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Pseudointegricorpus clarireticulatum (Samoilovitch) Takahashi: morphology and ultrastructure

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Dispersed tricolpate pollen of *Pseudointegricorpus clarireticulatum* (Samoil.) Takah. from the Upper Maastrichtian in Zeya-Bureya Basin, Amur (Heilongjiang) River area, Russian Far East/China has been studied with light and electron microscopy. Pollen size, pole outlines and the shape of equatorial projections show some variation within the species. The exine is striate-reticulate, semitectate and columellate. The species is characterised by highly complex structures that have harmomegathic function and include equatorial projections, endexinous thickenings, difference in the thickness of the infratectum, foot layer and endexine throughout the pollen grain, and equatorial furrows. Exine layers taper towards colpi regions while they break abruptly in furrow regions. The furrows could have helped to shed the exine quickly and enabled pollen germination. A non-extended region with a small cavity in the ectexine was observed in the equatorial region. We think that this region is characteristic of most Triprojectate species.

Keywords: Triprojectates; ultrastructure; harmomegathy; pollen

Introduction

Triprojectate pollen grains are known for their unique morphology and stratigraphic significance. A detailed history of studies of this group along with original data and extensive discussion are presented in Farabee (1993). This group comprises up to about 13 genera, although there is disagreement between different workers about which taxa belong to the group. The botanical affinities of most (if not all) members of this group are unknown and suggested ecological niches and pollination syndromes need to be supported by additional evidence. The only work on *in situ* Triprojectate pollen does not address these questions (McIver et al. 1991). At present, pollen grains of this morphology are studied mostly with light microscopy (LM), although scanning electron microscopy (SEM) data are gradually accumulating (see review in Farabee 1993; Hofmann and Zetter 2007; Samant et al. 2013). Information on the sporoderm ultrastructure available to date is scarce (Skvarla et al. 1988; Farabee 1990, 1993). Understanding of the Triprojectate pollen group, complete with systematic and phylogenetic position, ecology and functional aspects of the unique morphology, requires in-depth knowledge of their fine structure.

In this study, we present data on Triprojectate pollen from the Upper Maastrichtian of the Zeya-Bureya Basin, Amur (Heilongjiang) River area, Russian Far East/China. These pollen grains display equatorial furrows in addition to three meridional colpi and dominate in the pollen spectrum among other Triprojectate species (Markevich et al. 2011). Pollen grains similar in general morphology were first described as members of the genus *Integricorpus* by Mtchedlishvili (1961). In the same year, Funkhouser and Chlonova referred other pollen grains with equatorial furrows to *Aquilapollenites novacolpites* Funkhouser and *A. reticulatus* Chlonova. It was later noted that some *Integricorpus* species have equatorial furrows/colpi/fissures (e.g. Stanley 1970) and Samoilovich (1965) described pollen grains of *Integricorpus clarireticulatus* Samoilovitch with three equatorial furrows and conspicuous striate–reticulate exine sculpturing. Tschudy (1969) placed these pollen grains in *Aquilapollenites*, but in doing this she extended the definition of this genus too much. Takahashi (in Takahashi and Shimono 1982) established a new genus *Pseudointegricorpus* for pollen grains with differently developed equatorial furrows.

For this study, we have investigated a number of dispersed pollen grains of *Pseudointegricorpus clarireticulatum* (Samoil.) Takah. Individual pollen grains were first studied with LM and the same grains were subsequently studied in SEM and transmission electron microscopy (TEM). In addition, some pollen grains have been studied with confocal laser scanning microscopy (CLSM). The main goal was to document the morphological variability within the species and to obtain as much information as possible on its morphology and ultrastructure.

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Material and methods

The material comes from the Furao Formation, borehole XHY2008, in the vicinity of the Xiaoheyan of Jiayin in China, Amur (Heilongjiang) River area, in the Zeya-Bureya Basin (Markevich et al. 2011). The studied pollen grains belong to the *Aquilapollenites stelkii–P. clarireticulatum* assemblage of late Maastrichtian age. Thirty-five pollen grains of *P. clarireticulatum* (Samoil.) Takah. were picked up from the residue and each pollen grain was studied with LM; of those, 31 pollen grains were subsequently studied with SEM and 11 with TEM.

Pollen grains were photographed in glycerine slides on a Zeiss Axioplan-2 microscope (LM), then removed from the slides and after rinsing in ethanol they were mounted on SEM stubs on the emulsive face of small pieces of film. The pieces were affixed to the stub using nail polish. The stubs were sputter coated with gold. Pollen grains were observed and photographed under a Tescan SEM at the A.A. Borissiak Paleontological Institute, Russian Academy of Sciences. For TEM, some pollen grains were removed from the stubs and embedded in epoxy resin following the protocol of Meyer-Melikian et al. (2004). Pollen grains were sectioned with an LKB ultramicrotome Leica UC6. The ultrathin sections were post-stained with lead citrate and uranyl acetate for some pollen grains, and examined under Jeol 100 B and Jeol 1011 TEMs at the laboratory of electron microscopy, biological faculty, Lomonosov Moscow State University. Some sections were studied unstained.

Pollen grains have also been studied with an LSM 780 CLSM at the Core Centrum ‘Cell and Molecular Technologies in Plant Science’ in the Komarov Botanical Institute, Russian Academy of Sciences (St. Petersburg, Russia). Unstained pollen was mounted in glycerin slides and analysed using a 63×10 oil immersion objective and a 561-nm laser. The 3D pollen reconstructions were produced using the Zen 2011 imaging software.

Pollen size measurements were made in LM. The terminology used follows Hesse et al. (2009), except for some terms taken from original descriptions by other authors.

Results

The *P. clarireticulatum* pollen grains are isopolar to subisopolar, tricolpate, with three equatorial and two well-developed polar projections (Figures 1–3). The polar axis is 37–73 μm (mean = 61 μm), and the equatorial diameter (including equatorial projections) is −26 to 55 μm (mean = 37 μm). Meridional colpi extend to the full length of equatorial projections and reach the body (e.g. Figure 4(a),(c),(f)–(h))). Three equatorial (lateral) furrows are present, extending between the tips of adjacent equatorial projections (e.g. Figures 1(k),(s),(z),(ab), 2(m),(s), 3 and 4(a),(d)–(h)). Many specimens are broken into half along the equatorial furrows (Figure 4(i)) or just above endexinous thickenings (Figure 1(y)–(ad)). The pollen body is cylindrical or sub-ellipsoidal in equatorial view (Figures 1 and 2), with poles either broadly rounded (Figure 2(f)–(j),(r)–(t)) or with one rounded pole and the other one narrower and somewhat conical (e.g. Figures 1 (g)–(l),(s)–(x) and 3). Equatorial projections are short, V or U shaped. The exine sculpture is striate–reticulate with the striae oriented mostly perpendicular to the polar axis and becoming oriented parallel to the polar axis at the poles and near equatorial furrows (Figures 4 and 5).

The exine is semitectate, about 1.5–2.0 μm thick, and consists of a thick tectum, short collumelae and a well-developed foot layer and endexine (Figures 6 and 7). The thickness of the foot layer and endexine differs throughout the pollen grain and, in the regions of polar projections, they are not completely adjoined to each other at places and the foot layer is sometimes discontinuous (Figures 6 (j),(m) and 7(k)). In the central part of the pollen grain, the exine is slightly thickened at the expense of the infratectum, foot layer and endexine. In this region, the infratectum appears to contain columellar elements of different sizes, the foot layer increases in thickness and so does the endexine (Figure 6(c),(d),(f)). There is a non-extended region where the distance between the tectum and foot layer increases and a small cavity with loosely arranged infratectum elements can be observed (Figure 7 (a),(l)). The endexine is more electron dense than the ectexine and appears to be homogeneous. The endexine is thinnest in the regions of the polar projections and becomes thicker towards the equatorial projections (Figures 6(b)–(d),(g),(j),(m) and 7(k),(l)). Endexinous thickenings are formed at the polar ends of the colpi (Figures 1(a),(b),(k),(y)–(ab), 2(a),(b),(p) and 3(e),(f)). Towards the colpi the ectexine elements taper and then disappear; the endexine becomes thinner at the tip of the equatorial projections (Figures 6(f) and 7(b),(e)–(j)). Over the furrows, there is a break in all exine layers (Figure 6 (aj)–(d),(i),(k),(l)), and the endexine seems to be slighly thickened in the regions adjacent to the furrows (Figure 6 (c),(d),(k),(l)). Regions with endexine present at the bottom of equatorial furrows were observed (Figure 6(h)); these regions probably reflect the true ultrastructure of the furrows, while regions displaying a break in all exine layers may be the result of endexine breaking during fossilisation.

Although the LM, SEM and TEM data, considered together, are much more informative about the structure of these pollen grains than CLSM data, the CLSM is useful in enhancing the general understanding of the structure of this type of complex pollen and of the thickness differences of exine layers throughout the pollen. In turn, this makes the subsequent examination of the pollen with SEM and TEM much easier. In addition, CLSM provides

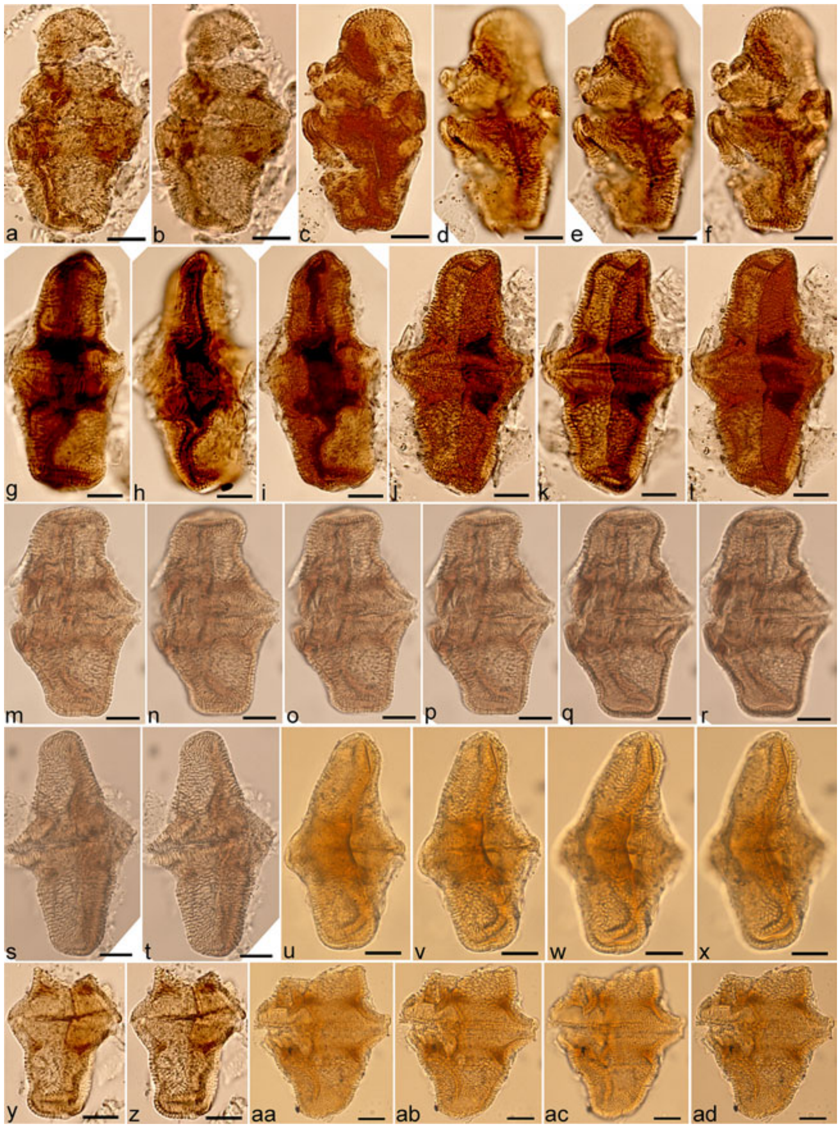

Figure 1. (Colour online) LM of pollen (p) with different foci. (a, b) p#1; (c–f) p#2; (g–i) p#3; (j–l) p#4; (m–r) p#5; (s, t) p#6; (u–x) p#7; (y, z) p#8, broken pollen grain; (aa–ad) p#9, broken pollen grain. Scale bar: 10 μm.

better resolution of exine sculpturing and pollen wall stratification than LM.

Discussion

Comparison of the general pollen morphology with related species

Pollen grains referred to *P. clarireticulatum* were previously included in genus *Integricorpus* Mtchedlishvili, 1961. The genus *Integricorpus* with the type species *I. bellum* Mtchedlishvili was established for rather large isopolar tricolpate pollen grains with cylindrical or ellipsoidal body, three equatorial projections and reticulate exine (Mtchedlishvili 1961). Samoilovitch (1965) described the new species *Integricorpus clarireticulatus* Samoilovitch from the Maastrichtian–Danian of Yakutia, Sakha Republic, Russian Federation. The diagnosis of the genus reads: ‘Pollen grains rather large to large, isopolar or subisopolar, with three equatorial and three meridional colpi/furrows. Body ellipsoidal, large, with three equatorial projections. Projections angled to the body, narrow, not long. Exine rather thin, clavate, reticulate, not tectate’ (Samoilovitch 1965, p. 123; our translation). The diameter in polar view is 49.7 μm, the polar axis in equatorial view is 56.8 μm and the equatorial diameter (including projections) is 54.7 μm. Samoilovitch notes that one of the polar projections is somewhat pointed and the other is rounded, and that the sculpture is clearly reticulate with coarse lumina in the mesocolpia, while they decrease in size and stretch near equatorial furrows. Concerning the

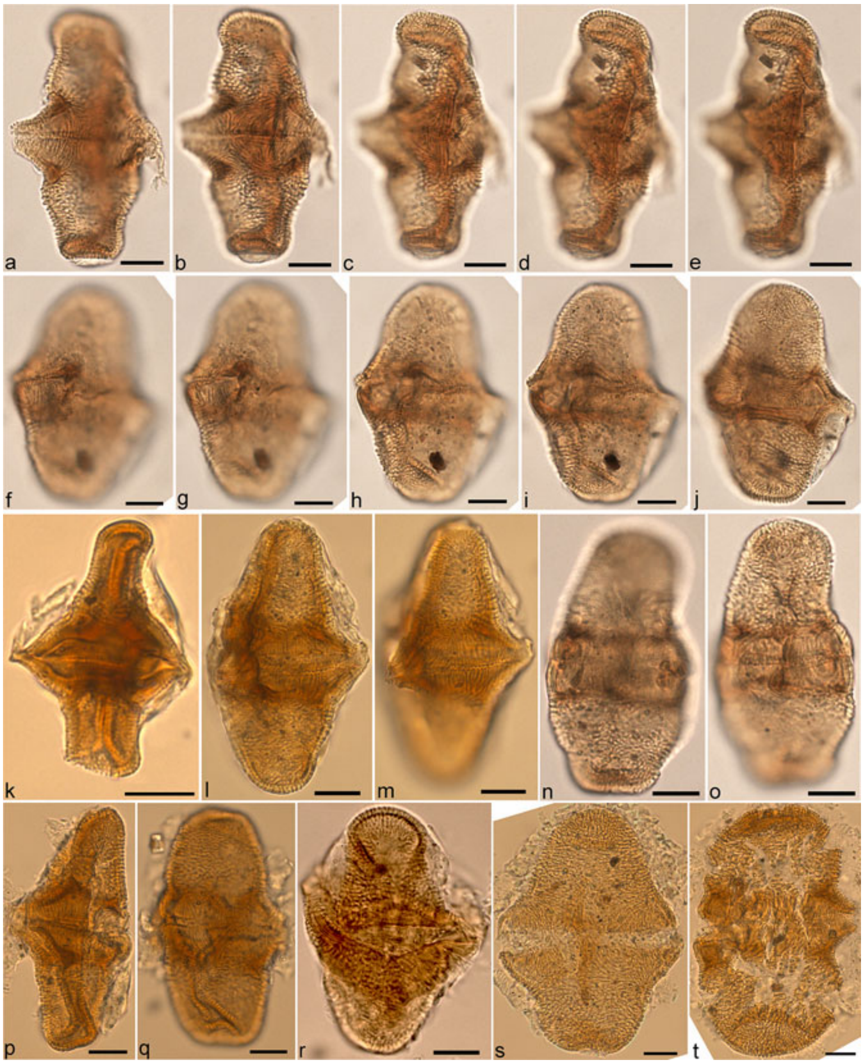

Figure 2. (Colour online) LM of pollen (p) with different foci. (a–e) p#10; (f–j) p#11; (k) p#12, the smallest pollen grain among the studied material; (l, m) p#13; (n, o) p#14; (p) p#15; (q) p#16; (r) p#17; (s) p#18, elliopsoid pollen, rarely found for this species; (t) p#19, elliopsoid pollen, rarely found for this species. Scale bar: 10 μm.

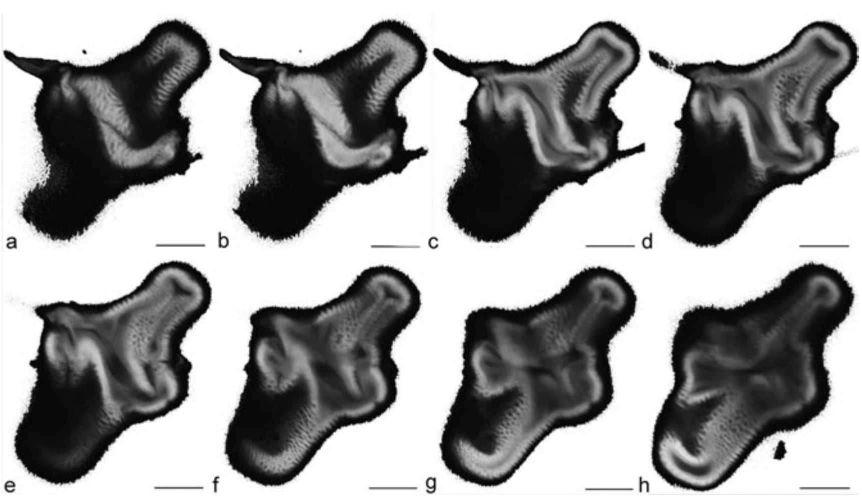

Figure 3. CLSM of pollen #20. (a–h) Different optical sections of the same pollen grain. Scale bar: 10 μm.

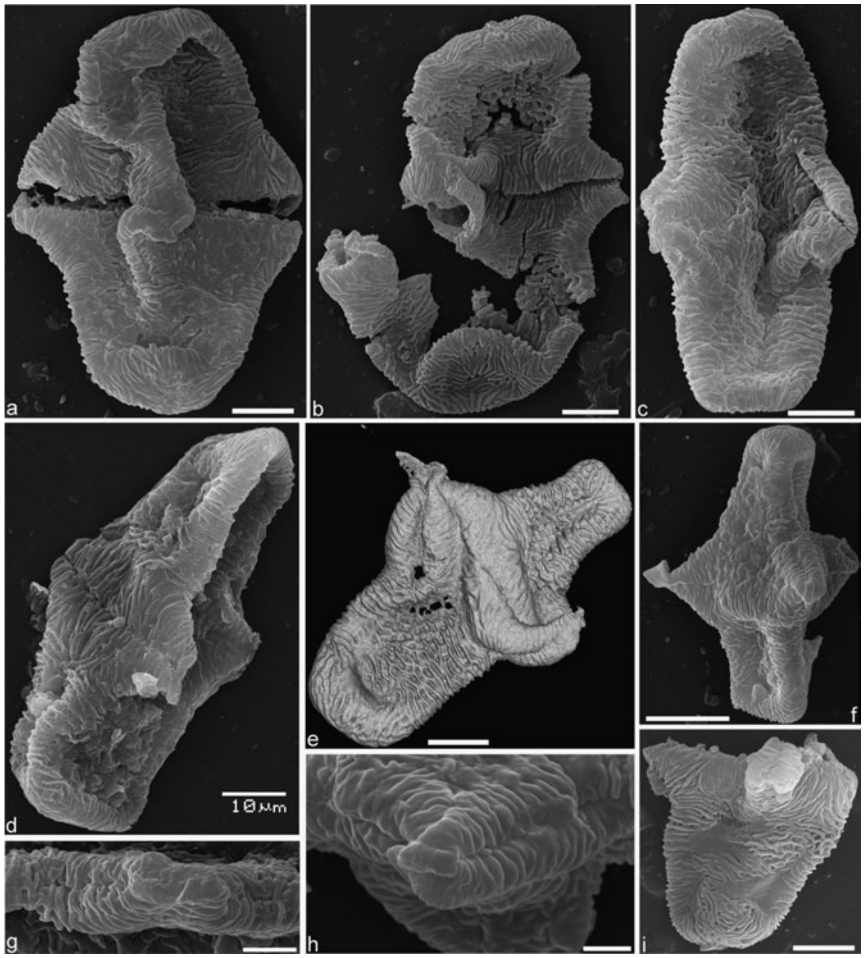

Figure 4. Exine sculpturing. (a–d), (f–i) SEM and (e) CLSM. (a) p#18; (b) p#19; (c) p#16; (d) p#3; (e) p#20; (f) p#12; (g) p#15, equatorial projection with a colpus; (h) p#12, equatorial projection with a colpus; (i) p#21, half of a pollen grain. Scale bar: 10 µm for (a–f), (i), 5 µm for (g) and 2 µm for (h).

exine structure, she writes that the exine layers are not clearly distinct and notes the presence of clavate sexine, very thin endonexine and ectonexine which thickens towards the equatorial projections and wedges out to their tips and at the poles. The terms 'endonexine' and 'ectonexine' are used following Erdtman (1952). Samoilovitch also specifies that Mtchedlishvili (1961) did not observe equatorial furrows when establishing the genus *Integricorpus*, but they were observed in *I. clarireticulatus* and the separated halves of *I. bellum* pollen grains, very likely broken due to thinned areas, support the presence of equatorial furrows. Unfortunately, Samoilovitch based her description on only four pollen grains from three outcrops and, thus, could not characterise a significant range of morphological variability. There is a complete translation of the diagnosis, description, remarks, etc. of *I. clarireticulatus* from Russian into English in Stanley (1970) and in Tschudy's (1969) work; the latter transferred this species to the genus *Aquilapollenites*.

Tschudy (1969) found pollen grains of this species (*I. clarireticulatus sensu* Samoilovitch) in two localities (Lower Campanian of Montana, USA, and Upper Cretaceous of Alaska, USA) and provided a description based on 40 specimens. The polar axes are from 28 to 44 µm, and equatorial diameters (including equatorial projections) are from 29 to 42 µm. She described poles as 'broadly rounded' (in contrast to Samoilovitch's note about difference pole outlines). Tschudy also noted that the colpi occupy the full length of equatorial projections, extending onto the body for about three-fourths the distance to the poles, while Samoilovitch (1965) did not report the length of colpi. The equatorial projections of the pollen illustrated in Tschudy (1969) appear slightly longer than those in the pollen grains studied here. Other characters are quite similar in both works, including the sculpture pattern changing towards the equatorial furrows, a feature also illustrated with SEM images for some of Tschudy's pollen. Overall, the studied pollen grains fit this

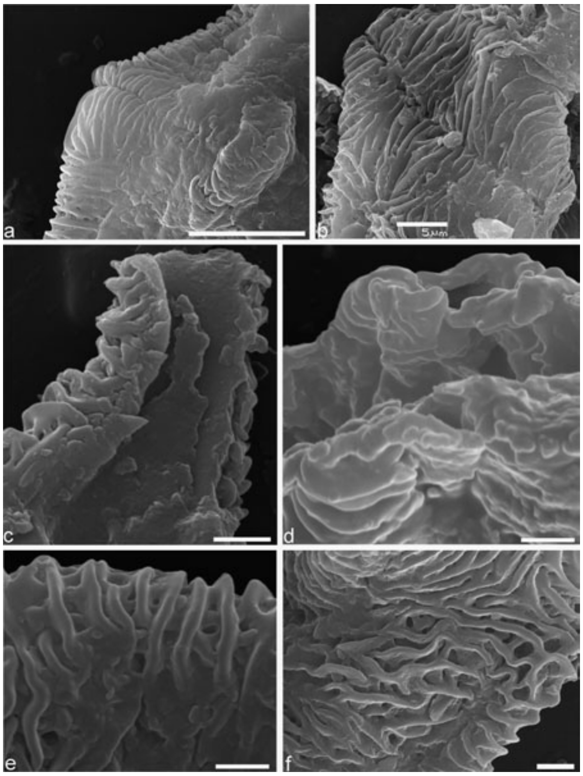

Figure 5. Exine sculpturing. (a–f) SEM: (a) p#11, equatorial region, equatorial furrow and meridional colpus can be seen; (b) p#3, equatorial region, equatorial furrow is seen; (c) p#22, broken in half equatorial projection, inner surface of the pollen wall is seen; (d) p#23, tips of two equatorial projections; (e) p#15, exine sculpturing on the polar projection; (f) p#21, exine sculpturing at the transition between equatorial and polar projections. Scale bar: 10 μm for (a), 5 μm for (b) and 2 μm for (c–f).

description: the polar axis is from 26 to 55 μm, and the equatorial diameter (including equatorial projections) is from 49 to 73 μm; polar projections in many pollen grains are indeed unequal, having pointed/rounded outlines, while other pollen grains have similar rounded polar projections; the colpi extend to the length of equatorial projections or are slightly more onto the pollen body (all having quite similar length), but never as long as Tschudy (1969) described, the sculpture changes in the equatorial region with striations becoming perpendicular to equatorial furrows. A lot of pollen grain halves or pollen broken in the equatorial region are also present. The slight difference in size between our pollen and that studied by Tschudy (1969) and Samoilovitch (1965) can be easily explained by differences in chemical processing or preservation, or may be due to the greater number of specimens observed in this study. Another possible explanation is that Tschudy described two or more species under *Aquilapollenites clarireticulatus* (Samiolovitch) Tschudy, as discussed by Hofmann and Zetter (2007).

Pseudointegricorpus clarireticulatum and *Integricorpus* species, which are quite similar to *Pseudointegricorpus* and are present in the studied assemblage, were assigned by Farabee (1993) to two morphotypes: isopolar strioreticulate lateral-furrowed and isopolar strioreticulate short-colpate. As noted above, the pollen grains under study have colpi extending to the length of the equatorial projections and the pollen grains of the same species studied by Tschudy (1969) are characterised by rather long colpi extending on the polar projections, whereas Samoilovitch (1965) did not mention colpus length at all when establishing the species! Nevertheless, the presence of lateral (equatorial) furrows allows us to assign our pollen grains, as well as *A. clarireticulatus* (Tschudy 1969) and *I. clarireticulatus* (Samoilovitch 1965), to the isopolar strioreticulate lateral-furrowed type *sensu* Farabee (1993). Other published species with equatorial furrows (located between equatorial projections) are *A. novacolpites* Funkhouser, *A. medeis* Srivastava, *A. oblatus* Srivastava, *A. validus* Srivastava and *A. hermanii* Hofmann et Zetter. Takahashi (Takahashi and Shimono 1982) transferred the first four species to the new genus *Pseudointegricorpus* with the type species *P. novacolpites* (Funkhouser) Takahashi, which he established for 'isopolar to sub-isopolar pollen grains [...] with well or poorly developed equatorial projections [...] and with equatorial exine fissures (or colpi) short or long'. *Aquilapollenites hermanii* has been described later, in 2007, and probably should also be transferred to *Pseudointegricorpus*. This would group all Triprojectate pollen grains with meridional (lateral) furrows within the genus *Pseudointegricorpus*. Aside from the above-mentioned species, Takahashi and Shimono (1982) included in this genus pollen grains previously published as *Aquilapollenites reticulatus* Chlonova, 1961, which had been subsequently transferred to *A. chlonovae* (Chlonova) by Srivastava (1968). In addition, Takahashi and Shimono (1982) described three new species (*Pseudointegricorpus kokufuense* Takahashi et Shimono, *P. protrusum* Takahashi et Shimono, *P. fragile* Takahashi) and one *Pseudointegricorpus* sp.

Chlonova (1961) did not mention the presence of equatorial furrows for her *A. reticulatus*, although they are clearly visible in her drawings (Plate XIV, fig. 107,107(a)). Main differences between *A. reticulatus* and the pollen studied here are in the exine sculpture (judging from the drawings), shape of equatorial projections and aperture type. The latter is described as rounded pores 3–6 μm in diameter (the pores described by Chlonova are also discussed in the section 'Fine structure and probable botanical affinity of the studied pollen grains'). Another species described in the same publication, *Aquilapollenites abscissus* Chlonova, is quite similar to the pollen grains studied here, judging from the drawings (Chlonova 1961, Plate XV, fig. 109(a)) – the architecture and exine sculpture whose pattern changes in the equatorial region – but it is unclear whether equatorial furrows are present and the description is too short to allow for conclusive comparison.

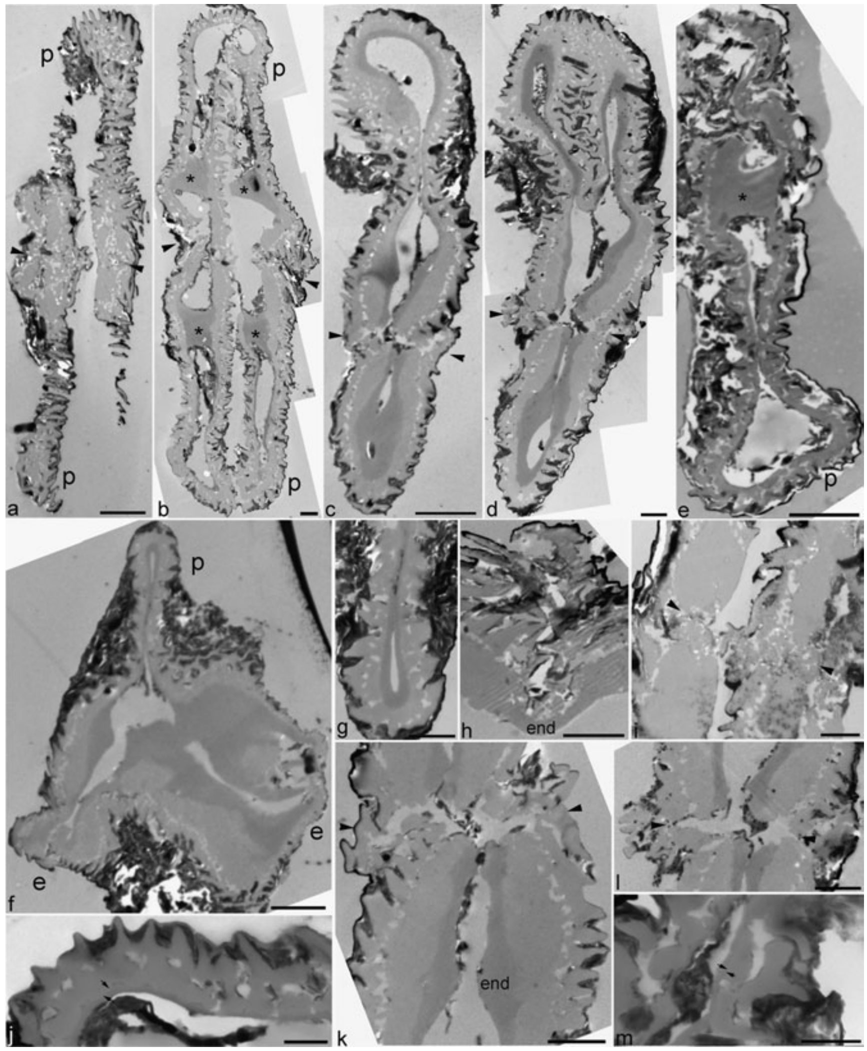

Figure 6. TEM, longitudinal sections. (a, b, h, i) p#24; (c, d, f, g, k, l) p#25; (e, j, m) p#15. (a) Section through the pollen grain, tips of sculpture elements of the pollen and the tip of equatorial projection are sectioned; (b) section through the pollen grain, endexinous thickening and equatorial furrows are cut; (c, d) slightly oblique sections through the same pollen grain on different depths, equatorial furrows are cut, different thickness of the endexine and foot layer through the pollen grain can be observed; (e) section through the pollen grain, endexinous thickening is seen; (f) slightly oblique sections through the same pollen grain, one polar projection and two equatorial projections are cut, endexinous thickening and colpus structure can be seen; (g) exine structure in the region of polar projection; (h) region of equatorial furrow, the endexine is seen on the furrow bottom; (i) region of the equatorial projection, equatorial furrow is cut; (j) exine structure in the region of polar projection with discontinuous foot layer and thin endexine, sometimes the foot layer and endexine do not closely adjoin to each other; (k, l) equatorial furrow is cut, slightly thickened endexine is seen near the furrow break; (m) exine structure in the region of polar projection with discontinuous foot layer and thin endexine. Scale bar: 5 μm for (a, c, e, f), 2 μm for (b, d, g–i, k, l) and 1 μm for (j, m). Arrowheads point to regions of equatorial furrow, asterisks mark endexinous thickenings, arrows indicate border between foot layer and endexine; end, endexine; e, equatorial projection; p, polar projection.

Aquilapollenites novacolpites described by Funkhouser (1961), which was transferred to *Integricorpus novacolpites* by Stanley (1970), differs from our specimens by the shape and greater length of equatorial projections and the three pairs of demicolpi (vs. three colpi in our species) which extend to a greater degree on the polar projections. Several species described by Srivastava (1968) differ from our material in overall pollen shape, shape and greater length of equatorial projections, long meridional colpi, probably exine sculpture and short equatorial furrows restricted within the equatorial projections for *Aquilapollenites medeis* and *A. validis*. Interestingly, Baksi and Deb (1976) described *Aquilapollenites bengalensis* Baksi et Deb with short equatorially oriented colpi (in contrast to usual meridional orientation in other species) at equatorial projections. Samant et al. (2013) also observed *A. bengalensis* with SEM, noting that the equatorial colpi are so short that they are comparable to

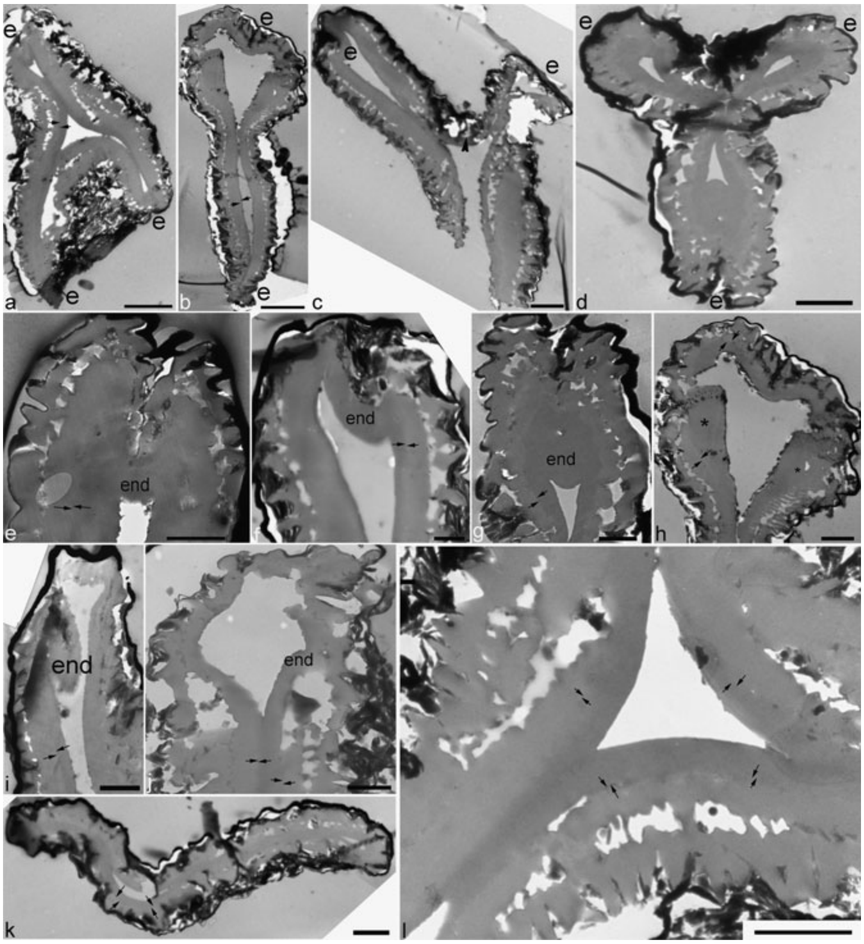

Figure 7. TEM, tangential section. (a, j, l) p#7; (b, c, f, h) p#9; (d, e, g, i) p#12; (k) p#16. (a) Section through three equatorial projections, region with a cavity between tectum and foot layer can be observed; (b) section through two equatorial projections, endexinous thickenings can be seen; (c) section through three equatorial projections, a region of equatorial furrow cut; (e–j) colpus regions at different depths; (k) region of polar projection; (l) higher magnification of the region with a cavity in the ectexine. Scale bar: 5 μm for (a, b), 2 μm for (d, h, k, l), 1.25 μm for (i) and 1 μm for (c, e–g, j). Arrowhead points to region of equatorial furrow, asterisks mark endexinous thickenings, arrows indicate border between foot layer and endexine; end, endexine; e, equatorial projection.

the 'pores' of *Aquilapollenites* species described by Chlonova (1961).

Pseudointegricorpus kokufuense is very similar to our pollen grains and, as noted by Takahashi and Shimono (1982), it differs from *P. clarireticulatum* in 'morphological forms of the central body and the equatorial projections'. *Pseudointegricorpus protrusum* is also quite similar to *P. kokufuense* and *P. clarireticulatum* but characterised by larger size (with polar axis 73–100 μm). *Pseudointegricorpus fragile* is easily distinguished by its finely punctate or 'chagrenate' (apparently meaning shagreen or shagranate termed in Kremp 1965) exine sculpture, also it has longer colpi than our specimens. *Pseudointegricorpus* sp. described in Takahashi and Shimono (1982) differs from our specimens in larger colpi, short equatorial furrows and exine sculpture. Furthermore, poorly developed endexinous thickenings were reported for *P. kokufuense*, *P. protrusum* and *P. fragile*, while in *Pseudointegricorpus* sp. they are well developed (Takahashi and Shimono 1982). In other *Pseudointegricorpus* species, the endexinous thickenings (costae, meridionally thickened nexine of contact areas) are well developed, although Tschudy (1969) observed for *A. clarireticulatus* pollen grains with endexinal costae both present and lacking. Endexinous thickenings are well developed in all our pollen grains.

On the whole, there are significant differences within the genus *Pseudointegricorpus*, although the presence of lateral (equatorial) furrows is a useful character for quickly distinguishing between species of this genus. Differences or similarities in outlines of polar projections (pointed/rounded), as well as pollen size, seem to be rather less useful in distinguishing species [as done for some species by Takahashi and Shimono (1982)] if not corroborated by additional characters. Endexinous thickenings and their position towards the apertures are characteristic for the Triprojectate group as a whole, but the preservation of this layer is not always satisfactory, so that possible differences in the extent of this character are hard to use in distinguishing taxa within the group. Nevertheless, the endexinous thickenings are well preserved and located at the colpi ends in all our specimens.

Fine structure and probable botanical affinity of the studied pollen grains

Very little TEM information is available on pollen grains in the Triprojectate group. Skvarla et al. (1988) and Farabee (1990, 1993) studied several species of isopolar (*Aquilapollenites amicus* Sriv., *A. conatus* Norton and *A. quadricretaceus* Chlonova) and heteropolar (*A. quadrilobus* Rouse, *Mancicorpus notabile* N. Mtch., *M. rostratus* Sriv., *M. vancampoi* Sriv. and *Mtchedlishvilia canadiana* Sriv.) morphotypes, all from other types than our pollen grains. *Aquilapollenites amicus* belongs to the isopolar strioreticulate long colpate type and is very similar in ectexine structure to *P. clarireticulatum*, but scarce illustrations and the absence of a detailed description preclude extensive and conclusive comparison. The similarity in exine sculpture is nevertheless conspicuous.

Our *P. clarireticulatum* pollen grains are characterised by very complex morphology and ultrastructure. Although they all belong to the same type, some variation in shape and size is present (especially Figure 2(k),(s),(t)), with the pollen body exhibiting a more cylindrical or ellipsoid shape, and polar projections rounded or pointed. This variation is probably due in part to different degrees of the pollen compression, and in part to natural morphological variability within the species. The size variation reported here falls within the range known for this species. Exine sculpturing is quite uniform in all studied pollen grains: striate–reticulate, with rather long anastomosing striae and lumina between them. This pattern is visible in both LM and SEM and, at the ultrastructural level, it forms a semitectate sporoderm.

The apertures are three colpi without any trace of endoapertures (ora). The apertures of Triprojectate pollen grains were described as colpi, demicolpi and colpoids and, for some of the species, tricolporate pollen grains were observed. For *Pseudointegricorpus* species, equatorial (lateral) colpi/furrows/fissures, termed 'furrows' in this study, are present. These furrows are not considered true apertures by many authors (including us) and apparently have harmomegatic function. The structure of true apertures was discussed by several authors who expressed different views. Mtchedlishvili (1961) and Chlonova (1961) described Triprojectate species with pores. Their material was examined by Stanley (1970) who considered that all of these species do not have pores, but colpoids. Demicolpi described by Funkhouser (1961) for several *Aquilapollenites* species are represented by three pairs of meridionally oriented apertures, but it is difficult to tell with confidence from Funkhouser's LM photos whether three colpi or three pairs of demicolpi are present.

Stanley (1970) believed that 'demicolpi' can occur by splitting of endexinous thickenings in any species of Triprojectate group, and Farabee (1993) considered the 'demicolpi' as long colpi with a constriction at the tip of the equatorial projections. Stanley (1961, 1970) also proposed that true apertures are colpoids which are meridionally oriented and always located only at the distal ends of equatorial projections, while the rest of the colpi (as we call them) are pseudoapertures or exine fissures, represented by a 'more or less predetermined rupture in a thick part of the exine that is internally initiated and which may reach the outer surface in some cases'. Stanley also wrote that they 'may go from the end of the colpoids along the polar edges of the projections toward the body and in some cases they can continue on to the body almost reaching the poles of the pollen grain'. And he also admitted for meridionally oriented exine fissures (what we call colpus ends in this case), that

> at other times there is a distinct invagination which starts on the projections and continues well on to the body, often reaching the poles and in this case the break in the exine may start from the exterior rather than the interior of the grain. (Stanley 1970)

This is a rather complicated explanation and the observations were made in LM, but it is difficult to examine all these fine details at such low magnifications. Stanley himself writes that 'this is not certain'. In our material, we did not see this pattern and considered our apertures to be colpi.

Farabee (1993) distinguished short and long colpate types in his material and he referred to the structures termed colpoid apertures by Stanley as short colpi, although there is evidently no clear correspondence between the two terms, as Stanley considered all true apertures in Triprojectate group to be colpoids with differently developed 'exine-fissures'. Here we do not use the term colpoid and treat all our pollen grains as having colpi of the same length which extend onto the whole equatorial projection region.

Equatorial furrows (or equatorial colpi, as they were referred to by some authors) were also interpreted by Stanley (1970) as pseudoapertures or exine fissures and he considered them to represent ruptures in a thick portion of endexine. Furthermore, in spite of the presence of sculpture differentiation in these regions, Stanley thought that these are 'tears' and they are 'always initiated from the interior and ragged-edged, and it may or may not penetrate the ektexine reaching the surface'. He also thought that sometimes there are several fine fissures parallel and staggered to one another. In our material, we consistently observed one furrow between equatorial projections and, in our opinion, Stanley's (1970, Pl. 3, figs 8 and 9) illustrations show two margins of one open furrow. Farabee (1993) stated that interpretation of these (lateral or equatorial) furrows as apertures is not supported by EM examinations.

We believe that although these furrows are indeed not true apertures, a differentiation of the exine sculpturing in this region, along with their consistent presence (both supported by EM observations; Figures 4(a),(b),(d)–(f), 5 (a),(b), 6(a)–(d),(h),(i),(k),(l) and 7(c)), precludes their interpretation as ruptures, or 'a result from tension produced when the equatorial projections are forced against the body' as suggested by Stanley (1970). Most probably, the sporoderm of living pollen grains in the region of the furrows consisted primarily of intine. These furrows may have helped in changing the volume of a partly hydrated/dehydrated pollen grain without damaging its living content (harmomegathy). These structures could also have helped to shed the exine quickly and enabled the pollen tube growth.

Equatorial projections and endexinous thickenings (when present/preserved) are thought to be one of main features of the Triprojectate group, and evidently appearance of these characters in this group is interrelated. Differences in the thickness of the foot layer and endexine in the polar and equatorial regions, furrows and the non-extended region of thickened and loose infratectum, along with endexinous thickenings (one of the functions of which is believed to prevent the wall of Triprojectate pollen from collapsing), comprise a highly complex set of characters, which undoubtedly bears harmomegathic functions. However, the full functional significance of these features and their relationship to the overall ecology of the parent plants are unresolved. The non-extended region with a cavity in the ectexine or an increased ectexine with loosely arranged infratectum which is located near the endexinous thickenings, not mentioned previously for Triprojectate pollen, may also be a characteristic feature of this group, associated with the presence of the endexinous thickenings. We observed a similar structure in another Triprojectate species (*Aquilapollenites stelckii* Sriv., unpublished data) and the same structure can be observed in Farabee's (1993, figs 65 and 66) illustrations, but detailed documentation of these structures requires additional ultrastructural studies of Triprojectate species.

Pollen grains of the Triprojectate group were compared with members of Pinaceae, Apiaceae, Morinaceae, Dipsacaceae, Rubiaceae, Proteaceae, Sapindaceae, Loranthaceae, Santalaceae, Olacaceae, Caprifoliaceae, Elaeagnaceae and Simarubaceae by different authors. Although Pinaceae and other gymnosperms were quickly excluded, the angiosperms keep being considered and reconsidered without any conclusive outcomes on the true relation to any members of the Triprojectate group. The most common comparisons involve Loranthaceae, Santalaceae and, to a somewhat lesser extent, Apiaceae species (e.g. Stanley 1970; Erdtman 1971; Jarzen 1977), whereas another current opinion considers similarity to Proteaceae species [especially concerning findings of tetrads of *Integricorpus reticulatus* (Mtchedlishvili) Stanley (Farabee and Skvarla 1988) and *Aquilapollenites* species (Catterall and Srivastava 1985) which are arranged according to Garside's law]. However, it is now becoming more clear that the set of characters present in pollen of the Triprojectate group, and especially in the species studied here, is quite distinct from any modern plant lineage. The still scarce information available on the fine structure of pollen in this extinct group prevents from the formulation of hypotheses on their possible ancestors, and additional information is needed for resolving the taxonomic affinities of the plants that produced pollen grains grouped in the Triprojectate type.

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