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Sporoderm development in *Pratia begonifolia* Lindl. (Lobeliaceae, Asterales)

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Summary: The sporoderm development of *Pratia begonifolia* (Lobeliaceae) was studied. Some differences in electron density of the premeiotic callose wall (callose I) and the post-meiotic callose wall (callose II) were observed. Apertures start their development in the middle tetrad period. This is later than in related Campanulaceae. During the tetrad period the columellae are formed first, followed by the tectum and at last the foot layer. Contrary to Campanulaceae, in *P. begonifolia* a difference in electron density between the foot layer and the endexine can be seen in the late post-tetrad stage. Intine deposition occurs earlier in ontogeny of *P. begonifolia* than in Campanulaceae.

Keywords: Lobeliaceae, *Pratia begonifolia*, pollen development, palynology

The family Lobeliaceae (which is often placed as subfamily of Campanulaceae) contains more than 30 genera which can be clearly distinguished using morphological characters of reproductive and vegetative structures (Lammers 2007). However, pollen grains are very uniform, tricolporate and elliptic. They can be divided in two groups depending on their size. Small pollen grains with a polar axis of 22–29 µm prevail among the species of the genera *Lobelia* and *Laurentia*. *Lobelia sessilifolia* and *Laurentia petraea* as well as the representatives of *Cyphocarpus, Downingia, Grammatotheca,* and *Isotoma* are characterized by large pollen grains with a polar axis of 30–47 µm. In all members of investigated Lobeliaceae colpi are long (sometimes they merge on the poles), wide-opened, and deeply immersed. Endoapertures are small, poorly discernible or often indiscernible. The exine is semitectate and 2–3 µm thick. Sculpture is striate (with long or short and sometimes smoothed elements of the surface) up to reticulate in some species of *Isotoma, Lobelia,* and *Palmerella*. Between the striae, the exine surface possesses many foveolae and perforations of different sizes which are sometimes poorly distinguished (Zolala et al. 2009; Avetisyan 1985; Dunbar 1973, 1975). Verrucae are generally not characteristic for Lobeliaceae but they occur in *Isotoma anemonifolius* and *Laurentia petraea*. The aperture membrane is always granulated (Zolala et al. 2009; Dunbar 1975). *Pratia* has pollen grains which are characteristic for most Lobeliaceae. They are medium sized (polar axis 25–30 µm) with well-expressed endoaperture (ora) and typical striate-perforate sculpture.

Lobeliaceae have tetrasporangiate anthers. The centrifugally developing locule wall consists of epidermis, endothecium with fibrous thickenings, one ephemeral middle layer, and secretory tapetum with binuclear cells. Microsporogenesis corresponds to the simultaneous type. Tetrads are usually tetrahedral and less frequently isobilateral (Sladkov & Grevtsova 1991). In Lobeliaceae mature pollen grains are usually two-celled (Batygina 1987; Ermakov 1990) with amyloid grains, but also three-celled pollen grains were described by Poddubnaya-Arnoldi (1982). Pollen development of angiosperms is traditionally divided in two periods: a tetrad and post-tetrad period. The tetrad period starts at the meiosis of the mother cell of the microspore. At the

same time callose deposition begins under the cellulose membrane of mother cells. One of the main functions of the callose is the protection of young microspores. Soon after the development of tetrads the primexine starts developing between the callose and the microspore plasmalemma (Gabaraeva 1987). During this period initiation of fundamental elements of the exine occurs. Gabaraeva (1987) distinguishes sequences of initiation as follows: 1) tectum, columellae and foot layer are developed gradually in the tetrad period; 2) the foot layer develops first, followed by the forming of the columellae and the tectum; 3) columellae and tectum are established at the same time whereas the foot layer is formed later.

The next period is a free microspore period. After a quick callose dissolution microspores are released into the anther locule. The callose dissolution occurs by means of the enzyme callase which is produced by tapetal cells. After the destruction of callose the first mitotic division of the nucleus happens and thus the development of the pollen grain (Poddubnaya-Arnoldi 1976; Reznikova 1985). The pollen grain gradually increases in volume. Small vacuoles in the protoplast decrease in number and aggregate to form a larger vacuole (Kosenko 2004). Before the dehiscence of anther locules and the dispersal the pollen grains are dehydrated and the large vacuole disappears.

Sporoderm development of Lobeliaceae species was not described in detail so far. Pollen grains of the related Campanulaceae are porate or colpate with many subtectal spines (Zolala et al. 2009). Sporoderm development of three Campanulaceae species with porate and colpate was described (Zolala et al. 2008). Only in the related Asteraceae the sporoderm development of several species with spinose and spinulose tricolporate pollen was described (Meyer-Melikyan et al. 2004).

Materials and methods

For the research of the sporoderm development pollen grains of *Pratia begonifolia* Lindl. were collected in the greenhouse of the Botanic Garden of Moscow State University. Pollen morphology and sporoderm development were studied by light microscopy (LM), scanning electron microscopy (SEM), and transmission electron microscopy (TEM).

For developmental studies the material was selected under LM. The anthers were separated from buds and squashed in a drop of water to examine the different developmental stages of the pollen.

For TEM studies buds of different sizes were fixed in 2.5% glutaraldehyde in 0.15 M phosphate buffer (pH 7.3) with saccharose addition for 2 hours at room temperature followed by the post-fixation in 1% OsO_4 for 10 hours at +4°C. Using a series of different concentrations of ethanol, the material was transferred to 70% ethanol and contrasted by a saturated solution of uranil acetate in 70% alcohol at +4°C (10 hours). Then the material was dehydrated by gradual transfer to absolute ethanol, 50% acetone and 50% absolute ethanol, 100% acetone, and finally in a mixture of Epon and acetone. Dehydrated buds were embedded in Epon (Weakley 1972) for 24 hours at room temperature and then exposed to a temperature of +62°C for 48 hours.

Semithin sections (5–7 µm) without any contrasting were studied by means of a LM MBI-3 for detecting the stages of pollen development in the anther locule. Ultrathin sections were obtained using a LKB Ultratome. The sections were contrasted according to Reynolds (Geyer 1977).

Examination of the samples was carried out by means of the electron microscopes JEM-100-B and JEM-1011 in the Laboratory of Electron Microscopy of Biological Faculty of Moscow University.

Mature pollen without special treatment was mounted on SEM stubs by nail polish. These stubs were coated with gold and examined by means of JSM and CamScan in the Laboratory of Electron Microscopy of Biological Faculty of Moscow University.

Results

Microspore mother cells

When microspore mother cells begin to deposit the callose under the primary cell wall they loose their original angular outlines and become isodiametric. Their cytoplasm is rich of organelles. On our sections it shows a higher electron density and many membranous elements belonging to various organelles. A lot of vacuoles can be recognized under the plasmalemma which they fit into and outpour their content under the primary cell wall. The plasmalemma has a strong undulate outline due to actively built vesicles. The primary cell wall is well distinguished underneath the layer of the initial callose enclosing the mother cell (Fig. 1, 1).

Early tetrad period (primexine matrix formation)

After the meiosis (which is of a simultaneous type) four microspores are formed and then they immediately begin to develop their own (secondary) callose wall (Fig. 1, 2: K2).

On our sections microspores show a rounded outline, a higher electron-density, many membraneous elements, different organelles, and small vacuoles. Microspores are surrounded by an irregularly thickend callose consisting of two parts: the outer primary callose formed by the mother cell and the inner secondary callose formed by each microspore (Fig. 1, 2: K1, K2). Primexine matrix starts to develop on the plasmalemma surface by small globules. These globules are homogeneous, often rounded or oblong, electron lucent, but denser than the callose (Fig. 1, 3–6).

Middle tetrad period (primexine formation)

The rounded microspores get an irregular shape with undulate contour. They are surrounded by the irregularly thickend callose, their content is electron dense. The primexine matrix becomes finely granulated by elements with a higher electron density. The primexine consists of well developed electron dense columellae and a thin tectum (Fig. 1, 7). Columellae rest on the plasmalemma. The tectum is irregular in thickness. In the areas of future apertures the primexine only consists of electron dense globules which continue the tectum (Fig. 1, 8). The electron dense content of the primexine matrix is characterized by a considerable thickness.

In this stage degraded microspores were observed. They have a normally developed primexine, but there are a lot of vacuoles of different sizes and shapes, aggregations of electron dense granules and membranous elements in the cytoplasm.

Late tetrad period (aperture formation)

Microspores have an irregular outline (Fig. 1, 10). The microspore content is electron dense. Vacuoles become smaller and decrease in number. The callose appears to be homogeneous and

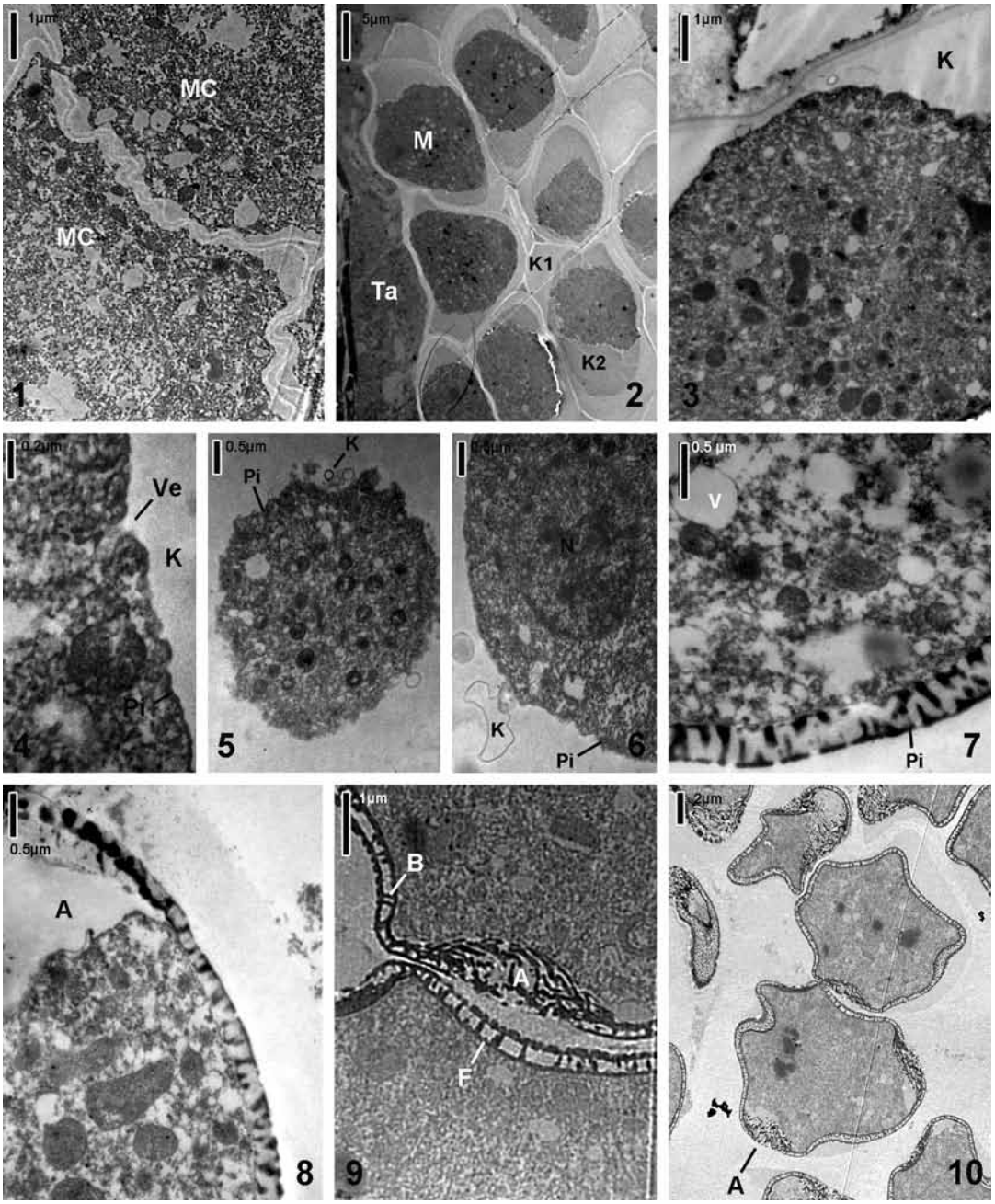

Figure 1. *Pratia begonifolia* TEM: 1 – microspore mother cells, 2–6 – early tetrad period, 7–8 – middle tetrad period, 9–10 – late tetrad period; A – aperture, B – baculum, F – foot layer, K – callose, M – microspore, MC – microspore mother cell, N – nucleus, Pi – primexine, Ta – tapetum, V – vacuole, Ve – vesicle.

begin to reduce. The primexine matrix gradually disappears, but its granules and lamellae persist between the columellae. The primexine consists of well-developed columellae and tectum. On paradermal sections the tectum consists of homogeneous areas penetrated by perforations and the columellae look like the sporopollenin globules. Columellae rest on a solid layer whose inner part appears to be electron denser. Neither endexine nor foot layer can be distinguished. In

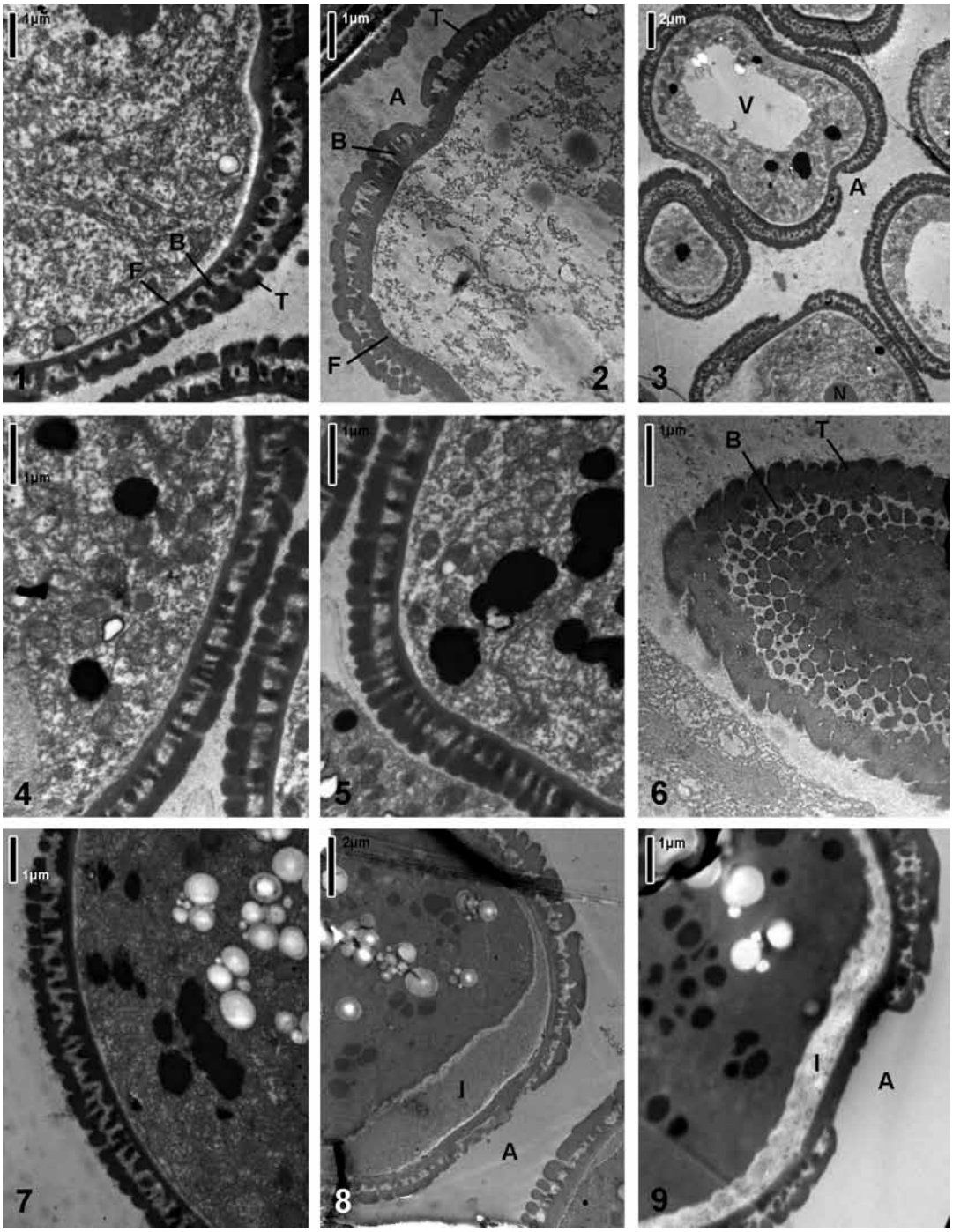

Figure 2. *Pratia begonifolia* TEM: 1–3 – early post-tetrad period, 4–6 – late post-tetrad period, 7–9 – mature pollen grain; A – aperture, B – baculum, F – foot layer, I – intine, N – nucleus, T – tectum, V – vacuole.

the areas of developing apertures, the inner primexine layer considerably thickens and a lot of electron dense, highly undulate and convoluted lamellae of different length can be seen in the layer in the midst of the electron lucent matrix. The lamellae are mainly arranged parallel to the plasmalemma (Fig. 1, 9).

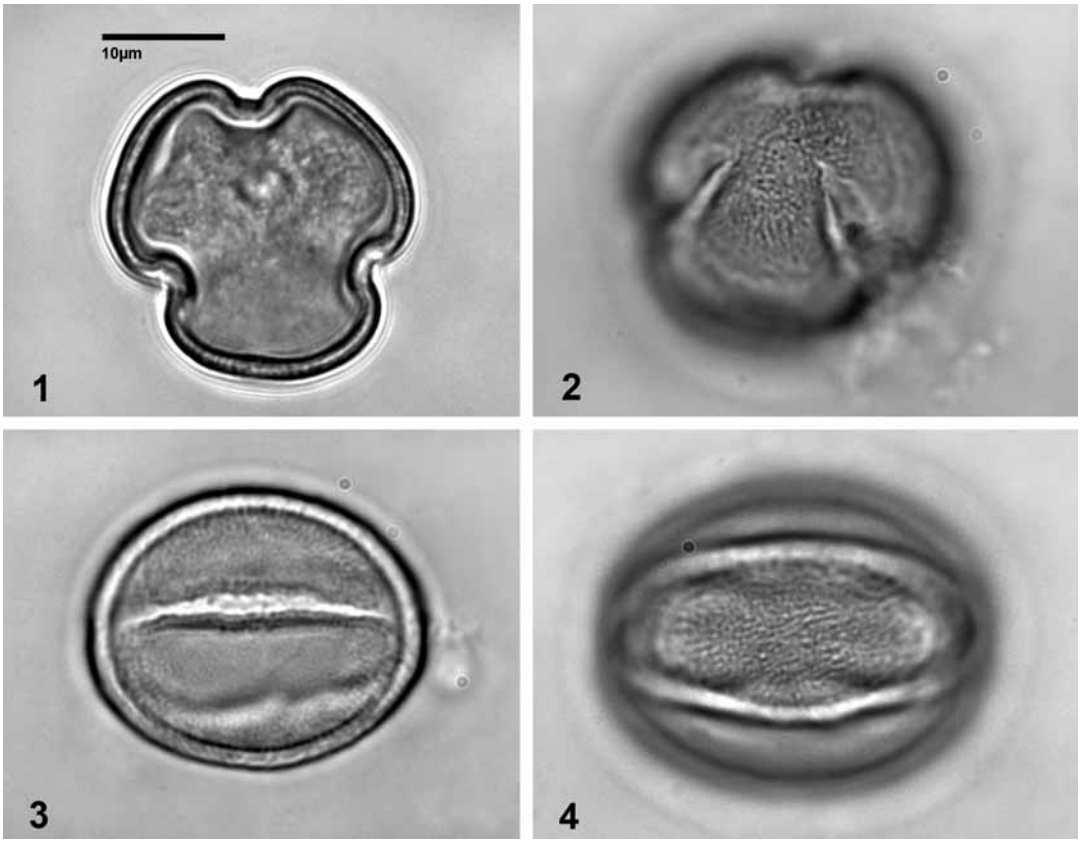

Figure 3. *Pratia begonifolia* LM: 1 – polar view, optical cross-section, 2 – polar view, ornamentation, 3 – equatorial view, furrow, 4 – equatorial view, ornamentation.

Early post-tetrad period (endexine formation)

Microspores appear like mature pollen. They are rounded trilobate in polar position and rounded in equatorial position. There are a lot of electron lucent and few electron dense vacuoles which begin to form one large central vacuole in the microspore cytoplasm (Fig. 2, 3). The callose completely dissolves and all exine elements considerably increase and become electron dense. Columellae become longer and thicker. They correspond to shape and size of the mature elements. The tectum thickens and forms a characteristic sculpture which appears wavy on our sections. The foot layer is well developed, continuous in the non-aperture areas, and discontinuous around apertures. It is slightly lighter than the endexine and the border between them could not always be traced. The endexine is disposed all over the perimeter of the pollen grain. It has a similar electron density like the foot layer and they could hardly be distinguished (Fig. 2, 1–2). In the areas of colpi the endexine consists of densely packed lamellae whereas endoapertures are formed by the matrix with loose, wavy lamellae.

Late post-tetrad period (intine formation)

Microspores appear to be mature, they are rounded trilobate in polar view and rounded in equatorial one. There is a large central vacuole and a number of lipid drops in the cytoplasm (Fig. 2, 4–5). Sporoderm looks like mature pollen grains, it is round-3-laciniated in polar position and rounded in equatorial position. The exine is completely developed, the ektexine and endexine

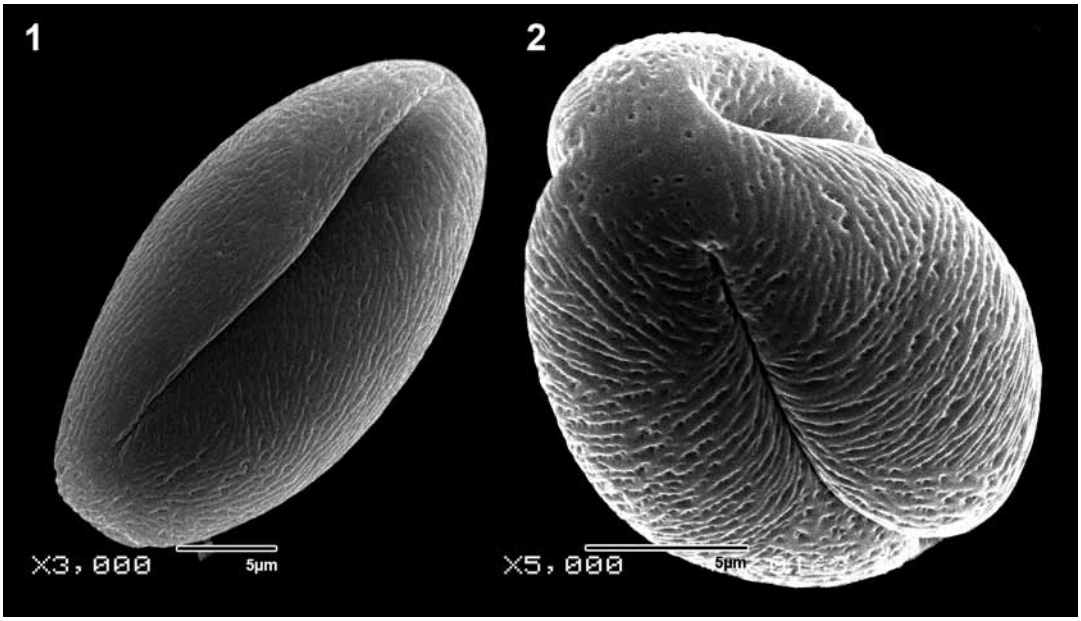

Figure 4. *Pratia begonifolia* SEM (grains collapsed): 1 – equatorial view, 2 – polar view.

show characteristics of mature pollen structure on radial and paradermal / oblique sections (Fig. 2, 5–6). Apertures have a complex structure. The outer part is formed by loose endexine lamellae, the inner one by an electron lucent intine which can also be revealed under the endexine in non-aperture regions.

Mature pollen in light microscopy

Pollen grains are tricolporate, oblate spheroidal (Fig. 3, 1–4). P / E = 1.15. They are triangular in polar view and rounded in equatorial one. Polar axis is 25–30 µm, equatorial diameter is 23–26 µm. Colpi are shallow, short, narrow, 22–25 µm long. Ends of colpi are pointed. The distance between the ends of colpi in the polar area is 5–7 µm. Mesocolpium width is 15–18 µm. The exine is psilate, ~1.5 µm thick, tectate, two-layered.

Sculpture of mature pollen in scanning electron microscopy

The sculpture is striate with numerous foveolae and perforations of different sizes. Aperture membrane is granulate (Fig. 4, 1–2).

Sporoderm ultrastructure of non-aperture region of mature pollen in transmission electron microscopy

A lot of lipid drops and electron lucent starch globules were observed in the microspore cytoplasm. The intine is thin, with granules (Fig. 2, 7). The endexine is homogeneous and almost 1.5–2 times thicker than the intine. The border between the endexine and ektexine is clearly visible. The ektexine consists of three layers. The foot layer is thicker than the endexine, homogeneous, irregular in thickness, and towards the apertures it becomes discontinuous and disappears under the apertures. Height of columellae is almost equal to the foot layer thickness. The columellae continue directly to the tectum without any transitional layers. The tectum is slightly thicker than the columellae. It is penetrated by sparse narrow perforations whose number increases towards

the apertures. Some of the inner ektexinal spaces are filled with fibrous contents which don't reach the pollen surface (Fig. 2, 7).

Sporoderm ultrastructure in the aperture region

Apertures are formed by a thickened (up to 10 times) multi-layered intine (Fig. 2, 8–9) and a slightly thickened endexine. The outer intine layer is thin, electron lucent, and not thickened. The middle intine layer is considerably thickened, electron dense, and granulate-lamellate. The inner intine layer is identical to the outer one. Towards apertures, the endexine thickens and becomes loosely lamellate. Electron lucent spaces between the lamellae are small. In the aperture regions the ektexine dissappears earlier than the endexine. Endoapertures are formed by a considerably thickened intine solely.

Discussion

Cell wall structure and function in general and some of its parts undergo considerable changes during pollen ontogeny. On early stages it provides a selective delivery (inflow) of nutrients into the tetrads of microspores from the tapetum. The wall influences the shaping, aperture structure, and exine ultrastructure. On late ontogenetic stages pollen sculpture, outer ektexine layers, and intine are formed. During pollen development the sporoderm often undergoes changes which can conceal its original structure and complicates homologization of the layers. This causes difficulties in revealing phylogenetic relationships because it is difficult to distinguish foot layer and endexine in Campanulaceae–Lobeliaceae.

First stages of mother cell and microspore development in *Pratia begonifolia* differs from other studied Campanulaceae species by clear differentiation of the callose of different origin. The pre-meiotic callose wall is electron lucent whereas the post-meiotic one is electron dense. This could be caused by differences in the chemical content of the callose I and II.

Pratia begonifolia is a member of the family Lobeliaceae. Pollen development has been studied by Zolala et al. (2008) in several representatives of the closely related Campanulaceae: *Campanula rapunculoides*, *C. cordifolia*, and *Platycodon grandiflorum*. Despite of their close phylogenetic positions there are differences in pollen development. Apertures in the tetrad period are more developed in Campanulaceae. Also the cavity under the primexine indicating the place of future apertures is wider. Therefore, we conclude a later aperture development in *Pratia begonifolia* compared to Campanulaceae. In Asteraceae aperture formation occurs considerably earlier (early tetrad stage) than in Campanulaceae and Lobeliaceae. Besides, in Asteraceae the primexine is not deposited in the aperture regions whereas in Campanulaceae and Lobeliaceae some primexine elements are present in the aperture region.

Mature pollen of *Pratia begonifolia* has the same aperture type as Asteraceae but differs from the related Campanulaceae (Zolala et al. 2008). In *Pratia begonifolia* columellae are formed during the tetrad stage at first, followed by the tectum and at last the foot layer.

Our data accomplish those obtained by Gabaraeva (1987) and enable us to separate another sequence of primexine elements deposition in the tetrad period. On the late post-tetrad stage the difference in electron density between the foot layer and the endexine in *Pratia begonifolia* can be clearly seen. In most Campanulaceae species this difference is very small until mature pollen stage. The endexine presence in the Campanulaceae can be traced only by its lamellate structure

during ontogeny (early developmental stages). The remnants remain at the aperture margins in mature pollen.

Intine deposition occurs earlier in *Pratia begonifolia* than in Campanulaceae. Thus, the scenario of sporoderm development better corresponds with the taxonomic position than with pollen morphology.

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