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The pollen morphology of the Catesbaeeae-Chiococceae-*Exostema* complex as recently treated by Delprete (1966) was examined with LM and SEM. The group is remarkably stenopalynous; typical representatives have medium sized, 3-colpate pollen with a perforate tectum covered with microspines. The inner nexine ornamentation is pronounced and offers more variation than the sexine pattern. A typology of the inside structures is presented based on LM observations and SEM observations of sectioned grains.

Orbicules are common and numerous in the Catesbaeeae and *Exostema*-group; for most genera of the Chiococceae confirmation is needed of orbicule presence. All orbicules observed are relatively large (1–4 μm) and spiny.

Pollen and orbicule morphology proved to be a powerful tool to delimit the Catesbaeeae-Chiococceae-*Exostema* complex. The overall delimitation of the complex is corroborated with our pollen data. The genera *Mastixiodendron* and *Placocarpa*, however, can be excluded from the complex based on their pollen morphology. *Mastixiodendron* has 3-colporate, perforate pollen without microspines and the endocolpi are fused into an endocingulum. Pollen of *Placocarpa* is reticulate and 3-colporate with perpendicular endocolpi.

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The systematic value of pollen morphological characters in the mostly tropical family Rubiaceae has been convincingly demonstrated (Robbrecht 1988 for general information, and inter alia Johansson 1987, Andersson 1993, Persson 1993, Huysmans et al. 1998, Delprete 1999*a*). Pollen data are now commonly incorporated in cladistic analyses designed to investigate the phylogenetic relationships within Rubiaceae (Rova & Andersson 1995, Andersson 1996, Andreasen & Bremer 1996). Unfortunately, phylogenetic studies are rarely complemented by a careful palynological investigation. The characters used are often 'classics' taken from the literature (as number of apertures, pollen type, sexine pattern), and not necessarily those that show a cladistically useful variation within the study group. The fact that most Rubiaceae pollen have interesting and systematically significant patterns at the inner nexine was first recognised by Van Campo (1978: 306): "...chez les Rubiaceae, les endosculptures sont particulièrement nettes, même en Mph dans la région aperturale où elles peuvent d'ailleurs fournir des caractères de diagnose." Lobreau-Callen (1978) discussed the complex structure of the mostly compound apertures in Rubiaceae. Since these works, the variation and taxonomic value of nexine ornamentation as well as endoapertures have been assessed for several tribes: Anthospermeae and Paederieae (Robbrecht 1982, 1985), Gardenieae (Keddam-Malplanche 1985), Coptosapelteae (Huysmans et al. 1993), Psychotrieae (Jansen et al. 1996),

Isertieae (Bosser & Lobreau-Callen 1998). In the palynological study of Huysmans et al. (1998) of the tribe Isertieae nexine characters were included in the cladistic analysis.

Many Rubiaceae groups are palynologically poorly known; one of them is the group dealt with in the present paper. In the traditional tribal concepts used in the Rubiaceae (Hooker 1873, Schumann 1891, Bremekamp 1952, 1966; Verdcourt 1958, Robbrecht 1988) the core of the genera studied here were placed in the Condamineeae, a tribe with many-seeded capsular fruits. Molecular data (Bremer & Jansen 1991, Bremer 1992) indicated that a part of these genera (Condamineeae subtribe Portlandiinae) are related to the Chiococceae, with fleshy fruits and one-seeded locules. This relationship was supported by floral features, among them filaments fused into a tube adnate to the base of the corolla-tube (but several genera of the Portlandiinae have free filaments attached at the base of the corolla and some genera of Chiococceae have filaments inserted on top of the ovary). Bremer (1992), based on the results of the analysis, proposed a wide concept for the Chiococceae, including the Portlandiinae.

Delprete (1996) re-evaluated the two tribes by means of cladistic analyses using morphological features. One pollen character only (pollen exine in surface view) was included in his data matrix. He concluded that the Catesbaeeae (an uncertain tribe in Robbrecht's classifications of the Rubiaceae in: 1988, 1993*b*) belong to the same alliance. He

reduced the Condamineeae sensu stricto to the Rondeletieae and included its subtribe Portlandiinae in the Catesbaeeae, wherein two informal groups were recognized (*Catesbaea*-group and *Portlandia*-group). The results of the analysis did not support the wide delimitation of the Chiococceae by Bremer. *Exostema*, transferred from Cinchoneae to Chiococceae sensu lato in the above cited works of Bremer, was treated by Delprete as an informal group closely related to these two tribes. In the '*Exostema*-group', Delprete also included *Badusa* and *Morierina*. This entire alliance is the subject of the present paper and is here called the Catesbaeeae-Chiococceae-*Exostema* complex, further abbreviated as the CCE-complex. It is interesting to note that in Delprete's study (1996), in the two cladistic analyses with *Joosia* and *Cinchona* as outgroup, the synapomorphic character that supported the CCE-complex as a monophyletic group was echinate pollen.

In this study we also included *Molopanthera*, because it was considered as a member of the *Portlandia*-group sensu lato by Robbrecht (1993*b*). Delprete (1996), however, excluded it from the Catesbaeeae on the basis of flower structure, dorsal anther attachment, and peltate seeds with fringed margins.

For a more extensive review of the CCE-complex, morphological descriptions of tribes and groups, and genera included, we refer to and follow Delprete (1996).

Pollen of few genera of the study group was investigated previously by Darwin (1977; *Mastixiodendron*-SEM) and Aiello (1979; *Portlandia* and *Isidorea*-SEM, related genera only descriptive). McDowell (1996) illustrated his treatment on the taxonomy of *Exostema* with SEM micrographs of pollen of two species. One SEM micrograph of *Coutarea* pollen was published by Rova & Andersson (1995). General palynological studies were performed by Delprete in order to code the sexine ornamentation for his phylogenetic analysis, but the SEM micrographs were not published.

In the present paper we aim to give an account of the pollen morphology in the CCE-complex, using LM and SEM, focusing primarily on the nexine ornamentation and secondarily on the tapetal orbicules. The systematic value of pollen characters in the circumscription of this complex, together with the intergeneric variation was evaluated.

MATERIAL AND METHODS

This study is based on 45 specimens from 33 species of 20 genera. Observations were made on dried material from the following herbaria: BM, BR, L, S, and US (see appendix Specimens Investigated). For details on treatment and equipment used, see Huysmans et al. (1998). Because the pollen apertures of certain taxa appeared damaged after standard chemical treatment, acetolysis at room temperature during four and six minutes was tried on pollen of *Bikkia retusiflora* in order to evaluate the effect of acetolysis compared to non-acetolysed grains. The damage to the pollen aperture increased proportionally with the increasing length of the treatment. All observations and illustrations are of standard acetolysed grains unless stated otherwise.

The ornamentation of the inner surface of the pollen exine was studied systematically with LM. Acetolysed pollen of selected specimens was cut with a freezing microtome (Ames Lab-Tek

Cryostat) at $-20°C$. The thickness of the sections was adjusted to $\pm$ half of the pollen diameter. Sections were collected with a brush and transferred to a stub.

Length of polar axis (P) and length of equatorial diameter (E) were measured on ten grains in equatorial view with LM using a camera lucida. Other measurements were made on SEM micrographs. Terminology follows Punt et al. (1994). Shape classes in equatorial view are according to Nilsson & Praglowski (1992: 40).

We have used the term 'nexine ornamentation' for structures that partly may also be called 'endoapertures'. According to the glossary of Punt et al. (1994), an endoaperture is "an aperture in the inner layer of the sporoderm, often the inner aperture of a compound aperture", and an aperture is defined as "a specialized region of the sporoderm, that is thinner than the remainder of the sporoderm and generally differs in ornamentation and/or structure". To enable descriptions of the complete inside ornamentation of the pollen grains, we have chosen the more general term 'nexine ornamentation' instead of 'endoapertures'.

RESULTS

General pollen descriptions

The Catesbaeeae-Chiococceae-*Exostema* complex is a remarkably stenopalynous group. The pollen morphological variation within the complex is very limited at first external sight, and its pollen is also highly characteristic and differs from other Rubiaceae. Palynological descriptions are given for the tribes, and detailed information on generic level and reference to figures are presented in Table I. The heading x/y (studied/total number of genera) informs on the relative number of genera studied for each group. Nexine ornamentation and orbicules are described separately.

Catesbaeeae (Catesbaea-group: 1/3; Portlandia-group: 7/12; Figs. 1–12)

The pollen grains of members of Catesbaeeae studied were always radially symmetrical, isopolar and medium-sized (P 17–48 μm, E 20–50 μm). Grains were 3-colpate with spiny colpus membranes. If an opening in the middle of the colpus membrane was present, it was a probable artefact caused by too aggressive acetolysis. All species investigated had a perforate tectum with microspines.

In *Catesbaea*, the only genus investigated of the "*Catesbaea*-group" (Figs. 1–4), four out of five species studied show a tectum densely beset with more or less blunt and short spines; the minute perforations in the tectum were much less numerous than the echinate processes. Pollen of *Catesbaea spinosa* (Figs. 1, 2), however, is easy recognisable by fewer, longer spines, and irregularly spaced perforations with a protruding rim (crateriform perforations).

In the "*Portlandia*-group", two genera differ slightly from the general pollen type: *Bikkia* pollen has a subtriangular amb and relatively short ectocolpi (Fig. 6), and in *Coutarea* pollen the ectocolpus margins are only vaguely defined.

Chiococceae (8/14; Figs. 13–17)

Pollen of Chiococceae was also medium-sized (P 15–29 μm, E 18–30 μm) and 3-colpate with spiny colpus membranes. It had a sexine ornamentation similar to that of the

Table I. *Some variable pollen morphological characters for each genus studied in Catesbaeeae, Chiococceae and Exostema-group.*
Tribe assignment is according to Delprete (1996), CAT.C: Catesbaeeae "Catesbaea-group", CAT.P: Catesbaeeae "Portlandia-group", CHI: Chiococceae, EXO: Exostema-group. RON: Rondeletieae. (x/y): x number of species studied, y total species number. O: oblate, SO: suboblate, S: spheroidal, SP: subprolate, P: prolate. Numbers in nexine-column refer to types in text.

| Tribe	Genus	Size		Shape	Eq. view	Pollen apertures	Wall ornamentation		Fig.
		P (μm)	E (μm)	P/E			Sexine	Nexine (type)	
CAT.C	*Catesbaea* (5/15)	17-(20.9)-27	20-(23.5)-28	0.75-(0.90)-1.00	SO-S	3-colpate	perforate, microechinate	3	1-4, 37, 38
CAT.P	*Bikkia* (3/20)	20-(27.8)-35	24-(28.5)-34	0.74-(0.97)-1.21	O-SO-S-SP	3-colpate	perforate, microechinate	4	5, 6, 40, 41
	Coutaportla (1/3)	26-(28.0)-30	24-(29.0)-33	0.93-(0.97)-1.08	S	3-colpate	perforate, microechinate	4	9, 10, 28
	Coutarea (1/7)	32-(37.9)-48	30-(36.9)-50	0.93-(1.03)-1.17	S-SP	3-colpate	perforate, microechinate	2	11, 12
	Hintonia (3/4)	21-(25.5)-30	23-(28.0)-32	0.81-(0.91)-1.0	SO-S	3-colpate	perforate, microechinate	1+2	7, 8, 35
	Isidorea (3/12)	18-(22.9)-28	22-(26.7)-33	0.74-(0.87)-1.05	O-SO-S	3-colpate	perforate, microechinate	2	36
	Nernstia (1/1)	20-(20.7)-22	25-(25.4)-26	0.80-(0.81)-0.88	SO-S	3-colpate	perforate, microechinate	1	
	Portlandia (1/5)	29-(30.0)-33	26-(28.9)-32	1.00-(1.04)-1.18	S-SP	3-colpate	perforate, microechinate	2	
CHI	*Asemnantha* (1/1)	24-(26.0)-29	23-(25.7)-28	0.93-(1.01)-1.12	S	3-colpate	perforate, microechinate	2	
	Ceratopyxis (1/1)	23-(24.6)-27	25-(26.3)-28	0.88-(0.94)-0.96	S	3-colpate	perforate, microechinate	1	31, 32
	Chiococca (2/20)	18-(22.4)-27	20-(24.9)-30	0.85-(0.90)-1.00	SO-S	3-colpate	perforate, microechinate	1+2	13, 14, 26
	Erithalis (2/10)	18-(20.0)-23	22-(25.3)-30	0.70-(0.80)-0.88	O-SO-S	3-colpate	perforate, microechinate	2	15
	Placocarpa (1/1)	20-(22.0)-24	25-(26.0)-27	0.80-(0.85)-0.92	SO-S	3-colporate	reticulate	5	16, 29, 43
	Salzmannia (1/1)	15-(16.3)-18	18-(19.0)-21	0.80-(0.86)-0.90	SO-S	3-colpate	perforate, microechinate	3	
	Scolosanthus (1/11)	18-(19.4)-20	22-(23.3)-24	0.78-(0.83)-0.87	SO	3-colpate	perforate, microechinate	2	
CHI?	*Mastixiodendron* (2/7)	20-(25.5)-30	24-(28.3)-33	0.79-(0.90)-1.04	SO-S	3-colporate	perforate	6	17, 30, 42
EXO	*Badusa* (1/3)	26-(26.9)-29	23-(24.6)-26	1.04-(1.06)-1.13	S	3-colpate	perforate, microechinate	1	21, 22, 25, 33, 34
	Exostema (2/40)	33-(36.8)-40	30-(35.4)-39	1.00-(1.04)-1.10	S	3-(4)-colpate	perforate, microechinate	1+3	18, 27
	Morierina (1/2)	24-(25.4)-27	18-(20.4)-22	1.19-(1.25)-1.33	SP-P	3-colpate	perforate, microechinate	3	19, 20, 39
RON	*Molopanthera* (1/1)	14-(14.7)-16	14-(15.6)-17	0.88-(0.95)-1.00	S	3-colporate	microreticulate, no supratectal processes	7	23, 24
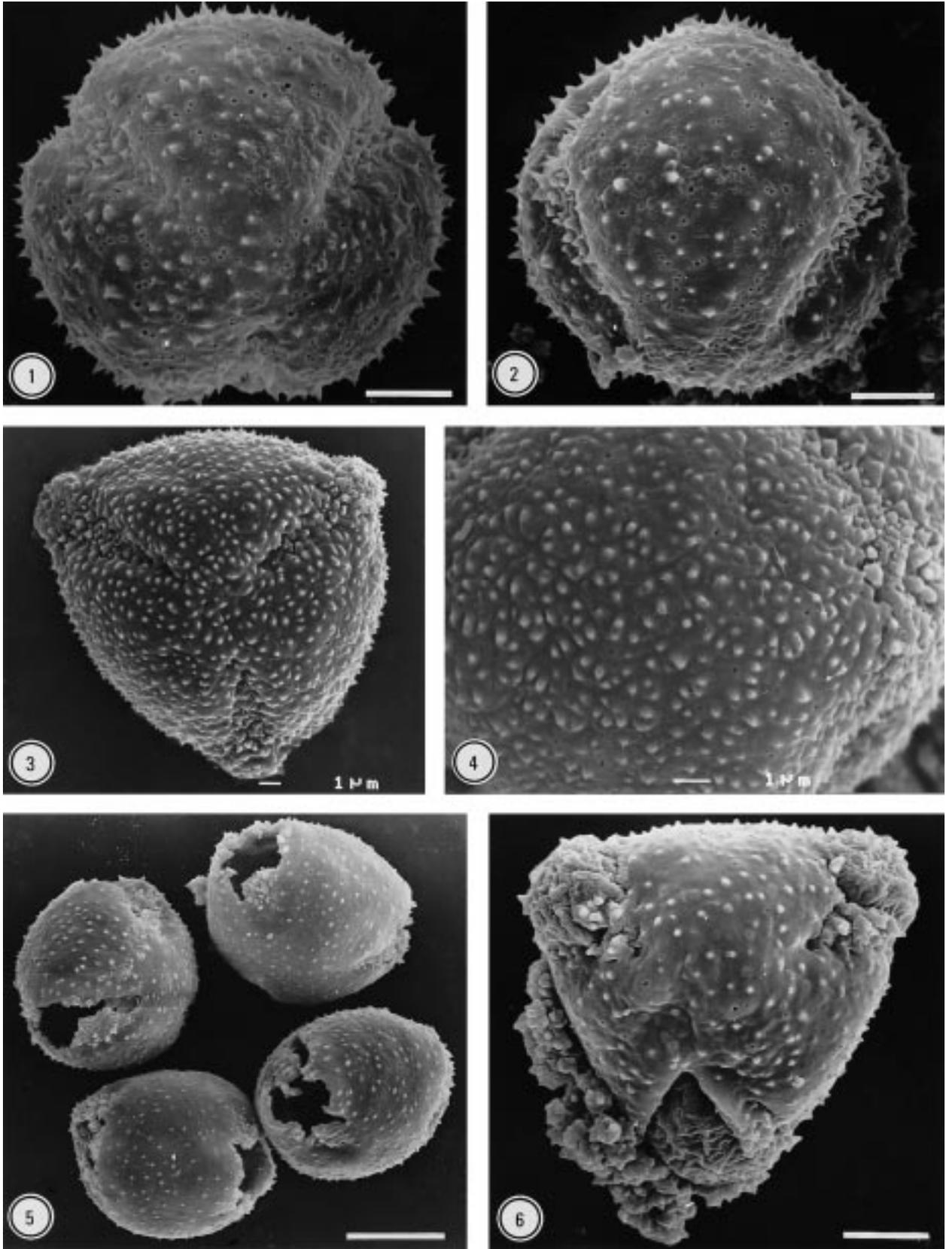
Figs. 1–6. Pollen grains of Catesbaeeae (SEM). 1–2. *Catesbaea spinosa*. (1) Polar view of tricolpate grain; (2) Mesocolpial view, note the spiny colpus membranes. 3–4. *Catesbaea glabra*. (3) Polar view of subtriangular, tricolpate grain; (4) Detail of tectum in mesocolpium with small perforations and short, blunt microspines. 5–6. *Bikkia retusiflora*. (5) Damaged apertures on standard acetolysed grains; (6) Grain of same specimen after 4 min. acetolysis on room temperature, intact apertures and spiny orbicules on its surface at the left. Scale bars: 5 μm in Figs. 1, 2 & 6; 10 μm in Fig. 5.
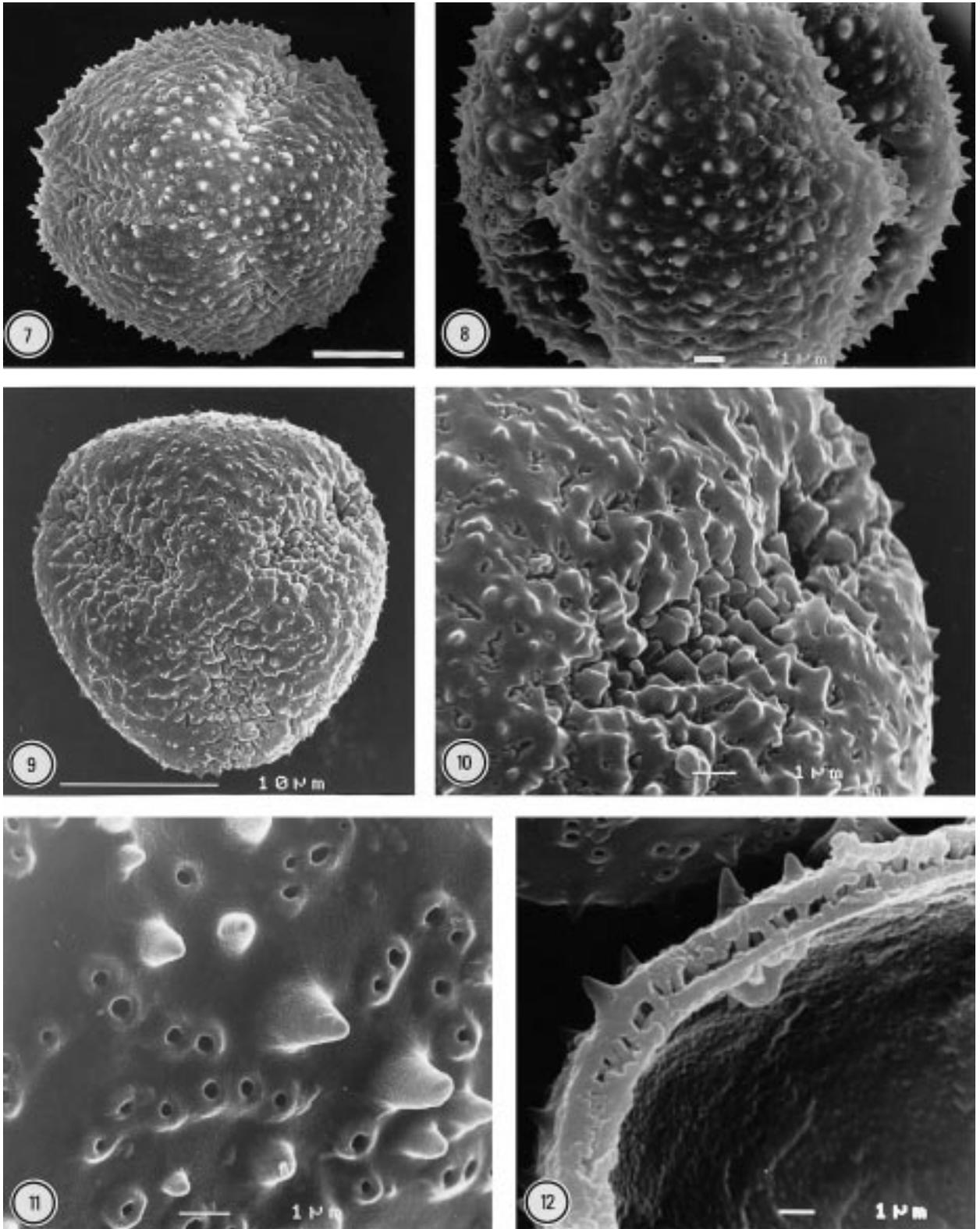
Figs. 7–12. Pollen grains of Catesbaeeae (SEM). 7–8. *Hintonia latiflora.* (7) Polar view of tricolpate grain. Scale bar: 5 μm; (8) Meso-colpial view, note crateriform perforations in tectum and microspines. 9–10. *Coutaportla guatemalensis.* (9) Polar view, three colpi rather vaguely delimited; (10) Detail of colpus in polar view, colpus membrane spiny, tectum with irregular, elongated perforations and microspines. 11–12. *Coutarea hexandra.* (11) Detail of tectum on pole with crateriform perforations and microspines; (12) Cross section through exine on pole with well developed columellae layer.
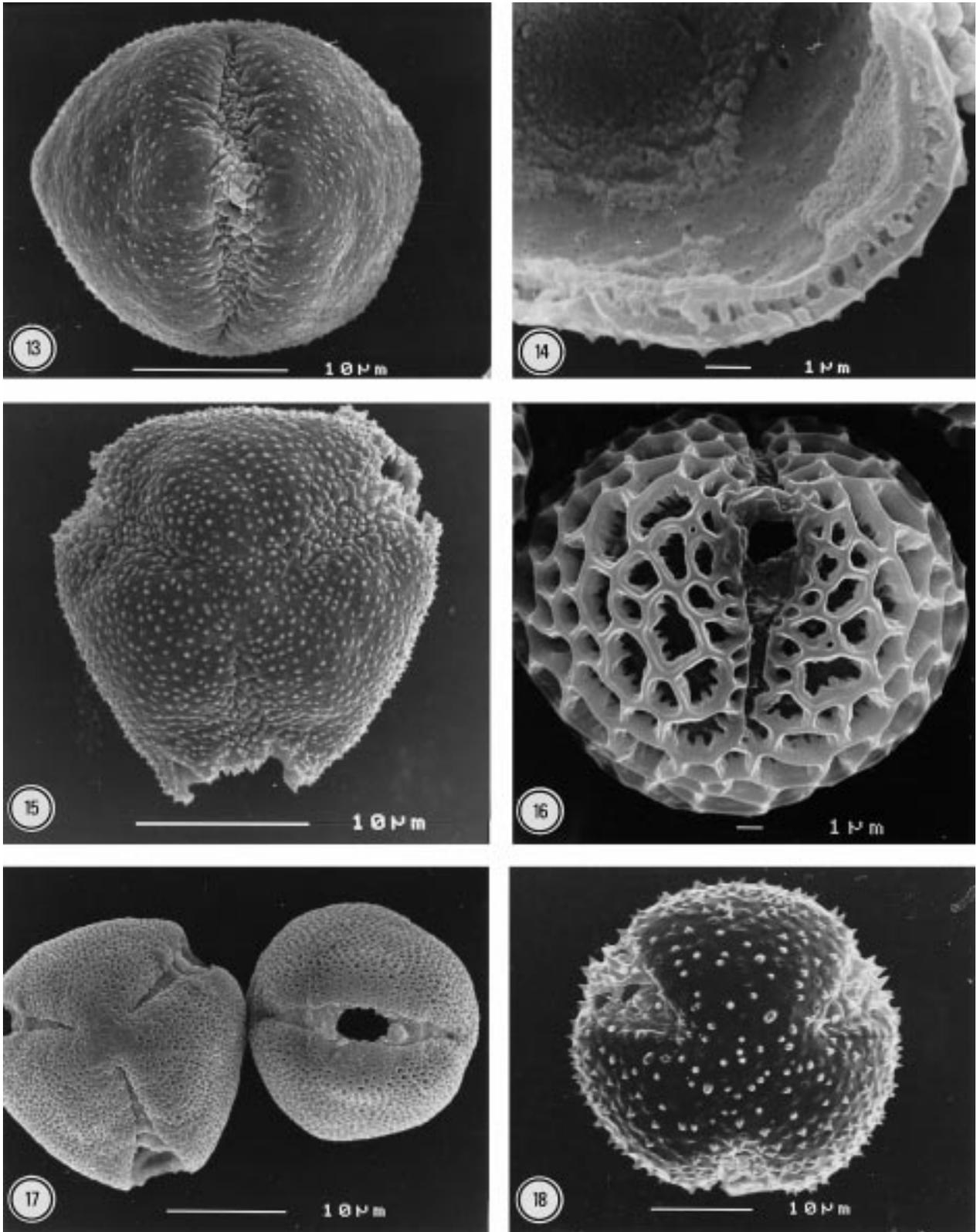
Figs. 13–18. Pollen grains of Chiococceae and *Exostema* (SEM). 13–14. *Chiococca phaenostemon.* (13) Equatorial view of colpus with spiny colpus membrane; (14) Cross section through exine, note inside ornamentation with granular patches on a smooth, perforated layer. 15. *Erithalis odorifera.* Polar view of tricolpate grain with damaged apertures by acetolysis. 16. *Placocarpa mexicana.* Equatorial view of aperture (colporate), reticulate tectum with double muri. 17. *Mastixiodendron pachyclados* var. *tomentosum.* Polar and equatorial view of perforate and tricolporate grains. 18. *Exostema elegans.* Polar view of tricolpate grain with spiny and perforate sexine.
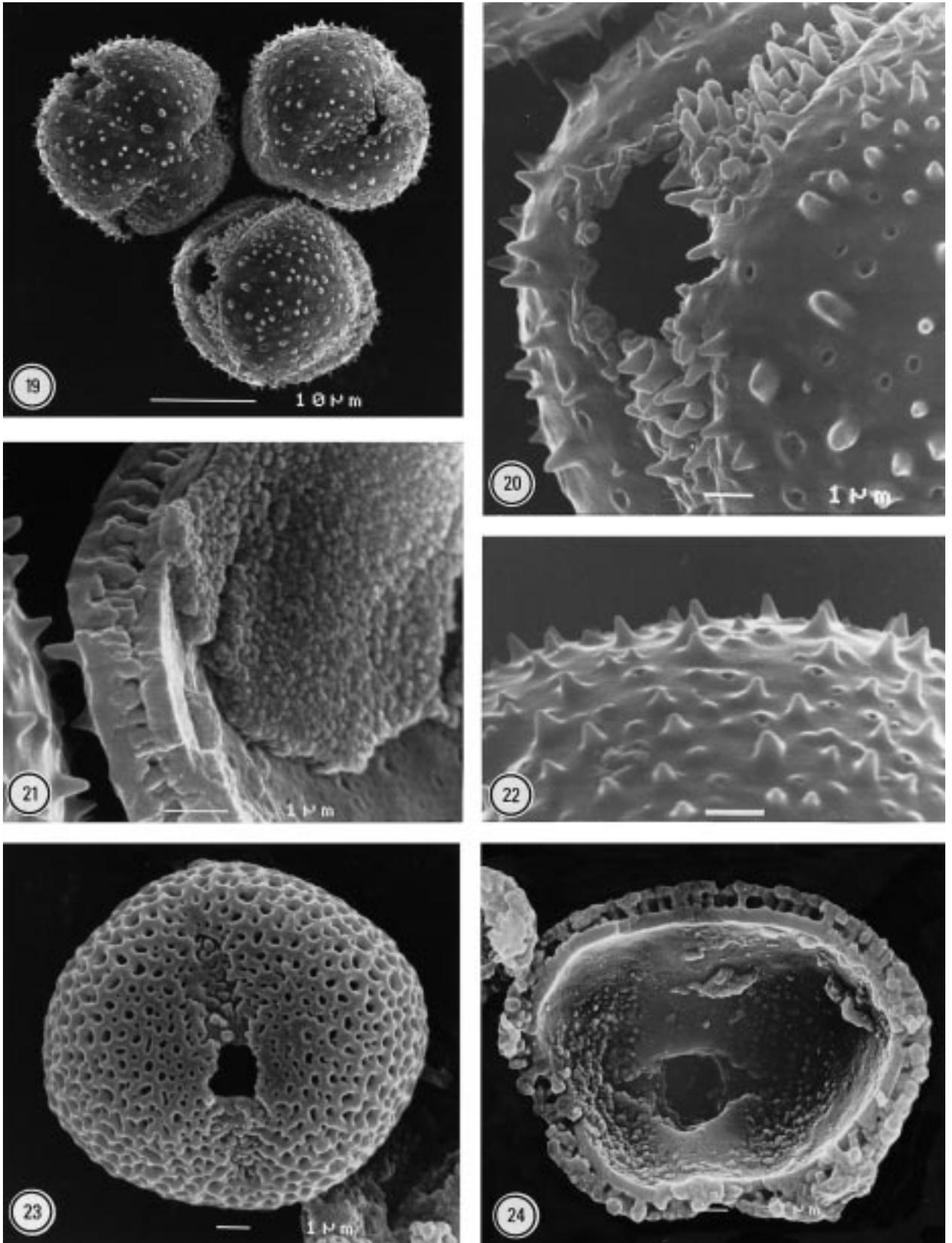
Figs. 19–24. Pollen grains of *Exostema*-group and *Molopanthera* (SEM). 19–20. *Morierina montana*. (19) Three grains in different orientations, apertures damaged by acetolysis; (20) Detail of aperture with spiny colpus membrane. 21–22. *Badusa corymbifera*. (21) Cross section through exine and view on nexine ornamentation with granular layer on top of smooth, perforated layer; (22) Detail of spiny tectum on pole, note crateriform perforations. Scale bar: 1 μm. 23–24. *Molopanthera paniculata*. (23) Equatorial view of aperture (colporate) with fragments of sexine on colpus membrane, tectum perforatum without supratectal processes; (24) Inside ornamentation, short endocolpus and kidney-shaped granular fields in mesocolpia (Type 7).

Catesbaeeae, that is perforate and microechinate. Nevertheless, two of the genera of this group deviate considerably from this general description both in external as internal pollen characters:

– *Placocarpa* (Figs. 16, 43) has reticulate pollen grains with muri bordered by granules and supported by long columellae; the three ectoapertures are narrow colpi, and in the granular nexine endocolpi with small extensions are situated.
– *Mastixiodendron* (Figs. 17, 42), which was only tentatively included in the tribe (Delprete 1996), has pollen grains with a tectum perforatum without any supratectal elements. The three compound apertures are composed of a narrow acute ectocolpus, a large lolongate mesoporus surrounded by an annulus, and an endocingulum with small, acute extensions.

Exostema-group (3/3; Figs. 18 – 23)

Pollen of this group is very similar to those of Catesbaeeae and most of the Chiococceae in having 3-colpate pollen with spiny colpus membranes and with a perforate and microechinate sexine (P 24 – 40 μm, E 18 – 31 μm).

Molopanthera (Figs. 23, 24)

Pollen morphology of this genus provides further evidence to justify its transfer from the *Portlandia*-group sensu Robbrecht (1993*b*) to the Rondeletieae (Delprete 1996, 1999*b*): the 3-colporate pollen grains are microreticulate without any supratectal processes. The mesoporus is as broad as the ectocolpus and has slightly protruding margins. Pollen grains are also smaller (14 – 17 μm) than the average within the study group and have characteristic H-shaped endostructures (Fig. 18).

Nexine ornamentation

Extra attention was paid to the morphological structures at the inside of the pollen grains. Their variation was studied systematically by LM (Figs. 31 – 43) and by additional SEM observations of freeze sectioned pollen grains (Figs. 24 – 30). The endopatterns were often very distinct at LM, but less so in e.g. *Catesbaea* and *Coutarea*.

Ornamentation types

Seven types of nexine ornamentation were identified for all species studied. Four types were recognized in the CCE-complex sensu stricto (excl. *Placocarpa*, *Mastixiodendron* and *Molopanthera*).

(1) *"Endocingulum" with extensions fused at poles (Figs. 25, 31 – 34).* – This type is the most pronounced one in the group. In the granular inner layer, wide and straight cut-aways occur in a fairly constant pattern. This pattern can be described as an endocingulum with a single, broad extension in each mesocolpium. These extensions meet at the poles and form a star-shaped thinning that is well visible at LM. Near each ectoaperture, four smaller extensions are present,

two at each side of the endocingulum, which fuse most commonly with the mesocolpial bands near the poles. In the cut-aways a smooth, perforated layer becomes visible (in *Coutarea* small granules occur). In the star-shaped thinning at the poles, small patches of the granular layer may be present (e.g. *Ceratopyxis*, Fig. 31).

Observed in: Catesbaeeae "*Portlandia*-group" (*Hintonia latiflora*, *Nernstia*);

Chiococceae (*Ceratopyxis*, *Chiococca nitida*); *Exostema*-group (*Badusa*, *Exostema caribaeum*).

(2) *Endocolpi with diverging ends, star at poles and endocracks in mesocolpium (Figs. 26, 35 – 36).* – There are three endocolpi perpendicular to the ectocolpi, with diverging, often branching ends. On the latitudinal sides of the endocolpi band-like cut-aways in the granular layer occur; these bands fuse at the poles appearing at the LM as a star-shaped thinning (Fig. 36). In the mesocolpia, short endocracks are situated at random. Where the granular inner layer is missing, a smooth and perforated layer is visible.

Observed in: Catesbaeeae "*Portlandia*-group" (*Coutarea*, *Isidorea*, *Hintonia standleyana* and *H. octomera*, *Portlandia*); Chiococceae (*Asemnantha*, *Chiococca phaenostemon*, *Erithalis*, *Scolosanthus*)

(3) *Endocolpi, each with four extensions (Figs. 27, 37 – 39).* – Three endocolpi occur perpendicular to the ectocolpi and each with four acute extensions, two at each side pointing towards the poles. The extensions do not fuse at the poles, so there is no star-shaped thinning at the poles. The structure as seen in equatorial view often resembles an animal hide with four strong paws and a small head and tail. In *Salzmannia*, however, we observed endocolpi with diverging ends but without the four clear extensions pointing towards the poles. Endocracks occur in the mesocolpia. The cut-aways in the granular layer show a coarse lower layer without perforations.

Observed in: Catesbaeeae "*Catesbaea*-group" (*Catesbaea*); Chiococceae (*Salzmannia*); *Exostema*-group (*Exostema elegans*, *Morierina*).

Although all species investigated of *Catesbaea* were classified under type 3, the nexine ornamentation is quite variable even within a single specimen. 'Animal hide'-like endoapertures (type 3) are obviously most common but endocolpi with acute or diverging ends occur as well, mostly in combination with endocracks in the mesocolpia and at the poles. A clear star-shaped thinning at the poles as in our type 1 and 2 is lacking.

(4) *Endocracks (Figs. 28, 40 – 41).* – All over the inside of the grain, irregular endocracks occur in the granular layer, revealing a perforated lower layer. At LM no star-shaped structures were seen at the poles. A zone without endocracks below the ectocolpi is often present, which gives the impression of costae ectocolpi at LM. In a revision of *Bikkia* of New Caledonia, Jérémie & Hallé (1976) mentioned the occurrence of endocracks in their concise pollen descriptions (not illustrated). The endocracks can form an endoreticulation, especially in *Bikkia*.

Observed in: Catesbaeeae "*Portlandia*-group" (*Bikkia*, *Coutaportla*).

The three remaining types were each observed in one
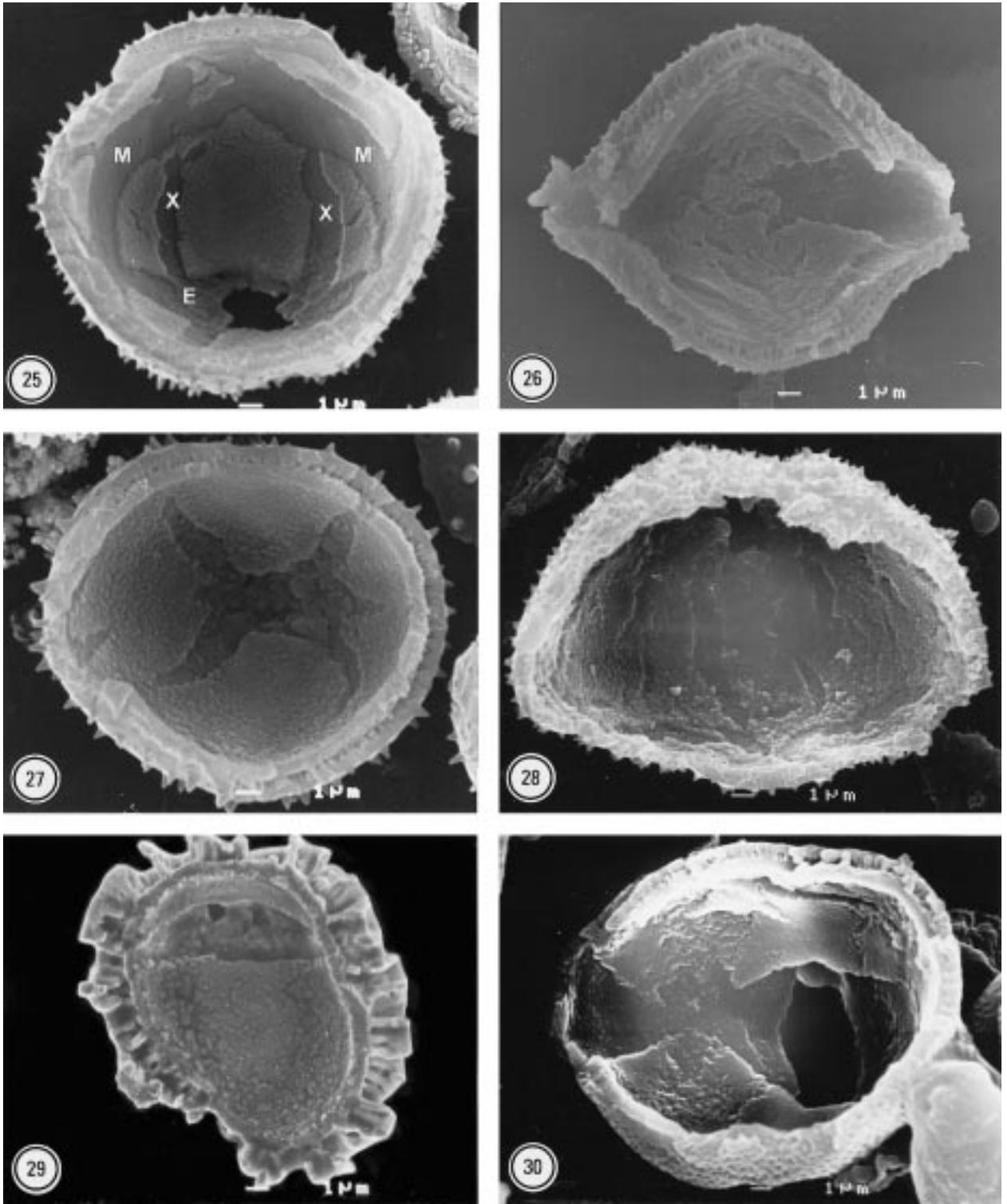
Figs. 25–30. Types of inside ornamentation in CCE-complex as delimited in text (SEM). (25) Type 1 in *Badusa corymbifera*, E = endo-cingulum, X = extension, M = mesocolpial band. (26) Type 2 in *Chiococca phaenostemon*. (27) Type 3 in *Exostema elegans*. (28) Type 4 in *Coutaportla guatemalensis*. Note that poles of grain are at left and right side. (29) Type 5 in *Placocarpa mexicana*. (30) Type 6 in *Mastixiodendron pachyclados* var. *tomentosum*.

genus only. The external pollen morphology of the genera concerned, indicated already that they do not fit within the main pollen type of the CCE-complex (see above).

(5) *Endocolpus with short extremities (Figs. 29, 43)*. – Endocolpi occur in the granular inner layer, perpendicular to the ectocolpi. Short extremities are common on their

Figs. 31–43. Types of inside ornamentation in CCE-complex (LM). All pictures are taken with an oil immersion lens (100x) and relative proportions were respected. 31–34. Type 1. 31–32. *Ceratopyxis verbenacea*. (31) Polar view showing fusion at pole of three mesocolpial extensions of endocingulum with triangular patch of granular inner wall layer in centre; (32) Equatorial view on mesocolpium showing endocingulum with two extensions running perpendicular toward poles. Note small patch of granular inner layer where mesocolpial extensions meet the endocingulum. 33–34. *Badusa corymbifera*. (33) Polar view showing fusion at pole of three mesocolpial extensions of endocingulum; (34) Two pollen grains showing endostructures beneath the ectocolpus at different angles. In grain to the

sides. No perforations were observed in the lower wall layer that is revealed by the endocolpi.

Observed in: Chiococceae (*Placocarpa*).

(6) *Endocingulum (Figs. 30, 42)*. – In one genus a broad endocingulum occurs in the almost smooth to granular inner part of the nexine. In the mesocolpia the borders of the endocingulum are rarely parallel and straight. It indeed appears as three endocolpi with diverging ends are fused on the equator. No perforations occur in the lower wall layer.

Observed in: ?Chiococceae (*Mastixiodendron*)

(7) *Short endocolpus and granular fields in mesocolpia (Fig. 24)*. – Very short endocolpi with acute ends occur under the mesopori. At both ends of the endocolpi, kidney-shaped granular fields are found parallel with the polar axis (only visible at SEM). The granules are lying on top of the exceptionally smooth inner nexine.

Observed in: Rondeletieae (*Molopanthera*)

Orbicules

No special preparations for observation of orbicules were made for this study; however, orbicules were often abundant, so that they were observed between the pollen grains and were observed with LM and SEM. Orbicules were observed in all genera studied of the Catesbaeeae; for *Nernstia* and *Portlandia* confirmation of orbicule presence is desirable. For the genera studied of the Chiococceae, orbicules were observed only in *Salzmannia*. In the *Exostema*-group orbicules were numerous in all taxa investigated.

Orbicules in the study group are relatively large (1–4 μm); they lie free on the tapetal membrane and are characteristically spiny, often with small perforations in their wall. According to the orbicule typology for the Cinchonoideae (Rubiaceae) presented by Huysmans et al. (1997), the orbicules of the CCE-complex belong to Type I (spiny orbicules). Orbicules of three species, which are relevant here, were illustrated: *Bikkia retusiflora* (their Figs. 4, 31), *Catesbaea glabra* (their Figs. 5, 6), and *Hintonia standleyana* (their Figs. 7, 8).

DISCUSSION

Pollen morphological features

The inner surface of the nexine is hardly ever completely smooth in Rubiaceae (e.g. Van Campo 1978, Keddam-Malplanche 1980, 1985; Huysmans et al. 1993, 1998, Jansen et al. 1996). In the CCE-complex, there is generally a granular inner layer with a smooth, often perforated layer

underneath. TEM-observations are needed to decide if these layers correspond with the endexine and the foot layer respectively (in progress). In an ontogenetic investigation of the pollen wall of *Rondeletia odorata* (Rubiaceae; El-Ghazaly et al. 2000) it is shown that the granular inside of the mature, acetolysed exine most likely corresponds to the "membranous granular layer", a wall layer that is formed proximally to the endexine, prior to intine formation. If this is also true for the CCE-complex, the smooth layer may correspond to the endexine. Our current knowledge of pollen wall ultrastructure in Rubiaceae is unsufficient to draw any conclusions on the nature of the morphologically different layers of the sporoderm. TEM-images of pollen exines were published for few genera only, mainly in systematic papers (Abadie & Keddam-Malplanche 1975, Johansson 1987, Igersheim & Weber 1993, Weber & Igersheim 1994, Endress et al. 1996, Tilney & van Wyk 1997).

El-Ghazali (1990) provided a typology of endoapertures based on a LM study of 350 species of angiosperms. Twenty-four types were recognized, but these are difficult to correlate with the ornamentation patterns described in the present study.

The nexine ornamentation in Rubiaceae is mostly constant at generic level (e.g. Huysmans et al. 1993, 1998; Persson 1993; Delprete 1999 a, b). This seems to be the case in the study group as well, and we believe that the systematic value of these structures is underrated. In the CCE-complex exceptions are limited to *Exostema* where type 1 and 3 co-occur, and to *Hintonia* and *Chiococca* where both type 1 and 2 were found. *Catesbaea* which shows an intrageneric and also an intraspecific variation in nexine ornamentation was mentioned above.

Orbicules

The study group is a good example of the much cited parallelism between pollen and orbicule morphology within the same species (e.g. El-Ghazaly & Jensen 1986, El-Ghazaly 1989, Hesse 1986, Clément & Audran 1993). It is striking that spiny orbicules were only found in species with (micro)echinate pollen (see also Huysmans et al. 1997). For Rubiaceae this means that so far spiny orbicules were only found in the CCE-complex. Because of the strong parallelism in ornamentation between pollen sexine and orbicular wall as observed in this study and their common presence in the CCE-complex, orbicle morphology might offer additional morphological characters to distinguish the CCE-complex as a monophyletic group.

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right, the endocingulum with four smaller extensions can be seen. The grain to the left is slightly tilted toward the pole where fusion of the mesocolpial bands can be seen, the smaller extensions fuse with these mesocolpial bands close to the pole. 35–36. Type 2. (35) *Hintonia standleyana*. Equatorial view on ectocolpus. The endocolpus having diverging, branching ends. (36) *Isidorea pungens*. Polar view showing star-shaped thinning. 37–39. Type 3. (37) *Catesbaea grayi*. Two grains in equatorial view, slightly tilted toward pole. Endocolpi perpendicular to ectocolpi with two out of four extensions visible; (38) *Catesbaea spinosa*. Equatorial view on ectoaperture, showing endocolpus with diverging, branching ends; (39) *Morierina montana*. Equatorial view on ectoaperture, showing endocolpus with acute ends and with four extensions running toward poles. 40–41. Type 4. *Bikkia retusiflora*. (40) Polar view, endocracks in inner granular layer; (41) Endocracks in mesocolpium. 42. *Mastixiodendron plectocarpum*. Endocingulum. 43. *Placocarpa mexicana*. Endocolpus.

Systematic relationships

Several molecular studies within the family Rubiaceae have recently been undertaken, among them those using *trn*L-F (Rova et al. 1997) and *rps*16 (L. Andersson, in progress) sequence data. In addition, Delprete et al. (in progress) started a multidisciplinary study that focuses on the phylogeny of the Catesbaeeae-Chiococceae-*Exostema* complex, using *trn*L-F, ITS1&2, and 5S-NTS sequences, and morphological, anatomical and palynological studies. The molecular phylogenies obtained in the studies above indicated are in strong overall agreement in recognizing the CCE-complex as a monophyletic group.

Pollen morphology proved to be a powerful, additional tool in delimiting the CCE-complex. The characteristic pollen features of the complex that are not found so far in any other group of the Rubiaceae, permit pollen morphology to be a test for any hypothesis on phylogeny and its implementation in a classification. Pollen features apparently represent several synapomorphies supporting the complex as a monophyletic group: simple colpate apertures, perforate and microechinate tectum, and a smooth, often perforated layer under the granular inner nexine. However, a cladistic analysis is necessary to evaluate the pollen characters in this respect.

Our results clearly demonstrate that *Mastixiodendron* and *Placocarpa* could be excluded from the CCE-complex based on their pollen morphology.

The anomalous genus *Mastixiodendron*, originally assigned to Cornaceae (Melchior 1925), has semi-superior ovaries and is probably the only genus of the Rubiaceae with free petals (see Piesschaert et al. 1997: 219). Its tentative placement in the Chiococceae (Darwin 1977) has been accepted by Delprete (1996) without inclusion in the analysis. The genus therefore is in need of reinvestigation. Its 3-colporate pollen with a smooth perforate tectum and an endocingulum fits well into the Rubiaceae but definitely not in the CCE-complex.

The monotypic genus *Placocarpa* was accommodated in the Chiococceae by Robbrecht in 1988, but later (1993*b*) he treated the genus in incertae sedis. Delprete (1996) restored its original position in the Chiococceae. Its reticulate, 3-colporate pollen grains with granules bordering the muri, very narrow ectocolpi and distinct endocolpi suggest a placement near the Hedyotideae.

The pollen morphology of *Molopanthera* provides further evidence to justify the transfer of the genus from the *Portlandia*-group sensu Robbrecht (1993*b*) to the Rondeletieae by Delprete (1996, 1999*b*). It has none of the characteristic pollen features of the complex.

SPECIMENS INVESTIGATED

Specimens of which pollen grains were sectioned with a freezing microtome for SEM observations are indicated by an asterisk.

Catesbaeeae "*Catesbaea*-group"

Catesbaea glabra Urb.: DOMINICAN REPUBLIC, Liogier 11607* (US).
C. grayi Griseb.: CUBA, Wright 371 (BR).
C. melanocarpa Urb.: ANTIGUA, Box 776 (US).
C. parvifolia DC.: HAITI, Leonard & Leonard 13333 (US).
C. spinosa L.: CUBA, Wilson & Brother Leon 11603 (US).

Catesbaeeae "*Portlandia*-group"

Bikkia campanulata Schltr.: NEW CALEDONIA, Fallen, Suprin & Favier 120 (L).
B. grandiflora Reinw. ex Blume: IRIAN JAYA, Versteegh BW4989 (L).
B. retusiflora Schltr.: NEW CALEDONIA, Mac Kee 21703 (US).
Bikkia sp.: NEW CALEDONIA, Deplanche 850 (BR).
Coutaportla guatemalensis (Standl.) Lorence: MEXICO, Alush Méndez 6078* (BR).
Coutarea hexandra (Jacq.) K.Schum.: NICARAGUA, Stevens 3361 (BM); PANAMA, de Nevers, Herrera, Charnley & Díaz 5746 (BM); BRAZIL, Silva 59730 (S), Smith & Klein 14076 (S); BELIZE, Dwyer 10960* (L).
Hintonia latiflora (Sessé & Moç.) Bullock: MEXICO, Hinton et al. 8123 (BR), Hinton et al. 15948* (US), Hinton et al. 15960 (US).
H. octomera (Hemsl.) Bullock: MEXICO, Gaumer 682 (BR).
H. standleyana Bullock: MEXICO, Hinton et al. 7676 (US); GUATEMALA, Contreras 7996 (US).
Isidorea leptantha Urb.: DOMINICAN REPUBLIC, Pater Fuertes 634 (BR).
I. pedicellaris Urb. & Ekm.: HAITI, Ekman 8251* (S).
I. pungens (Lam.) B.L. Robins.: HAITI, Ekman 2413* (S); DOMINICAN REPUBLIC, Pater Fuertes 665 (BR).
Nernstia mexicana (DC.) Urb.: MEXICO, Carranza 737 (BR).
Portlandia gypsophila Macfadyen ex Griseb.: CUBA, Wright 259 (BR)

Chiococceae

Asemnantha pubescens Hook.f.: GUATEMALA, Contreras 1365 (S).
Ceratopyxis verbenacea (Griseb.) Hook.f.: CUBA, Ekman 16553; Shafer 13517* (BM).
Chiococca nitida Benth.: FRENCH GUYANA, Billiet & Jadin 2015 (BR).
C. phaenostemon Schlecht.: COSTA RICA, Wilbur 27233 (BR).
Erithalis fruticosa L.: GUADELOUPE, Billiet & Jadin 5104 (BR).
E. odorifera Jacq.: GUADELOUPE, Billiet & Jadin 5049* (BR)
Placocarpa mexicana Hook.f.: MEXICO, Botteri 913 (BM).
Salzmannia nitida DC.: BRAZIL, Bamps 5062 (BR).
Scolosanthus versicolor Vahl: VIRGIN ISLANDS, St. Thomas, Eggers 191 (BR).
Mastixiodendron pachyclados (K.Schum.) Melch. var. *tomentosum* S. Darwin: IRIAN JAYA, Schram BW1929* (BR).
M. plectocarpum S. Darwin: IRIAN JAYA, Kalkman BW8516 (L).

Exostema-group

Badusa corymbifera A.Gray: TONGA, Yuncker 16090* (BM); *Fiji,* Smith 7438* (L).

Exostema caribaeum (Jacq.) Roem. & Schult.: DOMINICA, Ernst 1561 (BM).

E. elegans Krug & Urb.: DOMINICAN REPUBLIC, Miguel Fuertes 1940* (L).

Morierina montana Vieill.: NEW CALEDONIA, Veillon 4804 (L).

Rondeletieae

Molopanthera paniculata Turcz.: BRAZIL, Loino 75* (US), Williams & Assis 6861 (BR).

REFERENCES

Abadie, M. & Keddam-Malplanche, M. 1975. Etude au microscope électronique à transmission du sporoderme de deux espèces de Rubiaceae. – Societé Botanique Française, Coll. Palynologie 122: 89–91.

Aiello, A. 1979. A reexamination of *Portlandia* (Rubiaceae) and associated taxa. – Journal of the Arnold Arboretum 60: 38–126.

Andersson, L. 1993. Pollen characteristics of the tribes Calycophylleae, Cinchoneae, and Hillieae (Rubiaceae). – Nordic Journal of Botany 13: 405–417.

Andersson, L. 1996. Circumscription of the tribe Isertieae (Rubiaceae) – In: 2nd Int. Rubiaceae Conf., Meise 1995. Proc. (ed. E. Robbrecht, C. Puff & E. Smets), pp. 139–164. – Opera Botanica Belgica 7. Natl.Bot.Gard.Belg., Meise.

Andreasen, K. & Bremer, B. 1996. Phylogeny of the subfamily Ixoroideae (Rubiaceae) – In: 2nd Int. Rubiaceae Conf., Meise 1995. Proc. (ed. E. Robbrecht, C. Puff & E. Smets), pp. 119–138. – Opera Botanica Belgica 7. Natl.Bot.Gard.Belg., Meise.

Bosser, J. & Lobreau-Callen, D. 1998. *Landiopsis* Capuron ex Bosser, genre nouveau de Rubiaceae de Madagascar. – Adansonia 20: 131–137.

Bremekamp, C. E. B. 1952. The African species of *Oldenlandia* L. sensu Hiern et K. Shumann. – Verh. Afd. Nat., Ser. 2. 18. Kon. Ned. Akad. Wet., Amsterdam..

Bremekamp, C. E. B. 1966. Remarks on the position, the delimitation and the subdivision of the Rubiaceae. – Acta Botanica Neerlandica 15: 1–33.

Bremer, B. 1992. Phylogeny of the Rubiaceae (Chiococceae) based on molecular and morphological data. Useful approaches for classification and comparative ecology. – Annals of the Missouri Botanical Garden 79: 380–387.

Bremer, B. & Jansen, R. K. 1991. Comparative restriction site mapping of chloroplast DNA implies new phylogenetic relationships within the Rubiaceae. – American Journal of Botany 78: 198–213.

Clément, C. & Audran, J-C. 1993. Cytochemical and ultrastructural evolution of orbicules in *Lilium*. – Plant Systematics and Evolution, Suppl. 7: 63–74.

Darwin, S. P. 1977. The genus *Mastixiodendron* (Rubiaceae). – Journal of the Arnold Arboretum 58: 349–381.

Delprete, P. G. 1996. Evaluation of the tribes Chiococceae, Condamineeae and Catesbaeeae (Rubiaceae) based on morphological characters – In: 2nd Int. Rubiaceae Conf., Meise 1995. Proc. (ed. E. Robbrecht, C. Puff & E. Smets), pp. 165–192. – Opera Botanica Belgica 7. Natl.Bot.Gard.Belg., Meise.

Delprete, P. G. 1999a. *Riodocea* (Rubiaceae, Gardenieae), a new genus from the Brazilian Atlantic forest. – Brittonia 51: 15–23.

Delprete, P. G. 1999b. Rondeletieae (Rubiaceae), Part I – Flora Neotropica Monographs 77. N.Y. Bot.Gard., Bronx, NY.

Delprete, P. G. & , M. Nee. 1997. The enigmatic genus *Wernhamia* S. Moore is a synonym of *Simira* (Rubiaceae, Rondeletieae). – Brittonia 49: 303–308.

El-Ghazali, G. E. B. 1990. An illustrated key to endoaperture morphology. – Review of Palaeobotany and Palynology 63: 227–231.

El-Ghazaly, G. 1989. Pollen and orbicule morphology of some *Euphorbia* species. – Grana 28: 243–259.

El-Ghazaly, G. & Jensen, W.A. 1986. Studies of the development of wheat (*Triticum aestivum*) pollen. I. Formation of the pollen wall and Ubisch bodies. – Grana 25: 1–29.

El-Ghazaly, G., Huysmans, S. & Smets, E. 2000. Pollen development in *Rondeletia odorata* (Rubiaceae). – American Journal of Botany (In Press).

Endress, M. E., Sennblad, S., Nilsson, L., Civeyrel, M., Chase, S., Huysmans, E., Grafström, B. & Bremer, 1996. A phylogenetic analysis of Apocynaceae s.str. and some related taxa in Gentianales. A multidisciplinary approach – In: 2nd Int. Rubiaceae Conf., Meise 1995. Proc. (ed. E. Robbrecht, C. Puff & E. Smets), pp. 59–102. – Opera Botanica Belgica 7. Natl.Bot.Gard.Belg., Meise.

Hesse, M. 1986. Orbicules and the ektexine are homologous sporopollenin concretions in Spermatophyta. – Plant Systematics and Evolution 153: 37–48.

Hooker, J. D. 1873. Ordo LXXXIV. Rubiaceae – In: Genera plantarum ad exemplaria imprimis in herbariis kewensibus servata defirmata. Vol.2 (ed. G. Bentham & J. D. Hooker), pp. 7–151. – Reeve/Williams & Norgate, London.

Huysmans, S., Robbrecht, E. & Smets, E. 1993. Endoaperture morphology in the Coptosapelteae (Rubiaceae-Cinchonoideae) – In: 2nd Symp. Morph. Anat. & Syst., Salzburg 1993. Kurzfassungen (ed. D. Fürnkranz & H. Schantl), Beitr. 66. – Salzburg University, Salzburg.

Huysmans, S., El-Ghazaly, G., Nilsson, S. & Smets, E. 1997. Systematic value of tapetal orbicules: a preliminary survey of the Cinchonoideae (Rubiaceae). – Canadian Journal of Botany 75: 815–826.

Huysmans, S., Robbrecht, E. & Smets, E. 1998. A collapsed tribe revisited: pollen morphology of the Isertieae (Cinchonoideae-Rubiaceae). – Review of Palaeobotany and Palynology 104: 85–113.

Igersheim, A. & Weber, M. 1993. "Pollen bud" formation in *Ophiorrhiza* (Rubiaceae). An ultrastructural reinvestigation – In: Advances in Rubiaceae Macrosystematics (ed. E. Robbrecht), pp. 51–59. – Opera Botanica Belgica 6. Natl.Bot.Gard.Belg., Meise.

Jansen, S., Robbrecht, E. & Smets, E. 1996. The systematic value of endexine ornamentation in some Psychotrieae pollen (Rubiaceae-Rubioideae). – Grana 35: 129–137.

Jérémie, J. & Hallé, N. 1976. Le genre *Bikkia* (Rubiaceae-Condamineae) en Nouvelle-Calédonie. – Adansonia 2 Sér. 15: 341–355.

Johansson, J. T. 1987. Pollen morphology of the tribe Morindeae (Rubiaceae). – Grana 26: 134–150.

Keddam-Malplanche, M. 1980. Etude palynologique comparative des espèces lianescentes dans les genres *Sherbournia* et *Proterandia* (Rubiacées-Gardéniées). – Adansonia, 2 Sér. 19: 429–434.

Keddam-Malplanche, M. 1985. Le pollen et les stomates des Gardéniées (Rubiacées) du Gabon. Morphologie et tendances évolutives. – Mémoirs de Museum Nationale de Histoire Naturelle, Sér. B, Nouv. Sér. 29: 1–109.

Lobreau-Callen, D. 1978. L'Aperture composée des Rubiaceae. – Annales des Mines de Belgique 2: 167–173.

McDowell, T. 1996. *Exostema* (Rubiaceae): taxonomic history, nomenclature, position and subgeneric classification – In: 2nd Int. Rubiaceae Conf., Meise 1995. Proc. (ed. E. Robbrecht, C. Puff & E. Smets), pp. 277–295. – Opera Botanica Belgica 7. Natl.Bot.Gard.Belg., Meise.

Melchior, H. 1925. Die Cornaceen Papuasiens. – Botanische Jahrbücher 60: 167–174.

Nilsson, S. & Praglowski, J. (Eds.) 1992. Erdtman's handbook of palynology, 2nd ed. – Munksgaard, Copenhagen.

Persson, C. 1993. Pollen morphology of the Gardenieae-Gardeniinae (Rubiaceae). – Nordic Journal of Botany 13: 561–582.

Piesschaert, F., Robbrecht, E. & Smets, E. 1997. *Dialypethalanthus fuscescens* Kuhlm. (Dialypetalanthaceae): the problematic taxonomic position of an Amazonian endemic. – Annals of the Missouri Botanical Garden 84: 201–223.

Punt, W., Blackmore, S., Nilsson, S. & Le Thomas, A. 1994. Glossary of pollen and spore terminology. – LPP Found.-Ser.No.1. LPP Foundation, Utrecht.

Robbrecht, E. 1982. Pollen morphology of the tribes Anthospermeae and Paederieae (Rubiaceae) in relation to taxonomy. – Bulletin van de Nationale Plantentuin van België 52: 349–366.

Robbrecht, E. 1985. Further observations on the pollen morphology of the South African genus *Carpacoce* (Rubiaceae-Anthospermeae). – Review of Palaeobotany and Palynology 45: 361–371.

Robbrecht, E. 1988. Tropical woody Rubiaceae. Characteristic features and progressions. Contributions to a new subfamilial classification. – Opera Botanica Belgica 1. Natl.Bot.Gard.Belg., Meise.

Robbrecht, E. 1993*a* [1994]. Introduction – In: Advances in Rubiaceae Macrosystematics (ed. E. Robbrecht), pp. 7–18. – Opera Botanica Belgica 6. Natl.Bot.Gard.Belg., Meise.

Robbrecht, E. 1993*b* [1994]. Supplement to the 1988 outline of the classification of the Rubiaceae, index to the genera – In: Advances in Rubiaceae Macrosystematics (ed. E. Robbrecht), pp. 173–196. – Opera Botanica Belgica 6. Natl.Bot.Gard.Belg., Meise.

Rova, J. H. E. & Andersson, L. 1995. A reevaluation of the tribes Hippotideae and Tammsieae (Rubiaceae). – Nordic Journal of Botany 15: 269–284.

Rova, J. H. E., Andersson, L., Delprete, P. G. & Albert, V. A. 1997. Macrophylogeny of the Rubiaceae from *trn*L-F sequence data. Abstract of the AIBS meetings, Canada. – American Journal of Botany 84 (Suppl.): 227.

Schumann, K. 1891. Rubiaceae – In: Die natürlichen Pflanzenfamilien. Vol.4, P.4 (ed. A. Engler & K. Prantl), pp. 1–156. – W. Engelmann, Leipzig.

Tilney, P. M. & A. E., van Wyk 1997. Pollen morphology of *Canthium*, *Keetia* and *Psydrax* (Rubiaceae: Vanguerieae) in southern Africa. – Grana 36: 249–260.

Van Campo, M. 1978. La face interne de l'exine. – Review of Palaeobotany and Palynology 26: 301–311.

Verdcourt, B. 1958. Remarks on the classification of the Rubiaceae. – Bulletin van de Nationale Plantentuin van België 28: 210–290.

Weber, M. & A., Igersheim 1994. "Pollen buds" in *Ophiorrhiza* (Rubiaceae) and their role in pollenkitt release. – Botanica Acta 107: 257–262.