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Pollen of the *Spermacoce* (Rubiaceae) species from the Northern Territory of Australia: morphology and taxonomic significance

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Abstract. Complementary to the revision of the genus *Spermacoce* in the Northern Territory of Australia, pollen morphology of 48 of the 53 native *Spermacoce* species from the Northern Territory has been investigated by scanning electron and light microscopy. There is considerable variation for most diagnostic pollen characters. The average equatorial diameter (E) ranges from 10.5 to 41.7 µm. Grains are invariably colporate with the apertures situated at the equator (being zonocolporate). The number of apertures varies from 3 to 17. The endoaperture is generally an endocingulum, often with a secondary thinning at the ectocolpus; one species has endocolpi. The sexine is usually perforate, but psilate, foveolate, and (micro)reticulate patterns were also found. Supratectal elements are present as granules or microspines scattered over the whole surface or confined to a region around the ectoapertures. The inner nexine surface is granular, often with irregular grooves (endocracks).

The pollen morphological variation observed allows the distinction of four pollen types. Three of these types are not yet recorded in other palynological studies of *Spermacoce*. Pollen characters are often useful to delimit species and groups of related species.

Introduction

Numerous authors have acknowledged the value of pollen characters to unravel the phylogeny of Angiosperms. One of the first pollen morphological records for the family Rubiaceae is from Vermoesen (1922), who stressed the taxonomic importance of pollen features in the tribe Gardenieae. Bremekamp (1952) pioneered the use of pollen characters on a large scale to hypothesise relationships between genera as well as tribes of Rubiaceae. Subsequent studies dealing with the systematics of the family often included pollen morphological data (Verdcourt 1958; Lewis 1965; Bremekamp 1966).

With the more general use of scanning electron microscopy (SEM) to examine pollen grains, additional useful characters were discovered (e.g. endopatterns, special sexine patterns such as the double reticulum), and a few studies demonstrated the value of pollen data at tribal and generic level (e.g. Lobreau-Callen and Leroy 1980; Keddam-Malplanche 1985; Johansson 1987). In 'Tropical Woody Rubiaceae', a standard work for the Rubiaceae, Robbrecht (1988) gave a short summary of the pollen morphological variation within the family, being well aware that his survey was far from complete. In the subsequent years more and more systematic studies included pollen data and a few pollen treatments at the tribal level were published, for example Isertieae by Huysmans *et al.* (1998), Catesbaeeae–Chiococceae–*Exostema* complex by Huysmans *et al.* (1999) and Coccocypseleae by Piesschaert *et al.* (2000).

Most Rubiaceae have pollen grains with three apertures only. In the more derived clades of the family, however, we often notice an increase in the number of apertures. The herbaceous tribe Rubieae for example is characterised by 5–13-zonocolpate grains (Huysmans *et al.* 2003). In the Spermacoceae *s. str.*, the clade to which *Spermacoce* L. belongs, pluricolporate pollen grains dominate, but 3-colporate ones also occur. The genus *Spermacoce* is by far the most variable member of the alliance. This can partly be explained by the fact that it outnumbers the other genera in species, and that its pollen morphology is relatively well studied, but other well-documented, relatively large genera, such as *Galianthe* Griseb., show far less variation (Dessein 2003).

Pire (1996) and Dessein *et al.* (2002*a*) evaluated the value of pollen characters in the genus *Spermacoce* for the American and African representatives respectively. The two

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studies illustrate the extreme pollen morphological variation at species level. Prior to the present study, 17 different pollen types had been recognised within *Spermacoce* and many species can be identified based on their pollen grains only (cf. Dessein *et al*. 2002*a*, 2002*b*, 2003). Consequently, pollen is often useful in making taxonomic decisions at the species level.

Pollen of the Australian *Spermacoce* species has never been subjected to modern palynological research. The present paper partially fills this scientific gap by presenting the pollen morphology of the Australian *Spermacoce* species from the Northern Territory (NT); the study is complementary to the revision of these species by Harwood and Dessein (2005).

The following questions are addressed. (1) Does pollen morphology contribute to unravelling the systematic relationships between the species? (2) Is pollen of the Australian species different from pollen morphology of the other *Spermacoce* species? (3) Does the pollen morphological variation reflect functional adaptations?

Material and methods

This study is based on herbarium material from the Northern Territory Herbarium (DNA), with many duplicates deposited in the National Botanic Garden of Belgium (BR); 76 specimens from 48 species were investigated (for *S. inaperta* F.Muell., only observations in Hoyer medium were made, therefore it is not included in Table 2). The specimens investigated are listed in Table 1.

Most pollen data provided in this study are based on observations of acetolysed pollen grains, which were obtained by following Reitsma's 'wetting agent' method (Reitsma 1969). Since flowers and anthers of the study group are often rather small, *c*. ten mature flower buds were needed to yield enough pollen material. The buds were first hydrated in an Agepon solution (1 : 200). After dissection of the anthers and separation of the pollen grains from the remaining flower material by sifting with a micrometer sieve (diameter 60 or 90 μm), the pollen grains were dehydrated in pure acetic acid. The pollen grains were then acetolysed for 10 min in a heating block at approximately 90°C. Pollen of some species (e.g. pollen type 1) were acetolysed for 8 min only. After acetolysis the sample from each specimen was split into two sub-samples, one part for light microscopic (LM) and the other for scanning electron microscopic (SEM) observations and storage. Pollen for SEM was rinsed in ethanol 70% or 96%, pipetted on a stub, and left to dry. Prior to observations by SEM, the stubs were coated with gold with a SPI-MODULE™ sputter coater. Observations and digital images were made under a Jeol JSM 5800 LV microscope (JEOL-USA Inc., Peabody, MA).

Grains for LM studies were mounted in Kaiser's glycerin jelly and sealed with paraffin. The slides were observed using a Leitz Dialux 20 with a ×100 oil immersion or ×40 objective lens. Equatorial diameter (E) was measured under LM in at least ten mature pollen grains. It was often not possible to obtain accurate measurements of the polar axis (P) under LM, since many grains are suboblate and hence only visible in polar view. All other measurements were made on SE-micrographs with Carnoy software (Schols *et al*. 2002; http://www.kuleuven.ac.be/bio/sys/carnoy; verified 18 April 2005). To express the relative length of the colpi, the length colpi/polar axis ratio multiplied by 100 (= LC/P × 100) was used.

To investigate the inside of the pollen grains, the grains were broken by shaking a suspension of pollen and glass beads as described by Huysmans *et al*. (1994).

For *S. inaperta* flowering material was insufficient to allow acetolysis. Hydrated pollen grains were directly mounted on a slide and fixed with Hoyer's gum. Hoyer's gum was prepared as described by Robbrecht (1988); 30 g of Arabic gum was soaked in 50 mL distilled water, then 200 g chloral hydrate was added and the mixture stood for several days until it became clear, and then 20 g of glycerin was added.

Pollen terminology follows Punt *et al*. (1994).

Results

Characters and character states

This section provides the different character states observed for the important pollen features. The variation observed is then summarised in four pollen types, which are compared with the pollen types observed among African and American representatives of the genus in the discussion. Table 2 summarises the variation observed at species level.

Polarity and symmetry

Pollen of Australian *Spermacoce* species from the NT are all isopolar and radially symmetric.

Size

Pollen investigated is usually medium-sized (mean E between 30–40 μm). *Spermacoce brachystema* R.Br. ex Benth., *S. congestanthera* Harwood, *S. dolichosperma* Harwood, *S. graniticola* Harwood, *S. leptoloba* Benth., *S. lignosa* Harwood, *S. membranacea* R.Br. ex Benth., *S. platyloba* Harwood, *S. pogostoma* Benth., *S. reticulata* Harwood, and *S. rupicola* Harwood, however, have small pollen grains (mean E-values ranging from 10.5 to 24.2 μm). The size variation is considerable, even within a single specimen, or between specimens of one single population, and often exceeds 20%. A remarkable size variation has been observed in *S. fabiformis* Harwood: one specimen (Harwood 1034) has small pollen grains (mean E = 21.6 μm), while another collection (Harwood 837) has pollen grains that are almost double in size (mean E = 38.2 μm).

Shape

Pollen shape in equatorial view is expressed by the ratio of the polar axis (= P) and the equatorial diameter (= E). For almost all species, the P/E-ratio varies between 0.88 and 1.14, the pollen grains being spheroidal (e.g. Figs 3*C*, *E*, 5*B*, *C*). Suboblate pollen grains (P/E between 0.75 and 0.88) are found in e.g. *S. pessima* Harwood (Fig. 5*A*). Subprolate grains (P/E between 1.15 and 1.33) are observed in *S. brachystema* and *S. dolichosperma* (Fig. 1*F*, *G*), and sporadically in *S. leptoloba*, *S. platyloba*, *S. reticulata*, and *S. rupicola*. Oblate pollen grains (P/E between 0.50 and 0.75) are found in *S. stenophylla* F.Muell. (Fig. 2*B*, *I*) and *S. inaperta*. It should be noted, however, that the P/E value is strongly influenced by the maturity of the pollen grains. Pollen grains that are not fully mature are often subprolate, while they are spheroidal

Table 1. Specimens examined including pollen type and figure references

Specimens are housed in the Northern Territory Herbarium (DNA), often with duplicates at the National Botanic Garden of Belgium (BR)

Species	Collection	Type	Figures
S. aequabilis Harwood	*Harwood 1037*	20c	
S. aurantiseta Harwood	*Harwood 1072*	20c	6*C*
S. auriculata F.Muell.	*Harwood 612*	20c	6*A*, 7*D*
	Harwood 1071	20c	
S. brachystema R.Br. ex Benth.	*Michell & Carrow 1339A*	1	
S. brevicilia Harwood	*Harwood 1091*	20c	
	Harwood 781	20c	
	Harwood 1065	20c	4*D*
S. brevidens Harwood	*Harwood 1074*	20c	
	Brennan 4286	20c	
S. breviflora F.Muell. ex Benth.	*Harwood 852*	20c	
	Harwood 619	20c	
	Harwood 1070	20c	6*D*
	Harwood 1056	20c	
	Harwood 618	20c	
	Harwood 555	20c	
S. calliantha Harwood	*Harwood 1005*	20c	
	Michell 2643	20c	
	Harwood 1031	20c	6*B*, 7*B*
	Michell 2639	20c	
S. cardiophora Harwood	*Lazarides 8002*	20c	
S. caudata Harwood	*Harwood 1160*	20c	
S. congestanthera Harwood	*Harwood 1073*	20a	4*B*, 5*D*, 6*F*
S. constricta Harwood	*Michell & Yates 2180*	20c	
S. discreta Harwood	*Cowie 8252*	20c	
S. diversistyla Harwood	*Harwood 1154*	20a	
	Harwood 1179	20a	
S. dolichosperma Harwood	*Harwood 1066*	1	1*F*, *G*
S. elaiosoma Harwood	*Harwood 1156*	20c	
	Harwood 807	20c	
S. erectiloba Harwood	*Harwood 729*	20c	4*A*, 5*B*
	Harwood 812	20c	
S. erosa Harwood	*Harwood 1153*	20c	
S. erythrosepala Harwood	*Harwood 1082*	20c	
S. fabiformis Harwood	*Harwood 1034*	20c	
	Harwood 837	20c	
	Harwood 829	20c	7*E*, *F*
	Harwood 639	20c	
	Harwood 851	20c	
	Harwood 823	20c	
	Michell 2628	20c	
S. gilliesiae (Specht) J.R.Clarkson	*Harwood 799*	20c	
S. graniticola Harwood	*Harwood 598*	19	3*A*–*F*
S. hillii (Chippend.) Govaerts	*Harwood 796*	20c	
	Harwood 792	20c	
S. inaperta F.Muell.	*Cowie & Booth 6192*	18	
S. juncta Harwood	*Cowie 2873*	20c	
S. laevigata F.Muell.	*Harwood 1085*	20c	
S. lamprosperma Harwood	*Harwood 989*	20b	5*C*, 6*E*, 7*C*
S. latimarginata Harwood	*Harwood 789*	20c	
S. leptoloba Benth.	*Harwood 1036*	1	
S. lignosa Harwood	*Harwood 1151*	20c	
S. membranacea R.Br. ex Benth.	*Cowie 6547*	20c	
S. occultiseta Harwood	*Harwood 1030*	20c	
S. omissa J.R.Clarkson	*Harwood 1088*	20c	
	Harwood 733	20c	

Table 1. (*continued*)

Species	Collection	Type	Figures
S. pessima Harwood	*Cowie & Dunlop 8484*	20c	5*A*
S. phaeosperma Harwood	*Harwood 609*	20c	4*C*
S. phalloides Harwood	*Harwood 594*	20c	
S. platyloba Harwood	*Harwood 1068*	1	
S. pogostoma Benth.	*Harwood 1069*	1	1*A*, *E*
S. protrusa Harwood	*Cowie 3599*	20c	
S. redacta Harwood	*Harwood 810*	20c	
	Harwood 1041	20c	
S. reticulata Harwood	*Michell 2627*	1	1*C*, *D*
S. retitesta Harwood	*Harwood 786*	20c	
S. rupicola Harwood	*Harwood 830*	1	
	Harwood 600	1	1*B*, *H*, *I*
S. scabrisina Harwood	*Harwood 610*	20c	
	Harwood 552	20c	
S. stenophylla F.Muell.	*Egan 1936*	18	2*A*–*E*, *H*, *I*
	Harwood 1035	18	2*F*, *G*
S. suprahila Harwood	*Harwood 1083*	20c	7*A*
S. tectanthera Harwood	*Harwood 794*	20c	
	Harwood 1067	20c	
S. trichosiphonia Harwood	*Harwood 1075*	20c	

or suboblate when they are fully mature. Furthermore, pollen grains that are not fully hydrated may also tend to be subprolate. It was also observed that pollen shape measured under LM differs slightly from the observations made under SEM. Pollen that is oblate spheroidal (P/E between 0.88 and 1) under LM often appears suboblate under SEM. This can partly be explained by the fact that suboblate pollen grains in the glycerin jelly will tend to orient their short polar axis perpendicular to the glass, and so these pollen grains will only be visible in polar view and cannot be used to determine the equatorial shape, while spheroidal pollen grains will be oriented in all directions. Consequently, the majority of pollen grains that are visible in equatorial view under LM are spheroidal.

In polar view, the pollen grains show a circular outline (e.g. Fig. 3*A*, *F*), often somewhat lobed due to the sunken colpi (Fig. 4*A*–*D*). *Spermacoce stenophylla* and *S. inaperta* are the only exceptions, being triangular in outline (Fig. 2*A*, *H*).

Aperture position

The apertures are invariably situated at the equator. Pantoaperturate pollen grains as observed among African and American *Spermacoce* species are not present. In *S. stenophylla* and *S. inaperta*, the apertures are situated in the middle of the sides (planaperturate; Fig. 2*A*, *H*) when observed in polar view.

Aperture type

Pollen grains of all species investigated are colporate; the ectoaperture is invariably a colpus, the endoaperture an endocinglum (Figs 1*I*, 3*E*, 7*A*–*C*, *F*), except for *S. stenophylla* where an endocolpus is observed (Fig. 2*F*, *I*). Sometimes there is an additional thinning in the endocingulum at the ectocolpi (Fig. 7*B*, *C*). Where the ectoaperture and the endoaperture overlap, a gap is often observed in the colpus membrane.

The ectocolpi are slit-like and their relative length varies between approximately 30 in e.g. *S. stenophylla* and *S. omissa* J.R.Clarkson and approximately 75 in e.g. *S. hillii* (Chippend.) Govaerts and *S. lamprosperma* Harwood. LC/P × 100 shows much variation within a single specimen; even on a single pollen grain short and long colpi have been observed.

Aperture margo

In *S. brachystema*, *S. dolichosperma*, *S. graniticola*, *S. leptoloba*, *S. platyloba*, *S. pogostoma*, *S. reticulata*, *S. rupicola* and *S. stenophylla* the sexine around the apertures is distinctly differentiated from the remainder of the sexine by the presence of supratectal elements (granules or microspines). These margines can be well delimited as in *S. pogostoma* (Fig. 1*E*) or rather vague as in *S. dolichosperma* (Fig. 1*F*). *Spermacoce graniticola* has a continuous girdle of supratectal elements at the equatorial zone (Fig. 3*C*, *D*). In other species, e.g. *S. pessima*, the perforations of the sexine bordering the apertures are larger than for the remaining sexine (Fig. 5*A*).

Number of apertures

The number of apertures ranges from three in *S. stenophylla* (Fig. 2*A*, *H*), *S. inaperta*, *S. pogostoma* (Fig. 1*A*), and *S. rupicola* to 17 in *S. brevicilia* Harwood. Most

Table 2. Some variable pollen morphological characters for each species studied

Species are arranged by pollen type as discussed in the text. Number in parentheses after each species is the number of specimens studied (see Table 1 for list of specimens studied). Shape as seen in equatorial view: O = oblate; SO = suboblate; OS = oblate spheroidal; S = spheroidal; SP = subprolate. Colpi length: short = LC/P × 100 < 40; medium = LC/P × 100 between 40 and 60; long = LC/P × 100 > 60

Type	Species	# Colpi	E (µm)	Shape	Colpi length	Endoaperture	Sexine	Supratectal elements
1	*S. brachystema* (1)	6–7	19–(20.7)–24	SP	medium to long	endocingulum	psilate-perforate	margo of granules
1	*S. dolichosperma* (1)	5–6	19–(20.3)–21	SP	medium	endocingulum	psilate	margo of granules
1	*S. leptoloba* (1)	? 7	10–(10.5)–11	S–SP	?	endocingulum	perforate	margo of granules
1	*S. platyloba* (1)	7–8	12–(12.3)–13	S–SP	medium	endocingulum	perforate	margo of granules
1	*S. pogostoma* (1)	3	22–(24.2)–26	S	long	endocingulum	psilate	margo of granules
1	*S. reticulata* (1)	6	14–(15.0)–16	S–SP	long	endocingulum	psilate	margo of granules
1	*S. rupicola* (2)	3	17–(18.6)–21	S–SP	medium to long	endocingulum	psilate	margo of granules
18	*S. stenophylla (brevi)* (1)	3	33–(36.4)–40	O	short	endocolpus	(micro)reticulate	margo of granules
18	*S. stenophylla (longi)* (1)	3	27–(29.3)–31	O	short	endocolpus	(micro)reticulate	margo of granules
19	*S. graniticola* (1)	? 9–10	21–(21.5)–22	S	short	endocingulum	reticulate	margo of microspines
20a	*S. congestanthera* (1)	7–9	17–(19.0)–21	S	medium	endocingulum	(micro)reticulate	granules
20a	*S. diversistyla* (2)	9–12	25–(29.8)–33	SO–S	medium	endocingulum	(micro)reticulate	microspines
20b	*S. lamprosperma* (1)	(11–)12–15	35–(38.2)–40	SO–S	long	endocingulum	perforate/foveolate	absent
20c	*S. aequabilis* (1)	10–13	27–(30.9)–37	S	medium	endocingulum	perforate	microspines
20c	*S. aurantiseta* (1)	12–14	33–(35.6)–39	SO	medium	endocingulum	perforate	microspines
20c	*S. auriculata* (2)	11–13	31–(38.5)–45	S	medium to long	endocingulum	perforate	microspines
20c	*S. brevicilia* (3)	11–13(–17)	33–(38.9)–50	SO–S	medium to long	endocingulum	perforate	microspines
20c	*S. brevidens* (2)	8–9	25–(28.6)–32	S	long	endocingulum	perforate	microspines
20c	*S. breviflora* (6)	10–12	28–(31.6)–37	SO–S	medium to long	endocingulum	perforate	microspines
20c	*S. calliantha* (4)	11–15	33–(37.9)–45	SO–S	medium to long	endocingulum	perforate	microspines
20c	*S. cardiophora* (1)	7–8	33–(34.2)–36	S	long	endocingulum	perforate	microspines
20c	*S. caudata* (1)	8	29–(32.2)–35	S	medium	endocingulum	perforate	microspines
20c	*S. constricta* (1)	12–14	34–(36.6)–40	S	?	endocingulum	perforate	microspines
20c	*S. discreta* (1)	8–9	29–(29.7)–32	S	medium to long	endocingulum	perforate	microspines
20c	*S. elaiosoma* (2)	10–13	30–(34.9)–42	OS–S	medium	endocingulum	perforate	microspines
20c	*S. erectiloba* (2)	8–9	33–(37.5)–42	S	long	endocingulum	perforate	microspines
20c	*S. erosa* (1)	9–10	26–(28.7)–32	S	medium	endocingulum	perforate	microspines
20c	*S. erythrosepala* (1)	9–11	30–(31.5)–35	S	long	endocingulum	perforate	microspines
20c	*S. fabiformis* (7)	(9–)11–12(–13)	19–(31.4)–41	(SO–)S	medium	endocingulum	perforate	microspines
20c	*S. gilliesiae* (1)	13–15	38–(40.8)–44	S	medium to long	endocingulum	perforate	microspines
20c	*S. hillii* (2)	10–12	31–(34.6)–39	S	long	endocingulum	perforate	microspines
20c	*S. juncta* (1)	9–12	32–(34.7)–37	S	long	endocingulum	perforate	microspines
20c	*S. laevigata* (1)	13–14	37–(39.4)–43	S	medium to long	endocingulum	perforate	microspines
20c	*S. latimarginata* (1)	10–12	29–(30.9)–33	S	?	endocingulum	perforate	microspines
20c	*S. lignosa* (1)	8	20–(20.5)–21	S	medium	endocingulum	perforate to microreticulate	microspines
20c	*S. membranacea* (1)	9	23–(23.7)–25	S	medium to long	endocingulum	perforate	microspines
20c	*S. occultiseta* (1)	10–12	25–(30.8)–34	S	long	endocingulum	perforate	microspines
20c	*S. omissa* (2)	7–9	22–(28.7)–37	S	short	endocingulum	perforate	microspines
20c	*S. pessima* (1)	10–12	23–(24.4)–25	SO	medium	endocingulum	perforate	microspines
20c	*S. phaeosperma* (1)	10–11	35–(37.3)–41	S	long	endocingulum	perforate	microspines
20c	*S. phalloides* (1)	11–13	33–(34.6)–36	S	medium	endocingulum	perforate	microspines
20c	*S. protrusa* (1)	9–12	33–(33.9)–35	SO	medium	endocingulum	perforate	microspines
20c	*S. redacta* (2)	8–10	25–(28.0)–31	S	medium to long	endocingulum	perforate	microspines
20c	*S. retitesta* (1)	11–13	28–(31.8)–33	SO–S	medium	endocingulum	perforate	microspines
20c	*S. scabrisina* (2)	10–12	29–(31.4)–34	SO–S	medium	endocingulum	perforate	microspines
20c	*S. suprahila* (1)	12–14	38–(41.7)–43	SO–S	medium to long	endocingulum	perforate	microspines
20c	*S. tectanthera* (2)	(10–)11–13	37–(40.5)–44	(SO)–S	long	endocingulum	perforate	microspines
20c	*S. trichosiphonia* (1)	10	23–(25.4)–29	S	long	endocingulum	perforate	microspines

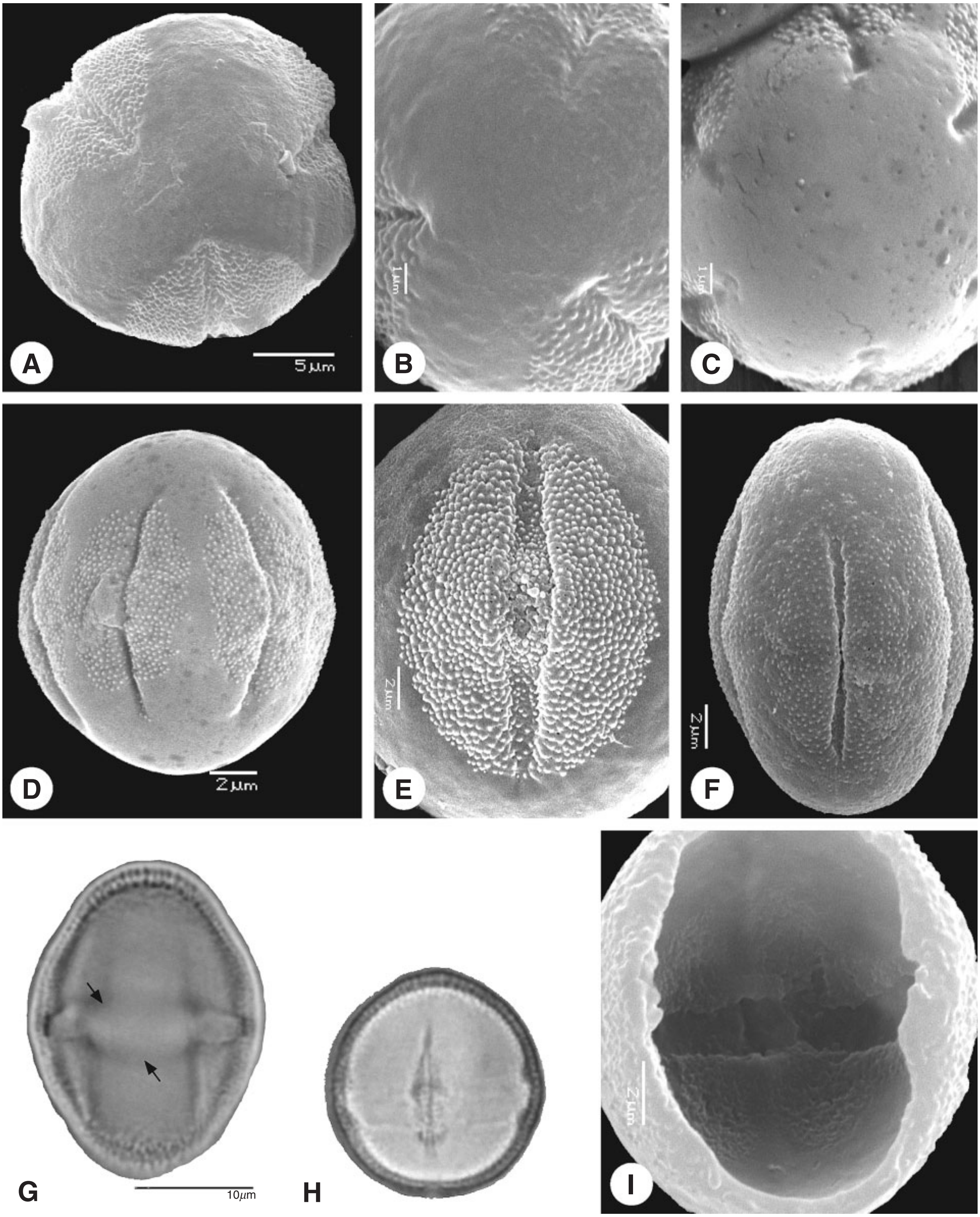

Fig. 1. SEM (*A–F*, *I*) and LM (*G*, *H*) photographs illustrating Type 1 pollen in NT species of *Spermacoce*. (*A*) *S. pogostoma*, polar view of 3-colporate pollen grain. (*B*) *S. rupicola*, detail of apocolpium of 3-colporate pollen grain. (*C*, *D*) *S. reticulata*: (*C*) detail of apocolpium of 6-colporate pollen grain; (*D*) equatorial view of pollen grain, notice the presence of granules around the ectoapertures. (*E*) *S. pogostoma*, detail of ectocolpus. (*F*, *G*) *S. dolichosperma*: (*F*) equatorial view of pollen grain, notice the presence of granules around the ectoapertures; (*G*) equatorial view of pollen grain, notice brighter zone indicating position of endocingulum (arrows). (*H*, *I*) *S. rupicola*: (*H*) equatorial view of pollen grain; (*I*) inner side of pollen grain showing endocingulum.

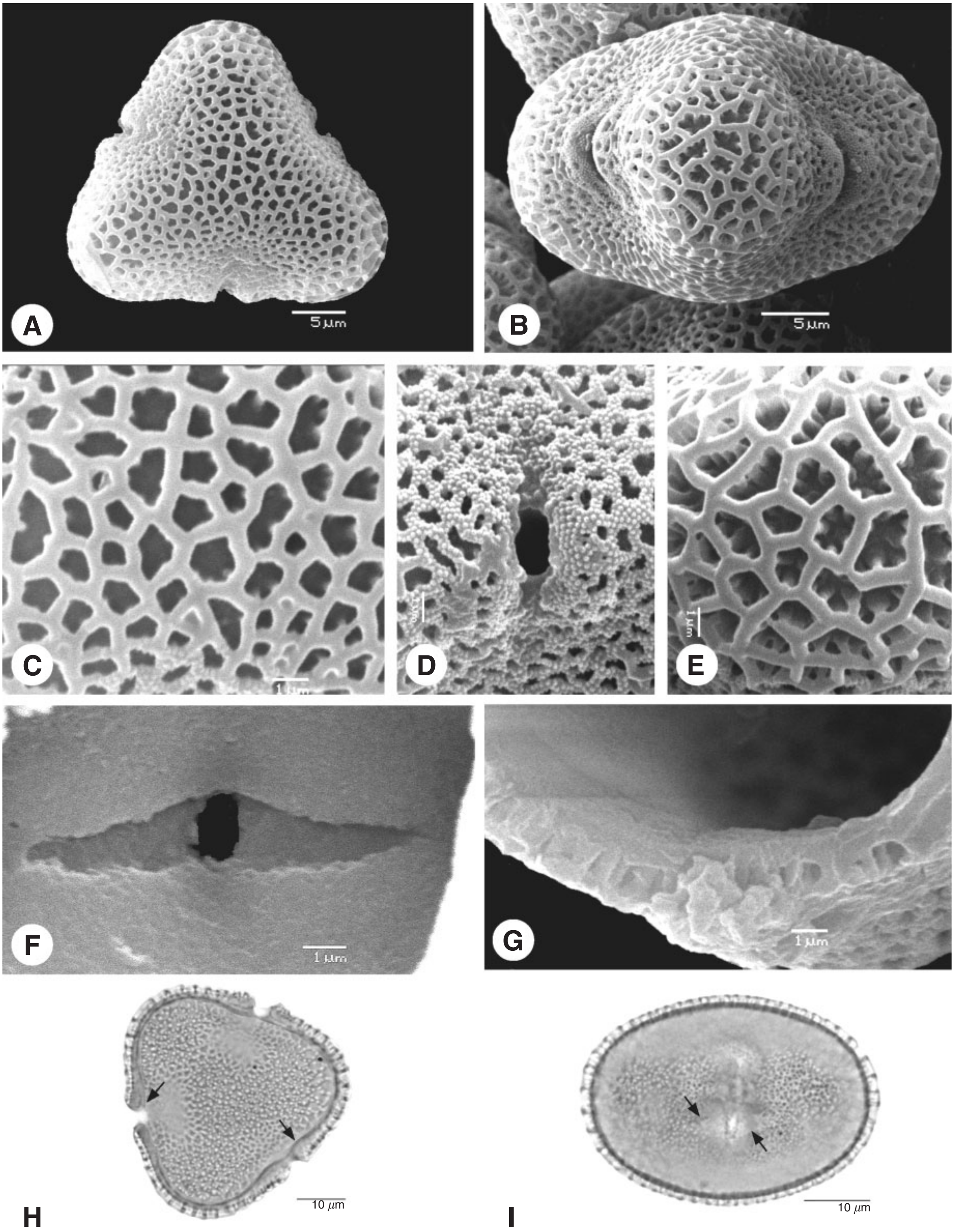

Fig. 2. SEM (*A–G*) and LM (*H*, *I*) photographs illustrating Type 18 pollen as observed in *Spermacoce stenophylla*. (*A*) Polar view of 3-colporate pollen grain. (*B*) Equatorial view of pollen grain. (*C*) Detail of apocolpium showing reticulate sexine. (*D*) Detail of ectocolpus, notice the presence of granules around the aperture. (*E*) Detail of mesocolpium showing reticulate sexine. (*F*) Detail of the inner side of pollen grain showing endocolpus. (*G*) Detail of pollen wall. (*H*) Polar view of pollen grain, notice the thickening of the nexine around the apertures (arrows). (*I*) Equatorial view of pollen grain, notice the somewhat brighter zone perpendicular to the ectocolpus indicating the position of the endocolpus (arrows).

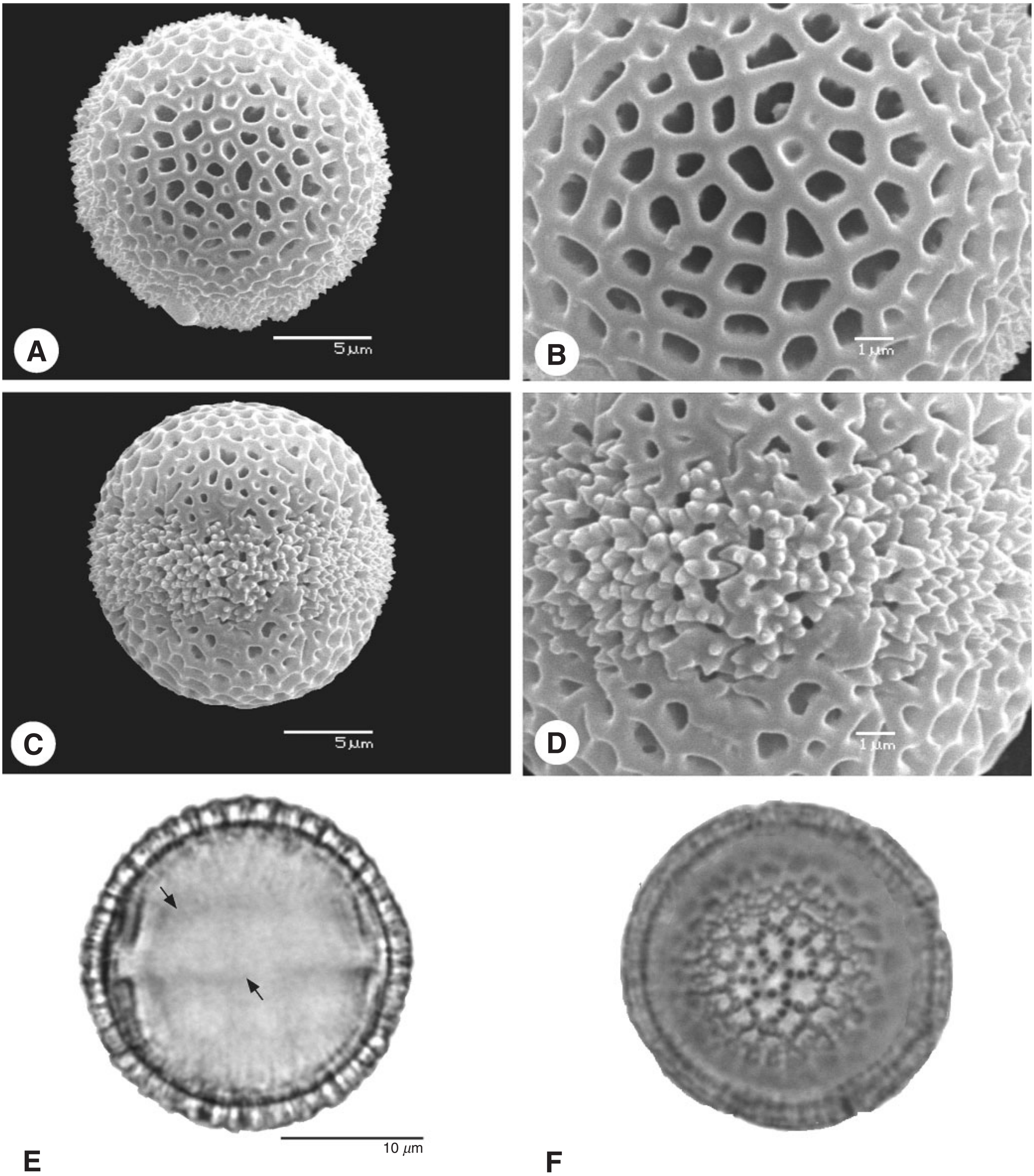

Fig. 3. SEM (*A–D*) and LM (*E*, *F*) photographs illustrating Type 19 pollen as observed in *Spermacoce graniticola*. (*A*) Polar view of pollen grain, notice that the ectoapertures are hardly visible. (*B*) Detail of apocolpium showing reticulate sexine. (*C*) Equatorial view of pollen grain, notice girdle of granules at the equator. (*D*) Detail of equatorial zone. (*E*) Equatorial view, notice the brighter zone indicating the presence of the endocingulum (arrows). (*F*) Polar view of pollen grain.

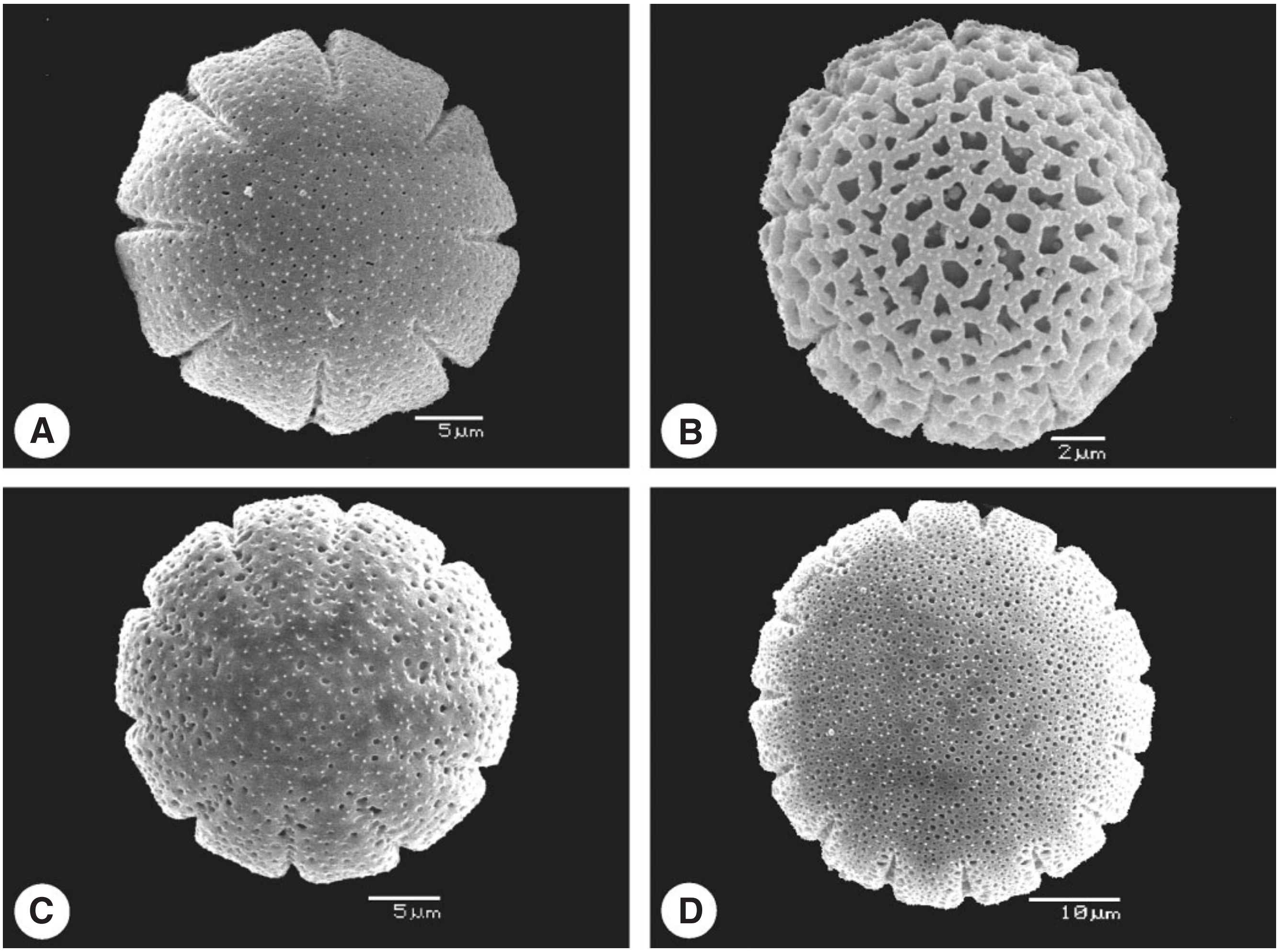

Fig. 4. SEM photographs illustrating variation in polar views of Type 20 pollen grains among NT species of *Spermacoce*. (*A*) *S. erectiloba*, polar view of 8-colporate pollen grain. (*B*) *S. congestanthera*, polar view of 9-colporate pollen grain. (*C*) *S. phaeosperma*, polar view of 10-colporate pollen grain. (*D*) *S. brevicilia*, polar view of 15-colporate pollen grain.

species have 10–13 apertures. There is a positive correlation between the size of the pollen grains and the number of apertures ($r^2 = 0.38$; $P < 0.01$). This correlation was also found among pollen of the African *Spermacoce* species (Dessein *et al*. 2002*a*). The pollen grains of *S. stenophylla* are somewhat deviant: notwithstanding their large size, they have only three apertures.

The pluriaperturate condition is typical for the tribe Spermacoceae *s. str.*, and is considered apomorphic within the family Rubiaceae. The variation in the number of apertures in individual species is rather limited (with the exception of *S. brevicilia*), which makes it a good taxonomic character at species level.

Pollen wall stratification

A nexine layer, a row of columellae, and a tectum with supratectal elements build up the exine (Figs 2*G*, 7*D*). The thickness of the different layers varies strongly between pollen grains of a single specimen, but is also variable within a single grain. The nexine bordering the ectocolpi is often thickened (cf. Fig. 2*H*). In the apocolpium, the nexine varies between 0.44 and 1.07 µm, the columellae between 0.28 and 1.30 µm and the tectum between 0.4 and 0.71. Given the large intraspecific variation, the systematic value is limited.

Tectum

The tectum of most species is perforated (Fig. 6*A–C*), the perforations not exceeding 1 µm in diameter, and the space between the perforations wider than the perforations. The perforations are rounded or somewhat angular, isodiametrical or irregular, rarely elongated, and larger perforations are often intermingled with smaller ones (Fig. 6*C*, *D*). There are only slight differences between the perforations of the apocolpium and those of the mesocolpia. As noted above, the perforations bordering the colpi, however, tend to be

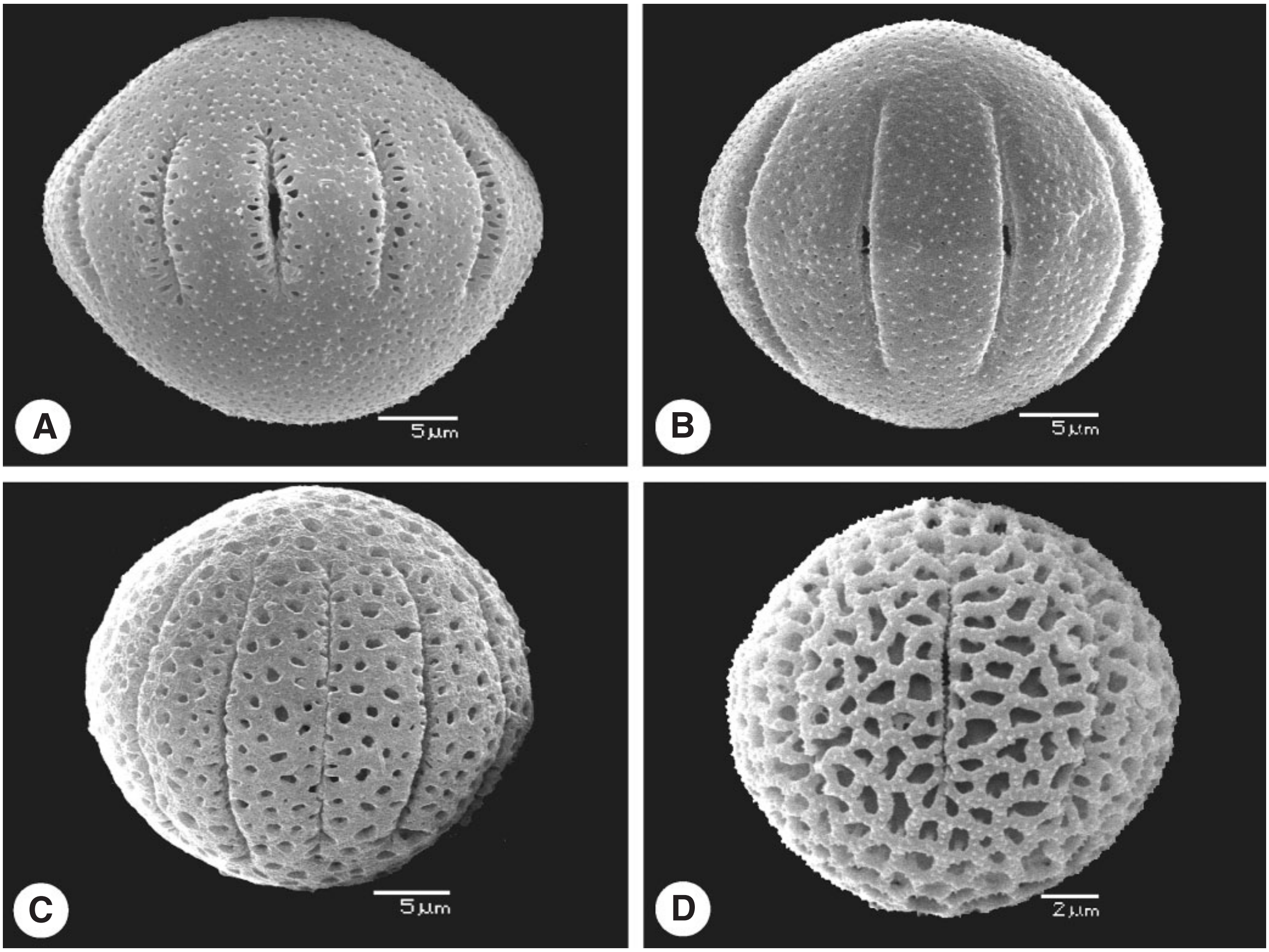

Fig. 5. SEM photographs illustrating variation in equatorial view of Type 20 pollen grains among NT species of *Spermacoce*. (*A*) *S. pessima*, equatorial view, notice the relatively short colpi and the larger perforations bordering the apertures. (*B*) *S. erectiloba*, equatorial view, notice the long colpi. (*C*) *S. lamprosperma*, equatorial view, notice the absence of granules. (*D*) *S. congestanthera*, equatorial view, notice the reticulate tectum with granules.

larger. In *S. lamprosperma* the tectum tends to be foveolate, in which perforations exceed 1 µm but with muri wider than the perforations (Fig. 6*E*). In this species, the borders of the perforations show a distinct slope. *Spermacoce diversistyla* Harwood, *S. congestanthera*, *S. graniticola*, and *S. stenophylla* have a microreticulate to reticulate tectum in which the lumina are wider than the bordering muri (Figs 2*C–E*, 3*B*, 6*F*). *Spermacoce lignosa* Harwood has a tectum intermediate between perforate and microreticulate. *Spermacoce brachystema*, *S. dolichosperma*, *S. pogostoma*, *S. reticulata*, and *S. rupicola* apparently have few or no perforations at the smooth apocolpium (= psilate) (Fig. 1*B–C*).

Supratectal elements

At the apocolpium, most species have supratectal elements. These may be granules or microspines. The number of supratectal elements per 25 μm^2 varies from five to more than 30 and seems to be rather constant for a given species. More data are needed, however, to confirm this. *Spermacoce lamprosperma* lacks supratectal elements (Fig. 6*E*), while *S. brachystema*, *S. dolichosperma*, *S. graniticola*, *S. platyloba*, *S. pogostoma*, *S. reticulata*, *S. rupicola* and *S. stenophylla* only have supratectal elements around the ectoapertures.

Endopatterns

Two endopatterns can be described. The first type characterises most species and shows a granular nexine surface with narrowly triangular or linear shaped bands of thinned nexine at the mesocolpia. These bands usually connect to the endocingulum (Fig. 7*A–C*, *F*) and have regular (Fig. 7*A*) or irregular (Fig. 7*B*, *C*) margins. In between these extensions of the endocingulum, the nexine

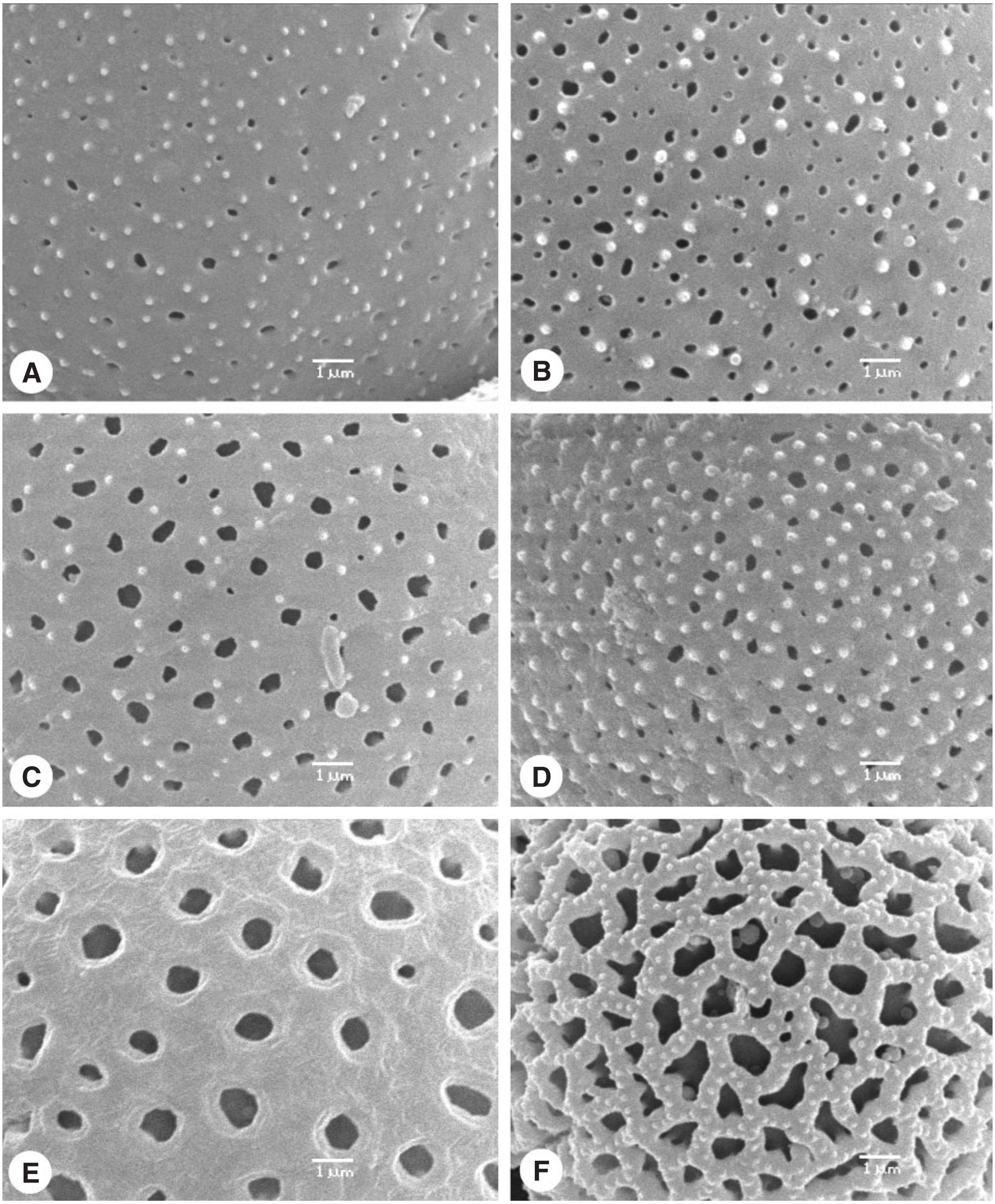

Fig. 6. SEM photographs illustrating variation in tectum of Type 20 pollen grains among NT species of *Spermacoce*. (*A*) *S. auriculata*, detail of apocolpium showing perforate tectum with small perforations and relatively densely beset with granules/microspines. (*B*) *S. calliantha*, detail of apocolpium showing perforate tectum with relatively large perforations and sparsely beset with microspines. (*C*) *S. aurantiseta*, detail of apocolpium showing perforate tectum with large perforations intermingled with smaller ones and sparsely beset with microspines. (*D*) *S. breviflora*, detail of apocolpium showing perforate tectum with large perforations intermingled with smaller ones and densely covered with microspines. (*E*) *S. lamprosperma*, detail of apocolpium showing foveolate tectum without supratectal elements. (*F*) *S. congestanthera*, detail of apocolpium showing reticulate tectum with the muri densely beset with granules/microspines.

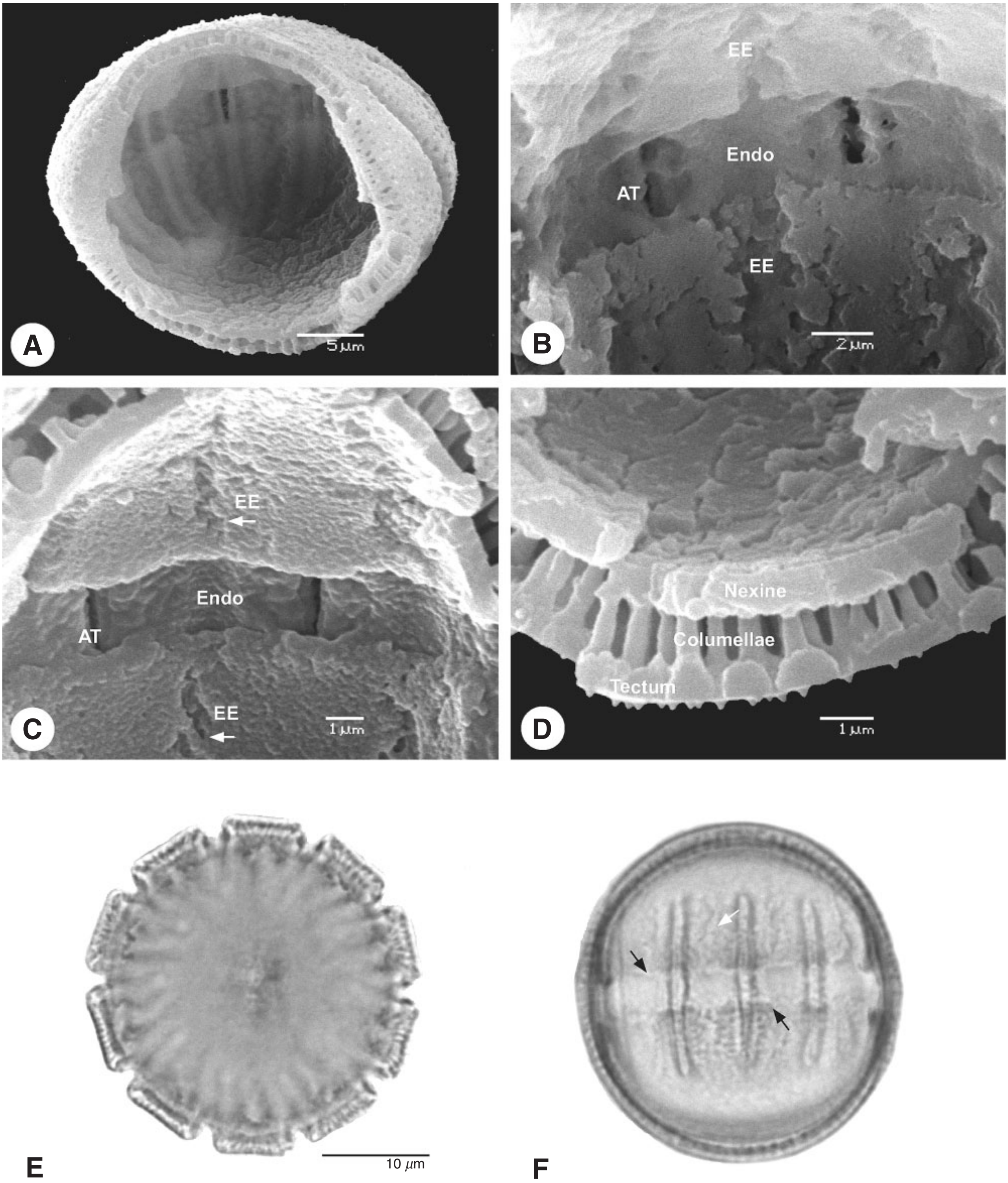

Fig. 7. SEM (*A*–*D*) and LM (*E*–*F*) photographs showing endopatterns observed among Type 20 pollen grains of NT species of *Spermacoce*. (*A*) *S. suprahila*, inside view of broken pollen grain showing endocingulum, extensions of endocingulum, and endocracks. (*B*) *S. calliantha*, detail of inside of pollen grain showing endocingulum (endo), additional thinning of endocingulum at the ectoapertures (AT), and irregular extensions of endocingulum in the mesocolpia (EE). (*C*) *S. lamprosperma*, detail of inside of pollen grain showing endocingulum with indistinct extensions in the mesocolpia. (*D*) *S. auriculata*, view at broken pollen wall showing nexine, a row of columellae and a perforated tectum with microspines. (*E*, *F*) *S. fabiformis*: (*E*) polar view of pollen grain; (*F*) equatorial view of pollen grain, notice the brighter zones indicating the position of the endocingulum (black arrows) and the extensions of the endocingulum (white arrow).

is granular and mostly continuous, rarely with a few endocracks. At the apocolpium, the nexine is granular with numerous distinct endocracks (Fig. 7*A*). The second type has a continuous granular nexine, with no or obscure endocracks (Fig. 1*I*).

Pollen types

Four pollen types can be recognised amongst the NT species. One type is identical to Type 1 described by Pire (1996) and Dessein *et al*. (2002*a*). The three other pollen types have not yet been recorded in other palynological studies of *Spermacoce,* and as numbering of the pollen types continues from the previous studies (Pire 1996; Dessein *et al*. 2002*a*), these are indicated as Type 18, 19 and 20.

Key to pollen types of Northern Territory species of *Spermacoce*

1. Pollen triangular in polar view, 3-colporate; endoaperture an endocolpus .... Type 18
   Pollen more or less circular in polar view, 3- or pluricolporate; endoaperture an endocingulum 2
2. Distinct margo of granules around ectoapertures; pollen small (mean E < 25 µm) 3
   No distinct margo of granules, supratectal elements being absent or scattered over the whole pollen surface; pollen usually larger E ≥ 25, sometimes E = 19–25 µm 4
3. Tectum perforate or psilate at the apocolpium .... Type 1
   Tectum reticulate at the apocolpium .... Type 19
4. Tectum microreticulate to reticulate .... Type 20a
   Tectum perforate or foveolate 5
5. Tectum perforate to foveolate, without supratectal elements .... Type 20b
   Tectum perforate with supratectal elements .... Type 20c

Type 1 (Fig. 1)

Pollen 3–8-zonocolporate; mean E 10.5–24.2 µm; equatorial shape spheroidal or subprolate; polar outline circular. Ectocolpi medium to long (LC/P × 100 = 45–70), slit-like, with a margo of granules. Endoaperture a narrow endocingulum without horns at the mesocolpia. Tectum perforate or ? psilate, supratectal elements absent from apocolpium. Inner nexine surface granular without distinct endocracks.

Species: *S. brachystema*, *S. dolichosperma*, *S. leptoloba*, *S. platyloba*, *S. pogostoma*, *S. reticulata*, *S. rupicola*.

Remark

This pollen type is also found among American and African representatives of *Spermacoce*, such as *S. confusa*, *S. exilis*, *S. natalensis*, *S. ocymoides*, and *S. tenuior*.

Type 18 (Fig. 2)

Pollen 3-zonocolporate and planaperturate; mean E 29.3–36.4 µm; equatorial shape oblate; polar outline triangular. Ectocolpi short (LC/P × 100 = *c.* 30), slit-like, with a margo of granules, exine somewhat protruding around the ectoaperture due to thickened nexine. Endoaperture an endocolpus with acute ends. Tectum microreticulate to reticulate, supratectal elements absent from apocolpium. Inner nexine surface granular without distinct endocracks.

Species: *S. stenophylla*, *S. inaperta*.

Type 19 (Fig. 3)

Pollen ? 9–10-zonocolporate; mean E 21.5 µm; equatorial shape spheroidal; polar outline circular. Ectocolpi short (LC/P × 100 = 25–30), slit-like, with a margo of granules. Endoaperture a narrow endocingulum without extension horns. Tectum reticulate, supratectal elements absent from apocolpium. Inner nexine surface unknown.

Species: *S. graniticola*.

Type 20 (Figs 4–7)

Pollen 7–17-zonocolporate; mean E 19.0–41.7 µm; equatorial shape suboblate to spheroidal; polar outline circular. Ectocolpi relatively long (LC/P × 100 = 40–75), sometimes shorter in *S. omissa*, slit-like, lacking margo of granules. Endoaperture a broad endocingulum with narrowly triangular or linear shaped extensions (of which the margins are distinct or rather diffuse) at the mesocolpia that are often continuous with the endocracks. Tectum perforate or more rarely (micro)reticulate with microspines uniformly present, rarely foveolate and lacking supratectal elements. Inner nexine surface granular with distinct endocracks at the apocolpium and granular without or with few endocracks in between the extensions of the endocingulum.

Based on differences of the tectum three subtypes can be described.

Type 20a: tectum microreticulate to reticulate with supratectal elements. Species: *S. congestanthera*, *S. diversistyla*.

Type 20b: tectum foveolate lacking supratectal elements. Specie: *S. lamprosperma*.

Type 20c: tectum perforate or somewhat microreticulate with supratectal elements. Species: *S. aequabilis* Harwood, *S. aurantiseta* Harwood, *S. auriculata* F.Muell., *S. brevidens* Harwood, *S. brevicilia*, *S. breviflora* F.Muell. ex Benth., *S. cardiophora* Harwood, *S. calliantha* Harwood, *S. caudata* Harwood, *S. constricta* Harwood, *S. discreta* Harwood, *S. elaiosoma* Harwood, *S. erectiloba* Harwood, *S. erosa* Harwood, *S. erythrosepala* Harwood, *S. fabiformis*, *S. gilliesiae* (Specht) J.R.Clarkson, *S. hillii*, *S. juncta* Harwood, *S. laevigata* F.Muell., *S. latimarginata*, *S. lignosa*, *S. membranacea*, *S. occultiseta* Harwood, *S. omissa*, *S. pessima*, *S. phalloides* Harwood, *S. phaeosperma* Harwood, *S. redacta* Harwood, *S. retitesta* Harwood, *S. protrusa* Harwood, *S. scabrisina* Harwood, *S. suprahila* Harwood, *S. tectanthera* Harwood, *S. trichosiphonia* Harwood.

Remark

Pollen of *S. omissa* does not fully fit in this pollen type. Its colpi are relatively short (LC/P × 100 = 25–35) and the nexine is distinctly thickened towards the apertures. These differences are considered too small, however, to diagnose another pollen type.

Discussion

Pollen of Australian v. *African and American Spermacoce species*

The pollen morphological variation observed among Australian *Spermacoce* species is considerable. The identification of four distinct pollen types in a group of only 48 species of one single genus is rather unusual within the family Rubiaceae, and even within angiosperms such variation is only sporadically observed. Compared with the pollen variation observed among African or American *Spermacoce* species, however, the variation is rather limited. From a similar number of African species, for example, nine pollen types were found (Dessein *et al*. 2002*a*).

Pollen type 1, characterised by small, often subprolate, pollen grains with a distinct margo of granules around the ectoapertures, is also found among African and American *Spermacoce* species. It was hypothesised (Dessein *et al*. 2002*a*; Dessein 2003) that this type of pollen grain could represent the basal pollen type within the genus *Spermacoce*. Indeed, this pollen type most resembles the pollen found in other Rubiaceae, and the other pollen types observed within the genus are easily derived from this type (cf. Pire 1996). Pollen types 19 and 20, exclusively found among Australian species, are also easily derived from this type. Pollen type 18, however, is very atypical for the genus. The combination of the oblate equatorial shape, the relatively small dimensions, the short colpi, and the presence of endocolpi, has so far not been found in any other member of the genus.

Systematic value

Pollen morphology is a useful character in the systematics of the tribe Spermacoceae. Especially at the lower taxonomic levels (genera, subgenera, species) it often helps to delimit troublesome taxa, including some Australian taxa.

The informal groupings of species by Harwood and Dessein (2005) are partly supported by our pollen observations. All species of the 'basal' group (*S. brachystema*, *S. dolichosperma*, *S. pogostoma*, *S. reticulata*, and *S. rupicola*), characterised by small flowers with hairs inside near the base of the corolla lobes, and with the stigma and anthers included, possess Type 1 pollen grains. Pollen and DNA studies by Dessein (2003) suggest that this group of species is basal within the Australian clade, an opinion that is in agreement with the evolutionary pollen trends proposed by Pire (1996) and Dessein *et al*. (2002*a*). Within the 'basal' group, *S. pogostoma* and *S. rupicola* are the only two species with three apertures. *Spermacoce reticulata* is at first sight very similar to *S. rupicola*, but differences in seed morphology set it apart. This is confirmed by pollen differences (six *v.* three colpi).

The 'linkage' group of Harwood and Dessein (2005) places together species with a mix of characters from the 'basal' group and those of the more derived groups. This is reflected in the pollen morphology, some species having primitive, others more derived pollen. *Spermacoce leptoloba* and *S. platyloba* have Type 1 pollen grains and feature the smallest grains of the study group. They are considered more closely related to each other than to any other species. Amongst other things, they are the only two species of this group with a persistent obturator on their seeds. *Spermacoce graniticola* is the only species with Type 19 pollen grains. This pollen type has a margo of microspines similar to what is observed in Type 1 pollen, but the colpi are short and the tectum is reticulate. *Spermacoce graniticola* differs from the species characterised by Type 1 pollen mainly by its exserted stamens. *Spermacoce lignosa* and *S. membranacea* possess Type 20 pollen grains.

The 'appendages', 'divided lobes', and 'appendages lost' groups of Harwood and Dessein (2005) are all characterised by pollen type 20. All four species with divided corolla lobes (*S. brevidens*, *S. cardiophora*, *S. caudata*, *S. erectiloba*) have relatively few apertures (7–9). *Spermacoce erectiloba* has much longer lobes than the other three, and has the largest pollen grains. It is somewhat remarkable that this group hypothesised to be derived is characterised by relatively few apertures, a condition often related to more primitive taxa.

Pollen variation among the other species characterised by Type 20 pollen is not obviously related to any other important morphological characters (e.g. there is no relationship between pollen data and presence or absence of appendages, appendage type, seed coat surface, obturator persistent on seed or not).

Of the unplaced species, *S. stenophylla* and *S. inaperta* are characterised by pollen type 18. They are closely related, having almost identical corollas. They differ in capsule dehiscence and seed type, but these differences have not been expressed in pollen. Their distinctive pollen type indicates that these species may have been isolated before the radiation of the other species took place. The presence of a margo of granules, a feature also present in Type 1 pollen, suggests that they evolved directly from an ancestor characterised by Type 1 pollen. The fact that this pollen type is found in two closely related species featuring a different fruit type—*S. stenophylla* has capsules, while *S. inaperta* has schizocarps—demonstrates the danger of making taxonomic groupings based on fruit characters only. Two other unplaced species, *S. congestanthera* and *S. omissa*, have a somewhat special pollen morphology. The pollen of the former species is morphologically different in having a distinct (micro-)reticulate tectum, a feature also observed in *S. diversistyla*. *Spermacoce congestanthera* differs from the other NT *Spermacoce* species by its basifixed anthers and single linear stigma. *Spermacoce omissa* has a very different type of capsule dehiscence and its pollen differs from the typical Type 20 pollen grains by the much shorter colpi.

How to explain this pollen morphological variation?

It is difficult to estimate the functional aspects of the pollen morphological variation observed. In the following paragraphs an attempt is made, nevertheless, to point to some aspects that may or may not influence the different pollen characters.

Pollen size

Among the NT *Spermacoce* species, it appears that differences in pollen size do not always reflect functional adaptation. Relatively large pollen size variation is observed within a single specimen and between specimens of the same species. Several factors, including flower size, style length, pollen vector size, mineral nutrition, and water supply, may cause differences in pollen size (for an overview, see Muller 1979). For many Rubiaceae, pollen size variation is associated with heterostyly, in which the short-styled morph usually has larger pollen grains than the long-styled morph (Ganders 1979). The only Australian *Spermacoce* species that shows distinct heterostyly is *S. multicaulis* Benth., but this species is endemic to Queensland and not studied here. In *S. stenophylla*, the anthers may be overtopped by the style, but the opposite situation, anthers overtopping the style, is more common. This flower dimorphism is not comparable with other heterostylous species of the Rubiaceae because the anthers are invariably well exserted, and only the position of the style is variable. Pollen of both morphs was investigated and it turned out that pollen of the long-styled morph is larger than that of the short-styled morph (mean E 36.4 µm *v.* 29.3 µm), but comparable size variation is observed among genuine isostylous species, hence no sound conclusions can be drawn.

Size variation can also be attributed to the growth conditions of the specimens, with better conditions meaning larger grains (Muller 1979). This rule does not hold for the NT species. In species with both southern and northern collections, the southern collections often have larger pollen grains, but there are exceptions. The southern areas have less rainfall, and generally speaking, vegetation is not as dense or as tall. Following the rule of Muller (1979), you would expect the northern collections to have larger pollen grains. However, for *S. fabiformis*, the two most southerly collections have E values of 35–39 µm (Harwood 639) and 31–40 µm (Harwood 837), the most northerly collection 19–23 µm (Harwood 1034), and those in between (Harwood 829, 851, 823, Michell 2628) have a size in between; for *S. hillii*, the southern collection is 35–38 µm (Harwood 792), and the northern collection 30–35 µm (Harwood 796); for *S. stenophylla* the southern collection is 33–40 µm (Egan 1936), the northern collection 27–31 µm (Harwood 1035); for *S. auriculata* the southern collection is 38–45 µm (Harwood 612), and the northern collection 31–37 µm (Harwood 1071). *Spermacoce omissa* is a notable exception; the southern collection is 22–25 µm (Harwood 733), and the northern collection 29–37 µm (Harwood 1088). *Spermacoce calliantha* and *S. breviflora*, for both of which there are several collections over a large area to compare, do not show any pattern.

In Type 1 pollen grains, on the contrary, the small pollen size (mean E ranging from 10.5 to 24.2 µm) might be an adaptation to a specific pollinator. The species characterised by this pollen type have very short corolla tubes (often shorter than 1 mm) and the anthers and style are included in the tube. In most other NT *Spermacoce* species the anthers and style are exserted, and it is possible that those species with short tubes and included anthers and style have different pollen vectors.

Number of apertures

The number of apertures is, as mentioned above, positively correlated with the pollen size. This is not surprising, since the larger the pollen grains the larger the surface where the colpi may initiate. Estimating the possible functional aspects of the number of colpi is difficult. More colpi may facilitate germination, but does the difference between 10 and 17 colpi really matter? Most probably it does not. A possible explanation may be found in the harmomegathic function of the colpi. Harmomegathy is the process by which pollen grains and spores change in shape to accommodate variations in the volume of the cytoplasm caused by changes in hydration. The invagination of the aperture membrane is known as an important harmomegathic mechanism (cf. Thanikaimoni 1986). It seems reasonable to assume that a larger volume requires more apertures to carry out this function.

Aperture length

The length of the apertures is rather variable within a single species. There is no direct evidence that it is an important feature, and it possibly also plays a role in the harmomegathic function of the pollen grains.

Sexine ornamentation

Variation in sexine ornamentation may indicate differences in pollinators. In general, psilate pollen grains are thought to be adapted to wind-pollination or beetles, while grains with a rough surface are typical for insect-pollinated species (cf. Punt 1986). Pollen of all Australian *Spermacoce* species is thought to be transported by insects. There are few data to show correlation between a certain sexine pattern and a pollinator, mainly because detailed observations are lacking. Grayum (1986) gives one of the best-documented examples: he found that among Araceae, beetles pollinate species with a psilate sexine, while species with spinose pollen grains are more often pollinated by flies.

Summary

The pollen morphology is important in the systematics of the Australian species of *Spermacoce*, particularly in defining many of them as distinct from African and American species and also in the clarification of groups of species. It is sometimes useful in distinguishing between morphologically similar species. The morphological variation exhibited by the pollen is poorly understood in relation to functional adaptation, primarily because little is known about pollination of the group.

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