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POLLEN MORPHOLOGY OF *GYPSOPHILA LARICINA* L. AND TAXONOMIC IMPORTANCE

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ABSTRACT. Pollen morphology of *Gypsophila laricina* L. were investigated using light microscopy (LM), scanning electron microscopy (SEM), and transmission electron microscopy (TEM). Pollen morphology features this taxon was determined based on palynological studies. Pollen grains are spheroidal and periporate. The exine structure is intectate The exine sculpture is clavate-microechinate ornamentation. The operculum exists in the form as a whole in this taxon. The exine consists of 2 parts; the upper part is the thick ectexine and the lower part is the thin endexine. The endexine is thin and continuous.

1. INTRODUCTION

The Caryophyllaceae is a large, cosmopolitan family of 86 genera, and about 2200 species of herbs and small shrubs [9, 22], including over 470 species, of which 32 of the genera exist as native species in Turkey [1, 2, 5-7, 13-17, 21, 24, 27, 29, 31, 37, 39-41]. *Gypsophila* L. (Caryophyllaceae) is a predominantly Eurasian genus. It is not just among the largest genera of the subfamily Silenoideae, but is also one of the most polymorphic. It occurs in the north-temperate part of the Old World; mainly between the latitudes of 30° and 60°. Most *Gypsophila* species are concentrated in a very small part of the geographic area of distribution, which may accurately be called the main variation centre of the genus and includes Turkey, Caucasia, northern Iraq, and northern Iran. In all, 75 of the 126 *Gypsophila* species are represented in this region and 49 of them are endemic to the area. Each of the 3 subgenera and all 8 sections of the genus are represented in this centre of diversity [8]. *Gypsophila* consists of 60 species, of which 35 are endemic to Turkey [2, 3, 14, 23, 25].

The morphological features of some *Gypsophila* species pollen grains have been previously studied [4, 8, 9, 28, 33, 45]. There has been no comprehensive study of this taxon of the genus are represented in this centre of diversity [8]. In the present study the pollen morphology of *Gypsophila laricina* were investigated.

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The purpose of this study was to use LM, SEM, and TEM to determine the extent to which morphological differences affect pollen morphology of *G. laricina.*

2. Materials And Methods

Pollen material was obtained from the Eskişehir Osmangazi University Faculty of Science and Art Herbarium (OUFE). For LM pollen grains were prepared according to the methods of Wodehouse (1959) and Erdtman (1960). LM observations of non acetolysed and acetolysed pollen were made using a Prior microscope. Non-acetolysed and acetolysed pollen were photographed with a Spot in-sight colour digital camera on an Olympus microscope equipped with an apochromatic 100× oil immersion objective and compensating 10× eyepieces. Pollen diameter, pore diameter, distance between 2 pori, and exine and intine thickness measurements were made with 50 pollen grains so that the resulting data would follow Gaussian curves [30]. Pollen from each species was mounted on stubs using double-sided adhesive tape. Each sample was coated with a 100-A-thick layer of gold in a Polaron SC7620 rotating and tilting vacuum coating apparatus for 60 s, and scanned using a JEOL 5600 LV SEM with 20-kV accelerating voltage [42, 43]. For TEM acetolysed pollen grains were stained with 2% OsO_4 and with uranyl acetate, dehydrated, and embedded in epon-araldite, according to the method described by Skvarla (1966). Ultrathin sections of pollen grains were obtained with a glass knife in Reichert Ultracut-R microtome. Post-staining was performed with lead citrate for 5 min [34] and the sections were examined with a JEOL 1220. Terminology for pollen morphology proposed by Skvarla (1966), Erdtman (1969), Walker (1974a, 1974b), Charpin et al. (1974), Faegri and Iversen (1975), and Punt and Hoen (1995) was used. In all, 5 pollen grain specimens were measured using SEM and TEM.

Specimens investigated— *G. laricina* Schreb. [Syn: *Gypsophila sphaerocephala* var. *cappadocica* (Boiss. &Balansa) Boiss.] 27.07.2012, İrano-Turanien, Endemic, LC, OUFE: 18011.

3. Results And Discussion

Gypsophila pollen grains ranged in size range from 28.74 to 31.22 μm in non-acetolysed pollen and from 23.17 to 24.48 μm in acetolysed pollen. The structure of the exine is tectate. Mean exine thickness between the regions of the pores varies from 1.74 to 1.85 μm. It tapers gradually towards the pore endings. Ornamentation is clavate-microechinate in *G. laricina*. The exine consists of 2 parts: the upper part is the thick ectexine and the lower part is the thin endexine. The tectum, with spinules, is thick with thin lines cutting across it at intervals. Infratectal columellae hang down from the tectum. Being thick and short, infratectal columellae do not branch out. The foot layer is continuous and is always thinner than the tectum. The endexine is thin and continuous. The operculum covers the entire pore and is surrounded by an annulus. The ectexine of the pore membrane is fairly thin. There are spinules in the form of convex cones that vary from 5-23 in number over the operculum, according to the species. The endexine is thick, with a large granule extending under the operculum and the thin ectexine of the pore membrane. LM investigations show that the pollen grains are spheroidal, polyporate, intectate and clavate (Figure 1).The comparative results of LM, SEM and TEM are given in below (Table 1, Table 2).

In *G. laricina*, the exine is caveate, and the endexine is thin and continuous. The intectal layer is thick and clavate-microechinate in the interspine regions. The pollen surface is covered with clavae and spinules. Clavae are 1.12 μm long × 1.36 μm wide and spinules are 1.1 μm long × 0.86 μm wide. There are 22.76 spinules 100 μm^2 and the distance between 2 spinules is 2.6-3.1 μm. The intectum is 1.5 μm, the foot layer is 0.25 μm, and the endexine is 0.125 μm. The foot layer is continuous and the foot layer/endexine ratio is 5:1. The ectexine/endexine ratio is 11:1. The operculum is entire. The pore and operculum are covered with clavae and spinules. Mean operculum length is 3.11 μm and the endexine is continuous on the operculum (Figure 1).

Our results show that the pollen of investigated *Gypsophila* taxon are polyporate and spheroidal. The exine sculptures of *G. laricina* were observed to be intectate with clavate-microperforate ornamentation. *G. laricina* was assumed to be more evolutive species. It has been reported that the aperture features and exine structures are among the essential criteria for determination of phylogenetic relationships of *Gypsophila* species [12, 26, 36, 42, 43]. Caryophyllaceae pollen grains are sub-oblatesubprolate (if 3-colpate), and spherical or ± rounded polyhedral (if porate or pantocolpate) [9].

Morphological features of exine layers have been reported to be the features that best explain the nature of the phylogenetic relationship between taxa [12, 26, 36, 42, 43]. The exine structure of all the species analysed was caveate. The fact that such cavea were present between the foot layer and columella in pollen grains seems to suggest that the species analysed have a more evolutive feature. In other words, the presence of cavea has been accepted as a progressive evolutionary characteristic, according to pollen terminology [32].

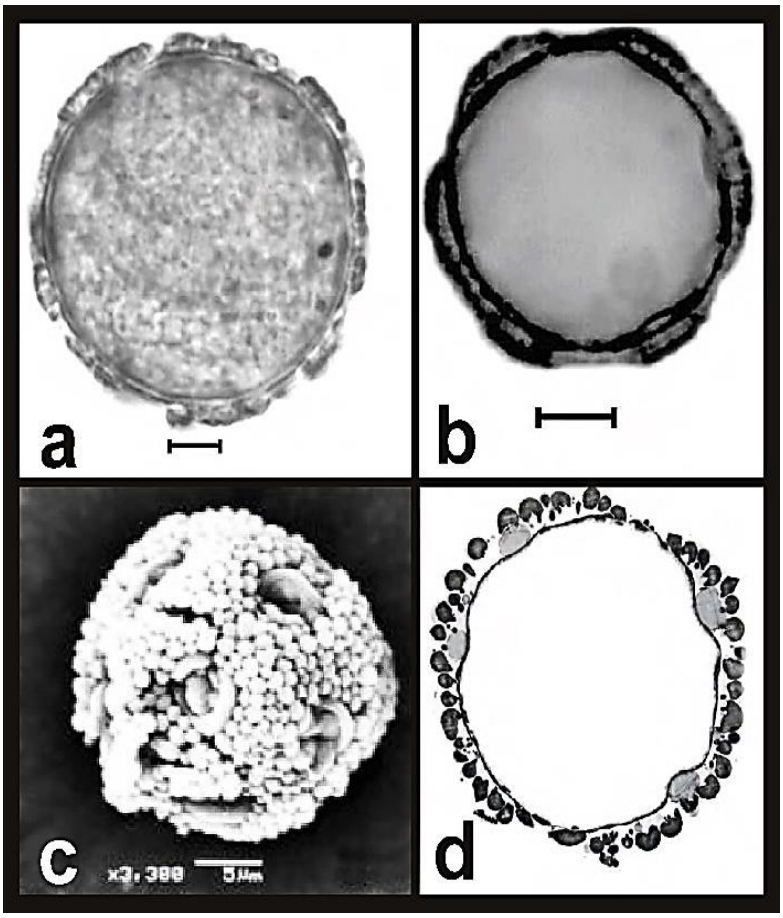

FIGURE 1. a. Non-acetolysed pollen, LM (bar: 5 µm), b. Acetolysed pollen, LM (bar: 5 µm), c. Ornamentation of the pollen, SEM (bar: 5 µm), d. Transverse section of the exine structure, TEM (bar: 2 µm).

TABLE 1. Morphological parameters of *Gypsophila laricina* pollen (LM).

Taxon	A (µm)	B (µm)	A/B	pa (µm)	pb (µm)	p (µm)	Ex (µm)	I (µm)	i (µm)
G. laricina (N)	31.22 ± 3.56	28.74 ± 2.84	1.19	non-meas.	non-meas.	5.82 ± 0.77	1.74 ± 0.30	0.92 ± 0.23	non-meas.
G. laricina (Ac)	24.48± 1.27	23.17 ± 1.62	1.08	non-meas.	non-meas.	4.54 ± 0.58	1.85 ± 0.70	non-meas.	non-meas.

N: Non-acetolysed pollen grains (LM); Ac: acetolysed pollen grains (LM); A: long axis; B: short axis; p: pore; pa: pore length; pb: pore width; Ex: exine at the thickest area; I: intine at the thickest area; i: intine; non-meas.: non-measurable.

TABLE 2. Morphological parameters of *Gypsophila laricina* pollen (SEM & TEM).

Taxon	Pollen Type	Pollen Shape	Ornamentation	Tectum	Columella	Annulus
G. laricina	periporate	spheroidal	clavate- intectate	intectate	single- layered	1.32
Taxon	**Spinules (100 µm^2)**	**Distance of two spinules**	**Footlayer/Endexine**	**Ectexine/Endexine**	**Operculum length(µm)**	**Exine N-Ac (µm)**
G. laricina	22.76	2.6-3.1	5/1	11/1	3.11	1.66-1.84

While LM revealed that the operculum only had a granulate structure, detailed SEM and TEM showed that the operculum was wrapped in both granules and spinules. The difference in measurements was attributed to the fact that all the species analysed had a genetic difference, which seems to comply with the claim that the pollen sculpture types have valid morphological features in taxonomy [12]. Columellae were single-layered and the foot layer was continuous in all the species

analysed. This feature of the foot layer is considered to be primitive in plant phylogenetics. An imperforate exine, fewer pores, and absence of spinules on the tectum of pollen are generally accepted as primitive characteristics of pollen grains [36, 38, 42, 43]. The endexine was thin and continuous in *Gypsophila* taxa.

Palynological findings also emphasise evaluative levels. *G. laricina* belongs to the section Capituliformes and differs from the other taxa. Moreover, morphological features of these taxa differed from those of other taxa. Future studies could determine the categorisation of this section within the species of *Gypsophila* after a thorough investigation of the remaining 6 taxa belonging to the section Capituliformes. The differences in pollen morphology of *G. laricina* could be an indication of their genetic differences. Cronquist (1968) reported that pollen sculpture types have valid morphological features in taxonomy. Thus, the taxonomic value of these taxa in *Gypsophila*, as well as their pollen morphology, could be a distinguishing criterion. In conclusion, morphological structures of pollen seem to be useful for differentiating taxa; thus, it is suggested that they could be of benefit in taxonomical studies.

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