19772*	37.45	±2.17	32.5–42.5	38.6	± 2.09	35–42.5	me	0.97	os	17	29.75	5
AAD 20722	34.95	±2.26	30–40	34.5	± 2.69	30–40	me	1.01	sp	15–17	25.45	4.31

TABLE 3. Continued

Taxa and specimens	***P* (µm)**			***E* (µm)**			**Pollen size**	***P/E* ratio**	**Shape**	**Colpi n**	**Colpi length (µm)**	**Colpi width (µm)**
	M	SD	V	M	SD	V						
P. turcica												
AAD 20390	34.8	±2.19	30–37.5	37	± 3.77	27.5–42.5	me	0.94	os	8–10	27.25	5.75
AAD 20391	36.6	±2.93	32.5–45	37.5	± 3.94	30–42.5	me	0.97	os	8–10	33	5.75
P. venulosa												
AAD 20794	39.26	±4.16	32.5–47.5	43.23	± 3.94	37.5–52.5	la	0.9	os	9–10	27. 5	5.1
AC 001	40.5	±3.87	35–50	43.65	± 3.61	35–52.5	la	0.92	os	10–11	25.25	7.5
AC 002	48.92	±2.61	40–52.5	48.79	± 2.77	40–55	la	0.92	os	12–13	25.25	7.5
P. vulgaris												
AAD 19849	30.85	±3.29	27.5–40	29.55	± 4.3	25–40	me	1.04	sp	18–19	24	3.75
AAD 20628	36.44	±3.33	27.5–40	37.74	± 3.29	30–42.5	me	0.96	sp	19–20	26.36	3.4
AAD 20743	34.27	±2.11	30–37.5	35.57	± 3.82	27.5–42.5	me	0.96	os	16–18	22.25	3.75
P. species nova AAD 20983	29.57	±1.94	25–35	31.74	± 3.08	27.5–37.5	me	0.93	os	18–19	22	4.5

Abbreviations: *P* – polar axis, *E* – equatorial diameter, M – mean value, SD – standard deviation, V – variation, n – number, me – medium, la – large, os – oblate-spheroidal, sp – spheroidal.

TABLE 3. Continued

Taxa and specimens	Mesocolpium ramification	Apocolpium diameter (µm)	Apocolpial lumens width (µm)	Exine pattern LM	Exine thickness (µm)	Intine thickness (µm)
P. alpestris						
AAD 20754		23.4–26.52		psi	2	<1
P. anatolica	+					
AAD 20856		20.28–24.96	3–7	psi	2	1
AAD 20648		32.76–34.32	2.5–15	psi	2	<1
AAD 20382		17.16–18.72	1.56–6.24	psi	2	1
P. azizsancarii						
AAD 19902		14.04–17.16		psi	1	<1
AAD 19906		17.16–20.28		psi	1	<1
AAD 19909		15.6–17.16		psi	1	<1
P. comosa	+					
AAD 20384		26.52–29.64	5–10	psi	2	1
AAD 20421		34.32–37.44	1.56–4.68	psi	2	1
AAD 20422 et al.		23.4–24.96	3.12–6.24	psi	2	<1
P. fadimeana						
AAD 20946		15.6–17.16		psi	2	<1
P. hohaneckeriana	+			psi	2	1
AAD 11415		7.8–9.36				
P. inexpectata #						
AAD 20373-ZUA		14.04–15.6		psi	2	1
P. major						
AAD 20914		14.04–23.4	1–5	psi	2, 3	<1

TABLE 3. Continued

Taxa and specimens	Mesocolpium ramification	Apocolpium diameter (µm)	Exine pattern LM	Exine thickness (µm)	Intine thickness (µm)
P. monspeliaca *AAD 6748*		15.6–17.16	psi	2	1
AAD 20797		15.6–21.84	psi	2	1
P. myritifolia *AAD 20975*		17.16–24.96	psi	2, 3	1
P. papilionacea # *AAD 20406-YK*		14.04–15.6	psi	2	1
AAD 20746		14.04–15.6	psi	2	<1
P. peshmenii # *AAD 20798*		10.92–23.4	psi	2	1
P. pruinosa ssp. *pruinosa* *AAD 20301-KŞ*	+	15.6–20.28	psi	2	<1
AAD 21159		15.6–18.72	psi	2	<1
AAD 20418		10.04–17.16	psi	2	<1
AAD 20818		15.6–17.16	psi	2	<1
P. pruinosa ssp. *megaptera* *AAD 20574*	+	15.6–17.16	psi	2	1

TABLE 3. Continued

Taxa and specimens	Mesocolpium ramification	Apocolpium diameter (µm)	Apocolpial lumens width (µm)	Exine pattern LM	Exine thickness (µm)	Intine thickness (µm)
P. supina	+					
AAD 19772		17.16–21.84		psi	2	<1
AAD 20722		15.6–18.72		psi	2	<1
P. turcica						
AAD 20390		12.48–14.04		psi	2	<1
AAD 20391		12.48–15.6		psi	2	<1
P. venulosa						
AAD 20794		21.84–24.96	4–10		2	1
AC 001		28.08–32.76	4–10	psi	2	<1
AC 002		34.32–35.88	4–9	psi	2	<1
P. vulgaris						
AAD 19849		14.04–15.6		psi	2	<1
AAD 20628		24.96–26.52		psi	2	<1
AAD 20743		17.16–18.72		psi	2	<1
P. species nova	+					
AAD 20983		15.6–17.16		psi	2	<1

Abbreviation: psi – psilate. Symbol + present.

TABLE 4. Details of pollen surface features of Turkish *Polygala* in SEM.

Taxa	Mesocol Exine pattern	Colpus memb	Pore memb	Microper near/in mesocol	Psi apocol	Apocolpial Lumen	Lumen wall	Small depression	Depression wall	Depression width (µm)
P. alpestris	fn str	ga	gra					gra	psi	1–4
P. anatolica	fn str	psi	psi			gra	rug			
P. azizsancarii	fn str	oper	oper		+					
P. comosa	psi	gra	psi	+		gra	psi			
P. fadimeana	psi	gra	oper	+	+			gra	psi	<1–1
P. hohaneckeriana	rug	per	per		+					
P. inexpectata	fn str	per	per					gra	rug	<1
P. major	psi	gra-per	gra-per	+		gra	psi			
P. monspeliaca	rug	gra-per	oper					gra	rug	1–2.5
P. myritifolia	rug, str	gra-per	gra-per		+					
P. papilionacea	rug	gra-per	gra-per		+					
P. peshmenii	str	oper	oper		+					

Abbreviations: mesocol – mesocolpium, memb – membrane, fn str – finely striate, gra – granulate, microper – microperforation, oper – covered with operculum, per – perforate, psi – psilate, psi apocol – psilate apolcolpium; without lumens or small depressions, rug – rugulate, str – striate. Symbol + present.

TABLE 4. Continued

Taxa	Mesocol exine pattern	Colpus memb	Pore memb	Microper near/ in mesocol	Psi Apocol	Apocolpial Lumen	Lumen Wall
P. pruinosa ssp. *pruinosa*	psi	oper	oper	+	+		
P. pruinosa ssp. *megaptera*	psi	oper	oper	+	+		
P. supina	psi	gra-per	gra-per		+		
P. turcica	rug	psi	psi		+		
P. venulosa	psi	psi-gra	psi-gra			gra	psi
P. vulgaris	fn str	oper, gra-per	oper, gra-per		+		
P. species nova	psi	gra	oper		+		

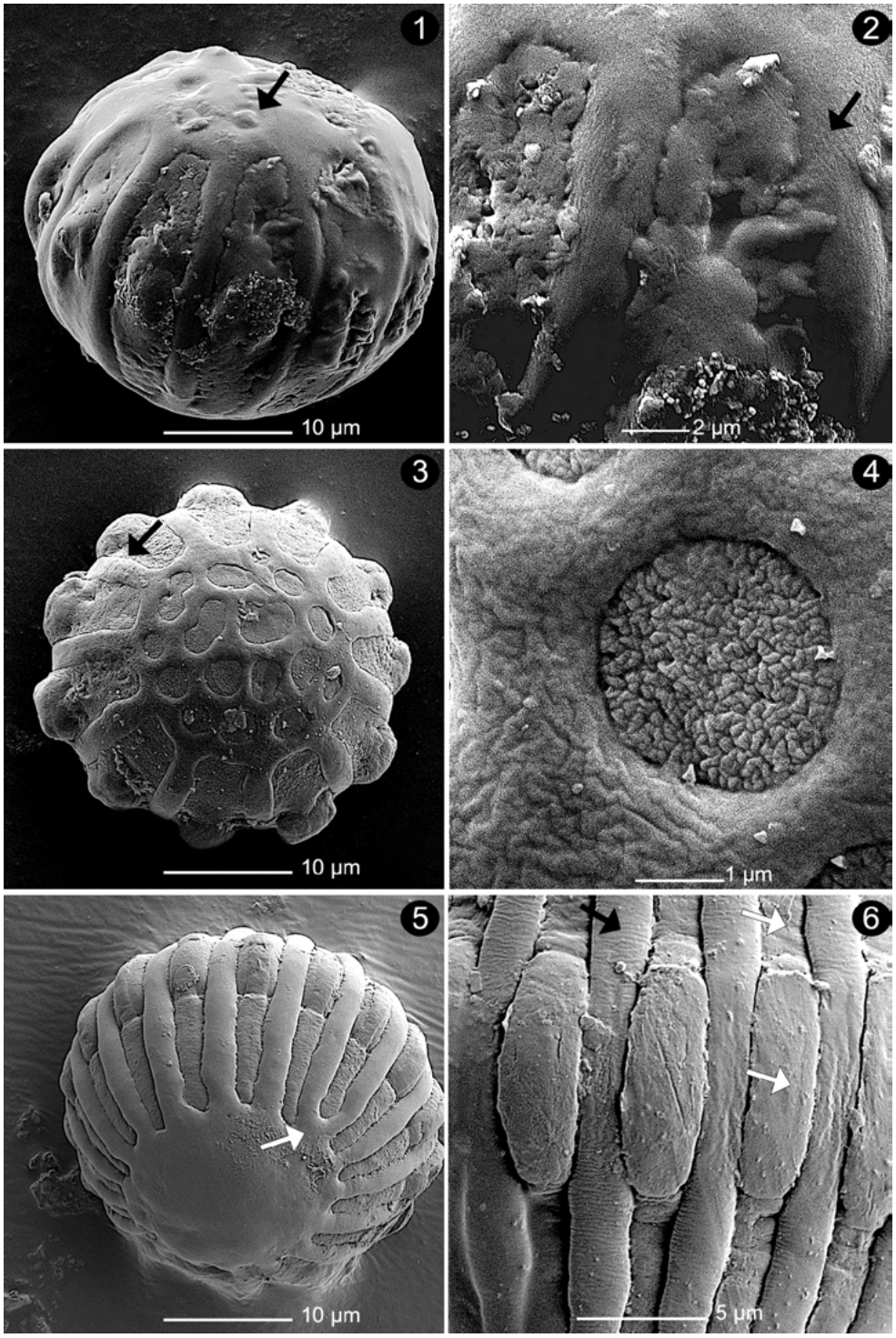

Plate 1. Pollen micrographs of *Polygala*. **Figs. 1-2.** *P. alpestris* (**1**) small depressions with granules and psilate walls (one arrowed) at apocolpium, (**2**) detail of finely striate mesocolpia (one arrowed), granulate colpi membranes, and a granulate pore membrane. **Figs. 3-4.** *P. anatolica* (sample from Sakarya province) (**3**) lumina at apocolpium, finely striate mesocolpia (one arrowed), colpi and pores with psilate membranes, (**4**) detail of a granulate lumen with rugulate wall at apocolpium. **Figs. 5-6.** *P. azizsancarii* (sample *AAD 19906*) (**5**) psilate apocolpium and mesocolpium ramification (arrowed), (**6**) detail of finely striate mesocolpium (black arrow), colpus and pore covered with opercula (white arrows) in equatorial view.

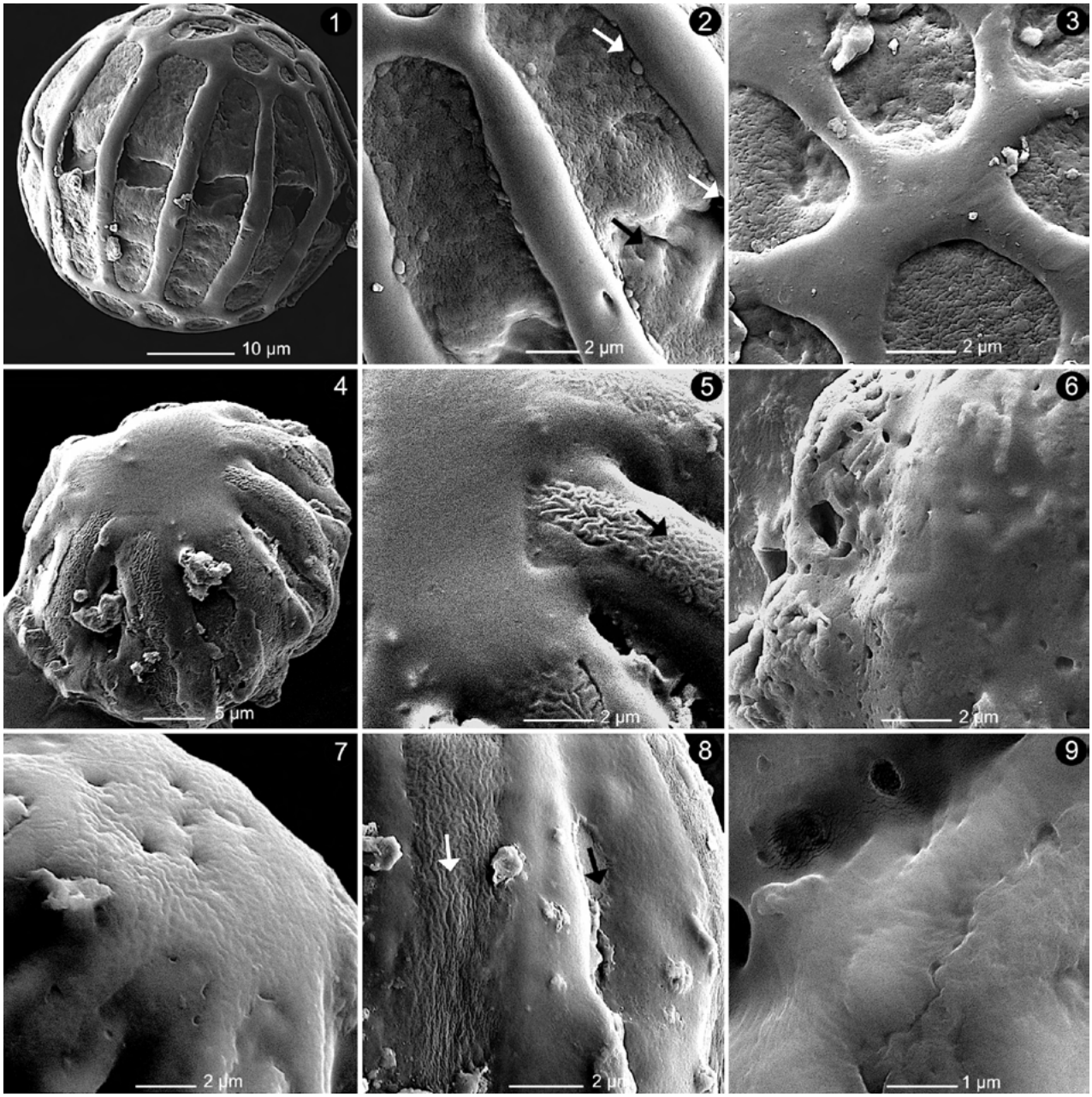

Plate 2. Pollen micrographs of *Polygala*. **Figs. 1-3.** *P. comosa* (sample from Sivas province, *AAD 20422 et al.*) (**1**) equatorial view, (**2**) detail of psilate mesocolpia, colpus with granulate membrane (white arrows) and pore with psilate membrane (black arrow), (**3**) detail of granulate lumens with psilate walls at apocolpium. **Figs. 4-6.** *P. hohaneckeriana* (**4**) psilate apocolpium, (**5**) detail of rugulate mesocolpium (arrow), (**6**) detail of perforate colpus and pore membranes. **Figs. 7-9.** *P. inexpectata* (**7**) small depressions with rugulate walls at apocolpium, (**8**) detail of a finely striate mesocolpium (white arrow) and a colpus (black arrow), (**9**) closer view of perforate aperture membrane.

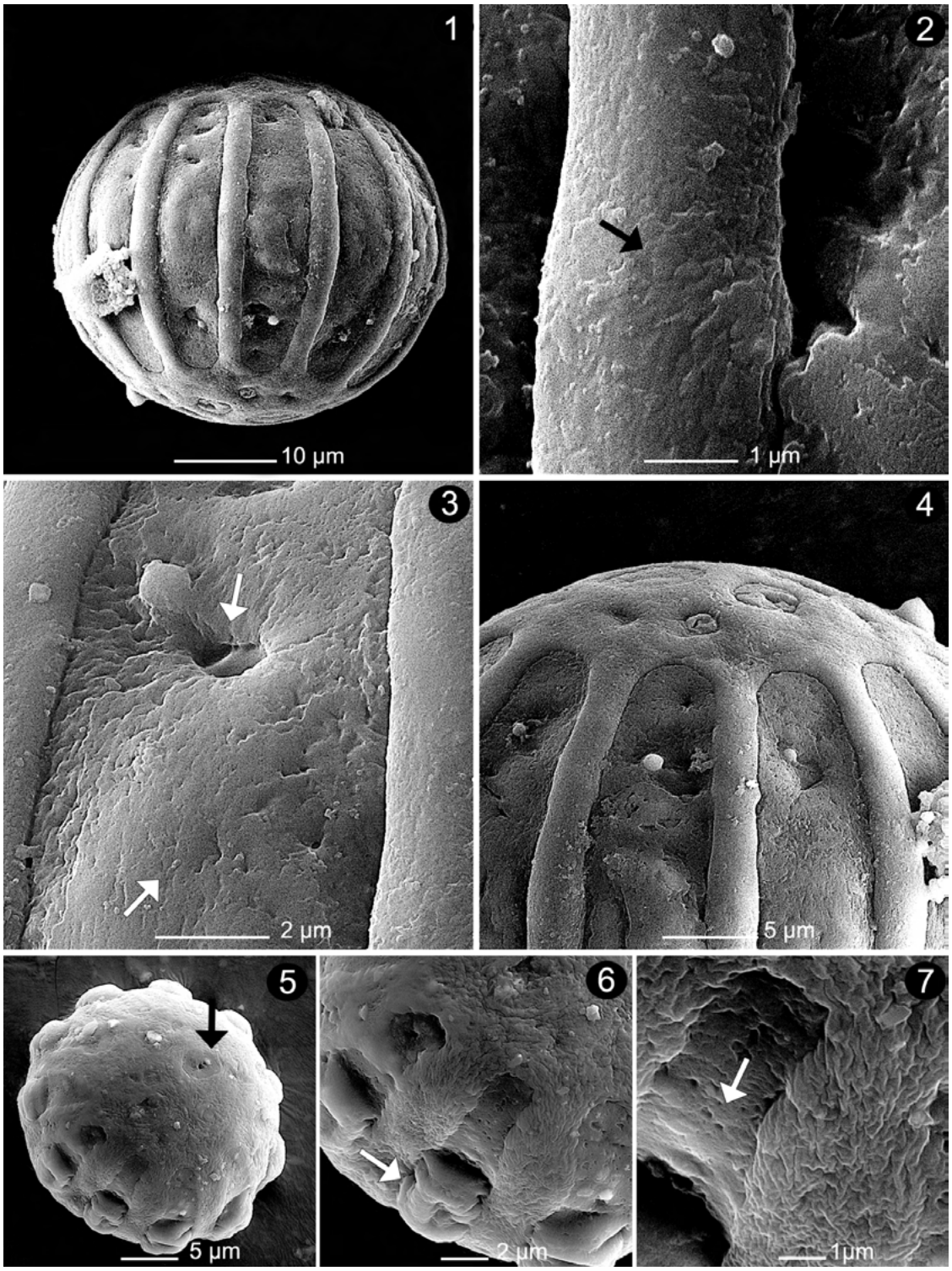

Plate 3. Pollen micrographs of *Polygala*. **Figs. 1-4.** *P. major* (**1**) equatorial view, (**2**) psilate mesocolpium (arrow), (**3**) detail of a colpus and pore with granulate-perforate membranes (arrows), (**4**) closer view of granulate lumens with psilate walls at apocolpium. **Figs. 5-7.** *P. monspeliaca* (sample from Muğla province) (**5**) granulate small depressions at apocolpium (one arrowed), (**6**) rugulate depression wall at apocolpium, mesocolpia, colpi, and pores covered with opercula (one arrowed), (**7**) detail of rugulate mesocolpia and a colpus with granulate-perforate membrane (arrowed).

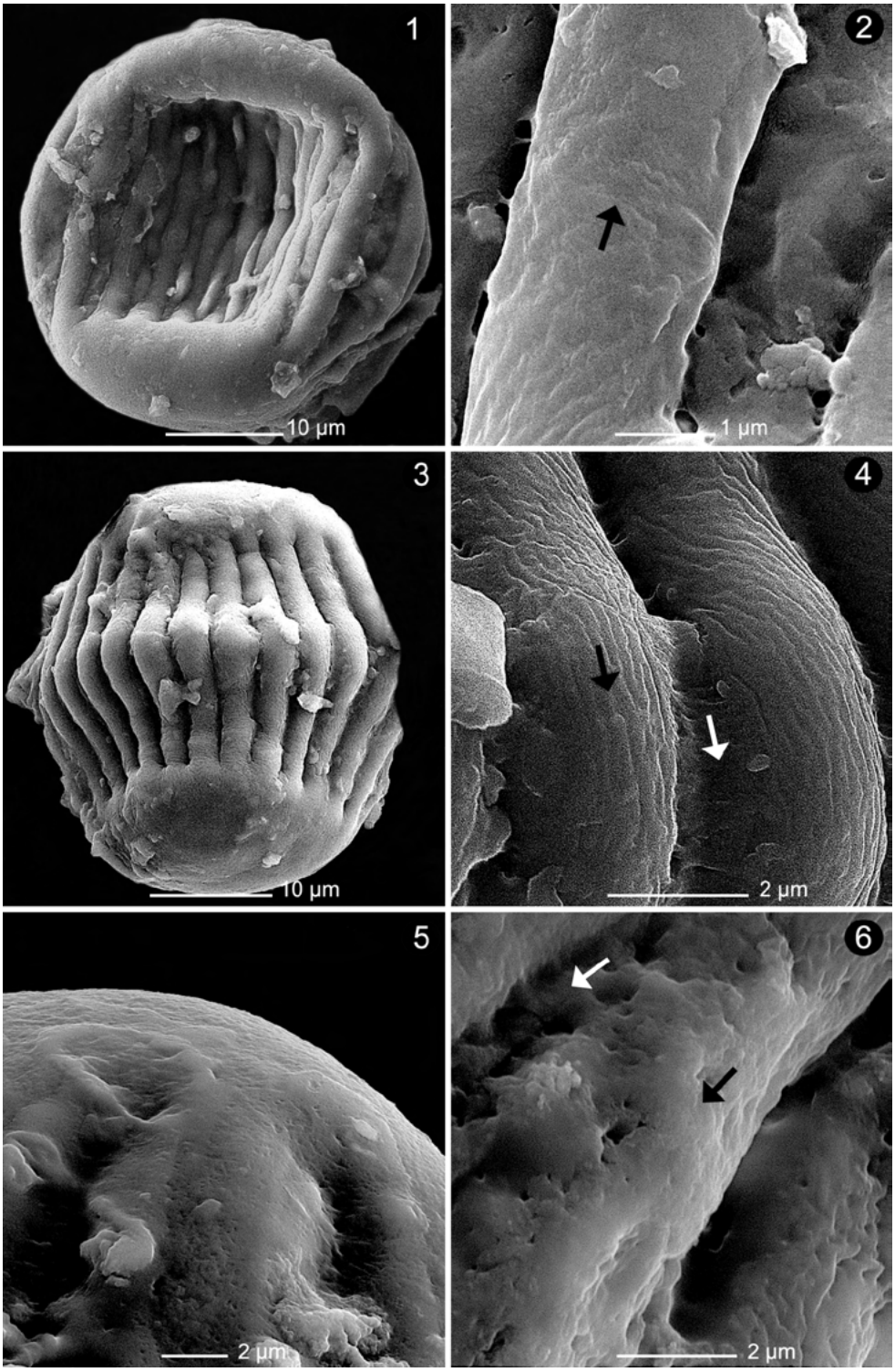

Plate 4. Pollen micrographs of *Polygala*. **Figs. 1-4.** *P. myrtifolia* (**1-2**) smaller pollen grain, collapsed, (**1**) psilate apocolpium, (**2**) detail of rugulate mesocolpium (arrowed) and granulate-perforate colpi membrane, (**3-4**) larger pollen grain, (**3**) psilate apocolpium, (**4**) detail of striate mesocolpia (black arrow) and granulate-perforate colpus and pore membranes (white arrow). **Figs. 5-6.** *P. papilionacea* (sample from Iğdır province) (**5**) psilate apocolpium, (**6**) Detail of rugulate mesocolpia (black arrow) and aperture with granulate-perforate membranes (white arrow).

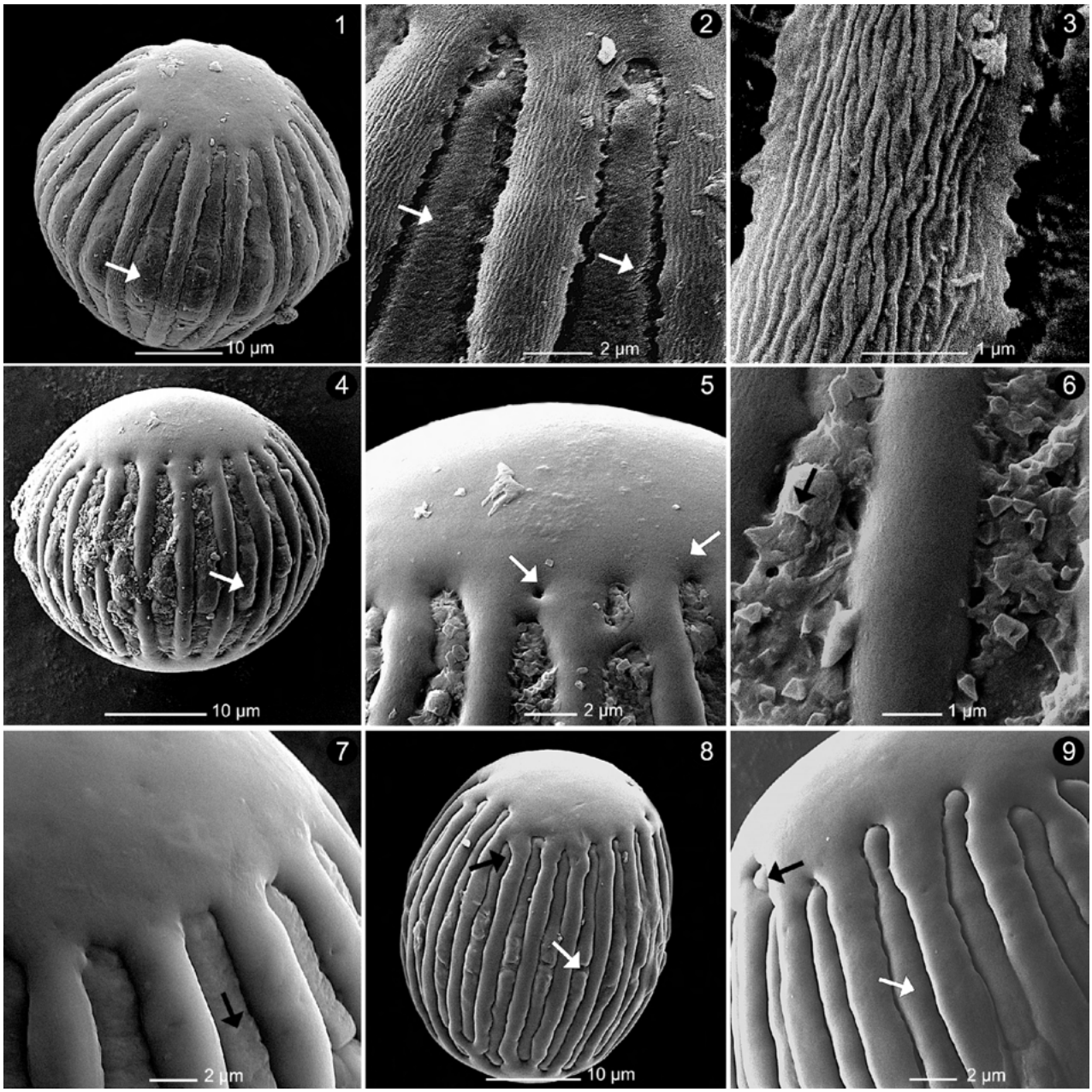

Plate 5. Pollen micrographs of *Polygala*. **Figs. 1-3.** *P. peshmenii* (**1**) psilate apocolpium and opercula on colpi and pores (one arrowed), (**2**) detail of colpi covered with opercula (arrows), (**3**) detail of striate mesocolpium exine pattern. **Figs. 4-7.** *P. pruinosa* subsp. *pruinosa* (**4-6**) sample from Erzincan province, *AAD 20818*, (**4**) general view and pores with opercula (one arrowed), (**5**) psilate apocolpium and microperforations near mesocolpia (arrows), (**6**) detail of psilate mesocolpium and colpi covered with opercula (one arrowed), (**7**) apertures covered with opercula (one arrowed), sample from Nevşehir province. **Figs. 8-9.** *P. pruinosa* subsp. *megaptera* (**8**) mesocolpium ramification (black arrow), and colpi and pores with opercula (white arrow), (**9**) psilate apocolpium, a microperforation near psilate mesocolpia (black arrow), and opercula on colpi (white arrow).

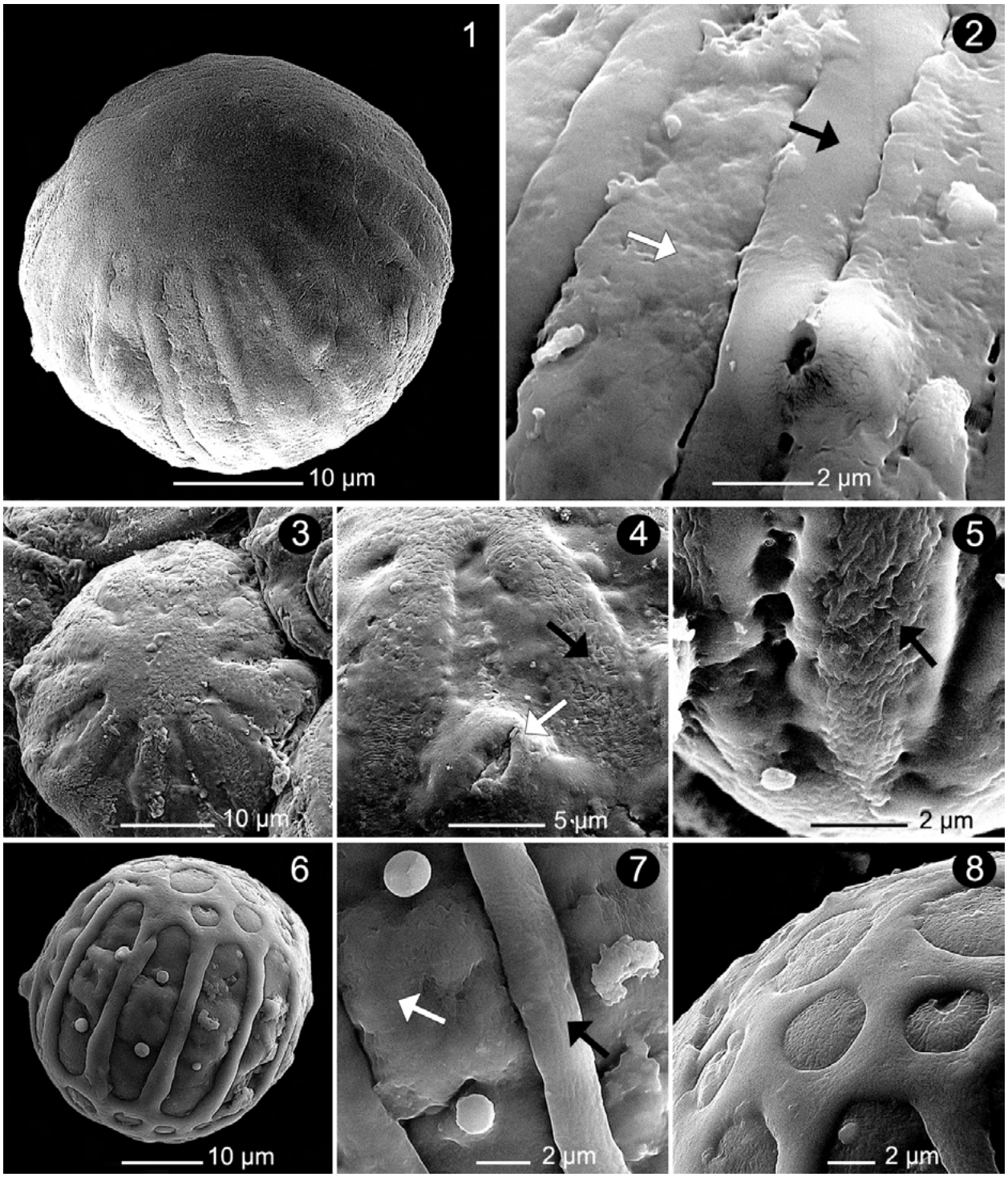

Plate 6. Pollen micrographs of *Polygala*. **Figs. 1-2.** *P. supina* (sample from Bursa province) (**1**) psilate apocolpium, (**2**) detail of psilate mesocolpia (black arrows) and colpus and pore with granulate-perforate membranes (white arrows). **Figs. 3-5.** *P. turcica* (**3-4**) sample *AAD 20390*, (**3**) psilate apocolpium, (**4**) rugulate mesocolpium (black arrow), apertures with psilate membrane (white arrow), (**5**) detail of rugulate mesocolpium (arrow), sample *AAD 20391*. **Figs. 6-8.** *P. venulosa* (sample from N. Cyprus, *AC 002*) (**6**) equatorial view, (**7**) detail of psilate mesocolpium (black arrow), colpus and pore with psilate-granulate membranes (white arrow), (**8**) detail of granulate lumens with psilate walls at apocolpium.

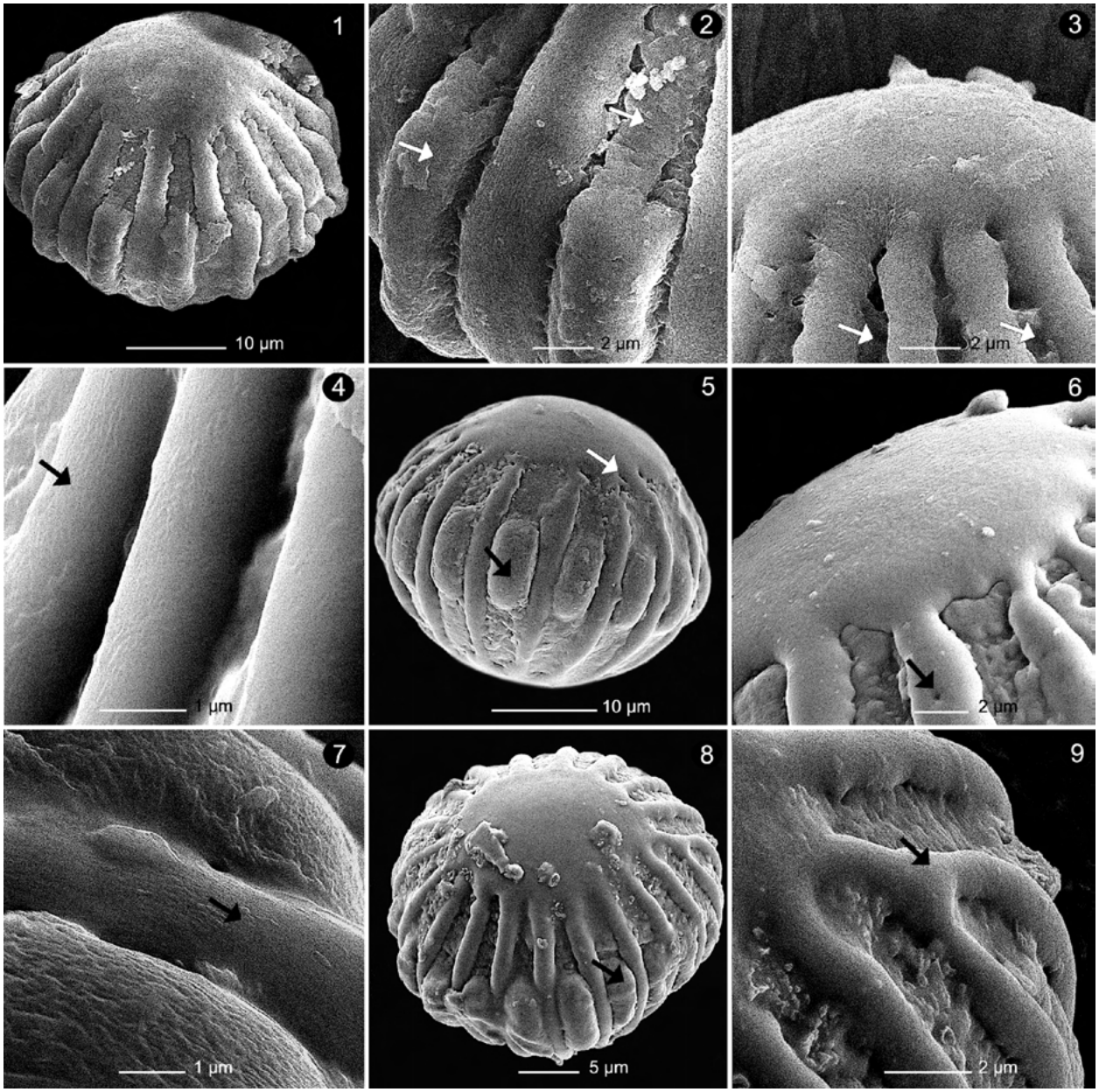

Plate 7. Pollen micrographs of *Polygala*. **Figs. 1-4.** *P. vulgaris* (**1-2**) sample from Kastamonu province, (**1**) psilate apocolpium, (**2**) colpus and pore covered with opercula (arrows), (**3-4**) sample from Erzurum province, *AAD 20743*, (**3**) granulate-perforate colpi membranes (arrows), (**4**) detail of finely striate mesocolpia (one arrowed). **Figs. 5-7.** *P. fadimeana* (**5**) pollen grain with psilate apocolpium, microperforation near mesocolpium (white arrow), colpus with granulate membrane, and pore covered with operculum (black arrow), (**6**) detail of psilate apocolpium and psilate mesocolpia with microperforation (white arrow), (**7**) detail of psilate mesocolpium exine pattern (arrow) and two pores covered with opercula. **Figs. 8-9.** *Polygala species nova* (**8**) psilate apocolpium, pores covered with opercula (one arrowed), (**9**) detail of ramification (arrow) and colpus with granulate membrane.

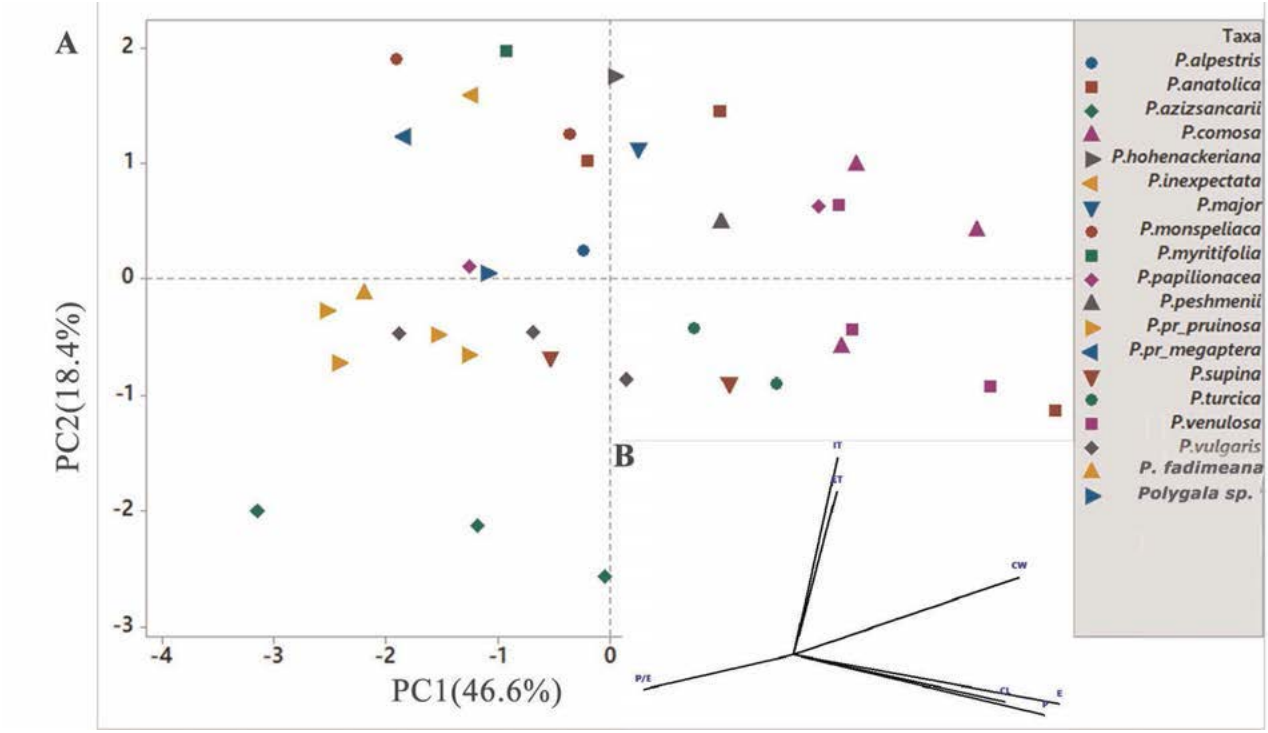

Plate 8. (**a**) Principal component analysis (PCA) performed with the pollen quantitative data from *Polygala* taxa, (**b**) Vector loading of quantitative traits. For abbreviations of the vectors see Table 5.

TABLE 5. Results of the first two dimensions of PCA based on seven quantitative pollen characters.

Variable	PC1 (46.6%)	PC2 (18.4%)
P	**0,497**	-0,214
E	**0,526**	-0,174
P/E	**-0,296**	-0,123
CL	**0,418**	-0,166
CW	**0,446**	0,272
ET	0,086	**0,573**
IT	0,087	**0,692**

Abbreviations: P – polar axis, E – equatorial diameter, P/E – polar axis/equatorial diameter, CL – colpi length, CW – colpi width, ET – exine thickness, IT – intine thickness.
The components loaded most highly for each character are given in bold.

The UPGMA phenogram of pollen morphology indicates that Turkish *Polygala* taxa are seperated into three main clusters (A, B and C) at the highest taxonomic distance of 0.45 (Plate 9). Cluster A encloses most *Polygala* taxa: *P. azizsancarii*, *P. hohenackeriana*, *P. myrtifolia*, *P. papilionacea*, *P. peshmenii*, *P. pruinosa* subsp. *pruinosa*, *P. pruinosa* subsp. *megaptera*, *P. supina*, *P. turcica*, *P. vulgaris*, and *P. fadimeana* along with the proposed species, *Polygala* sp. nov. All taxa in cluster A show distinct ornemantation that is characterized by a psilate apolcolpium. The second cluster comprises only three taxa, *P. alpestris*, *P. inexpectata* and *P. monspeliaca* having ornemantation characterized by a psilate or rugulate depression wall at the apocolpium. Cluster C contains the remaining four species: *P. anatolica*, *P. comosa*, *P. major* and *P. venulosa*, with granulate apocolpial lumens.

DISCUSSION

This study enlightens the pollen morphology of many species of the genus *Polygala* growing in Turkey. In addition, the pollen grains of nine species and one subspecies along with one proposed species from the genus are examined in detail for the first time, including, *P. inexpectata*, *P. papilionacea*, *P. pruinosa* subsp. *pruinosa*, *P. pruinosa* subsp. *megaptera*, *P. supina*, *P. venulosa* and the

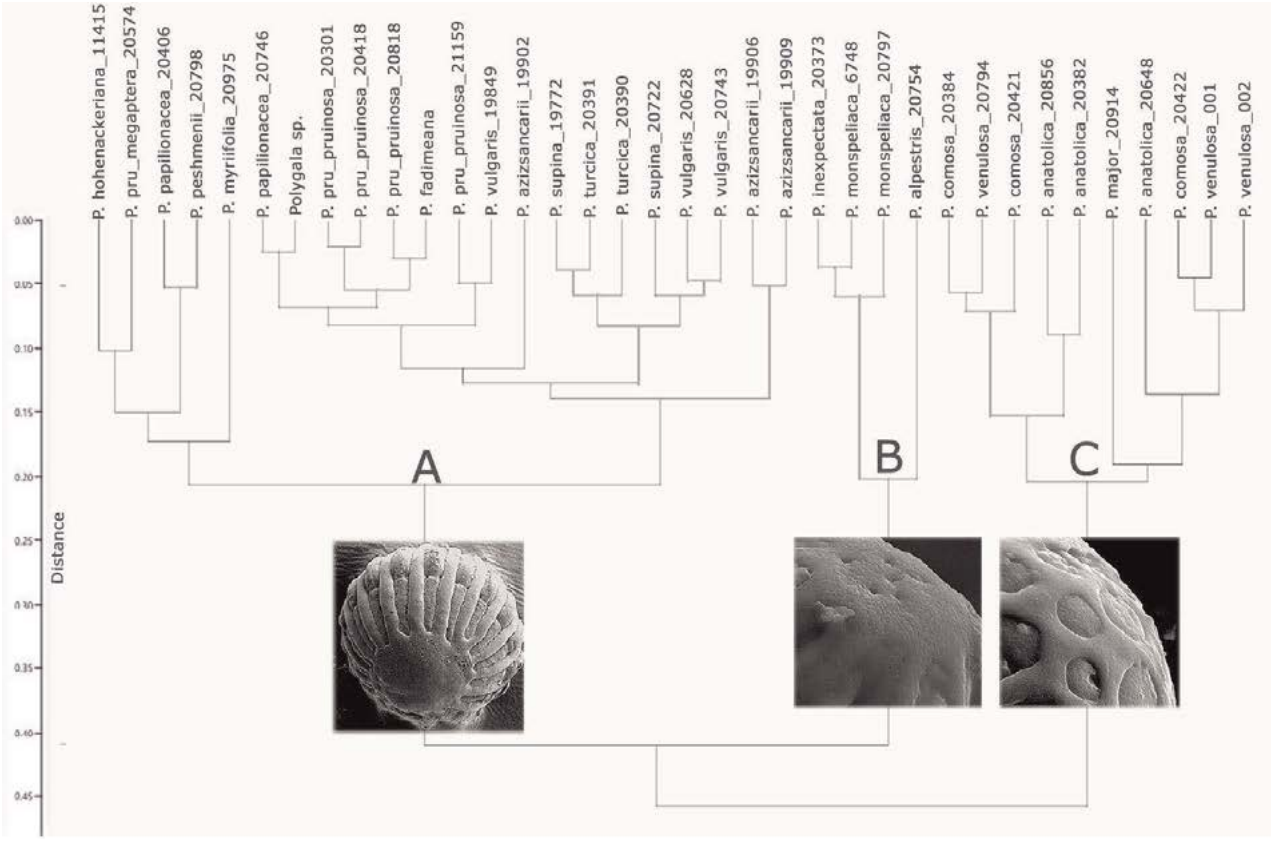

Plate 9. Cluster analysis performed based on selected pollen characters from the 35 *Polygala* specimens using the unweighted pair group method with arithmetic averages (UPGMA) employing the Gower coefficient.

endemic species *P. azizsancarii, P. fadimeana*, *P. peshmenii*, *P. turcica*, and *Polygala* sp. nov. (AAD 20983).

MULTIVARIATE DATA ANALYSES AND TAXONOMIC IMPLICATIONS

The pollen grains of the Turkish *Polygala* species show some similar morphological characters to those previously recorded for the genus (e.g., Furness and Stafford, 1995; Telitsina et al., 2019; Sarvi et al., 2022) - basically, regarding isopolarity, and the polyzonocolporate aperture system. Some characters, such as pollen size and colpi length, overlap in the species of the genus examined in this study. Although low, there is intraspecific variation in some taxa, comprising *P. anatolica*, *P. azizsancarii, P. comosa, P. monspeliaca*, *P. papilionacea, P. pruinosa* and *P. vulgaris*, in the pollen size, pollen shape, apocolpium diameter or in the aperture membrane characters. These are related to intraspecific diversities in several taxonomic features, such as morphology and the ploidy level.

Quantitative pollen morphological differences among the species belonging to two genera, *Polygaloides* Hall (DC.) Schb. and *Rhinotropis* (S.F. Blake) J.R. Abbott, with *Polygala* subgen. *Chodatia* Paiva, have been revealed (Castro et al., 2009). Meanwhile, it has been concluded that within the genus *Polygala* further pollen morphological distinctions for lower (species) level taxonomic discriminations are not possible because of significant overlaps in the range of variation for most characters as well as intraspecific diversifications (Furness and Stafford, 1995; Banks et al., 2008; Castro et al., 2009). Thus, the results of the PCA results presented inhere are in general consistent with this conclusion.

The multivariate analyses conducted in this study allow to determine qualitative pollen features that are more informative to distinguish *Polygala* taxa. These numerical analyses of pollen morphological characters have been previously performed to assess relationships in the family Polygalaceae, and *Polygala* has a high degree of pollen morphological variation amongst the sec-

tions (e.g., Persson, 2001; Forest et al., 2007; Banks et al., 2008; Castro et al., 2009).

In *Polygala*, the observed variations of pollen characters, especially in the ornamentation, indicate useful characters in this study. It is somewhat clear that pollen morphology alone is not enough to evaluate the taxonomic relationships within *Polygala*, while the variations of pollen characters can provide some diagnostic knowledge for the intraspesific taxonomic treatment. The next step would be to construct a well-supported molecular phylogenetic tree and to map the pollen morphology onto the tree, and to understand pollen evolution within *Polygala*. For this purpose, a phylogenetic study on the genus is being currently conducted by the authors.

COMPARISON OF THE POLLEN MORPHOLOGICAL RESULTS WITH PREVIOUS STUDIES IN THE SECTION *POLYGALA*

Although there are some similarities between the results of the present study and those recorded in previous studies of the same taxa in the section *Polygala* from other parts of the world, some discrepancies are found, mainly in the size of pollen grains, apocolpium character, colpi number, and the exine pattern in the mesocolpium. Pollen morphological differences are noticed in *P. alpestris*, *P. anatolica*, *P. comosa*, *P. hohaneckeriana*, *P. major*, *P. monspeliaca*, *P. myrtifolia*, and *P. vulgaris*, reflecting intraspecific diversities within the genus.

Pollen size fluctuations are widely linked to the methodology or mounting medium types, or the degree of hydration (Furness and Stafford, 1995; Castro et al., 2009; Hesse et al., 2009). Furthermore, pollen variability within a species is related to one of several factors, such as ploidy and functional specialization within individuals (Heubl, 1984; Borsch and Wilde, 2000).

Karyological studies show that there are polyploid individuals in *P. anatolica* and *P. vulgaris* (Heubl, 1984; Paiva, 1998). Sarvi et al. (2022) reported large pollen grains with a maximum diameter of 55 µm in one Iranian specimen of *P. anatolica*, whereas Telitsina et al. (2019) reported medium sized pollen grains with maximum diameters of 40 and 45 µm in two Caucasian specimens of the species. In our study, it is shown that two specimens of *P. anatolica* from Sakarya and Antalya provinces have medium sized pollen grains, whereas one specimen from Ardahan province has large pollen grains (Table 3). This can be correlated with the larger size of flowers and vegetative parts measured on the specimen of Ardahan than in other Turkish specimens of the species. There is unfortunately no karyological information about these Turkish specimens or the specimens from Iran or the Caucasus. Regarding other pollen morphological characters in *P. anatolica*, the three Turkish specimens have apocolpial lumens without gemmae (Plate 1, Figs. 3, 4) as in the Caucasian specimens (Telitsina et al., 2019), whereas the Iranian specimen has gemmate apocolpial lumens (Sarvi et al., 2022). It seems that gemmae are generally absent, appearing only occasionally in some individuals. Additionally, unlike the psilate mesocolpium exine pattern in the Caucasian and Iranian specimens, a finely striate mesocolpial exine pattern is noticed in the Turkish specimens (Plate 1, Fig. 3).

Polygala major has a narrow distribution area in north-eastern and eastern Turkey (Cullen, 1965). Recently, fifteen taxa, with variable morphological characters andwhich are found from central and southern Europe through the Balkans to southwest Asia and Caucasia, have been placed in the *Poygala major* complex, which is newly synonymized under *P. major*, based on the molecular evidence and main diagnostic morphological characters (Lyskov et al., 2019). One specimen of *P. major* was collected from Artvin in north-eastern Turkey. It shares similar pollen morphological characters with a neighboring Caucasian specimen (Telitsina et al., 2019), except the presence of both medium-sized and large pollen grains with different exine thickness from the same pollen sac in the Turkish individual, being 3 and 2 µm, respectively.

In all three Turkish specimens of *P. vulgaris*, the pollen apocolpial area is psilate without lumens or small depressions (Plate 7, Figs. 1, 3), and it has more than 14 colpi, but other pollen studies on this species reveal apocolpial lumens, with fewer colpi (Erdtman, 1969; Heubl, 1984; Villnueva and Ramos, 1986; Furness and Stafford, 1995; Paiva, 1998; Svojtka and Halbritter, 2005; Banks et al., 2008). The species, which is widespread in Eurasia as well as in North America, has been treated as a group, called the *Polygala vulgaris*-group, with some subspecies and local populations (Heubl, 1984). However, combined with karyological and molecular analyses, some ongoing taxonomic studies on the Turkish representatives of *P. vulgaris* by the present

authors point to diversification within a single species, with several polyploids. Notably, the psilate apocolpium pattern and a higher colpi number (16-20) are constant in the pollen grains of this species under investigation. These characters seem to be common in the individuals of Turkish *P. vulgaris* because they are recorded in one specimen from the north-western part of the country, at a lower altitude locality (in Kastamonu province), and in two specimens from its eastern part, at higher altitudes (in Erzurum province).

It was previously noted that morphologically *P. alpestris*, *P. comosa* and *P. vulgaris* are closely related taxa (Cullen, 1965; Davis et al., 1988). Furness and Stafford (1995) have placed *P. alpestris* and *P. comosa* within the *P. vulgaris* type in the Northwest European pollen key, with fewer than 14 colpi, apocolpial lumens, and the pollen diameter smaller than 45 µm. However, the pollen of *P. vulgaris* in Turkey shows some significant differences that may allow its separation from *P. alpestris* and *P. comosa*. In addition, our ongoing phylogenetic analyses suggest that these three species are distantly related.

Apocolpial lumens/depressions have been previously observed in *P. alpestris* and *P. comosa* (Heubl, 1984; Furness and Stafford, 1995; Paiva, 1998; Telitsina et al., 2019), which are also present in the individuals of the species examined in this study (Plate 1, Fig. 1; Plate 2, Figs. 1, 3). Contrary to the previous reports about the psilate mesocolpium exine pattern in *P. alpestris*, a finely striate exine is observed in the mesocolpium of the Turkish representatives of the species (Plate 1, Fig. 2). In addition, *P. comosa* specimens examined in the present study have larger pollen grains than those of *P. alpestris* and *P. vulgaris*, with a maximum diameter of 45 µm or over 45 µm, showing variation within the species, which is the case for its morphological characters, such as inflorescence and flower color variations. It also has larger grains than those of the European and Caucasian representatives from the species described in Furness and Stafford (1995) and in Telitsina et al. (2019), respectively. Heubl (1984) has reported that *P. comosa* is characterized by the diploid chromosome number 2n=34, but since there are no chromosome counts for the Turkish individuals examined here, it is impossible to make further comments.

Concerning *P. hohaneckeriana*, Sarvi et al. (2022) describe lumens with gemmae in the apocolpium and the presence of an aperture operculum in one western Iranian specimen. However, in our study, a single specimen from this species, with a restricted distribution area, from eastern Turkey, closely neighboring Iran, shows no such structures. It has apocolpium without lumens and no operculum (Plate 2, Figs. 4-6). This intraspecific pollen morphological variation for the species native to the Caucasus and the Middle East may be correlated with polymorphic taxonomic characters of the species.

In the three specimens of *P. monspeliaca* examined here, small depressions in the apocolpium and a rugulate mesocolpium exine pattern are observed (Plate 3; Figs. 5, 7). Apocolpial depressions have also been reported for one Iranian specimen of the species, but with a psilate mesocolpium (Sarvi et al., 2022). On the other hand, in a study on the Iberian Peninsula (western European) species, Villnueva and Ramos (1986) have described an apocolpium with lumina and a psilate mesocolpium. These may again reflect intraspecific pollen morphological variations over a wider geographical region.

For the cultivated South-African species *P. myrtifolia*, Paiva (1998) has given a mean pollen size of 51.32 µm (P) and 50.04 µm (E) and noted apocolpial lumens, whereas Halbritter et al. (2016) have documented large and very large pollen grains, ranging greatly from 51 to 100 µm in diameter, with no apocolpial lumens. In these previous studies, the mesocolpium has been characterized by a psilate exine pattern. The specimen examined here has pollen grains from a single pollen sac ranging in size from 30 to 50 µm, and psilate apocolpium (Plate 4, Figs. 1, 3). The mesocolpial pattern is rugulate in smaller pollen grains (Plate 4, Fig. 2) with a thicker exine layer (3 µm), whereas it is striate in larger ones (Plate 4, Fig. 4) with a thinner exine layer (2 µm). These variable pollen characters in *P. myrtifolia* must be assessed with caution when they are used in morphological analyses.

POLLEN APERTURE NUMBER AND ITS PROBABLE SIGNIFICANCE IN THE TURKISH *POLYGALA*

The pollen aperture number and its variability within species and among species are of significance in taxonomic as well as in evolutionary studies, and functional perspectives into apertures are also needed in such investigations (Castro et al., 2009). The number of ectoapertures is evaluated as one of interesting pollen characteristics of the genus *Polygala*, and it is polymorphic in several

taxa (Castro et al., 2009; Banks et al., 2008). In this study, marked polymorphism in the aperture number is observed in *P. anatolica*, ranging from 10 to 21, which can be correlated with morphological variability in the species as mentioned above. This is expected because it is a cosmopolitan species occurring in diverse habitats in most parts of Turkey. A rise in the pollen aperture number is probably linked to the selective advantage (Furness and Rudall, 2004), increasing the number of pollen tube germination sites and the efficiency of pollination (Dajoz et al., 1991).

In the eudicotyledons, one of the most notable pollen morphological evolutionary trends is an increase in the aperture number (Walker and Doyle, 1975), and thereby potentially increasing the fertilization rate (Furness and Rudall, 2004). Such increase is in fact characteristic of the whole family Polygalaceae (Banks et al., 2008). This is especially a critical case for animal-pollinated species (Dajoz et al., 1991). In *Polygala*, there are several species pollinated by a variety of bees (e.g., Brantjes, 1982; Castro et al., 2008a, 2008b, 2013; Aygören Uluer et al., 2021, 2022). Cleistogamy (a self-fertilization type) is also known in the genus (Heubl, 1984). Pollination biology in *P. vulgaris*, *P. myrtifolia* and *P. alpestris* has been studied (Heubl, 1984; Westerkamp and Weber, 1997; De Cock et al., 2018), but there is unfortunately no detailed information about pollination ecology in other species of the genus growing in Turkey. Large-flowering individuals (races) in the former species (Heubl, 1984) as well as in the second species (Westerkamp and Weber, 1997) are adapted to insect pollination, whereas the third species with small flowers is predominantly self-pollinated (autogamous) (Heubl, 1984). In the present study, it is revealed that the insect-pollinated species, *P. vulgaris* and *P. myrtifolia* have more ectoapertures (16-20 and 19-20, respectively) than the self-pollinated species *P. alpestris* with 9-10 ectoapertures, thus correlating with a potentially high germination site number in the former taxa for cross-pollination.

In addition to pollen tube formation and germination functions, pollen apertures play some other important roles, including protection of the male gametophyte from dehydration and from pathogens attacks, allowing transport of stigma recognition agents, and harmomegathy (expansion and contraction of the pollen in response to external conditions) (Wodehouse, 1935; Muller, 1979; Punt, 1986; Dajoz et al., 1991; Furness and Rudall, 2004; Wang and Dobritsa, 2018). Colpus number variabilities in different species of *Polygala* from Turkey may also be linked to such crucial functions of apertures. However, detailed investigations are needed in the native Turkish *Polaygala* species to understand pollination biology in the field and to determine the relationships between the aperture number and the function(s).

CONCLUSION

This study has provided detailed pollen data for *Polygala* species, including the subspecies and proposed species, from Turkey, thus enabling an overall review of their pollen characters. Pollen apocolpial features as well as pollen and colpus sizes (P, E, P/E, CL and CW) are taxonomically useful characters, based on multivariate analyses. However, pollen morphology is insufficient to evaluate taxonomic relationship at the species level.

AUTHORS' CONTRIBUTION

The authors designed the study conception. AAD acquired funding to conduct the research into the taxonomy and phylogeny of the species of *Polygala* in Turkey. AAD and ZUA performed field studies to collect specimens. EOD prepared pollen slides and specimen stub samples and did light and scanning electron microscopic studies. ZUA performed cluster analysis and principal component analysis. EOD drafted and wrote the manuscript. ZUA and AAD provided comments on multivariate data analyses and taxonomic implications, morphological characters and habitats of the species, as well as on preliminary results of ongoing phylogenetic analyses. The authors declare that there is no conflict of interest.

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