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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.

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