

POLLEN OF NIVENIOIDEAE AND ITS PHYLOGENETIC IMPLICATIONS

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ABSTRACT - Phylogenetic analyses clarify the evolution of pollen morphology and its systematic significance in *Iridaceae*. According to the classification of Goldblatt (1991), the subfamily *Nivenioideae* consists of six genera: *Nivenia* (9 spp.), *Klattia* (3 spp.) and *Witsenia* (1 sp.) in the southwestern Cape (South Africa), *Aristea* (ca. 50 spp.) in Africa and Madagascar, *Patersonia* (ca. 20 spp.) in Australia, and *Geosiris* (1 sp.), a saprophyte endemic to Madagascar. The first three genera form a monophyletic group with monosulcate pollen and reticulate sculpturing, as in the basal genus *Isophysis*, which forms its own subfamily. Pollen of *Aristea* is highly diverse, with plesiomorphic monosulcate types and derived dizonasulcate, trisulcate to spiraperturate and sulcate types, which correspond to three large clades within the genus. The microreticulate, monosulcate type found in *Geosiris* also resembles the basic type in African *Aristea*, consistent with its suggested position near the base of the subfamily. The genus *Patersonia* is unique among *Nivenioideae* in having inaperturate pollen convergent with that of certain *Ixioideae* and *Iridoideae* (*Diplarrhena*).

KEY WORDS - *Nivenioideae*, palynology, phylogeny

INTRODUCTION

Within the Monocotyledons, the *Iridaceae* represent a relatively large family of some 65 genera and approximately 1800 species, distributed widely throughout the world, but with a concentration in the southern hemisphere. Goldblatt's (1991) cladistic analysis of the family included characters from morphology, anatomy, embryology, cytology and chemistry, and led to the recognition of four subfamilies: *Isophysidoideae* (comprising only the Tasmanian genus *Isophysis* T. Moore with an inferior ovary), *Nivenioideae*, *Iridoideae* and *Ixioideae*. Recent systematic treatments, such as that of Dahlgren *et al.* (1985), considered the Madagascan saprophyte *Geosiris* an isolated family or a subfamily of *Iridaceae*; Goldblatt (1990) suggested that it is close to *Aristea* Sol. ex Aiton in the subfamily *Nivenioideae*. Thus, this subfamily *Nivenioideae* comprises six genera: *Nivenia* Vent. (9 species), *Klattia* Baker (3 species) and *Witsenia* Thunb. (1 species), all three endemic to the south-western Cape in

South Africa, *Patersonia* R. Br. (20 species, present only in Australia, New Guinea, Borneo and Sumatra), *Geosiris* Baill. (1 species, endemic to Madagascar), and the largest genus *Aristea* Ait. (50+ species, with an African-Madagascan distribution).

PALYNOLOGY

The palynological works of Radulescu (1970) and Schulze (1971), using optical microscopy were the first to show the pollen grain diversity of this subfamily, in particular that of *Aristea*. However, optical microscopy and especially acetolysis treatment of such delicate pollen masked characters or led to errors in interpretation. Pollen studies were undertaken using the scanning electron microscope (SEM) by Goldblatt and Manning (1989) for *Nivenia*, *Klattia* and *Witsenia*, and completed by Goldblatt and Le Thomas (1992) for the other genera of *Nivenioideae*. The results are summarised here.

Nivenia (Goldblatt and Manning, 1989): the pollen grain is more or less spherical and its diameter varies from 44 to 71 μm . It is monosulcate, with a very large aperture and a smooth, flat apertural membrane. The exine is reticulate, the lumina large in some species such as *N. capitata* (Klatt) Weim. and *N. corymbosa* (Benth. & Hook) Bak., or microreticulate as in *N. concinna* N.E.Br. When the lumina are sufficiently large, the presence of verrucae is evident on the foot layer of the exine. The surface of the walls is finely granular, and their sides are sometimes irregularly sculpted as in *N. binata* Klatt.

Klattia (Goldblatt and Manning, 1989): the pollen grain is very similar to that of several species of *Nivenia*, subspherical with a size range of 63 to 80 μm , a reticulum of large lumina of variable size, but with completely smooth walls.

Witsenia (Goldblatt and Manning, 1989): the pollen grain is subspherical, about 81 μm diameter, differing from the two preceding genera by its aperture with diffuse margins. The ornamentation is microreticulate, the walls finely verrucose and surmounted by large suprategical gemmae.

Geosiris (Goldblatt and Le Thomas, 1992): the pollen grain is heteropolar, small, about 28 μm long, with a smooth apertural membrane, a distinct margin and a microreticulate tectum.

Patersonia: Two species have been studied, *P. sericea* R.Br. (Goldblatt and Le Thomas, 1992) and *P. fragilis* Asch. & Graeb. (unpublished). The pollen grain is spherical, about 78 μm diameter, and shows significant differences with those of the preceding genera. It is inaperturate, which is unusual in *Iridaceae*, with the exception of several *Ixioideae* and the genus *Diplarrhena* in the *Iridoideae* (Rudall and Goldblatt, this volume), where it is similarly characterised by an intectate exine, consisting of more or less uniformly distributed verrucae, upheld by short columellae.

Aristea (Goldblatt and Le Thomas, 1992; Le Thomas, Suarez-Cervera and Goldblatt, 1996; Goldblatt and Le Thomas, 1997): this genus, the largest in the subfamily, is also that which presents the greatest pollen diversity. Thirty two species have been studied using SEM and eight by transmission electron microscopy (TEM). The pollen grains are generally dispersed as monads with the exception of three species, *A. ecklonii* Bak., *A. ensifolia* Muir ex Wein. and *A. pusilla* (Thunb) Ker, where the tetrad stage is prolonged, showing clearly that the aperture is distal. The pollen grain can be divided into two size classes, one medium (between 45 and 64 μm) and the other with an equatorial diameter of 69-98 μm . The types of aperture are particularly variable and in many cases are difficult to define using the classical terminology of Punt *et al.* (1994), because of the fragility of the exine. Thus, they have been

studied without acetolysis or observed after being critical-point-dried. According to these apertural characters, four principle pollen types have been defined:

1. Monosulcate pollen (Figs. 1, 2, 3), comparable to that of the other genera in the subfamily (with the exception of *Patersonia*) which represents the plesiomorphic condition of the family. This type is present in five species of the section *Racemosae*. It is characterised by a large furrow, with distinct margins, a smooth membrane lacking any trace of exine elements, with the exception of two species with tetrads. Exine ornamentation may be reticulate, microreticulate, rugose or, exceptionally, areolate in *A. macrocarpa* Lewis. (trichotomosulcate pollen is found in *A. fimbriata*, the aperture being restricted to a single face of the grain.)

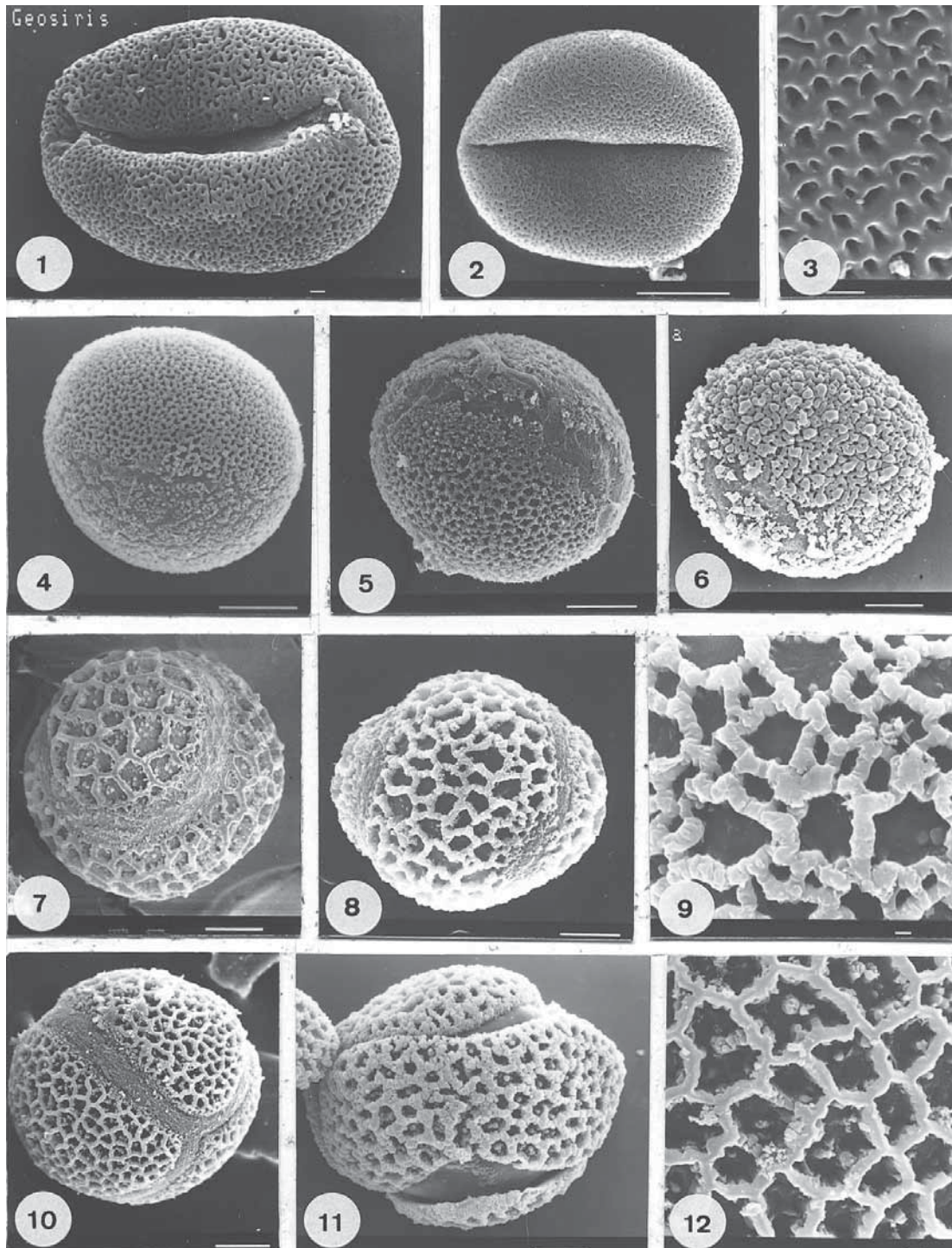
2. Sulcate pollen (Figs. 4 - 6), which includes subspherical pollen, either zonosulcate or disulcate, the distinction in certain cases being difficult to establish because of the variation in a single species, population or individual, in particular in the Madagascan species. This is the commonest type in the genus, found in 19 African and six Madagascan species. In this type, the aperture membrane is densely covered by more or less disorganised elements of the exine. In certain Madagascan species, these aperture elements can form a continuous band constituting a pantoperculum. The margins are always diffuse and irregular, and the limits of the aperture are difficult to distinguish, they are often confluent to form a zonosulcate aperture. The ornamentation of the exine is equally very variable, most often rugose, to rugose-areolate, with smooth or more or less sculpted walls.

3. Disulcate pollen (Figs. 7 - 9), described for the first time in the angiosperms (Le Thomas *et al.*, 1996) is present in all species of the section *Pseudaristea*. The spherical pollen grains have a distinct aperture at each pole, the aperture membrane is generally smooth, and there is a loose reticulum with large, irregularly shaped lumina and scattered basal verrucae. The walls are narrow and smooth as in *A. teretifolia*, or sculpted as in *A. cantarophila*. In *A. spiralis* (L.F.) Ker, the grains are also dizonosulcate, but the apertures are less well defined, having diffuse margins and scattered exine elements on the aperture membrane. In addition, the microreticulum is covered in large suprategal gemmae.

4. Trisulcate or more or less spiraperturate pollen (Figs. 10-12) where the grain is subspherical with well-delimited confluent furrows. This is found in four species of the section *Aristea*. The asymmetry of the exine plates of *A. africana* (L.) Hoffm. suggests that the aperture forms a distal furrow extending across the proximal pole. In *A. oligocephala* there is a clear tendency to spirally aperture. Exine ornamentation in the group is reticulate, with large lumina and prominent basal verrucae. The reticulum walls may be smooth as in *A. glauca*, but are ornamented in the other species.

Eight species belonging to these four pollen types have been studied using TEM: (1) *A. major* Andr., *A. macrocarpa*, *A. fimbriata*, (2) *A. woodii* N.E. Br., (3) *A. pauciflora* W. Dod. and (4) *A. africana*, *A. dichotoma* (Thunb.) Ker, *A. glauca*. This ultrastructural exploration merits further work to define additional exine characters and their introduction into cladistic analysis. However, it is useful to outline what is presently known:

1. The exine seems to lack endexine, although this is difficult to determine without ontogenetic study.
2. There are always three layers of ectexine: tectum, columellar infratectum and foot layer, but their structure and relative thickness are extremely variable.
3. The intine is always tripartite, of equal thickness or thicker than the exine. The exintine and the endintine are thin and fibrillar, whereas the middle layer is extremely thick and always channelled.



Figures 1-12. 1-3. - Monosulcate pollen grains: 1, *Geosiris aphylla* Baillon; 2, 3, *Aristea racemosa* Baker. 4-6, Sulcate pollen grains: 4, *A. abyssinica* Pax. 5, *A. montana* Baker. 6, *A. kitchingii* Baker. 7-9: Dizonasulcate pollen grains: 7, *A. teretifolia* Goldblatt & Manning. 8, 9, *A. cantarophila* Goldblatt & Manning. 10- 12: Trisulcate to spiraperturate pollen grains: 10, *A. glauca* Klatt. 11, 12, *A. oligocephala* Baker. Scale bars = 10 μ m

The ultrastructure of this last layer, studied in *A. major* during germination (Suarez Cervera *et al.*, 1998), showed that these tubules have a characteristic polygonal structure which suggests a specific protection mechanism for the pollen grains with a large aperture.

PHYLOGENY

A cladistic analysis of the genus *Aristea* (Goldblatt and Le Thomas, 1997) demonstrated that the basic pollen type for this genus is the same as that of the whole subfamily *Nivenioideae* (Fig. 13). It is heteropolar, monosulcate, and tectate-reticulate, as in *Isophysidoideae*, the most primitive subfamily of *Iridaceae* (Goldblatt, 1990). This pollen type is characteristic of *Aristea* section *Racemosae*, which belongs to the same clade as section *Aristea* with derived trisulcate pollen. It is also retained by *A. singularis* Wein (section *Singularis*), at the base of the same clade and in *A. schizolaena* Harv., which belongs to the clade with sulcate pollen. These three sections, *Racemosae*, *Aristea* and *Singularis* (assigned to subgenera by Goldblatt) together form a clade which is largely supported by fruit and seed characters. Dizonasulcate pollen is present in a clade which corresponds to the section or subgenus *Pseudaristea* and constitutes the sister group of the other species of *Aristea*. Finally, the third clade contains all the pollen which has been defined as sulcate and includes species from four other sections, which Weimarck (1940) united in the subgenus *Eucapsulares*.

In order to assess the evolution of pollen types in *Nivenioideae*, we mapped aperture characters onto a cladogram of family relationships, constrained to conform with results obtained by the molecular analysis of Reeves *et al.* (this volume). The ancestral aperture type is monosulcate (Fig. 1). The three derived pollen grain types in *Aristea* each have an

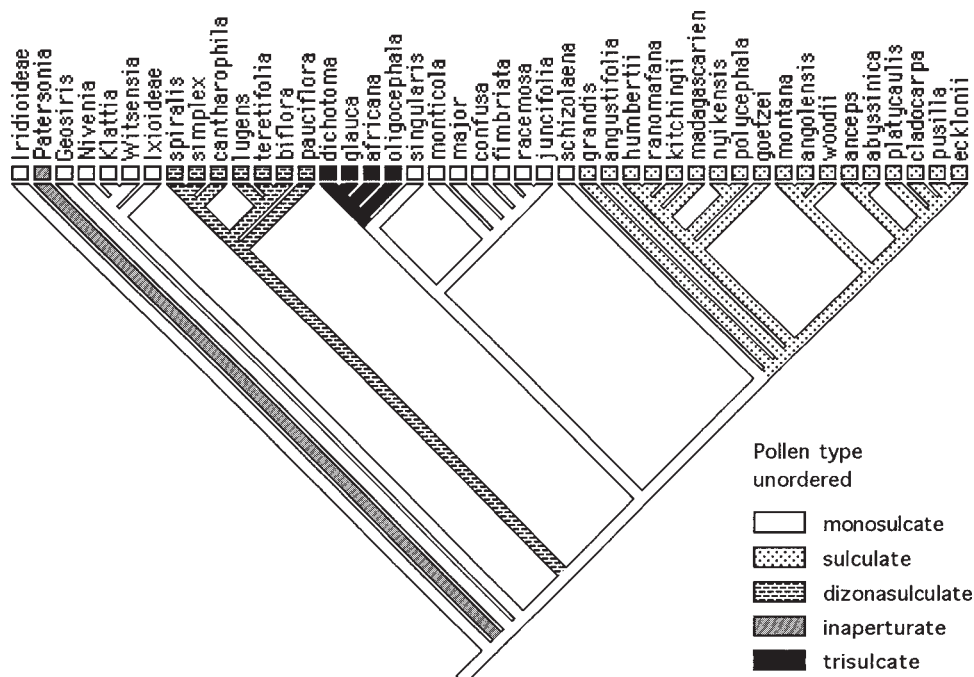


Figure 13 - Phylogenetic tree showing the distribution of aperture types in *Aristea*, established following a morphological and palynological analysis, with the basic topology of the tree constrained to conform with the results of the phylogenetic analysis of the family based on molecular data (Reeves *et al.*, this volume).

independent origin from a monosulcate ancestor. Pollen characters of subfamily *Nivenioideae* indicate that the subfamily is paraphyletic; the only morphological character uniting the subfamily is the type of inflorescence, a binate rhipidium.

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