



Sporoderm ultrastructure of some Devonian and Permian representatives of *Biharisporites* and their botanical affinity

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ABSTRACT

The megaspore genus *Biharisporites* has a very wide stratigraphic range, being recorded from the Devonian to the Cretaceous. However, *in situ* these megaspores are known only from the Middle–Upper Devonian, from archaeopteridalean sporangia. Post-Devonian producers of *Biharisporites* are so far unknown; the parent group can only be hypothesized from the spore morphology and ultrastructure, and from the composition of contemporaneous assemblages of macroremains. To contribute to the understanding of the botanical affinity of dispersed megaspores of the genus, we undertook a comparative ultrastructural study of several *Biharisporites* species from the Middle Devonian of Russia and the Lower Permian of India. Surprisingly, we found exclusively lycopsid variants of the sporoderm ultrastructure not only in the Permian spores, but also in the Devonian. Therefore, some megaspores of *Biharisporites* were produced by lycopsids even in the Middle Devonian. Megaspores of *Biharisporites* morphology have been produced by different groups of spore-bearing plants since the Middle Devonian, and the genus *Biharisporites* is heterogeneous.

KEYWORDS

dispersed megaspores; sporoderm ultrastructure; SEM; TEM; Middle Devonian; Lower Permian; Russia; India

1. Introduction

According to the initial diagnosis, the genus *Biharisporites* Potonié emend. Glasspool was established for dispersed megaspores with a circular to triangular amb, trilete rays of the scar exceeding 2/3 of the radius, distinct arcuate ridges, and numerous small coni at the apex (Potonié 1956). Later, Bharadwaj and Tiwari (1970) and Glasspool (2003, p. 19) added to the diagnosis the following information about the sculpture: ‘exine ornamented with coni, setae, or spinae of variable shape, size and density of distribution’ (Glasspool 2003). Furthermore, Glasspool (2003) removed from the diagnosis the information about the inner exosporium. Das et al. (2021, p. 5) re-emended the diagnosis by adding the following: ‘Inner body distinct, with or without cushions/pits’. We do not agree with this emendation, since inner exosporium features are difficult to evaluate in megaspores with a thick outer exosporium and a pronounced sculpture.

The type material of *Biharisporites* (type species *B. spinosus* (Singh) Potonié 1956) comes from the Lower Permian of Bihar, India. More than 30 species have been reported to date from Paleozoic and Mesozoic strata of all continents except Antarctica. The oldest *Biharisporites* species have been found in the Middle Devonian (e.g. Allen 1972; Marshall et al. 2007; present paper); and the youngest finds are dated to the Early Cretaceous (e.g. Batten and Kovach 1990; Tewari 2008). Most species of *Biharisporites* come from the Middle–Upper Devonian of North America (e.g. Allen 1972;

Chi and Hills 1976), Africa (Steenmans et al. 2011), Europe (e.g. Richardson 1965; Fuglewicz and Prejbisz 1981), and Asia (Marshall et al. 2007) and from the Permian–Triassic of Asia (e.g. Bharadwaj and Tiwari 1970; Feng et al. 2011; Joshi 2020) and South Africa (e.g. Glasspool 2003). Reports from the Carboniferous (e.g. Mune et al. 2012; Brazil), Jurassic (e.g. Batten and Kovach 1990; Poland) and Early Cretaceous (e.g. Tewari 2008, 2009; India) are sporadic and often unreliable.

Megaspores assignable to *Biharisporites* were extracted from the sporangia of archaeopteridalean plants, which are common in the Middle–Upper Devonian (e.g. Pettitt 1965; Phillips et al. 1972; Balme 1995), but post-Devonian producers of *Biharisporites* remained unknown. Feng et al. (2020) assigned to *Biharisporites* megaspores associated with the Permian isoetalean *Tomiostrabus sinensis* Feng. Although these spores resemble *Biharisporites* in general morphology, some of them possess a verrucate sculpture, which prevents their attribution to *Biharisporites*. Pant and Mishra (1986) hypothesized that megaspores (including *Biharisporites*) from the Upper Carboniferous and Permian strata of Lower Gondwana, India, belonged to lycopsids. This assumption was based on a frequent occurrence of heterospory in lycopsids and on the similarity in general morphology between these megaspores and *in situ* megaspores of lycopsids – in particular, the presence of spinae and processes in ornamentation. Pant and Mishra (1986, p. 16) believed that ‘*in situ* megaspores of no other group show such ornamentation’.

Although most Paleozoic megaspores were produced by heterosporous lycopsids (Scott and Hemsley 1996), the very existence of *in situ* archaeopteridalean megaspores that are similar to the dispersed genus *Biharisporites* contradicts the opinion expressed by Pant and Mishra (1986). Nevertheless, the similarity in general morphology between the archaeopteridalean megaspores and lycopsid megaspores allows us to assume that the Devonian megaspores of *Biharisporites* could have been produced not only by archaeopterids, but also by some lycopsids. We expect that a study of the morphology and ultrastructure will help to clarify the affinity of the megaspores of this genus. Therefore, the aim of the present study is to elucidate the morphology and wall ultrastructure of *Biharisporites* megaspores of different ages. For this purpose, we used a scanning electron microscope (SEM) and a transmission electron microscope (TEM) to study megaspores from the Middle Devonian of the Kursk Region of Russia and from the Lower Permian of Rajmahal Basin of India.

2. Previous research on the ultrastructure of some progymnosperm megaspores and *Biharisporites*-type megaspores

The ultrastructure of megaspores of *Biharisporites* type was first studied by Pettitt (1966) on *in situ* material from sporangia of *Archaeopteris* cf. *jacksonii* Dawson from the Upper Devonian of North America. According to Pettitt (1966), the sporoderm of the megaspores is two-layered: the outer layer is spongy (granular in transmitted light) and composed of three-dimensional, anastomosing sporopollenin units, and the inner layer is lamellate (laminated). The outer layer accounts for a greater part of the thickness of the wall. The structure of the outer layer of the megaspores studied by Pettitt has been interpreted in different ways. Doyle et al. (1975) interpreted the outer layer as granular, whereas Doyle (1978) and Doyle and Donoghue (1986) reinterpreted it as alveolar ('spongy alveolar'). Apparently, the difficulty of interpretation is caused by differences in the fossilization of megaspores and the mature state of the sporoderm.

Telnova and Meyer-Melikyan (1993) studied the ultrastructure of megaspores of *Biharisporites* type belonging to *Archaeopteris fimbriata* Nathorst (*Svalbardia fissilis* according to Orlova et al. 2016), *Archaeopteris* sp. 1, and *Archaeopteris* sp. 2. According to their paper, the sporoderm of the megaspores is similar to that described by Pettitt (1966). Orlova et al. (2020) described *in situ* megaspores of *Biharisporites* type from sporangia of the archaeopteridalean *Svalbardia* sp. as cavate with a much thicker outer granular layer and a thinner inner homogeneous layer. Towards the inner layer, the sporopollenin elements of the outer layer are less densely packed, and gaps between them are more spacious. An extended cavum is developed between the outer and inner layers.

Besides *Biharisporites*, archaeopteridalean sporangia are known to contain megaspores of *Contagisporites* type (Phillips et al. 1972; Jurina and Raskatova 2014). Marshall (1996) studied the distribution of miospores of a supposed progymnosperm affinity (*Rhabdosporites*, *Contagisporites*, and *Geminospora*) in the Middle Devonian strata of Scotland and

established a succession of related spore types – from *Rhabdosporites langii* of aneurophytalean affinity through 'early forms' of *Contagisporites* up to normal *Contagisporites* – whereas Wellman (2009) studied the ultrastructure of *Rhabdosporites langii* from the Middle Devonian of Scotland and substantiated a gradual evolutionary transition from *Rhabdosporites*-type spores of homosporous aneurophytaleans to spores of *Geminospora* and *Contagisporites* types of heterosporous archaeopterids.

Turnau et al. (2009) described for the first time the ultrastructure of dispersed megaspores of *Contagisporites optivus* and *Biharisporites? capillatus*. They interpreted as granular the sporoderm ultrastructure of the previously described *in situ* megaspores of *Archaeopteris* (Pettitt 1966; Telnova and Meyer-Melikyan 1993). The sporoderm ultrastructure of *Contagisporites optivus* was described as similar to the sporoderm ultrastructure of *in situ* megaspores from sporangia of archaeopterids. The sporoderm of megaspores of *Biharisporites? capillatus* was described as bilayered, with the outer layer alveolate and the inner layer homogeneous. The outer layer greatly exceeds the inner layer in thickness. The spores are cavate. Turnau and colleagues provided no definite conclusion about the botanical affinity of megaspores of *Biharisporites? capillatus*.

In addition, Turnau et al. (2009) analyzed the published literature and hypothesized that megaspores recovered from archaeopteridalean sporangia in Phillips et al. (1972) were related to three types: the 'early forms' *Contagisporites*, *C. optivus*, and *Biharisporites*. They proposed that not completely mature megaspores of archaeopteridaleans may be closer to *Biharisporites* in terms of their morphology, whereas mature spores demonstrate *Contagisporites* morphology. They suggested that the immature megaspores of archaeopteridaleans described so far are of *Biharisporites* appearance, while the more mature ones are of *Contagisporites* morphology. We assume that the idea of Turnau et al. (2009) is correct, but it must be proved on the basis of extensive factual material. Research on the ultrastructure of the *Biharisporites*-type megaspores from post-Devonian deposits has not yet been carried out.

3. Materials and methods

Part of the studied material (*Biharisporites arcticus* var. *productus* Chi & Hills) comes from the Middle Devonian strata of the Shchigry-16 borehole. The borehole is situated near the village of Nizhnekrasnoe, 20 km west-northwest of Shchigry, Kursk Region, Russia (Figure 1). The material was collected from sandstones of the Yastrebovskaya Formation (Figure 2) from a depth interval of 150.75–154.9 m. This formation corresponds to the lower part of the Pashiyen Regional Stage (Reshenie 1990). The base of the Pashiyen Regional Stage on the Eastern European Platform corresponds to the lower boundary of the Upper Givetian Stage coinciding with the Taghanic Event (Sobolev and Evdokimova 2008). Givetian (Middle Devonian) conodonts from the Shchigry-16 borehole were studied by Nazarova and Kononova (2020). According to their paper, conodonts are absent in the deposits of the Yastrebovskaya Formation of the Shchigry-16 borehole. Kanarkina et al. (2019) identified the following Late Givetian

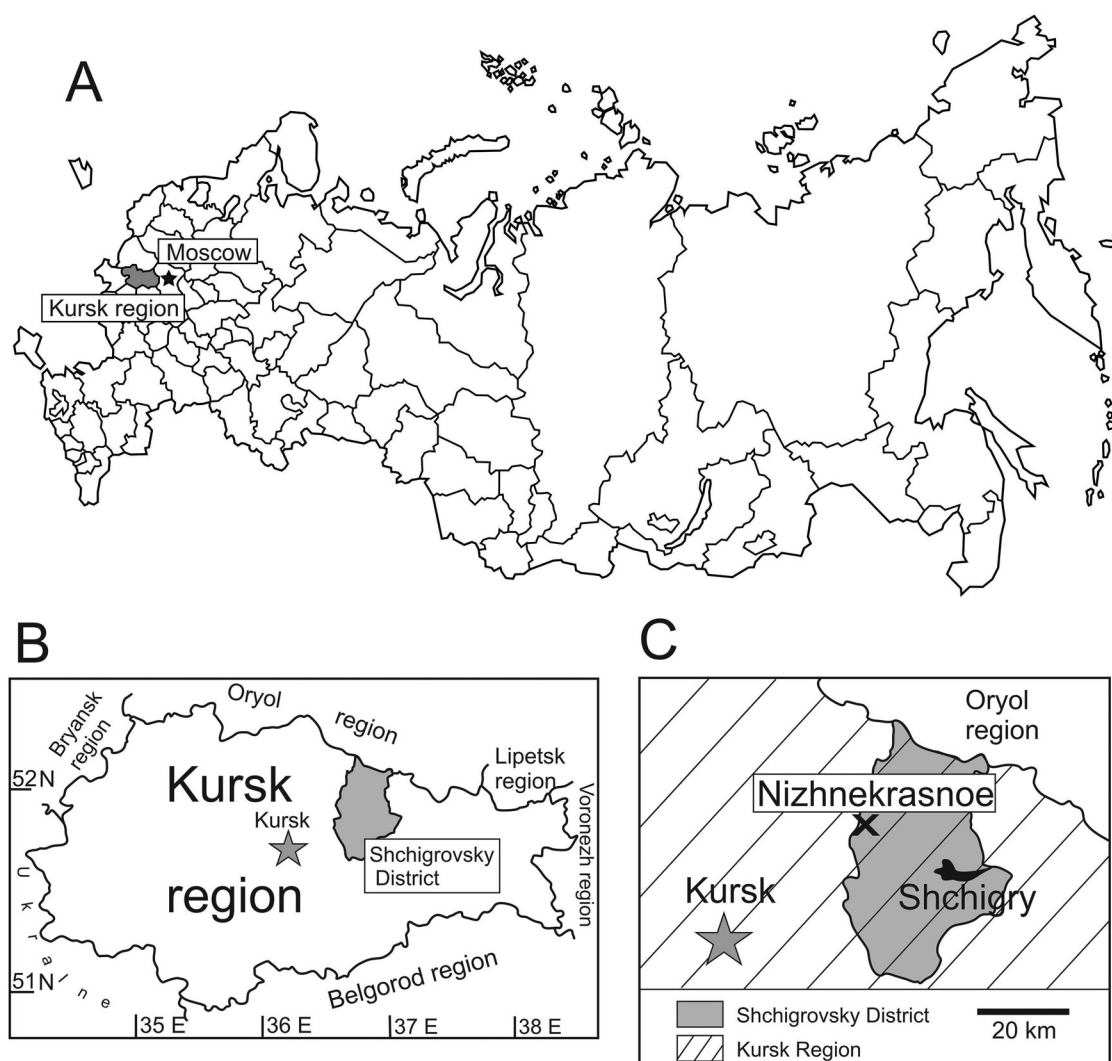


Figure 1. Location of the Shchigry-16 borehole (Nizhnekrasnoe). Source: Author.

assemblage of megaspores from this borehole: *Biharisporites capillatus* Fuglewicz & Prejbisz, *B. arcticus* Chi & Hills, *Biharisporites* sp., *Corystisporites acutispinosus* (Fuglewicz & Prejbisz) Turnau, *Granditetraspora zharkovae* Arkhangelskaya & Turnau, *Heliotriletes longispinosus* Fuglewicz & Prejbisz, and *Hystricosporites furcatus* Owens et al.

The rest of the studied material (four megaspores of *Biharisporites*) is from the Lower Permian strata (Figure 4) of the Rajmahal opencast mine, Hura Coalfield, Rajmahal Basin, India (Figure 3). The megaspores were collected from carbonaceous shales of the Barakar Formation (Coal Seam II; Singh and Singh 1996). The description of the Barakar Formation of the Hura Coalfield is given by Singh and Singh (1996).

The Devonian megaspores were isolated from the rock by hydrogen peroxide (55%) maceration followed by treatment with hydrofluoric acid (HF) and hydrochloric acid (HCl) (Oshurkova 2001). The Permian megaspores were obtained by HF and HCl maceration following Raevskaya and Shurekova (2011). The organic residue was sieved at 15 µm and then cleaned by ultrasonic dispersion at 35 kHz (Elmasonic Ultrasonic Cleaner).

Spores of *Biharisporites* were picked from the organic residue using a needle in reflected light under an Olympus CZ-

6045 stereomicroscope at the Department of Paleontology of the Lomonosov Moscow State University (MSU), mounted on SEM stubs and coated with gold and palladium. SEM observations were accomplished under a TESCAN VEGA-II XMU SEM (accelerating voltage 30 kV) at the Borissiak Paleontological Institute of the Russian Academy of Sciences (PIN).

The sporoderm structure of five species was studied in ultra- and semithin sections. In total, one Devonian and four Permian species were studied with these methods. The megaspores were embedded in a mixture of epoxy resins after Zavialova and Karasev (2017). The spores were polymerized for 2 days at 62 °C, oriented and cut. Sectioning was accomplished using a Leica EM UC6 ultramicrotome with a diamond knife at the PIN. Ultrathin sections (70 nm thick) were observed under a JEOL JEM-1011 fitted with a digital camera (ORIUS SC1000W) and processed using Digital Micrograph (GATAN) software (accelerating voltage 80 kV) at the electron microscopy laboratory of MSU, Biology Faculty.

Semithin sections 1.5 µm thick were observed under the SEM and in transmitted light under an Axioplan 2 Zeiss microscope at PIN. For SEM, the resin of semithin sections was dissolved using Maxwell's solution (Maxwell 1978) after Zavialova and Karasev (2017), with one modification: a

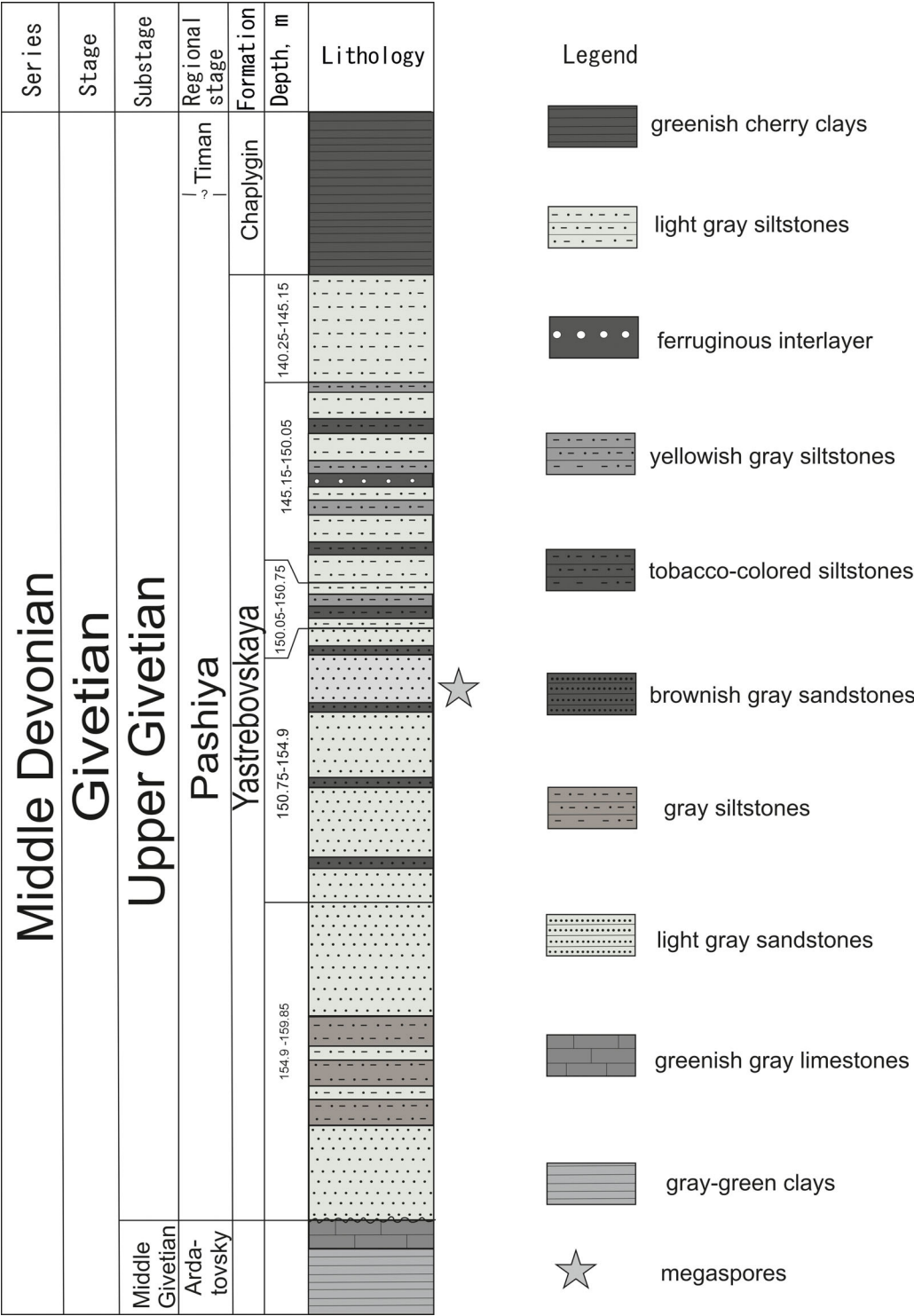


Figure 2. Stratigraphic column of the Shchigry-16 borehole (Yastrebovskaya Formation), and position of the megaspore samples.

Pipetman micropipette was used to handle semithin sections instead of an eyelash attached to a toothpick.

Polymerized blocks, grids with ultrathin sections, SEM and TEM micrographs, and stubs with semithin sections are stored at the Department of Paleontology (Faculty of Geology, MSU) under collection numbers 410 (Devonian species) and 409 (Permian species).

The term ‘laminate zones’ is used following Grauvogel-Stamm and Lugardon (2004). The term ‘laminae’ is used following Wellman (2002); however, when citing previous

studies, the terminology applied therein (which is sometimes different to that preferred herein) is used to avoid confusion.

4. Results

4.1. *Biharisporites arcticus* var. *productus* (Plate 1, figure 5; Plate 2; Supplementary Plates S1, S2; Figure 5)

The megaspore is trilete, with a circular amb, and about 400 µm in diameter. Labra are straight, raised, triangular in

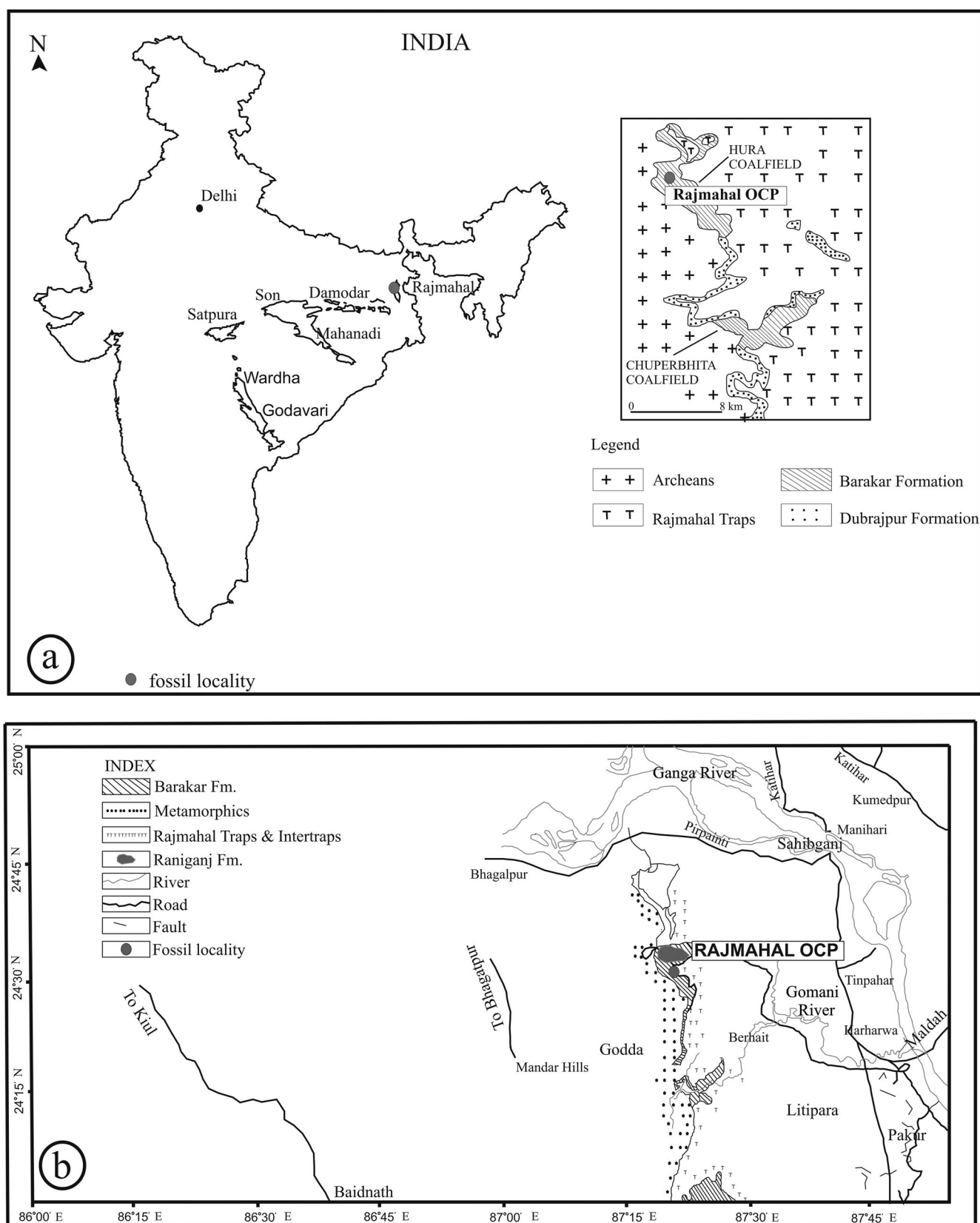


Figure 3. Location of the Rajmahal opencast mine and the fossil locality (after Eastern Coalfields Limited, India).

cross section, extending to half of the spore radius. The contact area is smooth, bounded by well-defined, high arcuate ridges. The proximal-equatorial and distal surfaces (Plate 1, figure 6) are ornamented with spines up to 60 μm in length.

The spinae are closely spaced, often curved or broken; the shortest spinae occur near the arcuate ridges.

The sporoderm is bilayered. The outer layer is composed of rod-like units that are curved and coiled. The rod-like units

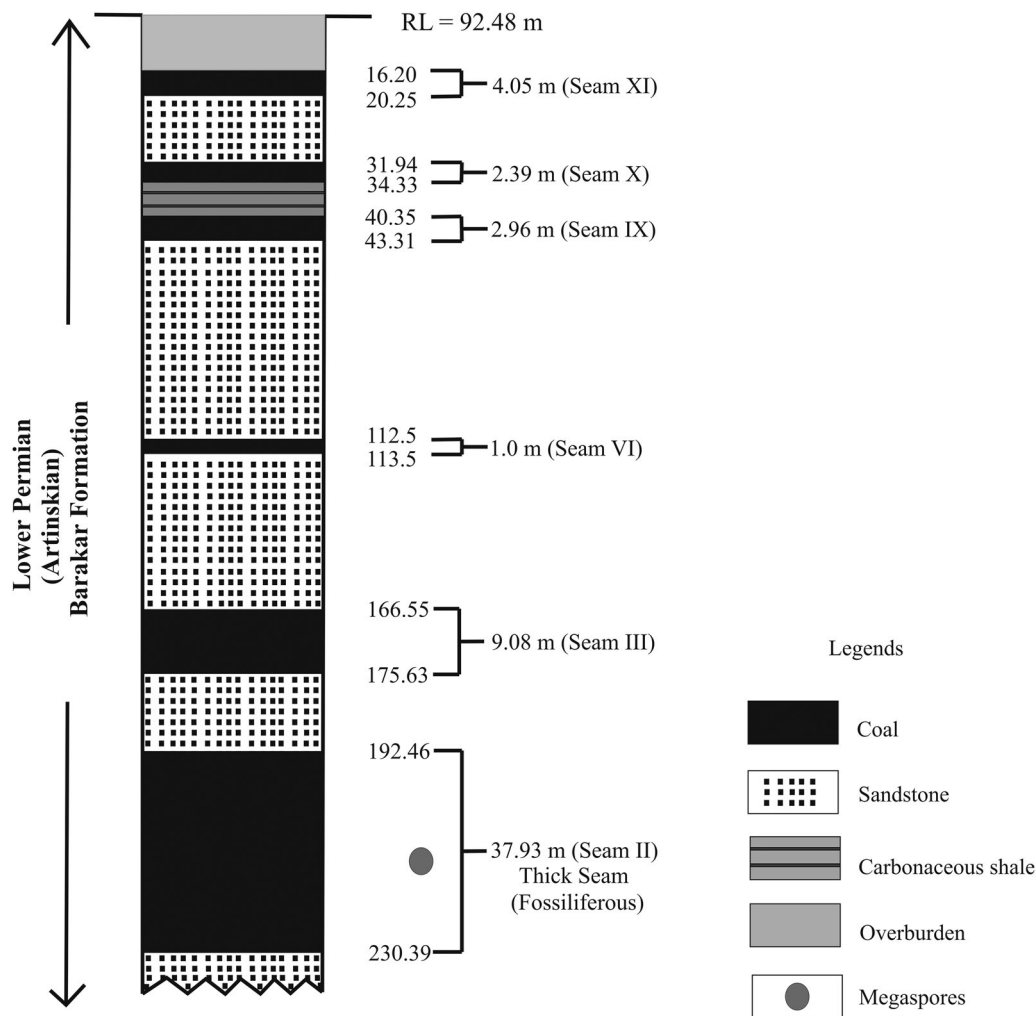


Figure 4. Stratigraphic column of the Rajmahal opencast mine (Barakar Formation), and position of the megaspore samples.

are round in cross section (Supplementary Plate S1, figure 4). The layer varies in thickness from $22\mu\text{m}$ in the contact area (Plate 2, figure 1, c.a.; Supplementary Plate S1, figures 1, 2) to $30\mu\text{m}$ in the apertural region (Plate 2, figure 1, asterisk, figure 2; Supplementary Plate S2, figure 1), and to $40\mu\text{m}$ in the distal region (Plate 2, figure 1; Supplementary Plate S1, figures 1, 3). The outer layer is divided into three sublayers (Plate 2, figure 3; Supplementary Plate S1, figures 2, 3). The sublayers differ in the size of their units and the gaps between them. In particular, the middle sublayer is characterized by the largest units and the largest spaces between them; the inner sublayer is characterized by the smallest units.

The units of the outer sublayer decrease in size toward the outside of the spore and become so small that they resemble granules (Supplementary Plate S1, figure 5). The sublayer is $10\mu\text{m}$ thick (not including the height of the sculptural elements) over the entire spore (Plate 2, figure 3; Supplementary Plate S1, figures 1–3), except the laesural region where it becomes thinner, down to $6\mu\text{m}$ (Plate 2, figures 1, 2; Supplementary Plate S2, figure 1). The sculptural elements are formed by densely spaced units (Plate 2, figure 3, white arrow; Supplementary Plate S1, figure 3, 5).

The middle sublayer is present only outside the contact area (Plate 2, figures 1, 2; Supplementary Plate S1, figures 1–3), wedging out at the boundary of the contact area (Plate 2, figure 1, w.o.; Supplementary Plate S1, figures 1, arrows, 2). The sublayer varies in thickness; it is thicker under sculptural elements (Plate 2, figure 3; Supplementary Plate S1, figure 3). This sublayer rises in such places, slightly penetrating into the bases of the sculptural elements. It reaches its maximum thickness under the sculptural elements and is equal to $20\mu\text{m}$, and the minimum thickness is half that. Structural elements of the middle sublayer are more stretched and flattened compared to those of other sublayers. Another feature of this sublayer is the vertical orientation of the spaces between the units.

The roughness left by the knife on the inner sublayer testifies to its high hardness (Supplementary Plate S1, figure 2, white arrows). However, it becomes softer near the inner layer. The thickness of the inner sublayer is $8\text{--}10\mu\text{m}$ and it is uniform outside the contact area, where it is $13\mu\text{m}$ thick reaching $23\mu\text{m}$ in the apertural region (Supplementary Plate S1, figure 1). In the distal part of the megaspore, the units are flattened and concentrically aligned. In the proximal part of the megaspore, the units are situated more randomly.

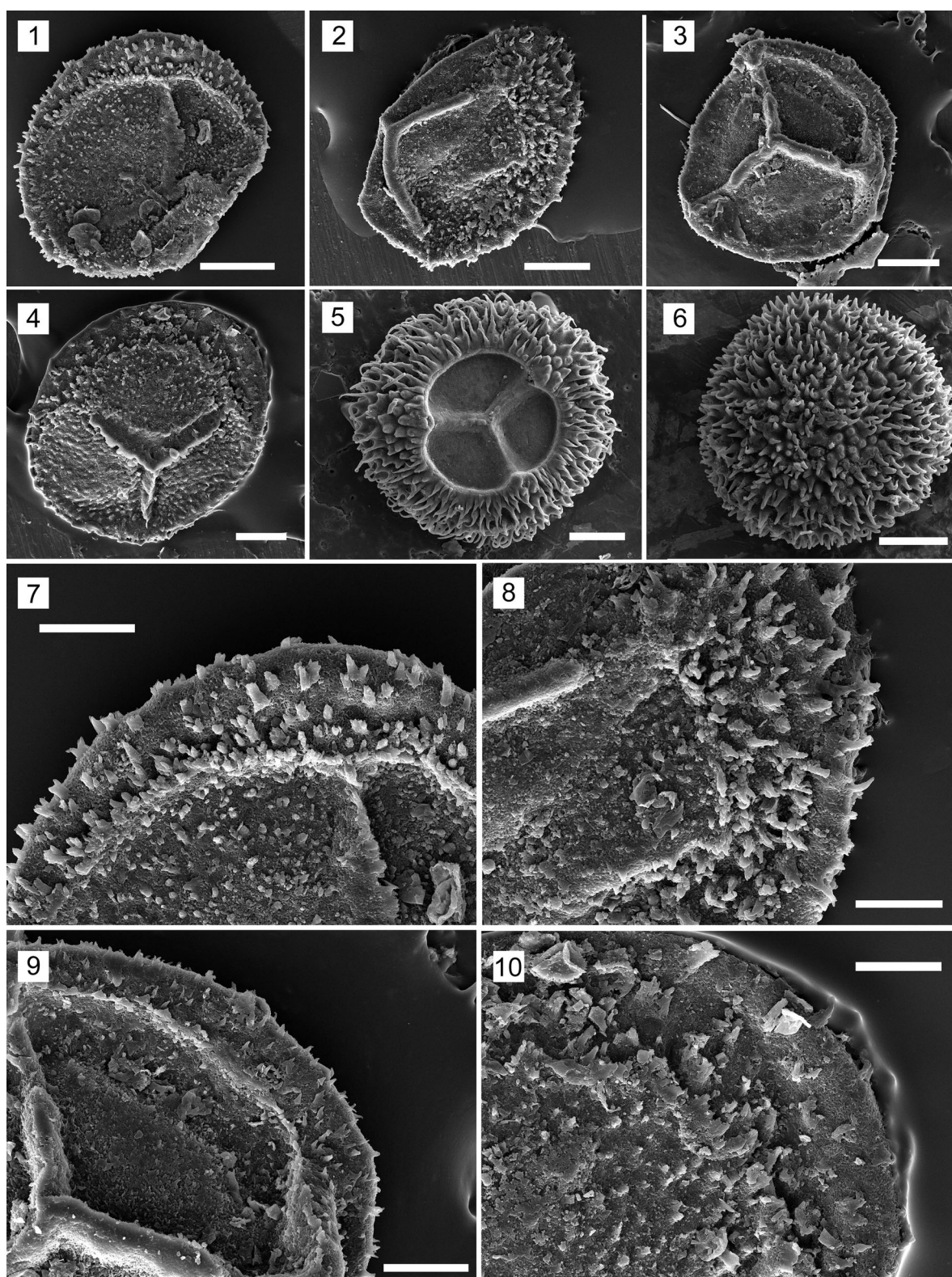


Plate 1. Images of megaspores of *Biharisporites* Potonié. All photomicrographs taken with a scanning electron microscope. 1–4, 7–10. Megaspores from the Lower Permian of the Rajmahal Basin, India. 5, 6. Megaspores from the Upper Givetian of the Kursk Region, Russia. 1. *Biharisporites* cf. *spinosus*, specimen 409-1, proximal face; the ultrastructure is shown in Plate 3 (figures 1–4), Supplementary Plate S3 (figures 1–3). 2. *Biharisporites boralii* Bajpai, specimen 409-2, proximal-equatorial view; the ultrastructure is shown in Plate 4 (figures 1–4), Supplementary Plate S4 (figures 1–4). 3. *Biharisporites* sp. 1, specimen 409-3, proximal face; the ultrastructure is shown in Plate 5 (figures 1–6), Supplementary Plate S5 (figures 1–3). 4. *Biharisporites* sp. 2, specimen 409-4, proximal face; the ultrastructure is shown in Plate 6 (figures 1–3), Supplementary Plate S5 (figures 4, 5). 5. *Biharisporites arcticus* var. *productus* Chi & Hills 1976, specimen 410-03, proximal face; the ultrastructure is shown in Plate 2 (figures 1–5), Supplementary Plate S1 (figures 1–5), and Supplementary Plate S2 (figures 1, 2). 6. *Biharisporites arcticus* var. *productus* Chi & Hills, specimen 410-04, distal face. 7. *Biharisporites* cf. *spinosus*, enlargement of figure 1 showing the surface of the spore. 8. *Biharisporites boralii*, enlargement of figure 2 showing the surface of the spore. 9. *Biharisporites* sp. 1, enlargement of figure 3 showing the surface of the spore. 10. *Biharisporites* sp. 2, enlargement of figure 4 showing the surface of the spore. Scale bar: 1–6 = 100 μ m; 7–10 = 50 μ m.

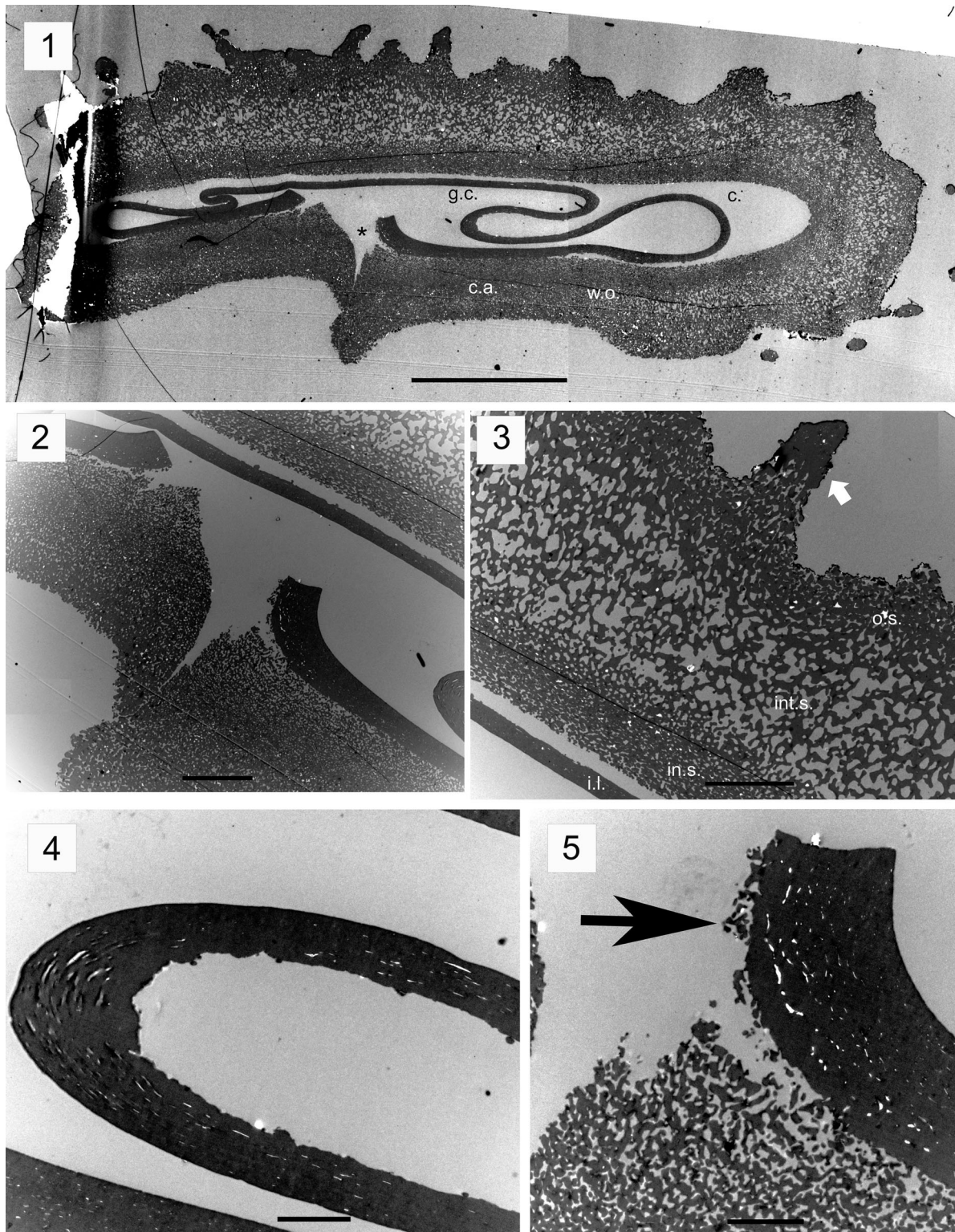


Plate 2. Megaspore ultrastructure of *Biharisporites arcticus* var. *productus* Chi & Hills, specimen 410-03 (Upper Givetian of the Kursk Region, Russia), all photomicrographs taken with a transmission electron microscope; the general morphology is shown in Plate 1 (figure 5). 1. Composite image of the section showing bilayered sporoderm, cavity between the layers (c.), gametophyte cavity (g.c.), and the contact area (c.a.); note the intermediate sublayer of the outer layer of the sporoderm (w.o.) that wedges out at the contract area and the apertural region (asterisk). 2. Enlargement of figure 1 showing the apertural region. 3. Fragment of the distal sporoderm, showing sublayers of the outer layer: outer sublayer (o.s.), intermediate sublayer (int.s.), inner sublayer (in.s.), and inner layer (i.l.). The white arrow indicates a sculptural element formed by the outer sublayer of the outer layer. 4. Fragment of the inner layer of the sporoderm showing gaps between the laminae that constitute this layer. 5. Enlargement of figure 2, which clearly shows the laminate structure of the inner layer of the sporoderm, as well as the cavity between the inner and outer layers near the ray of the trilete mark. The black arrow points to the units of the outer layer. Scale bar: 1 = 50 μm ; 2, 3 = 10 μm ; 4, 5 = 3 μm .

The inner layer is laminate (Plate 2, figures 4, 5; Supplementary Plate S2, figures 1, 2). The sections clearly show the gaps between the laminae that constitute this

layer. The layer is composed of approximately eight laminae. The layer is 2 μm thick distally, reaching 5 μm in the contact area and up to 10 μm under the aperture. The inner layer is

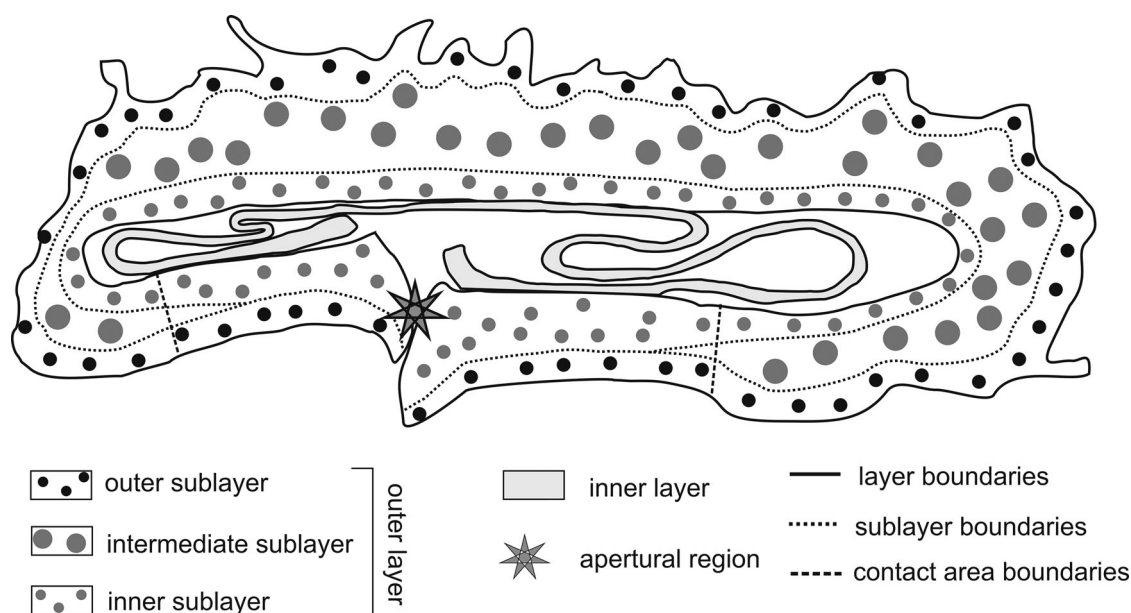


Figure 5. Diagrammatic representation of the sporoderm of *Biharisporites arcticus* var. *productus* Chi & Hills.

slightly more electron-dense than the outer layer. The difference in electron density suggests some differences in the chemical composition of sporopollenin.

The spore is cavate. The cavity is situated within the inner sublayer in the distal and proximal-equatorial areas (Plate 2, figure 1, c.; Supplementary Plate S1, figure 1) and is absent in the contact area (Plate 2, figure 2; Supplementary Plate S1, figure 1). Separated units of the outer layer can sometimes be seen attached to the inner layer (Plate 2, figure 5, black arrow).

4.2. *Biharisporites cf. spinosus* (Plate 1, figures 1, 7; Plate 3; Supplementary Plate S3; Figure 6)

The megaspore is trilete, with a circular amb, and about 300 μm in diameter. Labra are straight, raised, flat in cross section, extending to 2/3 of the spore radius. The contact area is ornamented with tiny coni, bounded by well-defined, high arcuate ridges. The proximal-equatorial and distal surfaces are ornamented with coni and spinae of different sizes. Their height ranges from 5 μm to nearly 20 μm . The specimen fits the diagnosis of *Biharisporites spinosus* in all characters except for more widely spaced sculptural elements; in particular, the distance between neighboring elements is greater than the element width.

The sporoderm is bilayered. The outer layer varies in thickness from 10 to 30 μm (Plate 3, figure 1; Supplementary Plate S3, figure 1). It consists of cylindrical units with a diameter of about 0.5 μm or less (Plate 3, figures 2–4; Supplementary Plate S3, figures 2, 3). The units of the proximal face (contact area) are extended wavy laminae (Plate 3, figures 2, 3). The units of the distal face are more compressed and ragged. The units are greatly reduced near the inner layer and arms of the proximal scar (Plate 3, figure 1, asterisk; Supplementary Plate S3, figure 1, asterisk). Bending and joining, the units form a three-dimensional network.

There is a large number of rounded elements in the plane of the section (Supplementary Plate S3, figure 3, arrowheads). These are transversely cut cylindrical units. The sculptural elements are homogeneous (Supplementary Plate S3, figure 1, white arrows). They have a base rooted in the outer wall layer.

In general, the inner layer appears homogeneous, but lamination is occasionally observed (Plate 3, figures 3, 4; Supplementary Plate S3, figures 2, 3). The thickness of the inner layer is less than 1 μm on the proximal wall and two or three times thinner on the distal wall, except in laminated zones (Plate 3, figure 3, black arrows, 4; Supplementary Plate S3, figure 1, black arrow, 2, white arrow). The inner layer is split into about six intervening laminae, each approximately 0.5 μm thick. This zone is about 5 μm long and 3 μm thick (at its thickest part). There are regions where cavities occur between the two wall layers (Supplementary Plate S3, figure 3, white arrows). It may be the result of mechanical damage.

4.3. *Biharisporites boralii* (Plate 1, figures 2, 8; Plate 4; Supplementary Plate S4; Figure 7)

The megaspore is trilete, with a circular amb, and about 400 μm in diameter. Labra are straight, raised, and circular in cross section; they extend to half of the spore radius and do not reach the arcuate ridges. The contact area is ornamented with tiny coni and bounded by well-defined, low arcuate ridges. The proximal-equatorial and distal surfaces are ornamented with spinae and bacules (which may represent broken spinae) up to 15 μm in length. Some spinae have split tips.

We have not detected a continuous inner sporoderm layer consistently lining the entire perimeter of the gametophyte cavity (Plate 4, figure 1; Supplementary Plate S4, figure 1), and we lean toward the conclusion that either the sections are situated in too peripheral an area of the spore, where

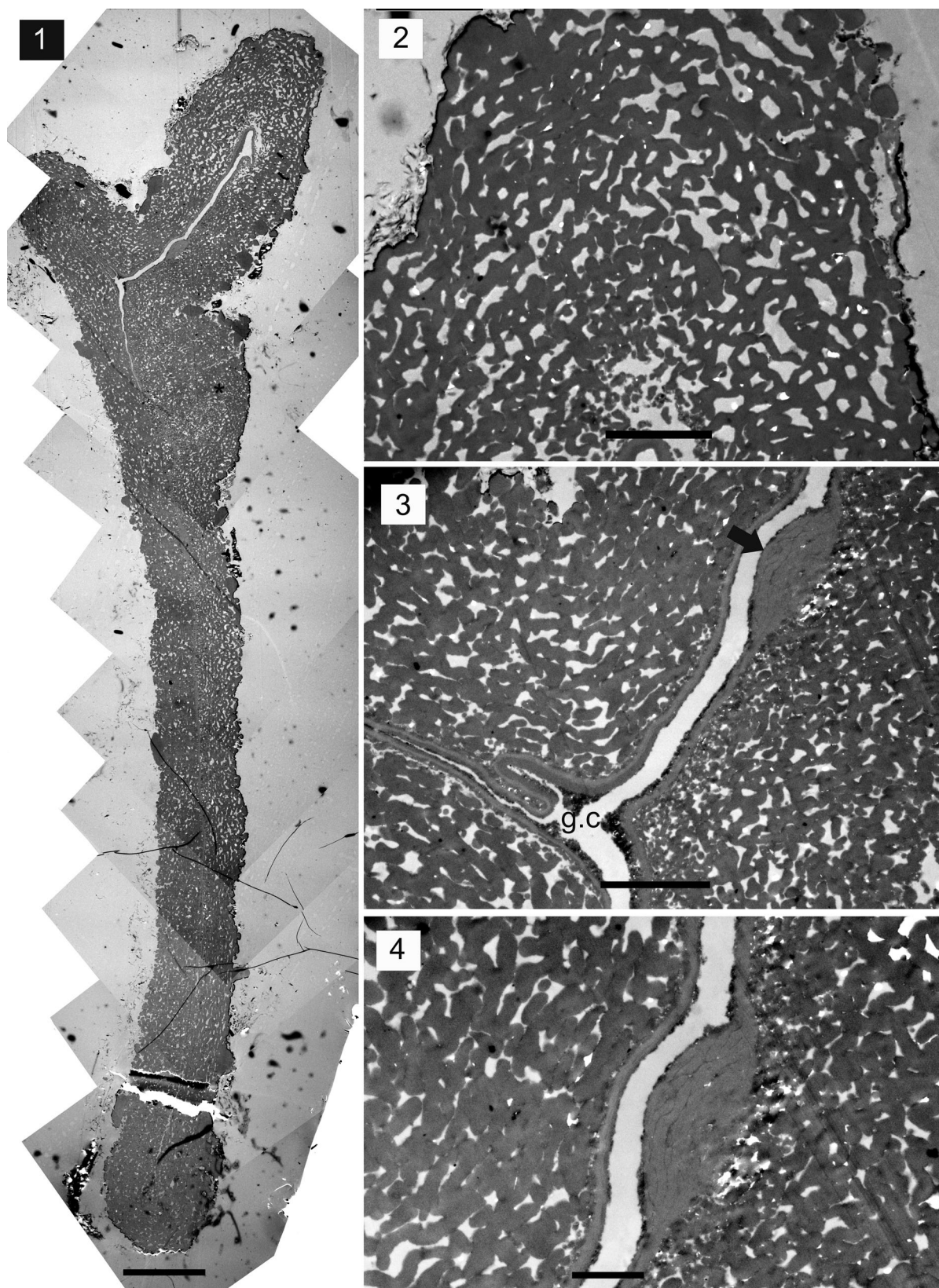


Plate 3. Megaspore ultrastructure of *Biharisporites* cf. *spinosus*, specimen 409-1 (Lower Permian of the Rajmahal Basin, India), all photomicrographs taken with a transmission electron microscope; the general morphology is shown in Plate 1 (figure 1). 1. Composite image of the section showing bilayered sporoderm. 2. Enlargement of figure 1 showing the structure of the outer layer. 3. Enlargement of figure 1 showing the gametophyte cavity (g.c.) and the inner layer splitting into a laminated zone (black arrow). 4. Enlargement of figure 3 showing the structure of the inner layer and the laminated zone. Scale bar: 1 = 20 μm ; 2, 3 = 5 μm ; 4 = 2 μm .

the inner layer is still lacking, or the layer was not preserved. Therefore, the observed sporoderm is interpreted as the outer layer alone (Plate 4, figures 1–4; Supplementary Plate

S4, figures 1–4). It is subdivided into a thicker outer sublayer consisting of larger units and a thinner inner sublayer consisting of smaller units.

The outer sublayer of the outer layer consists of extended cylindrical units with a diameter of about 1 µm (Supplementary Plate S4, figure 2–4). These units are slightly smaller near the inner border of the sublayer. The sublayer is 7–15 µm thick and reaches 20 µm only in the region of the proximal scar (Plate 4, figure 1, asterisk, figure 4). The units of the outer sublayer also form sculptural elements (Plate 4, figure 1, arrowheads).

The inner sublayer consists of smaller units of an obscure shape (Plate 4, figures 2–4, black arrows). It is about 1–2 µm thick, crumpled into folds (Plate 4, figure 3) and often separated from the outer sublayer by a narrow cavity (Supplementary Plate S4, figure 2). The proximal and distal portions of the sublayer are often joined together (Plate 4, figure 2, black arrow; Supplementary Plate S4, figure 4).

4.4. *Biharisporites* sp. 1 (Plate 1, figures 3, 9; Plate 5; Supplementary Plate S5, figures 1–3; Figure 8)

The megaspore is trilete, with a circular amb, and 360 µm in diameter. Labra are straight, raised, and circular in cross section, extending to 3/4 of the spore radius. The contact area is ornamented with tiny coni, bounded by well-defined, high arcuate ridges. The proximal-equatorial and distal surfaces are ornamented with coni and spinae up to 6 µm in length.

The sporoderm is bilayered (Plate 5, figures 1–5; Supplementary Plate S5, figures 1–3). The outer layer consists of extended cylindrical elements. The units of the proximal face (contact area) are fused and form a clearly distinguishable network, mostly radially oriented; near the inner layer, the units become more parallel to the surface. The units of the distal face are shorter and flattened parallel to the surface of the megaspore (Plate 5, figures 2–6; Supplementary Plate S5, figures 2, 3). The innermost part of the outer layer consists of small granular elements. The average thickness of the outer layer is 13 µm. It becomes thicker and reaches 26 µm in the laesural region. An arm of the trilete mark is expressed as a semicircular elevation on the sections (Plate 5, figure 1, white asterisk, figure 4, white asterisk). The sculptural elements are homogeneous (Plate 5, figure 6, black arrows; Supplementary Plate S5, figure 3, black arrow).

The inner layer is laminate, less than 1 µm thick proximally (Plate 5, figure 3, black arrow, figure 5, black arrow) and two times thicker distally (Plate 5, figure 3, white arrow, figure 5, white arrow). We observed two laminated zones near the sectioned arm of the trilete mark. There are probably more than two laminated zones (it is usually so in megaspores), but our sections included two of them. One of them is larger (Plate 5, figure 4, black arrow) and probably was cut in its central part; the other is small (Plate 5, figure 4, gray arrow) and probably was cut in its periphery. The large zone is about 6 µm long and 3 µm thick (Plate 5, figure 2, black asterisk), and the small zone is 3 µm long and 1 µm thick (Plate 5, figure 3, gray asterisk). The large zone is split into more than 10 intervening laminae. There are cavities between the inner and outer layers of the distal sporoderm (Plate 5, figure 1, white arrows) and between the proximal and distal parts of the outer layer in the equatorial part of

the megaspore (Plate 5, figure 1, black arrows). Thus, the spore is cavate.

4.5. *Biharisporites* sp. 2 (Plate 1, figures 4, 10; Plate 6; Supplementary Plate S5, figures 4, 5; Figure 9)

The megaspore is trilete, with a circular amb, and about 400 µm in diameter. The arms of the trilete mark are straight, raised, and flat in cross section, extending to 3/4 of the spore radius. The contact area is ornamented with tiny coni, bounded by well-defined, low arcuate ridges. The proximal-equatorial and distal surfaces are ornamented with coni and spinae. Their sizes range from very small to about 15 µm in height and 10 µm in diameter at the base. The sculptural elements are widely spaced over the entire surface of the spore, with the exception of the curvature region, where they are situated closer to each other.

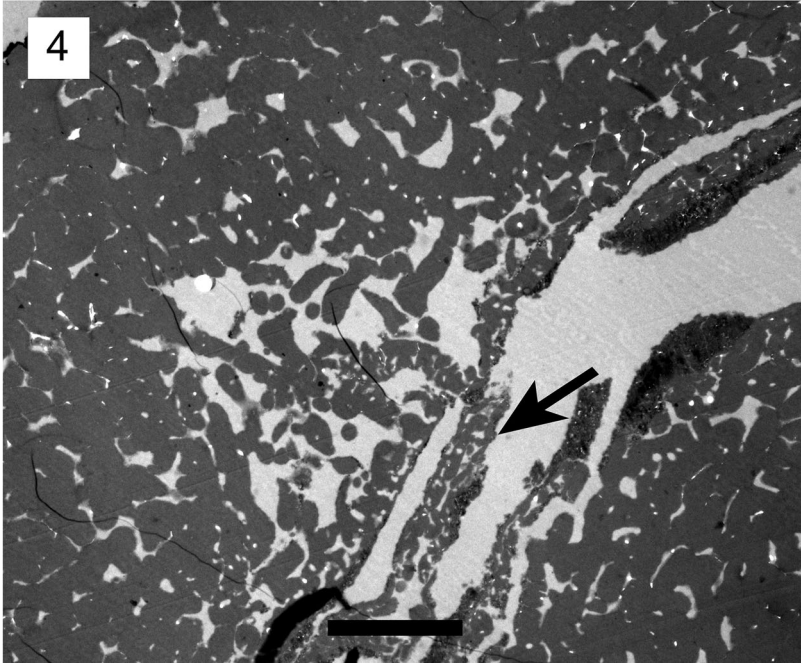
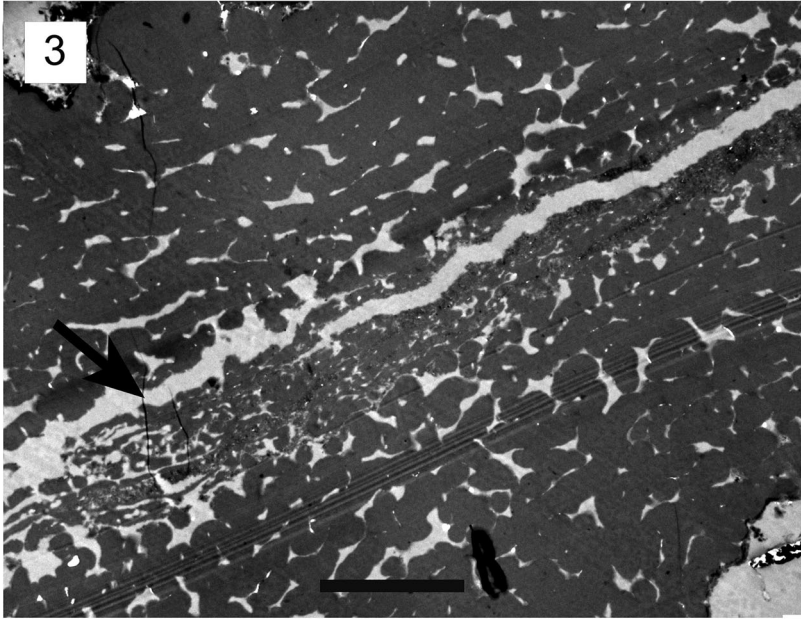
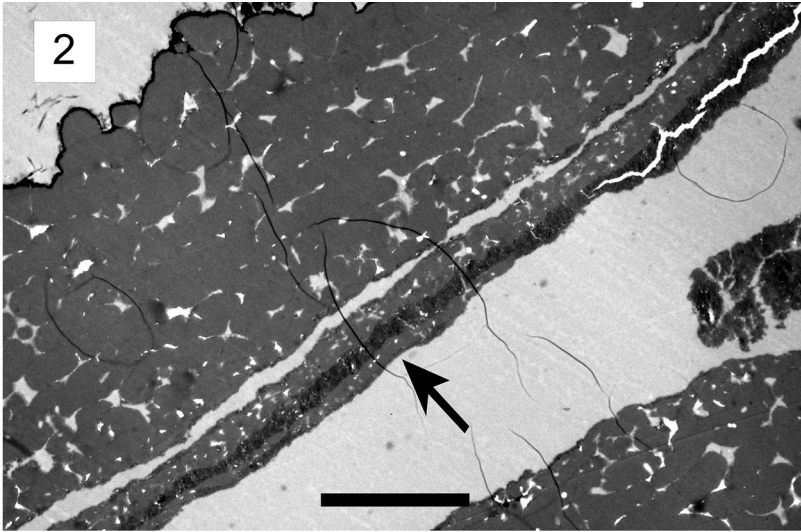
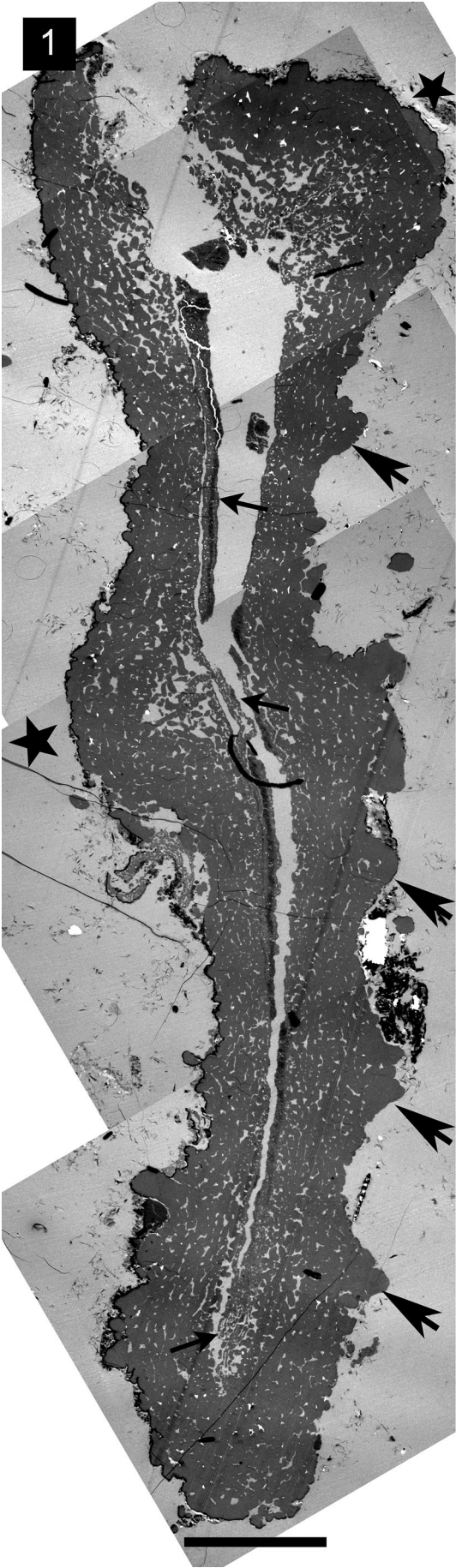
The sporoderm is bilayered (Plate 6, figures 1–3; Supplementary Plate S5, figure 4). The outer layer consists of elongated cylindrical units that are bent and connected together (Plate 6, figures 2, 3; Supplementary Plate S5, figure 5). The diameter of the units that constitute this layer is less than or equal to 1 µm. The diameter of the units diminishes markedly near the inner layer. The layer varies in thickness from 8 µm to 15 µm. The sculptural elements are formed by elements of the outer layer (Plate 6, figure 1, black arrows).

The inner layer is represented by a basal lamina (Plate 6, figures 2, 3, black arrows; Supplementary Plate S5, figure 4, black arrows), which is very thin. In the proximal part, the inner layer is attached to the outer one. In the distal part, there is a cavity between the layers (Plate 6, figure 2, black arrows). Often, the proximal and distal parts of the inner layer are joined together and pressed to the proximal sporoderm (Plate 6, figure 3, black arrow).

5. Discussion

We have obtained data on the sporoderm ultrastructure of *Biharisporites arcticus* var. *productus* from the Upper Givetian strata of Russia and *B. cf. spinosus*, *B. borealii*, *B. sp. 1*, and *B. sp. 2* from the Lower Permian sedimentary rocks of India. The megaspore sporoderm of *Biharisporites* of all the species is bilayered, with an outer layer that is much thicker than the inner one. The outer layer is composed of cylindrical units that bend and join to form a complex three-dimensional network.

The outer layer of *B. arcticus* var. *productus* has a different structure not only within and outside the contact area, but also at its different depths (at different distances from the sporoderm surface). The outer layer of *B. sp. 1* and *B. cf. spinosus* has a different structure within and outside the contact area. In the Permian species, the units are closely packed, mostly elongated, and parallel to the surface of the megaspore; units are distributed quite regularly along the wall of the megaspore, but noticeably decrease in size near the inner layer. In the outer layer of *B. borealii*, an inner sublayer is distinguishable that consists of small units; this sublayer is separated from the rest of the layer by a cavity.



The sculptural elements represent a continuation of the outer layer in all species studied. However, the sculptural elements in *B. boralii* have a denser structure than the outer layer. The sculptural elements are homogeneous in *B. cf. spinosus* and *B. sp. 1*.

The inner layer is laminate in *B. arcticus* var. *productus* and *B. sp. 1* and homogeneous in *B. cf. spinosus*, and is represented by a basal lamina in *B. sp. 2*. In *B. boralii* the inner layer could not be seen, probably because either the sections passed in a very peripheral area of the spore or the preservation of the spore is of insufficient quality. Laminated zones have been observed in the inner sporoderm layer of the Permian *B. cf. spinosus* and *B. sp. 1*.

As reviewed above, the sporoderm of the *in situ* archaeopteridalean megaspores of the *Biharisporites* type consists of two layers: a much thicker outer layer that is interpreted as granular or alveolar ('spongy alveolar'), and a thinner inner layer that is lamellate (laminate) or homogeneous. Thus, the sporoderm structure of the megaspores studied here is not identical to that of the *in situ* megaspores of the *Biharisporites* type described earlier (Pettitt 1966; Telnova and Meyer-Melikian 1993; Orlova et al. 2020).

Both groups of megaspores (*in situ* megaspores of the *Biharisporites* type studied earlier and dispersed ones studied in the present work) have a two-layered sporoderm with a thicker outer layer and a thinner inner layer. The inner layer in both groups is lamellate (laminate) or homogeneous. However, we detected laminated zones in this layer in spores of two Permian species. Laminated zones are absent in archaeopteridalean megaspores of *Biharisporites* type (Pettitt 1966; Telnova and Meyer-Melikian 1993; Orlova et al. 2020) and dispersed megaspores of *Biharisporites? capillatus* (Turnau et al. 2009), *B. arcticus* var. *productus* and *B. sp. 2* (present study). Laminated zones occur in megaspores of some fossil lycopsids and microspores of some fossil and extant lycopsids (Lugardon et al. 1999; Grauvogel-Stamm and Lugardon 2004; Wellman et al. 2009; Orlova et al. 2017). Most of the megaspores that possess laminated zones are from fossil lycopsids of the isoetalean and possibly selaginellalean lineages, indicating that megaspores with laminated zones are characteristic of the Isoetales and, less confidently, of the Selaginellales (Grauvogel-Stamm and Lugardon 2004). The presence of such zones in *B. cf. spinosus* and *B. sp. 1* indicates their affinity with lycopsids – and, moreover, more likely with the isoetalean lineage than the selaginellalean one. The thickness of the inner layer is not uniform in both *B. cf. spinosus* and *B. sp. 1*. However, the inner layer is thicker proximally in *B. cf. spinosus* and distally in *Biharisporites* sp. 1. Similar ratios of the thickness of the inner layer as in *B. cf. spinosus* (the distal part is two to three times thinner than the proximal one) are observed in megaspores of Triassic

age such as *Pleuromeia sternbergii* which also possess laminated zones (Grauvogel-Stamm and Lugardon 2004).

A basal lamina (we detected it in *Biharisporites* sp. 2) is also not a characteristic feature of archaeopteridalean megaspores, but it is common in the Upper Carboniferous lepidodendroid megaspores (Scott and Hemsley 1996).

The dispersed spores studied by us differ from *in situ* archaeopteridalean megaspores of *Biharisporites* type by the structural elements of the outer layer. There are granules in the archaeopteridalean megaspores and rod-like units that bend and join together to form a three-dimensional network in the dispersed megaspores in the present study. The outer layer of *B. arcticus* var. *productus* is subdivided into three sublayers. The outer and inner sublayer units are similar in their small size. The units of the outer sublayer are rounded and resemble granules. The units of the inner sublayer are angular; distally, they are elongated and parallel to the surface of the megaspore. The middle sublayer is present only outside the contact area; its units are angular, separated by large unclosed cavities. A similar structure of the outer layer is observed in megaspores of *Setosisporites praetextus* (Zerndt) Potonie & Kremp from the Upper Carboniferous of the UK (Hemsley and Scott 1991). Its outer sporoderm is divided into three regions: an outer granular region, a spongy middle region with numerous cavities, and an inner laminate region. This structure is very similar to the structure of the outer layer of *Biharisporites arcticus* var. *productus* outside the contact area. It should be noted that megaspores of the *Setosisporites praetextus* type were extracted from a cone of the isoetalean *Bothrodendrostrobus watsonii* (Chaloner 1967).

Units of the outer layer resembling the units of the middle sublayer of *Biharisporites arcticus* var. *productus* were documented in the sporoderm of Carboniferous megaspores of *Lagenosporites nudus* (Taylor 1990, plate IV, 19), where angular elements separated by prominent open cavities form a net. The network constitutes the largest part of the wall thickness. Units of the inner portion of the outer layer are arranged parallel to the surface. The inner layer of *L. nudus* megaspores is a basal lamina, unlike *Biharisporites arcticus* var. *productus*, with its laminate inner layer. The structure of the inner sublayer within the contact area of *B. arcticus* var. *productus* is similar to the *Caboehonicus* type of the ultrastructure of Mesozoic selaginellalean megaspores erected by Kovach (1994, fig. 4). According to Kovach, units of this type have a distinct angular character, rather than being rounded as in most of the laterally fused (type of ultrastructure erected by Taylor 1989) patterns.

Units of the outer layer of the sporoderm of *Biharisporites* cf. *spinosus* are represented by wavy laminae, which are slightly finer and shorter near the apertural region. In other areas, they also vary in length, but this is probably due to varying orientation of the elements toward the plane of the

Plate 4. Megaspore ultrastructure of *Biharisporites boralii* Bajpai, specimen 409-2 (Lower Permian of the Rajmahal Basin, India), all photomicrographs taken with a transmission electron microscope; the general morphology is shown in Plate 1 (figure 2). 1. Composite image of the section showing the arms of the proximal scar (asterisks), inner sublayer (black arrow), and sculptural elements that are composed of the outer layer (black arrowheads). 2. Fragment of the section showing sublayers of the outer layer; the inner sublayer is indicated with a black arrow. 3. Fragment of the sporoderm in the equatorial area; note the inner sublayer (black arrow). 4. The apertural region. Black arrow points to the inner sublayer. Scale bar: 1 = 20 µm; 2–4 = 5 µm.

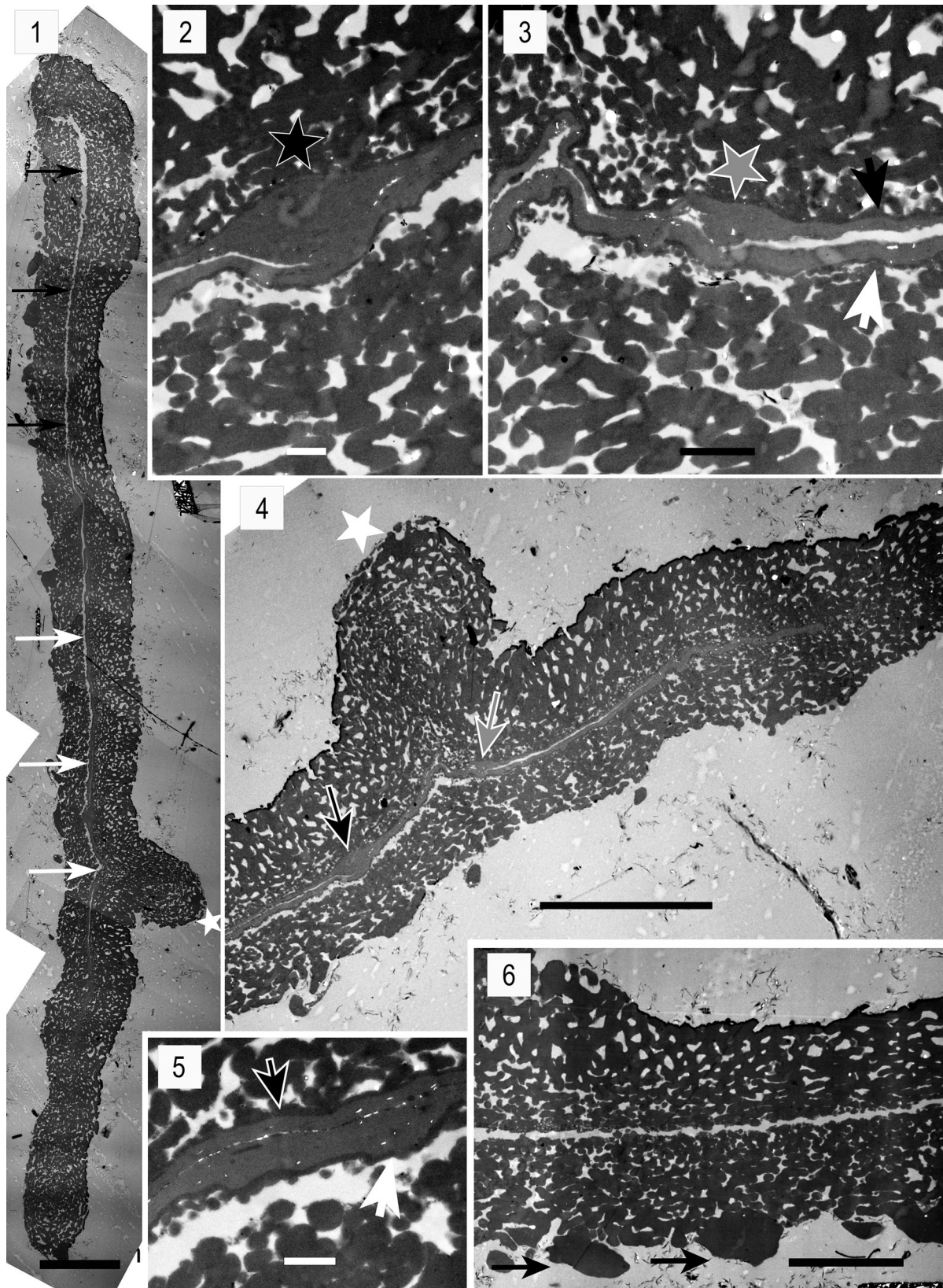


Plate 5. Megaspore ultrastructure of *Biharisporites* sp. 1, specimen 409-3 (Lower Permian of the Rajmahal Basin, India), all photomicrographs taken with a transmitted electron microscope. 1. Composite image of the section showing the sporoderm, the apertural region (asterisk), and cavities between the inner and outer layers of the distal sporoderm (white arrows) and between the proximal and distal face of sporoderm in the equatorial part of the megaspore (black arrows). 2. Enlargement of figure 1 showing a laminated zone probably cut in its central part (black asterisk). 3. Enlargement of figure 1 showing a laminated zone presumably cut at its periphery (gray asterisk), and the distal (white arrowhead) and the proximal (black arrowhead) parts of the inner layer. 4. The apertural region (asterisk). The black arrow points to a laminated zone presumably cut at its central area, the gray arrow points to a laminated zone presumably cut in its periphery. 5. Enlargement of figure 1 showing thickness of the distal (white arrow) and the proximal (black arrow) parts of the inner layer. 6. Fragment of the section showing the structure of sculptural elements (black arrows). Scale bar: 1, 4 = 20 μm ; 2, 5 = 1 μm ; 3 = 2 μm ; 6 = 10 μm .

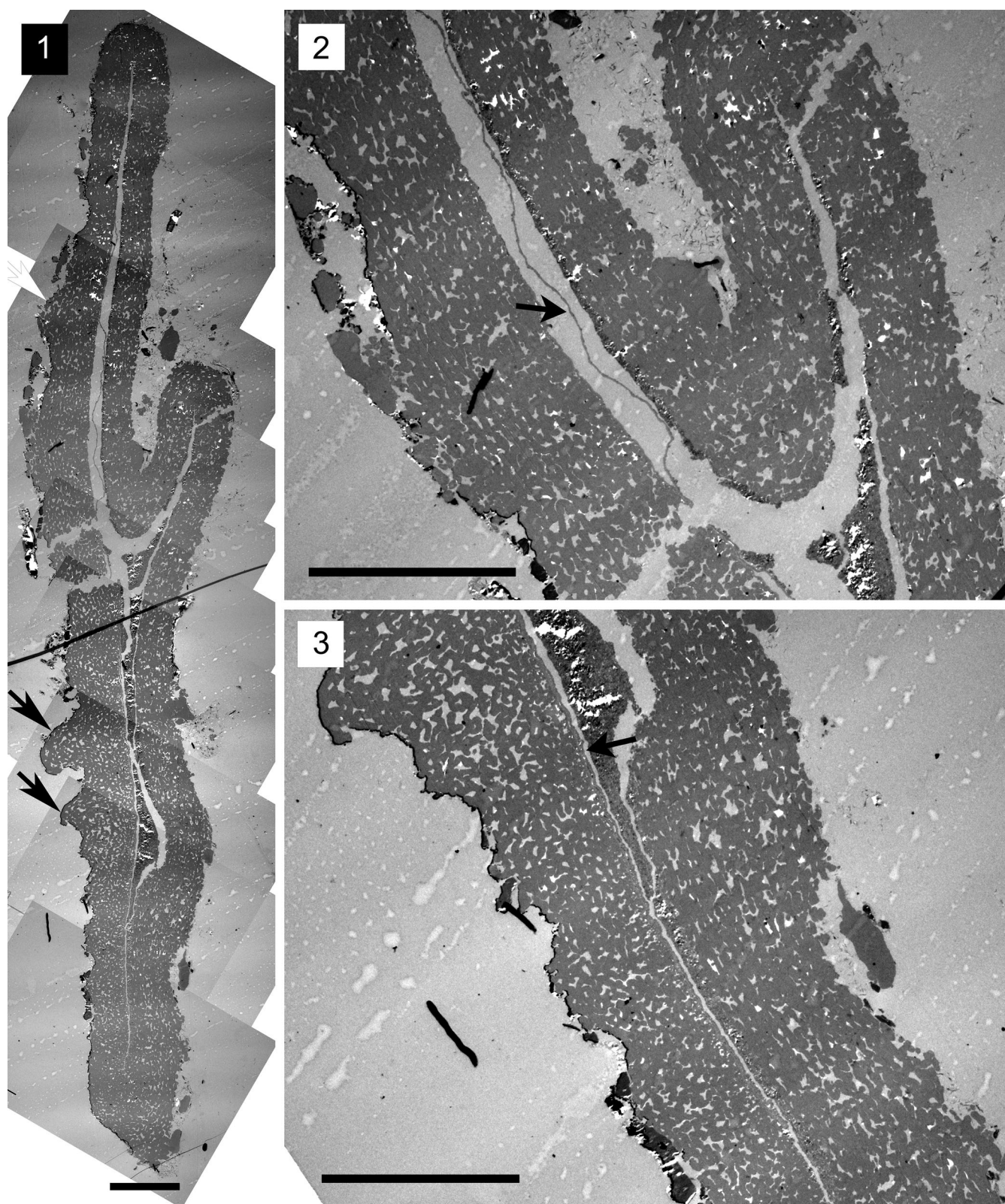


Plate 6. Megaspore ultrastructure of *Biharisporites* sp. 2, specimen 409-4 (Lower Permian of the Rajmahal Basin, India), all photomicrographs taken with a transmitted electron microscope. 1. Composite image of the section showing the sporoderm and the structure of sculptural elements (black arrows). 2. Fragment of the section. The black arrow points to the inner layer. 3. Fragment of the section showing the structure of the outer layer and the inner layer (black arrow). Scale bar: 20 μ m.

section. It is not entirely clear whether the distal and proximal units are principally different or merely appear different because the section passed through different areas of the units and they are oriented differently toward the plane of

the section. The outer layer of the proximal face of *B. cf. spinosus* (Plate 3) is most similar to the structure of the outer layer in the laesural region of the megaspore of the *Valvisporites auritus* type from the Upper Devonian of Ohio,

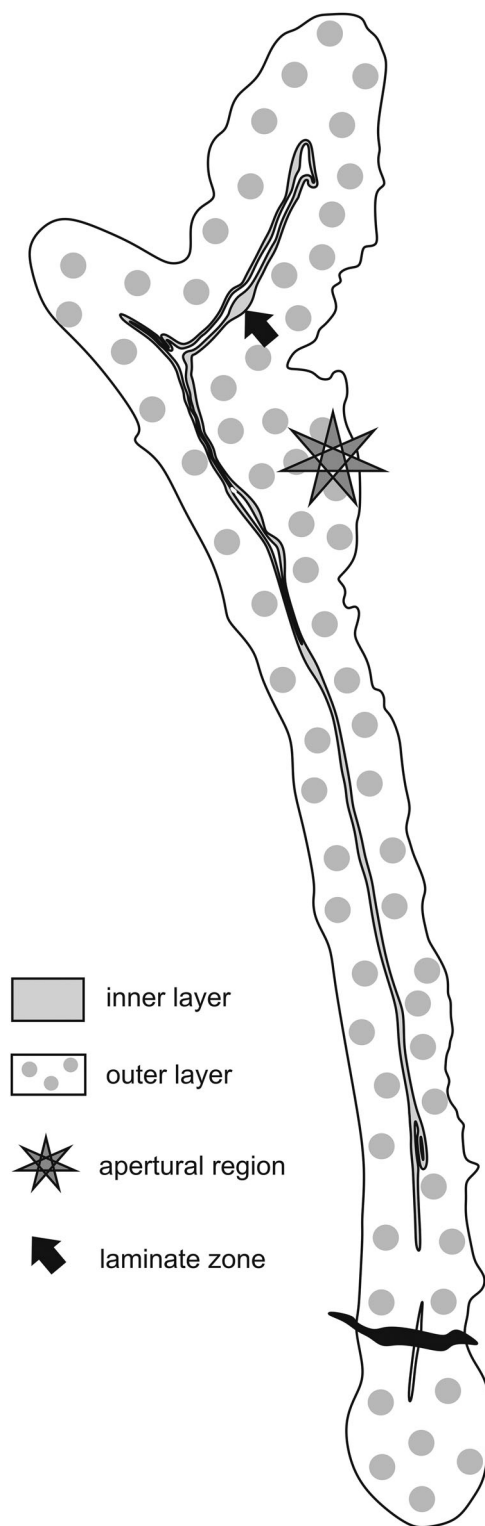


Figure 6. Diagrammatic representation of the sporoderm of *Biharisporites cf. spinosus*.

USA (Bek et al. 2009, plate VI, 1). The following was written about the structure of the laesural region: 'The inner layer and the overlying elements of the outer layer form some small irregular wrinkles, but do not show any special apertural differentiation' (Bek et al. 2009).

In general, the outer layer of Devonian *Valvisporites auritus* is described as consisting of elements closely packed in the innermost part and more separated and widened and

irregularly shaped toward the outer surface. In *Biharisporites cf. spinosus*, the entire outer layer is formed by densely packed elements, especially on the distal face, probably due to compression. However, the outer layer of Pennsylvanian megaspores of *Valvisporites auritus* (Taylor 1990) is described as consisting of plate-like laminae oriented parallel to the megaspore surface and often appressed to one another due to the high degree of compression. This structure of the outer layer is more similar to that of *Biharisporites cf. spinosus*.

The structures of the inner layer of *B. cf. spinosus* and *Valvisporites auritus* are different. The inner layer of *Biharisporites cf. spinosus*, with the exception of the laminated zones, is homogeneous with barely noticeable delamination. The inner layer of the *Valvisporites auritus* type is described as partly amorphous–partly laminate, without registered laminated zones (Bek et al. 2009). However, similar zones are present in the Carboniferous megaspores of *Valvisporites*, for example in *V. sculptus* (Glasspool et al. 2009). Most species of *Valvisporites* are from the Carboniferous. In the Carboniferous, megaspores of *Valvisporites* were produced by sub-arborescent isoetalean lycopsids of the genera *Chaloneria* and *Polysporia* (Bek et al. 2008).

The proximal and distal faces of the megaspore of *Biharisporites sp. 1* differ in structure. As in the case of *B. cf. spinosus*, it is not entirely clear whether this difference is caused by different types of structural elements or different cut planes of elements of the same type. Ultrastructurally, the outer layer of the proximal sporoderm of *Biharisporites sp. 1* has features in common with that of the Mesozoic *Nathorstisporites hopliticus* of isoetalean affinity (Kempf 1971): the units are small in the vicinity of the inner layer, but become thicker and irregularly oriented closer to the surface. Kempf (1971) interpreted the units as threads, which are fine and concentrically arranged far from the surface and become thicker and radially arranged toward the sporoderm surface. The sporoderm of *Biharisporites sp. 1* is much more compressed than the sporoderm of *Nathorstisporites hopliticus*, and small elements far from the surface resemble granules. *Biharisporites sp. 1* shares some structural features with Carboniferous megaspores of *Setosisporites hirsutus* var. *brevispinosa* (Kempf 1973; Hemsley 1992). The outer layer has the same structural pattern: small units at the base, parallel inner units, and an outer radially oriented network. Spores similar to *Setosisporites hirsutus* have been found in sporangia of the lycopsid *Porostrobis canonbiensis* (Balme 1995).

Hemsley (1997) reflected on the impact of protoplast expansion on the sporoderm structure in lycopsid megaspores. The sporoderm looks different in cases where it formed simultaneously with the expansion of the spore versus when sporoderm deposition stopped before the fully expanded size of the spore was reached. Different layers of the sporoderm will be stretched and compressed to different degrees. Hemsley (1997) mostly focused on deeper and outer layers of the sporoderm. We think that the differences in size

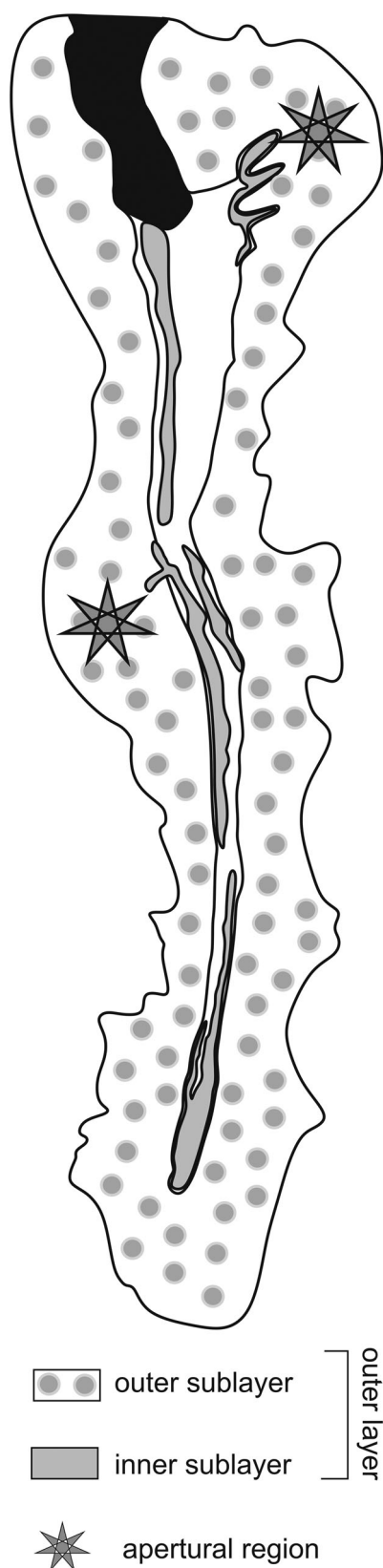


Figure 7. Diagrammatic representation of the sporoderm of *Biharisporites borali* Bajpai.

and orientation of structural elements that we observed between the proximal and distal sporoderms in our material may be explained similarly: some areas of the sporoderm might have been affected to a greater degree than others by the pressure of the developing protoplast.

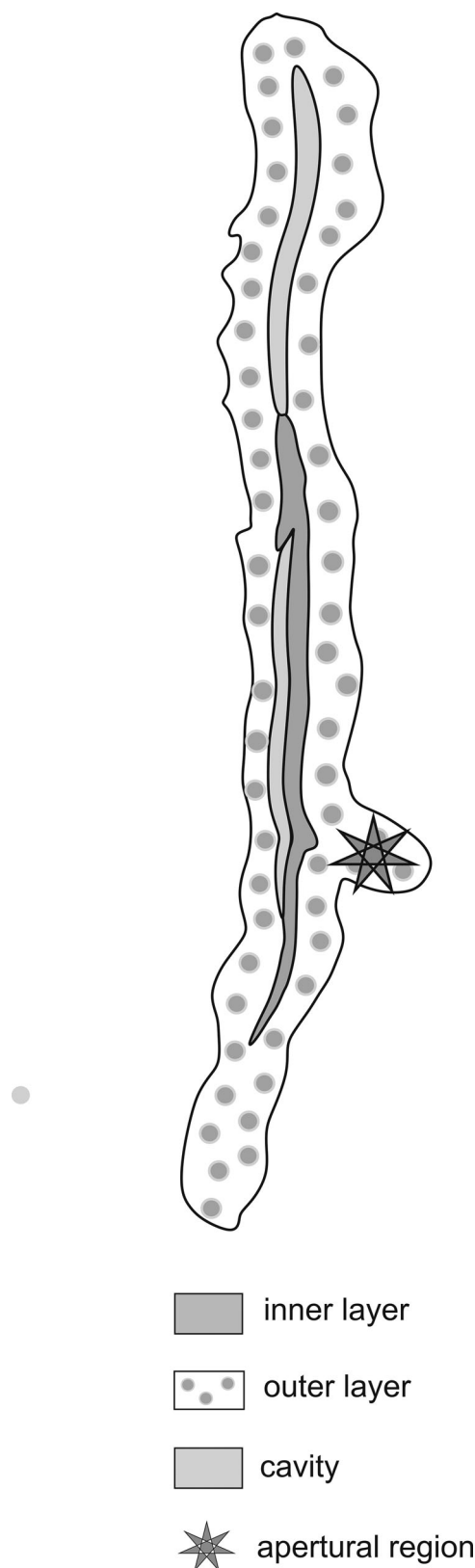


Figure 8. Diagrammatic representation of the sporoderm of *Biharisporites* sp. 1.

6. Conclusions

In general, the ultrastructure of the outer layer of the Early Permian *Biharisporites* studied herein resembles the laterally fused type of *Selaginella* reported by Taylor (1989). The laterally fused type is composed of spherical or rod-like units



Figure 9. Diagrammatic representation of the sporoderm of *Biharisporites* sp. 2.

with various degrees of lateral fusion. Some of the units are joined to form more plate-like units, while others are rod-shaped for most of their length. However, in two of the four megaspores studied herein the Early Permian species of *Biharisporites*, laminated zones are present in the inner layer,

which may indicate an isoetalean affinity. The ultrastructure of the outer layer of the Middle Devonian *Biharisporites* differs from that of the Early Permian *Biharisporites* in the variety of shapes and sizes of units in different parts of the layer. However, in general, it also looks like the laterally fused type reported by Taylor (1989). Despite the generally similar ultrastructure of the sporoderm, there are differences between the studied species of *Biharisporites* in the structure of both the inner layer of the sporoderm and the outer one, which suggests that megaspores of these species might have been produced by different lycopsids.

The Middle Devonian megaspores studied here differ from *in situ* archaeopteridalean megaspores of *Biharisporites* type by the structural elements of the outer layer. These are granules in the archaeopteridalean megaspores and rod-like units that bend and join together to form a three-dimensional network in the dispersed megaspores studied here. Therefore, some megaspores of *Biharisporites* were already produced by lycopsids even in the Devonian. We think that the difference in the elements that constitute the outer layer of the sporoderm is important for differentiation between lycopsid and archaeopteridalean megaspores, but often it is not easy to discern the type of these elements in such megaspores. Thus, Doyle et al. (1975) interpreted as granular the outer layer of archaeopteridalean megaspores studied by Pettitt (1966), but later re-interpreted the same ultramicrographs as showing a spongy alveolar sporoderm (Doyle and Donoghue 1986). Turnau et al. (2009) failed to draw conclusions about the type of structural element of the outer layer of megaspores of *Biharisporites? capillatus* and, mostly because of this, refrained from interpretation of these spores either as archaeopteridalean or lycopsid. Archaeopteridaleans and lycopsids are two unrelated groups, but we face a challenging case of convergence as far as their spores are concerned.

The ultrastructural data on *in situ* archaeopteridalean megaspores of the *Biharisporites* type and on dispersed *Biharisporites* studied in the present paper lead to the conclusion that megaspores of *Biharisporites* morphology have been produced by different groups of spore-bearing plants since the Middle Devonian, and, thus, the genus *Biharisporites* is heterogeneous. This points to the necessity for a taxonomic revision of the genus, but for now the amassed information is insufficient to undertake this properly. Currently, we cannot differentiate between lycopsid and archaeopteridalean megaspores of the *Biharisporites* type by means of LM or SEM, but it would be awkward to include ultrastructural characters in the diagnoses so long as TEM is not routinely used.

Megaspores of *Biharisporites* morphology often prevail in megaspore assemblages of the Givetian in the Voronezh Dome. For instance, the specimen of *Biharisporites arcticus* var. *productus* that was ultrastructurally studied in the present paper comes from an assemblage dominated by various *Biharisporites*, which reach 80% of the total (Kanarkina et al. 2019). Prior to our study, all Devonian *Biharisporites* were interpreted as archaeopteridalean megaspores, since such spores were found *in situ* in sporangia of these plants. Such

a megaspore assemblage would have allowed one to assume that archaeopteridalean plants dominated the hinterland. Our results show that at least some Devonian *Biharisporites* were produced by lycopsids, but we do not know how common these lycopsids were. Nonetheless, the information already available points to the presence of lycopsids among plants that produced this assemblage. Thus, even now the findings are important for more accurate reconstructions of the vegetation of the past using palynological data.

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Disclosure statement

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Supplementary information for ‘**Sporoderm ultrastructure of some Devonian and Permian representatives of Biharisporites and their botanical affinity**’ by Kanarkina A., Zavialova N., Orlova O., Joshi A.

Plate S1. Megaspore ultrastructure of *Biharisporites arcticus* var. *productus* Chi et Hills 1976, specimen 410-03 (Upper Givetian of the Kursk Region, Russia), SEM, the general morphology is shown in Plate 1 (figure 5).

1. Composite image of the section showing bilayered sporoderm, cavity between the layers, and the intermediate sublayer of the outer layer of sporoderm that wedges out (white arrows).
2. Fragment of the contact area showing the outer layer with its sublayers: outer sublayer (o.s.), intermediate sublayer (int.s.), and inner sublayer (in.s.) and cavity (c.) between the inner and outer layers. White arrows point to the roughness left by the knife on the inner sublayer.
3. Fragment of the distal wall showing the sublayers of the outer layer: outer sublayer (o.s.), intermediate sublayer (int.s.), and inner sublayer (in.s.).
4. An area of the inner sublayer showing the constituting elements.
5. Fragment of the distal wall showing the structure of the sculptural element: outer sublayer (o.s.) and intermediate sublayer (int.s.).

Scale bar (1) 50 μm , (2, 3, 5) 5 μm , (4) 1 μm .

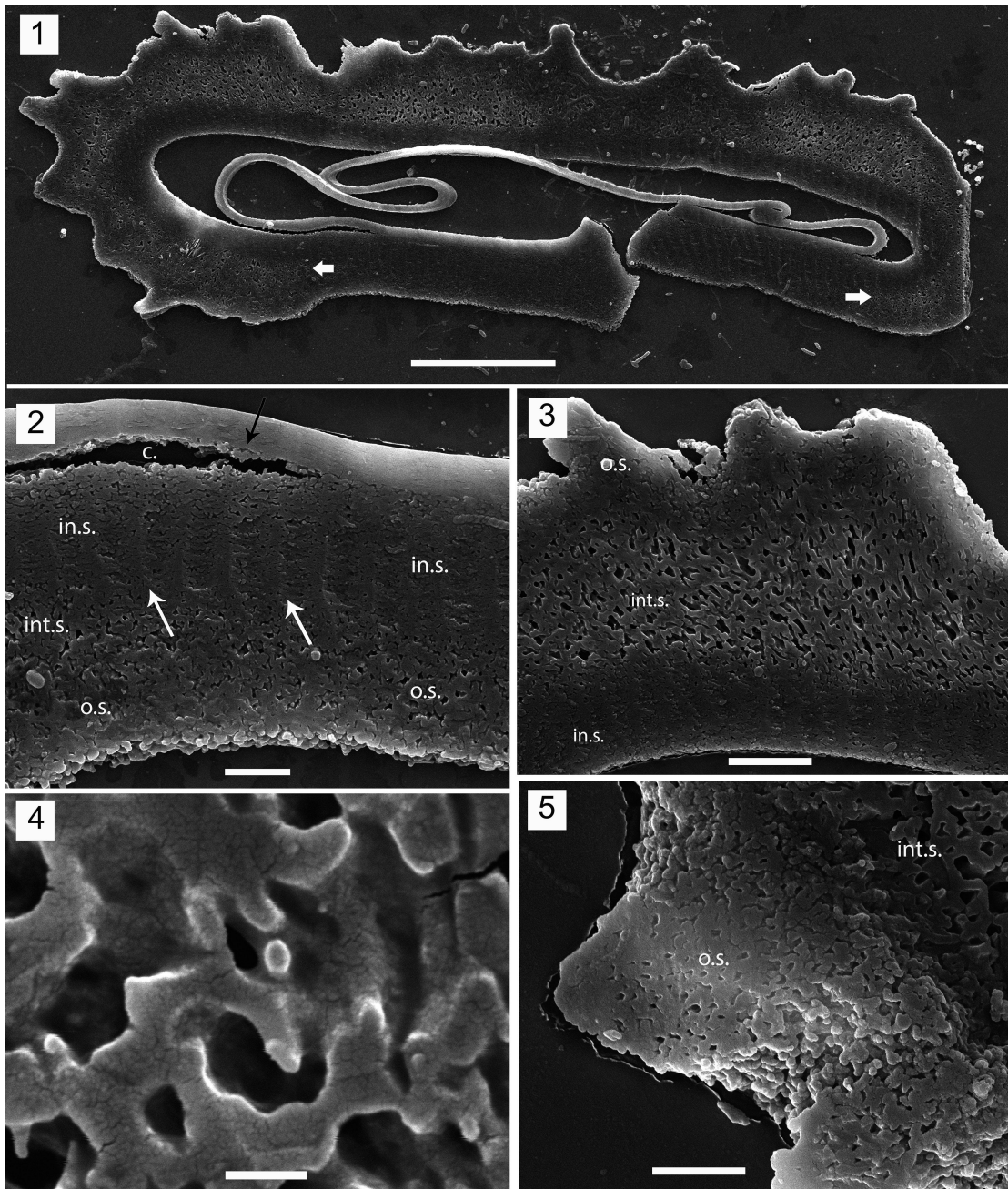


Plate S2. Megaspore ultrastructure of *Biharisporites arcticus* var. *productus* Chi et Hills 1976, specimen 410-03 (Upper Givetian of the Kursk Region, Russia), SEM, the general morphology is shown in Plate 1 (figure 5).

1. The apertural region.

2. Enlargement of Plate S2 (figure 1) showing the structure of the inner (i.l.) and outer (o.l.) layers near the ray of the trilete mark.

Scale bar (1) 10 μm , (2) 5 μm .

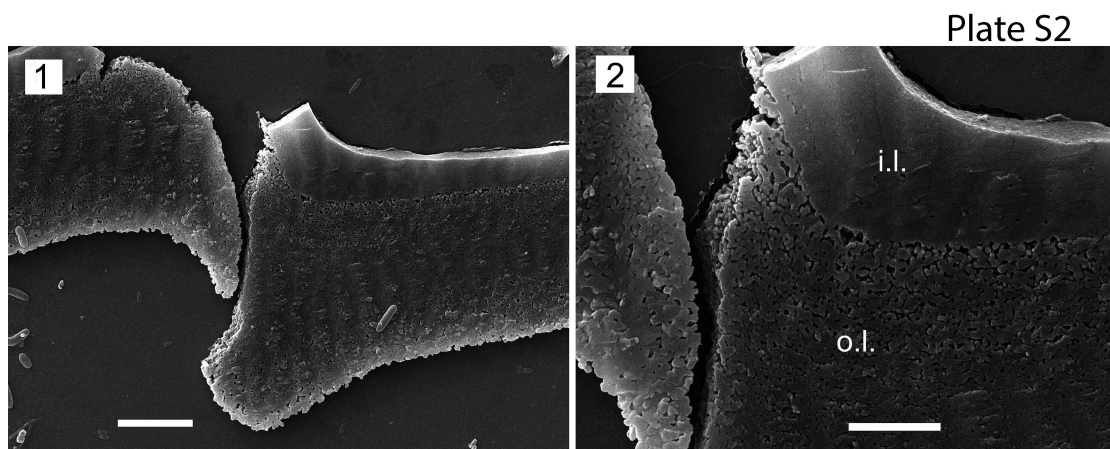


Plate S3. Megaspore ultrastructure of *Biharisporites* cf. *spinosus*, specimen 409-1 (Lower Permian of the Rajmahal Basin, India), SEM, the general morphology is shown in Plate 1 (figure 1).

1. Composite image of the section showing bilayered sporoderm, apertural region (asterisk), sculptural elements (white arrows), and the multilamellate zone (black arrow).

2. Enlargement of Plate S3 (figure 1) showing the structure of the outer layer and the multilamellate zone (white arrow).

3. Enlargement of Plate S3 (figure 1) showing the units of the outer layer (arrowheads) and cavities between the inner and the outer layers (white arrows).

Scale bar (1) 20 μm , (2) 10 μm , (3) 5 μm .

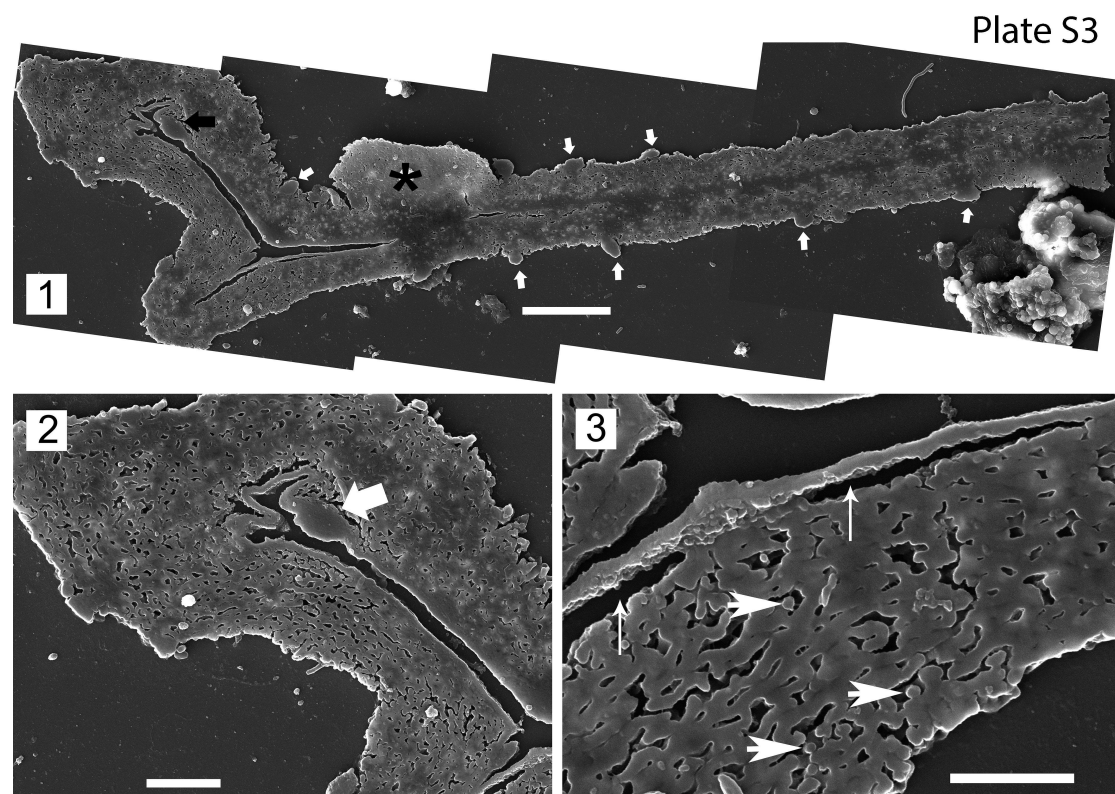


Plate S4. Megaspore ultrastructure of *B. boralii* Bajpai 2003, specimen 409-2 (Lower Permian of the Rajmahal Basin, India), SEM, the general morphology is shown in Plate 1 (figure 2).

1. Composite image of the section; the apertural region is indicated by asterisk.
2. Fragment of the sporoderm in the equatorial area, showing the division of the outer layer into outer sublayer (o.s.) and inner sublayer (in.s.), note cavities (c.) between the sublayers.
3. The apertural region; outer (o.s.) and inner (in.s.) sublayers; their constituting elements are clearly visible.
4. Enlargement of Plate S4 (figure 1) showing the structure of the outer layer.

Scale bar (1) 10 μm , (2-4) 5 μm .

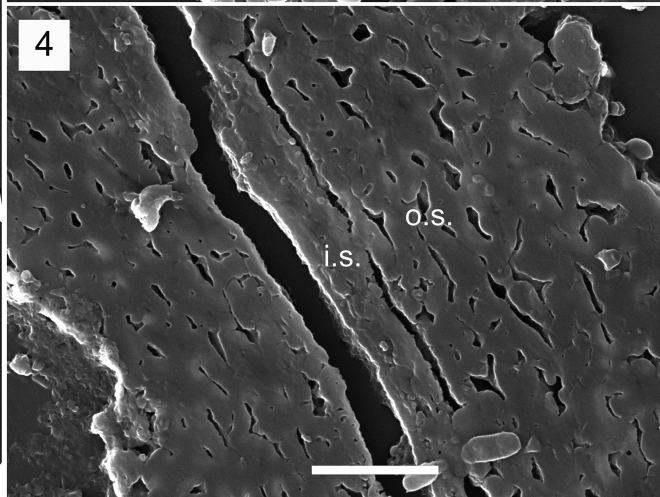
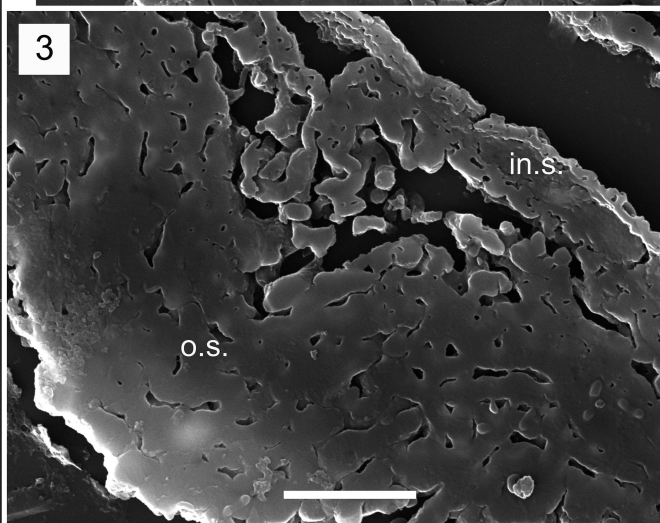
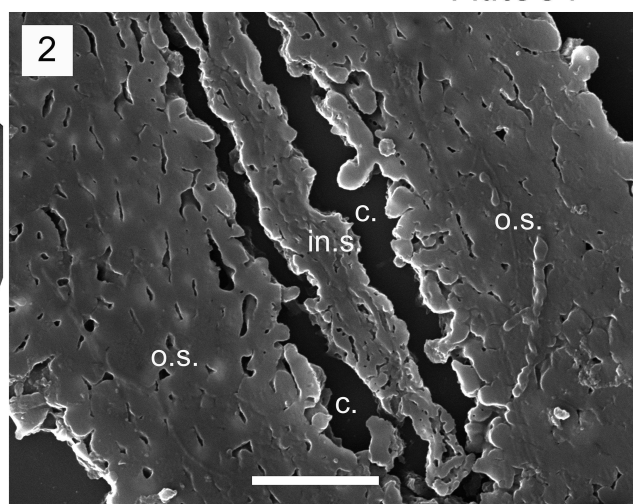
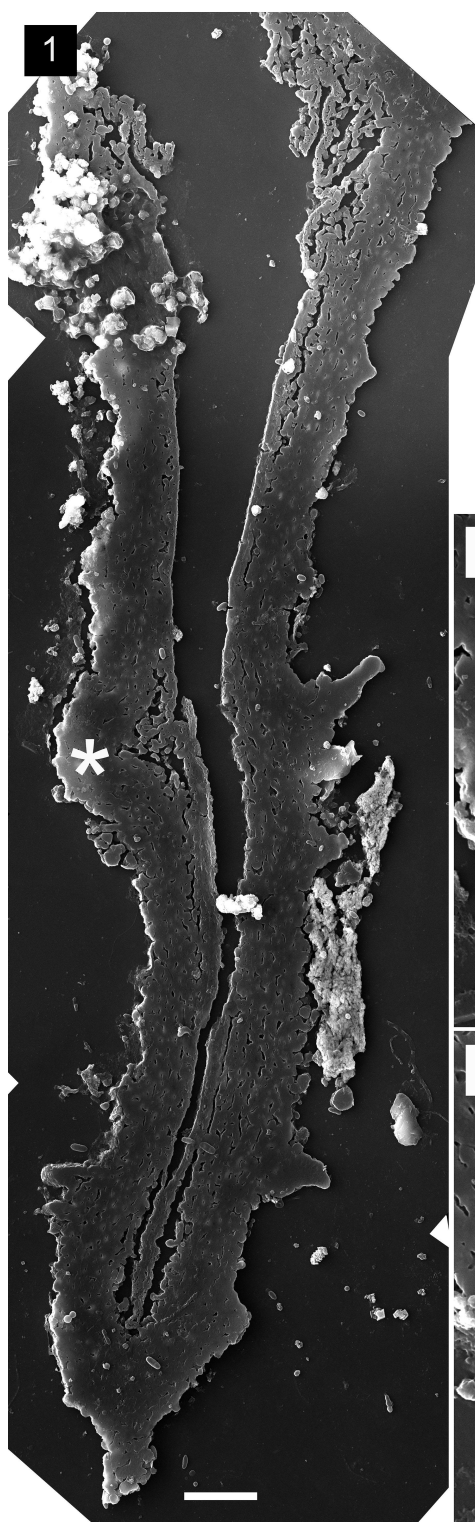


Plate S5. Megaspore ultrastructure in SEM sections.

1-3. *Biharisporites* sp. 1, specimen 409-3 (Lower Permian of the Rajmahal Basin, India).

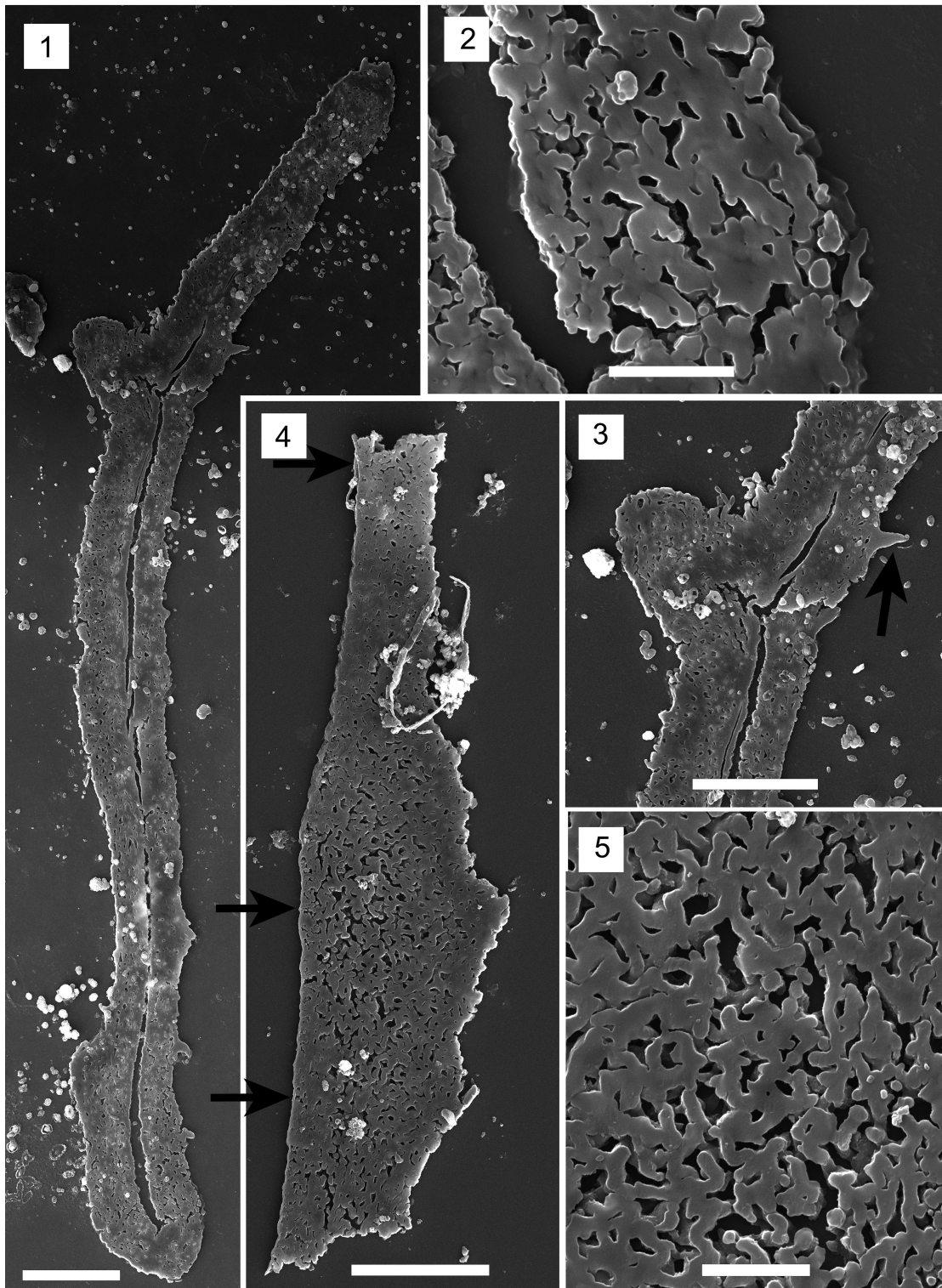
1. Composite image of the section showing bilayered sporoderm.
2. Enlargement of Plate S5 (figure 1) showing the structure of the outer layer of the sporoderm.

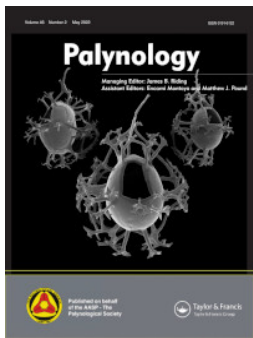
3. The apertural region. The black arrow points to a sculptural element.

4, 5. *Biharisporites* sp. 2, specimen 409-4 (Lower Permian of the Rajmahal Basin, India).

4. Fragment of the section showing bilayered sporoderm. The black arrow points to the inner layer.
5. Enlargement of Plate S5 (figure 4) showing the structure of the outer layer of the sporoderm.

Scale bar (1) 25 μm , (2, 5) 5 μm , (3, 4) 20 μm .







Sporoderm ultrastructure of some Devonian and Permian representatives of *Biharisporites* and their botanical affinity

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