

## Exine Ultrastructure of *Fritillaria* (Liliaceae Juss)

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### ABSTRACT

Palynological data in *Fritillaria* obtained by LM, SEM and TEM are elucidated infra-genus positions of subgenus within *Fritillaria*. Ten pollen grains from eight species of six subgenera of *Fritillaria* were examined using light microscopy, scanning electron microscopy and transmission electron microscopy. The exine surface of pollen grains in *F. camschatcensis* (subgenus *Liliorhiza*) and *F. eduardii* (subgenus *Petilium*) is macroreticulate, in *F. zagrica* Stapf. (subgenus *Fritillaria*) and *F. stenantha* (subgenus *Rhinopetalum*) and *F. persica* L. (subgenus *Theresia*) reticulate, in *F. gibbosa* (subgenus *Rhinopetalum*) and in *F. imperialis* (subgenus *Petilium*) is foveate-rugulate, but in *F. sewerzowii* (subgenus *Korolkowia*) is pisilate to reticulate. Ultrastructural characteristics appear to be valuable for pollen morphological data at species level, but there are not enough data for such conclusions for higher taxonomical levels (section, subgenus, and genus).

**KEY WORDS:** Caput; Columella; Ectexine; Exine ornamentation; *Korolkowia*; Microrelief; *Petilium*; Pollen grain; *Rhinopetalum*; Tectum.

### INTRODUCTION

Genus *Fritillaria* L. is a large genus of the monocot plants which according to Kosenko (1991), including more than 100 species incorporated in 6 subgenus following the opinion as shown in Table 1. Some authors (Baranova and Zaharjeva, 1981; Losina-Losinskaya, 1935; Vvedensky, 1941, 1963; Pasii, 1971) are of the opinion that their subgenera are really a separate genus. For example: *Rhinopetalum*, *Korolkowia*, *Petilium*. The structure of pollen grains sporoderm does not provided give the bases for allocation of these genera from genus *Fritillaria*, but these data are coordinated with the opinion of other researchers (Baker, 1874, Rix, 1977, Rechinger, 1990) that suggest the distinguishing within genus *Fritillaria* some subgenus: *Fritillaria*, *Rhinopetalum*, *Theresia*, *Petilium*, *Korolkowia*, *Liliorhiza*.

Some species of *Fritillaria* using palynological data obtained with LM have been made (Zaklinskaya, 1950; Ikuse, 1956, Wang et al. 1960, Nair and Sharma (1965), Melikyan and Avakyan 1975, Schulze 1980, Tarasevich 1983). Several Caucasian species of *Rhinopetalum* (Melikyan and Avakyan, 1979) have been investigated with LM and SEM. Ryabkova (1987) investigated the morphology of pollens from *Fritillaria olgar* Vved., *Petilium eduardii* (Regel) Vved. and 4 species of *Rhinopetalum* Fisch. ex Alexand. and *Korolkowia sewerzowia* (Regel) Regel using LM and also *Fritillaria regelii* Losinsk. using SEM. The pollen characters of *Fritillaria* were studied in detail using LM and SEM (Kosenko, 1991, Kosenko, 1999). The main features of the pollen grains, for subgenus allocation are the type of ultrasculpture of a pollen grain and the structure of a sulcus membrane (Kosenko, 1991). Therefore, Kosenko (1991) and Ryabkova (1987) considered these taxons in a rank of separate genera. Pollen morphology of 27 species in genus *Fritillaria* from China were examined under LM and SEM (Ping et al., 1991). *Fritillaria michailovskyi* Fomin and *Fritillaria armena* Boiss. from Turkey using both SEM and TEM (Pehlivan & Özler, 2002), and also *Fritillaria caucasica* Adams using TEM (Meyer, 1975). These isolated TEM investigations do not allow to make some conclusions concerning a genus as a whole.

Although term of caput (expanded apex (head) of a columella) used by Edrtmen (1952) as an architectural element on top of a columella, its measurement as palynological character is not very consideration.

The aims of the current study are elucidating infra-genus positions of subgenus within *Fritillaria* and the systematic position of the genus by means of palynological data obtained by LM, SEM and TEM (height and width of caput thickness and the form, length and width of columella, foot layer thickness) in *Fritillaria* and also provide a correlation between taxonomy and detailed pollen-morphological characteristics.

### MATERIALS AND METHODS

Examined species of *Fritillaria* L. and their subgenus and synonymous (Kosenko, 1991) are given in Table 1. Pollen grains of 10 species were investigated (Table 2, 3). Pollens for this study were taken from the collections in the Main Botanical Garden Moscow (MHA), Russia; Komarov Botanical Institute of the Russian

Academy of Science, St. Petersburg (LE); Biological Faculty of Lomonosov Moscow State University (MW), Russia; Research Institute of Forests and Rangelands (RIFR), Tehran, Iran; Razi University, Kermanshah, Iran. In addition fresh material obtained from the Botanical Garden of MGU, Moscow, Russia.

Table 1- Examined species of *Fritillaria* L. and their subgenus and synonymous (Kosenko, 1991)

Subgenus	species	Synonyms
<i>Fritillaria</i>	<i>F. zagraca</i> Stapf.	
<i>Rhinopetalum</i> (Fisch.) Baker	<i>F. gibbosa</i> Bioss.	<i>Rhinopetalum gibbosum</i> (Boiss.) A. Les. Et Vved
<i>Rhinopetalum</i> (Fisch.) Baker	<i>F. stanathera</i> Regel	<i>Rhinopetalum stanatherum</i> Regel
<i>Theresia</i> (C. Koch) Baker	<i>F. persica</i> L.	
<i>Liliorhiza</i> (Kellog) Baker	<i>F. camschatcensis</i> (L.) Ker. Gawl	
<i>Petilium</i> (L.) Baker	<i>F. eduardii</i> Regel	<i>Petilium eduardii</i> (Regel) Vved.
<i>Petilium</i> (L.) Baker	<i>F. imperialis</i> L.	
<i>Korolkowia</i> (Regel) Baker	<i>F. sewerzowii</i> Regel	<i>Korolkowia sewerzowia</i> (Regel)

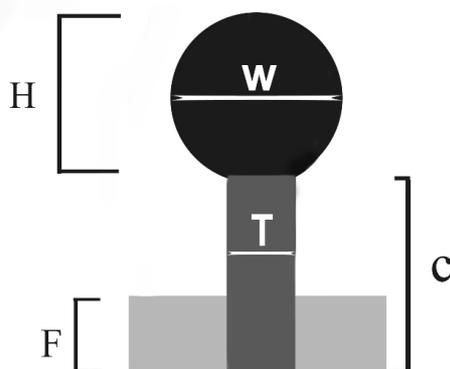


Fig. 1. Diagram of the ultrastructural exine of all pollen grain and measuring parts of them. Abbreviations: C = length of columella, F = foot layer thickness, H = height of caput, T = width of columella, W = width of caput.

For LM observations, pollens were acetolysed following the technique of the Erdtman (1960) and mounted in glycerine jelly. Slides were prepared for LM by mounting pollen in glycerin jelly. Size measurements were taken based on 25 pollen grains; the values of P (polar axis length) and E (equatorial diameter) were measured and the P/E ratios were calculated. Measurements were recorded using both a 40 × objective, and a crossed micrometer eyepiece graticule. The pollen data for all species examined are summarized in Table 2.

For SEM studies unacetolysed pollen grains were attached to aluminum stubs and sputter coated (Eiko IB-3 ION COATER) with Au/Pb and examined under Hitachi S-405 A scanning electronic microscope (Fig. 2–3) (Table 2).

Table 2- Pollen morphological parameters of the investigated *Fritillaria* species using LM and SEM *F. sewerzowii*\* from Russia, *F. sewerzowii*\*\* from kazakhstan., *F. sewerzowii*\*\*\* from Uzbekistan

Taxon	P (μm)	E (μm)	P/E	exine ornamentation	Width of muri (μm)	Lumina size (μm)
<i>F. zagraca</i>	27 – 37	43 – 55	0.6	reticulate	0.3 – 0.7	0.1 – 1.3
<i>F. gibbosa</i>	26 – 33	40 – 53	0.7	foveate-rugulate	0.2 – 0.5	0.05 – 0.2
<i>F. stanathera</i>	23 – 35	45 – 60	0.6	reticulate	0.4 – 1.1	0.2 – 1.4
<i>F. persica</i>	26 – 35	42 – 51	0.6	reticulate	0.2 – 0.9	0.1 – 1.2
<i>F. camschatcensis</i>	28 – 40	43 – 60	0.7	macroreticulate	0.7 – 1.3	0.3 – 4.4
<i>F. eduardii</i>	40 – 51	61 – 78	0.7	macroreticulate	0.3 – 1.3	0.2 – 3.0
<i>F. imperialis</i>	42 – 35	62 – 87	0.5	foveate-rugulate	0.3 – 0.7	0.1 – 0.4
<i>F. sewerzowii</i> *	28 – 38	38 – 58	0.7	pisilate-foveate or foveate-microreticulate		0.05 – 0.2
<i>F. sewerzowii</i> **	33 – 47	56 – 65	0.7	foveate-rugulate	0.1 – 0.6	0.05 – 0.1
<i>F. sewerzowii</i> ***	37 – 45	53 – 62	0.7	Rugulate-microreticulate or reticulate	0.3 – 0.7	0.1 – 0.7

For TEM pollen grains were fixed in 1% osmium tetroxide and stained with a solution of Uranyl-acetate in 70 % alcohol and lead citrate (Reynolds, 1963), then dehydrated in an ethanol series and embedded in Epon mixture (Epon 812, Epon Härter DDSA, Epon Härter MNA) according to the standard method of Weakley

(1977). Ultrathin sections of the pollen grains were obtained by a glass knife (LKB 8800 Ultratome III), and lead citrate ((Reynolds, 1963). Observations were made using a JEOL JEM\_100B transmission electronic microscope. All measurements on TEM micrographs have been made in standard vision in several pollen grains. Height and width of caput thickness and its form, length of columella (from under foot layer until down of caput) and width of columella, foot layer thickness in ultrastructural exine of all pollen grains in *Fritillaria* were measured (Fig. 1, 5) (Table 3), and also diagrams of exine were drawn with two caput of columella for every investigated species as Fig. 4.

Descriptive terminology follows Kremp (1967) and Punt et al., 2007

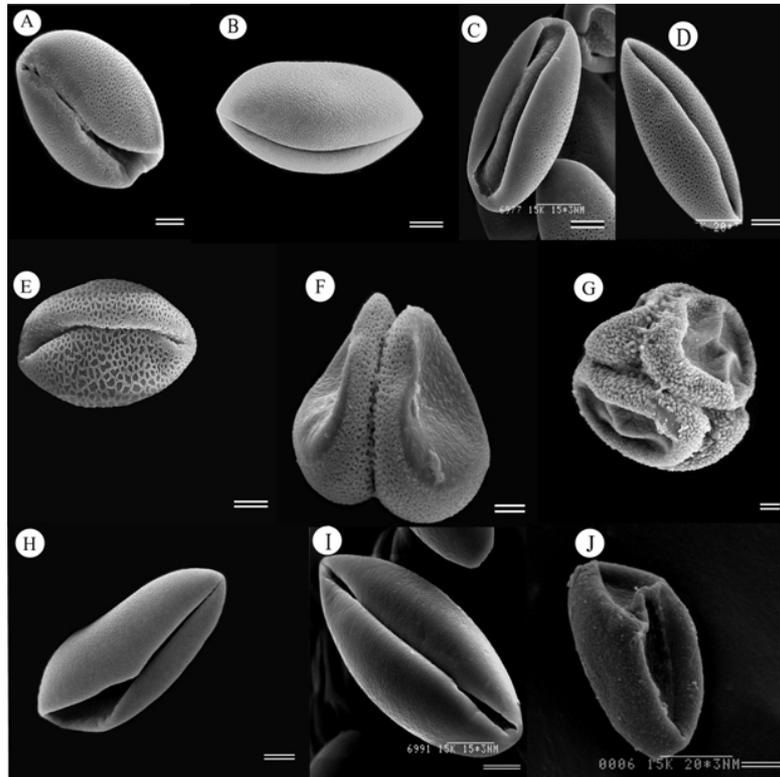


Fig. 2. Scanning electron micrographs of monosulcate pollen of *Fritillaria* in distal view: (A) *F. zagrica*, (B) *F. gibbosa*, (C) *F. stenantha*, (D) *F. persica*, (E) *F. camtschatscensis*, (F) *F. eduardii* in dyad form, (G) *F. eduardii* in tetrad form, (H) *F. imperialis*, (I) *F. sewerzowii* from Russia, (J) *F. sewerzowii* from kazakhstan. Scale bar: (B, E, F, G, J) = 10  $\mu\text{m}$ , (D, I) = 15  $\mu\text{m}$ , (A, C, H) = 20  $\mu\text{m}$ .

## RESULTS

Pollen grains used in this investigated are monosulcate, sulcus large, reaching end of grains, almost equal to equatorial diameter. The forms of pollen grains of all species used are basically similar i.e. oblate-spheroidal. Outline of pollen grains at equatorial position in all investigated species is oblate. The ratio P/E (The ratio of the length of the polar axis (P) to the equatorial diameter (E)) = 0/5 – 0/7 (Table 3). For all species single pollen grains are characteristic (Fig. 2 A-E, F-H), only pollen grain *F. eduardii* are shown collected in dyads (from Petersburg) (Fig. 2 F) or tetrad (from Moscow) (Fig. 2 G).

### Subgenus *Fritillaria*

#### *F. zagrica*

Exine surface is reticulate, the sulcus edge is tubercular and surface of sulcus membrane is occasionally granulate. Muri are continuous and 0.3-0.7  $\mu\text{m}$  wide. Lumina 0.1-1.3  $\mu\text{m}$  in diameter (Table 2, Fig. 2A, 3A ).

The sporoderm consists of tryphine, ectexine, a discontinuous and very thin endexine, intine. The ectexine consists of a tectum, columellae and foot layer. The form of caput is spheroidal (0.5  $\mu\text{m}$  in diameter) to elliptical. The surface of muri (Microrelief) is smooth (Table 3, Fig. 4A, 5A).

### Subgenus *Rhinopetalum*

#### *F. gibbosa*

Exine surface is similar in sculptural features to that of the sulcus edge, foveate-rugulate with the surface of the sulcus membrane striate-granulate. Muri are fractional, twisting with a diameter range of 0.2-0.5  $\mu\text{m}$ . Lumina are almost uniformly 0,05 – 0,2  $\mu\text{m}$  in diameter, rounded to elliptical or rounded-polygonal (Table 2, Fig. 2B, 3B).

The sporoderm consists of tryphine, ectexine, a discontinuous and very thin endexine, intine. The ectexine consists of a tectum, columellae and foot layer. The form of caput of columella is elliptical (oblate-spheroidal) with a length of 0.7  $\mu\text{m}$  and width of 0.5  $\mu\text{m}$ . The surface of the muri (microrelief) is smooth (Table 3, Fig. 4B, 5B).

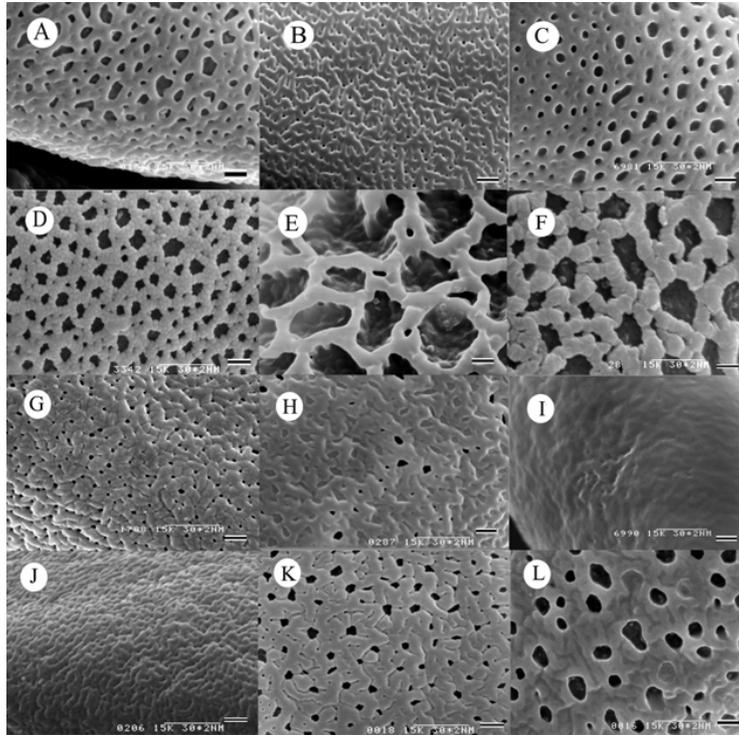


Fig. 3. Scanning electron micrographs of exine ornamentation of pollen in *Fritillaria*: (A) *F. zagrica*. Reticulate exine; (B) *F. gibbosa*. Foveate-rugulate exine; (C) *F. stanathera*. Reticulate exine; (D) *F. persica*. Reticulate exine; (E) *F. camschatcensis*. Macroreticulate exine; (F) *F. Eduardii*. Macroreticulate exine; (G) *F. Imperialis*. Foveate-rugulate exine; (H) *F. sewerzowii* from Russia. Foveate-microreticulate exine; (I) *F. sewerzowii* from Russia. Psilate-foveate exine; (J) *F. sewerzowii* from kazakhstan. Foveate-rugulate exine; (K) *F. Sewerzowii* from Uzbekistan. Rugulate-microreticulate exine. (L) *F. Sewerzowii* from Uzbekistan. Reticulate exine. Scale bar = 1  $\mu\text{m}$ .

*F. stanathera*

Exine surface reticulate, with the sulcus edge being foveate and surface of sulcus membrane macrotuberculate-plicate. Muri solid with 0.4 – 1.1  $\mu\text{m}$  wide. The Lumina are 0.2 – 1.4  $\mu\text{m}$  in diameter (Table 2, Fig. 2C, 3C).

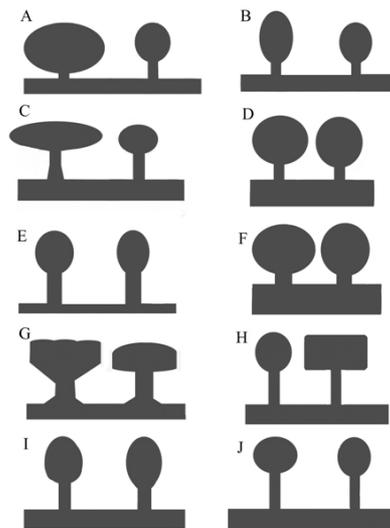


Fig. 4. Diagrams of ultrathin section in exine of the pollen grains in *Fritillaria*: (A) *F. zagrica*; (B) *F. gibbosa*; (C) *F. stanathera*; (D) *F. persica*; (E) *F. camschatcensis*; (F) *F. Eduardii* from Petersburg; (G) *F. Imperialis*; (H) *F. sewerzowii* from Russia; (I) *F. sewerzowii* from kazakhstan; (J) *F. Sewerzowii* from Uzbekistan.

The sporoderm consists of tryphine, ectexine, a discontinuous and very thin endexine, and intine. Tryphine is located both outside of the columellae and between them in two layers: internal fine-grained; external electronically more transparent and homogeneous.

The ectexine consists of a tectum, columellae and foot layer. The caput form of columella is flattened – spheroidal (height 0.3  $\mu\text{m}$ , width 0.4  $\mu\text{m}$ ). The surface of the muri (microrelief) is smooth. Endexine is very thin and discontinuous, formed by separate electron dense bar (Table 3, Fig. 4C, 5C).

Subgenus *Theresia*

*F. persica*

Exine surface reticulate, with the sulcus edge microreticulate and surface of sulcus membrane is uniformly microgranulated. Muri are fractional with a diameter range of 0.2 – 0.9  $\mu\text{m}$ . Lumina is 0.1-1.2  $\mu\text{m}$  in diameter (Table 2, Fig. 2D, 3D ).

The sporoderm consists of tryphine, ectexine, a discontinuous and very thin endexine, and intine. Ectexine consists of a tectum, columellae, foot layer. The form of caput of columella is ellipsoidal or spheroidal (0.9-1.1  $\mu\text{m}$  diameter). The surface of the muri (microrelief) is irregular, tubercular (Table 3, Fig. 4D, 5D).

Subgenus *Liliorhiza*

*F. camschatcensis*

Exine surface is macroreticulate, with the sulcus edge is tuberculate and surface of the sulcus membrane is macrotuberculate-plicate. Muri are continuous and 0.7 – 1.3  $\mu\text{m}$  wide. Lumina are 0.3 – 4.4  $\mu\text{m}$  in diameter and variable in size (0.3 – 4.4  $\mu\text{m}$  in diameter) (Table 2, Fig. 2E, 3E).

The sporoderm consists of tryphine, ectexine, a discontinuous and very thin endexine, and intine. Ectexine consists of tectum, columellae and foot layer. The form of columella caput is spheroidal or flattened – spheroidal (length 0.5 – 0.6  $\mu\text{m}$ ; width 0.6 – 0.8  $\mu\text{m}$ ). The surface of the muri (microrelief) is smooth (Table 3, Fig. 4E, 5E).

Subgenus *Petilium*

*F. eduardii* Regel

Exine surface macroreticulate with the sulcus edge is microreticulate and surface of sulcus membrane is unequally macrotuberculate. Muri are fractional 0.3-1.3  $\mu\text{m}$  wide. Lumina are highly variable in size (0.2 – 3.0  $\mu\text{m}$  in diameter) (Table 2, Fig. 2F, 2G, 3F).

The sporoderm consists of thick electronic dense tryphine located outside of columellae, ectexine, a discontinuous and very thin endexine, and intine.

Ectexine consists of a tectum, columellae, foot layer. The form of columella caput is spheroidal or flattened – spheroidal (length 0.7 – 1.0  $\mu\text{m}$ ; width 0.7–1.4  $\mu\text{m}$ ). The surface of muri (Microrelief) is smooth (Table 3, Fig. 4F, 5F).

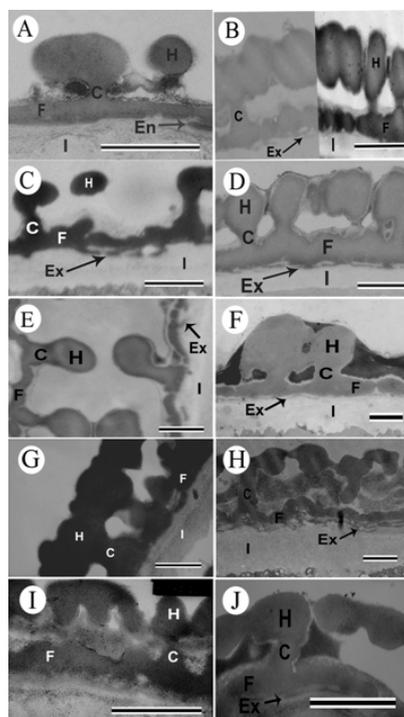


Fig. 5. Ultrathin section of the pollen grains in *Fritillaria*: (A) *F. zagrica*; (B) *F. gibbosa*; (C) *F. stenantha*; (D) *F. persica*; (E) *F. camschatcensis*; (F) *F. eduardii* from Petersburg; (G) *F. Imperialis*; (H) *F. sewerzowii* from Russia. lamellarous endexine; (I) *F. sewerzowii* from kazakhstan; (J) *F. sewerzowii* from Uzbekistan. discontinuous endexine. (C) = columellae, (H) = caput, (Ex) = endexine, (F) = foot layer; (I) = intine. Scale bar = 1  $\mu\text{m}$ .

*F. imperialis*

Exine surface is foveate-rugulate, with sulcus edge is granulate and surface of the sulcus membrane irregularly granulate. Muri are fractional with 0.3 – 0.7 µm wide. Lumina show little variation in size (0.1-0.4 µm in diameter) (Table 2, Fig. 2H, 3G).

The sporoderm consists of tryphine, ectexine, a discontinuous and very thin endexine, and intine. Ectexine consists of tectum, columellae and foot layer. The form of columella caput is spheroidal to irregular and wide-rectangular or indeterminate (length 0.6 – 1.0 µm; width 0.7 – 1.2). The surface of the muri (microrelief) is almost smooth (Table 3, Fig. 4G, 5G).

Subgenus *Korolkowia**F. sewerzowii*\* (from Russia)

Exine surface is pislite-foveate or foveate-microreticulate, with the sulcus edge being foveate and surface of sulcus membrane macrotuberculate. Muri are solid with a diameter range of 0.1 – 0.7 µm. Lumina are 0.1 – 0.7 µm in diameter (Table 2, Fig 2 I, 3H, 3I).

The sporoderm consists of tryphine, ectexine, a discontinuous and very thin or lamellarous endexine, intine. Ectexine consists of a tectum, columellae, foot layer. The form of columella caput is ellipsoidal or spheroidal to irregular and rectangular or indeterminate length 0.4 - 0.5 µm; width 0.3 - 0.6 µm). The surface of the muri (microrelief) is almost smooth (Table 3, Fig. 4H, 5H).

*F. sewerzowii* \*\* (from Kazakhstan)

Exine surface is foveate-rugulate, with the sulcus edge is foveate and surface of sulcus membrane macrotuberculate-plicate. Muri are solid with a diameter range of 0.1 – 0.6 µm. Lumina are almost uniformly 0.1 µm in diameter (Table 2, Fig 2J, 3J).

The sporoderm consists of tryphine, ectexine, and intine. Ectexine consists of a tectum, columellae, foot layer. Endexine is very slight and is not conspicuous. The caput of each columellae differs in form (spheroidal or flattened – spheroidal or elliptical or pyramidal to irregularly rectangular or indeterminate) (length 0.4 - 0.5 µm; width 0.3 - 0.4 µm). The surface of the muri (microrelief) is almost smooth (Table 3, Fig.4I, 5I).

*F. sewerzowii* \*\*\* (from Uzbekistan)

Exine surface is rugulate-microreticulate or reticulate, with the sulcus edge is foveate and surface of sulcus membrane is macrotuberculate. Muri are irregular and fractional and also variable in diameter size (0.1 to 2.6 µm). Lumina is 0.1 – 1.6 µm in diameter (Table 2, Fig.3K, 3L).

The sporoderm consists of tryphine, ectexine, a discontinuous and very thin or lamellarous endexine, intine. Ectexine consists of a tectum, columellae, foot layer. The form of columella caput is spheroidal to flattened (length 0.4 - 0.5 µm, width 0.6 - 0.8 µm). The surface of the muri (microrelief) is almost smooth (Table3, Fig 4J, 5J).

Table 3- Measurements of sporoderm in *Fritillaria* species investigated using TEM

*F. sewerzowii*\* from Russia, *F. sewerzowii*\*\* from kazakhstan., *F. sewerzowii*\*\*\* from Uzbekistan.

Abbreviations: P = polar axis length; E = equatorial axis length; O = exine ornamentation: re = reticulate; fo-ru = foveat –rugulate; ma-r = macroreticulate; mc-r = microreticulate.

Taxon	Exine thickness (µm)	Caput thickness (height; width) (µm)	Length of columella (µm)	Width of columella (µm)	Foot layer thickness (µm)
<i>F. zagrica</i>	0.9 – 1.1	0.4 – 0.5; 0.4 – 0.7	0.4 – 0.5	0.2 – 0.3	0.2 – 0.3
<i>F. gibbosa</i>	1.5 – 1.7	0.7 – 0.9; 0.5 – 0.6	0.6 – 0.8	0.2 – 0.3	0.3 – 0.4
<i>F. stenanthera</i>	1.0 – 1.2	0.3 – 0.4; 0.5 – 0.9	0.7 – 0.9	0.3 – 0.4	0.2 – 0.3
<i>F. persica</i>	1.9 – 2.1	0.9 – 1.1; 0.9 – 1.1	0.8 – 1.0	0.3 – 0.4	0.5 – 0.7
<i>F. camschatcensis</i>	1.1 – 1.4	0.5 – 0.6; 0.6 – 0.8	0.4 – 0.5	0.3 – 0.5	0.1 – 0.2
<i>F. eduardii</i>	1.6 – 2.3	0.7 – 1.0; 0.7 – 1.4	0.8 – 1.0	0.3 – 0.5	0.5 – 0.8
<i>F. imperialis</i>	1.3 – 2.7	0.6 – 1.0; 0.7 – 1.2	0.6 – 1.0	0.3 – 0.5	0.4 – 0.5
<i>F. sewerzowii</i> *	0.9 – 1.2	0.4 – 0.5; 0.3 – 0.6	0.5 – 0.6	0.3 – 0.4	0.2 – 0.4
<i>F. sewerzowii</i> **	0.9 – 1.3	0.4 – 0.5; 0.3 – 0.4	0.5 – 0.6	0.2 – 0.3	0.2 – 0.3
<i>F. sewerzowii</i> ***	1.0 – 1.6	0.4 – 0.5; 0.6 – 0.8	0.7 – 0.9	0.3 – 0.4	0.2 – 0.3

## DISCUSSION

Structure of pollen grains of *Fritillaria* is very heterogeneous. The pollen of *F. eduardii* in different populations is in tetrads or dyads forms as also indicated by Kosenko (1991). Radulescu (1973) and Tarasevich (1983) using light microscopy have described exine surface in *Fritillaria imperialis* as rugulate, but our study as well as that of Kosenko (1991) have established a pislite-foveate exine for this species.

Pollen grains of the species investigated are large and variable in size. In our data the sizes of *F. eduardii* and *F. Sewerzowii* pollen grains are much less specified (Kosenko 1991). However the

representative subgenus *Petilium*, possess larger pollen grains, than other species of *Fritillaria*. Exine ornamentation of the pollen grains in studied species is reticulate to reticulate-rugulate.

The sculpture of surface of pollen grains from the specimen collected from different regions differs in the form and structure of the exine surface. For example, structures of the muri in *F. camschatcensis* from several habitats are very twisted and is different the specimen of the same species collected from others habitats ([Kosenko, 1991], [Kosenko, 1999]) (Fig. 3E).

In addition, *F. sewerzowii* is a heterogeneous species. Ryabkova (1987) considered exine surface of pollen grains in this species as microreticulate, also Kosenko (1991) has noted the presence of microreticulate exine in specimens from four different locations. We have established the presence of microreticulate exine in samples from two places (the sample cultivated in Moscow in the Botanical Garden of the Moscow State University and Uzbekistan), and also the intermediate ornamentation from psilate-foveate until rugulate-microreticulate were observed. In addition to the mentioned cases, samples of pollen from this plant in Kazakhstan has a foveate-rugulate exine. Besides, it was noted by us for the first time that the surface of the muri (microrelief) *F. persica* is irregular, tubercular. Among all investigated species, the representative pollens have different sculpture formation. Only the subgenus *Theresia* possess a unique sculpture.

According to Nair and Sharma (1965), the exine ornamentation of pollen grains of 26 species taxed in Sect. *Fritillaria* and *Liliorhiza* belong to transitive from zono-reticulate type to pan-reticulate one, but that of *F. Karelinii* Baker in Sect. *Theresia* belongs to psilate type and the pollen of *F. Karelinii* may be regarded as a type more advanced than others.

Ultrastructure of pollen grains of the investigated species revealed a classical well advanced monocotyledon columella of ectexine with a well expressed on a cut caput of columella; inconspicuous feebly marked or absent endexine and thick stratified intine.

Walker (1974) has shown 4 such structure tectums in angiosperm: tectate-imperforate, tectate-perforate, semitectate and inectate. Nilsson and Praglowski (1992) have offered the name semitectate for dividing tectum into sites. Thomas (1980) has shown various type of tectums on cuts and has illustrated free elements in tectum representatives of family *Annonaceae* which we used in this study as columella with caput. Baranova (1985) described three morphological types on the basis of the number, shape and arrangement of the types of columella forming the muri: 1. muri formed by rectangular columellae; 2. muri formed by rounded columella; 3. muri formed by separated rounded and polygonal columellae. Kosenko (1996) has shown different free elements in order *Liliales* Lindley. We have found out in the investigated species semitectate, that will be coordinated to data Pehlivan and Özler (2002) for two Turkish *Fritillaria*. We have revealed the minimal thickness foot layer in the pollen grain *F. camschatcensis* and the elliptical (oblate-spheroidal) caput form in columella *F. gibbosa* differs from the caputs (spheroidal to indeterminate) of columellae in all of the other studied species.

As a result of this investigation, unique endexine structure has been revealed in *F. stenantha* (Subgenus *Rhinopetalum*) which differs from that of other species of *Fritillaria* by the presence of a separate electron dense bar.

Distinctions in the various stage of development endexine at pollen grains from different populations *F. Sewerzowii* from discontinuous and very thin to lamellarous endexine. The exine structures in our study do not coincide with data of Simpson (1985) which has noted the presence of lamellate inner foot layer at *Philydrella pygmaea* Caruel (*Philydraceae*). Other authors have shown that, the presence and structure of endexine in different genera Liliaceae: *N. thomsonianum* (Royle) Stapf., *Nomocharis pardanthina* Franch., *Cordiocrinum cardatum* (Thunb.) Makino. and several *Lilium* endexine continuous and sufficient thickness, and in *Notholirion hyacinthinum* (E.H.Wilson) Stapf. discontinuous and thin ((Maassoumi, 2004a), (Maassoumi, 2004b), (Maassoumi, 2004c)). In *Erythronium* (Takahashi, 1987), *Amana* (Maassoumi, 2004d), *Gagea* (Maassoumi, 2005) and *Tulipa* (Harly, 2003) no endexine exists.

## Conclusions

The analysis of the results obtained show, that endexine in the investigated species of *Fritillaria* is very thin or absolutely non-existent.

The form and diameter of columella caput can be used as a good pollen-morphological characteristics for semitectate type of tectum.

Attributes of ultrastructure have not shown any correlation with subdivision into subgenera *Fritillaria*. However still there is not enough data from TEM for final conclusions.

The available Palynological data provide evidence for the heterogeneous character of the genus *Korolkowia* with respect to the exine ornamentation.

## Appendix

### Specimen examined

The following specimens were included in the study:

*Fritillaria.camschatcensis* (L). Ker. Gawl JAPAN: Hokkaido, Kiritoppu, Hammanaka-cho, Atscukeshi-gumgune, 19 Jun. 1986, N. 36281. *Fritillaria. eduardii* Regel.: RUSSIA, The Botanical garden of the Komarov Institute botanica, 20 April 1884, R. Kamelin. *Fritillaria imperialis* L. IRAN: Kermanshah, 10 May 2002, S.M. Maassoumi, N. 2422 (RUH). *Fritillaria gibbosa* Boiss. IRAN: Tehran, 30.March. 1975, R.Vendelbo. *Fritillaria persica* L.: IRAN, Loristan, Narabad, 3 May.1975, Vendelbo and Asadi. *Fritillaria sewerzowii* Regel.: KAZAKHSTAN, Jambolcki area, mountain range of Karatao. 4 May 1984, I.Rusanovich, A.Shvetsov. *Fritillaria sewerzowii* Regel. RUSSIA: Moscow, The Botanical garden of the Lomonosov Moscow State University(MGU), 30 April 2002, E.V.Kljukov. *Fritillaria sewerzowii* Regel. UZBEKISTAN: Chatkal., 11 April 1975, Z.Alfereva, S. Udinitceva. *Fritillaria stenantha* Regel.: RUSSIA: Moscow, The Botanical garden of MGU 30 April 2002, E.V. Kljukov. *Fritillaria zagrica* Stapf.: IRAN: Khorasan, 26 March 1997, S.M. Maassoumi, N. 2423 (RUH).

#### Footnotes

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