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Three *Aquilapollenites* species from the late Maastrichtian of China: New data and comparisons

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ABSTRACT

Three species, *Aquilapollenites quadricretaceus*, *A. stelckii*, and *A. amicus*, from the late Maastrichtian of China have been studied using light, scanning, and transmission electron microscopy. Rare specimens of *Aquilapollenites quadricretaceus* and *A. stelckii* with reduced polar and/or equatorial projections were observed. *A. quadricretaceus* and *A. stelckii* are more similar to each other in exine ultrastructure than they are to *A. amicus*, which has a thicker foot layer and thin infratectum. A comparison with extant Lythraceae pollen revealed characters similar to those of triprojectate pollen, probably resulting from convergent evolution: prolate pollen with protruding equatorial ora and pores, frequent occurrence of striate and reticulate exine sculpturing, consistent presence of endexine, and infratectal columellae that are mostly simple and not branched. The colporate condition is rare in Triprojectacites and occurs in species with rather short equatorial projections, which suggests that the protruding part of the pollen grain, corresponding to the equatorial projections seen in Triprojectacites, might have been replaced functionally by the ora in tricolpate pollen like that of extant angiosperms. The possible adaptation of some Triprojectacites species to a wetland environment is suggested based on comparison with extant pollen.

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1. Introduction

Triprojectacites are one of the stratigraphically important and diverse groups of fossil angiosperm pollen for which the exact botanical affinity or, more precisely, affinities still remain enigmatic. The polyphyletic nature of the group has often been discussed, and the triprojectate morphology has been considered a convergent feature unifying several systematic groups. Information on the Triprojectacites group comes from dispersed pollen grains (with rare exception) and is mostly at the transmitted light microscopic (LM) level. There are few works using scanning electron microscopy (SEM) and even fewer applying transmission electron microscopy (TEM) (see reviews in Farabee, 1993; Tekleva et al., 2015). For an adequate comparison with modern and fossil angiosperms and determination of the botanical affinities of Triprojectacites species, it is necessary to study at least the main morphotypes of this huge group with SEM and TEM. At the present state of knowledge, more studies are still required to reach this goal.

In this paper, we present studies of three species belonging to the Triprojectacites group from the late Maastrichtian of China using LM, SEM, and TEM. We also discuss previous and new comparisons of this

group with modern angiosperms and make some suggestions as to the ecology of their parent plants.

2. Material and methods

The material comes from the uppermost Cretaceous Furao Formation, borehole XHY2008, in the vicinity of Xiaoheyuan in Jiayin County, China, Amur (Heilongjiang) River area, in the Zeya-Bureya Basin (Markevich et al., 2011). The studied pollen grains belong to the *Aquilapollenites stelckii*-*Pseudointegricorpus clarireticulatum* assemblage of late Maastrichtian age. Thirty-four pollen grains identified as *Aquilapollenites quadricretaceus* Chlonova were studied, 17 identified as *A. stelckii* Srivastava, and 10 identified as *A. amicus* Srivastava. The pollen grains were picked from the residue and each pollen grain was studied with LM and SEM; some of them were further studied with TEM. Pollen grains were photographed in glycerine slides on a Zeiss Axioplan-2 microscope (LM) equipped with an AxioCam 105 digital camera, and then processed for SEM and TEM according to the methods described in Zavialova et al. (2018). Some images were processed with Helicon Focus 6.6.1 software. The pollen grains were observed and photographed under a TESCAN VEGA-II XMU SEM (accelerating voltage 20 kV) at the Borissiak Paleontological Institute, Russian Academy of Sciences. The pollen grains were sectioned with a Leica EMUC6 ultramicrotome equipped with a diamond knife. The ultrathin sections were

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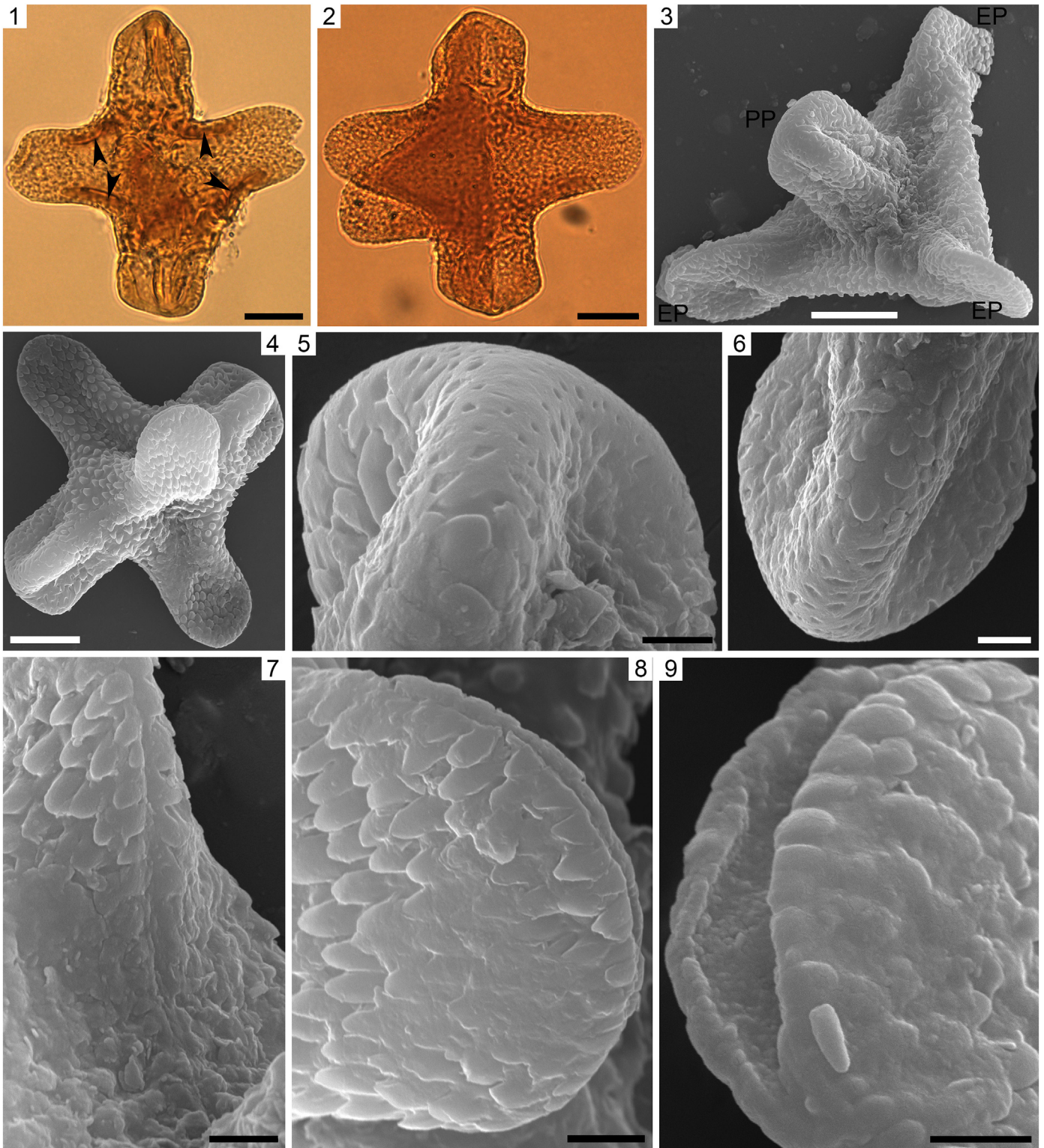


Plate I. Sp. 1, *Aquilapollenites quadricretaceus*, LM and SEM. 1, 2. LM, equatorial view, different focal levels. 3. SEM, polar view. 4. SEM, equatorial view. 5, 6. SEM, close-ups of polar projections. 7–9. SEM, close-ups of a colpus with an aperture membrane (seen in 9). Arrowheads indicate endexine thickenings. Scale bars: 10 μ m (1–4), 2 μ m (5–9).

post-stained with lead citrate and uranyl acetate for some pollen grains, and examined under Jeol 100 B and Jeol 1011 TEMs (accelerating voltage 80 kV) at the laboratory of electron microscopy, Biological Faculty, Lomonosov Moscow State University. The Jeol 1011 TEM was equipped with a side-mounted Orius SC1000W digital camera (11 megapixels,

effective 8.5 megapixels); Digital Micrograph v. 2.0 (Gatan) software was used. Some sections were studied unstained.

Pollen grains of fresh material of *Trapa natans* L. (Lake Parovoye, Nizhniy Novgorod Region, Russia (N 55.669056, E 43.549256)) were treated following Zavialova et al. (2018) for extant material, with

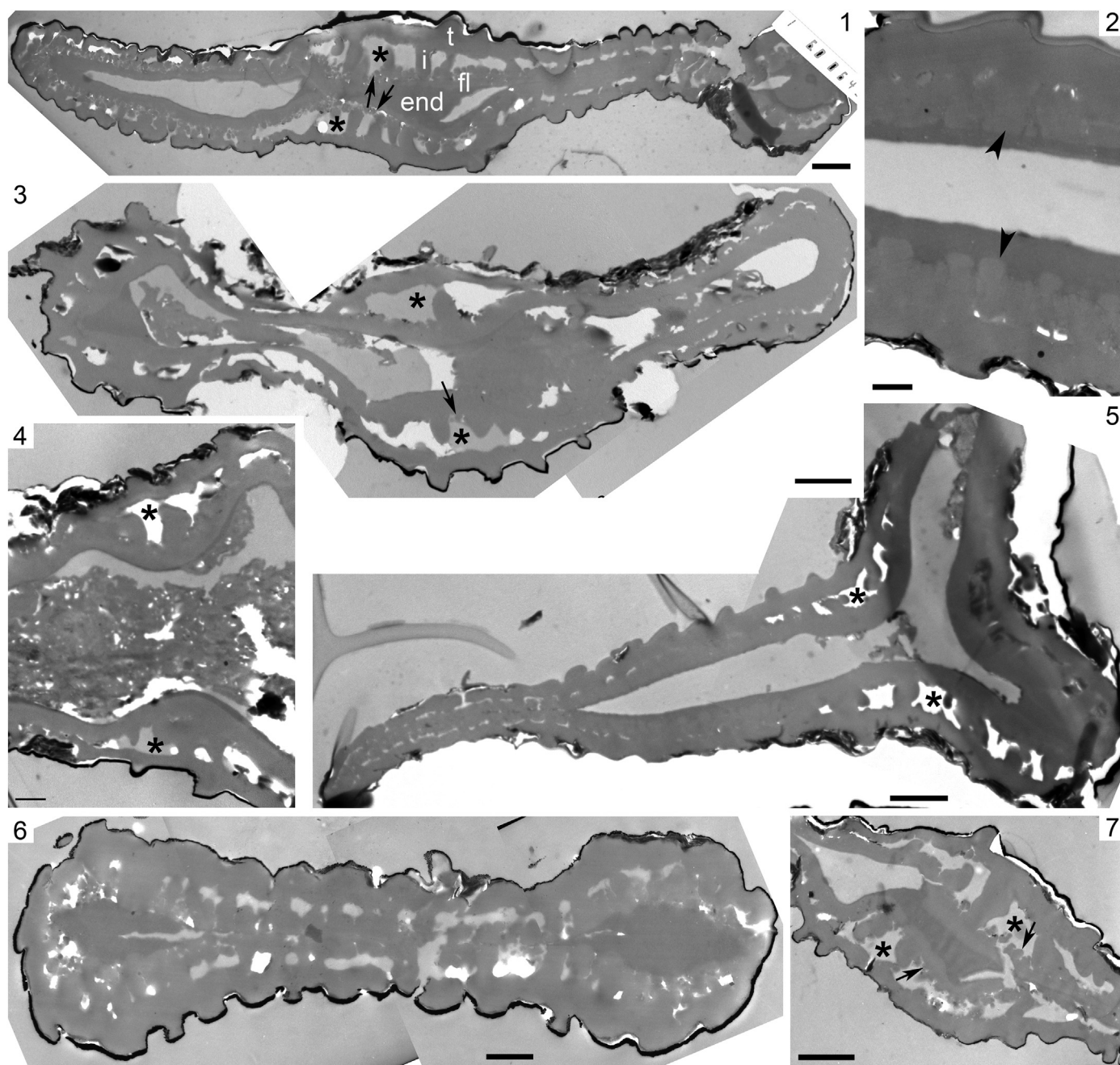


Plate II. Sp. 1, *Aquilapollenites quadricretaceus*, TEM, transverse sections. 1, 3, 5, 6. Sections of whole pollen grains at different levels. 2. Non-apertural region, showing a protruding outline of the inner surface of the foot layer (arrowheads). 4, 7. Close-ups of a region with an increased infratectum thickness. Asterisks indicate areas with increased infratectum thickness, arrows indicate small granules, t – tectum, i – infratectum, fl – foot layer, end – endexine. Scale bars: 1 μm (1, 4), 0.5 μm (2), 2 μm (3, 5), 0.67 μm (6), 1.25 μm (7).

Table 1

Pollen measurements in light (LM) and scanning electron (SEM) microscopy. Pollen measurements in light (LM) and scanning electron (SEM) microscopes.

Pollen characters	Average (min-max)	<i>A. quadricretaceus</i>	<i>A. stelckii</i>	<i>A. amicus</i>
Polar axis LM/SEM		48.3 (40.4–54.0)/46.2 (37.3–52.0)	42.5 (36.1–52)/44.0 (36.9–50.0)	36.1 (31.0–40.0)/34.2 (31.7–38.5)
Equatorial diameter LM/SEM		48.3 (40.0–55.3)/47.3 (41.4–53.0)	40.9 (35.0–56.0)/43.9 (35.8–52.7)	29.3 (23.0–36.7)/25.4 (14.9–32.4)
Polar projection length LM/SEM		16.7 (12.0–20.7)/16.0 (9.7–20.4)	15.4 (11–22.8)/15.4 (11.2–19.5)	13.4 (9.6–16.7)/14.1 (9.5–17.7)
Polar projection width LM/SEM		13.0 (9.6–17.3)/12.4 (8.2–15.7)	9.9 (6.4–15.3)/10.8 (7.2–12.7)	14.8 (10.0–23.3)/15.0 (12.4–19.7)
Equatorial projection length LM/SEM		18.0 (13.6–23.3)/17.6 (12.1–21.0)	15.1 (9.6–20.0)/15.5 (11.3–19.5)	6.9 (5.3–9.0)/5.5 (3.8–8.8)
Equatorial projection width LM/SEM		12.6 (8.0–16.0)/12.2 (8.9–14.5)	10.0 (6.8–13.0)/10.7 (7.7–14.1)	6.4 (5.2–7.5)/6.3 (5.1–9.2)

osmium tetroxide. Pollen grains were photographed in LM with a Nikon Eclipse Ci equipped with a Nikon DS-Vi1 digital camera. Dry pollen grains were observed with a Camscan-S2 SEM (accelerating voltage 20 kV) in SEI mode. The ultrathin sections were studied unstained under JEM-1400 Flash and JEM-1011 (acceleration voltage 80 kV) TEMs with an ORIUS SC1000W digital camera at the laboratory of electron microscopy, Biological Faculty, Lomonosov Moscow State University.

Pollen measurements were made under LM, SEM, and TEM. The morphological terminology used follows Hesse et al. (2009), except for some terms taken from original descriptions by other authors. In this paper we use *Aquilapollenites* as a generic name for our species, while Braman (2013) made new combinations for *A. quadricretaceus*, *A. stelckii*, and *A. amicus*, transferring them to *Triprojectus quadricretaceus* (Chlonova) Braman, *Parviprojectus stelckii* (Srivastava) Braman, and *Parviprojectus amicus* (Srivastava) Braman, respectively.

3. Results

3.1. *Sp. 1, Aquilapollenites quadricretaceus* (Table 1, Plate I, 1–9, Plate II, 1–7, Plate VII, 1, 3)

3.1.1. LM

Pollen grains are tricolpate and isopolar and often appear four-lobed in equatorial view (Plate I, 1). The colpi reach the pollen body. Polar and equatorial projections are of more or less the same length and width; in general the polar projections are slightly shorter and wider than the equatorial ones, but the difference is insignificant (see Table 1). The equatorial projections have rounded ends; the polar ones may also have rounded ends, although most often one of them is triangular while the other has a rectangular outline (Plate I, 1). The exine sculpturing is granulate except at the ends of the polar projections, where it appears foveolate (Plate I, 2). The exine is two-layered, with endexine thickenings typical for most triproctate pollen (Plate I, 1). The exine is thickened at the edges of the polar projections, giving them the above-mentioned peculiar outlines (Plate I, 1, 2). One pollen grain with the polar projections/polar axis reduced in length was observed (Plate VII, 3).

3.1.2. SEM

The exine sculpturing is densely microechinate in the equatorial region and on part of the polar projections and microreticulate on the apical parts of the polar projections (Plate I, 3–6). The microechini have rounded tips; their distribution becomes sparser in the transition from the equatorial region to the polar projections (Plate I, 3–6). On the equatorial projections (especially their distal parts) the microechini are at an acute angle to the exine surface, oriented with their tips towards the pollen body (Plate I, 8). The exine surface between the microechini is psilate with very rare perforations (Plate I, 7–9). The lumina on the polar projections tend to be smaller towards the top of the projections, often reduced to the size of perforations or absent, resulting in a psilate surface (Plate I, 5, 6). The colpus margin (or margin of the equatorial projections) is ornamented by microechini arranged in a row (Plate I, 7–9). The colpi extend just to the pollen body or somewhat further onto the polar projections (Plate I, 7). The aperture membrane is granular (Plate I, 9).

3.1.3. TEM

The non-apertural exine is from 1.1 to 2.5 μm thick, tectate, columellate, with a thin tectum (except for protruding elements that form the microechinate pattern) and foot layer; the infratectum is also thin all around the pollen except in the equatorial regions, where it greatly increases in thickness (Plate II, 1, 3–5, 7); the exine in these thickened regions is from 3.2 to 5.0 μm thick. The tectum is about 0.3–0.4 μm thick without and up to 0.9–1.0 μm thick with the

protruding elements that form the microechinate pattern. The infratectum thickness is about 0.2–0.4 μm , except in the equatorial regions where it is thicker; the columella width is about 0.2–0.4 μm , and in the lower part of the equatorially thickened infratectum small granules about 0.06–0.17 μm in diameter are observed (Plate II, 1, 3, 7). The foot layer is 0.3–0.5 μm thick. At places, a protruding outline of the inner surface of the foot layer was observed (Plate II, 2). The endexine is more electron-dense than the ectexine and appears homogeneous (Plate II, 1, 2, 6, 7). The non-apertural endexine is thin, usually 0.05–0.2 μm , and thickens towards the apertural regions (Plate II, 1, 3, 6, 7).

3.2. *Sp. 2, Aquilapollenites stelckii* (Table 1, Plate III, 1–8, Plate IV, 1–7, Plate VII, 2, 4)

3.2.1. LM

Pollen grains are tricolpate and isopolar or subsipolar. The colpi are short. Polar and equatorial projections are similar in length and width, although one polar projection is often slightly longer than the other, and the equatorial projections sometimes appear somewhat wider than the polar ones (Table 1, Plate III, 1, 2, Plate VII, 2). Polar and equatorial projections have rounded ends, although one of the polar projections often shows a rectangular outline (Plate III, 1, 2). The exine pattern appears strioreticulate in LM, with striations oriented parallel to the polar axis. The exine is two-layered; endexine thickenings are weakly expressed.

3.2.2. SEM

The exine sculpturing is striate with striations oriented parallel to the polar axis. The striations are regular and tile-like on the equatorial projections (Plate III, 3, 6), becoming slightly interwoven on the body and polar projections (Plate III, 4, 5). Colpi are short and occupy only the tips of the equatorial projections (Plate III, 7, 8). The colpus margin is solid. The aperture membrane is probably granular. One pollen grain in which polar and equatorial projections were reduced in length was observed (Plate VII, 4).

3.2.3. TEM

The non-apertural exine is from 1.4 to 3 μm thick, tectate, columellate, with a thin tectum (excluding regions with protruding elements forming the striate pattern), thick infratectum, and relatively thick foot layer (Plate IV, 3–6). The tectum is about 0.1–0.4 (rarely 0.6) μm thick without and 0.5–1.5 μm with the protruding elements forming striations. The infratectum thickness is from 0.5 to 1.1 (rarely 1.8) μm ; the columella width is about 0.3–0.4 μm , and rarely branched columellae are observed (not shown); in the lower part of the infratectum small granules about 0.08–0.17 μm in diameter are often observed (Plate IV, 1, 2, 7). The foot layer is 0.4–0.6 μm thick. The endexine is more electron-dense than the ectexine and appears homogeneous (Plate IV, 2, 3, 5). The non-apertural endexine is thin, usually 0.01–0.05 μm , sometimes 0.1–0.2 μm ; it thickens towards the apertural regions (Plate IV, 2).

3.3. *Sp. 3, Aquilapollenites amicus* (Table 1, Plate V, 1–8, Plate VI, 1–4)

3.3.1. LM

Pollen grains are tricolporate and isopolar. The colpi are long, reaching the polar regions and continuing up to half the height of the polar projection (Plate V, 1, 2). The ora are not always clearly visible. The polar projections are much longer and wider than the equatorial ones, with rounded ends. The equatorial projections are small and auriculate, with rounded ends (Plate V, 1, 3). The exine sculpturing on the body and polar projections is reticulate, with lumina mostly about 1 μm in diameter, although lumina of different sizes up to 2 μm can be observed; the exine sculpturing on the equatorial projections is striate

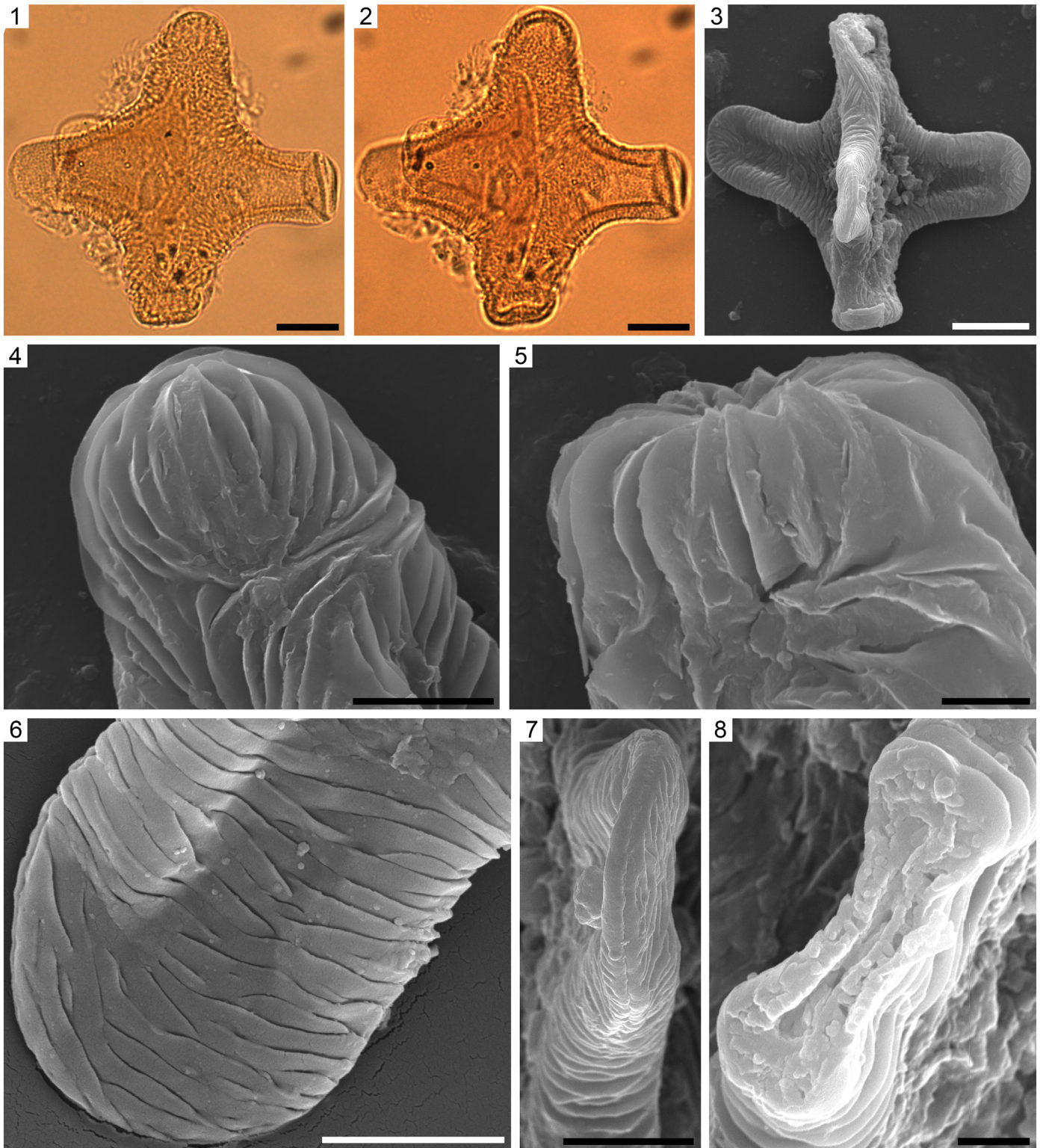


Plate III. Sp. 2, *Aquilapollenites stelckii*, LM and SEM. 1, 2. LM, equatorial view, different focal levels. 3. SEM, equatorial view. 4, 5. SEM, close-ups of polar projections. 6. SEM, close-up of equatorial projection. 7, 8. SEM, close-ups of colpi. Scale bars: 10 μm (1–3), 5 μm (4, 6, 7), 2 μm (5, 8).

(Plate V, 1, 3). The exine is two-layered, with endexine thickenings typical for most triprojectate pollen.

3.3.2. SEM

The exine sculpturing is reticulate on the body and polar projections and striate on the equatorial projections (Plate V, 4–7). The lumina are

mostly 1–1.5 μm in diameter, rarely less than 1 μm or up to 2 μm . The muri are rather narrow, about 0.2–0.4 μm wide. On the equatorial projections the striations appear to radiate from more or less one area; the width of the striations is comparable with that of the muri. The colpi extend well onto the polar projection, occupying about half of its height (Plate V, 5, 8).

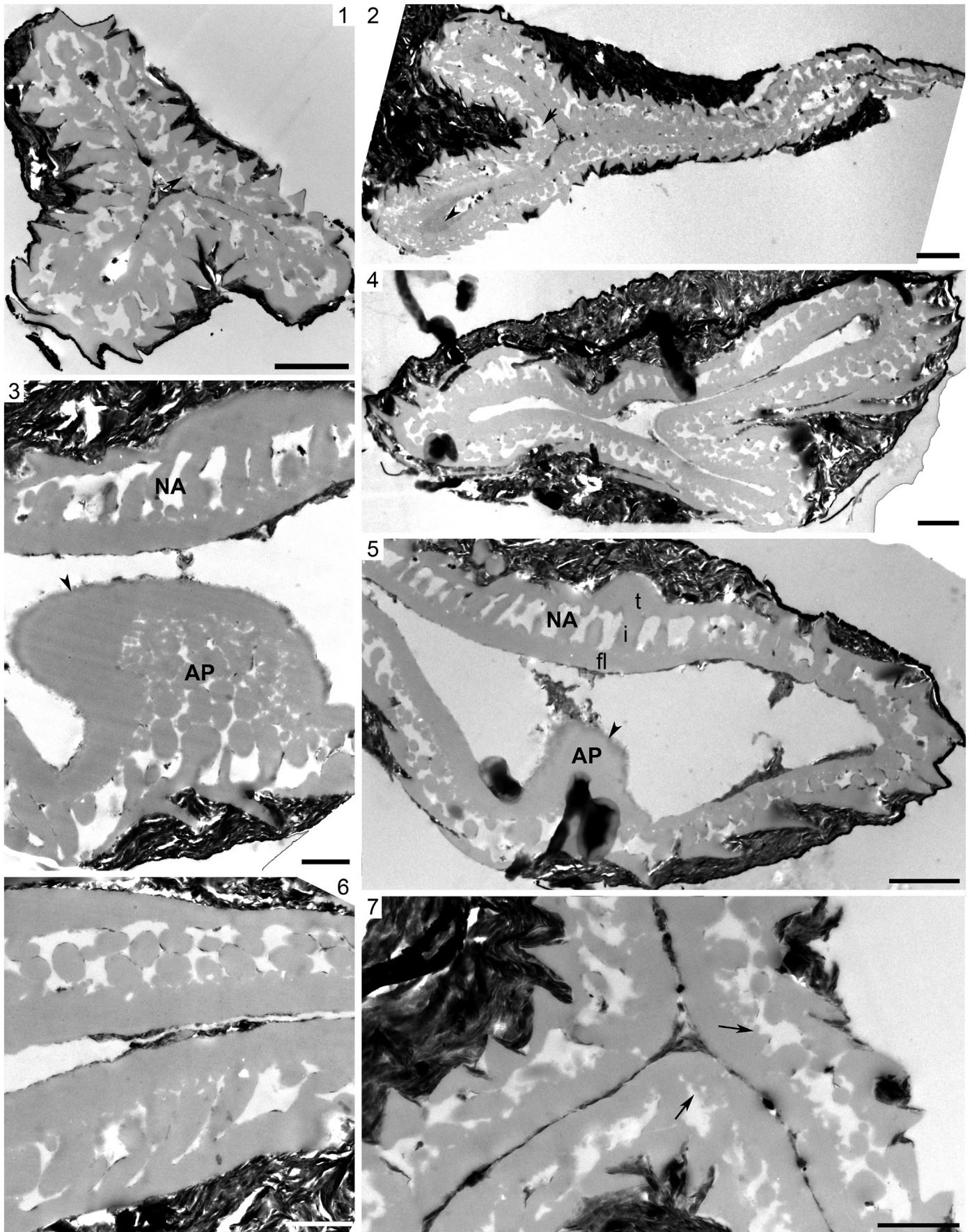


Plate IV. Sp. 2, *Aquilapollenites stelckii*, TEM, transverse sections. 1, 2, 4. Sections of whole pollen grains at different levels. 3, 5. Close-ups of part of a pollen grain with apertural and non-apertural regions. 6, 7. Non-apertural region. AP – apertural region, NA – non-apertural region; arrows indicate small granules, arrowheads indicate endexine. Scale bars: 2 μm (1, 2, 4, 5), 1 μm (3, 6, 7). t – tectum, i – infratectum, fl – foot layer.

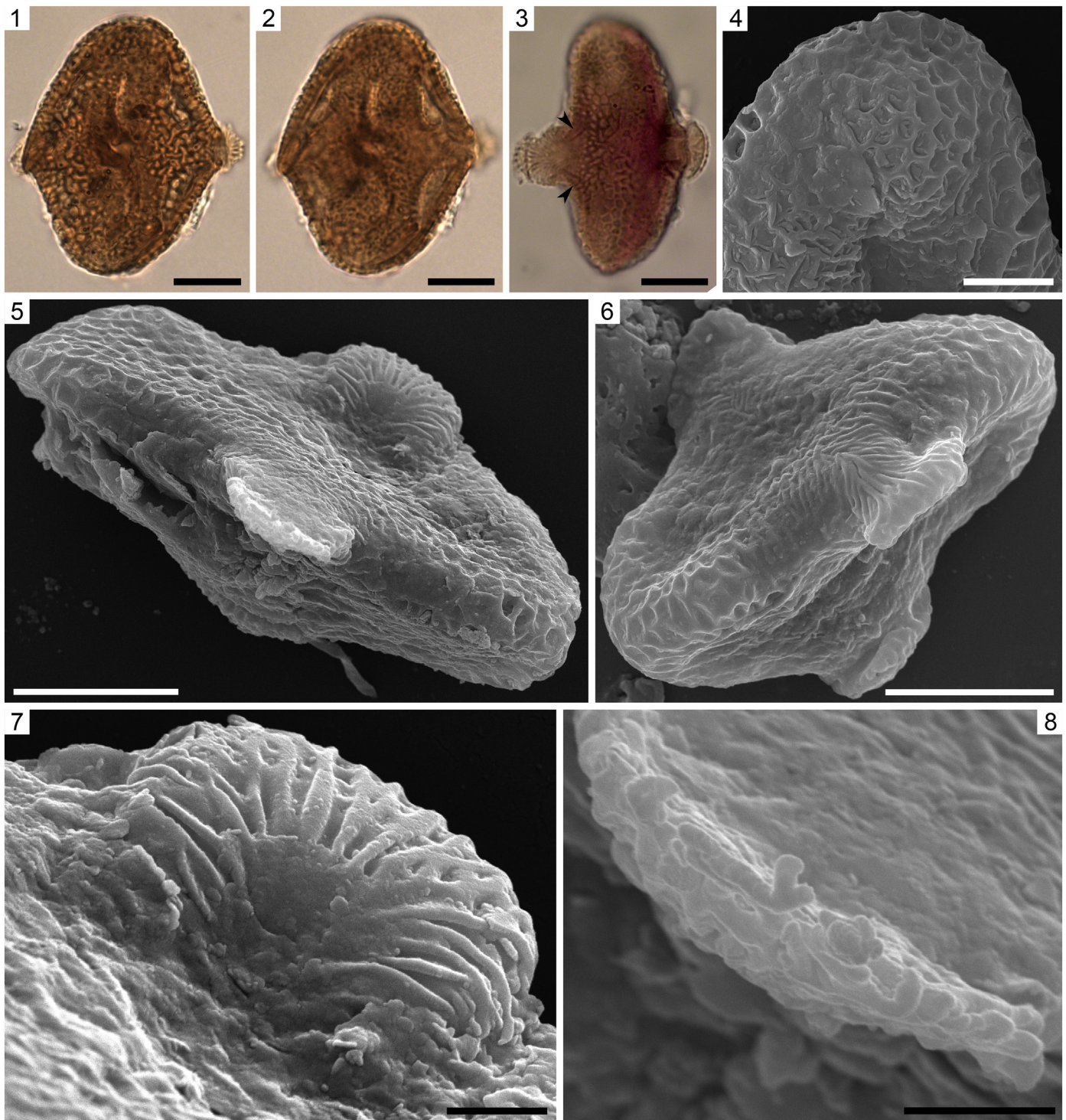


Plate V. Sp. 3, *Aquilapollenites amicus*, LM and SEM. 1, 2. LM, equatorial view, different focal levels. 3. LM, focus on equatorial projections. 4. SEM, close-up of a polar projection. 5, 6. SEM, equatorial view. 7. SEM, equatorial projection. 8. SEM, colpus. Arrowheads indicate endexine thickenings. Scale bars: 10 μm (1–3, 5, 6), 5 μm (4), 2 μm (7, 8).

3.3.3. TEM

The exine is about 2.0–2.5 μm thick, semitectate, columellate, with a thick tectum, thin infratectum, and relatively thick foot layer (Plate VI, 1, 2, 4). The tectum is about 0.7–1.4 μm thick, the columella height is 0.2–0.6 μm , and the columella width is

0.2–0.4 μm . The foot layer is about 0.5–0.9 μm thick. The endexine is more electron-dense than the ectexine and appears homogeneous (Plate VI, 1, 3). The non-apertural endexine is about 0.1–0.2 (rare) μm thick; it thickens towards the apertural regions (Plate VI, 1).

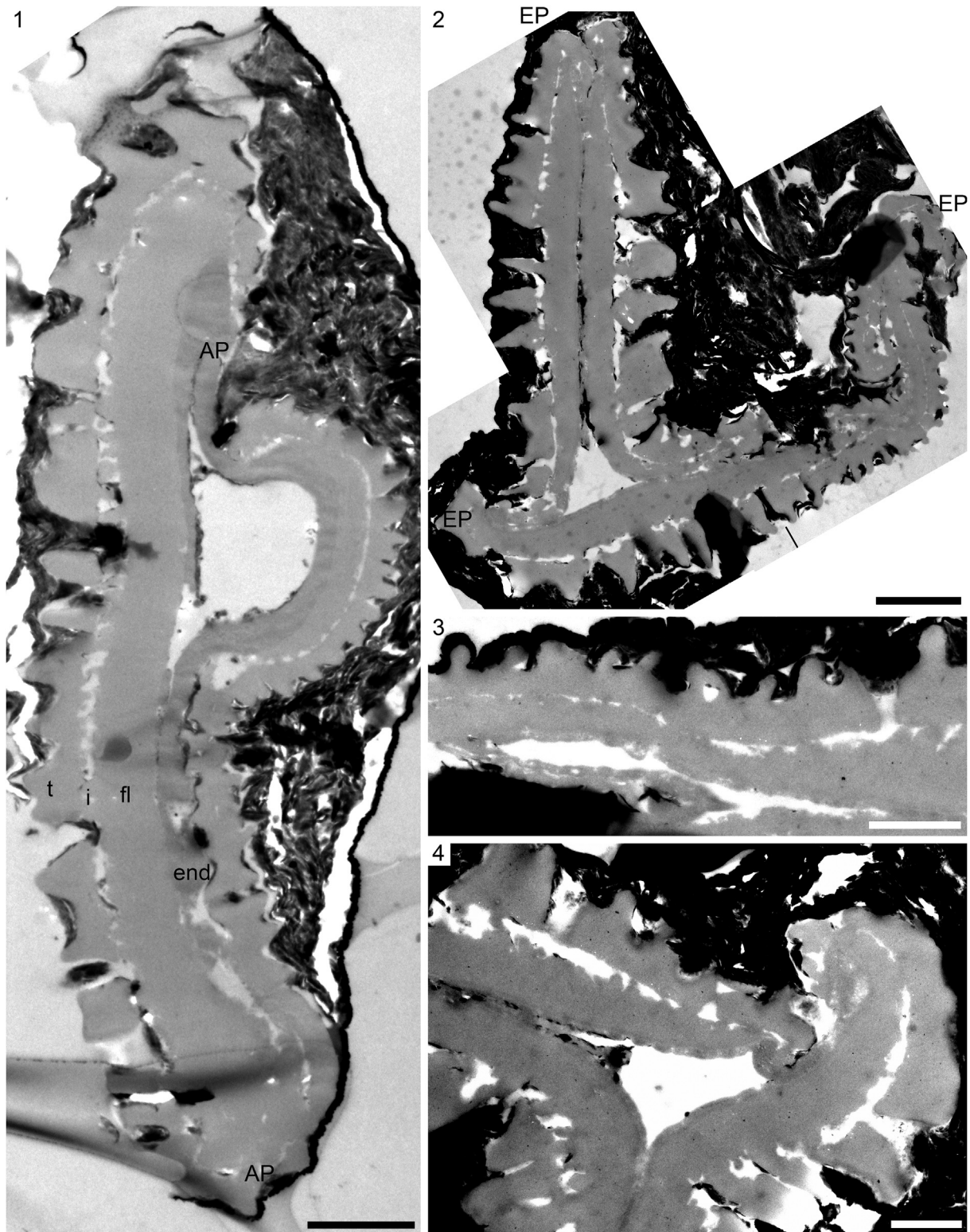


Plate VI. Sp. 3, *Aquilapollenites amicus*, TEM. 1. Longitudinal section of a whole pollen grain, cut through one equatorial projection, endexine thickening towards the apertural regions (AP). 2–4. Transverse sections. 2. Section of a whole pollen grain, cut through three equatorial projections (EP). 3. Close-up of apertural region. 4. Close-up of a central part of the pollen. Scale bars: 2 μm (1, 2), 1 μm (3, 4). t – tectum, i – infratectum, fl – foot layer, end – endexine.

4. Discussion

4.1. Comparison of the studied species with similar *Triprojectacites* taxa

When found in the pollen spectra, the studied pollen species 1, 2, and 3 were determined as *Aquilapollenites quadricretaceus*, *A. stelckii*,

and *A. amicus*, respectively. *Aquilapollenites quadricretaceus* and *A. stelckii* occur relatively often, while pollen grains of *A. amicus* are rare. The palynological assemblage was previously studied by Markevich et al. (2011), and a taxon list was presented in table 3 there; however, *Aquilapollenites quadricretaceus* and *A. amicus* were absent in the taxon list for this sample. The *Triprojectacites* group of this

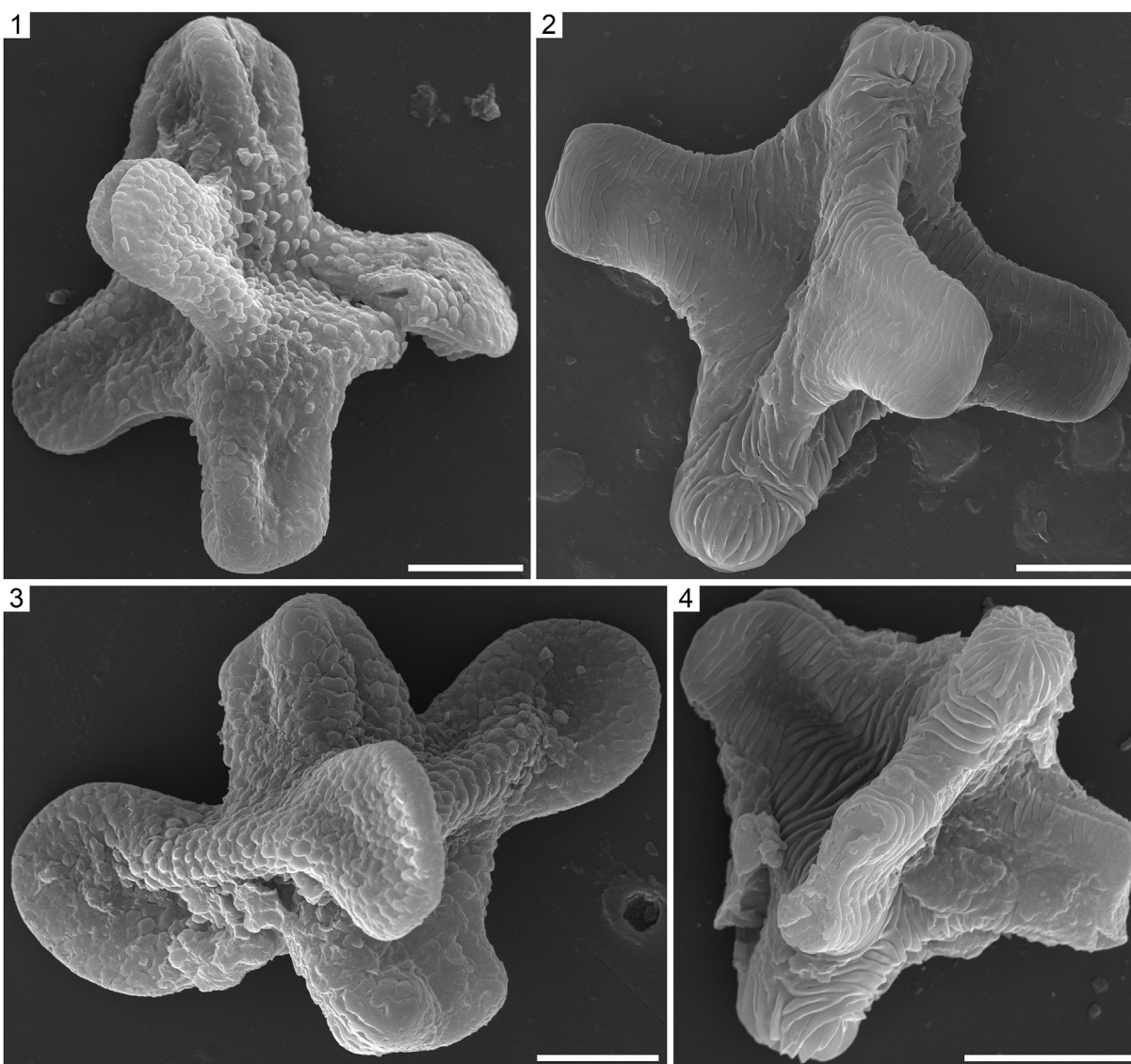


Plate VII. Pollen grains with normal (1, 2) and reduced (3) polar and (4) polar and equatorial projections. 1, 3. *Aquilapollenites quadricretaceus*. 2, 4. *Aquilapollenites stelckii*. Scale bars: 10 μ m.

sample comprises *Cranwellia striata* Srivastava, *Aquilapollenites stelckii*, *A. cruciformis* Mtchedlishvili, *A. conatus* Norton, *A. subtilis* Mtchedlishvili, *A. procerus* Samoilovich, *A. rhombicus* Samoilovich, *Aquilapollenites* sp., *Integricorpus bellum* Mtchedlishvili, *Pseudointegricorpus clarireticulatum* (Samoilovich) Takahashi, and *Parviprojectus reticulatus* Mtchedlishvili (Markevich et al., 2011). While the presence of *A. stelckii* corresponds well with previous work, the absence of the other two species in the list needs explanation.

The first explanation could be that we misidentified our sp. 1 and sp. 3. However, one of the few works presenting SEM of triprojectate pollen, by Farabee (1993), shows SEM photos of *A. quadricretaceus* and *A. amicus* that are indistinguishable from our sp. 1 and sp. 3, respectively, which supports our determinations. Sp. 1 fits well with the diagnosis of *A. quadricretaceus* except for the recognition that the exine pattern, while appearing granular at standard working magnifications ($\times 400$ – $\times 600$), is seen to be reticulate at higher ones (with immersion). Another difference is that the polar axis of *A. quadricretaceus* was described as longer (58 (52–64) μ m) by Chlonova (1961). While a difference in pollen size can be explained by pollen variability and a large range of pollen size characterizing a species, exine sculpturing is among the more crucial characters. However, this recognition of the

reticulate nature of the exine has been ignored or rejected by many authors who indicate the presence of *A. quadricretaceus* in their palynological assemblages based on verrucate/granulate sculpture. For example, Braman's (2013) comments on *A. quadricretaceus* are "The sculpture appears to be verrucate to granulate on the specimens observed in the present study rather than the reticulate one suggested in the original description." As seen under SEM, the exine of apical parts of the polar projections of the studied species has microreticulate to foveolate sculpture (Plate I, 3–6). This may be one of the reasons for the reticulate exine pattern indicated in Chlonova's description of *A. quadricretaceus*. Or the reticulation seen by Chlonova may have represented the infratectal structure, and not the sculpture pattern of the pollen. Or the originally described pollen grains of *A. quadricretaceus* may have indeed had a reticulate exine, and the attribution of verrucate forms to this species needs reconsideration. On the other hand, for those researchers who take the reticulate exine pattern as a crucial character, such pollen might have been described as *A. subtilis*. And this may explain the situation we face with our material. The diagnosis of *A. subtilis* also fits our sp. 1, and the described pollen size matches nicely our measurements. However, Mchedlishvili (1961) indicated that *A. subtilis* differs from other species of the genus in having a thinner exine and especially

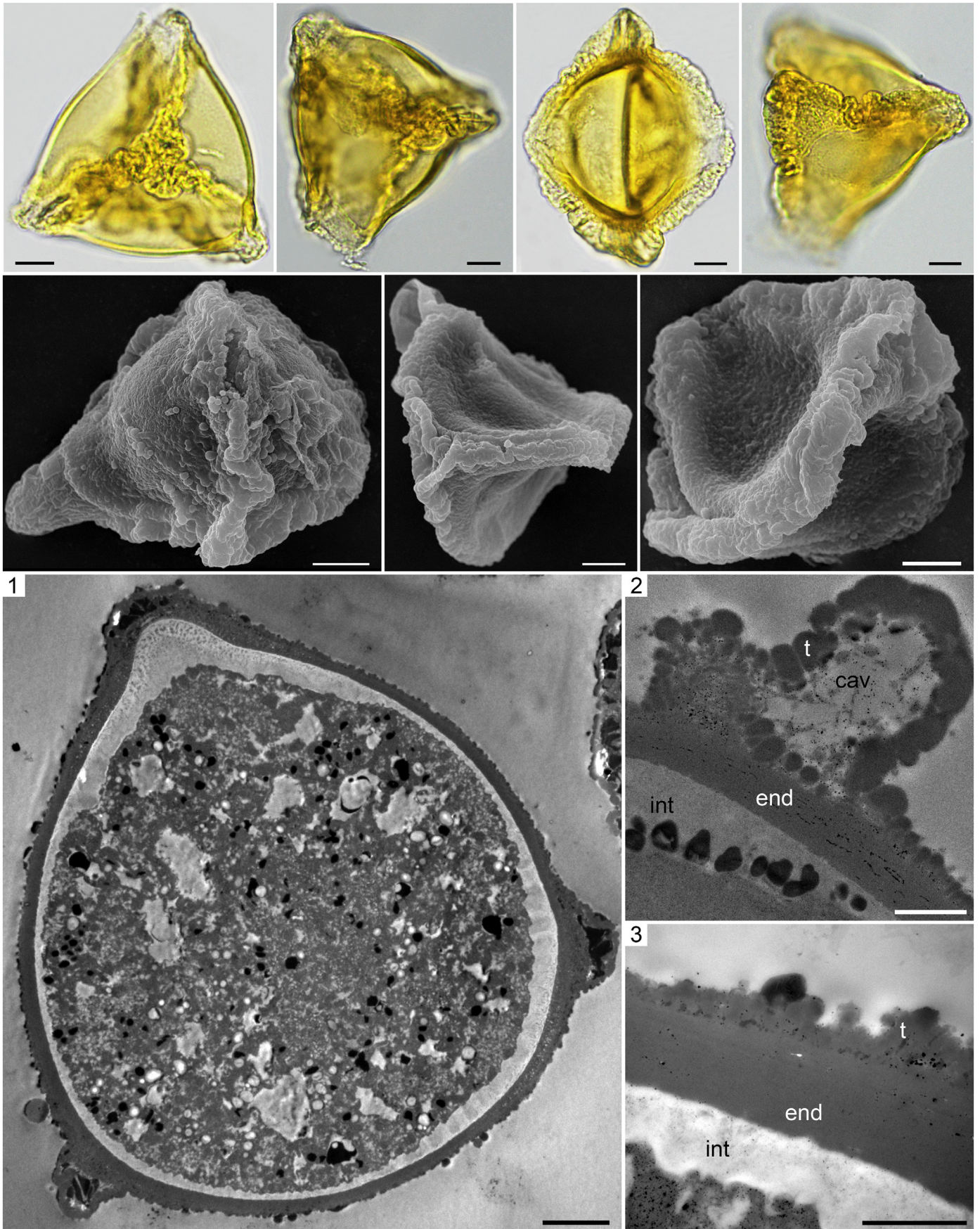


Plate VIII. *Trapa natans*, 1–4 LM, 5–7 SEM, 8–10 TEM. 1, 2. Polar view, optical section. 3. Equatorial view, optical section. 4. Polar view, surface. 5. Equatorial view. 6. Polar view. 7. A meridional crest. 8. Section of a whole pollen grain. 9. Non-apertural region. 10. Apertural region. Scale bars: 10 μm (1–7), 5 μm (8), 2 μm (9), 1 μm (10). t – tectum, cav – cavity, end – endexine, int – intine.

weakly developed nexine thickenings. This remark refutes determination of our sp. 1 as *A. subtilis*, as our sp. 1 has a rather thick exine and well-developed endexine thickenings. Braman (2013) treated *A. subtilis* and *A. nenjiangensis* Yu, Guo et Mao as synonyms, although the diagnosis and photos of *A. nenjiangensis* (Yu et al., 1983) show notable differences from *A. subtilis* and our studied species, so we will not discuss *A. nenjiangensis* further. Other listed *Aquilapollenites* species also differ from our sp. 1. *A. procerus* differs in having reticulate sculpture and equatorial projections that are usually longer than the polar ones; *A. cruciformis* and *A. insignis*, in having wider and longer equatorial projections relative to the polar ones (Mchedlishvili, 1961; Samoilovitch, 1965). The most plausible explanation is that *A. quadricretaceus* was incorrectly entered in table 3 of Markevich et al. (2011) as *A. cruciformis*, as the latter appeared in the table twice and *A. quadricretaceus* was mentioned as occurring in Furoo Formation pollen spectra in the text.

The most interesting feature of our sp. 2 (*A. stelckii*) concerns its exine sculpture. It was initially described as retipilate by Srivastava (1968), then Farabee (1993) considered it strioreticulate. In LM the exine pattern indeed appears strioreticulate on the polar projections and body and striate on the equatorial projections. However, in SEM it is clear that the exine sculpture on both the polar and equatorial projections is striate, although the striation patterns are different. This shows once again the importance of SEM (and TEM) studies for correct description and interpretation.

Our sp. 3 is characterized by tricolporate pollen grains. However, the ora are often unclear and, for this reason, our sp. 3 could have been determined as *Parviprojectus reticulatus* (tricolpate pollen) in Markevich et al. (2011) or *Aquilapollenites reticulatus* Stanley (Stanley, 1961), which Braman (2013) treated as a synonym of *Parviprojectus reticulatus*. The overall description of *Parviprojectus reticulatus* fits our sp. 3 (except for the absence of ora), although the equatorial projections are notably longer (9.7–15.6 µm versus 5.3–9.0 µm in our pollen and 5–10 µm in *A. reticulatus* as described by Stanley (1961)). We think this is a rather crucial character for distinguishing these species and not considering them synonyms. The description of *A. reticulatus* by Stanley (1961) fits well our sp. 3 except for the tricolpate condition of *A. reticulatus*, in contrast to the tricolporate pollen of our sp. 3. Another similar species with tricolpate pollen, *Parviprojectus dolium* Samoilovitch, has short equatorial projections (2.2–6.4 µm), but in *P. dolium* they are notably shorter; besides, the exine sculpturing is microreticulate with more uniformly sized lumina, and there is no mention of whether the exine sculpturing of the equatorial projections is striate; from the description it is supposed to be microreticulate as well. The description of *A. amicus* perfectly matches our sp. 3. In addition, our species looks quite similar to *Aquilapollenites amicus* illustrated by Farabee (1993) with SEM. The equatorial projections for this species figured in Braman (2013) are longer, but this may fall in the range of infraspecific variability. *A. amicus* and *A. reticulatus* (Stanley, 1961) appear to differ only in the presence of endoapertures in *A. amicus*. So, the absence of *A. amicus* in the taxon list of Markevich et al. (2011) could be a result of either its misidentification as *A. reticulatus* or *Parviprojectus reticulatus*, or its rare occurrence in the pollen spectra; in other words, our studies revealed this species when it had been missed in previous work.

We observed one pollen grain of *Aquilapollenites quadricretaceus* and one pollen grain of *A. stelckii* with a reduced length of the polar and/or equatorial projections. This character (ratio of polar and equatorial projections) is crucial for distinguishing some Triprojectacites species at the LM level, so these finds again urge researchers to provide at least SEM observations in studies of dispersed pollen of this group for a clear interpretation. *Aquilapollenites quadricretaceus* and *A. stelckii* show more similarity to each other in their exine ultrastructure than they do to *A. amicus*; the latter differs notably in its thicker foot layer and thin infractum.

So far, TEM has been applied to a very limited number of fossil triprojectate species: *Aquilapollenites amicus*, *A. conatus* Norton,

A. quadricretaceus, *A. quadrilobus* Rouse, *Mancicorpus notabile* Mchedlishvili, *M. rostratus* Srivastava, *M. vancampoi* Srivastava, *Mtchedlishvilia canadiana* Srivastava, and *Pseudointegricorpus clarireticulatum* (Skvarla et al., 1987; Farabee, 1990, 1993; Tekleva et al., 2015). Unfortunately, except for *Pseudointegricorpus clarireticulatum*, there is no detailed description of the species studied in TEM and the photos are few, so it is difficult to use these data effectively. The available TEM photos of *A. quadricretaceus* and *A. amicus* are in good accordance with our data for our spp. 1 and 3.

4.2. Comparison with pollen grains of modern angiosperms

Previous works suggested similarities of Triprojectacites members with species from Apiaceae, Rubiaceae, Proteaceae, Sapindaceae, Loranthaceae, Santalaceae, Olacaceae, Caprifoliaceae s. lat. (including Morinaceae s. str., Dipsacaceae s. str., Caprifoliaceae s. str.), Elaeagnaceae, and Simaroubaceae (see review in Tekleva et al., 2015). The most “popular” comparisons include Apiaceae, Santalaceae, Loranthaceae, Morinaceae, and Proteaceae; the latter group is especially attractive in light of the two known finds of triprojectate tetrads arranged according to Garside's law (Catterall and Srivastava, 1985; Farabee and Skvarla, 1988). Among the above-mentioned families, besides Proteaceae, some Olacaceae members also have tetrads arranged according to Garside's law. Farabee (1991) made a detailed comparison of the main suggested modern taxa using SEM and found the most resemblance of some Triprojectacites taxa to some species of Santalaceae and Apiaceae. He also stated that the similarity of *Morina* (Morinaceae) and *Aptandra* (Olacaceae) in pollen morphology to triprojectates results from convergence as an adaptation to particular environments. The exine ultrastructure in the families compared to fossil triprojectate pollen is quite diverse: columellate (Apiaceae, Dipsacaceae, Olacaceae, Rubiaceae, Sapindaceae, Simaroubaceae), columellate, granular, or homogeneous (Proteaceae), spongy or granular (Morinaceae), and granular-columellate or columellate with a granular matrix between the columellae (Loranthaceae) (Feuer, 1978, 1986, 1989, 1990; Feuer and Kuijt, 1980; Verlaque, 1983; Blackmore and Cannon, 1983; Dettmann and Jarzen, 1990, 1996; Milne, 1994; El-Ghazaly et al., 2001; Sauquet and Cantrill, 2007; paldat.org, n.d.). So, each case should be carefully considered in terms of the exine ultrastructure; there are groups with a rather specific structure of the foot layer (e.g., Proteaceae), without a foot layer (Morinaceae), with branched columellae (Dipsacaceae s.str.), etc.

In the present paper, we present some new comparisons with species from Lythraceae. Although the choice of this family may seem strange and surprising, there are several reasons that led us to the comparison. On the one hand, there is still very little information on the detailed morphology (in SEM) and exine ultrastructure (TEM) of Triprojectacites species, which impedes further reliable comparison with the above-mentioned, “most popular,” extant groups of angiosperms. This will have to wait for the accumulation of factual data on different Triprojectacites genera and species. On the other hand, Braman (2013) presented a short review of hypotheses on palaeoecology of the Triprojectacites group; among them Srivastava (1973) suggested their preference for marshy, paludal environments, while there are also some different ideas from other authors. Tekleva et al., 2019a, 2019b suggested the possibility of an aquatic or semiaquatic (wetland) environment for some Triprojectacites species. In this context, we decided to make a comparison with a new family, Lythraceae, which attracted our attention by having several similar features in light of the aforesaid. Lythraceae are a widespread large family with numerous species occupying aquatic and wetland environments; among them there are dominant members in wetland biocenoses, including woody species with a relatively high percentage of pollen productivity and high possibility of being represented in a pollen spectrum (Graham, 2006). Pollen grains of Lythraceae species show trends towards complexity and thickening of the exine in particular parts of the wall

(Graham et al., 1985, 1987, 1990), as in some studied Triprojectacites species. In Lythraceae pollen as well as in Onagraceae and Myrtaceae (Kriebel et al., 2017), a trend towards ornamentation and projections in the regions near the apertures is conspicuous; this character is also somewhat reminiscent of pollen structure in Triprojectacites members. Although pollen grains of Lythraceae do not possess such clearly expressed projections, there is still some similarity in overall morphology. Most probably, these features are of convergent origin, but at this stage of knowledge we need an accumulation of as much data as possible for comparison. There are a number of taxa in Lythraceae that show equatorial structures somewhat similar to triprojectate morphology: *Trapa*, *Decodon*, *Didiplis*, *Lafoensia*, *Sonneratia*, *Duabanga*, and *Lourtella*. Among them, *Trapa* and the similar extinct genus *Hemitrapa* show the most outstanding morphology: large pollen, prolate-spheroidal, triangular amb, with three protruding apertures (short colpi/colpate to porate) and three meridional crests; the exine is differently sculptured – psilate, perforate to rugulate and verrucate, depending on the pollen region and/or species (Mohr and Gee, 1990; Zetter and Ferguson, 2001; Plate VIII, 1–7). *Decodon* has medium-sized prolate pollen with a circular amb, tricolporate apertures, colpi meridionally elongated, exine psilate to scabrate; in SEM faint elongated depressions parallel to the colpi are seen, representing incipient/residual pseudocolpi or slight folds (Lieux, 1983; Graham et al., 1985; Volkova, 2014). *Didiplis* has small oblate-spheroidal pollen with a triangular to oval-triangular amb, exine protruding in the mesocolpal region, tricolporate apertures, colpi meridionally elongated, exine striate to rugulate in the mesocolpal region (Graham et al., 1985). *Lafoensia* has medium-sized prolate to subprolate pollen with a circular or slightly lobate amb, tricolporate apertures with six pseudocolpi, meridionally elongated, exine scabrate to finely verrucate and psilate-punctate in the equatorial zone of the mesocolpal ridge, psilate at the poles (Graham et al., 1987). *Duabanga* has small prolate to prolate-spheroidal pollen with an oval amb, triplicate apertures, pores equatorially arranged and slightly protruding, exine scabrate to finely verrucate in mesocolpal regions and psilate to areolate at the poles (Graham et al., 1990). *Sonneratia* has medium-sized prolate pollen with an irregular amb, tricolporate apertures (or triplicate with pores located in elongated apertural fields), with three pseudocolpi, colpi meridionally elongated, exine scabrate to finely verrucate, psilate on the mesocolpal ridges and areolate at the poles (Muller, 1978; Graham et al., 1990; Mao et al., 2009). *Lourtella* has small prolate to prolate-spheroidal pollen with a circular amb, tricolporate apertures, colpi meridionally elongated, exine scabrate to finely verrucate (Graham et al., 1990). Another member of this family, *Cuphea* (Graham and Graham, 1971; Graham et al., 1985), bears some similarity to *Cranwellia* in the Triprojectacites group, which is not considered in the present paper but provides more basis for a comparison with Lythraceae. The exine ultrastructure in Lythraceae species shows great diversity, although it is mostly columellate; often the endexine is well-developed and rather thick towards the apertural regions; this thickening begins far from the apertural regions and is often combined with reduction of the foot layer, sometimes also beginning almost at the polar regions (Graham et al., 1985, 1987, 1990; Volkova, 2014). Such an endexine-foot layer condition was not observed in our Triprojectacites taxa, despite their characteristic endexine thickenings. An increased thickness of the infratectum like that observed in our *Aquilapollenites quadricretaceus* has not been indicated in any Lythraceae species. The columellate infratectum of Lythraceae species shows a great diversity, sometimes resembling the columellate infratectum of the studied species (as well as many other dicot pollen grains). *Trapa* pollen grains show a completely different exine ultrastructure from our pollen (Plate VIII, 8–10). Pollen morphology of Lythraceae species does not coincide with that of our *Aquilapollenites* species; however, there are similar trends in some features characteristic both for Lythraceae and the species under study: protruding equatorial ora and pores in prolate pollen, frequent occurrence of a striate and reticulate exine pattern, consistent presence of endexine, infratectal columellae mostly simple and not

branched. At least one species, *Pseudointegricorpus clarireticulatum*, has meridional furrows similar to the pseudoapertures of Lythraceae. The colporate condition is rare in Triprojectacites species and occurs in species with rather short equatorial projections, which raises the idea that the protruding part of the pollen grain, which corresponds to the equatorial projections of Triprojectacites, might have been replaced functionally by the ora in tricolporate pollen like that of extant angiosperms.

The fossil record of Lythraceae pollen shows that after the first finds in the Late Cretaceous the family rapidly diverged and became widely distributed by the end of the Paleocene (Muller, 1978; Grimsson et al., 2011; see reviews in Graham, 2013 and Volkova, 2014). Volkova (2014) studied and analyzed different “pseudoapertural” structures and, in particular, the possible path of evolutionary development of the pseudocolpi in Lythraceae and concluded that the pseudocolpi are newly formed structures and not a result of aperture reduction.

In the Lythraceae taxa considered above we observe varying degrees of protrusion of the apertures or equatorial parts of the wall. The recent idea of a possibly aquatic or semiaquatic (wetland) environment for at least some *Wodehouseia* and Triprojectacites species (Tekleva et al., 2019a, 2019b) gets some support from these new comparisons, based on the ecology of some of the above-mentioned taxa of Lythraceae (*Trapa*, *Hemitrapa*, *Decodon*, *Didiplis*, *Sonneratia*). Although *Wodehouseia* pollen exhibits quite different pollen morphology and ultrastructure, it is often found in the same pollen spectra as triprojectates. Its botanical affinity is also unknown and suggested comparisons with systematic groups vary greatly, but there are also a number of aquatic or semiaquatic taxa with somewhat similar morphology (e.g., *Lemna*, *Myriophyllum* (Alwadi, 2008)). It is possible that such morphologies resulted from highly specialized adaptation to wetland or aquatic environments. This does not imply that such morphology was exclusively an adaptation to wetland or aquatic environment. What we consider probable is that this morphology reflects a high degree of specialization, and the range of possible environments to which it was adapted could have included several types.

Together with other previous comparisons of the Triprojectacites group, we suggest that the development of such morphological features shows an adaptation to the environment rather than a phylogenetic character. This idea was repeatedly suggested in a number of earlier works (see review in Farabee, 1993). However, in order to prove this a detailed study of many more Triprojectacites members using SEM and TEM is needed that would also make possible an adequate comparison with possible modern relatives of members of this group.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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