Pollen morphology of the monotypic genus *Poellnitzia* (Alooideae: Asphodelaceae)

G.F. Smith*
Department of Plant and Soil Sciences, Potchefstroom University for Christian Higher Education, Potchefstroom, 2520 Republic of South Africa

A.E. van Wyk
Department of Botany, University of Pretoria, Pretoria, 0002 Republic of South Africa

Accepted 23 December 1991

Poellnitzia Uitewaal (subfamily Alooideae, family Asphodelaceae) has a history of taxonomic confusion and doubtful generic delimitation. It has previously been variously combined with, and separated from, four other genera of the Alooideae. In this study, the taxonomic significance of its pollen morphology was assessed by means of scanning electron microscopy. Plants collected from two populations in the Robertson area, where *Poellnitzia* is endemic, were included in the survey. *Poellnitzia* is homogeneous in its pollen morphology; grains are shed as monads, are bilaterally symmetrical, medium in size and have a perforated tectum. The mean pollen size of *Poellnitzia* (36.4 µm) is greater than that of *Chortolirion* and *Haworthia*. However, no marked differences in pollen symmetry, shape, aperture features and fine structure between *Poellnitzia* and other genera of the Alooideae could be detected. This is in striking contrast to the strong macromorphological evidence for separating *Poellnitzia* from the other Alooideae genera. The similarity in pollen morphology merely supports the inclusion of *Poellnitzia* within the subfamily.

Poellnitzia Uitewaal (subfamilie Alooideae, familie Asphodelaceae) het 'n geskiedenis van taksonomiese verwarring en onduidelike genusafbakening. Dié monotipiese genus is voorheen as 'n spesie van vier ander Alooideae-genusse geklassifiseer. In hierdie ondersoek is die taksonomiese waarde van stuifmeelmorfologie met behulp van die skandeerelektronmikroskoop ondersoek. Plante wat in twee populasies in die Robertson-omgewing versamel is, is in die ondersoek ingesluit. *Poellnitzia* is endemies tot die Robertson Karoo. Stuifmeelkorrels van *Poellnitzia* is vry, monosulkaat, bilateraal-simmetries, medium-groot en het 'n geperforeerde tektum. Die gemiddelde stuifmeelkorrelgrootte van *Poellnitzia* (36.4 µm) is groter as dié van *Chortolirion* en *Haworthia*. Op grond van stuifmeelkorrelsimmetrie, -vorm, -apertuur en -skulptuur bestaan daar egter geen beduidende verskille tussen *Poellnitzia* en die ander Alooideae-genusse nie. Stuifmeel van die spesies stem bykans volkome ooreen en dit is onmoontlik om op grond van palinologiese kenmerke tussen die genusse te onderskei. Stuifmeel weerspieël dus geensins die makromorfologiese kenmerke aan die hand waarvan *Poellnitzia* van die ander Alooideae-genusse onderskei kan word nie.

Keywords: Alooideae, Asphodelaceae, palynology, *Poellnitzia*, pollen, scanning electron microscopy

*To whom correspondence should be addressed.

Introduction

In a reclassification of the inclusive family Liliaceae (*sensu lato*), Dahlgren *et al.* (1985) reinstated the family Asphodelaceae, as well as several other segregate families. Within the Asphodelaceae two subfamilies, namely Asphodeloideae and Alooideae, were recognized. The Alooideae constitutes a natural assemblage of more or less succulent-leaved taxa which were previously placed in the Aloineae, one of 28 tribes recognized by Hutchinson (1959) in the family Liliaceae (*sensu lato*). According to Rowley (1976), 27 generic names are available for taxa included in the subfamily Alooideae. However, only seven genera are currently upheld. This reflects to some extent the considerable difficulties with generic delimitation in the subfamily. Widely recognized genera include: *Aloe* L., *Astroloba* Uitewaal, *Chortolirion* Berger, *Gasteria* Duval, *Haworthia* Duval, *Lomatophyllum* Willdenow and *Poellnitzia* Uitewaal. Of these, *Chortolirion* and *Poellnitzia* warrant monotypic generic status (Bayer 1972; Smith 1988), but are included under *Haworthia* by Obermeyer (1973) and Dyer (1976).

Specimens on which the name *Poellnitzia rubriflora* (L. Bol.) Uitewaal is based, were originally described in the genus *Apicra* Haworth *non* Willdenow as *A. rubriflora* (Bolus 1920). However, in terms of its red flowers and aberrant floral morphology, this species differs considerably from other members of *Apicra*. This prompted Uitewaal (1940) to afford the taxon monotypic generic status as *Poellnitzia rubriflora*. Parr (1971, 1972) transferred *Poellnitzia* to the genus *Haworthia* whereas Rowley (1981) claimed that it has more characters in common with *Aloe*. In a revision of the smaller genera of the Alooideae currently undertaken by one of us (G.F.S.), the genus *Poellnitzia* will be reinstated with *P. rubriflora* (probably including *Apicra jacobseniana* Von Poellnitz) the only species. It is a low-growing, caulescent, succulent herb with densely leaved stems up to 250 mm long. The ovate leaves are pungent-acuminate and up to 40 mm long. *Poellnitzia* has a restricted geographical distribution in the south-western Cape Province of South Africa. It is a floristic component of the Robertson Karoo which is one of the drier winter rainfall

areas bordering the Fynbos Biome.

Palynological evidence for the Alooideae is fragmentary and incomplete (Majumdar 1972; Yuhl & Majumdar 1981; Smith 1988, 1991). Since the pollen of *Poellnitzia* has not previously been examined by scanning electron microscopy (SEM), nor illustrated, the aim of the present study was to explore whether this source of evidence might provide clues to its intergeneric relationships, including possible additional support for its segregation from *Aloe*, *Astroloba* (a new name for *Aprica* Haw. *non* Willd.) and *Haworthia*.

Materials and Methods

For SEM study, pollen of *P. rubriflora* was obtained from mature flowers on dried herbarium specimens kept in the herbarium of the Potchefstroom University for CHE (PUC), from post-anthesis flowers of specimens grown in the greenhouse of the Department of Plant Sciences and from FAA fixed material. The flower morphology of *Poellnitzia* is unique in the Alooideae, in that the tips of the perigone members of mature flowers are connivent and reduplicate-valvate. Thus, especially in the case of greenhouse-grown plants, it is unlikely that post-anthesis flowers may have been contaminated by pollen from other plants. All the specimens examined originated from two populations about two kilometres apart on the farm 'Langverwacht' (34°06'S, 20°02'E), 180 m altitude, approximately 24 km from Robertson towards Bonnievale, south-western Cape Province, South Africa (*G.F. Smith 174, G.F. Smith 177*, PUC).

During this study acetolysis was initially employed. Use of this method unfortunately resulted in the loss of large numbers of pollen grains, and in changes in shape and volume of the acetolysed grains (Smith & Tiedt 1991). For these reasons two non-destructive techniques were employed for preparing pollen of *Poellnitzia* for SEM. It should, however, be noted that use of the osmium tetroxide method of Smith and Tiedt (1991) does not give results which are directly comparable with results from acetolysed pollen, particularly with regard to measurements of grain size.

For examining exine sculpture, pollen grains were prepared using the filter technique of Bredenkamp and Hamilton-Attwell (1988) (herbarium material and fixed material). Filter membranes containing pollen samples were air-dried in a desiccator from 90% ethanol and attached to electron microscope stubs using conductive carbon cement. For determining pollen grain dimensions, symmetry and shape, the osmium tetroxide method of Smith and Tiedt (1991) was used. Freshly collected post-anthesis anthers were placed in a Petri dish containing small drops of 2% OsO_4. The lid of the Petri dish was replaced and it was left in a fume hood for 24 h. The OsO_4 drops were then removed and the samples were left to air-dry. The fixed plant material was subsequently mounted onto electron microscope stubs and, along with the material prepared by means of the filter technique, sputter-coated with a thin layer of Au/Pd (60:40) and examined with a Cambridge Stereoscan 250S SEM. Pollen dimensions were measured from scanning electron micrographs. The descriptive terminology used mainly follows that of Erdtman (1966, 1969).

Results

The pollen of *Poellnitzia* is fairly uniform in its size, shape and apertures. Its morphology is described below, including the range of variability of the samples examined.

Pollen grains are shed as monads. Grains are bilaterally symmetrical (Figures 1A, 1B) and heteropolar with an inaperturate proximal wall (Figures 1A, 1C) and a distal, monosulcate aperture (Figure 1B, 1C). The sulcus is well-defined and usually about equal to the long equatorial axis. Aperture margins are usually very narrow, but conspicuous (Figures 1B, 1F). Grain size, long axis: (33 –) 36.4 (– 42) μm. The amb is pyriform, occasionally elliptical.

The exine surface is semitectate; coarsely perforated (Figures 1D – 1F). The sexine is discontinuous; tectum perforatum *sensu* Praglowski and Punt 1973).

Figure 1E shows a fractured distal wall of a pollen grain exposing the infratectal columellae and a thin footlayer, agreeing with the state in *Haworthia* (Yuhl & Majumdar 1981) and *Chortolirion* (Smith 1988). As in the case of *Chortolirion* (Smith 1988), distinct areas of a coarse (centre of both the proximal and distal faces of the pollen grain) and a fine (ends of longest equatorial axis; areas bordering sulcus) reticulum are present in *Poellnitzia* pollen (Figures 1A, 1B, 1F) (see also Roth *et al.* 1986).

Discussion

Morphologically, the pollen of *Poellnitzia* is similar to that described for other genera of the Alooideae (Smith 1988, 1991). As in other Alooideae, pollen grains of *Poellnitzia* are shed as monads, and are bilaterally symmetrical, medium in size (dominant equatorial axis), heteropolar and monosulcate with a pyriform amb and a perforated tectum. They also agree with other members of the subfamily in that the ektexine is composed of a foot layer, infratectal columellae and tectum. The mean pollen size of *Poellnitzia* is, however, greater than that of *Haworthia* and *Chortolirion* (Smith 1988), although not markedly so. In both *Poellnitzia* (this study) and *Haworthia* (Yuhl & Majumdar 1981) there is an abrupt transition from a perforated sexine to a smooth surface lacking perforations in the apertural region. Such apertural margins have not been observed in *Chortolirion* (Smith 1988).

Although geographical isolation may have played a role in the diversification of the Alooideae, (e.g. *Poellnitzia* which is endemic to the Robertson Karoo), specialization through isolation has not resulted in the concomitant evolution of distinctive pollen morphological features useful as generic indicators [see also Takahashi (1987) on *Erythronium* L. (Liliaceae) and Rogers (1985) on *Cliococca* Babington (Linaceae)].

Owing to the lack of palynological variation amongst genera of the Alooideae, pollen morphological features are difficult to apply taxonomically at the generic level. Hence, the lack of differentiating characters between the pollen of *Poellnitzia* and other alooid genera does not necessarily invalidate or justify the proposed treatment of *Poellnitzia* as a separate genus. However, a recent cladistic analysis of the subfamily (Smith & Van Wyk 1991), based on a wide range of vegetative and reproductive characters, has shown that *Poellnitzia* cannot be realistically included in any other genus of the Alooideae. Pollen morphological similarities indicate support for the claimed monophyly of the subfamily. Despite the lack of support from palynological

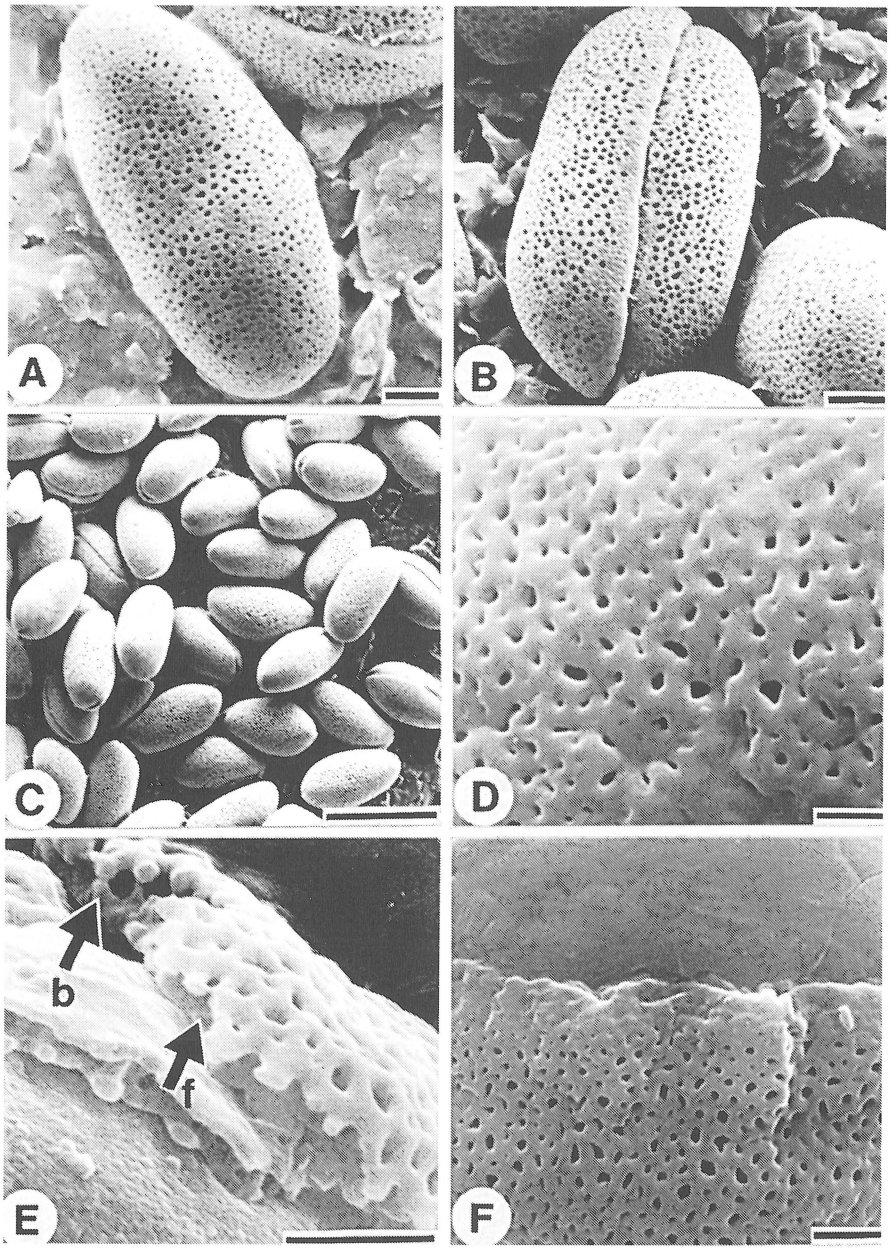

Figure 1 Scanning electron micrographs of pollen of *Poellnitzia rubriflora*. (A) Proximal surface. (B) Distal face showing sulcus. (C) Group of pollen grains showing uniform symmetry, tectal ornamentation and aperture shape/length within a sample. (D) Detail of tectum surface in a non-apertural region showing perforations and smooth muri. (E) Fractured sexine layer exposing the columellae (b) and foot layer (f). (F) Detail of distal tectum surface in the apertural region showing the transition from coarse to fine perforations and the presence of a smooth area bordering the sulcus. Figures 1A – 1C from *Smith 174*, prepared for SEM using the osmium tetroxide technique of Smith and Tiedt (1991); Figures 1D – 1F from *Smith 177*, prepared for SEM using the filtering technique of Bredenkamp and Hamilton-Attwell (1988). Scale bars: 5 μm in Figures 1A, 1B; 40 μm in Figure 1C; 2 μm in Figures 1D – 1F.

evidence so far, it is recommended that *Poellnitzia* be regarded as a monotypic genus with *P. rubriflora* as the only species.

Acknowledgements

We thank Dr L.R. Tiedt, Laboratory for Electron Microscopy, Potchefstroom University for CHE, for technical assistance. Mr Ben Engelbrecht, curator of the Karoo Botanic Garden, Worcester, is thanked for hospitality extended to one of us (G.F.S.) while he did field work in the Robertson Karoo. Financial support from the Potchefstroom University for CHE is gratefully acknowledged.

References

BAYER, M.B. 1972. Reinstatement of the genera *Astroloba* and *Poellnitzia* Uitew. (Liliaceae–Aloineae). *Nat. Cactus Succ. J.* 27: 77 – 79.

BOLUS, H.M.L. 1920. Novitates Africanae: *Apicra rubriflora* L. Bolus (Liliaceae–Aloineae). *Ann. Bolus Herb.* 3: 1 – 14, Pl. 2d.

BREDENKAMP, C.L. & HAMILTON-ATTWELL, V.L. 1988. A filter technique for preparing pollen for scanning electron microscopy. *Pollen Spores* 30: 89 – 94.

DAHLGREN, R.M.T., CLIFFORD, H.T. & YEO, P.F. 1985. The families of the Monocotyledons. Structure, Evolution and Taxonomy. Springer-Verlag, Berlin.

DYER, R.A. 1976. The genera of southern African flowering

plants. Vol. 2, Gymnosperms and Monocotyledons. Department of Agricultural Technical Services, Pretoria.

ERDTMAN, G. 1966. Pollen morphology and plant taxonomy, Angiosperms. An introduction to palynology, 1. Hafner Publishing Company, New York.

ERDTMAN, G. 1969. Handbook of palynology : Morphology – taxonomy – ecology: An introduction to the study of pollen grains and spores. Munksgaard, Copenhagen.

HUTCHINSON, J. 1959. The families of flowering plants. Vol. 2, Monocotyledons, 2nd edn. Clarendon Press, Oxford.

MAJUMDAR, S.K. 1972. Ultrastructure of *Astroloba* and *Haworthia* pollen wall. In: 30th Ann. Proc. Electron Microscopy Soc. Am., ed. C.J. Arceneaux, pp. 220 – 221. Claitor Publishing Division, Louisiana.

OBERMEYER, A.A. 1973. *Aloe, Chamaealoe, Haworthia, Astroloba, Poellnitzia* and *Chortolirion*. *Bothalia* 11: 119.

PARR, C.A.E. 1971. Revision of the genus *Astroloba*. Part 2. *Bull. Afr. Succ. Pl. Soc. London* 6: 195 – 197.

PARR, C.A.E. 1972. Revision of the genus *Astroloba*. Part 4. *Bull. Afr. Succ. Pl. Soc. London* 7: 89.

PRAGLOWSKI, J. & PUNT, W. 1973. An elucidation of the microreticulate structure of the exine. *Grana* 13: 45 – 50.

ROGERS, C.M. 1985. Pollen morphology of the monotypic genus *Cliococca* (Linaceae). *Grana* 24: 121 – 123.

ROTH, J.L., WALKER, J.W. & WALKER, A.G. 1986. Pollen morphology and evolution of the lilialean complex. *Am. J. Bot.* 73: 745 (abstract).

ROWLEY, G.D. 1976. Generic concepts in the Aloineae. Part 2. Generic names in the Aloineae. *Nat. Cactus Succ. J.* 31: 54 – 56.

ROWLEY, G.D. 1981. Re-name that succulent. *Cactus Succ. J. Gt Br.* 43: 2.

SMITH, G.F. 1988. A scanning electron microscopic investigation of the pollen morphology of *Chortolirion* Berger (Aloineae; Liliaceae). *S. Afr. J. Sci.* 84: 428 – 430.

SMITH, G.F. 1991. Studies on the reproductive biology and palynology of *Chortolirion* Berger (Asphodelaceae: Alooideae) in southern Africa. *Taxon* 40: 61 – 73.

SMITH, G.F. & TIEDT, L.R. 1991. A rapid, non-destructive osmium tetroxide technique for preparing pollen for scanning electron microscopy. *Taxon* 40: 195 – 200.

SMITH, G.F. & VAN WYK, B.-E. 1991. Generic relationships in the Alooideae (Asphodelaceae). *Taxon* 40: 557 – 581.

TAKAHASHI, M. 1987. Pollen morphology in the genus *Erythronium* (Liliaceae) and its systematic implications. *Am. J. Bot.* 74: 1254 – 1262.

UITEWAAL, A.J.A. 1940. Een nieuw geslacht der Aloineae. *Succulenta* 21: 25 – 30.

YUHL, D.E. & MAJUMDAR, S.K. 1981. Light and electron microscope studies of *Haworthia* (Liliaceae) pollen. *Phyton* 41: 203 – 209.