

ARE THE GENERA HALLEA AND MITRAGYNA (RUBIACEAE-COPTOSAPELTEAE) POLLEN MORPHOLOGICALLY DISTINCT?

S. HUYSMANS¹, E. ROBBRECHT² & E. SMETS¹

SUMMARY

Recent literature is controversial as regards the segregation of *Hallea* and *Mitragyna*, and pretends that the two genera show pollen morphological differences. In the present study the pollen morphology of all ten species of the complex is described on the basis of light and scanning electron microscopy (including examination of broken grains, which were obtained with a technique never applied in palynology, viz. shaking with glass beads).

The two genera have 3-zonocolporate grains with compound apertures (endoapertures are always H-shaped, sometimes incompletely so). While *Hallea* showed to be stenopalynous (sexine always tectate-perforate), *Mitragyna* is more variable (sexine microreticulate or tectate-perforate), and several of its species have pollen similar to that of *Hallea*.

Numerical analysis was used to evaluate the palynological observations in the light of the macromorphological variation in the complex. It is concluded that both *Hallea* and *Mitragyna* deserve generic recognition, but are not fully distinct pollen morphologically.

INTRODUCTION

Mitragyna (s.l.) is a medium-sized palaeotropical genus occurring in Africa (4 spp.) and Asia (6 spp., from India and Sri Lanka to Vietnam and southwards through the Malay Archipelago to New Guinea); it is absent from Madagascar. *Mitragyna* is one of the rubiaceous genera once transferred to the Naucleaceae, which are characterized by congested, spherical, head-like inflorescences; this family is now generally accepted to be polyphyletic and included in the Rubiaceae in all present-day systems (see Robbrecht, 1993a: 20). *Mitragyna* (s.l.) placed in the subtribe Mitragyninae Havil. was transferred to the tribe Cinchoneae by Ridsdale (1978). Andersson & Persson (1991) emended the old concepts of the tribe Cinchoneae placing the Mitragyninae and some other genera in the tribe Coptosapelteae Bremek. ex Darwin emend L. Andersson & C. Persson. For comments on this delimitation of the Coptosapelteae, see Robbrecht (1993b: 175).

Recently, the genus *Mitragyna* has received considerable attention. Leroy (1975) segregated the genus *Hallea*, including three of the four African species. However, he was not followed by Ridsdale (1978), who made a worldwide revision of the group, inter alia because the variation within the related genus *Uncaria* is greater than the differences between *Hallea* and *Mitragyna* s.s. In 1985 Leroy defended again the

1) Laboratory of Plant Systematics, Katholieke Universiteit Leuven, Kardinaal Mercierlaan 92, B-3001 Heverlee, Belgium.

2) Nationale Plantentuin van België, Domein van Bouchout, B-1860 Meise, Belgium.

generic status of *Hallea*, mainly on tree architectural criteria. A few years later, *Hallea* was adopted in the Rubiaceae instalment in the 'Flora of tropical East Africa' (Verdcourt, 1988: 447), because "the separation of *Hallea* on rather small but constant floral characters is supported by palynology, wood and leaf anatomy, and inflorescence development", and because "there are also some differences in the spectrum of alkaloids." The palynological evidence was based on a light and scanning electron microscopic study by Leroy (1975) of only two species: the African species *Mitragyna inermis* and *Hallea ciliata* (= *H. ledermannii*).

When the first of us decided to undertake a global palynological investigation of the Coptosapelteae (sensu Andersson & Persson, 1991), based on an examination of representative species of all the genera of this tribe, the *Mitragyna/Hallea* problem was considered interesting enough for a profound palynological study including all ten species.

The present paper intends to give a full pollen morphological documentation of the species of *Mitragyna* s.s. and *Hallea*. The taxonomic value of these data is then assessed by a numerical analysis in which palynological data are combined with macro-morphological features.

MATERIAL AND METHODS

The present pollen morphological study is based on herbarium material of all ten species of *Mitragyna* s.l. The collections examined are listed below, with reference to the illustrations. The synonymy given is restricted to names under *Hallea* and *Mitragyna*, except for *Hallea ledermannii*; *H. ledermannii* published by Leroy (1985), a few months before Verdcourt's combination (1985), is not valid because of incomplete basionym reference. For full synonymy, see Ridsdale (1978).

Hallea ledermannii (K. Krause) Verdc. [*Adina ledermannii* K. Krause; *Mitragyna ledermannii* (K. Krause) Ridsd.; *Hallea ciliata* (Aubrév. & Pellegr.) Leroy; *Mitragyna ciliata* Aubrév. & Pellegr.]: Liberia, *Bos* 2645 (BR) (Fig. 1e); Ivory Coast, *Leeuwenberg* 2639 (L) (Figs. 1d, f, g; 6a; 7a).

Hallea rubrostipulata (K. Schum.) Leroy [*Mitragyna rubrostipulata* (K. Schum.) Havil.]: Rwanda, *Bridson* 185 (BR) (Figs. 1a, b; 7b).

Hallea stipulosa (DC.) Leroy [*Mitragyna stipulosa* (DC.) Kuntze]: Zaire, *Breyne* 4660 (BR) (Fig. 1c).

Mitragyna diversifolia (Wall. ex G. Don) Havil.: Thailand, *Maxwell* 85-827 (L) (Figs. 2a-c; 6b).

Mitragyna hirsuta Havil.: Thailand, *Smitinand* 10887 BKF 46243 (L) (Figs. 2d-f; 6d; 7c).

Mitragyna inermis (Willd.) Kuntze: Togo, *Warnecke* 247 (BR) (Figs. 3a-c; 6e; 7d).

Mitragyna parvifolia (Roxb.) Korth.: India, collector unknown (L) (Figs. 3d-f; 6f).

Mitragyna rotundifolia (Roxb.) Kuntze: Thailand, *Maxwell* 88-1145 (L) (Figs. 4a-c; 6g).

Mitragyna speciosa (Korth.) Havil.: Borneo, *Kostermans* 7693 (L) (Figs. 4d-f; 7e).

Mitragyna tubulosa Havil.: South India, *Ridsdale* 110 (L) (Figs. 5a-d; 6c; 7f).

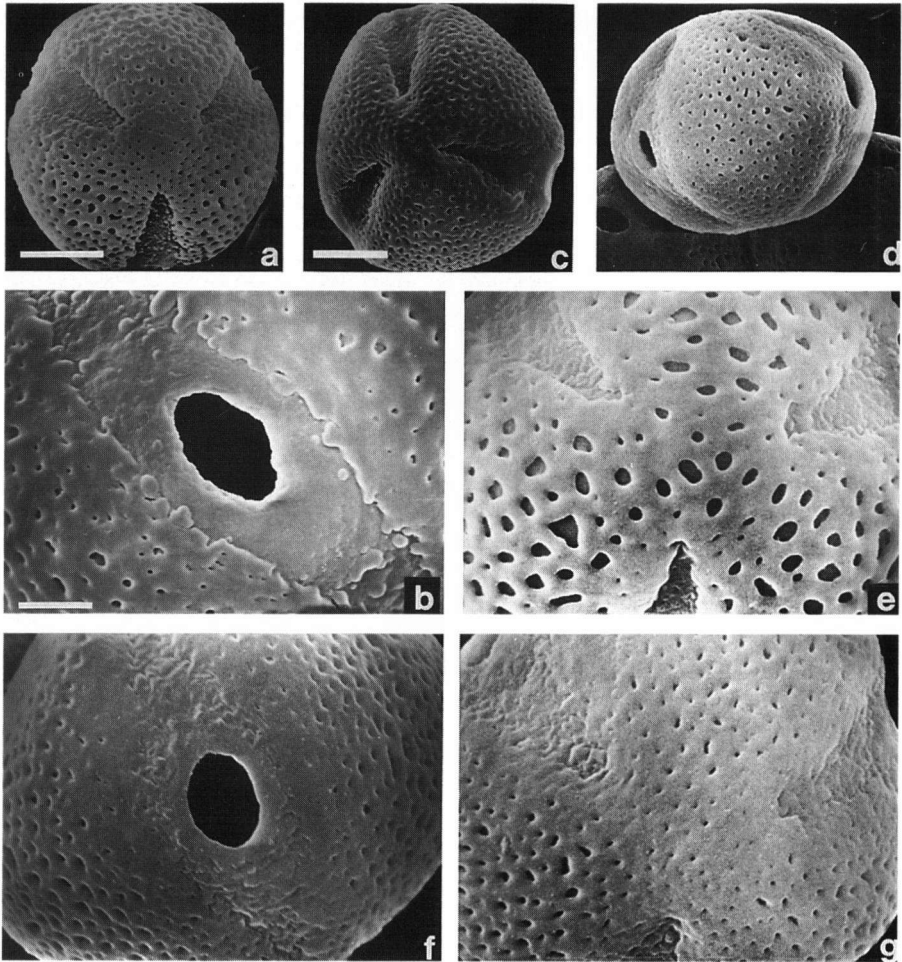


Fig. 1. *Hallea* (a, b: *H. rubrostipulata*; c: *H. stipulosa*; d–g: *H. ledermannii*). — a, c: polar view; b, f: ectocolpus; d: equatorial view; e, g: apocolpium. — Scale bar on a = 5 μm ; scale bar on b = 2 μm , also for e–g; scale bar on c = 5 μm , also for d.

All samples were acetolysed according to Reitsma's (1969) 'wetting agent' method. Pollen descriptions are based on light microscopy (LM) and scanning electron microscopy (SEM). The glycerine jelly slides have been observed with a Leitz Dialux 20. Acetolysed grains for SEM have been suspended in ethanol, air dried on a stub and coated with gold, using a Balzers SCD 020 sputter coater, and observed with a Philips SEM 501.

Measurements of the length of the polar (P) and equatorial axis (E) were made in about ten fully developed grains per specimen under oil immersion at a $\times 1000$ magnification. All other measurements were made on scanning electron micrographs.

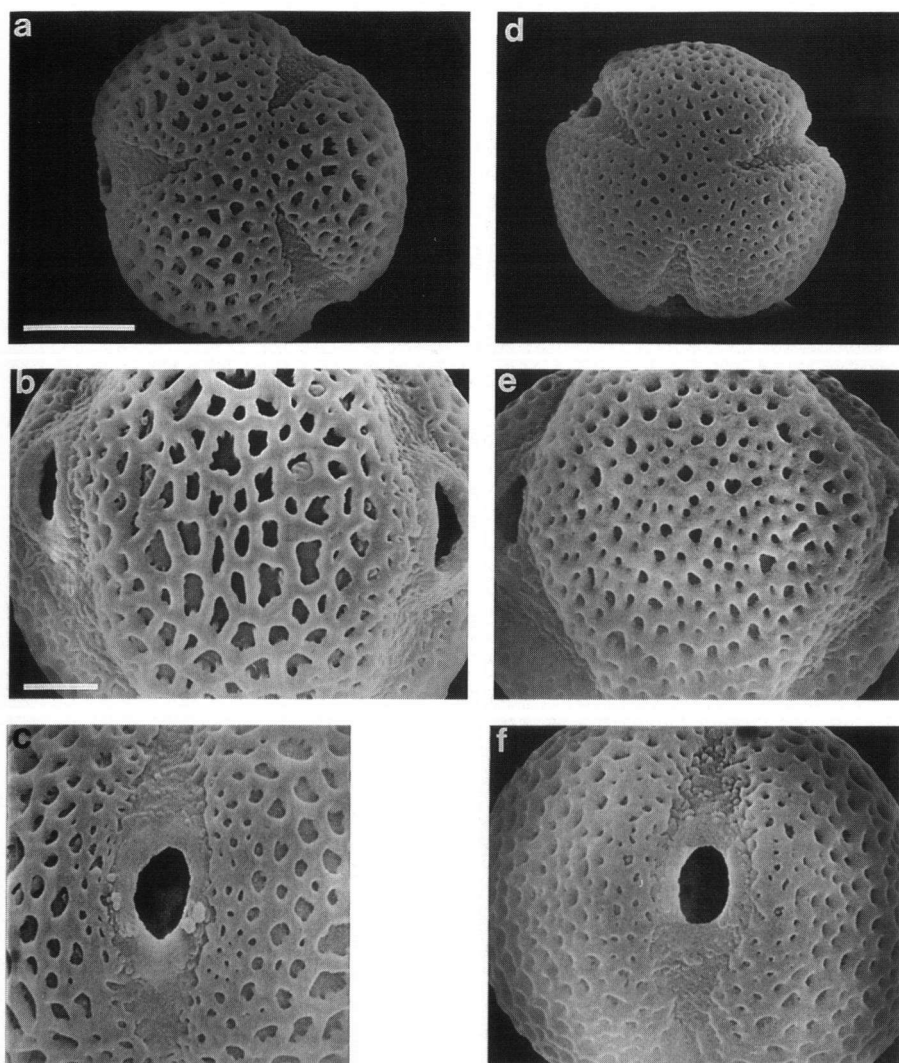


Fig. 2. *Mitragyna* (a–c: *M. diversifolia*; d–f: *M. hirsuta*). — a, d: polar view; b, e: mesocolpium c, f: ectocolpus. — Scale bar on a = 5 μm , also for d; scale bar on b = 2 μm , also for c, e, f.

In our opinion, characters at the inner surface of the exine have, at least in Rubiaceae, a great systematic value. For this reason broken pollen grains of all investigated species were observed with SEM. To obtain broken grains we applied a technique that, as far as we know, was never described in palynological literature, viz. shaking a pollen suspension with glass beads (Huysmans et al., 1993): 0.4 ml pollen suspension in acetone and c. 0.5 ml glass beads (1 mm in diameter) were agitated together in a small test tube by a Vortex; 50 to 70 seconds of shaking was found to be effective.

After checking the number of broken grains with LM, a few drops of the suspension were brought on a stub for SEM observation.

For the palynological terminology we refer to Punt et al. (1994); shape classes in equatorial view are adopted from Erdtman (1971). The interpretation of the apertural system follows Lobreau-Callen (1978).

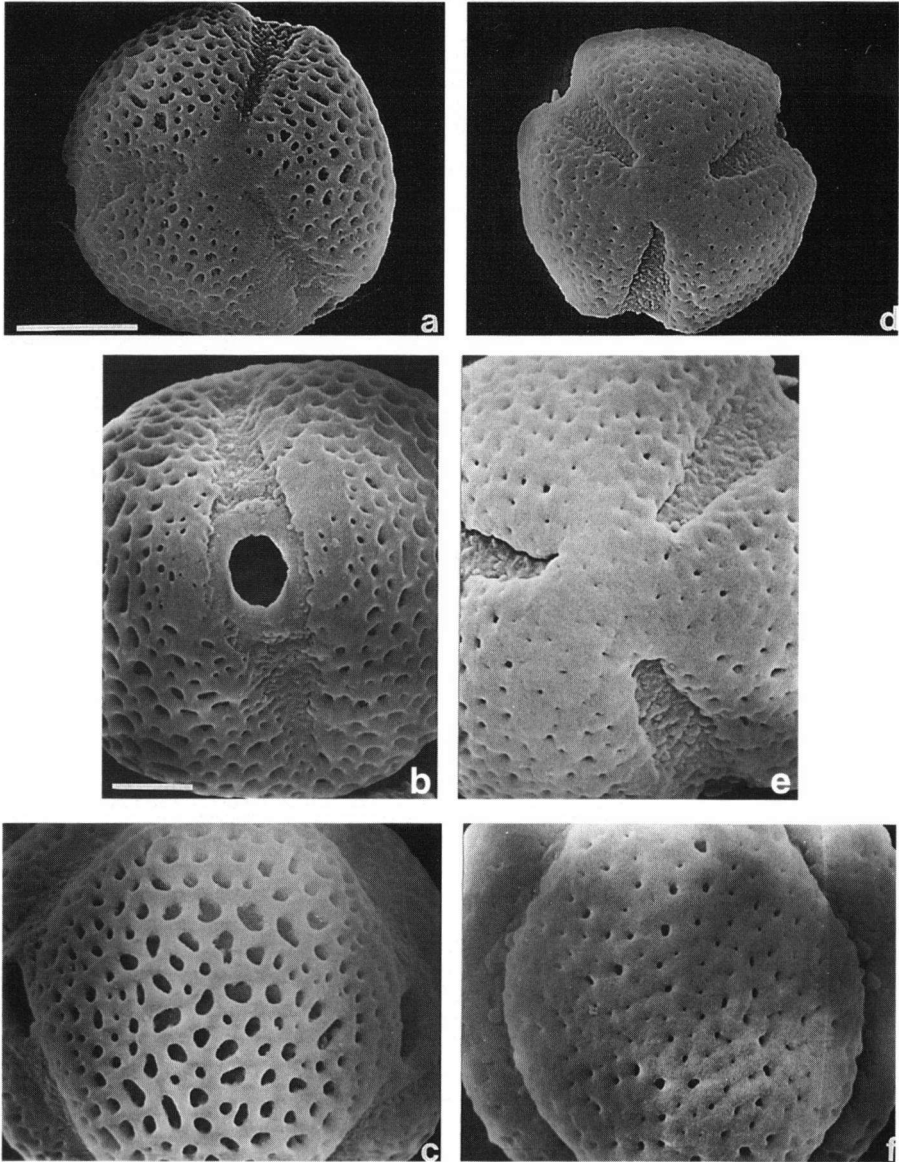


Fig. 3. *Mitragyna* (a–c: *M. inermis*; d–f: *M. parvifolia*). — a, d: polar view; b: ectocolpium; e: apocolpium; c, f: mesocolpium. — Scale bar on a = 5 μm , also for d; scale bar on b = 2 μm , also for c, e, f.

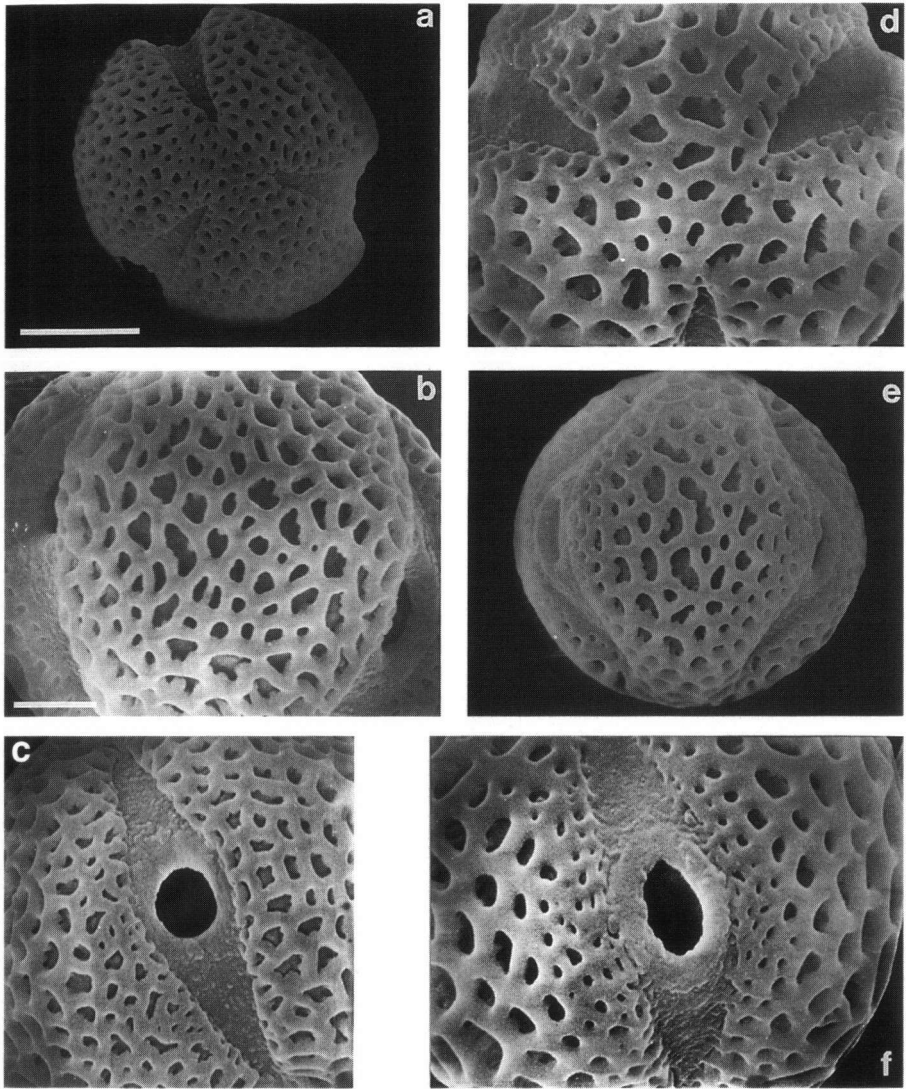


Fig. 4. *Mitragyna* (a–c: *M. rotundifolia*; d–f: *M. speciosa*). — a: polar view; b: mesocolpium; c, f: ectocolpus; d: apocolpium; e: equatorial view. — Scale bar on a = 5 μm , also for e; scale bar on b = 2 μm , also for c, d, f.

Macromorphological data were gathered in the first place from the keys and descriptions by Ridsdale (1978); in this revision, however, only four out of the ten species were described. Additional information was obtained from protologues and floristic literature (Haviland, 1897; Koorders & Valetton, 1902; Pitard, 1922; Hallé, 1966; Leroy, 1975; Verdcourt, 1988) as well as from personal observations (dissections of BR specimens).

The data, both palynological and macromorphological, were encoded estimating frequencies of character states, and submitted to a numerical analysis (SYSTAT hierarchical clustering with average linkage and Pearson's correlation coefficient; Wilkinson, 1988). The acronyms used for the OTU's in tables and figures are the first three letters of the generic name combined with the first three letters of the specific name.

PALYNOLOGICAL CHARACTERS

General morphology

Mitragyna s.l. has small, isopolar and radially symmetrical pollen grains. The polar axis ranges from 14 to 22 μm , the equatorial axis from 15 to 25 μm . In equatorial view, the shape of the grains varies from suboblate to prolate-spheroidal (P/E 0.75 to 1.06). The outline in polar view (= amb) is mostly circular; *Hallea stipulosa* has a subtriangular outline with convex sides.

The aperture system is always 3-zonocolporate; the compound apertures consist of three parts which are located in different wall layers. The *ectoaperture* is a wide colpus with a granular, slightly sunken membrane and distinct margins which are often irregular. The ends of the colpi are acute, obtuse or intermediate; the apocolpium

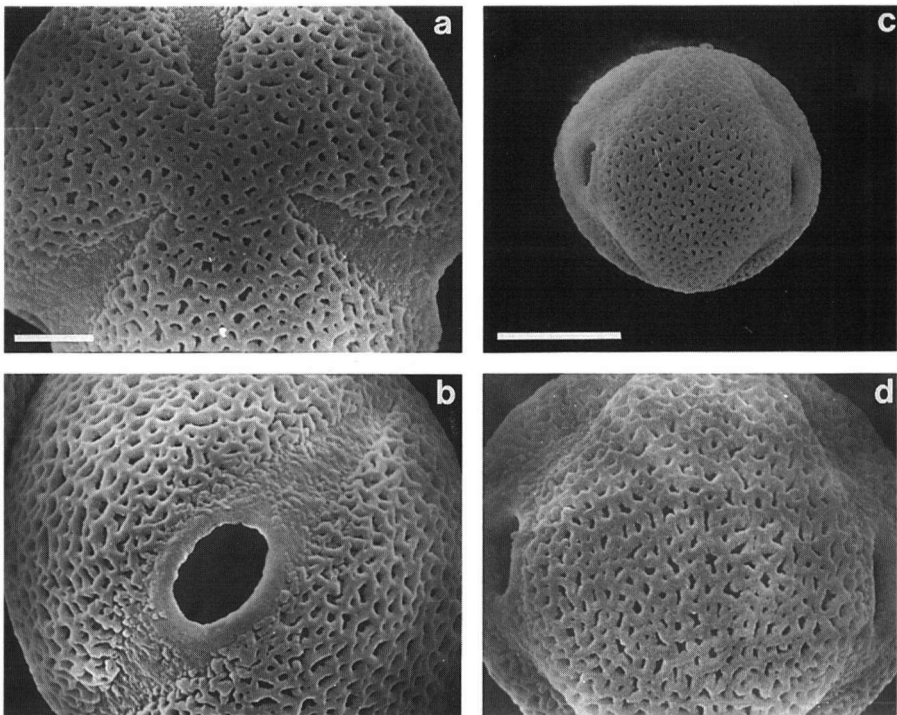


Fig. 5. *Mitragyna* (*M. tubulosa*). — a: apocolpium; b: ectocolpus; c: equatorial view; d: mesocolpium. — Scale bar on a = 2 μm , also for b, d; scale bar on c = 5 μm .

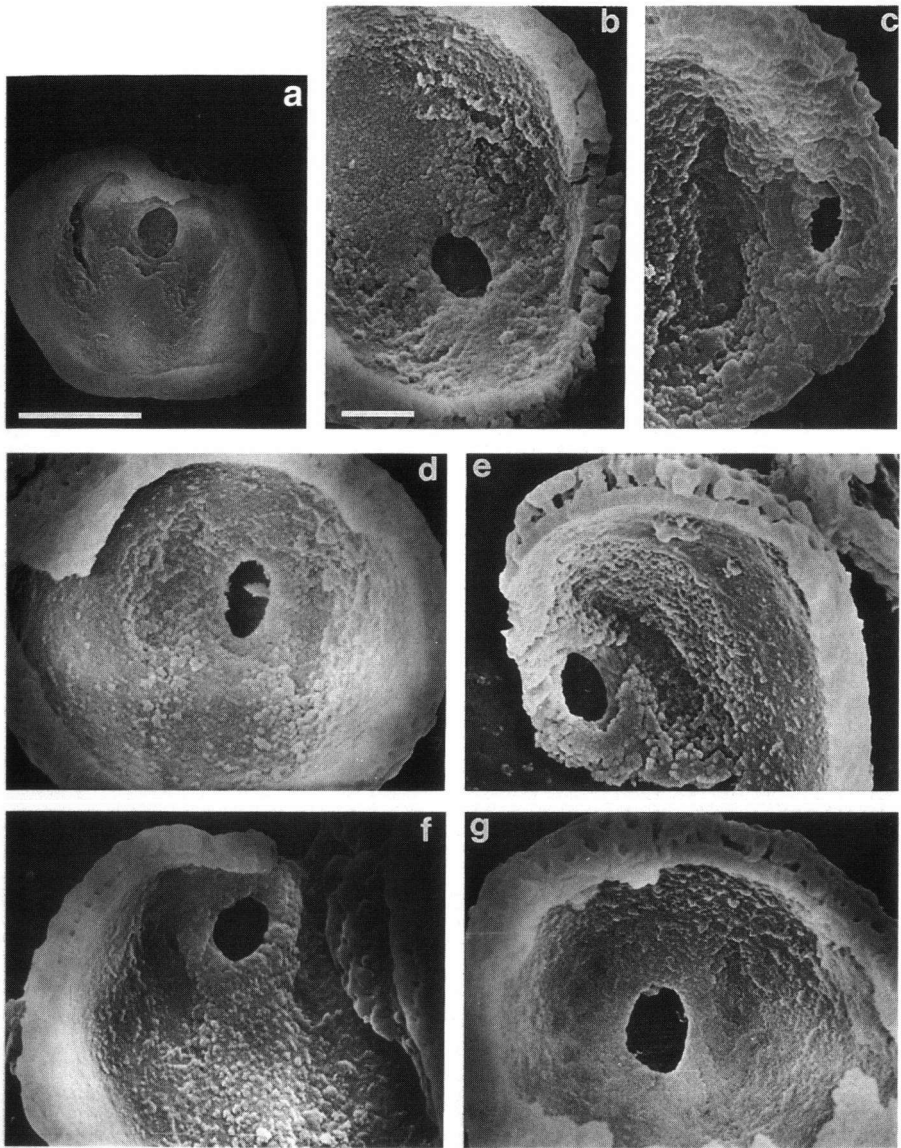


Fig. 6. Broken grains to show H-shaped endoapertures and inner surface of nexine. —a: *Hallea ledermannii*; b: *Mitragyna diversifolia*; c: *M. tubulosa*; d: *M. hirsuta*; e: *M. inermis*; f: *M. parvifolia*; g: *M. rotundifolia*. — Scale bar on a = 5 μ m; scale bar on b = 2 μ m, also for c–g.

index varies from 0.17 to 0.42. The *mesoaperture* is a lolongate porus, mostly surrounded by a \pm smooth *aspis*. The term ‘*aspis*’ is preferred above the more general ‘*annulus*’ because the differentiated area surrounding the pore is always a thickening of the exine. A *costa* (thickening of the nexine) surrounding the mesoaperture at the inside of the grain occurs in six species. All species show a H-shaped cut-away of

the nexine which is the *endoaperture*. The downstrokes of the H are parallel with the ectocolpus; in some species, the H may be incomplete, i.e. the horizontal, equatorial connection may be weak (diffuse margins) or even absent. In *Mitragyna parvifolia* e.g., the equatorial connection is missing. The H-shaped *endoaperture* is reduced to a kidney-shaped cut-away at both sides of the mesoaperture, ± 3 times as long as the diameter of the mesoporus. The surface of the *endoapertures* is often more coarsely scabrate than the rest of the inner side of the nexine.

The sexine is tectate-perforate to microreticulate with short columellae (observed in the centre of the mesocolpium). The lumina or perforations tend to be larger in the centre of the mesocolpium and decrease in diameter towards the poles and the ecto-

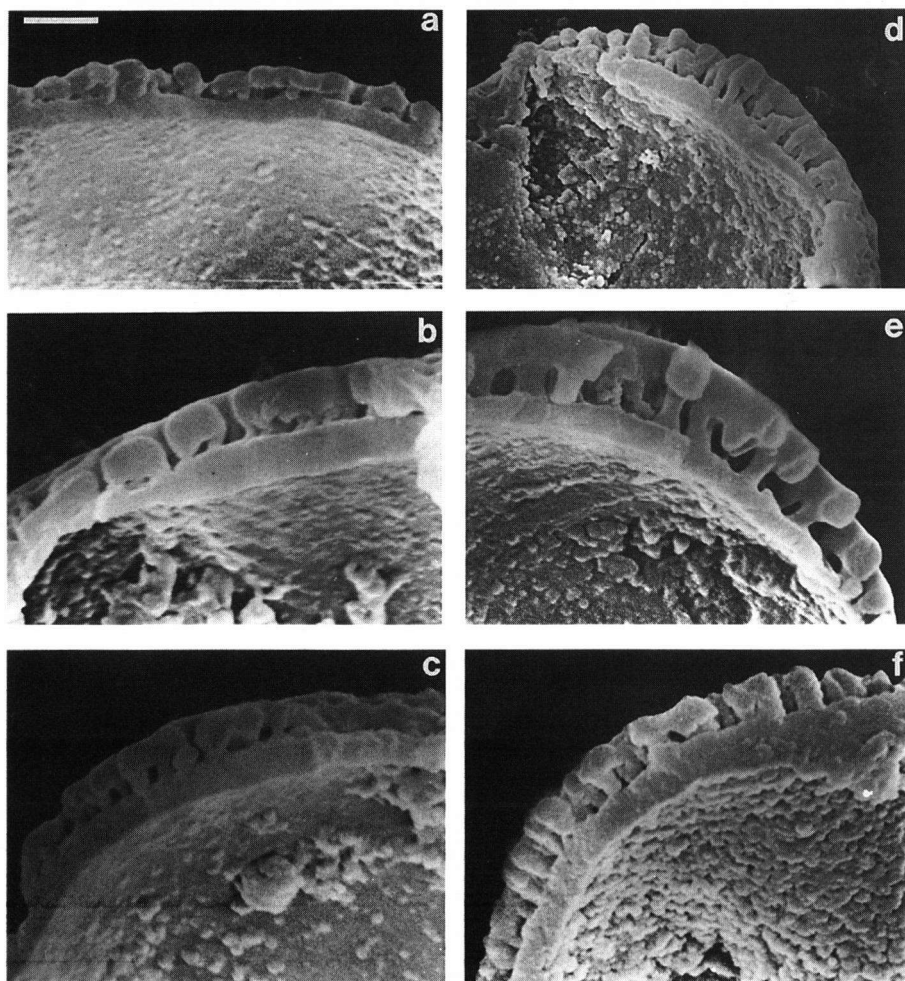


Fig. 7. Details of broken grains to show structure of exine and inner surface of nexine. — a: *Hallea ledermannii*; b: *H. rubrostipulata*; c: *Mitragyna hirsuta*; d: *M. inermis*; e: *M. speciosa*; f: *M. tubulosa*. — Scale bar on a = 1 μ m, also for b–f.

Table 1. Overview of pollen morphological features of *Mitragyna* s.s. ('dot' = dot-like perforation). Characters considered to be diagnostic for *Mitragyna* s.s. and *Hallea* by Leroy (1975) are printed in bold. All measurements are in μm .

	Mitdiv	Mithir	Mitine	Mitpar	Mitrot	Mitspe	Mittub
P	14 (15.6) 17	14 (14.2) 15	15 (15.9) 17	16 (17.1) 18	15 (15.7) 17	17 (18.7) 20	16 (16.4) 17
E	16 (17.1) 19	15 (16.1) 17	17 (17.9) 18	16 (17.5) 18	17 (17.6) 19	19 (20.4) 22	17 (18.0) 19
P/E	0.84 (0.91) 0.94	0.82 (0.88) 0.94	0.83 (0.89) 0.94	0.94 (0.98) 1.06	0.83 (0.89) 0.94	0.86 (0.92) 0.95	0.89 (0.91) 0.94
amb	circular	circular	circular	circular	circular	circular	circular
apocolpium index	0.27-0.30	0.32-0.42	0.17-0.23	0.23-0.25	0.24-0.32	0.30-0.35	0.24-0.27
width ectoaperture	2.5-3.2	2.0-2.5	2.5	1.3-2.0	2.3-2.5	2.5-2.8	3-2
margins ectoaperture	distinct-irregular	distinct-irregular	distinct-regular	distinct-regular	distinct-regular	distinct-regular	distinct-irregular
ends ectoaperture	mostly acute	obtuse to acute	obtuse to acute	mostly obtuse	acute	acute	mostly obtuse
width mesoaperture	1.3-1.7	1.2	1.2-1.3	1.0	1.5	1.2-1.3	1.3-1.8
height mesoaperture	2.3	1.8	1.7-1.8	1.3-1.5	1.7-1.8	1.8-2.3	2.5-2.7
aspis	smooth	\pm smooth	\pm smooth	absent	smooth	\pm smooth	smooth
costa	—	—	—	coarse	if present, coarse	if present, coarse	coarse
endoaperture	H-shaped	H-shaped	H-shaped	H-shaped	H-shaped	H-shaped	H-shaped
equatorial connection of endoaperture	as wide as mesoaperture	unclear or absent	irregular	absent	unclear or absent	unclear or absent	as wide as mesoaperture
sexine	microreticulate	tect.-perforate	microreticulate	tect.-perforate	microreticulate	microreticulate	tect.-perforate
max. \emptyset lumina apocolpium	0.5	0.3	0.2	dot	0.3	0.7	dot
max. \emptyset lumina mesocolpium	1.0	0.3	0.8	0.3	0.7	1.0	0.2
width muri	\pm 0.3	—	\pm 0.3	—	\pm 0.3	\pm 0.5	—
inner surface nexine	scabrate	scabrate	scabrate	scabrate	scabrate	scabrate	scabrate
columellae layer	0.17	0.17	0.33	0.17	0.33	0.50	0.17
tectum	0.42	0.33	0.50	0.33	0.42	0.50	0.33
columellae layer/tectum ratio	0.40	0.52	0.66	0.52	0.79	1.00	0.52
sexine	0.59	0.50	0.83	0.50	0.75	1.00	0.50
nexine	0.42	0.42	0.50	0.33	0.50	0.33	0.42
sexine/nexine ratio	1.4	1.2	1.7	1.5	1.5	3.0	1.2

Table 2. Overview of pollen morphological features of *Hallea*. Presentation as Table 1.

	Halled	Halrub	Halsti
P	15 (16.6) 18	19 (19.6) 22	14 (15.5) 18
E	18 (19.3) 21	21 (23.0) 25	17 (18.7) 20
P/E	0.79 (0.86) 0.95	0.80 (0.85) 0.92	0.75 (0.83) 0.95
amb	circular	circular	subtriangular
apocolpium index	0.27–0.33	0.29	0.25–0.26
width ectoaperture	2.8–3.3	3.7	3.0–3.3
margins ectoaperture	diffuse to distinct-irregular	distinct-irregular	distinct-irregular
ends ectoaperture	obtuse to acute	obtuse	obtuse
width mesoaperture	1.5–1.7	1.7–2.0	1.5–2.0
height mesoaperture	2.2–2.5	3.0–3.3	2.0–2.5
aspis	± smooth	smooth	smooth
costa	—	coarse	thick, coarse
endoaperture	H-shaped	H-shaped	H-shaped
equatorial connection of endoaperture	wider than mesoaperture	wider than mesoaperture	wider than mesoaperture
sexine	tect.-perforate	tect.-perforate	tect.-perforate
max. ∅ lumina apocolpia	0.5	dot	dot
max. ∅ lumina mesocolpia	0.8	0.5	0.3
width muri	—	—	—
inner surface nexine	scabrate	scabrate	scabrate
columellae layer	0.17	0.17	0.12
tectum	0.50	0.50	0.44
columellae layer/tectum ratio	0.34	0.34	0.27
sexine	0.67	0.67	0.57
nexine	0.50	0.50	0.44
sexine/nexine ratio	1.3	1.3	1.3

apertures, except for pollen of *M. hirsuta*. The lumina/perforations of *M. diversifolia* and *M. tubulosa* are often elongate and angular; they are rounded in all other species. The muri are simplicolumellate. Any supracteal processes are absent; the very fine granulation, observed in only one case (*M. parvifolia*), is interpreted as an artifact. The inner surface of the nexine is always scabrate, but the density and the size of the elements may differ locally. In *Hallea rubrostipulata*, *Mitragyna parvifolia*, and *M. tubulosa*, the scabrae are more densely spaced opposite the ectocolpi.

The palynological characters for each species are given in Tables 1 and 2. Table 3 lists the pollen characters and their states retained for our numerical analysis.

Table 3. Palynological characters and their states observed in *Mitragyna* s.l., with their coding used in Table 5.

Grain size	very small (10–18 μm)	SISMa
	small (18–25 μm)	SISMb
Shape equatorial view	prolate-spheroidal (1.00–1.14)	SHPS
	spheroidal (1.00)	SHS
	oblate-spheroidal (1.00–0.88)	SHOS
	suboblate (0.88–0.75)	SHSO
Shape polar view (amb)	circular	AMBCI
	triangular	AMBTR
Apocolpium index	small (< 0.35)	AIMS
	large (> 0.35)	AILR
Ectoaperture width (% of E)	narrow (< 14%)	ECNA
	wide (> 14%)	ECWI
Ectoaperture margin	diffuse	ECDF
	distinct	ECDI
Ends of ectoaperture	acute	ECAC
	obtuse	ECOB
Dimensions mesoaperture	small (< 9 μm^2)	MESM
	large (> 9 μm^2)	MELR
Aspis	absent	ASAB
	present	ASSM
Costa surrounding mesoaperture	present	MECOA
	absent	MECOP
Sexine	tectate-perforate	SEXTP
	microreticulate	SEXMR
Perforations smaller towards poles	yes	PFPS
	no	PFPL
Columellae layer/tectum ratio	= 1	COL
	< 1	COLS
Sexine/nexine ratio	< 2	WALa
	> 2	WALb

*Notes on species:**Hallea ledermannii* (K. Krause) Verdc.

The pollen of *H. ledermannii* was previously described by Leroy (1975: 86, pl. 12/8–12/14, LM and SEM). The material he studied (no voucher specimen cited and maybe not acetolysed) has slightly smaller grains with narrower ectocolpi. The porus that we have called the mesoaperture is described as the endoaperture. Leroy nowhere mentioned the existence of a third aperture.

We observed a slight intraspecific variation between the two examined specimens as regards the size of the mesoaperture and the diameter of the lumina (compare Fig. 1e with Fig. 1g): both are larger in *Bos* 2645.

Mitragyna inermis (Willd.) Kuntze

The pollen of *M. inermis* was already described and illustrated by Leroy (1975: 84, pl. 12/1–12/7, LM and SEM). Except about the width of the ectocolpi the same remarks can be made as for *Hallea ledermannii*.

Table 4. Macromorphological characters and their states observed in *Mitragyna* s.l.: characters retained for the numerical analysis and their coding.

Architecture	monopodial	MON
	sympodial	SYM
Leaf-blades	medium-sized (up to 14 × 9 cm)	LEAMS
	large (> 14 × 9 cm)	LEAL
Interfloral bracteoles	linear	IBL
	linear-spathulate	IBLS
	spathulate	IBS
Interfloral bracteoles reaching	well below calyx lobes	IBWB
	up to calyx lobes	IBSA
	beyond calyx lobes	IBA
Calyx	truncate to repand	CALT
	with short obtuse lobes	CALO
	with triangular + interstitial lobes	CALT
	with spathulate lobes	CALS
Margins of calyx lobes	ciliate	CALC
	glabrous	CALG
Corolla tube	long ($\geq 2 \times$ length of corolla lobes)	COTL
	short ($< 2 \times$ length of corolla lobes)	COTS
Corolla tube	hypocrateriform	COTH
	narrowly infundibular	COTI
Corolla throat	hairy	COTRH
	glabrous	COTRG
Throat hairs	not protruding	TRNP
	conspicuously protruding	TRP
Corolla lobes	with appendage	COAP
	without appendage	COWAP
Corolla lobes outside	hairy	COLOH
	glabrous	COLOG
Corolla lobes inside	ciliate along midrib	COLIC
	hairy/pubescent	COLIH
	glabrous	COLIG
Anthers	partially protruding from corolla tube	ANPP
	conspicuously protruding from corolla tube	ANP
Stigma	\pm isodiametric	STISO
	elongate	STEL
Calyx on fruits	persistent	CALP
	subpersistent	CALSP

MACROMORPHOLOGICAL CHARACTERS AND THEIR CODING

The palynological data set is fairly complete. On the contrary, our set of macromorphological characters is limited. Ridsdale's (1978) revision of *Mitragyna* and *Uncaria* contains an extensive discussion of the architecture of these plants, but otherwise hardly describes their macromorphological characteristics. We have tried as much as possible to gather extra information, though from a limited number of specimens. The following survey is especially intended to introduce the characters and their states retained in our numerical analysis; these are summarized in Table 4.

Vegetative characteristics

Mitragyna species are (often large) trees or more rarely shrubs with medium-sized to large leaves and very apparent foliaceous interpetiolar stipules.

Tree architecture was thoroughly discussed by Leroy (1975) who argued that *Mitragyna* s.s. fundamentally differs from *Hallea* in having the inflorescences terminal on lateral twigs; the flowering twig has maintained its vegetative capacity, as its axillary buds sometimes develop into new branches (sympodial growth). In *Hallea*, on the contrary, the inflorescences are axillary on lateral twigs and the architecture is monopodial. Ridsdale (1978) studied the architecture of the Asiatic species (*Mitragyna tubulosa* in the field); he compared these observations with herbarium material of the African *Hallea* and concluded that all species of *Mitragyna* have a similar ramification of the plagiotropic branches, including those segregated into *Hallea* by Leroy. In 1985 Leroy reported field observations on the architecture of *Hallea ledermannii*; he convincingly corroborated the absence of sympodial growth in its plagiotropic branches and thus confirmed the architectural differences between *Hallea* and *Mitragyna*.

Inflorescences

Inflorescences are compact perfectly spherical heads. The number of heads per branch strongly varies, from 1 to 15 (30), but so gradually that we could not retain it for the numerical analysis.

Each flower is surrounded by numerous (> 10) hairy interfloral bracteoles which mostly have a characteristic spatulate shape. In a few species, the bracteoles are linear or have a transitional shape. The relative length of the bracteoles varies greatly; they are well visible when they reach beyond the calyx lobes, but may also be hidden between the ovaries.

Flowers

Calyx and corolla are pentamerous and morphologically very variable (Leroy, 1975: pl. 1). They provide the features generally used to distinguish between the species.

The calyx is truncate to repand or provided with distinct lobes. In *Hallea rubrostipulata*, the calyx lobes are narrowly triangular and alternate with five much smaller interstitial lobes¹.

¹) Erroneously called an 'epicalyx' by Verdcourt (1988); this type of calyx is rare in Rubiaceae and hitherto only reported from *Sherbournia* and *Strumpfia* (Robbrecht, 1988).

The corolla is infundibuliform or hypocrateriform, with short triangular lobes with valvate-induplicate aestivation. In the species placed in *Hallea*, the lobe-tips are sometimes provided with characteristic short to linear appendages, somewhat reminiscent to those observed in the related genera *Pausinystalia* and *Corynanthe* (Hallé, 1966: pl. 9, 10).

The gynoecium is bicarpellate. The style ends in a mitre-shaped stigma of very variable size and shape, from \pm isodiametric to strongly elongated. In *Hallea*, the whole exterior part of the stigma is papillary, while in *Mitragyna* s.s. the papillary parts are limited to the tip and sometimes to the base of the 'mitre'. The cylindrical disk is deeply sunken. Placentation is characteristic of the Coptosapelteae: each locule is provided with a pendulous placenta covered with numerous ascendingly imbricate ovules.

Fruits and seeds

The ovaries are completely free on the receptacles of the heads and develop into \pm elongated capsules dehiscing into 4 valves adhering at the base. Each locule contains numerous seeds provided with an apically triangular and basally forked wing.

Differences between species are small and concern especially the persistency of the calyx and the size of the fruit (length varying from c. 5 to c. 15 mm).

NUMERICAL ANALYSIS

Our observations established 14 pollen morphological characters (30 states) within the *Mitragyna/Hallea*-complex. On the other hand we were able to document variation in 16 macromorphological characters (37 states).

The following cluster analyses were performed: 1) with palynological characters, 2) with macromorphological characters, and 3) with both macromorphological and palynological characters. In the two latter cases, the number of species was restricted to eight because *Mitragyna speciosa* and *M. diversifolia* were insufficiently documented macromorphologically.

The purely palynological clustering (Fig. 8A) resulted in a distinct separation of one species, *Mitragyna speciosa*; this species has indeed several unique states as regards the relative thickness of its exine layers, columellae/tectum and sexine/nexine ratio (Table 5); in fact these states are all related to the large absolute thickness of the columellae layer. The separated position of *M. speciosa* should thus not be overrated. The remaining species are divided into two clusters. The first cluster groups the African species of *Hallea*, with one Asiatic species of *Mitragyna* s.s. (*M. tubulosa*); these species have larger pollen with larger mesoapertures, two features mostly but not always associated with a tectum perforatum and blunt ectoapertural ends. The second cluster contains the five remaining species of *Mitragyna* s.s.; among these species *M. parvifolia* occupies a rather isolated position, because it is the only one with more prolate pollen and without aspides.

The macromorphological clustering and the combined palynological-macromorphological analysis yielded similar phenograms: two groups, corresponding with

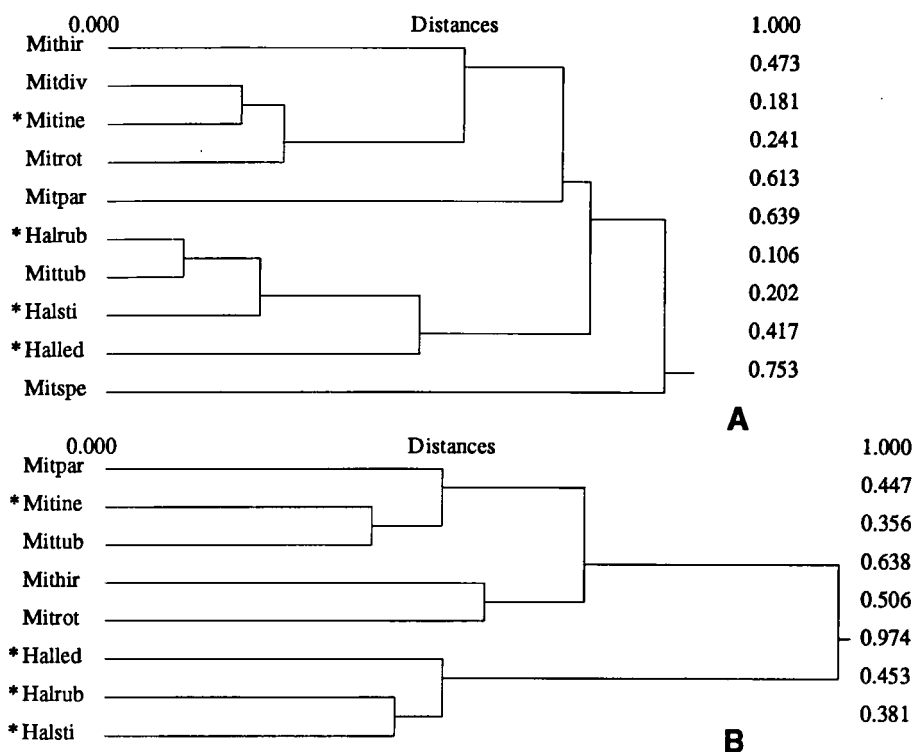


Fig. 8. Hierarchical clustering (using average linkage and 1-Pearson's correlation coefficient) of *Hallea* and *Mitragyna* species (African species marked with an asterisk, other ones from tropical Asia): A, using palynological characters; B, using palynological and macromorphological characters. Data matrices in Tables 5 and 6. Distances are a measure of phenetic similarity between species or clusters of species.

Mitragyna s.s. and *Hallea* (Fig. 8B). This is of course not astonishing and results from the strong correlation between certain (especially floral) characters as discussed by Leroy (1975). The weight of this macromorphological evidence is so high that distinction between *Hallea* and *Mitragyna* s.s. is equally corroborated by the combined analysis.

DISCUSSION AND CONCLUSION

Leroy (1975) concluded (from an examination of a single species from each genus) that *Mitragyna* s.s. and *Hallea* can be distinguished palynologically, viz. by reticulate pollen with a thicker ectexine versus tectate-perforate pollen with a thinner ectexine. We have observed both microreticulate and tectate-perforate grains in *Mitragyna* s.s., and found that the sexine thickness of *Mitragyna* s.s. continuously varies from 0.5 to 1.0 μm . This range includes the sexine thicknesses (0.57–0.67 μm) that we observed in *Hallea*. It is thus clear that Leroy's statement is a simplification, which illustrates the danger of studying single representatives.

Table 5. Matrix of frequencies of states of palynological characters used in the cluster analysis. Symbols of states explained in Table 3.

	Halled	Halrub	Halsti	Mitdiv	Mithir	Mitine	Mitpar	Mitrot	Mitspe	Mittub
SISMa	0	0	10	90	100	100	100	90	0	50
SISMb	100	100	90	10	0	0	0	10	100	50
SHPS	0	0	0	0	0	0	20	0	0	0
SHS	0	0	0	0	0	0	20	0	0	0
SHOS	35	30	20	90	80	90	60	70	90	100
SHSO	65	70	80	10	20	10	0	30	10	0
AMBCI	100	100	0	100	100	100	100	100	100	100
AMB ¹² R	0	0	100	0	0	0	0	0	0	0
AISM	100	100	100	100	50	100	100	100	100	100
AILR	0	0	0	0	50	0	0	0	0	0
ECNA	0	0	0	0	0	0	50	0	0	0
ECWI	100	100	100	100	100	100	50	100	100	100
ECDF	100	0	0	0	0	0	0	0	0	0
ECDI	0	100	100	100	100	100	100	100	100	100
ECOB	50	100	100	0	50	50	100	0	0	100
ECAC	50	0	0	100	50	50	0	100	100	0
ASAB	0	0	0	0	0	0	100	0	0	0
ASSM	100	100	100	100	100	100	0	100	100	100
MESM	0	0	0	0	100	100	100	100	50	0
MELR	100	100	100	100	0	0	0	0	50	100
MECOA	100	0	0	100	100	100	0	0	0	0
MECOP	0	100	100	0	0	0	100	100	100	100
SEXTP	100	100	100	0	100	0	100	0	0	100
SEXMR	0	0	0	100	0	100	0	100	100	0
PFPS	100	100	100	100	0	100	100	100	100	100
PFPL	0	0	0	0	100	0	0	0	0	0
COL	0	0	0	0	0	0	0	0	100	0
COLS	100	100	100	100	100	100	100	100	0	100
WALa	100	100	100	100	100	100	100	100	0	100
WALb	0	0	0	0	0	0	0	0	100	0

Hallea pollen has always a perforate tectum and is in general larger, while *Mitragyna* s.s. mostly possesses smaller microreticulate grains; however, several *Mitragyna* s.s. species have pollen similar to that of *Hallea*, viz. *M. hirsuta*, *M. parvifolia* and *M. tubulosa*. The differentiation of the pollen of the *Mitragyna/Hallea*-complex seems to be very low. This is in agreement with the remainder of the *Coptosapeltea* (pers. obs.). The tribe is in general stenopalynous; the grains are 3-colporate, except for *Coptosapelta*, which has 3-pororate grains. Only a few genera are pollen morphologically distinct. *Uncaria* has a characteristic striate-reticulate sexine, *Greeniopsis* and *Mussaendopsis* have colpus-shaped mesoapertures and endocinguli, while *Luculia* shows a reticulate sexine with long columellae.

Table 6. Matrix of frequencies of states of macromorphological characters used in the cluster analysis. Symbols of states explained in Table 4.

	Halled	Halrub	Halsti	Mithir	Mitine	Mitpar	Mitrot	Mittub
MON	100	100	100	0	0	0	0	0
SYM	0	0	0	100	100	100	100	100
LEAMS	0	0	0	50	100	100	0	100
LEAL	100	100	100	50	0	0	100	0
IBL	0	0	0	50	0	0	0	50
IBLS	0	0	0	50	0	0	100	50
IBS	100	100	100	0	100	100	0	0
IBWB	0	100	100	0	0	0	0	100
IBSA	100	0	0	100	0	0	50	0
IBA	0	0	0	0	100	100	50	0
CALT	0	0	100	0	100	100	0	100
CALO	100	0	0	0	0	0	100	0
CALT	0	100	0	0	0	0	0	0
CALS	0	0	0	100	0	0	0	0
CALC	100	100	0	0	0	100	0	0
CALG	0	0	100	100	100	0	100	100
COTL	0	100	0	100	100	100	0	100
COTS	100	0	100	0	0	0	100	0
COTH	0	0	100	50	100	50	50	100
COTI	100	100	0	50	0	50	50	0
COTRH	0	100	0	100	100	50	100	100
COTRG	100	0	100	0	0	50	0	0
TRNP	100	100	100	0	100	100	0	100
TRP	0	0	0	100	0	0	100	0
COAP	30	80	80	0	0	0	0	0
COWAP	70	20	20	100	100	100	100	100
COLOH	100	100	100	0	0	0	0	0
COLOG	0	0	0	100	100	100	100	100
COLIC	0	0	0	0	50	50	0	0
COLIH	100	100	100	100	0	0	100	0
COLIG	0	0	0	0	50	50	0	100
ANPP	100	100	100	0	0	0	0	0
ANP	0	0	0	100	100	100	100	100
STISO	100	100	100	0	0	0	0	0
STEL	0	0	0	100	100	100	100	100
CALP	100	100	100	100	100	0	0	100
CALSP	0	0	0	0	0	100	100	0

Leroy's generic segregation of *Hallea* from *Mitragyna* was based on convincing macromorphological evidence. It is true that the set of supporting characters is limited: architecture (summarized above) and some floral features (summarized in Leroy, 1975: pl. 1). The correlation of advanced characters supporting *Hallea* is strong, however. As the generic distinction is also confirmed by chorology (*Hallea* is strictly limited to the African rain forest area while the sole representative of *Mitragyna* s. s. in Africa is Soudanian), we are inclined to accept the segregation of the two genera. Ridsdale's (1978: 57) statement that the "variation within *Uncaria* [is] greater than that found between *Hallea* and *Mitragyna* s. s." is hardly an argument against this, but more an expression of the general fact that larger genera (*Uncaria* is a rather large and widespread palaeotropical genus with 35 species) tend to be more variable (compare with *Tricalysia*, *Gardenia* or *Rothmannia* in the Rubiaceae–Gardenieae; Robbrecht & Puff, 1986: 131).

Leroy offered two phylogenetic explanations: in 1975 he considered *Mitragyna* s. s. and *Hallea* as two branches diverging from a common ancestor, while in 1985 he rather believed *Hallea* to be the descent of an extinct African *Mitragyna*. Is there any palynological evidence to support these hypotheses? It is plausible to consider the microreticulate pollen of *Mitragyna* s. s. as derived compared to the tectate-perforate pollen of other *Mitragyna* species and *Hallea* (Walker & Doyle, 1975: 684; Keddam-Malplanche, 1985: 30). Consequently, the ancestral stock of the complex probably possessed tectate-perforate grains, which were 'replaced' by the more advanced microreticulate pollen in a restricted number of species of *Mitragyna* s. s. This scenario is in agreement with both hypotheses.

In conclusion, we accept the generic recognition of *Hallea* and *Mitragyna* s. s. on the basis of tree architectural and flower morphological features presented in the past, but found hardly any convincing palynological evidence to support it. The presumed evolution of the ornamentation of the pollen wall corroborates the phylogenetic scenarios offered by Leroy.

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REFERENCES

- Andersson, L., & C. Persson. 1991. Circumscription of the tribe Cinchoneae (Rubiaceae) – a cladistic approach. *Pl. Syst. Evol.* 178: 65–94.
- Erdtman, G. 1971. Pollen morphology and plant taxonomy: angiosperms. Hafner Publ. Co., New York.
- Halli, N. 1966. Famille des Rubiacées (1ère partie). *Flore du Gabon* 12: 278 pp.
- Haviland, G.D. 1897. A revision of the tribe Naucleaeae (Nat. Ord. Rubiaceae). *J. Linn. Soc.* 33: 1–94, pl. 1–4.
- Huysmans, S., E. Smets & E. Robbrecht. 1993. Endoaperture morphology in the Coptosapelteae (Rubiaceae–Cinchonoideae). In: D. Fürnkranz & H. Schantl (eds.), *Kurzf. "11. Symp. Morph., Anat., Syst."* Salzburg 1993. Beitr. nr. 66.

- Keddam-Malplanche, M. 1985. Le pollen et les stomates des Gardéniées (Rubiaceées) du Gabon. Morphologie et tendances évolutives. *Mém. Mus. Hist. Nat., N.S., sér. B, Bot.* 29: 109 pp., 16 pl.
- Koorders, S.H., & Th. Valeton. 1902. Bijdrage n° 8 tot de kennis der boomsoorten op Java. *Meded. 's Lands Plantentuin* 59: 285 pp.
- Leroy, J.-F. 1975. Taxogénétique: Étude sur la sous-tribu des Mitragyninae (Rubiaceae-Naucleaceae). *Adansonia, sér. 2*, 15: 65–88.
- Leroy, J.-F. 1985. L'ontogénèse dans le genre *Hallea* Leroy (Rubiaceae-Mitragyninae), genre de grande importance forestière en Afrique tropicale. *C. R. Acad. Sci. Paris, sér. 3*, 300: 221–226.
- Lobreau-Callen, D. 1978. L'aperture composée des Rubiaceae. *Ann. mines Belg.* 2: 167–173.
- Pitard, J. 1922–24. Rubiacées. In: F. Gagnepain (ed.), *Flore générale de l'Indo-Chine*: 20–442. Paris, Masson.
- Punt, W., S. Blackmore, S. Nilsson & A. Le Thomas. 1994. Glossary of pollen and spore terminology. Utrecht, Lab. Palaeobot. Palynol., LPP Contr. Ser. 1: 71 pp.
- Reitsma, T. 1969. Size modifications of recent pollen grains under different treatments. *Rev. Palaeobot. Palynol.* 9: 175–202.
- Ridsdale, C.E. 1978. A revision of *Mitragyna* and *Uncaria*. *Blumea* 24: 43–100.
- Robbrecht, E. 1988. Tropical woody Rubiaceae. *Opera Bot. Belg.* 1: 272 pp.
- Robbrecht, E. 1993a. On the delimitation of the Rubiaceae. A review. *Opera Bot. Belg.* 6: 19–30.
- Robbrecht, E. 1993b. Supplement to the 1988 outline of the classification of the Rubiaceae. Index to genera. *Opera Bot. Belg.* 6: 173–196.
- Robbrecht, E., & C. Puff. 1986. A survey of the Gardenieae and related tribes (Rubiaceae). *Bot. Jahrb.* 108: 63–137.
- Verdcourt, B. 1985. A new combination in *Hallea* (Rubiaceae–Cinchoneae). *Kew Bull.* 40: 508.
- Verdcourt, B. 1988. Cinchoneae. In: D.M. Bridson & B. Verdcourt, *Rubiaceae (part 2)*: 445–457. *Fl. Trop. East Africa*. Rotterdam, Balkema.
- Walker, J.W., & J.A. Doyle. 1975. The bases of angiosperm phylogeny: palynology. *Ann. Missouri Bot. Gard.* 62: 664–732.
- Wilkinson, L. 1988. *Systat: the system for statistics*. Evanston, IL: Systat, Inc.