Pollen morphology, ultrastructure and taphonomy of the Neuradaceae with special reference to *Neurada procumbens* L. and *Grielum humifusum* E.Mey. ex Harv. et Sond.

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ABSTRACT

Pollen morphology and sporoderm ultrastructure of modern *Neurada procumbens* L. and *Grielum humifusum* E.Mey. ex Harv. et Sond. were studied using light (LM) and electron (SEM and TEM) microscopy. Additionally late Holocene pollen of the *Grielum*-type was studied using LM. Systematic and ecological aspects have been discussed for the family Neuradaceae. The pollen grains of the studied species are characterized by similarities in size, shape, aperture type and differences in exine sculpture (reticulate semitectate exine in *Neurada* and finely reticulate to foveolate in *Grielum*) and sporoderm ultrastructure. The cavea in the exine is situated between the ectexine and endexine which are connected near the aperture region only. A combination of the palynological characters of the Neuradaceae (semitectate exine, rather loose columellae, interrupted foot layer, the cavea in the exine) increases the pollen plasticity, allowing considerable changes of the pollen grain volume but still remains insufficient to survive sharp fluctuations in hydration level.

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1. Introduction

Neurada L. and *Grielum* L. along with *Neuradopsis* Bremek. et Oberm. constitute the family Neuradaceae Link, which was formerly placed within the order Rosales (Hutchinson, 1973; Cronquist, 1981; Takhtajan, 1997). Recently the Neuradaceae was included in the order Malvales (Alverson et al., 1998; Judd and Olmstead, 2004; Takhtajan, 2009).

Neurada, prostrate annuals or perennials, includes one species, *Neurada procumbens* L., with two varieties that are sometimes described as separate species — *Neurada al-eisawii* Barsotti, Borzatti et Garbari and *Neurada austroafricana* Schinz. It is distributed in arid regions of Northern Africa, Arabia, Syria, Northern Iran, Afghanistan, Pakistan and India (Ronse Decraene and Smets, 1996; Bayer, 2003).

Grielum, a creeping annual dwarf herb, is present with four species in southern Africa. *G. grandiflorum* (L.) and *Grielum humifusum* (Thunb. var. *humifusum*, Thunb. var. *parviflorum*) occur in the fynbos biome and are distributed from Namaqualand to the Cape peninsula and between Namibia and the southwestern Cape respectively (Germishuizen and Meyer, 2003; Trinder-Smith, 2003; Manning and Goldblatt, 2007; Manning, 2008). *G. sinuatum* Licht ex. Burch can be found in the Northern Cape, e.g. in the Kgalagadi Gemsbok Park (van Rooyen et al., 1988). *G. cuneifolium* Schinz is another southern African prostrate annual herb (Germishuizen and Meyer, 2003).

Neuradopsis is a prostrate annual dwarf herb, and grows in Namibia, the Northern Cape and Botswana (Dyer, 1975; Germishuizen and Meyer, 2003). *Neurada austroafricana* (Schinz) Bremek. et Oberm., and *N. bechuanensis* Bremek. et Schweik. are typical elements of arid regions and grow e.g. in Namibia and the Kgalagadi Gemsbok Park between Botswana and South Africa (van Rooyen et al., 1988; Germishuizen and Meyer, 2003).

Fideliids, ground-nesting bees, e.g. *Parafidelia* species, *Fidelia (Fideliopsis) major*, *F. fasciata*, and *F. hessei*, are the pollinators of *Grielum* and *Neuradopsis* (Whitehead, 1984; Whitehead and Eardley, 2003; Gess and Gess, 2004).

The unusual aperture type and exine structure for *Neurada* and *Grielum* under the light microscope were noted by several authors (Demchenko, 1966; Erdtman, 1966, 1992; Perveen, 2000; Bayer, 2003) but no ultrastructural studies were undertaken.

Erdtman (1956) notes that these two genera, *Neurada* and *Grielum*, have unique pollen grains. They are oblate (24 × 44 μm; 16 × 30.5 μm, excluding sexine in *Grielum* and 15 × 29.5 μm in *Neurada*), triangular

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or sometimes rounded rhomboid from polar view. Pollen grains are occasionally meridional tricolpate (syncolpate: colpi with two endoapertures); they are probably characterized by six semicolpi, merging by three in poles; sometimes tricolporate pollen might occur (*Neurada procumbens* var. *orbicularis*). The nexine is with six, sometimes eight (in *Neurada*) ora, each os corresponds with each ray of trilete sexine openings. The sexine is described as microreticulate (Erdtman, 1986). Erdtman (1966) suggests that *Grielum* has perisaccate pollen, i.e. with a saccus around the corpus, the saccus with two-polar trilobate openings (corresponding with meridional semicolpi). In contrast, *Neurada* has a sexine with the rudiment saccus (subsaccate sensu Erdtman, 1986), especially in the equatorial angular parts. Later Erdtman (1992) described pollen grains in the peculiar *Neurada* group (*Neurada*, *Grielum*) as a unique type — the dizonorate nexine, with two zones of three ora, is surrounded by a sexinous envelope with two-polar apertural parts. Each part consists of three meridional furrows which anastomose at the poles. From the poles they extend into low latitudes (somewhat nearer the equator than the latitude at which the underlying ora occur). Especially in old acetolysed pollen grains, the sexine is separated from the nexine in a manner reminiscent of sacci.

Demchenko (1966) has studied four *Grielum* species and one *Neurada* species. In general her description agrees with that by Erdtman though she describes the pollen as trilete, with six or eight (in case of *Neurada*) ora and reticulate-asteroid sculpture. Trilete apertures (a term which is usually restricted to spores) are with rays, reaching the equator with ends narrow and pointed. Each os is located under each ray, the forth os which sometimes occurs in *Neurada* is located between the rays.

Perveen (2000) in his study of the flora of Karachi, Pakistan, describes pollen of *Neurada procumbens* as subsaccate.

Thus, those studies show that the Neuradaceae is characterized by a unique pollen type, though the authors have a number of discrepancies in the interpretation of pollen morphology and especially in the nature of the cavea between the exine layers (sexine and nexine supposedly). For this reason a detailed study of the palynomorphology of the Neuradaceae by means of the light and electron microscopy was undertaken in the current study. This gives a more complete view on the sporoderm ultrastructure and exine sculpturing and provides additional data for a better understanding of the systematic position of the Neuradaceae. Also it can be used to reveal possible adaptation to the ecological niche of the group expressed in pollen structure.

Here we present the pollen morphology and sporoderm ultrastructure of these genera. Additionally late Holocene samples from the Western Cape (South Africa) were palynologically investigated and pollen grains of the *Grielum*-type were positively identified, documented and measured. A comparison with recent pollen grains (herbarium) will give details of the pollen taphonomy of the Neuradaceae and will probably help to solve the question if the formation of the saccus-like structure was caused by preservation or sample treatment (acetolysis, mounting in glycerine jelly, see Erdtman, 1992).

2. Materials and methods

2.1. Herbarium specimens/treatment and documentation

Extant pollen grains were obtained from the herbarium of the Botanical Institute, St-Petersburg (*Neurada procumbens* L. and *Grielum humifusum* E.Mey. ex Harv. et Sond.) and acetolysed according to Erdtman (1969) for light microscopy (LM). Ten pollen grains were measured for each species.

For scanning electron microscopy (SEM), untreated mature pollen grains were mounted on SEM stubs (covered with nail varnish) and sputter coated with platinum–palladium. Pollen grains were observed and photographed under a Camscan SEM and a Hitachi S-405 SEM.

For transmission electron microscopy (TEM), individual pollen grains and fragments of sporangia were fixed with 1% OsO_4, dehydrated in an ethanol series, stained with uranyl acetate, dehydrated in acetone, and embedded in epoxy resin according to Meyer-Melikjan et al. (2004). Pollen grains were sectioned with an ultramicrotome LKB-3. The ultrathin sections were post-stained with lead citrate and examined under Jeol 100 B and Jeol 400 TEMs.

The terminology follows after Hesse et al., 2009.

2.2. Holocene specimens/treatment and documentation

During the investigation of sediments from a late Holocene core at Verlorenvlei (western coast South Africa, spanning ~600 years) 19 pollen grains of the *Grielum*-type were identified under the light microscope (1000x magnification) and photographically documented. The complete palynological and sedimentological results of the site will be presented elsewhere.

The samples (volume: 4 cm³) were chemically treated using 10% HCl, 10% KOH, 40% HF and acetolysis (Faegri and Iversen, 1989). Heavy liquid mineral separation was used to concentrate the palynomorphs. Pollen slides were prepared with glycerine jelly. The pollen reference collection at the University of the Free State, Bloemfontein as well as in the literature (Van Zinderen Bakker, 1953–1970; Erdtman, 1966) was used for the positive identification of *Grielum*-type pollen. Measurements of individual pollen grains as well as photographs were undertaken using the image analysis program AnalySIS 5.1 at the Bernard Price Institute for Palaeontology, University of the Witwatersrand, Johannesburg.

3. Description

3.1. Description of herbarium specimens

Neurada procumbens L.

In LM pollen grains are bipolar oblate (P/E = 0.5), trisindemicolporate (with semicolpi merging in three on both poles), with six ora (occasionally 8 ora occur), each os is situated under each colpus ray. Pollen is rounded triangular from the polar view, elliptic from the equatorial one (Plate I, 1–4). The polar axis (P) is 14.2 (10.9–17.6) μm, the equatorial diameter (E) is 29.9 (28.4–32.3) μm.

Colpi rays are distinct and pointed, almost reaching equatorial outline, 9.2 (4.5–12.3) μm long, 1.7 (1.3–2.2) μm wide. The colpus membrane is smooth. Ora are rounded, 2.8 (2.4–4.1) μm in diameter. Each os is situated under each colpus ray. The ora membrane is smooth. The exine is 2.4/4.1 (1.7–2.8/2.8–5.6) μm, two-layered with a cavea in the exine. The cavea is relatively small. The outer layer is about two times thinner than the inner one (Plate I, 2–4). The sculpture is reticulate, uniform in the apocolpium and mesocolpium areas (Plate I, 1). Sometimes small islands of exine can be observed in the aperture region (Plate II, 1, 3).

In SEM the exine sculpturing is reticulate including lumina of different shapes with sinuous walls ranging from triangular to polygonal. The muri thickness is about half of the lumina diameter, the muri surface is smooth (Plate II, 1–3, 6).

In TEM the pollen wall consists of the exine and intine, the exine includes the ectexine, endexine and small cavea up to 1 μm between the ectexine and endexine (Plate III, 5, 6). The ectexine is semitectate, 0.9 μm thick on average. The tectum is about 0.3 μm thick. The infratectum is columellate, about 0.37 μm thick, the columellae are 0.25 μm wide on average. The foot layer is discontinuous, about 0.25 μm thick. The endexine is homogeneous, less electron dense than the ectexine, uniformly about 0.35 μm thick in the non-aperture regions and thickening sharply towards the apertures up to 1.33 μm (Plate III, 5). Under the endexine a fibrillar single-layered electron

lucent intine is situated. The intine is about 0.22 μm in non-aperture region, thickens sharply up to 2.8 μm towards the apertures. Towards the apertures the ectexine reduces sharply, the foot layer disappears last of all, the ectexine and endexine are connected only in the regions near the apertures or otherwise there is a cavea between them.

Grielum humifusum E.Mey. ex Harv. et Sond.

In LM pollen grains are bipolar oblate (P/E = 0.7), trisindemicolporate, with six ora, each os is situated under each colpus ray. Pollen is rounded triangular from the polar view, elliptic from the equatorial one (Plate I, 5–8). The polar axis is 21.7 (19.4–23.7) μm, the equatorial diameter is 32.0 (29.5–34.2) μm.

Colpi rays are indistinct and rounded, 6.5 (4.3–8.8) μm long, 2.2 (0.9–3.2) μm wide. The colpus membrane is smooth. Ora are rounded, 2.8 (2.4–3.2) μm in diameter. Each os is situated under each colpus ray (Plate I, 6). The ora membrane is smooth. The exine is 6.0 (4.3–9.0) μm, consists of two external layers approximately equal in thickness, a cavea and an inner layer; the thickness of the inner layer is similar to that of the external ones (Plate I, 6, 8). The sculpture is finely reticulate and uniform in the apocolpium and mesocolpium areas (Plate I, 5, 7). Sometimes small islands of exine can be observed in the aperture region (Plate I, 5).

In SEM the exine sculpturing is finely reticulate to foveolate, the lumina are of different sizes, small, rounded or oval. The muri are thick, the muri thickness exceeds the lumina diameter, the muri surface is smooth (Plate II, 4, 5, 8). The colpi margins are crenate, they are represented by the broken muri so that the reticulum is interrupted with freely ending elements with a spinule on the top and the underlying exine is seen as a solid plate. The colpus membrane is granular (Plate II, 7).

In TEM the pollen wall consists of the exine and intine, the exine includes the ectexine, endexine and a large cavea up to 4 μm between the layers (Plate III, 1). The ectexine is semitectate to tectate, 1.0 μm thick on average. The tectum is about 0.41 μm thick. The infratectum is columellate, about 0.37 μm thick and the columellae are 0.24 μm wide on average. The foot layer is discontinuous, about 0.27 μm thick (Plate III, 3). The endexine is homogeneous, slightly less electron dense than the ectexine, about 0.39 μm thick, uniform in thickness in the non-aperture regions and somewhat thickened towards the apertures (Plate III, 4). Under the endexine a fibrillar single-layered electron lucent intine is situated. The intine is about 0.1 μm in non-aperture region, thickens sharply towards the apertures (Plate III, 2). Towards the apertures the ectexine reduces sharply in thickness and the foot layer disappears last of all. The ectexine and endexine are connected only in the regions near the apertures, otherwise there is a cavea between them (Plate III, 2, 4).

3.2. Description of Holocene specimens of the Grielum-type pollen (LM)

Nineteen pollen grains were observed in samples of the Verlorenvlei (Western Cape) sediments of which seventeen were eventually measured. About half of the pollen grains are strongly crumpled. In one case the endexine and the ectexine are completely separated by 17 μm (Plate I, 14). In another specimen only the ectexine was found. The majority of the *Grielum*-type pollen is oblate and in polar view triangular (Plate I, 9–11). In equatorial view pollen often appears elliptic (Plate I, 12, 13). Only four out of 17 pollen grains were seen and measured from equatorial view. All observed pollen grains have six ora and each os is located beneath the colpus ray (Plate I, 9–11). The polar axis is 17.1 (14.4–19.4) μm, the equatorial diameter is 29.0 (15.3–43.4) μm.

Colpi rays are rounded, sometimes feature sharp margins and resemble trilete marks of spores. The colpi rays are 9.3 (8.6–10.0) μm long, 1.2 (0.7–2.4) μm wide. The ora are circular to elliptic, 2.4 (1.0–3.7) μm in diameter. The os membrane is smooth. The exine is 4.6 (1.9–10.4) μm thick and consists of two approximately equally thick outer layers (the ectexine), a cavea and an inner layer (the endexine);

the endexine is thinner than the ectexine. The endexine is 0.5 (0.3–0.8) μm thick. The ectexine is 1.3 (0.6–2.6) μm thick. The cavea size, which separates the ectexine and endexine, differs throughout the pollen grain and can vary between 0.1 and 7.6 μm (average: 2.6 μm). The cavea decreases in size towards the apertures.

The sculpture is finely reticulate, lumina are mostly rounded — polygonal and of a similar size. Muri are clearly visible.

4. Discussion

4.1. Systematic position and morphology

The Neuradaceae is placed in the order Malvales (APG II, 2003; Judd and Olmstead, 2004; Takhtajan, 2009). Besides the Neuradaceae the order Malvales includes Bixaceae, Cistaceae, Cochlospermaceae (these three families were in the Bixales (Cistales) sensu Takhtajan, 1997), Dipterocarpaceae, Malvaceae (including Bombaceae, Sterculiaceae, Tiliaceae sensu Cronquist, 1981), Muntingiaceae, Sarcolaenaceae, Sphaerosepalaceae and Thymelaeaceae.

The majority of the species of these families are characterized by tricolpate or tricolporate pollen, less frequently porate, rarely syncolpate (*Lechea intermedia* Leggett ex Britton et Hollick). Some species of the families are characterized by oblate pollen (Sphaerosepalaceae, Dipterocarpaceae) as in *Neurada* and *Grielum*. Among them pollen of the Sarcolaenaceae is of an exceptional interest showing some similarity to *Neurada* and *Grielum* in aperture structure (Erdtman, 1992). Sarcolaenaceae pollen grains are gathered in tetrads. Within the family a tendency from relatively small (about 45 μm in diameter) to large (about 120–140 μm in diameter) tetrads could be seen. Interesting changes in aperture structure in different tetrads were observed. Small tetrads consist of tricolpate pollen with narrow short colpi and elongate ora. The colpi gradually become longer and finally are connected on the distal surface forming triangular apocolpia. Ora also undergo some changes. In the tetrads the intermixing of two ora belonging to adjacent pollen grains occurs and so six "double" ora are formed in six places where 12 semicolpi of two pollen meet. Thus, each pair of ora belongs to two pollen grains (Erdtman, 1992).

Characteristic features found in the Neuradaceae pollen were described in different taxa. Two endoapertures (ora) per each colpus and a separation of the sexine from nexine are known to occur in the tricolpate pollen of *Didymeles madagascariensis* Willd. (Didymelaceae). In several Euphorbiaceae species two endoapertures per colpus occur (*Glochidion arborescens* Blume, *Glochidion puberum* Hutch., *Agyneia bacciformis* A.Juss., *Breyniopsis pierrei* Beille, *Breynia nivosa* Small, *Breynia patens* Rolfe, *Sauropus androgynus* Merr.). The number of colpi in these species is 4–8, they are characterized by oblate pollen shape, though no colpi merging was described (Erdtman, 1956, 1992). Another family with species that show tricolpodiorate pollen is the Scrophulariaceae (*Capraria biflora* L., *Mimulus cardinalis* Douglas ex Benth. and *Mimulus cupreus* Regel). In this family species with syncolpate pollen also occur (*Aptosimum depressum* Burch., *Gratiola officinalis* L., *Melampyrum sylvaticum* L., *Pedicularis hirsuta* L.). Syncolpate and/or syncolporate pollen often occur in different groups — Balanaphoraceae (*Sarcophyte* Sparrm.), Caryocaraceae (*Caryocar brasiliense* A.St.-Hil.), Heteropyxidaceae, Lecythidaceae, Myrtaceae, Primulaceae (*Primula farinosa* L., *Primula vittata* Bureau et Franch., *Soldanella montana* Willd.), Sapindaceae, Scrophulariaceae, Simarubiaceae, rarely in Gentianaceae, Lamiaceae, Brassicaceae and some others (Erdtman, 1956; Patel et al., 1984; Chaw and Peng, 1987; Tsou, 1994; Anderberg and El-Ghazaly, 2000; Perveen, 2000; Wang et al., 2003; Song et al., 2004; Khan, 2005; Pire and Dematteis, 2007; Argül et al., 2008; Pinar et al., 2009; Wang et al., 2009). In one of the cases (*Lepeostegeres elmeri* Merr., Loranthaceae) three semicolpi merge on the poles as in *Neurada* and *Grielum*, but the apertures are simple and do not have any endoapertures (ora). Syncolpate pollen always combines with oblate pollen shape.

Despite a number of the species showing some similarity to pollen morphology of the Neuradaceae, on the whole the aperture type (syncolporate with semicolpi merging on both poles and an os per each colpus ray) and sporoderm ultrastructure (separation of the ectexine from endexine) revealed in *Neurada* and *Grielum* are quite peculiar and show no complete resemblance to any other angiosperm species while individual pollen features of the Neuradaceae occur in the plants of different taxonomic position.

Our data correspond well with that of other authors who studied *Neurada* and *Grielum* pollen in detail (Erdtman, 1956; Demchenko, 1966; Erdtman, 1986, 1992): they reported pollen grains with the same size range, aperture type and exine sculpture. The present study which includes SEM and TEM methods revealed more details about exine sculpture and sporoderm ultrastructure.

The exine is reticulate semitectate in *Neurada* and finely reticulate to foveolate in *Grielum*. The TEM study has revealed the exact position of the cavea in the exine: between the ectexine and endexine. Previous authors believed that the cavea was situated between the sexine and nexine, according to the terminology of Erdtman (1956, 1966; Demchenko, 1966, Reitsma 1970; Erdtman, 1986, 1992) and therefore between the infratectum (= collumelae) and the foot layer. The terms nexine and sexine are used in descriptions based on results obtained in transmitted light, but applying these terms to our TEM study the cavea is situated between nexine 1 (foot layer) and nexine 2 (endexine) in Erdtman's terminology. Thus, this distinguishes the Neuradaceae from saccate pollen, where the saccus is situated between the foot layer and infratectum. The cavea is well-pronounced in *Grielum* and relatively small in *Neurada*. The ectexine and endexine are connected near the aperture region only. Besides, the two genera show a difference in the intensity of the exine contrasting: it is more electron dense in *Neurada* than in *Grielum*.

4.2. Ecology and harmomegathic function

Neuradaceae plants occur in arid to semi-arid regions with extended dry seasons, e.g. *Grielum* in the winter rainfall region. The environmental conditions imply the presence of some adaptation (harmomegathic mechanisms) in pollen for the ecology. As Wodehouse (1935) pointed out in his classic text book, "harmomegathy" is a volume-change accommodation and a "harmomegathus (pl. harmomegathi)" — is an organ or mechanism which accommodates a semirigid exine to allow changes in volume. Payne (1972) was one of the first to study and analyze harmomegathic effects in different taxa with colpate, porate and colporate pollen; he emphasized that harmomegathi include not only apertures but all parts of the pollen wall and therefore "the entire grain constitutes a sort of higher order harmomegathus" (Payne, 1972). Harmomegathy allows the pollen grain to adapt to changes of osmotic pressure in the cytoplasm during dehydration or hydration (Hesse et al., 2009). According to Halbritter and Hesse (2004) and Hesse et al. (2009) the harmomegathic effect depends on various pollen morphological features to a different degree. For example, the presence of the pollenkitt, pollen size and exine ornamentation have a limiting influence on the ability of pollen grains to change their volume, while aperture condition (aperture type, length, etc) and sporoderm ultrastructure are quite important. Endoapertures, internal girdles as well as reticulate sculpture might influence the harmomegathic effect. In many cases it is the aperture type that determines the potential of the pollen grain to considerably change its volume. The exine is in general durable, but flexible and elastic (Halbritter and Hesse, 2004). In an extremely rigid exine the harmomegathic effect is weak (Hesse et al., 2009).

Described pollen types within the Neuradaceae (*Grielum humifusum*, *Grielum*-type, *Neurada procumbens*) are similar and share basic characteristics (oblate, 6 ora, each under a colpus ray, reticulate, columellate with a cavea between end- and ectexine). A combination of the semitectate exine, rather loose columellae, interrupted foot layer, along with the cavea in the exine apparently increases the plasticity in angiosperm pollen, allowing considerable changing of the pollen grain volume (harmomegathy, Hesse et al., 2009; Halbritter and Hesse, 2004). We suggest that the caveae observed in specimens of *Grielum* and *N. procumbens* between end- and ectexine possibly serve as harmomegathic mechanisms.

Nonetheless, this set of characters was proven to be insufficient (at least for *Grielum*) in some conditions. Von Hase et al. (2006) positively

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Plate I. LM photos of *Neurada procumbens* L. (1–4), *Grielum humifusum* E.Mey. ex Harv. (5–8) and Holocene pollen grains of *Grielum*-type (9–14).

1.   Polar view, exine sculpturing.
2.   Polar view, optical section, three ora visible.
3.   Equatorial view, exine sculpturing.
4.   Equatorial view, optical section, two ora visible.
5.   Polar view, exine sculpturing.
6.   Polar view, optical section, three ora visible.
7.   Equatorial view, exine sculpturing.
8.   Equatorial view, optical section, four ora visible.
9.   Polar view, exine sculpturing, three ora visible.
10–11.   Polar view, optical section, different foci, three ora visible.
12–13.   Equatorial view, optical section, different foci.
14.   Polar view, optical section, the end- and ectexine are completely separated and are observed in a distance of 17 μm. Scale bar — 10 μm.

Plate II. SEM photos of *Neurada procumbens* L. (1–3, 6) and *Grielum humifusum* E.Mey. ex Harv. (4, 5, 7, 8). (see on page 168)

1.   Polar view, scale bar 10 μm.
2.   Equatorial view, scale bar 10 μm.
3.   Aperture region, scale bar 3 μm.
4.   Equatorial view, scale bar 3 μm.
5.   Surface, scale bar 1 μm.
6.   Surface, scale bar 3 μm.
7.   Aperture region, scale bar 3 μm.
8.   Exine ultrastructure and endexine surface, scale bar 3 μm.

Plate III. TEM photos of *Grielum humifusum* E.Mey. ex Harv. (1–4) and *Neurada procumbens* L. (5, 6). (see on page 169)

1.   Section through pollen grain, scale bar 1 μm.
2.   Part of the sporoderm towards aperture region, scale bar 1 μm.
3.   Non-aperture region, scale bar 1 μm.
4.   Aperture region, scale bar 1 μm.
5.   Part of the sporoderm towards the aperture region, scale bar 0.7 μm.
6.   Non-aperture region, scale bar 1 μm. t — tectum, I — infratectum, fl — foot layer, cav — cavea, end — endexine, int — intine.

tested the hypothesis that nocturnal flower closure allows protection of pollen from winter rainfall in the fynbos biome. More than 40% of *Grielum humifusum* pollen was damaged due to exposure to moisture overnight. Damaged grains were described as burst, shriveled, or crumpled, the intine had ruptured or extruded from the apertures (Von Hase et al., 2006). Evidently a closure of the petals is necessary to protect *Grielum* pollen from extreme moisture. Interestingly Asteraceae species, featuring according to Halbritter and Hesse (2004) a rather rigid exine, do not show any obvious damage after hydration (Von Hase et al., 2006).
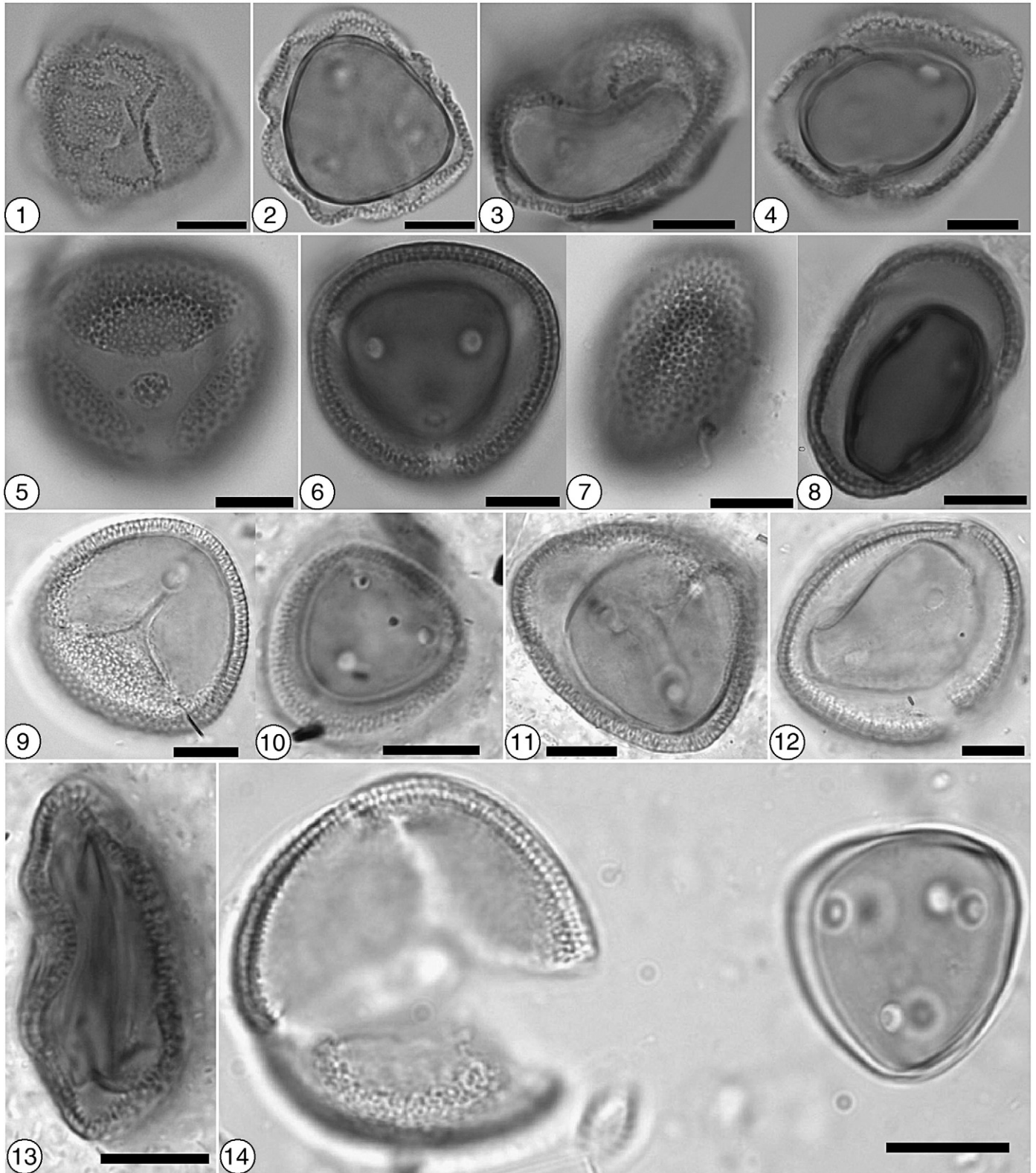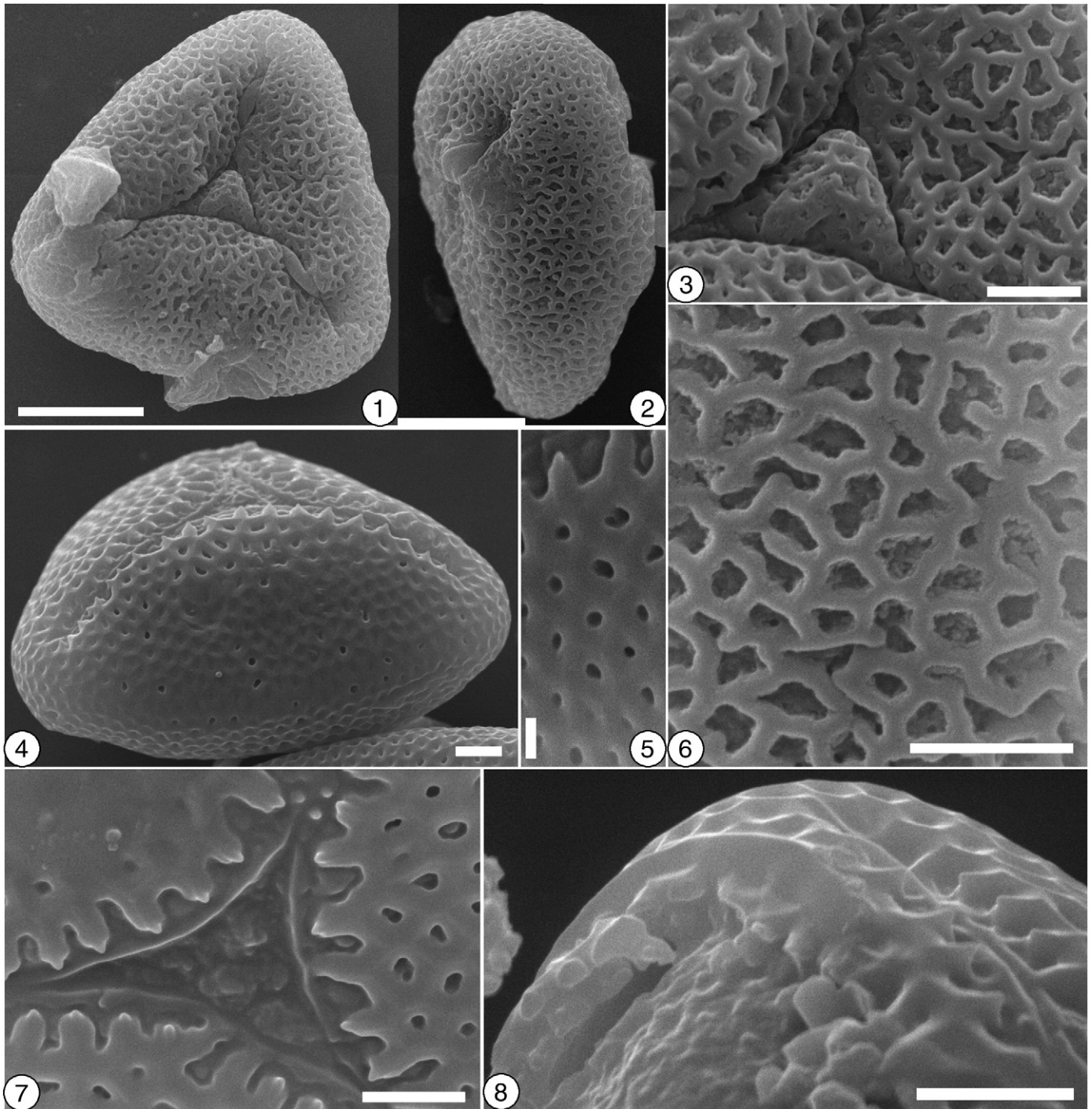

Plate I.

Plate II (*caption on page 166*).

We suggest several possible explanations for such an imperfection of the harmomegathic function. As was stated above this function in the exine is accomplished by the apertures in the first place. At the same time the transpiration also occurs mostly in the aperture regions. The studied taxa supposedly could reduce the aperture area (from tricolporate to six semicolpi merging in three on the poles) to decrease the water loss. This inevitably led to a considerable loss in the harmomegathy. The ability to change the pollen volume effectively without damage is much higher in tricolporate pollen compared with the aperture type of the Neuradaceae. Therefore this deterioration in harmomegathy should be corrected in a different way. Developing a rather large cavea between the ectexine and

endexine allows for a change in the volume of the pollen and preventing damage to the protoplast while changing the volume of the trisindemicolporate pollen. Meanwhile the exine layers are connected only in the regions near the apertures. On the one hand this increases the ability of the volume change but is also dangerous if there are sharp increases in hydration level. As was demonstrated by Von Hase et al. (2006) this caused the loss of pollen vitality in a substantial number of pollen. In contrast, Asteraceae pollen which are not sensitive to moisture exposure overnight, are characterized by mostly tricolporate pollen. Some of the asteraceous species are also caveate, though the cavea is situated in the ectexine, between the foot layer and infratectum. This ectexine construction though more rigid is
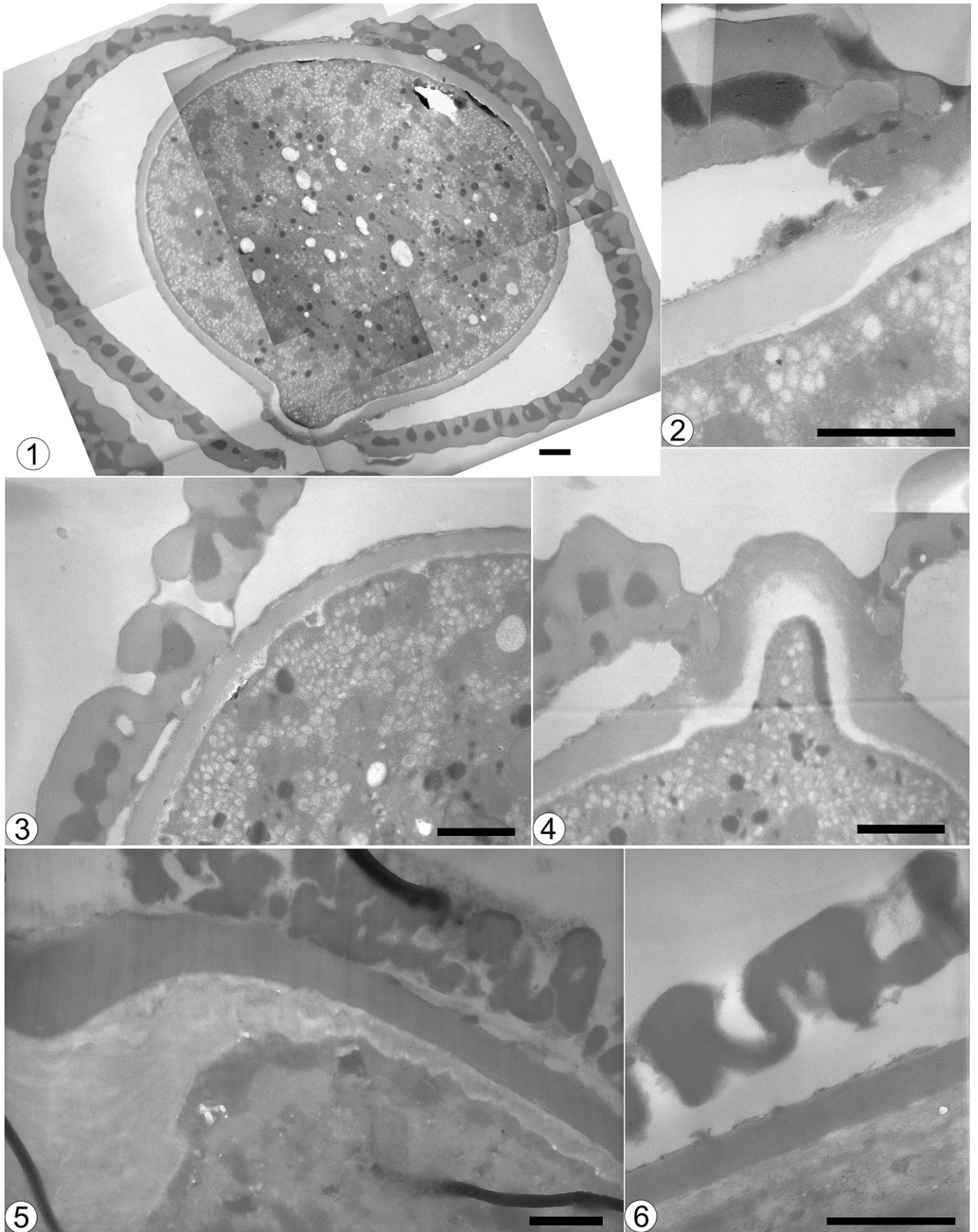
Plate III (caption on page 166).

also more stable, and in complexes with lamellate endexine and tricolporate condition appear to enhance harmomegathy.

Heavily damaged pollen grains were observed in the Holocene *Grielum*-type pollen, although SEM images of herbarium specimens of *Grielum* and *Neurada* also show partly crumpled pollen grains (Plate II, 8). Halbritter and Hesse (2004) and Hesse et al. (2009) mention that the harmomegathic effect in restricted form can also be observed in herbarium pollen and to some degree in fossil material. In the fossil material of Verlorenvlei, *Grielum*-type pollen grains are often crumpled and caveae between the endexine and ectexine are sometimes >7 μm wide. Apparently the pollen can be swollen to such a degree that the fragile connection between the two layers of the exine is broken so that separation between endexine and ectexine can occur during pollen preparation. It is unclear if swelling in glycerine jelly or the hydration of the pollen in the wet sediment is responsible for those effects. It should be also mentioned that acetolysis was used in the Verlorenvlei preparations and might have changed the pollen shape in the case of the *Grielum*-type.

Trisindemicolporate aperture type in its geometry is analogous to perinate trilete spores which are widely distributed among different groups, from mosses to ferns. The perisporium (an outer wall layer) is often detached from the exosporium and it can form the caveae, while near the aperture all the layers are connected (e.g. *Sphagnum* (Boros and Jarai-Komlodi, 1975), *Angiopteris*, *Gleichenia* (Devi, 1977)). The perisporium can often be found separately. To some extent these features are similar to the Neuradaceae. However, the apertures in spores do not carry out harmomegatic functions and serve only as a place for the germination (Tryon and Lugardon, 1990). Inefficiency of such a type of aperture and the inability to sufficiently compensate for it by the exine sculpturing, as well as exine structure could be the reasons for the rarity of this type of pollen amongst angiosperms.

5. Conclusions

The SEM and TEM studies reveal new details of exine sculpture, muri surface, aperture membrane, colpus morphology and sporoderm ultrastructure of the Neuradaceae. The studied pollen grains are characterized by similar sizes, shapes and aperture types and differences in exine sculpture (reticulate semitectate exine in *Neurada* and finely reticulate to foveolate in *Grielum*) and sporoderm ultrastructure. Further, the two genera differ in electron density of exine with *Neurada* showing stronger contrasts than *Grielum*. Our study shows that the cavea in the exine is situated between the ectexine and endexine which are connected near the aperture region only. The unusual aperture type characteristic for the Neuradaceae though helping to reduce transpiration processes in the pollen also reduces their harmomegathic ability. A combination of the semi-tectate exine, rather loose columellae, interrupted foot layer, and the cavea in the exine apparently increases the pollen plasticity in the Neuradaceae, allowing considerable change in the pollen grain volume but still remains insufficient to survive sharp fluctuations in hydration levels. Fossil pollen grains of the *Grielum*-type, observed in the late Holocene sediments from the estuarine lake Verlorenvlei in South Africa, often show pronounced caveae and in some cases even a total separation of endexine and ectexine. This supports the idea that endexine and ectexine in the pollen of the Neuradaceae are relatively loosely connected and may be enhanced either by pollen processing (acetolysis, mounting in glycerine jelly) and/or by hydration of pollen grains in the sediment after deposition.

Acknowledgements

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