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Pollen morphology of *Calochortus* Pursh and its systematic position in the Liliaceae family

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Abstract

Pollen grain of eight species belonging to *Calochortus* were taken from the Herbarium in Komarov Botanical Institute of the Russian Academy of Science (RAN) and were studied by Light Microscopy (LM) and Scanning Electron Microscopy (SEM). Pollen grains of all the studied species were single, heteropolar, bilaterally symmetrical, distal-monosulcate, flattened-spheroidal to oblate, in outline with a pole rounded-elliptical, with the equator - flattened. Surface ornamentation of pollen in this species Corrugated - punching but in *C. albus* is punching - Corrugated. Palynomorphological data showed that in Comparasion with other genera of Liliaceae, *Calochortus* is similar to some of species in *Tulipa* and *Fritillaria*. Cluster analysis revealed two main groups in the studied species. Therefore, present research has aided the Liliaceae family can be divided into 3 tribes as: Tulipeae, Calochortae, Lloydieae.

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Introduction

Calochortus genus first sex by Frederick Pursh (1814) classified and described in the first volume of plants in North America were described (Ownbey 1940). The genus can be divided into three sections and 12 subsections. In terms of taxonomic Melchior (1964) this item Calochortaceae the tribe and others in the tribe were Tulipeae (Hutchinson 1973, Elwan 2008). More over Govaerts *et al* (2011) listed 73 species of this genus in the Czech list Botanical Gardens Kew mention and their distribution in North America. Systematics of the genus belongs to the Liliaceae family. Its family, with 22 genera and 485 species in the world, in order Liliales, Liliidae seeds and seeds are Liliopsida (e.g., Zomlefer 1994). The classification system also phylogeny angiosperms (APG III) attributed the family as to the true Liliales (e.g., Chase and Reveal 2009). Numerous studies of chromosome features (Ness 1989), Anatomy and Embryology (Dahlgren *et al.*1985, Takhtajan, 1986, Weber and Wittmann, 2001, Watson and Dallwitz 1992) has described many of the features Calochortaceae family. Geographic, Chromosomal evolution and molecular studies of many different species of *Calochortus* by Patterson and Givnish (2003) was investigated. In addition, Elwan (2008) Morphology of leaves and seeds of 20 species of the genera of Liliaceae family (Liliales) including *Calochortus* the electron microscope electron (SEM) study and found that of the epidermis and cuticle of leaves and seeds of species of important The classification of these plants. By Kosenco (1987) using light and electron microscope (SEM) were studied in 27 species of the genus *Calochortus*. His research showed that most is gully single *Calochortus* species, but the species *C. kennedyi* porter of different types of valves and two single holes or gully is. *Calochortus* level has 4 types of decorations exin (grid, corrugated, punching, punching a button) is. The purpose of this study was pollen morphology of Liliaceae genus and classified communications in a Family place .

Materials and method

Pollen of eight species of the genus *Kamarof* Herbarium Botanical Institute of Russian Academy of Sciences (RAN) (Tab.1).

For LM observations

pollen was 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 ratio was calculated. (Figure 1 and Tab. 2).

For SEM studies

For samples with a scanning electron microscope (SEM) Not acetolysed pollen into the metal block (sample stub) and then transferred to the sputter coated with gold (Sputter coater BAL-TEC model SCDOOS manufacturing companies of Switzerland), the blocks covered with deposits of gold or platinum and transferred electron microscope to see them. The microscope scanning electron (SEM) model LEO 440i build England and model Hitachi S-405 A made in Japan micrograph electron enlarge 10000-1000 for procurement to help detail level exin such as the type of surface ornament, wall thickness Network The net-like wall distance networks from each other, and the pore structure were evaluated (Figure 2,3 and Tab.3).

Results

For LM observations

pollen of the Liliales order divided into binary (Dyad) and four (Tetrad). For example, *Fritillaria eduardi* Regel.Vved. Pollen is seen as a binary or quaternary Kosenco, (1991a, 1991b,1990, 1999), or the genus *Lilium candidum* L. A binary or (Dyad) is (Masoumi 2012), but pollen grains are individual species of *Calochortus*. In terms of size, the pollen of different species (42/2 -26/1 micrometers)(tab.2, Figure 1). pollen grains of all the studied species are single, heteropolar, bilaterally symmetrical, distal-monosulcate, flattened-spheroidal to oblate, in outline with a pole rounded-elliptical, with the equator – flattened (Tab.2).

For SEM studies

The smallest pollen grains of *C. albus* Douglas ex Benth and greatest *gunnisonii* S.Watson to be. Exine ornamentation in *Calochortus* is Corrugated-punching but in *C.albus* Douglas ex Benth is Punching –Corrugated (Tab.3 and Figure 2,3). In order evolution (Liliales) using pollen data of Maasoumi (2005) and Kosenco (1996) have been observed in surface decoration of the decorative

surface is Interlace earlier, as for example in *Lilium* surface ornamentation. For example, the walls of sample *C. albus* connected mesh networks, while the walls of the networks, the sample is separated by Kosenco wall thickness as well as in *C. elegans* Pursh mesh networks larger than the thickness reported by Kosenco (1987) for the species. Moreover, the parsimonious *Calochortus* using Patterson and Givnis (2003), as monophyletic is introduced. The results of pollen diversity of the species in this genus is discussed.(Figure 4).

Table 1. Position classification (derived from Ownbey, 1940) and location of species collected.

Section	subsection	species	location
Calochortus	Eleganti	<i>C. apiculatus</i> Baker	Missoula, Montana, A. Cronquist, 19/jun/1960
“	“	<i>C. coeruleus</i> S.Watson	California,Wosnessensky, 3/1841
“	“	<i>C. elegans</i> Pursh	Florida, T. J. Howell, 5/Agust/1881
“	Nitidus	<i>C. eurycarpus</i> S.Watson	Oregon, A. Cronquist, 6/jun/1953
“	“	<i>C. greenei</i> S.Watson	Washington, T. J. Howell, jun/1881
“	puchelli	<i>C. albus</i> Douglas exBenth	Mariposa,CA, H. B. Ertter, 23/April/1989
Mariposa	Gunnisoniani	<i>C. gunnisoni</i> S.Watson	Wyoming, C. L. Porter, 22/july/1969
“	venusti	<i>C. venustus</i> Douglas ex Benth.	California, M. Dayid, 23/May/1960

Table 2. Measurement of pollen wall features using an optical microscope.

Taxon	pollen size (µm)	E	P	P/E
<i>C. apiculatus</i> Baker	38.5±4.8	32.5-38.5-42.5	27.5-29.7-32.5	0.63
<i>C. coeruleus</i> S.Watson	27.9±3.2	22.0-27.9-32.6	15.3-18.7-20.2	0.63
<i>C. elegans</i> Pursh	38.0±3.2	33.8-38.0-42.5	20.0-21.5-25.0	0.57
<i>C. eurycarpus</i> S.Watson	37.7±3.4	34.4-37.7-39.5	18.3-22.9-24.5	0.61
<i>C. greenei</i> S.Watson	34.2±7.2	31.5-34.9-42.5	22.4-25.0-27.5	0.64
<i>C. albus</i> Douglas exBenth.	26.2±2.6	22.0-27.9-32.6	15.5-17.8-20.2	0.66
<i>C. gunnisoni</i> S.Watson	42.2±3.5	39.5-42.2-47.1	22.5-27.4-32.0	0.64
<i>C. venustus</i> Douglas ex	39.8±2.4	37.5-39.8-42.5	25.0-27.6-35.0	0.69

Table 3. Measurement of pollen wall detail using electron scanning electron microscope (SEM).

Taxon	Exineornamentation	Width of muri (µm)	Lumina size (µm)
<i>C. apiculatus</i> Baker	Corrugated-punching	0.3-0.7	0.3-0.4
<i>C. coeruleus</i> S.Watson	Corrugated-punching	0.2-0.6	0.1-0.3
<i>C. elegans</i> Pursh	Corrugated-punching	0.2-0.4	0.1-0.2
<i>C. eurycarpus</i> S.Watson	Corrugated-punching	0.2-0.8	0.3-0.6
<i>C. greenei</i> S.Watson	Corrugated-punching	0.2-0.6	0.3-0.8
<i>C. albus</i> Douglas exBenth.	punching -Corrugated	0.1-0.3	0.1-0.4
<i>C. gunnisoni</i> S.Watson	Corrugated-punching	0.2-0.5	0.2-0.8
<i>C. venustus</i> Douglas ex	Corrugated-punching	0.3-0.8	0.1-0.6

Discussion

Classification Tkthjan (1995) The Liliaceae family, sex Halongia Liliales-h family is one of the genera.

Maassoumi (2005) Pollen morphology of the genus by having a large lattice decorated with irregular wall and valve status, basic material presented in this

family. Tkhtjan also believe that sex is one of the ancestors of plants Liliales family Liliaceae in order. The evolutionary path of pollen in the genus *Lilium* we Halongia Liliaceae family after sex. The decorations made of coarse mesh grid is up, endexin a continuous layer of pollen grains is seen in this genus. *Lilium* is a genus of four evolutionary path. *Calochortus* genus by having smaller pollen of other genera and single mode sulcate punching the corrugated surface decoration could be close to *Gagea*. Also Elwan (2008) using UPGMA clustering on morphological characteristics of leaf Liliaceae family genera 20 species, genus *Calochortus* and *Gagea* two put together.

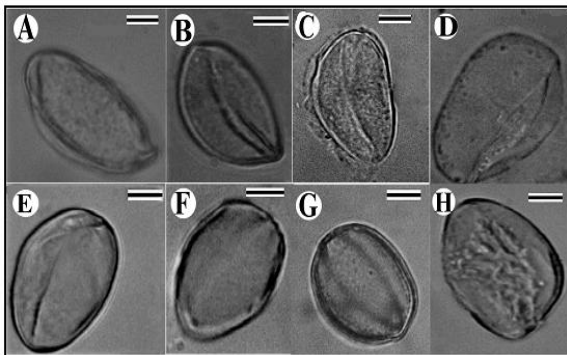


Fig. 1. Illustration of a light microscope (LM) of the overall shape of the pollen species:

(A) *C. apiculatus* Baker, (B) *C. coeruleus* S. Watson, (C) *C. elegans* Pursh, (D) *C. eurycarpus* S. Watson, (E) *C. greenei* S. Watson, (F) *C. albus* Douglas ex Benth., (G) *C. gunnisoni* S. Watson, (H) *C. venustus* Douglas ex Benth.

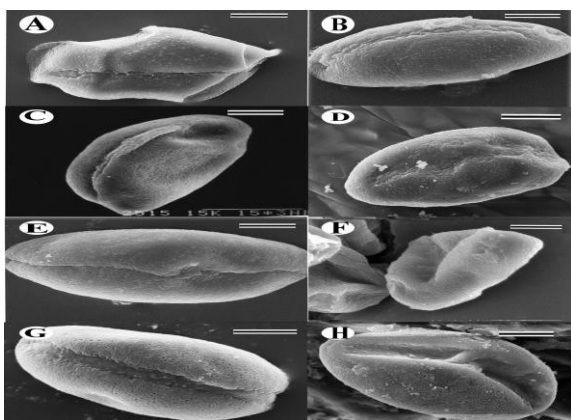


Fig. 2. Scanning electron microscope images (SEM) of the total pollen: 6(μm).

A) *C. apiculatus* Baker, B) *C. coeruleus* S. Watson, C) *C. elegans* Pursh, D) *C. eurycarpus* S. Watson, E) *C.*

greenei S. Watson, F) *C. albus* Douglas ex Benth., G) *C. gunnisoni* S. Watson, H) *C. venustus* Douglas ex Benth.

Danhgrdh morphology section of *Calochortus* Mariposa sector is very diverse. So in *Calochortus*, of *C. albus* of subsection called Puchelli, in a separate species, The subdivision is *C. eurycarpus* S. Watson Nitidus. Also *C. albus* and *C. coeruleus* S. Watson parts in this section is quite distinct from other species. Whereas in Mariposa species *C. gunnisoni* S. Watson of subsection *C. Gunnisoniani* and *C. venustus* Douglas ex Benth. Venusti in the subdivision are adjacent to each other.

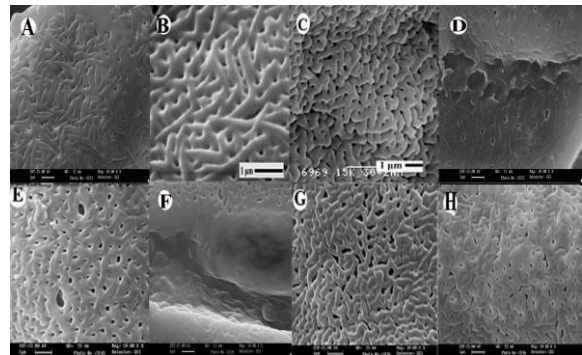


Fig. 3. Detail of pollen wall surface decoration using (SEM). 10(μm) A) *C. coeruleus* S. Watson, B) *C. albus* Douglas ex Benth., C) *C. elegans* Pursh, D) *C. apiculatus* Baker, E) *C. greenei* S. Watson, F) *C. venustus* Douglas ex Benth., G) *C. gunnisoni* S. Watson, H) *C. eurycarpus*.

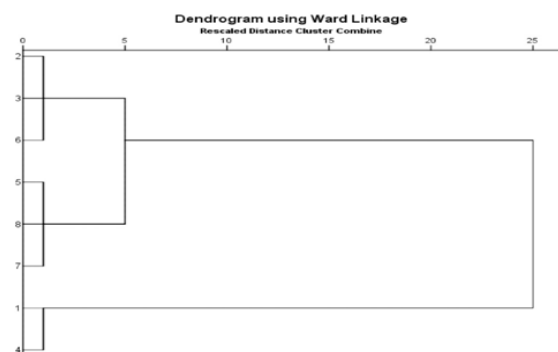


Fig. 4. Dendrogram of cluster analysis of the data using optical and electron.

1) *C. albus* 2) *C. elegans* 3) *C. eurycarpus* 4) *C. coeruleus* 5) *C. apiculatus* 6) *C. Greenei* 7) *C. gunnisoni* 8) *C. venustus* Hh.

Palynomorphological data showed that in Comparison with other genera of Liliaceae, *Calochortus* is similar to some of species in *Tulipa* and *Fritillaria*. Cluster analysis revealed two main groups in the studied species. the analysis of the pollen of species, including Liliaceae family is divided into 3 tribes: Tulipeae, Calochortae, Lloydieae.

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