Pollen grain ultrastructure comparative of two species belonging to *Calochortus* Pursh (Liliaceae)

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**Abstract**

Pollen morphology of two genera of *Calochortus* was studied under transmission electron microscopy (TEM). The shape of caput in *Calochortus* is oblate spheroidal, flattened-spheroidal, rectangular that similar to caput of columella in other genera in Liliaceae. Absent endexine of *Calochortus* is advanced features in Liliaceae order, as well as monocotyledon plants. Also *Calochortus* has three layers intine, which consists of exintine, mesintine and endexine. The microreliefs on the surface of the muri in studied species *Calochortus* are smooth. The tectum to foot layer ratio (T/F) in studied species *Calochortus* is 1.6–2.6. This species was different from each other by shape and thickness of caput, length and width of columella and T/F ratio. The minimal thickness foot layer was in the pollen grain *C. albus* Douglas ex Benth. The elliptical (oblate-spheroidal) caput form is in *C. eurycarpus* Wats, but from the caput in *C. albus* is spheroidal to indeterminate. In additional to sporoderm feature in *Calochortus* was similar to some species of genera *Tulipa* and *Fritillaria*.

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Introduction

The works are previously done including Fritsche (1837) introduced exine and intine as two major layers of pollen grain. Also exine was divided into inner and outer layers (Fritsche, 1837). Praglowski and Raj (1979) described sculpture and structure of pollen grain (Punt et al., 2001) For the first time, these two words were separated by Potonia (1934). Despite the differences between the word "sculpture" and "structure", they have used similarly in many researches. Also Faegri and Iversen (1975) expressed their investigation of two terms separately (Faegri et al., 1975). Nilsson and Praglowski (1992) and Walker (1974) found types of tectum structure of pollen grain in angiosperms (Walker, 1974, Nilsson et al., 2002). In Liliales order, pollen grains are different. These differences include type of aperture, shape and exine ornamentation of pollen grain (Erdtman, 1952, Kosenko, 1992, Kuprianova, 1983, Maassoumi, 2005). Several studies have been made on different genera of this order.


Thus, evaluation of light microscopy and SEM of two species in Calochortus did not much differ from each other. What still needs to be investigated using of TEM that will indicate differentiations. The aim of current study was compared of pollen wall ultrastructure using TEM carefully.

Materials and methods

Plant material

Pollen grains of two species were taken from Komarov Botanical Institute of the Russian Academy of Science, St. Peters burg (LE), and Russia. Pollen grains for TEM were fixed in 1 % osmium tetroxide and stained with a solution of Uranylacetate in 70 % alcohol and lead citrate (Reynalds, 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 (Weakly, 1977). Ultrathin sections of the pollen grains were obtained by a glass knife (LKB 8800 Ultratome III) and lead citrate (Reynalds, 1963). Observations were made using a JEOL JEM_100B transmission electron microscope.

For TEM studies

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
Calochortus were measured (Tab 1, Fig. 1-2) and also diagrams of caput of columella were drawn for every investigated species as Fig. 3. Descriptive terminology follows Kremp (1967) and Punt *et al.* (2007) (Kremp, 1967, Punt *et al.*, 2007).

**Results**
Transmission electron micrographs of Sporoderm in pollen of investigated species of *C. Albus* and *C. eurycarpus* S indicates parameters.

The parameters are studied including: (surface of the muri, exine thickness, tectum thickness, form of caput, caput thickness, Length of columella, width of columella, foot layer thickness, none present of endexine, T/F ratio, intine thickness in region of sulcus, exintine thickness, mesintine thickness, endintine thickness).

Ectexine in *C. albus* Douglas ex Benth (With 0.42-0.59 μm thickness) and *C. eurycarpus* S. Watson (with 0.46-0.59 μm thickness) include Tectum (with 0.7-0.9 μm thickness), columellae and foot layer (Fig. 1 and 2). The shape of caput in *C. albus* is oblate spheroidal, rectangular flattened-spheroidal with 0.33-0.46μm width and in *C. eurycarpus* is Spheroidal, Oblate spheroidal and rectangular with 0.26-0.4μm width. In studied species, the microreliefs on the surface of the muri are smooth (Tab. 1; Fig. 1C; 2C) and the thickness in the sulcus zone is equal to 4.4 μm (Fig. 2B and 2C). Intine is composed of three layers, including: (i) Exintine, which is a layer in *C. albus* 0.10–0.20 μm and in *C. eurycarpus* 0.10–0.16 μm thickness with high electron density and a lot of channels inside; (ii) Mesintine, a layer with 0.33–0.53μm and 0.20–0.26 μm thickness (in *C. albus* and *C. eurycarpus* respectively) with low electron density and (iii) Endintine, which is a layer with 0.05–0.10 μm and 0.04–0.10 μm thickness (in *C. albus* and *C. eurycarpus* respectively) with a more electron density (Tab. 1; Fig. 1C, 1D and 2D).

**Table 1.** Measurements of sporoderm in Calochortus species investigated using TEM. Abbreviations: (M) more electron dense; (L) less electron dense.

<table>
<thead>
<tr>
<th>Taxon</th>
<th><em>C. Albus</em> Douglas ex Benth</th>
<th><em>C. eurycarpus</em> Wats.</th>
</tr>
</thead>
<tbody>
<tr>
<td>surface of the muri (microrelief)</td>
<td>smooth</td>
<td>smooth</td>
</tr>
<tr>
<td>Exine thickness (μm)</td>
<td>0.42–0.59</td>
<td>0.46–0.59</td>
</tr>
<tr>
<td>Tectum thickness (μm)</td>
<td>0.33–0.53</td>
<td>0.22–0.40</td>
</tr>
<tr>
<td>form of caput</td>
<td>Oblate spheroidal, rectangular</td>
<td>Spheroidal, Oblate spheroidal, rectangular</td>
</tr>
<tr>
<td>Caput thickness (μm)</td>
<td>0.16–0.26; 0.33–0.46</td>
<td>0.20–0.26; 0.26–0.40</td>
</tr>
<tr>
<td>Length of columella (μm)</td>
<td>0.26–0.33</td>
<td>0.26–0.33</td>
</tr>
<tr>
<td>Width of columella (μm)</td>
<td>0.10–0.26</td>
<td>0.13–0.20</td>
</tr>
<tr>
<td>Foot layer thickness (μm)</td>
<td>0.13–0.17</td>
<td>0.16–0.24</td>
</tr>
<tr>
<td>Endexine</td>
<td>absent</td>
<td>absent</td>
</tr>
<tr>
<td>T/F ratio</td>
<td>2.6</td>
<td>1.6</td>
</tr>
<tr>
<td>intine thickness in region of sulcus-less(μm)</td>
<td>0.8-1.4</td>
<td>0.5-0.7</td>
</tr>
<tr>
<td>exintine thickness (μm)</td>
<td>0.10–0.20 L</td>
<td>0.10–0.16 L</td>
</tr>
<tr>
<td>mesintine thickness (μm)</td>
<td>0.33–0.53 M</td>
<td>0.20-0.26 M</td>
</tr>
<tr>
<td>endintine thickness (μm)</td>
<td>0.05–0.10 L</td>
<td>0.04-0.1 M</td>
</tr>
</tbody>
</table>

**Discussion**
Baranova (1985) showed three type of caput of colomula in *Lilium* that the form caputs are similar to *Calochortus* (Baranova, 1985). One of the tectum structures in Angiosperms is semitectate (Walker, 1974, Nilsson *et al.*, 2002). In some genera of Liliales (e.g. genus of Fritillaria)
has semitectate pollen grain (Pehlivan et al., Kosenko, 1996). Also, tectum of investigated species of Calochortus is semitectate.

![Image](image-url)

**Fig. 1.** Transmission electron micrographs of Sporoderm in pollen of investigated species of *C. albus*. (C) = columellae, (F) = foot layer; (I) = intine, (T) = tectum, (Xi) = exintine, (Mi) = Mesintine, (Ei) = Endintine

We have revealed the minimal thickness foot layer in the pollen grain *C. albus* and the elliptical (oblate-spheroidal) caput form in columella in *C. eurycarpus* differs from the caputs (spheroidal to indeterminate). The surface of the muri (microrelief) in Subgenus of *Eriostemones* and *Leistemones* was microtuberculate tuberculcate respectively (Maassumi, 2008) but microrelief in studied species was smooth. In addition, lack of Endexine in *Calochortus* indicates its similarity to the genus *Tulipa, Amana* and *Gagea* (Maassoumi, 2005). The obtained data indicate the similarity of two species *C. albus* and *C. eurycarpus* in terms of ectexine thickness, the tectum to foot layer ratio (T/F) (Tab. 1) and different layers of intine (Fig. 1D and 2D).

![Image](image-url)

**Fig. 2.** Transmission electron micrographs of Sporoderm in pollen of investigated species of *C. eurycarpus*. (C) = columellae, (F) = foot layer; (I) = intine, (T) = tectum, (Xi) = exintine, (Mi) = Mesintine, (Ei) = Endintine

In addition, these two species were different from other species, by microreliefs on the surface of the muri, shape and thickness of caput, and the height and width of columellae (Tab. 1; Fig 1, 2 and 3).

![Image](image-url)

**Fig. 3.** Diagrams of ultrathin section in exine of the pollen grains in *Calochortus*: (A) *C.albus*; (B) *C. eurycarpus.*

Palynolomorphological data using TEM revealed more detail in comparison with SEM.

The shape of caput in *Calochortus* was oblate spheroidal, flattened-spheroidal, rectangular. The shape of caput of columella in *Calochortus* was similar to other genera in Liliales. Absent endexine in *Calochortus* is advanced features in Liliales order, as well as monocotyledon plants. *Calochortus* has three layers intine, which consists of exintine, Mesintine and endexine. The microreliefs on the surface of the muri in studied species of *Calochortus* were smooth. The tectum to foot layer ratio (T/F) in studied species of *Calochortus* was 1.6–2.6. Thus, palynolomorphological data showed that *Calochortus* was similar to some of species in *Tulipa* and *Fritillaria*.

**Appendix**

**Specimen examined**


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