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IN SITU POLLEN OF *ALASIA*, A SUPPOSED STAMINATE INFLORESCENCE OF *TROCHODENDROIDES* PLANT

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Abstract. Genus *Alasia* GOLOVNEVA, 2006 includes staminate inflorescences repeatedly found together with *Trochodendroides* type leaves and *Trochodendrocarpus* - *Joffrea* type infructescences and is considered as the most probable pollen organ of the *Trochodendroides* plant. In situ pollen grains were extracted from the anthers of two *Alasia* flowers and detached stamens of cf. *Alasia* from the Paleocene of Amur Province, Russian Far East. The morphology of the flowers was interpreted as the fascicles of stamens with dorsifixed introrse anthers adpressed to the subtending bract before exsertion. The pollen grains are small tricolpate with long colpi and a variably microreticulate to verrucate-scabrate ornamentation. *Alasia* differs from *Cercidiphyllum* in both staminate structures and pollen morphology suggesting hamamelid affinities.

■ Fossil angiosperms, floral morphology, pollen morphology, hamamelids, angiosperm evolution, Paleocene.

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Introduction

Trochodendroides BERRY is the most widespread and abundant leaf genus in the Late Cretaceous and Paleogene of northern Asia and North America evidently representing an ecologically dominant plant of the lowland arboreal vegetation. It comprises polymorphous long-petiolate ovate, obovate or cordate leaves with palmate acrodromous venation that before recognition of the extinct genus, were frequently misassigned to *Populus, Cocculus, Zizyphus*, *Cercidiphyllum* and other extant genera of similar leaf morphology (reviewed in Krassilov 1976). Variation in leaf shapes and venation might have been partly related to dimorphism of long and short leafy shoots. Reproductive structures repeatedly found in association with *Trochodendroides* are compound infructescences with racemose paracladia bearing free or paired many-seeded follicles, described under *Cercidiphyllum* SIEBOLD et ZUCCARINI, *Nyssidium* HEER, *Trochodendrocarpus* KRYSHTOFOVICH, and *Joffrea* CRANE et STOCKEY (Brown 1939, Kryshtofovich and Baikovskaya 1966, Krassilov 1976, Crane 1984, Crane and Stockey 1985). Their taxonomic affinities were sought with the Cercidiphyllaceae (Brown 1939, Crane and Stockey 1985) or the Hamamelidaceae (Krassilov 1976, Krassilov and Fotyanova 1995).

Inflorescences of spirally arranged bracts subtending staminate flowers have been found in association with *Trochodendroides* in a number of Cretaceous and Paleogene localities of northern Asia, but remained misunderstood until their revision by Golovneva (2006), who erected an inflorescence morphogenus *Alasia* for them. Similar structures seem to have been collected together with *Joffrea* from the Paleocene of North America (Crane and Stockey 1985).

Regular associations, often with *Trochodendroides* leaves alone, makes it fairly probable that *Alasia* represents staminate inflorescences of the *Trochodendroides* plant. However their interpretation remained ambiguous and no pollen grains have been so far extracted. We report *in situ* pollen grains and discuss staminate morphology of *Alasia* in relation to its taxonomic affinities. Hopefully, this study will assist in recognition of dispersed pollen grains of the *Trochodendroides* plant.

Material and methods

Material described in this paper came from the Paleocene coal-bearing Kivda deposits (age assignments after Krassilov 1976, Akhmetiev et al. 2002) of the Pioneer

Quarry, Raichikhinsk Coal Field in the Amur Province of the Russian Far East (Text-fig. 1), including:

(1) A detached bract with exserted stamens, collected by Lina Golovneva and deposited in the Botanical Institute, St. Petersburg, collection BIN No. 1537, specimen and counterpart No. 44, figured in Golovneva (2006, pl.2, fig. 1) and re-figured here in Pl. 1, figs 1-3;

(2) A disintegrated bracteate inflorescence showing partly exserted anthers collected by Tatiana Kodrul and deposited in the Geological Institute, Moscow, collection GIN No. 4867, specimen No. PN4-81 (Pl. 1, figs 5, 6);

(3) Detached stamens similar to those of *Alasia* sp., here designated as cf. *Alasia* sp. from Kodrul's collection GIN No. 4867, specimen No. PN4-5 (Pl. 1, fig. 4).

All the specimens were borrowed and re-described by Krassilov and Kodrul in the Laboratory of Paleobotany, Paleontological Institute, Moscow and the Institute of Evolution, University of Haifa. Photographs were obtained with a digital camera Leica 420.

The plant remains are preserved as slightly ferruginous impressions with patches of compressed material that can be macerated. Small fragments of anthers were transferred to varnish film by dissolving the rock matrix in hydrofluoric acid, cleared in nitric acid and studied under SEM. Both specimens of intact flowers had dehisced anthers yielding unshed pollen stuck to the locules. The best preserved pollen grains, both scattered and in small clumps, were obtained from Kodrul's specimen 4867-PN4-81 (Pl. 2, figs 1-6; Pl. 3, figs 1-6; Pl. 4, figs 4-6). The grains are of the same morphological type, whereas the noticeable variations in size and ornamentation are ascribed to developmental, as well as preservational differences.

Transfers from the counterpart of Golovneva's specimen 1537-44 exposed a few recognizable grains and clumps of crumpled grains (Pl. 4, figs 1-3). In the detached stamens cf. *Alasia* sp., specimen 4867-PN4-5, the anthers had locules filled with masses of coalescent pollen grains that proved difficult to separate (Text-fig. 2A-D).

Description

Golovneva (2006) described *Alasia pojarkovae* (Krassilov) Golovneva, the holotype of which came from an outcrop of the lowermost Paleocene conglomerates with lenticular shales on the Belaya Gora (White Mountain), 12 km upstream from the mouth of the Bureya River. All the material in the protologue of this species (Krassilov 1976, re-figured in Golovneva 2006) is sterile, showing neither exserted stamens, nor even impressions of anthers on bracts, which regularly occur in *Alasia*. Attribution to *Alasia* was based on Golovneva's specimen 1537-44 of a bracteate flower from the Pioneer Quarry, Raichikhinsk Coal Field, found in a stratigraphically higher coal-bearing horizon. Although undoubtedly representative of *Alasia*, this specimen has been figured without being described and the characters of the flower did not enter the diagnosis of *Alasia pojarkovae* based on Krassilov (1976). The sterile material from Belaya Gora and fertile material from Pioneer Quarry differ not only in age and geography, but also in their shapes and dimensions, which makes their attribution to one and the same morphotaxon highly problematic. We therefore designate the Pioneer Quarry specimens as *Alasia* sp. and the detached stamens from the same locality as cf. *Alasia* sp.

Text-fig. 1. Map of the study area showing the Pioneer Quarry locality (49º39'53.9" N, 129º34'00.1" E).

Staminate flowers

Pl. 1, figs 1-6

Specimen GIN 4867- PN4-81 is a disintegrated spike of imbricate bracts, which are ovate, shortly pointed, broadly rounded at the base (Pl. 1, fig. 5). All the bracts are preserved with their convex abaxial face upwards showing a smooth surface with faint epidermal cell pattern, apparently lacking stomata. The subtended stamens were imbedded in the rock matrix, but in a few scales it was possible to uncover anthers basally protruding from under the adaxial impression (Pl. 1, fig. 6). The anthers are linear-elongate, curved away from the bedding plane, about 1.4 mm long, imprinted parallel to each other as they were adpressed to the bract before exsertion. Since the anthers are dehiscent, it is suggested that they were not or only slightly exserted at anthesis.

Specimen GIN 4867- PN4-5 (Pl. 1, fig. 4) represents two bithecate anthers and a theca of one more, about 1.4 mm long, imprinted nearly parallel to each other and somewhat curved, as in the intact flowers. A poorly preserved laminar impression above the anthers suggest, that they were also overlapped by a bract, as in the specimen 4867- PN4-81, and the dimensions are nearly the same. However, the thecae are preserved as solid bodies filled with pollen grains, perhaps being mechanically detached shortly before anthesis.

Specimen BIN 1537-44 is a solitary inflorescence bract with an exserted fascicle of about 20 stamens. The bract is preserved with its concave adaxial face upwards, with infolded margins, showing imprints of adpressed anthers marked by a series of parallel ridges (Pl. 1, fig. 1). The exserted stamens are irregularly spread without any evidence of differentiation within the fascicle. They are all equally developed, about 4 mm long, consisting of a short (1/4 of stamen length) stout filament and a massive anther. The anthers are elongate, slightly curved, having a connective with a short point and two large spindle-shaped thecae pointed at both ends. An enlarged stamen figure (Pl. 1, fig. 2) shows its anther being dorsally attached to the filament (dorsifixed), with a well marked dehiscence suture on the slightly concave ventral side (introrse). Because the anthers are larger than in specimen 4867-PN4-81, it is suggested that they represent a later developmental stage of an open inflorescence with the bracts separated and the flowers exserted before being shed from the axis.

Pollen grains

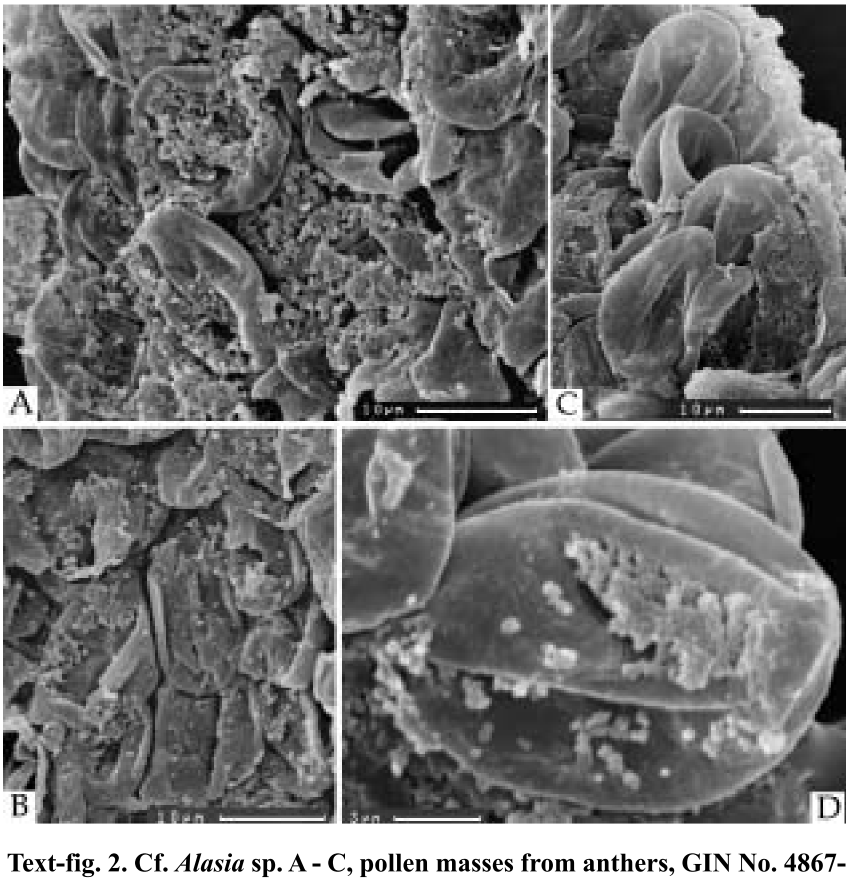
Pl. 2, figs 1-6; Pl. 3, figs 1-6; Pl. 4, figs 1-6; Text-fig. 2A-D; 4A

Pollen grains transferred from specimen 4867- PN4-81 (Pl. 2, figs 1-6; Pl. 3, figs 1-6; Pl. 4, figs 4-6) are the most representative of the species, showing a range of variation in dimensions and sculpture. They can be divided into groups of distinct and vague ornamentation, although the differences are gradational and at least partly developmental. Grains with distinct ornamentation (Pl. 2, figs 1-6; Pl. 3, figs 1-6) are typically elongate elliptical, polar axis 25–26 µm, equatorial axis 20.5–21 µm (ten measurements). The equatorial amb is somewhat irregularly curved, infolded at one or both poles. An obliquely compressed grain shows a nearly rounded equatorio-polar aspect with broadly convex mesocolpia and shallowly invaginate colpi converging towards the pole (Pl. 2, fig. 4). The colpi are long, mostly infolded, occasionally open, gaping in the middle to a width of 5 µm, covered with a coarsely granular membrane (Pl. 3, fig. 1).

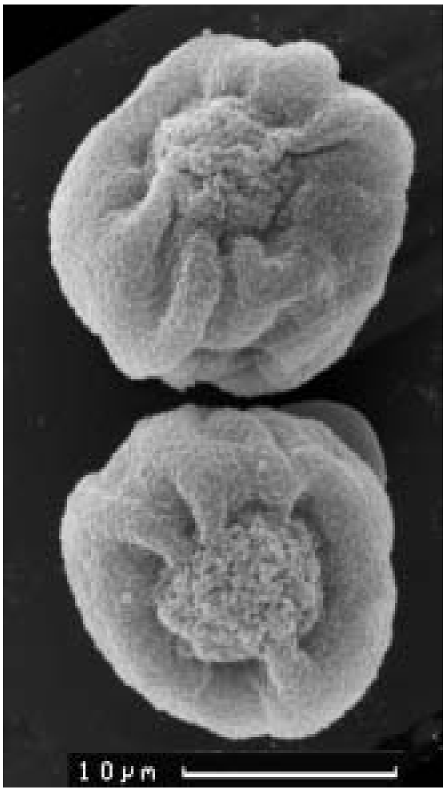
The sculptural pattern of mesocolpia is crochet-like, ranging from the microreticulate of dense rounded pits and broad cristate muri (Pl. 3, figs 3, 4) to the canaliculate of scattered pits and intricately ramified furrows cut between the verrucate-scabrate patches of positive relief (Pl. 3, figs 5, 6). Ornamentation tends to smooth out in the vicinity of colpi bordered by continuous stripes of verrucate-scabrate surface. Minute electronically reflective orbicules are scattered over the mesocolpia and on the borders of colpi.

The ratio of microreticulate and verrucate-scabrate areas varies both over the surface of a single grain and between the grains. In a somewhat larger than average, 29 x 28 µm, almost spherical grain (Pl. 3, figs 2, 5), about 2/3 of the surface is continuously verrucate-scabrate, with relatively small densely pitted areas. Such sculpture variants are transitional to the vague ornamentation of smaller grains, about 22.5 × 17. 5 µm, with long, but less prominent slit-like colpi and low patchily obliterated verrucate-scabrate ornamentation (Pl. 4, figs 4-6). Some show distinct differentiation of scabrate and smooth areas (Pl. 4, fig. 6). Orbicules are more abundant than in the distinctly ornamented grains, locally aggregated in dense masses.

An open anther transferred from the counterpart of BIN 1537-44 contains a few unshed pollen clumps and solitary grains struck to the wall (Pl. 4, figs 1-3). The pollen grains are compressed and somewhat crumpled, perhaps by desiccation before burial. The better preserved grains are broadly elliptical in equatorial aspect, polar axis 25–29 µm, short axis 24–26 µm (five measurements). The amb is smoothly rounded, often truncate at the poles. The colpi are infolded over their whole length. The apocolpia are marked by prominent, nearly straight or somewhat undulate, smooth or indistinctly verrucate ridges. The mesocolpia are about 12 µm wide, canaliculate, with irregularly lobed or digitate patches 2–3 µm wide, divided by narrow sinuous furrows. Ornamentation of the patches is verrucate-scabrate with hemispherical verrucae about 0.5–0.6 µm wide and globular scabrae about 0.2 µm in diameter. The irregularly scattered orbicules are spherical, of nearly the same size as the verrucae or occasionally larger, about 1–1.5 µm in diameter.



Text-fig. 2. Cf. *Alasia* sp. A - C, pollen masses from anthers, GIN No. 4867-PN4-81 (Pl. 1, fig. 4); D, surface features, with sculptured layer degraded to granular aggregates with tapetal spherules.



Text-fig. 3. *Cercidiphyllum japonicum* Sieb. et Zuc., Botanical Garden, Moscow: pollen grains from anthers.

In cf. *Alasia* sp., specimen 4867- PN4-5 (Text-fig. 2A-D), the thecae of detached anthers are filled with masses of several hundred coalescent pollen grains, some in discernible tetrade configurations. Individual grains are elliptical, broadly rounded or truncate and shallowly invaginate at both ends, or else ovate, with one end broadly rounded, the other narrowly rounded, polar axis 18.2–19 µm, equatorial axis 10.5–12 µm, mesocolpia 6–6.2 µm wide. The colpi nearly reach to the poles, are straight or gently curved. The mesocolpia are covered with granular aggregates with distinct orbicules. Exposed areas of exine are smooth with sparse pits, minutely dotted with punctuate prominences at the ends of infratectal rods.

The grains are smaller than those found in dehiscent anthers, obviously immature. They are comparable to the vaguely sculptured grains from dehiscent anthers (Pl. 3, figs 4-6), which are also considered to be underdeveloped. However, the distinctions in the surface features can be due not only to developmental differences, but also to a post-depositional degradation of surface material resulting in obliteration of sculpture and aggregation of structureless tapetal deposits.

As a result of this study, the diagnosis of *Alasia* Golovneva has to be emended by exclusion of species based on sterile material alone, such as *"Alasia" pojarkovae*, and by additional taxonomically important characters of stamens and pollen grains described above.

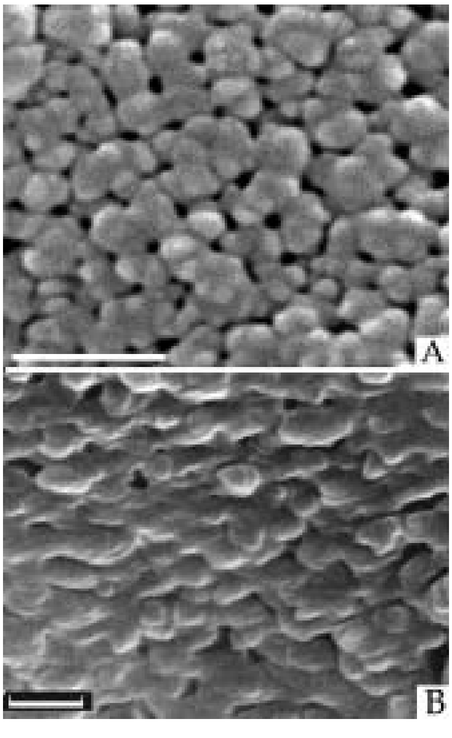
Alasia Golovneva, 2006 emend. nov.

Type: *Alasia jacutensis* (Sveshnikova) Golovneva (first described as *Araucarites jacutensis* Sveshnikova, 1967), Golovneva 2006, p. 1901.

Emended diagnosis: Inflorescences long spicate of imbricate deciduous bracts subtending axillary fascicles of numerous stamens. Bracts ovate with stamens appressed upon their concave adaxial face, shortly exserted at anthesis. Stamens free, simultaneously developing, with a short (1/4 of stamen length) stout filament and a massive dorsifixed bithecate anther, dehiscence introrse. Pollen grains tricolpate with long colpi, ornamentation microreticulate or canaliculate, on the positive elements additionally verrucate-scabrate, in immature grains vaguely developed, with abundant orbicules.

Discussion

In *Alasia* Golovneva, 2006, interpretation of the axillary staminate structures as pseudanthial inflorescences is based on an alleged analogy to *Cercidiphyllum* (in which such interpretation is also problematic) and is scarcely warranted by the material at hand. In extant *Cercidiphyllum*, staminate clusters develop from inflorescence buds that also produce a solitary leaf in the axil of a bud-scale (cataphyll). Before anthesis, staminate clusters are enclosed in the leaf stipules (unpublished observation by Krassilov). Association with the leaf is maintained until the appearance of a morphologically distinct growth point of a sympodially branching inflorescence and is a peculiar feature of *Cercidiphyllum* finding no analogy in *Alasia*, in which the stamens instead associate with large inflorescence bracts shed with their subtended flowers. There are also differences in the stamen morphology. *Alasia* has short filaments and massive anthers that, unlike those of extant *Cercidiphyllum*,



Text-fig. 4. A – *Alasia* sp., pollen ornamentation, compared with B – extant *Quercus castaneifolia* C.A. Mey (courtesy of Natalia Naryshkina, Institute of Biology and Soil Science, Vladivostok), with similar verrucate – scabrate elements. Scale bar 1 µm.

are dorsifixed rather than basifixed, introrse rather than latrorse (Endress 1993) and lack a distinctly protruding connective.

A more productive comparison can be made with hamamelids (*sensu* Cronquist 1981, Takhtajan 1987), typical of which are the catkin-like male spikes with solitary flowers or partial pseudanthial inflorescences, perianthless in the Myricaceae (Macdonald 1978), and sporadically in other families (Takhtajan 1987). The morphology of *Alasia* suggests that such simple androecial structures are of considerable antiquity.

A recent revision of the Hamamelidae as a coherent morphological group is based on the molecular phylogenetic reconstructions (Fishbein and Soltis 2004, Soltis 2005) assigning the Hamamelidaceae and Cercidiphyllaceae to the Saxifragales. This supposedly new phylogenetic arrangement actually corresponds to the views widely held in the XIX century (critically reviewed in Baillon 1871), but abandoned with further advances in morphological classification. The controversies can only be resolved by simultaneous revisions of both morphology and molecular data. However fossil evidence (e.g., Maslova et al. 2005) suggests an earlier appearance and diversification of the Hamamelidaceae as a probable stem group, rather than a sister group, of the Saxifragaceae and their morphological allies.

Pollen grains of *Alasia* are tricolpate with long colpi, whereas in all varieties (some separated as distinct species) of *Cercidiphyllum japonicum* the apertural type, although conventionally described as tricolpate with short colpi, is far more typically triporate (Text-fig. 4). A configuration of long colpi found in *Alasia* is fairly common in the Hamamelidaceae and Fagaceae, some members of which also show significant similarities in ornamentation. Irregular canaliculate furrows are a characteristic feature of pollen ornamentation in the Hamamelidaceae (Zavada and Dilcher 1986), whereas a similar association of scabrae and verrucae on the ripples of positive relief bounded by distinct or vague furrows is typical of many species of oaks (Van Benthem et al. 1984, Wang and Chang 1991) and the details may nearly coincide (Text-fig. 4). These features appear to be of certain phylogenetic significance and are potentially useful for detecting *Trochodendroides* plant pollen grains in paleopalynological assemblages. Our material shows that pollen grains could have been partly dispersed in an immature state, with ornamentation underdeveloped and prone to post-depositional obliteration. This possibility has to be taken into account in taxonomic assignment of dispersed pollen grains through the Late Cretaceous and Paleogene.

Acknowledgements

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Explanation to the plates

PLATE 1

Alasia sp. from the Kivda Beds, Paleocene of Pioneer Quarry, Raichikhinsk Coal Field, Amur Province.

1, 2. Detached staminate flower with a persistent inflorescence bract, BIN No. 1537-44.

3. Counterpart from which pollen grains were transferred, BIN No. 1537-44.
4. Detached stamens cf. *Alasia* sp., GIN No. 4867-PN4-5.
5, 6. Disintegrated bracteate inflorescence with stamens partly exserted, GIN No. 4867- PN4-81.

PLATE 2

Alasia sp., distinctly ornamented microreticulate – canaliculate pollen grains transferred from anthers, GIN No. 4867-PN4-5 (Pl. 1, figs 5, 6).

1. pollen clump.
2, 3. equatorial aspect, infolded at one pole and with invaginated colpi.
4. oblique equatorio – polar aspect.
5. ectexine hollow inside, showing inner surface.
6. example of transitional microreticulate – verrucate ornamentation.

PLATE 3

Alasia sp., pollen grains transferred from anthers, GIN No. 4867- PN4-81 (Pl. 1, figs 5, 6), distinctly ornamented microreticulate – canaliculate and transitional canaliculate verrucate – scabrate grains.

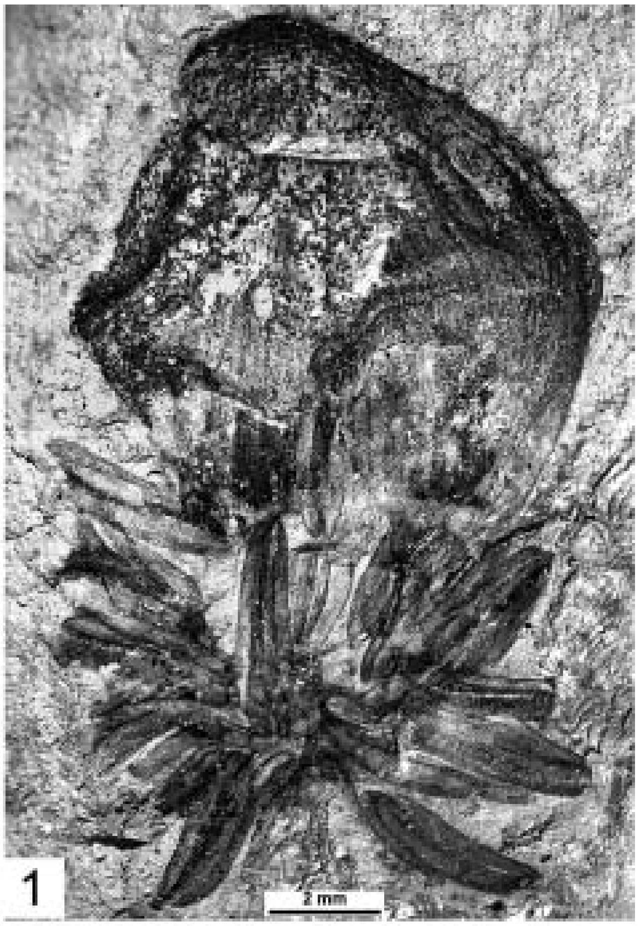
1. with open colpi showing a coarsely granular membrane.
2. with invaginate, partly open colpi.
3. Canaliculate, patchily verrucate – scabrate ornamentation of grain shown in Pl. 2, fig. 3.
4. Mainly microreticulate ornamentation of grain shown in Pl. 2, fig. 2.
5, 6. transitional, mainly verrucate – scabrate ornamentation of grains shown in this Plate, fig. 2 and Pl. 2, fig. 5.

PLATE 4

Alasia sp., pollen grains transferred from anthers.

1 – 3. From counterpart of BIN No. 1537-44 (Pl. 1, fig. 3):
1. Clumped grains.
2, 3. A larger grain with canaliculate patchily verrucate-scabrate ornamentation.
4, 5. From GIN No. 4867- PN4-81 (Pl. 1, figs 5, 6), vaguely ornamented with abundant spherules.
6. Transitional verrucate-scabrate (vs) to smooth with spherules (sm) ornamentation of grain shown in this Pl., fig. 5.

PLATE 1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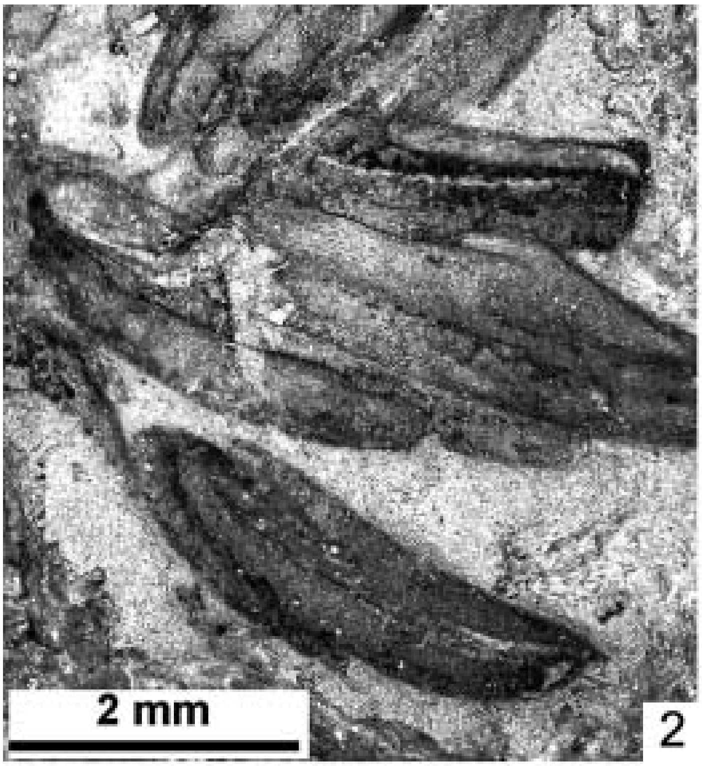
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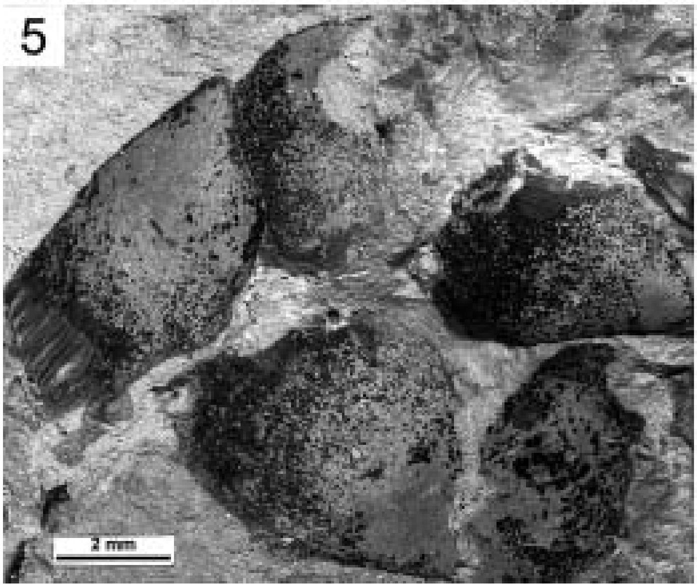
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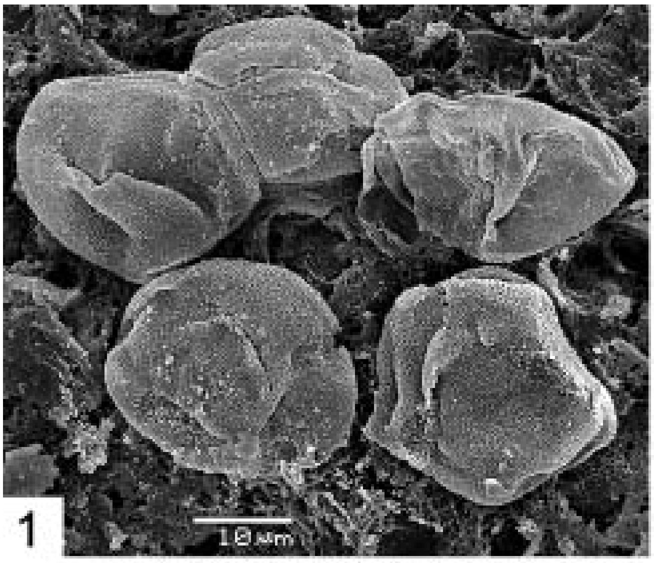
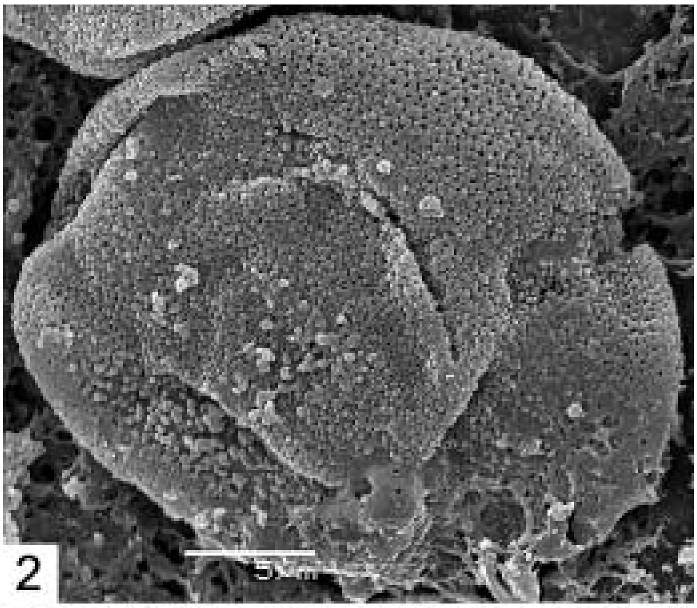
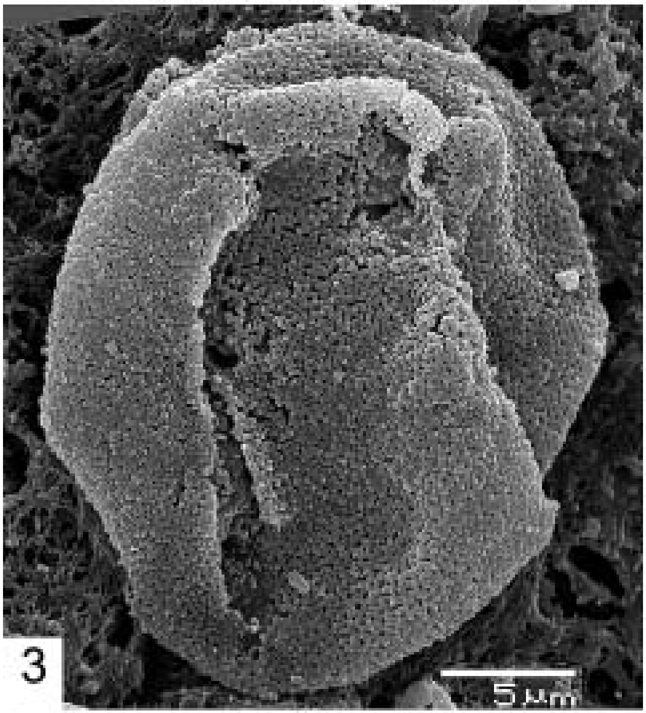
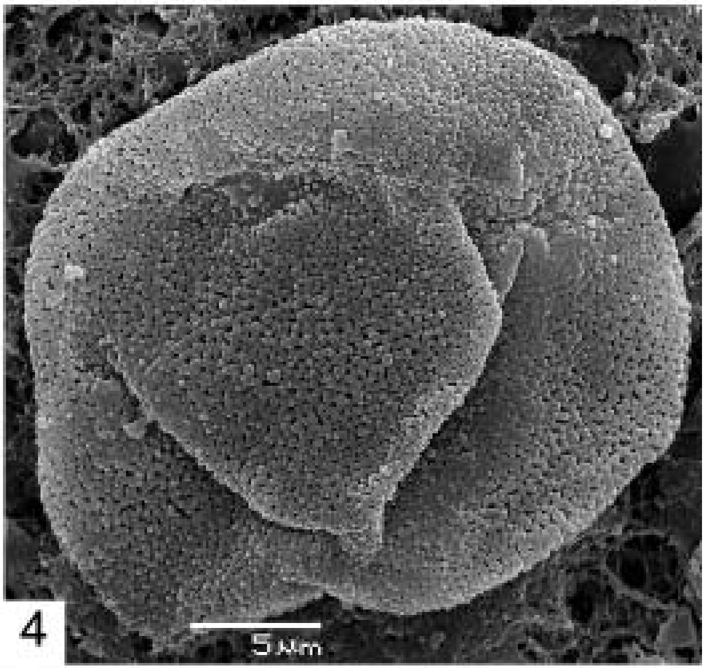
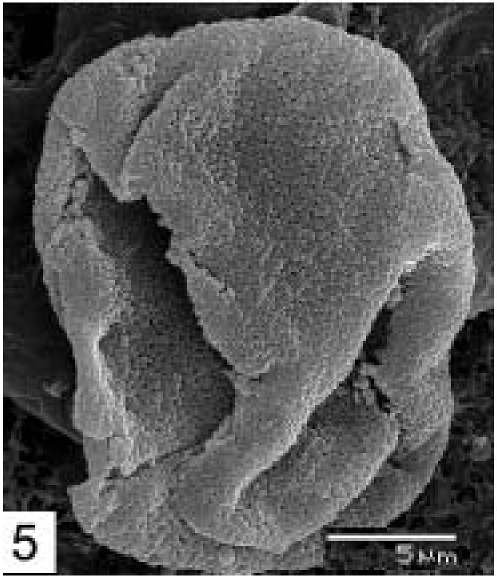
PLATE 2











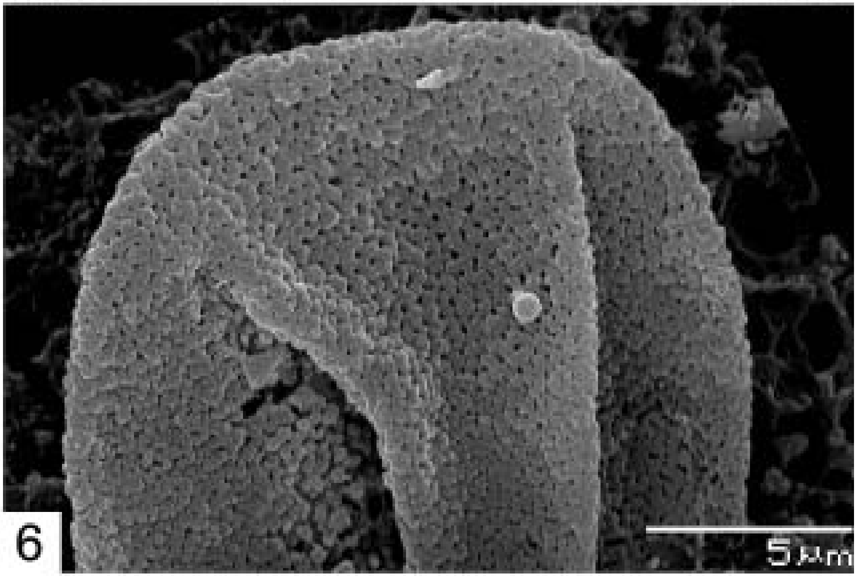
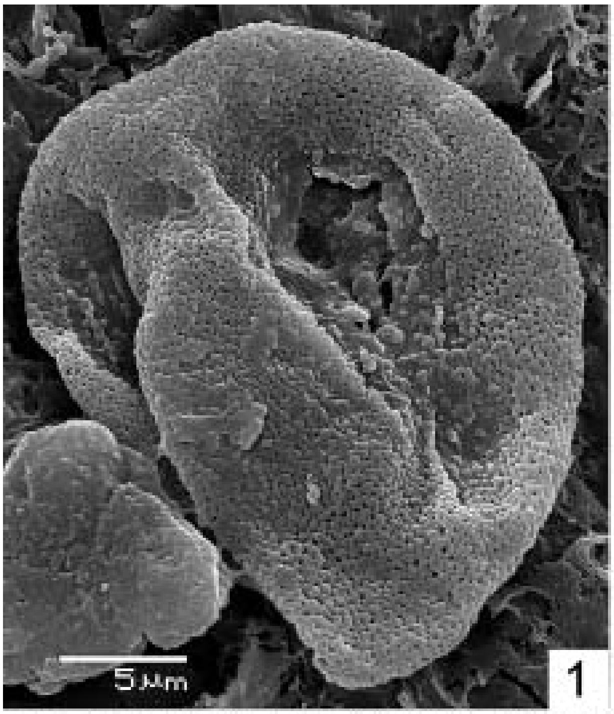


PLATE 3



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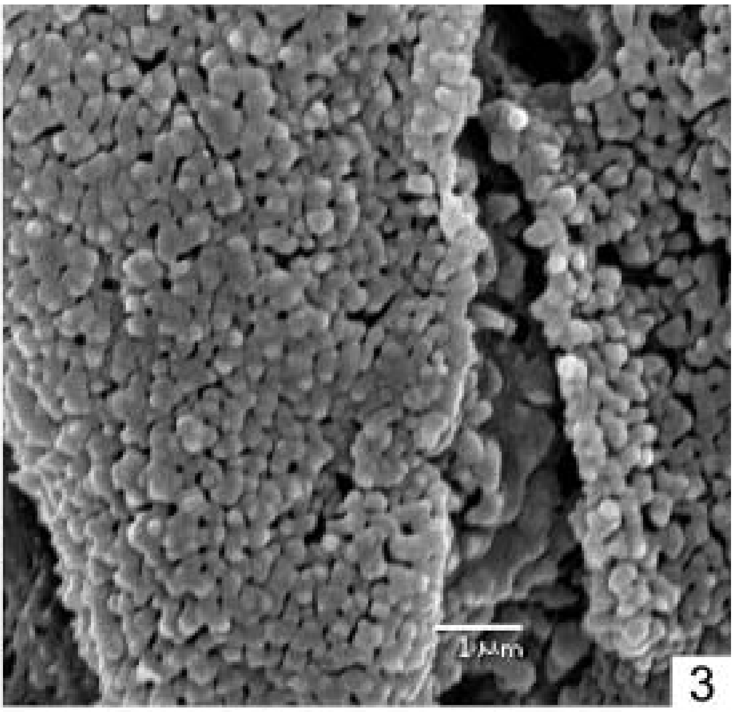

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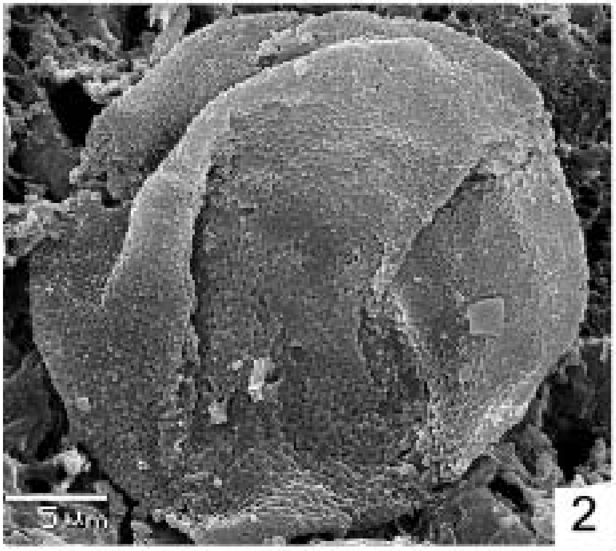

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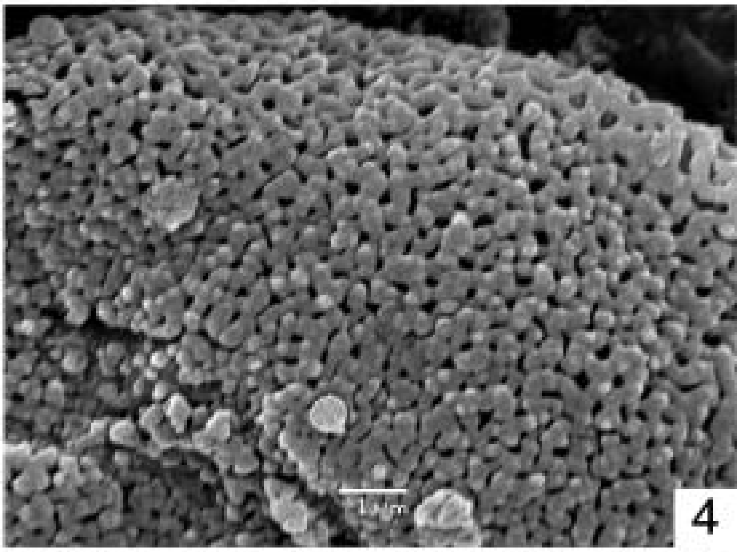

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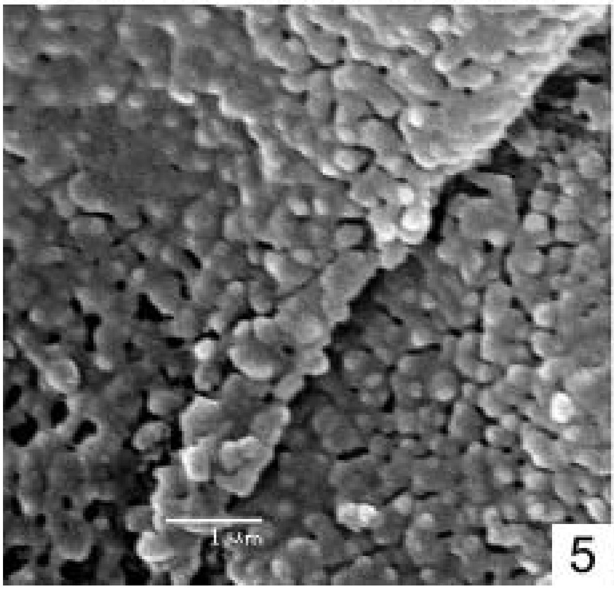

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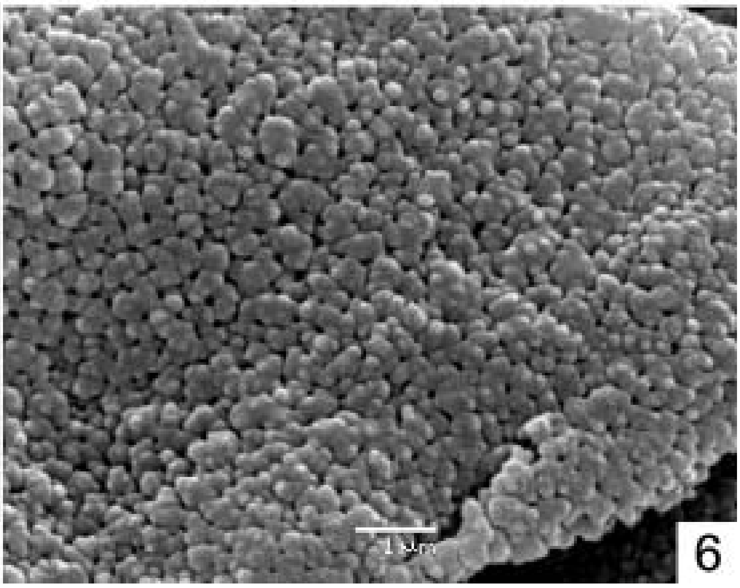

6

PLATE 4