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Exine morphology and ultrastructure of *Duplicisporites* from the Triassic of Italy

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A morphological study of dispersed Circumpolles pollen grains from the Upper Triassic of the Southern Alps has been initiated with the genus *Duplicisporites*. Individual pollen grains were studied by means of scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Seen with SEM the pollen surface is finely verrucate with low verrucae of different sizes. A sub-equatorial continuous rimula is clearly visible. The proximal trilete scar is small and indistinct. TEM images reveal a bi-layered exine. The ectexine is formed by numerous small, closely packed, granulae subdivided by irregularly-spaced cavities. In the region of the subequatorial canal, the ectexine becomes thinner, about 1/3 of the usual thickness. At places, the ectexine is slightly separated from the underlying endexine. The endexine is prominent and significantly darker than the ectexine. It is homogeneous and of constant thickness. On the basis of its older age, with respect to *Classopollis*, the present ultrastructural dataset provides information on the possible origin of cheirolepidiaceous-type morphology.

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The fossil pollen group Circumpolles is of interest to palynologists and evolutionary biologists due to its complex morphology and parallelisms to angiospermoid characters (Pocock et al. 1990). The relationship between *Classopollis*, the most advanced member of the group, and the family Cheirolepidaceae has been proven (Alvin 1982). However, early members of the Circumpolles group differ remarkably from typical *Classopollis* and have not yet been found in any cheirolepidaceous fructifications. Although scanning and transmission electron microscopy are valuable tools for evolutionary palaeobotany, available data on the exine ultrastructure of early members of Circumpolles are scarce (Medus 1977, Kedves 1994). To fill this gap in information the ultrastructure of some ancient members of the Circumpolles group has been studied. Upper Triassic units of Italy, Austria and Hungary have revealed rich palynological assemblages, which contain members of Circumpolles (De Zanche et al. 2000, Roghi 2004). Those from the Carnian of Italy served as the material for the present morphological study.

MATERIAL AND METHODS

Detailed examination of rich fossiliferous sediments (De Zanche et al. 1993, 2000; Roghi 2004) from the Upper Triassic of the Dolomites and Julian Alps provided the material for this study (Fig. 1).

The specimens studied come from sample 'SCS 16' collected from the mixed carbonate-clastic Heiligkreutz-S. Croce Formation (ex Dürrenstein Formation), in the type locality of the formation, near

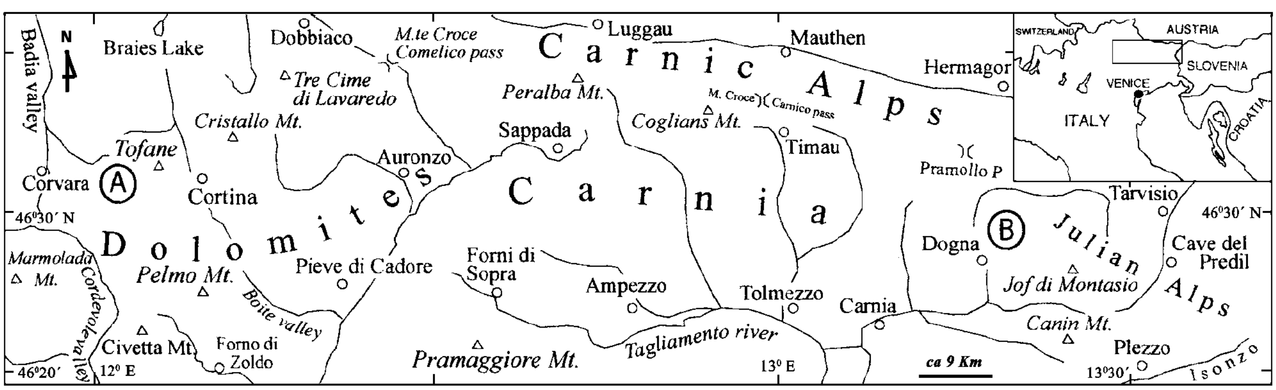

Fig. 1. Map of the study area with sample sites (A, B).

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the Heiligkreutz Hospiz (Badia Valley, Dolomites; Gianolla et al. 1998*a*, Keim et al. 2001, Koken 1913). Fossil resin, remains of plants, bivalves, and vertebrates (teeth and bones) have been discovered in sediments from this formation (Koken 1913, Dalla Vecchia & Avanzini 2002, Roghi et al. 2002). Cuticle analysis (Roghi et al. 2002) indicates the presence of plants referred to the Cheirolepidaceae (det. J. Van Konijnenburg-Van Cittert). The age of this formation is dated as Late Julian – Early Tuvalian (Carnian) by ammonoids and palynomorphs (Gianolla et al. 1998*b*, De Zanche et al. 2000, Roghi 2004).

The palynological assemblage from the Heiligkreutz-S. Croce Formation includes typical Upper Triassic elements such as trilete laevigate and ornamented spores, monosaccate and bisaccate pollen types, and Circumpolles. Quantitative analysis indicates the presence of conifers (33 – 46%), pteridosperms (29%), and lycopsids (13%) (Table I).

The pollen samples were macerated and treated with HCL, HF and, when necessary, HNO_3 (3 – 5 minutes). After washing and sieving, the residue was mounted in glycerol jelly. Slides are stored in the Dipartimento di Geologia, Paleontologia e Geofisica of Padova University. LM was carried out using a Leica DMLB microscope and Leica DFC 300 firewire cameras for photomicrography. For SEM the pollen grains were mounted on stubs, coated with gold-palladium and examined with a Hitachi S-405A SEM. For TEM pollen grains were removed from SEM stubs with a needle and embedded in epoxy resin (Meyer-Melikian & Telnova 1991). The pollen grains were sectioned with a LKB 3 ultramicrotome using a diamond knife, and then examined with a Hitachi H-600 TEM. The SEM and TEM negatives are held in the palaeobotanic laboratory of Palaeontological Institute (Moscow).

MORPHOLOGICAL DESCRIPTION

Several genera (*Duplicisporites*, *Paracirculina*, and *Camerosporites*) belonging to the group Circumpolles, and of supposed cheirolepidaceous affinity are common in the palynological assemblage and comprise about 10% of the total (Table I). For the morphological analysis *Duplicisporites* was chosen because of its abundance and good preservation. Pollen grains of this genus are circular to subtriangular in outline, with a rimula in the equatorial zone. The position of the rimula may vary resulting either in identical, or in different diameters and shapes of the hemispheres.

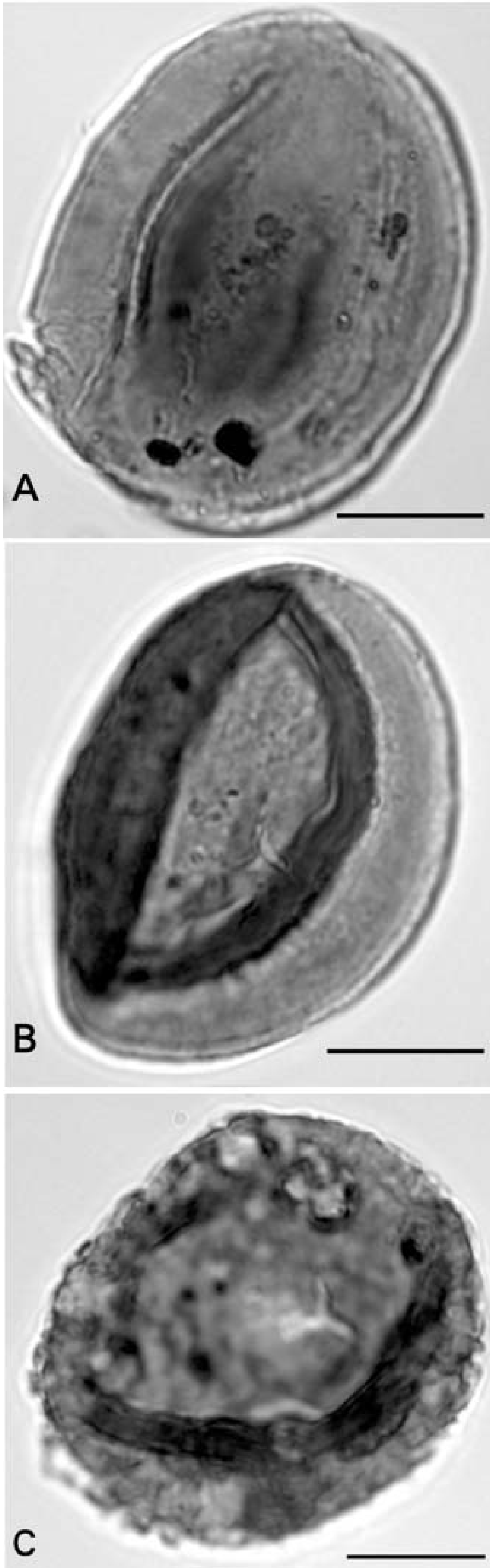
Duplicisporites granulatus **(Leschik) Scheuring 1970, (Fig. 2 A, B, 3A – D, 4A – E).**

Pollen grains are circular to subtriangular in outline. A small trilete mark with simple laesurae is often visible at the proximal pole (Fig. 2 B). In the equatorial zone a ring-like exine thinning forms a rimula (Fig. 2 A, B). Finely granulate to vermiculate ornamentation is observed. Specimen ‘SCS 16/6’ is 43 μm diameter (Fig. 2 A), and ‘SCS 16/8’ is 42 μm in diameter (Fig. 2 B).

SEM of the pollen surface is finely verrucate (Fig. 3A – D) with short, variably sized verrucae. A sub-equatorial continuous rimula is clearly distinguished. The proximal trilete scar is small and indistinct, with the margins closely pressed together. TEM reveals typical exine organization with a bi-layered wall (Fig. 4 A, B). The ectexine is formed by numerous, small, closely packed granulae subdivided by irregularly-spaced cavities. At the base of this layer the granulae are clearly visible (Fig. 4 D), while superficially they are fused into an indistinct tectum with an undulating margin (Fig. 4 C), which corresponds to the verrucate sculpture visible with SEM. In the region of the rimula, the ectexine becomes considerably thinner, about 1/3 of the usual thickness (Fig. 4 E). In places, the ectexine is slightly separated from the underlying endexine (Fig. 4 A). The endexine is prominent and significantly darker than the ectexine. It is homogeneous and of constant thickness.

Table I. *The pollen spectrum, botanical affinity, and quantitative data in sample SCS 16 of Heiligkreutz-S. Croce Formation (Dolomites).*

Species	%	Botanical affinity
Laevigate and ornamented spores of the genera *Calamospora, Todisporites, Concavisporites, Retusotriletes,* and *Uvaesporites*	13	lycopsids, filicopsids, and sphenopsids
Spiritisporites spirabilis Scheuring, 1970	<1	? filicopsida
Vallasporites ignacii Leschik (Kräusel & Leschik 1956)	8	conifers
Enzonalasporites vigens Leschik (Kräusel & Leschik 1956)	10	conifers
Patinasporites cf. *P. densus* (Leschik, 1956) Scheuring, 1970	2	conifers
Patinasporites densus (Leschik, 1956) Scheuring, 1970	1	conifers
Pseudoenzonalasporites summus Scheuring, 1970	1	conifers
Samaropollenites speciosus Goubin, 1965	1	conifers or pteridosperms
Ovalipollis pseudoalatus (Thiergart) Schuurman, 1976	8	?Cycadales, ?pteridosperms, ?conifers
Lunatisporites acutus Leschik (Kräusel & Leschik 1956)	4	pteridosperms (Peltaspermales), ?conifers, ?Podocarpaceae
Infernopollenites parvus Scheuring, 1970	<1	pteridosperms (Peltaspermales), ?conifers
Triadispora spp.	8	conifers (Voltziales)
Lueckisporites sp.	3	conifers (Majonicaceae)
alete bisaccate	29	pteridosperms (Peltaspermales), conifers
Duplicisporites continuus Praehauser-Enzenberg, 1970	1	conifers (Cheirolepidiaceae)
Paracirculina maljawkinae Klaus, 1960	3	conifers (Cheirolepidiaceae)
Duplicisporites verrucosus (Leschik) Scheuring, 1970	1	conifers (Cheirolepidiaceae)
Duplicisporites granulatus (Leschik) Scheuring, 1970	6	conifers (Cheirolepidiaceae)
Camerosporites secatus Leschik (Kräusel & Leschik 1956)	<1	conifers (Cheirolepidiaceae), ?pteridosperms



***Duplicisporites verrucosus* (Leschik) Scheuring 1970, (Fig. 2 C, 3E, F, 4F, G).**

Pollen grains are circular to subtriangular in outline. A small trilete mark with simple laesurae is often visible at the proximal pole. In the equatorial zone a rimula is present. Vermiculate and verrucate ornamentation is observed. Verrucae are up to 2.5 μm wide.

Diameter: specimen 'SCS 16/7', 44.5 μm (Fig. 2 C).

The surface is distinctly verrucate (Fig. 3 E, F). The exine consists of two layers (Fig. 4 F, G). The outer ectexinous layer is composed of large indistinct granulae (Fig. 4 G) while the inner endexinous layer is more homogeneous and darker than the ectexine, and of constant thickness (Fig. 4 G).

The two species differ in their surface sculpture and ectexine ultrastructure, but are identical in having a homogeneous endexine that is of constant thickness throughout the section and does not show any traces of lamellations (Fig. 4).

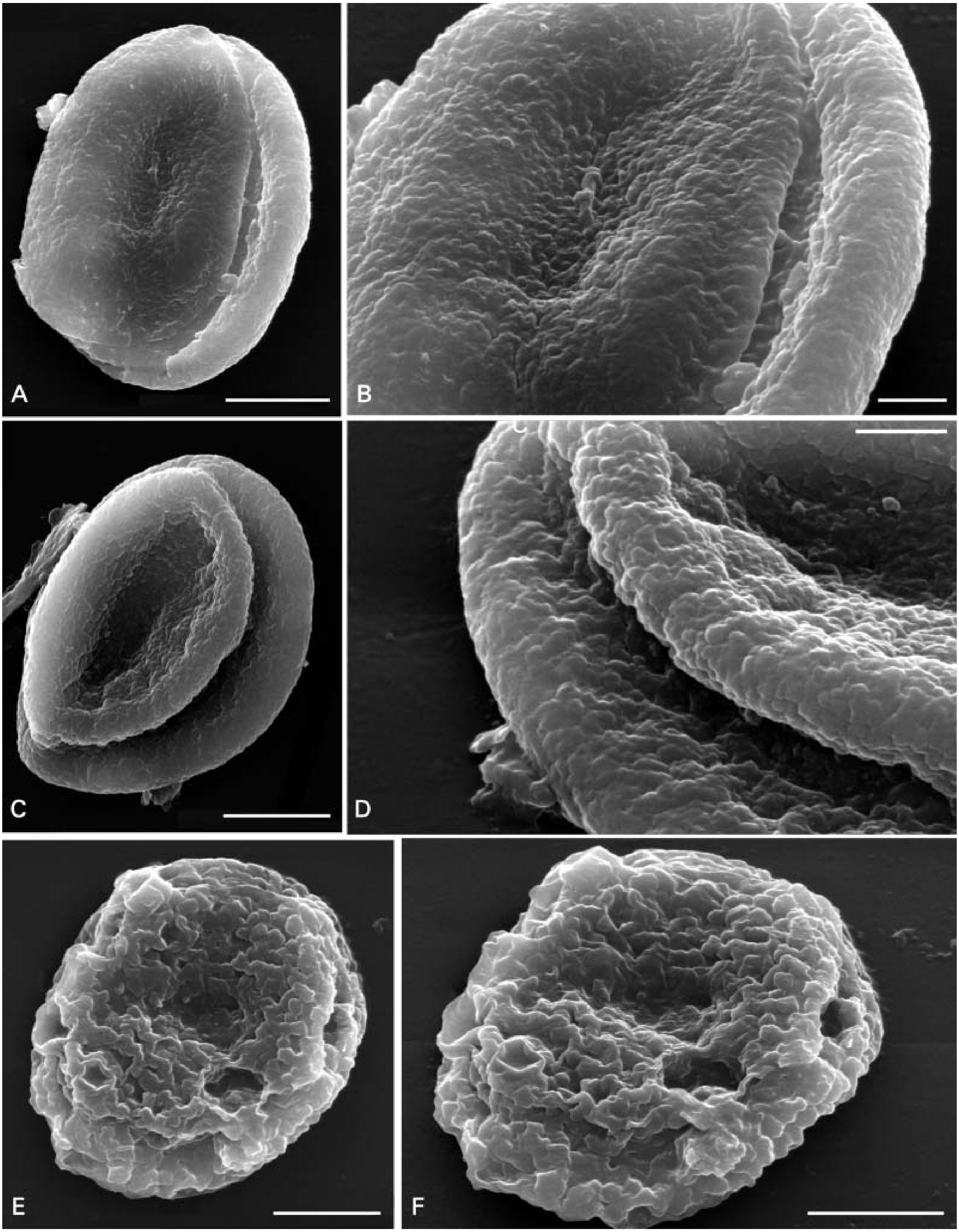
DISCUSSION

The data obtained allow us to carry out a comparison between primitive and advanced members of the Circumpolles group. There are two morphological peculiarities that are distinguishable in all published sections of *Classopollis* (to a greater or lesser extent) which have not been found in our material: the tectal complex and columellate ectexine. However, the columellate structure may have been derived from a granulate structure. Such a transformation has been hypothesized already for the exine of angiosperms (Walker 1976, Walker & Skvarla 1977). It is of interest to find some indications which imply an analogous transformation in an independent plant group that is known to show an angiospermous trend.

The presence of a rimula and its identical ultrastructural organization in both ancient and later members of the Circumpolles group substantiate the early appearance of this structure during the evolution of the group. In the area, the ectexine becomes much thinner at the expense of its intermediate layer (columellate in *Classopollis* or granulate in *Duplicisporites*), whereas, the thickness of the endexine does not change. Scheuring (1970) noticed the varying position of the rimula in Triassic Circumpolles taxa, and this is also the case in our material. However, in *Classopollis*, the rimula always occupies a subequatorial position and this allows us to consider the varying positions of the rimula as a primitive character.

Different types of endexine are known in *Classopollis*; multi-lamellate (Taylor & Alvin 1984, Pettitt & Chaloner 1964); composed of a single lamella (Rowley & Srivastava 1986) or, supposedly, homogenous (Zavialova 2003). The

Fig. 2. LM of *Duplicisporites granulatus* (Leschik) Scheuring, 1970 and *Duplicisporites verrucosus* (Leschik) Scheuring, 1970 from the Heiligkreutz-S. Croce Formation, Badia Valley, Dolomites, Italy. (A) *D. granulatus*, specimen 'SCS 16/6'. (B) *D. granulatus*, specimen SCS '16/8', note trilete scar. (C) *D. verrucosus*, specimen 'SCS 16/7', note trilete scar. Scale bars – 10 μm.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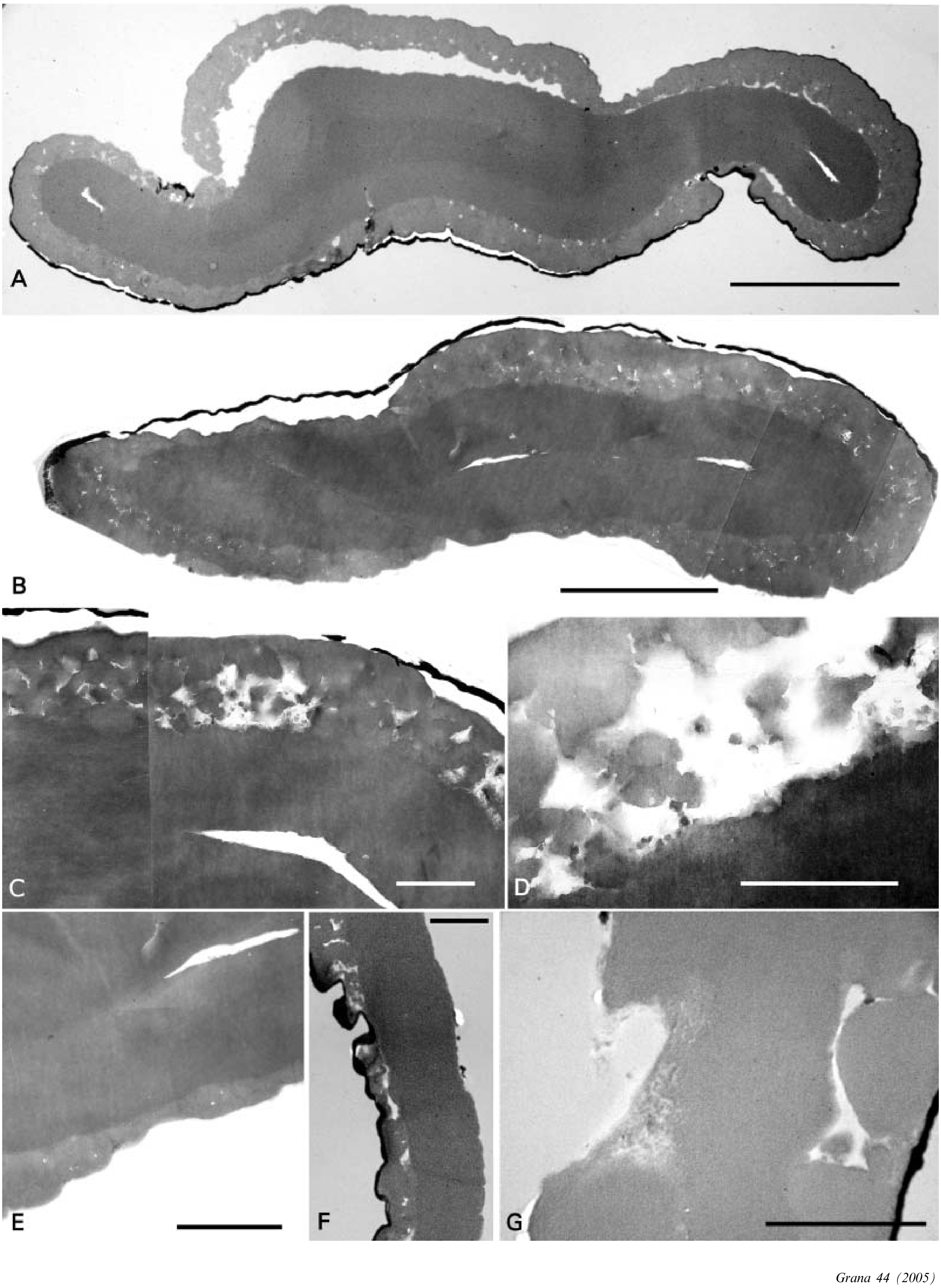
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Fig. 3. SEM of *Duplicisporites granulatus* (Leschik) Scheuring, 1970 and *Duplicisporites verrucosus* (Leschik) Scheuring, 1970 from the Heiligkreutz-S. Croce Formation, Badia Valley, Dolomites, Italy. **A, B.** *D. granulatus*, specimen 'SCS 16/6'. (A) A clearly distinguished continuous rimula. (B) Enlargement of Fig. 3 A showing the finely verrucate surface of the pollen grain. **C, D.** *D. granulatus*, specimen 'SCS 16/8'. (C) General view of the pollen grain showing the position of the rimula. (D) Enlargement of Fig. 3 C showing sculptural elements of varying size. **E, F.** *D. verrucosus*, specimen 'SCS 16/7'. (E) General view of the pollen grain; in the central region the sculptural elements are reduced and the proximal trilete scar can be distinguished – compare with Fig. 2 C. (F) Enlargement of Fig. 3 E, inclined at an angle of 30° to show the sculptural detail more clearly. Scale bars – 10 μm (A, C, E & F), 3 μm (B, D).

Fig. 4. TEM of *Duplicisporites granulatus* (Leschik) Scheuring, 1970 and *Duplicisporites verrucosus* (Leschik) Scheuring, 1970 from the Heiligkreutz-S. Croce Formation, Badia Valley, Dolomites, Italy. **A.** *D. granulatus*, specimen 'SCS 16/6', section through an obliquely compressed specimen – composite image; the exine is bi-layered, the inner layer (endexine) is more electron dense, the opposing walls are so compressed that only two small hollows remain to suggest the internal cavity of the pollen grain, the areas of thinned ectexine indicate the position of the rimula. **B – E.** *D. granulatus*, specimen 'SCS 16/8'. (B) Composite image showing a less central section of the pollen grain than seen in Fig. 4 A, note granular ultrastructure of the ectexine. (C) Enlargement of Fig. 4 B showing the granular ectexine and homogeneous endexine. (D) Enlargement of Fig. 4 C, note large and small granulae. (E) Notably thinned ectexine in the presumed rimula region. **F, G.** *D. verrucosus*, specimen 'SCS 16/7'. (F) Section showing the two layers of the exine. (G) A section of the exine under higher magnification. Scale bars – 3 μm (A, B), 1 μm (C), 0.5 μm (D), 1 μm (E–G).

ultra-micrographs of Medus (1977) show both a homogeneous endexine (Fig. 2.2, *Circulina*, form 2, Triassic) and an endexine having indistinct traces of lamellae (e.g., Fig. 2.3, *Circulina,* form 3, Triassic). The homogeneous endexine lacking any traces of lamellae observed in our material suggests a primitive state for the homogeneous exine in *Duplicisporites*.

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