

Pollen morphology of the genus *Lathyrus* (Fabaceae) section *Cicerula* in Thrace (European Turkey)

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We examined the pollen morphology of four taxa from the *Cicerula* section of *Lathyrus*, grown in the Thrace region (European Turkey), including *L. annuus* L., *L. gorgoni* Parl. var. *pilosus* C. C. Townsend, *L. cicera* L. and *L. hirsutus* L. The pollen grains are 3-zonocolporate of subprolate and prolate types (P/E=1.2378-1.4491), medium to large sized, elliptical or rectangular-obtuse-convex (equatorial view) and circular to slightly triangular-obtuse-convex (polar view). The ornamentation is reticulate.

Keywords: pollen, morphology, *Lathyrus*, *Cicerula*, Turkey

Introduction

Pollen morphology is not affected by environmental conditions; therefore it has been accepted as a reliable taxonomic marker in the identification of higher plants (AYTUG 1959). The genus *Lathyrus* L. (Fabaceae), comprising nearly 200 species with annual and perennial plants, is centered in Mediterranean countries and mainly distributed in the northern hemisphere and the highlands of tropical Africa (SEEN 1938; DAVIS 1970, 1988; HEYWOOD 1978; KUPICHA 1983). The flora of Turkey contains 64 species belonging to the sections *Orobus*, *Platystylis*, *Pratensis*, *Orobastrum*, *Orobon*, *Lathyrus*, *Cicerula*, *Aphaca*, *Nissolia* and *Clymenum*; a total of 24 of these are endemic (DAVIS 1970, 1988; GUNER et al. 2000).

The pollen morphology have been already investigated in *Lathyrus digitatus* (AYTUG 1967, AYTUG et al. 1971), *L. undulatus*, *L. sylvestris* and *L. ochrus* (GUNES and CIRPICI 1998). *L. sylvestris*, *L. pratensis*, *L. maritimus*, *L. nissolia* and *L. montanus* (MOORE et al. 1991), *L. grandiflorus*, *L. latifolius*, *L. sylvestris*, *L. tuberosus*, *L. alpestris*, *L. aureus*, *L. linifolius*, *L. niger*, *L. palustris*, *L. transsilvanicus*, *L. venetus* and *L. vernus* (TOSHEVA et al. 2004, TOSHEVA and TONKOV 2005), *L. emodii*, *L. cicera*, *L. humulis* and *L. pratensis* (PERVEEN and QAISER 1998). The authors indicated that the pollen grains are mainly tricolporatae. In addition to pollen type, a tectate type of exine structure and a reticulate

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type of sculpture were also reported in all *Lathyrus* species. Furthermore, MOORE et al. (1991) also noted that the endexine thickens 2–3 times around the colpus and the porus and form a large costae formation.

Material and methods

Four taxa of *Lathyrus* were collected from their natural habitats in the Thrace region of Turkey between 1996 and 1998. All collected specimen were identified and deposited at the herbarium of the Biology Department, Faculty of Arts and Sciences of the Marmara University (MUFE).

Pollen grains for LM examination were prepared following the standard procedure of WODEHOUSE (1959) and the acetolysis method (ERDTMAN 1960). Fifteen pollen characters were investigated by light microscope (LM). P (polar diameter), E (equatorial diameter) were measured at a magnification of 400 times and clg (colpus length), clt (colpus width), plg (porus length regarding the poles), plt (porus width regarding the equatorial diameter), t (one edge of polar triangle), exine thickness, intine thickness and costa were also measured under a magnification of 1000×. Structure (exine), sculpture (exine ornamentation) were also shown.

For SEM examination, pollen grains were coated with gold. The microphotographs were obtained using JEOL-JSM-5200 scanning electron microscope (SEM) at a magnification of 2000–5000×. The arithmetic means, standard deviation and variations were calculated for each characteristic. Standard deviations are not given for characteristics having no requirement for multiple measurements. In table 1, fresh pollen measurements are given in light and the fossilized pollen measurements are given in bold. To determine the structure and sculpture characteristics, the specimens specifically prepared with the acetolysis method were analyzed under LM microscopy. Reticulate type of sculpture was shown by photographs taken in SEM (Fig. 2). The pollen morphological descriptions follow the terminology of IVERSEN and TROELS-SMITH (1950), REITSMA (1970), MOORE et al. (1991) and PUNT et al. (1994).

Results

The following shapes of pollen were found in the four *Lathyrus species*: 3-zonocolporate type, a tectate type of exine structure and a reticulate type of sculpture, subprolate and prolate. Colpus width in fossilized grains appears to have decreased based on the values determined as described above. As the pollens fossilize they increase in the direction of the poles, that is they increase in length. The colpus measurements were done at the regions close to the pores. Although the colpus above the pores in *L. gorgoni* enlarged as much as the pore itself, the colpus above the pore narrowed to a line in *L. cicera*.

Lathyrus annuus L. (Tab. 1, Figs. 1, 2) A1(E). Edirne: Saçlımüsellim-Siğircik village road, 1 km from rice-field edges, 30 m, 25.05.1997, F. Gunes, MUFE 5392.

Pollen class: 3-zonocolporate.

Pollen group: Subprolate [P/E=1.112 (Wodehouse) and P/E=1.114 (acetolysis)]

Dimensions: Medium size [P × E = 36.679 × 33.085 (Wodehouse), 39.676 × 35.620 (acetolysis)].

Apertures: Ectoapertures – colpi: short, narrow, deep, with acute ends, thick costae along the margins of the colpi, colpus membrane covered by clearly visible granules; clg: 20.300 (Wodehouse), 32.5 (acetolysis) μm , clt: 1.8 (Wodehouse), 2.3 (acetolysis) μm . Endoapertures – pori : lalongate (Wodehouse), lolongate (acetolysis) methods., plg: 7.0 (Wodehouse), 12.6 (acetolysis) μm , plt: 9.0 (Wodehouse), 9.9 (acetolysis) μm and plg/plt= 0.8 (Wodehouse), 1.3 (acetolysis).

Outlines: Equatorial view – elliptic to rectangular-obtuse-convex; polar view circular

Ornamentation: reticulate; reticules distinct, big, irregular. Heads of single columellae are visible inside the lumina. Colpus area, porus and apocolpium are slightly reticulate. Ex/int (Wodehouse): 1/1

Exine (acetolysis): 2 μm thick, tectate – infrastructure, nexine/sexine = 1/1

Lathyrus gorgoni Parl. var. *pilosus* (Tab. 1; Fig. 1–4). A1(E) İstanbul: Büyükçekmece-highway way out, through road, meadow, 30 m, 11.05.1996, F. Gunes, MUFE 5007.

Pollen class: 3-zonocolporate.

Pollen group: subprolate [P/E=1.1426 (Wodehouse)] – prolate [P/E=1.3944 (acetolysis)].

Dimensions: Medium size [P \times E = 41.228 \times 36.081 (Wodehouse), 56.992 \times 40.872 (acetolysis)].

Apertures: Ectoapertures – colpi: long, narrow, deep, nearly reaching the poles, with acute ends, thick costae along the margins of the colpi, colpus membrane covered by clearly visible granules; clg: 34.266 (Wodehouse), 55.240 (acetolysis) μm , clt: 2.737 (Wodehouse), 3.383 (acetolysis) μm . Endoapertures – pori: lalongate to slightly lalongate, plg: 9.790 (Wodehouse), 13.440 (acetolysis) μm , plt: 11.437 (Wodehouse), 14.252 (acetolysis) μm and plg/plt= 0.856 (Wodehouse), 0.943 (acetolysis).

Outlines: Equatorial view – elliptic to rectangular-obtuse-convex; polar view circular

Ornamentation: reticulate; reticules distinct, big and irregular. Heads of single columellae are visible inside the lumina. Colpus area, porus and apocolpium are slightly reticulate.

Ex/int (Wodehouse): 1/2

Exine (acetolysis): 3 μm thick, tectate – infrastructure, nexine/sexine = 1/1

Lathyrus cicera L. (Tab. 1; Figs. 1–6). A1(E) Çanakkale: Gelibolu-Eceabat Road, 20 km. 0–10 m, 24.04.1998, F. Gunes and A. Cirpici, MUFE 5646.

Pollen class: 3-zonocolporate.

Pollen group: subprolate [P/E=1.321 (Wodehouse)] – prolate (P/E=1.574 (acetolysis)].

Dimensions: medium size [P \times E = 41.707 \times 31.581 (Wodehouse), 60.320 \times 38.324 (acetolysis)].

Apertures: Ectoapertures – colpi: long, slightly wide, nearly reaching the poles, with acute ends, thick costae along the margins of the colpi, colpus membrane covered by small granules; clg: 31.390 (Wodehouse), 58.804 (acetolysis) μm , clt: 4.031 (Wodehouse), 3.864 (acetolysis) μm . Endoapertures – pori: lalongate, plg: 4.379 (Wodehouse), 10.366 (acetolysis) μm , plt: 8.120 (Wodehouse), 14.868 (acetolysis) μm and plg/plt= 0.539 (Wodehouse), 0.6972 (acetolysis).

Outlines: Equatorial view – elliptic to rectangular-obtuse-convex; polar view circular to slightly triangular

Tab. 1. Mean pollen characteristics (M), structure and sculpture (ornamentation). σ – standard deviation, var. – variations of line numbers of pollen lamella, W – Wodehouse-method, A – asetolysis-method, P – polar diameter, E – equatorial diameter, P/E – pollen shape, Ex – exine thickness, Ex/Int – the ratio of exine to the intine, clg – colpus length, clt – colpus width, plg – porus length regarding the poles, plt – porus width regarding the equatorial diameter, plg/plt – porus shape, t – one edge of polar triangle.

	<i>Lathyrus annuus</i>			<i>Lathyrus gorgoni</i> var. <i>pilosus</i>			<i>Lathyrus cicera</i>			<i>Lathyrus hirsutus</i>		
	M	σ	var.	M	σ	var.	M	σ	var.	M	σ	var.
PW	36.679	± 1.617	11–13	41.228	± 1.597	13–16	41.707	± 1.354	13–15	38.976	± 2.504	13–14.5
A	39.676	± 2.590	12–17	56.992	± 2.257	20–24	60.320	± 3.247	20–25	52.832	± 2.463	18–23
E	33.085	± 1.462	10–13	36.081	± 1.476	11–14	31.581	± 0.974	10–11.5	32.799	± 1.227	10–11.5
	35.620	± 2.031	12–16	40.872	± 2.146	14–17	38.324	± 2.108	13–16	30.784	± 1.198	10–14
P/E	1.112	Subprolate		1.143	Subprolate		1.321	Subprolate		1.188	Subprolate	
	1.114	Subprolate		1.394	Prolate		1.574	Prolate		1.716	Prolate	
Ex	Not	Not		Ince	Not		Not	Not		Not	Not	
	measured	measured			measured		measured	measured		measured	measured	
	2			3			3			2		
Ect/end												
	1/1			1/1			1/1			1/1		
Ex/int	1/1	Not		½	Not		2/1	Not		1/1	Not	
		measured			measured			measured			measured	
clg	20.300	± 1.477	16–21	34.266	± 1.317	27–32	31.390	± 1.631	24–30	27.882	± 1.211	22–26
	32.523	± 2.343	20–26	55.240	± 3.553	35–44	58.804	± 3.493	39–47	47.796	± 2.761	29–38
clt	1.856	± 0.568	1–2	2.7377	± 0.950	1–5	4.031	± 0.733	2–5	3.712	± 0.843	2–4
	2.275	± 0.500	1–2	3.383	± 1.063	1–4	3.864	± 1.548	1–5	1.960	± 0.686	1–2
plg	7.018	± 0.498	5–7	9.790	± 0.903	7–10	4.379	± 0.710	3–5	8.294	± 0.611	6–8
	12.593	± 1.565	7–11	13.440	± 1.313	7–12	10.366	± 1.249	5–9	10.556	± 0.978	6–9

Tab. 1. – continued

	<i>Lathyrus annuus</i>			<i>Lathyrus gorgoni</i> var. <i>pilosus</i>			<i>Lathyrus cicera</i>			<i>Lathyrus hirsutus</i>		
	M	σ	var.	M	σ	var.	M	σ	var.	M	σ	var.
plt	9.048	± 0.696	7–9	11.438	± 0.957	8–12	8.120	± 0.935	5–9	10.701	± 0.661	8–10
	9.870	± 1.744	5–10	14.252	± 1.810	8–13	14.868	± 1.768	8–13	10.136	± 0.856	6–8
plg/plt	0.776	Not measured	Not measured	0.856	Not measured		0.539	Not measured	Not measured	0.775	Not measured	Not measured
	1.276	Not measured	Not measured	0.943	Not measured		0.697	Not measured	Not measured	1.041	Not measured	Not measured
t	Not measured	Not measured	Not measured	14.500	± 1.249	10–15	17.110	± 1.264	13–16	Not measured	Not measured	Not measured
Structure	Tectate- infra- structure			Tectate- infra- structure			Tectate- infrastruc- ture			Tectate- infra- structure		
Sculpture	Reticulate, distinct, big, irreg- ular			Reticulate, distinct, big and ir- regular			Reticulate, distinct, big and ir- regular			Reticulate, slightly, distinct, regular and small		

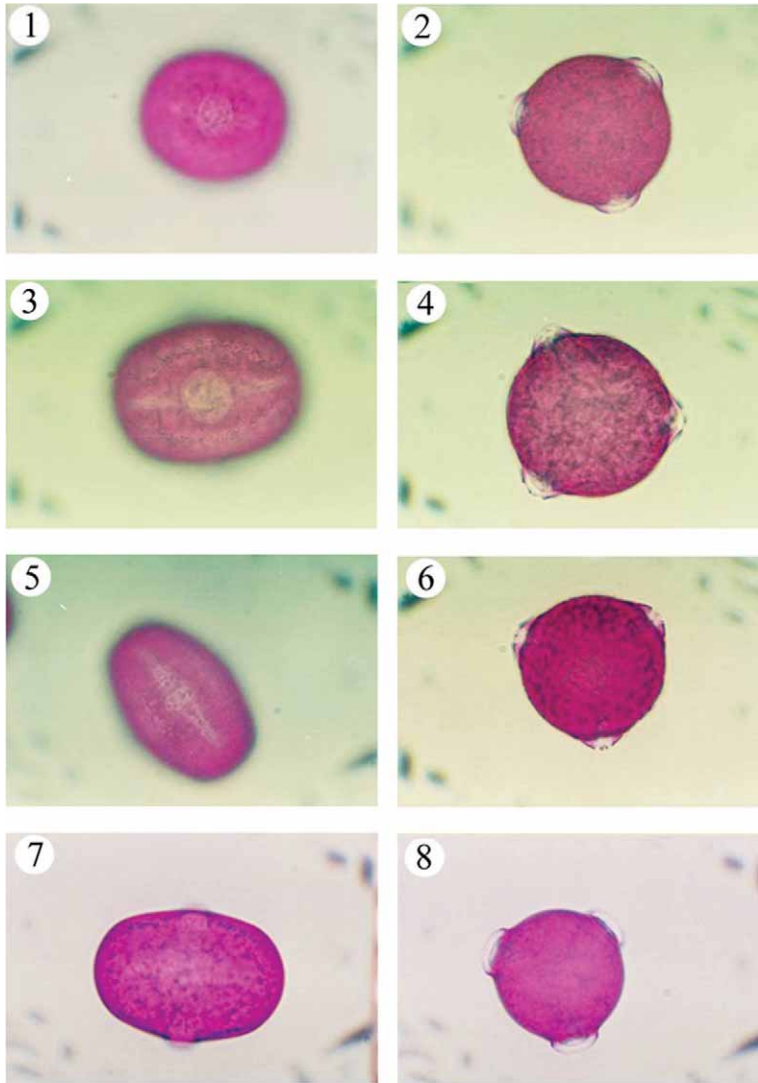


Fig. 1. Pollen grains of *Lathyrus annuus* (1, 2), *L. gorgoni* (3, 4), *L. cicera* (5, 6) and *L. hirsutus* (7, 8). 1, 3, 5, 7 – equatorial view; 2, 4, 6, 8 – polar view (LM). Bar denotes 15 μ m.

Ornamentation: Reticulate; reticules slightly distinct, big and irregular. Colpus area, porus and apocolpium are slightly reticulate.

Ex/int (Wodehouse): 2/1

Exine (acetolysis): 3 μ m thick, tectate – infrastructure, nexine/sexine = 1/1

Lathyrus hirsutus L. (Tab. 1, Figs. 1 – 8). A1(E) Edirne: Edirne-Doyran village entrance, through water channels, 60 m, 31.05.1998, F. Gunes and A. Cirpici, MUFE 5739.

Pollen class: 3-zonocolporate.

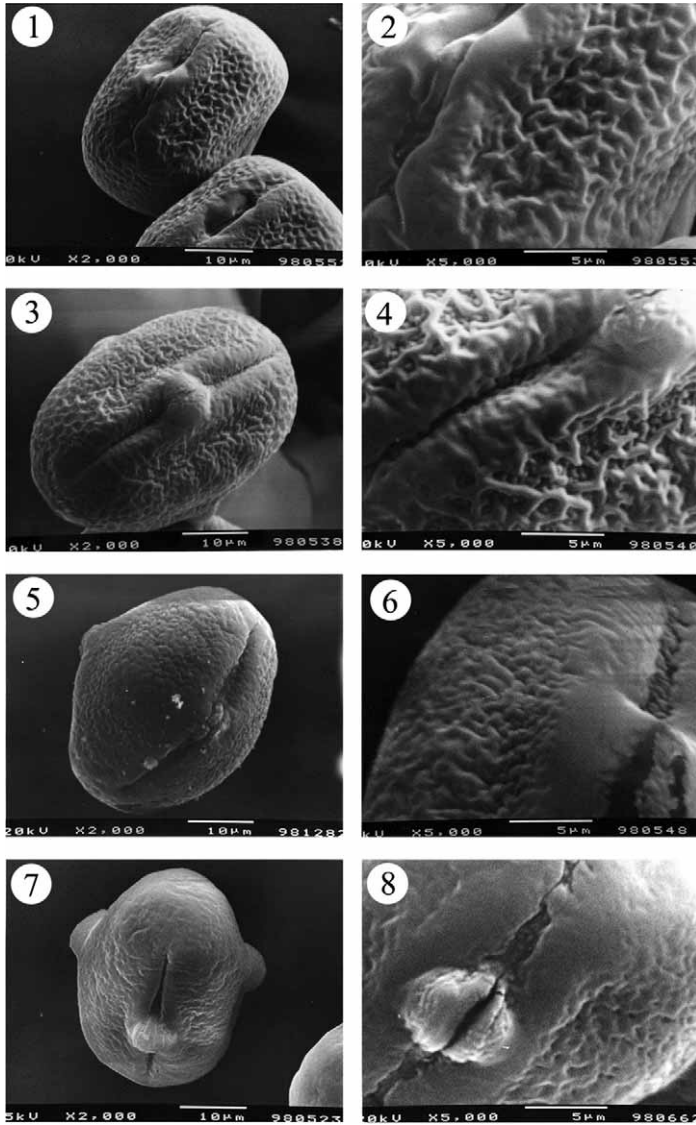


Fig. 2. Ornamentation in pollen grains of *Lathyrus annuus* (1, 2); *L. gorgoni* (3, 4); *L. cicera* (5, 6); *L. hirsutus* (7, 8) (SEM). 1, 3, 5, 7 – equatorial view; 2, 4, 6, 8 – polar view.

Pollen group: Subprolate [$P/E = 1.188$ (Wodehouse)] – prolate ($P/E=1.716$ (acetolysis)].

Dimensions: Medium size [$P \times E = 38.976 \times 32.799$ (Wodehouse), 52.832×30.784 (acetolysis)].

Apertures: Ectoapertures – colpi: long, slightly wide (Wodehouse), narrow (acetolysis), nearly reaching the poles (Wodehouse), with acute ends, thick costae along the margins of the colpi, colpus membrane covered by small granules; clg: 27.882 (Wodehouse), 47.796

(acetolysis) μm , clt: 3.712 (Wodehouse), 1.960 (acetolysis) μm . Endoapertures – pori: lalongate to circular, plg: 8.294 (Wodehouse), 10.556 (acetolysis) μm , plt: 10.701 (Wodehouse), 10.136 (acetolysis) μm and plg/plt= 0.775 (Wodehouse), 1.041 (acetolysis).

Outlines: Equatorial view – elliptic to rectangular-obtuse-convex; polar view circular
Ornamentation: reticulate; reticules slightly distinct, regular and small. Colpus area is psilate. Porus and apocolpium are slightly reticulate.

Ex/int (Wodehouse): 1/1

Exine (acetolysis): 2 μm thick, tectate – infrastructure, nexine/sexine = 1/1

Discussion

Pollen dimensions varied in three of the taxa; the exception is *L. annuus*. The subprolate type of pollen shape (P/E) was found in fresh pollens of *L. gorgoni*, *L. cicera* and *L. hirsutus*, but the prolate shape was found in fossilized pollens of those three species. In contrast to *L. gorgoni*, *L. cicera*, *L. hirsutus*, subprolate shape (P/E) was observed in both fresh and fossilized pollens of *L. annuus* (Tab. 1).

Pollen dimensions and shape differed in fresh and fossilized pollens (Tab. 1). The ratio of plg to plt in fresh pollens was 0.775 in *L. annuus*, 0.856 in *L. gorgoni*, 0.539 in *L. cicera*, and 0.775 in *L. hirsutus*. The values found in fossilized pollens were 1.276 in *L. annuus*, 0.943 in *L. gorgoni*, 0.697 in *L. cicera*, and 1.041 in *L. hirsutus*. Looking at the pore length, the ratio of plg to plt in fossilized pollens revealed an increase in the four species.

Although a tectate-infrastructure (exine) and a reticulate ornamentation were seen in all four taxa (IVERSEN and TROELS-SMITH 1950, AYTUG et al. 1971, MOORE et al. 1991, TOSHEVA and TONKOV 2005), our study showed that the reticulate size and its arrangement were distinct from each other (Tab. 1, Fig. 2) and that *L. annuus* possessed a distinct, big, and evenly shaped reticulation. A very distinct and unevenly shaped reticulation was also seen in *L. gorgoni*. Similar to these results in *L. gorgoni*, a very big distinct and uneven shaped reticulation was observed in *L. cicera*. A slightly distinct, evenly shaped and small sized reticulation was found in *L. hirsutus*. Although a reticulate pattern in all *Lathyrus* species is clearly distinct in the center of its pollens, a distinguishable reticulate characteristic appeared to be diminished in the split and holes of pollens. Reticulation was clearly distinguishable in the polar regions of *L. annuus*, *L. gorgoni* and *L. cicera*, whereas *L. hirsutus* (Fig. 2). Operculum above pores and colpus had a reticulated appearance.

The contribution of this study to previously reported data on the study of pollen morphology can be summarized as follows:

1. The pollen grains are 3-zonocolporate of subprolate – prolate type (P/E = 1.112 – 1.188 (Wodehouse), 1.114 – 1.716 μm (acetolysis)), medium to large in size. The smallest pollen grains belong to *L. annuus* (P×E = 36.679 × 33.085 μm in Wodehouse and Acetolysis) and the biggest to *L. cicera* (P/E = 41.707 × 31.581 [Wodehouse] – (P/E = 60.320 × 38.324 μm (acetolysis)). Equatorial view is elliptic to rectangular-obtuse-convex. The polar view in all pollen grains is circular.
2. The aperture system consists of ectoapertures – colpi and endoapertures – pori. The colpi are generally straight, narrow, with acute ends and thick costae along the colpus regions. There are small granules in colpus membrane. The pori are large, circular to lalongate.

3. The thickness of the exine is 1 μm in Wodehouse and 2–3 μm in acetolysate, the nexine as thick as the sexine.
4. The ornamentation is reticulate in all four taxa, but the size and the arrangement of the lumina are different from each other. The reticulate ornamentation is clear in mesocolpium, while the apocolpium, the colpus area and the porus are psilate or small.

Although pollens in observed taxa seemed to be of a type 3-zonocolporate characteristic, the shapes and largeness of apertures, the largeness of the polar triangle, the clearness of the aperture borders, the shape of the pollens (P/E), the thickness of the exine and the intine, played an important key for the diagnostic of the differentiation of the taxa AYTUG (1959).

In conclusion, it can be stated that the four taxa discussed, although belonging to the same section, can clearly be differentiated based on the palynological findings as described.

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