

Pollen morphology of the tribes Naucleaeae and Hymenodictyeae (Rubiaceae – Cinchonoideae) and its phylogenetic significance

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The tribe Naucleaeae has recently been recircumscribed on the basis of both morphological and molecular [*rbcL*, *trnT-F*, internal transcribed spacer (ITS)] evidence, and has been found to be the sister group of the tribe Hymenodictyeae Razafim. & B. Bremer. In order to find pollen morphological support for this new classification, the pollen and orbicules of 65 species, representing 23 Naucleaeae and the two Hymenodictyeae genera, were investigated by scanning electron and light microscopy. Naucleaeae pollen is very small (< 20 µm) to small (20–30 µm) and its shape in equatorial view is suboblate to spheroidal or, more rarely, subprolate. Three compound apertures are present, each comprising a long and narrow ectocolpus, a circular to slightly lolate mesoporus, and an often H-shaped endoaperture. The sexine ornamentation is perforate, rugulate, or (micro)reticulate, and supracteal elements are always absent. Apart from the variation in sexine ornamentation, the tribe is rather stenopalynous. The pollen of Hymenodictyeae is very similar to that of Naucleaeae. The H-shaped endoapertures often observed probably form a synapomorphy for the clade comprising Naucleaeae and Hymenodictyeae. Our pollen morphological observations are not in conflict with the widened delimitation of Naucleaeae. Unambiguous pollen support for the recent subtribal or generic concepts of Naucleaeae could not be found because of a lack of variation of pollen characters within the tribe. Orbicules are invariably present in the ten Naucleaeae taxa investigated. They are spheroidal and smooth or irregularly folded. © 2007 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2007, 153, 329–341.

ADDITIONAL KEYWORDS: compound apertures – endoapertures — orbicule morphology – palynology – sexine ornamentation.

INTRODUCTION

Naucleaeae, as circumscribed by Razafimandimbison & Bremer (2002), is a mainly palaeotropical tribe of the subfamily Cinchonoideae (Rubiaceae) comprising 26 genera and *c.* 180 species. The tribe's highest diversity is found in South-east Asia, followed by Madagascar and Africa. Only five species, representing two genera,

occur in Central, North, and South America. Members of Naucleaeae are morphologically well recognizable by the many-flowered globose inflorescences and the epigynous floral nectaries deeply embedded in hypanthia (Bremer, Andreassen & Olsson, 1995; Razafimandimbison & Bremer, 2001, 2002; Razafimandimbison, 2002).

Naucleaeae is sister to the tribe Hymenodictyeae Razafim. & B. Bremer (Razafimandimbison & Bremer, 2001). Razafimandimbison & Bremer (2001) described the latter tribe to accommodate the genera *Hymenodictyon* Wall. and *Paracorynanthe* R. Capuron, previ-

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ously placed by Andersson & Persson (1991) in their polyphyletic tribe Coptosapelteae. These genera differ from the Naucleaeae genera in having elongate inflorescences and lenticellate capsular fruits containing bilaterally flattened, elongate, accrescent placentas (S.G. Razafimandimbison & B. Bremer, unpubl. data). The Naucleaeae–Hymenodictyeae clade forms the sister group to the rest of Cinchonoideae (Andersson & Antonelli, 2005).

The results of the phylogenetic studies by Razafimandimbison & Bremer (2001, 2002), based on molecular [internal transcribed spacer (ITS), *rbcL*, and *trnT-F*] and morphological data, strongly suggested a much broader circumscription for Naucleaeae than previously proposed, including all members of Naucleaeae *sensu* Ridsdale (1978), together with *Cephalanthus* L., *Hallea* J.-F. Leroy, *Mitragyna* Korth., *Uncaria* Schreb. (as shown by Bremer *et al.*, 1995), and also *Corynanthe* Welw. and *Pausinystalia* Pierre ex Dupouy & Beille. Their analyses also showed that Naucleaeae can be subdivided into six highly supported and morphologically distinct subtribes: Breoniinae, Cephalanthinae, Corynantheinae, Naucleinae, Mitragyninae, and Uncarinae. A seventh subtribe, the Adiniinae, was only poorly supported. Razafimandimbison & Bremer (2002) also proposed changes to the delimitation of some of the Naucleaeae genera. *Corynanthe* was restricted to *Corynanthe paniculata* Welw., the other species of the genus being transferred to the reinstated genus *Pseudocinchona* A. Chev. ex Perrot. *Hallea* J.-F. Leroy (Leroy, 1975) was merged with *Mitragyna*, and *Neobreonia* Ridsdale with *Breonia* A. Rich. The generic delimitations and infratribal taxa adopted here are as circumscribed by Razafimandimbison & Bremer (2002).

The pollen morphology of Naucleaeae and Hymenodictyeae is very poorly documented. Only the pollen of *Hallea* and *Mitragyna* has been studied extensively. Leroy (1975) segregated the genus *Hallea* from *Mitragyna*, partly on the basis of palynological evidence from only two species. This study was later elaborated by Huysmans, Robbrecht & Smets (1994), who studied the pollen of all ten species of *Hallea* and *Mitragyna*. They concluded that *Hallea* and *Mitragyna* could not be split on the basis of pollen morphology, although they accepted the separation on the basis of other morphological evidence.

As pollen morphology has been proven to provide useful taxonomic characters in other groups of Rubiaceae (for example, Johansson, 1992; Andersson, 1995; Rova & Andersson, 1995; Andreasen & Bremer, 1996; Delprete, 1996; De Block & Robbrecht, 1998; Huysmans, Robbrecht & Smets, 1998; Huysmans *et al.*, 1999; Dessein *et al.*, 2002, 2005a), we decided to conduct a thorough investigation of the pollen and orbicule morphologies of Naucleaeae and its sister

Hymenodictyeae. This article addresses the following questions: (1) are the new delimitation of Naucleaeae (*sensu* Razafimandimbison & Bremer, 2002) and the sister group relationship between Naucleaeae and Hymenodictyeae supported by pollen data?; (2) are there any pollen morphological synapomorphies for the seven subtribes proposed by Razafimandimbison & Bremer (2002)?; and (3) are the newly proposed generic circumscriptions supported by pollen morphology?

MATERIAL AND METHODS

This study is based on the examination of 123 specimens, encompassing 65 species belonging to 25 genera of Naucleaeae and Hymenodictyeae. Some of these observations were extracted from the Master's thesis of Huysmans (1993); in the material list, these samples are indicated with an asterisk. *Mitragyna s.l.* (*Hallea* J.-F. Leroy included) was not investigated here, as extensive pollen data of *Mitragyna s.s.* and *Hallea* were published by Huysmans *et al.* (1994). No pollen observations were made for *Diyaminauclea* Ridsdale and *Khasiaclunea* Ridsdale as suitable material was lacking. Pollen samples were taken from specimens from the following herbaria: A, AS, BR, L, MO, P, S, TAN, and TEF (abbreviations according to Holmgren, Holmgren & Barnett, 1990). The identifications of all specimens used were verified.

All specimens were acetolysed following Reitsma's (1969) 'wetting agent' method. Prior to acetolysis (10 min in a heating block at 90 °C), flowers were hydrated in an Agepon solution (1 : 200) and dehydrated in pure acetic acid. After acetolysis, the pollen sample from each specimen was split into two subsamples, one part for light microscopy (LM) and the other for scanning electron microscopy (SEM) observations and storage. Pollen for SEM was rinsed in 70% ethanol, mounted on a stub, air-dried, and coated with gold for 2 min using a SPI-Module™ sputter coater. Pollen grains were broken by adding small glass beads to the pollen suspension and shaking it with a vortex, as described by Huysmans *et al.* (1994). Digital images were taken with a Jeol JSM 5800 scanning electron microscope. Grains for LM studies were mounted in Kaiser's glycerine jelly and sealed with paraffin. The slides were observed using a Leitz Dialux 20 light microscope with a ×100 oil immersion lens.

In order to check for the possible presence of orbicules on the inner locule wall of the anthers, entire anthers were critically point dried (Balzers, CPD 030) after hydration in Agepon solution and dehydration in an acetone series. The dried, entire anthers were mounted on stubs with double adhesive carbon strips before further preparation. If undehisced, a longitudinal section of each theca was made using a razor

blade. To be able to observe the inner locule wall, the pollen grains were gently removed using a cactus needle.

Both the polar axis diameter (P) and equatorial diameter (E) were measured under LM in at least ten mature pollen grains. P/E values were calculated for each measured pollen grain. All other measurements were performed on digital SEM images using CARNOY software (Schols *et al.*, 2002). Pollen terminology follows the online edition of Punt *et al.* (1994) at <http://www.bio.uu.nl/~palaeo/glossary/index.htm/>. Pollen size classes are as proposed by Dessein *et al.* (2005a): very small, < 20 μm ; small, 20–30 μm ; medium, 30–40 μm . For shape classes in equatorial view (P/E), we adopted the wide definitions of Nilsson & Praglowski (1992): suboblate, 0.75–0.88; spheroidal, 0.88–1.14; subprolate, 1.14–1.33; prolate, 1.33–2.00. Measurements of the lumina refer to the lumina at the mesocolpium. Tribal, subtribal, and generic concepts are as proposed by Razafimandimbison & Bremer (2002). After each genus in the 'Results' section, the number of species investigated relative to the number of estimated species is given in parentheses.

RESULTS

GENERAL POLLEN OBSERVATIONS

Naucleaeae and Hymenodictyaeae are relatively stenopalynous. Variation was mainly observed in sexine ornamentation and, to a lesser extent, in pollen size and shape, and aperture morphology. Table 1 summarizes the pollen morphological characters at a generic level.

Pollen is always shed as monads. The pollen size in Naucleaeae varied from very small (< 20 μm) to small (20–30 μm), although the majority of the genera had very small-sized pollen. The largest pollen grains were observed in *Adina* Salisb. and *Cephalanthus*. Pollen shape in equatorial view ranged from suboblate to subprolate, with pollen of most species and genera being spheroidal. Suboblate and subprolate pollen grains sometimes occurred in the same genus, as in *Breonia*, *Ludekia*, and *Nauclea*. A few prolate grains were observed in *Adina*. The amb was usually circular, more rarely subtriangular.

All Naucleaeae taxa investigated were three-colporate. Only in *Hymenodictyon floribundum* (Hochst. & Steud.) B. L. Robinson and *Neonauclea forsteri* (Seem. ex Havil.) Mer. were a few four-colporate pollen grains observed. From the outside, an ectocolpus and mesoporus were observed in all genera (Figs 1–6). In most cases, the ectocolpus was long and narrow. A relatively short ectocolpus was observed in *Myrmeconuclea* Merr. (Fig. 5) and some species of *Neonauclea* Merr. Because the colpi were usually long, the apocolpium

index was low (see Table 1). The colpus ends varied from acute (i.e. Figs 2, 3, 7) to obtuse (Figs 4, 11). The colpus membrane was coarse, granular, or smooth. The mesoporus was located in the middle of the colpus (at the equator), and was circular (Fig. 1) or somewhat longer than wide (Figs 2–6). The mesoporus was usually small and rarely exceeded 2 μm in diameter. A mesoporus exceeding 2 μm was observed in *Ochreinauclea* Ridsdale & Bakh. f. H-shaped endoapertures were observed in *Breonadia* Ridsdale, *Corynanthe*, *Haldina* Ridsdale, *Hymenodictyon*, *Neonauclea*, *Pausinystalia*, *Pseudocinchona*, and *Uncaria* (Fig. 18). The inner ornamentation of the sporoderm was not observed with SEM for all genera studied, and so H-shaped endoapertures might also occur in other genera. A distinct endoaperture seemed to be lacking in *Neonauclea* and *Nauclea*.

The sexine was semitectate and simpliculumellate (Figs 16–18). Supratectal elements were always absent. The most common sexine ornamentations in Naucleaeae were microreticulate (Figs 1–3, 5–7, 14), rugulate (Fig. 15), and perforate (Figs 8, 9, 11). A reticulate sexine pattern (lumina > 1 μm) was only observed in the genus *Ludekia* (Figs 4, 10) and in some *Pausinystalia* species. Usually, there was no differentiation of the sexine towards the poles or apertures (Figs 7, 10, 11–15). In some species, however, the lumina or perforation size was smaller towards the colpi and/or at the poles (Figs 8, 9). For all genera investigated, the inner surface of the nexine was granular (Figs 17, 18).

GENERAL ORBICULE OBSERVATIONS

Orbicules in Naucleaeae were small and spheroidal, or sometimes slightly lobed. A central indentation in the orbicule body was rare, but occurred in *Nauclea*. The surface of the orbicules was always smooth. Orbicules can lie freely upon the locule wall (Figs 19–21, 23, 24) or can be embedded (Fig. 22). According to the orbicule typology as proposed by Huysmans *et al.* (1997) and Vinckier, Huysmans & Smets (2000), they belong to type IV (irregularly folded orbicules) in *Burttidavya* Hoyle and type IIIa (smooth spheroidal orbicules without a central perforation) in all other genera investigated.

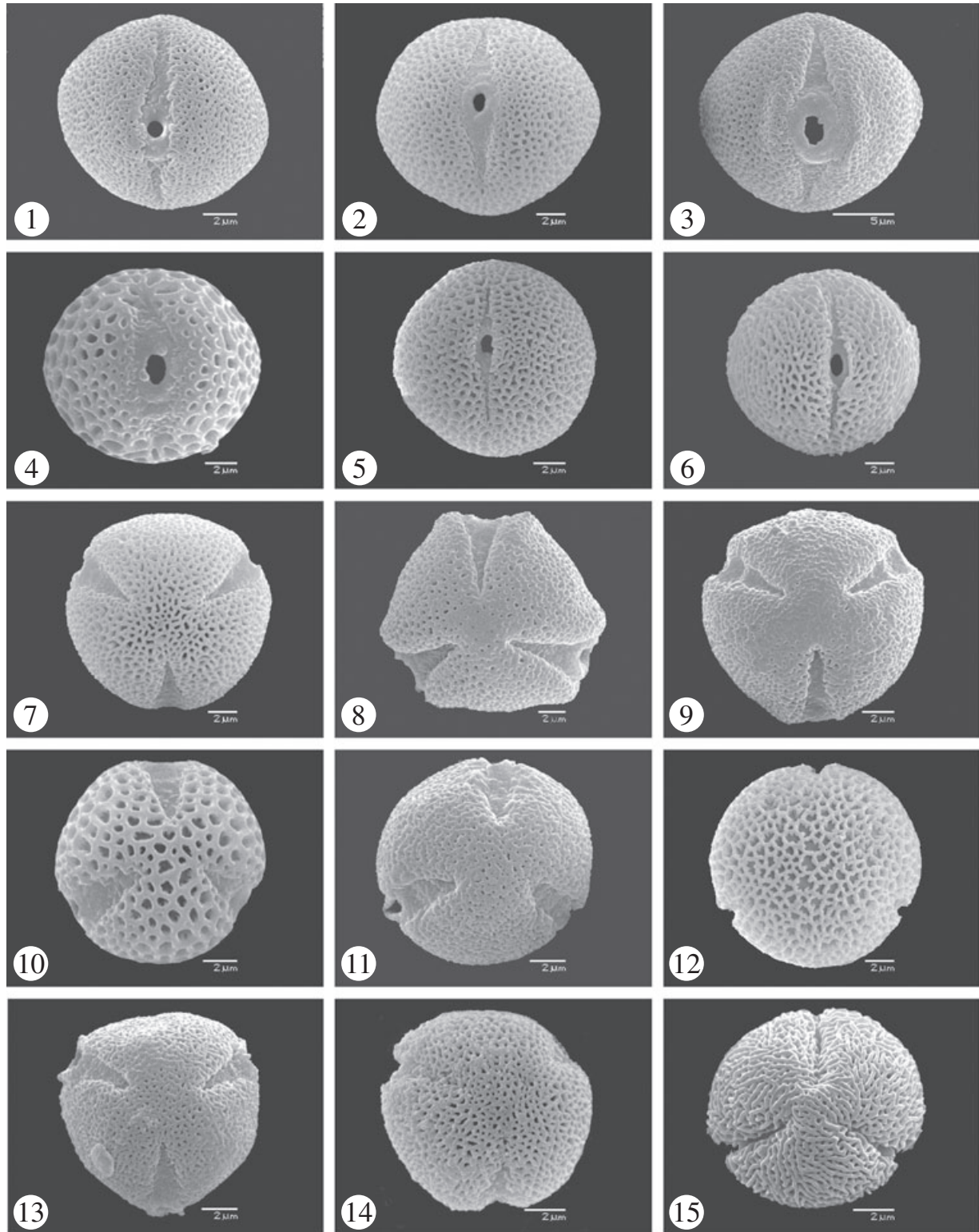
GENERIC POLLEN AND ORBICULE DESCRIPTIONS

Adina Salisb. (2/3) (Figs 1, 19): Pollen very small to small (P , 16–31 μm ; E , 15–24 μm); shape in equatorial view spheroidal, subprolate, or rarely prolate; amb circular to subtriangular. Apertures 3, colporate with long and narrow ectocolpi; colpus ends acute to obtuse; colpus membrane coarse to granular; mesoporus

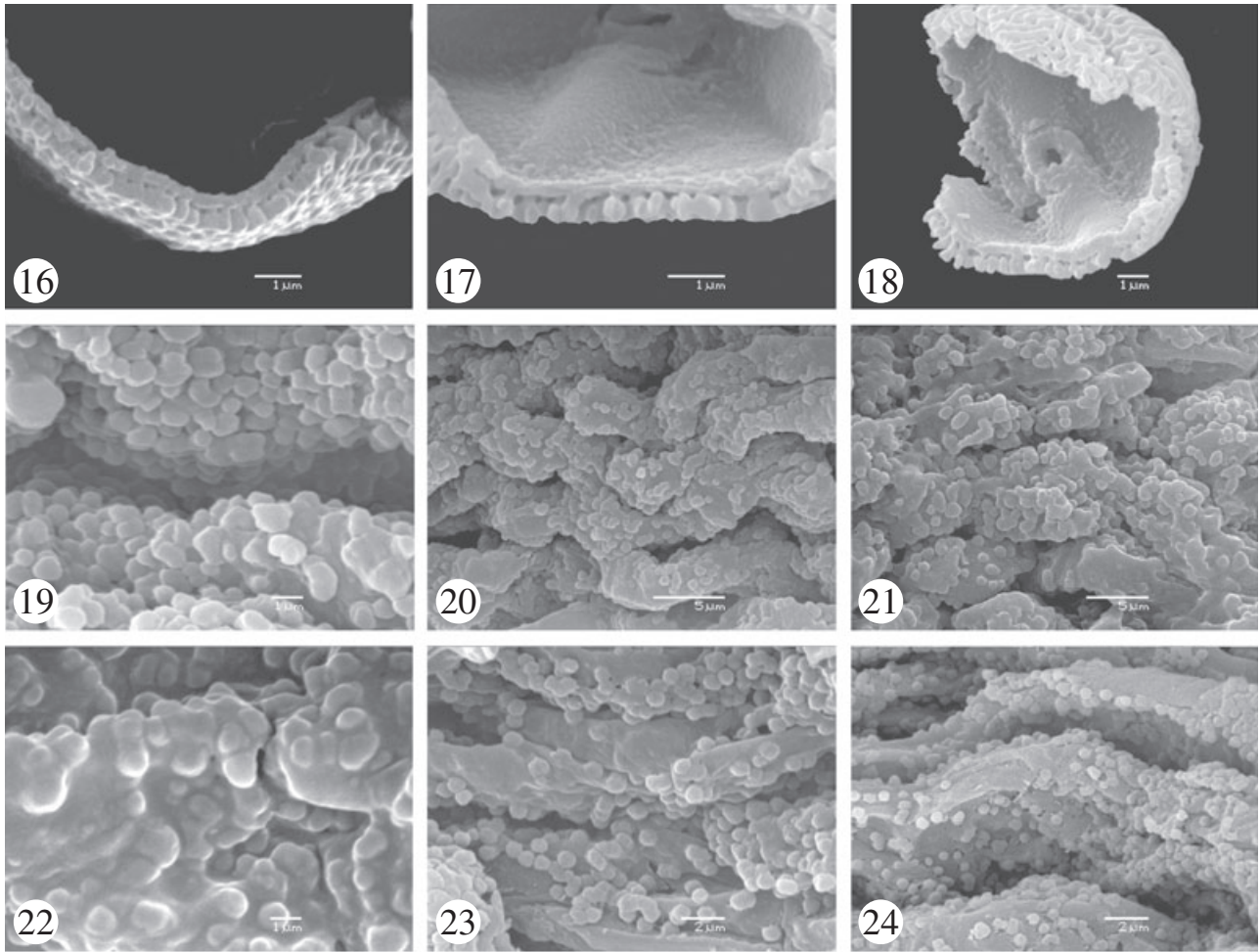
Table 1. Pollen morphological characters for each genus studied in the Naucleaceae and Hymenodictyeae

Genus	<i>P</i> value (μm)	<i>E</i> value (μm)	<i>P/E</i> value	Shape	Apocolpium index	Sexine ornamentation	Lumina size (μm)
Naucleaceae							
Subtribe Naucleinae							
<i>Burttidarya</i> (1/1)	16–(17.4)–19	18–(20.0)–23	0.80–(0.88)–1.00	SO–S	0.30	Microreticulate–rugulate	0.38–(0.65)–0.88
<i>Nauclea</i> (9/9)	13–(19.3)–25	16–(19.7)–24	0.72–(1.07)–1.18	SO–S–SP	0.36	Perforate–microreticulate	0.13–(0.35)–0.61
<i>Neolamarckia</i> (1/2)	13–(13.8)–15	14–(15.0)–16	0.87–(0.92)–1.00	SO–S	0.31	Microreticulate–rugulate	0.16–(0.22)–0.41
<i>Ochreinauclea</i> (1/2)	11–(11.6)–13	12–(12.5)–13	0.85–(0.93)–1.00	SO–S	0.31	Microreticulate	0.23–(0.40)–0.66
<i>Sarcocephalus</i> (2/2)	15–(16.5)–19	17–(18.0)–20	0.79–(0.89)–1.00	SO–S	0.31	Microreticulate–rugulate	0.28–(0.46)–0.67
Subtribe Adininae							
<i>Adina</i> s.s. (2/3)	16–(22.2)–31	15–(19.2)–24	0.94–(1.15)–1.41	S–SP–P	0.35	Rugulate–microreticulate	0.14–(0.29)–0.63
<i>Adinauclea</i> (1/1)	–	–	–	S	0.37	Rugulate	0.12–(0.19)–0.29
<i>Haldina</i> (1/1)	13–(14.5)–16	12–(13.9)–16	0.93–(1.09)–1.25	S–SP	0.25	Microreticulate	0.20–(0.32)–0.46
<i>Ludekia</i> (2/2)	11–(14.6)–19	11–(15.2)–21	0.85–(0.94)–1.15	(SO)–S(–SP)	0.30	(Micro)reticulate	0.65–(0.90)–1.23
<i>Metadina</i> (1/1)	11–(14.1)–19	12–(14.6)–17	0.81–(0.97)–1.13	SO–S	0.33	Perforate–microreticulate	0.13–(0.30)–0.60
<i>Myrmeconuclea</i> (3/3)	13–(17.5)–23	14–(18.4)–25	0.88–(0.93)–1.07	S	0.69	(Micro)reticulate	0.24–(0.51)–1.06
<i>Neonuclea</i> (8/65)	11–(17.0)–23	13–(17.7)–22	0.86–(0.96)–1.07	SO–S	0.45	Microreticulate	0.17–(0.39)–0.74
<i>Pertusadina</i> (2/4)	11–(13.0)–15	11–(13.4)–16	0.86–(0.98)–1.09	SO–S	0.45	Microreticulate	0.20–(0.40)–0.54
<i>Sinoadina</i> (1/1)	15–(16.4)–19	13–(14.9)–16	1.00–(1.10)–1.20	S–SP	0.32	Rugulate–striate	0.15–(0.31)–0.45
Subtribe Breoniinae							
<i>Breonadia</i> (1/1)	15–(16.1)–20	17–(17.9)–22	0.80–(0.90)–1.00	SO–S	0.33	Microreticulate	0.33–(0.48)–0.74
<i>Breonia</i> s.l. (4/20)	19–(20.4)–23	19–(20.2)–21	0.95–(1.01)–1.15	S–SP	0.39	Microreticulate	0.18–(0.32)–0.56
<i>Gyrostipula</i> (2/2)	13–(16.2)–19	13–(17.8)–21	0.81–(0.92)–1.00	SO–S	0.35	Microreticulate	0.14–(0.25)–0.54
<i>Janotia</i> (1/1)	11–(11.6)–13	12–(12.7)–13	0.85–(0.92)–1.00	SO–S	0.35	Perforate–microreticulate	0.22–(0.32)–0.55
Subtribe Cephalanthinae							
<i>Cephalanthus</i> (5/6)	20–(23.5)–25	19–(24.3)–27	0.88–(0.97)–1.09	S	0.27	Perforate	0.12–(0.35)–1.05
Subtribe Uncarinae							
<i>Uncaria</i> (5/34)	13–(15.9)–21	14–(15.3)–18	0.83–(1.04)–1.13	SO–S	0.37	Striato–microreticulate	0.17–(0.39)–0.65
Subtribe Corynantheinae							
<i>Corynanthe</i> (1/1)	12–(14.4)–16	13–(14.7)–16	0.87–(0.98)–1.07	(SO)–S	0.31	Perforate–microreticulate	0.11–(0.32)–0.50
<i>Pausinystalia</i> (3/5)	8–9	10–(12.8)–15	0.79–(0.91)–1.00	SO–S	0.36	Perforate–(micro)reticulate	0.20–(0.45)–1.19
<i>Pseudocinchona</i> (2/2)	13–(14.7)–18	14–(16.3)–19	0.83–(0.91)–1	SO–S	0.28	Perforate–microreticulate	0.10–(0.35)–0.70
Hymenodictyeae							
<i>Hymenodictyon</i> (4/26)	11–(17.3)–24	13–(17.6)–22	0.83–(0.97)–1.17	SO–S(–SP)	0.29	Perforate–microreticulate	0.12–(0.35)–0.80
<i>Paracorynanthe</i> (2/2)	15–(17.2)–18	17–(18.5)–20	0.84–(0.93)–1	SO–S	0.28	Perforate–microreticulate	0.10–(0.45)–0.83

–, data not available; *E*, equatorial diameter; *P*, prolate; *P*, prolate; *P*, polar axis diameter; S, spheroidal; SO, suboblate; SP, subprolate; x/y, number of species studied for each genus over total number of species in the genus.



Figures 1–15. Figs 1–6. Equatorial view of pollen grains. Figs 7–15. Polar view of pollen grains. Figure 1. *Adina pilulifera*. Figure 2. *Breonia macrocarpa*. Figure 3 *Cephalanthus tetrandra*. Figure 4. *Ludekia benardoi*. Figure 5. *Myrmeconuclea stipulacea*. Figure 6. *Uncaria africana*. Figure 7. *Breonia macrocarpa*. Figure 8. *Cephalanthus tetrandra*. Figure 9. *Gyrostipula comoriensis*. Figure 10. *Ludekia benardoi*. Figure 11. *Metadina trichotoma*. Figure 12. *Myrmeconuclea stipulacea*. Figure 13. *Neolamarckia cadamba*. Figure 14. *Pertusadina eurhyncha*. Figure 15. *Uncaria africana*.



Figures 16–24. Figs 16–18. Broken pollen grains, showing pollen wall stratification, H-shaped endoaperture, and granular endo-ornamentation. Figs 19–24. Orbicule morphology. Figure 16. *Cephalanthus salicifolius*. Figs 17, 18. *Uncaria africana*. Figure 19. *Adina rubella*. Figure 20. *Adinauclea fagifolia*. Figure 21. *Cephalanthus tetrandra*. Figure 22. *Gyrostipula comoriensis*. Figure 23. *Janotia macrostipula*. Figure 24. *Metadina trichotoma*.

circular to slightly lologate without distinct annulus; endoaperture unknown. Sexine pattern rugulate to microreticulate, without differentiation towards colpi or poles; supratectal elements absent; lumina 0.14–0.63 µm in diameter.

Orbicules present, 1 µm or less in diameter, spheroidal, sometimes slightly lobed, without central perforation, not embedded in the locule wall; surface smooth (Fig. 19).

Adinauclea Ridsdale (1/1) (Fig. 20): Pollen very small (no measurements); shape in equatorial view spheroidal; amb circular. Apertures 3, colporate with long ectocolpi; colpus ends acute; colpus membrane coarse; mesoporus not observed; endoaperture unknown. Sexine pattern distinctly rugulate with interwoven rugae, without differentiation towards colpi or poles; supratectal elements absent; lumina 0.12–0.29 µm in diameter.

Orbicules present, 1 µm or less in diameter, spheroidal without central perforation, sometimes aggregated, not or only slightly embedded in the locule wall; surface smooth (Fig. 20).

Breonadia Ridsdale (1/1): Pollen very small to small (*P*, 15–20 µm; *E*, 17–22 µm); shape in equatorial view suboblate to spheroidal; amb circular. Apertures 3, colporate with long and narrow ectocolpi; colpus ends acute; colpus membrane granular; mesoporus circular, without distinct annulus; endoaperture present, H-shaped. Sexine pattern microreticulate, without differentiation towards colpi, but lumina sometimes slightly smaller at poles; supratectal elements absent; lumina 0.33–0.74 µm in diameter.

Orbicules: no observations.

Breonia A. Rich. ex DC. (4/20) (Figs 2, 7): Pollen very small to small (*P*, 19–23 µm; *E*, 19–21 µm);

shape in equatorial view spheroidal to subprolate; amb circular. Apertures 3, colporate, with long and narrow ectocolpi; colpus ends mostly acute; colpus membrane granular; mesoporus circular to slightly lolongate with a distinct, more or less smooth annulus; endoaperture unknown. Sexine pattern microreticulate, without differentiation towards colpi or poles; suprategal elements absent; lumina 0.18–0.56 µm in diameter.

Orbicules present, 1 µm or less in diameter, often in aggregates of several orbicules; individual orbicules spheroidal, often slightly embedded in the locule wall; surface smooth.

Burttidavya Hoyle (1/1): Pollen very small to small (*P*, 16–19 µm; *E*, 18–23 µm); shape in equatorial view suboblate to spheroidal; amb circular. Apertures 3, colporate with long ectocolpi; colpus ends acute to obtuse; colpus membrane granular; mesoporus circular to slightly lolongate, sometimes with an annulus; endoaperture unknown. Sexine pattern microreticulate to rugulate, without differentiation towards colpi or poles; suprategal elements absent; lumina 0.38–0.88 µm in diameter.

Orbicules present, 0.80–1.90 µm in diameter, irregularly shaped, not embedded in the locule wall; surface folded (Huysmans *et al.*, 1997: figs 22, 23).

Cephalanthus L. (5/6) (Figs 3, 8, 16, 21): Pollen very small to small (*P*, 20–25 µm; *E*, 19–27 µm); shape in equatorial view spheroidal; amb circular to subtriangular. Apertures 3, colporate with long ectocolpi; colpus ends acute; colpus membrane granular; mesoporus lolongate with annulus; endoaperture unknown. Sexine pattern perforate, sometimes microreticulate, with perforations/lumina slightly smaller towards colpi and at poles; suprategal elements absent; perforations/lumina 0.12–1.05 µm in diameter (the largest lumina were measured in *Cephalanthus glabratus* K. Schum.).

Orbicules present, smaller than 1 µm in diameter, spheroidal, sometimes in aggregates, not embedded in locule wall; surface smooth (Fig. 21).

Corynanthe Welw. (1/1): Pollen very small (*P*, 12–16 µm; *E*, 13–16 µm); shape in equatorial view spheroidal or more rarely suboblate; amb circular with sunken colpi. Apertures 3, colporate, with long and narrow ectocolpi; colpus ends obtuse; colpus membrane granular; mesoporus lolongate, without distinct annulus; endoaperture H-shaped with costa. Sexine pattern perforate to microreticulate, without differentiation towards colpi or poles; suprategal elements absent; perforations/lumina 0.11–0.50 µm in diameter.

Orbicules: no observations.

Gyrostipula J.-F. Leroy (2/2) (Figs 9, 22): Pollen very small (*P*, 13–19 µm; *E*, 13–21 µm); shape in equatorial view suboblate to spheroidal; amb circular. Apertures 3, colporate, with long and narrow ectocolpi; colpus ends acute; colpus membrane granular; mesoporus circular to lolongate, without or with indistinct annulus; endoaperture unknown. Sexine pattern microreticulate without differentiation towards colpi or poles, sometimes with interwoven muri; suprategal elements absent; lumina 0.14–0.54 µm in diameter.

Orbicules present, 1 µm or less in diameter, sometimes in small aggregates, spheroidal, slightly embedded in the locule wall; surface smooth (Fig. 22).

Haldina Ridsdale (1/1): Pollen very small (*P*, 13–16 µm; *E*, 12–16 µm); shape in equatorial view spheroidal to subprolate; amb circular. Apertures 3, colporate, with long and narrow ectocolpi; colpus ends acute; colpus membrane granular but smooth around mesoporus; mesoporus circular to slightly lolongate without distinct annulus; endoaperture faint, H-shaped with costa; inner nexine surface granular. Sexine pattern microreticulate, without differentiation towards colpi, with the lumina somewhat smaller at the poles; suprategal elements absent; lumina 0.20–0.46 µm in diameter.

Orbicules: no observations.

Hymenodictyon Wall. (4/26): Pollen very small to small (*P*, 11–24 µm; *E*, 13–22 µm); shape in equatorial view suboblate to spheroidal, rarely subprolate; amb circular or subtriangular. Apertures 3 or exceptionally 4, colporate, with long and narrow ectocolpi; colpus ends obtuse; colpus membrane granular; mesoporus circular to slightly lolongate or lalongate with or without annulus; endoaperture faint, probably H-shaped, with costa. Sexine pattern perforate or microreticulate, sometimes with smaller perforations/lumina towards the colpi and at the poles; suprategal elements absent; perforations/lumina 0.12–0.80 µm in diameter.

Orbicules: no observations.

Janotia J.-F. Leroy (1/1) (Fig. 23): Pollen very small (*P*, 11–13 µm; *E*, 12–13 µm); shape in equatorial view suboblate to spheroidal; amb circular. Apertures 3, colporate, with long and narrow ectocolpi; colpus ends unknown; colpus membrane unknown; mesoporus unknown; endoaperture unknown. Sexine pattern microreticulate to perforate; suprategal elements absent; perforations/lumina 0.22–0.55 µm in diameter.

Orbicules present, smaller than 1 µm, spheroidal, sometimes in aggregates, not embedded in locule wall; surface smooth (Fig. 23).

Ludekia Ridsdale (2/2) (Figs 4, 10): Pollen very small (P , 11–19 μm ; E , 11–21 μm); shape in equatorial view spheroidal, rarely suboblate or subprolate; amb circular. Apertures 3, colporate, with relatively broad and long ectocolpi; colpus ends acute; colpus membrane coarse; mesoporus with rather distinct annulus; endoaperture unknown. Sexine pattern (micro)reticulate, without differentiation towards colpi or poles; supracteal elements absent; lumina 0.65–1.23 μm in diameter.

Orbicules present, 1 μm or less in diameter, spheroidal, often in aggregates, embedded in locule wall; surface smooth.

Metadina Bakh. f. (1/1) (Figs 11, 24): Pollen very small (P , 11–19 μm ; E , 12–17 μm); shape in equatorial view suboblate to spheroidal; amb circular. Apertures 3, colporate, with relatively broad ectocolpi; colpus ends obtuse to acute; colpus membrane coarse; mesoporus circular to lolongate with or without distinct annulus; endoaperture unknown. Sexine pattern perforate to microreticulate, sometimes with interwoven muri, without differentiation towards colpi or poles; supracteal elements absent; perforations/lumina 0.13–0.60 μm in diameter.

Orbicules present, smaller than 1 μm , spheroidal, never distinctly aggregated and lying freely upon locule wall; surface smooth (Fig. 24).

Myrmeconauclaea Ridsdale (3/3) (Figs 5, 12): Pollen very small to small (P , 13–23 μm ; E , 14–25 μm); shape in equatorial view spheroidal; amb circular. Apertures 3, colporate, with narrow and relatively short ectocolpi; colpus ends acute; colpus membrane usually smooth, sometimes slightly granular; mesoporus circular or slightly lolongate, without annulus; endoaperture indistinct. Sexine pattern microreticulate, without differentiation towards colpi or poles; supracteal elements absent; lumina 0.24–1.06 μm in diameter (the largest lumina were measured in *Myrmeconauclaea stipulacea* Ridsdale).

Orbicules: no observations.

Nauclea Korth. (9/9): Pollen very small to small (P , 13–25 μm ; E , 16–24 μm); shape in equatorial view spheroidal, suboblate, or subprolate; amb circular. Apertures 3, colporate, with long and narrow ectocolpi; colpus ends acute; colpus membrane coarse or granular; mesoporus circular to lolongate with indistinct annulus (mesoporus often remarkably large in *Nauclea officinalis*); endoaperture unknown. Sexine pattern microreticulate to perforate, often with interwoven muri, without differentiation towards colpi or poles; supracteal elements absent; perforations/lumina 0.13–(0.35)–0.61 μm in size (the largest lumina were found in *Nauclea*

parva Merr., the smallest ones in *Nauclea tenuiflora* Merr.).

Orbicules present, 0.60–1.10 μm in diameter, mostly spheroidal, sometimes with perforations, sometimes in small aggregates; surface smooth (Huysmans *et al.*, 1997: figs 24, 25).

Neolamarckia Bosser (1/2) (Fig. 13): Pollen very small (P , 13–15 μm ; E , 14–16 μm); shape in equatorial view spheroidal or sometimes suboblate; amb circular to subtriangular. Apertures 3, colporate with long ectocolpi; colpus ends obtuse or acute; colpus membrane granular; mesoporus lolongate with pronounced smooth annulus; endoaperture unknown. Sexine pattern microreticulate to slightly rugulate, without differentiation towards colpi or poles; supracteal elements absent; lumina 0.16–0.41 μm in diameter.

Orbicules: no observations.

Neonauclea Merr. (8/65): Pollen very small to small (P , 11–23 μm ; E , 13–22 μm); shape in equatorial view suboblate to spheroidal; amb circular to subtriangular. Apertures 3, colporate (in *Neonauclea forsteri*, a single four-colporate grain has been observed) with long or short (*Neonauclea borneensis* Ridsdale, *Neonauclea excelsa* Merr., *Neonauclea formicaria* Merr., and *Neonauclea forsteri*) and narrow ectocolpi; colpus ends acute; colpus membrane smooth or finely granular; mesoporus circular without annulus; endoaperture indistinct, H-shaped, with costa in *Neonauclea forsteri*. Sexine pattern microreticulate, without differentiation towards the colpi, sometimes changing to perforate at poles; lumina 0.17–0.74 μm in diameter (the largest lumina were found in *Neonauclea formicaria*).

Orbicules: no observations.

Ochreinauclea Ridsdale & Bakh. f. (1/2): Pollen very small (P , 11–13 μm ; E , 12–13 μm); shape in equatorial view suboblate to spheroidal; amb circular. Apertures 3, colporate, with long and narrow ectocolpi; colpus ends acute or obtuse; colpus membrane coarsely granular; mesoporus circular to lolongate, rather large (> 2 μm), usually with an annulus; endoaperture unknown. Sexine pattern microreticulate to slightly rugulate, with interwoven muri without differentiation towards colpi or poles, or lumina slightly smaller at poles; supracteal elements absent; lumina 0.23–0.66 μm in size.

Orbicules: no observations.

Paracorynanthe R. Capuron ex Bosser (2/2): Pollen very small (P , 15–18 μm ; E , 17–20 μm); shape in equatorial view spheroidal or sometimes suboblate; amb subtriangular. Apertures 3, colporate, with long and narrow ectocolpi; colpus ends acute or obtuse; colpus

membrane granular; mesoporus slightly lalongate with distinct annulus; endoaperture unknown. Sexine pattern perforate to microreticulate, with perforations/lumina smaller towards colpi and at the poles; supracteal elements absent; perforation/lumina 0.10–0.83 µm in diameter.

Orbicules: no observations.

Pausinystalia Pierre ex Dupouy & Beille (3/5): Pollen very small (*P*, 8–13 µm; *E*, 10–15 µm); shape in equatorial view suboblate or spheroidal; amb circular or subtriangular. Apertures 3, colporate with long ectocolpi; colpus ends obtuse; colpus membrane slightly granular; mesoporus circular, often not as wide as ectocolpus, without clear annulus; endoaperture distinct, H-shaped. Sexine pattern (micro)reticulate or perforate, with the lumina decreasing in size towards the colpi, sometimes also smaller at the poles; supracteal elements absent; lumina 0.20–1.19 µm in diameter (remarkably large lumina were observed in *Pausinystalia lane-poolei* (Hutch.) Hutch. ex Lane-Poole ssp. *lane-poolei*).

Orbicules: no observations.

Pertusadina Ridsdale (2/4) (Fig. 14): Pollen very small (*P*, 11–15 µm; *E*, 11–16 µm); shape in equatorial view spheroidal, sometimes suboblate; amb circular. Apertures 3, colporate with long and narrow ectocolpi; colpus ends acute; colpus membrane granular; mesoporus circular to slightly lalongate with indistinct smooth annulus; endoaperture unknown. Sexine pattern microreticulate, sometimes with interwoven muri, without differentiation towards colpi but often with slightly smaller lumina at poles; lumina 0.20–0.54 µm in diameter.

Orbicules: no observations.

Pseudocinchona A. Chev. ex E. Parrot (2/2): Pollen very small (*P*, 13–18 µm; *E*, 14–19 µm); shape in equatorial view spheroidal or suboblate; amb circular with sunken colpi. Apertures 3, colporate, with long and narrow ectocolpi; colpus ends obtuse or acute; colpus membrane granular; mesoporus lalongate, with smooth annulus; endoaperture H-shaped with costa. Sexine pattern perforate to almost microreticulate, with perforations smaller towards colpi and poles; supracteal elements absent; perforations/lumina 0.10–0.70 µm in diameter.

Orbicules: no observations.

Sarcocephalus Afzel. ex R. BR. (2/2): Pollen very small (*P*, 15–19 µm; *E*, 17–20 µm); shape in equatorial view suboblate to spheroidal; amb circular or subtriangular. Apertures 3, colporate, with long and narrow ectocolpi; colpus ends acute, sometimes slightly obtuse; colpus membrane granular; mesoporus

circular to lalongate, mostly with distinct annulus; endoaperture presumably lalongate in *Sarcocephalus pobeginii* Hua ex Pobég. Sexine pattern microreticulate to rugulate, with interwoven muri, without differentiation towards colpi or poles; lumina 0.28–0.67 µm in size.

Orbicules: no observations.

Sinoadina Ridsdale (1/1): Pollen very small (*P*, 15–19 µm; *E*, 13–16 µm); shape in equatorial view spheroidal to subprolate; amb circular. Apertures 3, colporate with long and narrow ectocolpi; colpus ends acute; colpus membrane granular; mesoporus circular to slightly lalongate, without annulus; endoaperture unknown. Sexine pattern rugulate to almost striate, without differentiation towards colpi or poles; lumina 0.15–0.45 µm in size.

Orbicules: no observations.

Uncaria Schreb. (5/34) (Figs 6, 15, 17, 18): Pollen very small (*P*, 13–21 µm; *E*, 14–18 µm); shape in equatorial view spheroidal to suboblate; amb circular with sunken colpi. Apertures 3, colporate with long and very narrow ectocolpi; colpus ends acute; colpus membrane granular with smooth zone around mesoporus; mesoporus lalongate, without annulus; endoaperture, H-shaped with costa. Sexine pattern microreticulate with interwoven muri or rugulate with slender and long striae on reticulum, without differentiation towards colpi or poles; supracteal elements absent; lumina 0.17–0.65 µm in size.

Orbicules: no observations.

DISCUSSION

TRIBAL LEVEL

Naucleaeae are fairly stenopalynous; especially in pollen size and shape, number and type of apertures, and nexine characteristics, the variation is limited. The only pollen character that varied considerably within the tribe was the sexine ornamentation. Perforate, rugulate, microreticulate, and reticulate sexine patterns were observed. The pollen morphologies of the genera that were transferred from Coptosapelteae *sensu* Andersson & Persson (1991) (*Mitragyna sensu* Ridsdale, *Uncaria*, *Corynanthe*, and *Pausinystalia*) and Cephalantheae (*Cephalanthus*) to Naucleaeae by Razafimandimbison & Bremer (2001) agreed well with the pollen found in Naucleaeae *sensu* Ridsdale (1978). The emendation of Naucleaeae proposed by Razafimandimbison & Bremer (2001, 2002) is thus not in conflict with the pollen morphological evidence. However, a pollen morphological synapomorphy for Naucleaeae could not be found. Indeed, most pollen features observed in Naucleaeae were also commonly found in

other genera of Rubiaceae. Tricolporate pollen was the basic pollen type in Rubiaceae (Dessein *et al.*, 2005a). A tendency towards pluriaperturate grains, as found in the tribes Psychotriaceae (Johansson, 1992), Iseritaceae (Huysmans *et al.*, 1998), Rubiaceae (Huysmans *et al.*, 2003), and Spermaceae (Dessein *et al.*, 2002, D2005b) was not seen in Naucleaceae.

The pollen morphology of Hymenodictyeae was very similar to that of Naucleaceae. The sister group relationship between the two tribes seemed to be supported by the shared presence of the H-shaped endoapertures. Our palynological evidence added support for merging Hymenodictyeae in Naucleaceae, as proposed by Andersson & Antonelli (2005).

The H-shaped endoapertures observed in both Naucleaceae and Hymenodictyeae have only been recorded in two distant Rubiaceae genera: *Burchellia* (Gardenieae, Ixoroideae) and *Molopanthera* (associated with Henriquezieae, Ixoroideae; Rova *et al.*, 2002) (Dessein *et al.*, 2005a).

Orbicules were found in all ten genera investigated. They were small, spheroidal, with a smooth surface, and could be aggregated and slightly embedded in the locular wall. The orbicules could also have a folded surface, as observed in *Burttidavya nyasica* (Naucleinae). According to the typology of Huysmans *et al.* (1997), Vinckier *et al.* (2000), and Vinckier & Smets (2002), the orbicules found in Naucleaceae can be classified as type IIIa (smooth, spheroidal orbicules without a central indentation) and type IV (angular, irregularly folded orbicules) in *Burttidavya*. Type IIIa orbicules were the most common type in both Cinchonoideae and Ixoroideae. They occurred in Pavetteae (Vinckier *et al.*, 2000), Cinchoneae, Iseritaceae, and Rondeletiaceae (Huysmans *et al.*, 1997). The irregularly folded orbicules of type IV have so far only been observed in *Burttidavya* of Naucleaceae and *Alibertia pilosa* of Gardenieae (Vinckier *et al.*, 2000).

INFRATRIBAL AND GENERIC LEVEL

The second aim of this article was to verify whether the recently modified subtribal concepts proposed by Razafimandimbison & Bremer (2002) were supported by palynological data. Therefore, we attempted to identify pollen morphological synapomorphies that supported the current division of Naucleaceae into seven subtribes. We also wished to determine whether the Naucleaceae genera could be distinguished on the basis of pollen morphology alone. Very few if any characters could be detected to support the subtribal and generic delimitations.

In the subtribe Naucleinae, three different sexine ornamentations were observed. Microreticulate patterns were most common, but a tendency towards rugulate patterns was observed in the genera *Burttidavya*,

Neolamarckia, and *Sarcocephalus*. The largest grains were found in *Nauclea*, where subprolate grains were also observed. The lumina size was largest in the genus *Burttidavya*.

Adininae was heterogeneous in pollen size (largest grains in *Adina*) and sexine ornamentation. The genus *Ludekia* was remarkable because of its larger lumina compared with the other genera. It was the only genus, together with *Pausinystalia* (Corynantheinae), in which a reticulate sexine pattern was detected. Pollen shape was variable and ranged from suboblate to prolate in *Adina*. The tendency towards rugulate patterns was also observed in this subtribe, namely in *Adina*, *Adinauclea*, and *Sinoadina*.

Breonia was exceptional in the subtribe Breoniinae because it had larger pollen than the other three genera (*Breonadia*, *Gyrostipula*, and *Janotia*), with a tendency towards a subprolate shape.

The single genus *Cephalanthus* in the subtribe Cephalanthinae had an equatorial diameter exceeding 25 µm. As a consequence, this subtribe was characterized by the largest pollen grains in the tribe.

Both *Mitragyna s.s.* and *Hallea* had the H-shaped endoapertures found in the other Naucleaceae and Hymenodictyeae taxa investigated. They could not be distinguished on the basis of their pollen morphologies. The subtribe Mitragyninae does not seem to have any pollen synapomorphies.

The genus *Uncaria* in the subtribe Uncarinae was characterized by a tendency towards rugulate sexine patterns and subprolate grains. Yet, it should be mentioned that only four of the 34 species of the genus *Uncaria* were studied.

Pausinystalia of the subtribe Corynantheinae had the smallest pollen in the tribe (not exceeding 15 µm). The pollen grains of the other two genera of this subtribe, *Corynanthe* and *Pseudocinchona*, were slightly larger. The sexine pattern was often (micro)reticulate.

The resurrection of the genus *Pseudocinchona*, making *Corynanthe* monospecific, as proposed by Razafimandimbison & Bremer (2002), was not supported or contradicted by pollen morphology.

CONCLUSION

Naucleaceae is a stenopalynous tribe, characterized by very small to small, tricolporate pollen with an ectocolpus, a circular to lolate mesoporus, and an often H-shaped endoaperture. Sexine patterns are perforate, (micro)reticulate, or somewhat rugulate. Orbicules are present; they are small, spheroidal, and with a smooth surface. They belong to type IIIa and type IV. Our pollen morphological observations are not in conflict with a recent widened delimitation of Naucleaceae. H-shaped endoapertures probably form a synapomorphy for the Naucleaceae–Hymenodictyeae clade. Pollen

morphology is of little value in distinguishing the subtribes and genera of Naucleaeae.

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SPECIMENS EXAMINED

Collections indicated with an asterisk were studied by Suzy Huysmans and presented in her Master's thesis (Huysmans, 1993). 'SF' stands for 'Service Forestier'.

Adina pilulifera (Lam.) Franch. ex Drake, *Clemens & Clemens* 3066 (A), Vietnam; *Tsang* 29239 (L.), Vietnam; ***Adina rubella*** Hance, *Wilson* 1949 (A), China.

Adinauclea fagifolia (Teijsm. & Binn. ex Havil.) Ridsdale, *Van Steenis* 1939 (A), Indonesia.

Breonadia salicina (Vahl) Hepper & J.R.I.Wood, *de Koning et al.* 8640 (BR), Mozambique; *Antanimilanja* 4587 (L.), Madagascar.

Breonia fragifera Capuron ex Razafim., *Perrier de la Bâthie* 14215bis (P), Madagascar; ***Breonia macrocarpa*** Homolle, *SF-18116* (P), Madagascar; ***Breonia richardsonii*** Razafim., *SF-14359* (TEF), Madagascar; ***Breonia decaryana*** Homolle, *Razafimandimbison* 257 (TAN), Madagascar.

Burttavya nyasica Hoyle, *Schlieben* 5581 (BR), Tanzania.

Cephalanthus glabratus (Spreng.) K. Schum., *Zardini & Valazques* 18861 (MO, AS), Paraguay;

Cephalanthus natalensis Oliv., *Bolus* 7679 (MO), South Africa; ***Cephalanthus occidentalis*** L., *Flores & Martinez* 1724 (MO); ***Cephalanthus salicifolius*** Humb. & Bonpl., *Meyerand & Rogers* 1889 (MO); ***Cephalanthus tetrandra*** (Roxb.) Ridsdale & Bakh. f., *collector unknown s. n.* (MO).

Corynanthe paniculata Welw., *Cabra* 43 (BR), R. D. Congo, *Dechamps* 92* (BR), R. D. Congo; *Donis* 2404* (BR), R. D. Congo; *Toussaint* 2194* (BR), R. D. Congo, *Wagemans* 2123* (BR), R. D. Congo.

Gyrostipula comoriensis J.-F. Leroy, collector unknown 16 (P), Comoro Islands; ***Gyrostipula foveolata*** (Capuron) J.-F. Leroy, *Razafimandimbison* 270 (TAN, MO), Madagascar; *SF-27633* (TEF), Madagascar.

Haldina cordifolia (Roxb.) Ridsdale, *Abdul Rashid Manhas s. n.* (BR-S.P. 862493), India; *Geesink et al.* 6726 (L.), Thailand; *Shaik Mokim s. n.* (L.), Burma.

Hymenodictyon biafranum Hiern, *Thomas* 422* (VR), Cameroon; ***Hymenodictyon decaryii*** Homolle, *Phillipson* 2778* (VR), Madagascar; ***Hymenodictyon orixense*** (Roxb.) Mabb., *Fox in PNH* 4795* (BR), Philippines; ***Hymenodictyon floribundum*** (Hochst. & Steud.) B. L. Robinson, *Runyingya* 603* (BR), Rwanda.

Janotia macrostipula (Capuron) J.-F. Leroy, *SF-2071* (TAN, MO), Madagascar.

Ludekia bernardoi (Merr.) Ridsdale, *Sulit* 22889 (A), Philippines; *Ramos* 1707 (L.), Philippines; ***Ludekia borneensis*** Ridsdale, *Yii Puan Ching* *S40171* (L.), Borneo.

Metadina trichotoma (Zoll. & Moritzi) Bakh. f., *Bapel* 1909 (S), Burma; *Merrill* 9383 (MO), Philippines; *Krukoff* 4128 (BR), Sumatra; *Parkinson* 4350 (MO), Thailand; *Maxwell* 88-228 (L.), Thailand.

Myrmeconauclea stipulacea Ridsdale, *Chai* *S37274* (L.), Malaysia; ***Myrmeconauclea strigosa*** (Korth.) Merr., *Slooten* 2229 (L.), Borneo; *Ridsdale* 1954 (L.), Malaysia; ***Myrmeconauclea sp.***, *Kostermans* 12872 (L.), Borneo.

Nauclea diderrichii (De Wild.) Merr., *Louis* 6123 (BR), R. D. Congo; *Letouzey* 5190 (BR), Cameroun; ***Nauclea officinalis*** (Pierre ex Pit.) Merr. & Chun, *Smitinand & Abbe* 6274 (L.), Thailand; ***Nauclea orientalis*** (L.) L., *Soejarto et al.* 8438 (L.), Philippines; *Kostermans & Kuswata* 68 (L.), Indonesia; ***Nauclea parva*** (Havil.) Merr., *Haviland* 134 (L.), Indonesia; ***Nauclea robinsonii*** Merr., *Mendoza* 61-450 (L.), Philippines; ***Nauclea subdita*** (Korth.) Steud., *Endert* 4085 (L.), Indonesia, *Thakur Rup Chad* 6173 (L.), India; ***Nauclea tenuiflora*** (Havil.) Merr., *Sauveur* 3348 (L.), New Guinea; ***Nauclea vanderguchtii*** (De Wild.) E. M. A. Petit, *Le Testu* 8308 (BR), Gabon; *Louis* 13736 (BR), R. D. Congo; ***Nauclea xanthoxylon*** (A. Chev.) Aubrév., *Tisserant* 931 (BR), R. D. Congo.

Neolamarckia cadamba (Roxb.) Bosser, *de Wilde & de Wilde* 16959 (MO), Indonesia.

Neonauclea bartlingii (DC.) Merr., *Mendoza* 18462 (L.), Philippines; ***Neonauclea borneensis*** Ridsdale, *Othman et al.* *S41394* (L.), Malaysia; ***Neonauclea calycina*** (Bartl. ex DC.) Merr., *Schmutz* 3370 (L.), Lesser Sunda; *Krukoff* 4098 (BR), Sumatra; ***Neonauclea excelsa*** (Blume) Merr., *de Wilde & de Wilde-Duyffes* 20499 (L.), Indonesia; ***Neonauclea formicaria*** (Elmer) Merr., *Madulid et al.* 117820 (L.), Philippines; ***Neonauclea forsteri*** (Seem. ex Havil.) Merr., *Maurias & collectors* 13859 (L.), Solomon Islands, *Moerenhout* 1831-4 (BR); *W. Beer's collectors* 7271 (L.), Solomon Islands; ***Neonauclea maluensis*** (Valeton) S. Moore, *Kalkman* B3688 (BR), New Guinea; ***Neo-***

nauclea obversifolia (Valeton) Merr. & L.M.Perry, *Kalkman BW3688* (L.), New Guinea.

Ochreinauclea maingayii (Hook. f) Ridsdale, *Kunstler 6424* (BR), Malaysia, *Boschproefstation 794* (L.), Indonesia.

Paracorynanthe antankarana Capuron ex J.-F. Leroy, *SF-28718** (P), Madagascar; *Paracorynanthe uropetala* Capuron, *SF-6798** (P).

Pausinystalia johimbe (Schumann) Pierre ex Beille, *Le Testu 9075** (BR), Gabon; *Zenker 1059** (WAG), Cameroon; *Zenker 2883** (BR), Cameroon; *Kennedy 2071** (BR), Nigeria; *Pausinystalia lane-polei* (Hutch.) Hutch. ex Lane-Poole *ssp. ituriense* (De-Wild.) Stoffelen & Robbr., *Bequaert 2543** (BR), R. D. Congo; *Gutzwiller 3753** (BR), R. D. Congo; *Gutzwiller 3720** (BR), R. D. Congo; *Le Testu 8029** (BR), Gabon; *Michelson 722** (BR), R. D. Congo; *Michelson 1014** (BR), R. D. Congo; *Michelson 1035** (BR), R. D. Congo; *Pierlot 792** (BR), R. D. Congo; *Pierlot 1048** (BR), R. D. Congo; *Pausinystalia lane-polei* (Hutch.) Hutch. ex Lane-Poole *ssp. lane-polei*, *Voorhoeve 133* (BR), Liberia; *Pausinystalia macroceras* (K. Schum.) Pierre ex Beille, *Bequaert 6694** (BR), R. D. Congo; *Gérard 3900** (BR), R. D. Congo; *Hart 289** (BR), R. D. Congo; *Hart 862** (BR), R. D. Congo; *Louis 4124** (BR), R. D. Congo; *Toussaint 2175** (BR), R. D. Congo; *Le Testu 8984* (BR), Gabon; *Pausinystalia talbotti* Wernham, *Thomas 6843** (BR), Cameroon; *Thomas & Nemba 5903** (WAG).

Pertusadina eurhyncha (Miq.) Ridsdale, *Boschproefstation 24 E3 P.T 844* (L.), Indonesia; *Dr King's Collector 7850* (BR), Malay Peninsula; *Pertusadina multiflora* (Havil.) Ridsdale, *Elmer 15428* (L.), Philippines.

Pseudocinchona mayumbensis (Good) Raym.-Hamet, *Louis 1737** (WAG), Gabon; *Louis 2095** (WAG), Gabon; *Wilks 1038** (WAG), Gabon; *Wilks 820** (WAG), Gabon; *Pseudocinchona pachyceras* A. Chev., *Evrard 1899** (BR), R. D. Congo; *Lebrun 1542** (BR), R. D. Congo.

Sarcocephalus latifolius (Sm.) E. A. Bruce, *Lebrun 2300* (BR), R. D. Congo; *Billiet & Jadin 4069* (BR), R. D. Congo; *Sarcocephalus pobeguini* Hua ex Pobég., *Malaisse 4608* (BR), R. D. Congo; *Bruneel 1906* (BR), R. D. Congo.

Sinoadina racemosa (Siebold & Zucc.) Ridsdale, *Pierot s.n. (BR-S.P. 862487)*, Japan; *von Siebold s.n.* (L.), Japan; *Textor s.n.* (L.), Japan.

Uncaria africana G. Don, *Mwangulango & Leliyo 371* (BR), Tanzania; *Friis et al. 7144* (BR), Ethiopia; *Friis et al. 565** (BR), Ethiopia; *Kisena 1472* (BR), Tanzania; *Toussaint 476* (BR), Ethiopia; *Evrard 2057* (BR), R. D. Congo; *Ridsdale, Letouzey 6111* (BR), Cameroon; *Le Testu 1138* (BR), Gabon; *Uncaria gambir* (Hunter) Roxb., *Corbisier-Baland 2055** (BR), R. D. Congo; *Uncaria guianensis* (Aubl.) J. F. Gmel.,

Taylor et al. 12870 (MO), Guiana; *Uncaria hirsuta* Havil., *Odashima 17849** (BR), Taiwan, *Uncaria talbotii* Wernham, *de Wilde 1076* (BR), Ivory Coast.

REFERENCES

- Andersson L. 1995. Tribes and genera of the Cinchoneae complex (Rubiaceae). *Annals of the Missouri Botanical Garden* **82**: 409–427.
- Andersson L, Antonelli A. 2005. Phylogeny of the tribe Cinchoneae (Rubiaceae), its position in Cinchonoideae, and description of a new genus, *Ciliosemina*. *Taxon* **54**: 17–28.
- Andersson L, Persson C. 1991. Circumscription of the tribe Cinchoneae (Rubiaceae) – a cladistic approach. *Plant Systematics and Evolution* **178**: 65–94.
- Andreasen K, Bremer B. 1996. Phylogeny of the subfamily Ixoroideae (Rubiaceae). In: Robbrecht E, Puff C, Smets E, eds. *Second International Rubiaceae Conference, Proceedings Opera Botanica Belgica 7*: 119–138.
- Bremer B, Andreasen K, Olsson D. 1995. Subfamilial and tribal relationships in the Rubiaceae based on *rbcL* sequence data. *Annals of the Missouri Botanical Garden* **82**: 383–397.
- De Block P, Robbrecht E. 1998. Pollen morphology of the Pavetteae (Rubiaceae, Ixoroideae) and its taxonomic significance. *Grana* **37**: 260–275.
- Delprete PG. 1996. Evaluation of the tribes Chiococceae, Candamineae and Catesbaeeae (Rubiaceae) based on morphological characters. In: Robbrecht E, Puff C, Smets E, eds. *Second International Rubiaceae Conference, Proceedings Opera Botanica Belgica 7*: 165–192.
- Dessein S, Harwood R, Robbrecht E, Smets E. 2005b. Pollen of the *Spermaceae* (Rubiaceae) species from the Northern Territory of Australia: morphology and taxonomic significance. *Australian Systematic Botany* **18**: 367–382.
- Dessein S, Huysmans S, Robbrecht E, Smets E. 2002. Pollen of African *Spermaceae* species (Rubiaceae): morphology and evolutionary aspects. *Grana* **41**: 69–89.
- Dessein S, Ochoterena H, De Block P, Lens F, Robbrecht E, Schols P, Smets E, Vinckier S, Huysmans S. 2005a. Palynological characters and their phylogenetic signal in Rubiaceae. *Botanical Review* **71**: 354–414.
- Holmgren PK, Holmgren NH, Barnett LC. 1990. Index Herbariorum. Part I: The herbaria of the world. *Regnum Vegetabile 120*, 8th edn. New York: New York Botanical Garden.
- Huysmans S. 1993. De pollenmorfologie van de Coptosapelteae (Rubiaceae – Cinchonoideae). Master's Thesis, Institute of Botany and Microbiology, K.U.Leuven.
- Huysmans S, Dessein S, Smets E, Robbrecht E. 2003. Pollen morphology of NW European representatives confirms monophyly of Rubieae (Rubiaceae). *Review of Palaeobotany and Palynology* **127**: 219–240.
- 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, Delprete P, Smets E. 1999. Pollen morphological support for the Catesbaeeae–Chiococceae–*Exostema*-complex (Rubiaceae). *Grana* **38**: 325–338.

- Huysmans S, Robbrecht E, Smets E. 1994.** Are the genera *Hallea* and *Mitragyna* (Rubiaceae-Coptosapelteae) pollen morphologically distinct? *Blumea* **39**: 321–340.
- 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.
- Johansson JM. 1992.** Pollen morphology in *Psychotria* (Rubiaceae, Rubioideae, Psychotrieae) and its taxonomic significance. A preliminary survey. *Opera Botanica* **115**: 5–71.
- Leroy JF. 1975.** Taxogénétiqne dans le genre *Hallea* sur la sous-tribu des Mitragyninae (Rubiaceae – Naucleeeae). *Adansonia Série 2* (15): 65–88.
- Nilsson S, Pragłowski J. 1992.** *Erdtman's handbook of palynology*, 2nd edn. Copenhagen: Munksgaard.
- Punt W, Blackmore S, Nilsson S, Le Thomas A. 1994.** *Glossary of pollen and spore terminology*. Utrecht: LPP Foundation.
- Razafimandimbison SG. 2002.** A systematic revision of *Breonia* (Rubiaceae – Naucleeeae). *Annals of the Missouri Botanical Garden* **89**: 1–37.
- Razafimandimbison SG, Bremer B. 2001.** Tribal delimitation of Naucleeeae (Rubiaceae): inference from molecular and morphological data. *Systematics and Geography of Plants* **71**: 515–538.
- Razafimandimbison SG, Bremer B. 2002.** Phylogeny and classification of Naucleeeae (Rubiaceae) inferred from molecular (ITS, rbcL, and trnT-F) and morphological data. *American Journal of Botany* **89**: 1027–1041.
- Reitsma T. 1969.** Size modifications of recent pollen grains under different treatments. *Review of Palaeobotany and Palynology* **69**: 23–47.
- Ridsdale CE. 1978.** A revision of the tribe Naucleeeae s.s. (Rubiaceae). *Blumea* **24**: 307–366.
- Rova JHE, Andersson L. 1995.** A reevaluation of the tribes Hippotideae and Tammsieae (Rubiaceae). *Nordic Journal of Botany* **15**: 269–284.
- Rova JHE, Andersson L, Delprete PG, Albert VA. 2002.** A *trnL-F* cpDNA sequence study of the Condamineae–Rondeletieae–Sipaneeae complex with implications on the phylogeny of the Rubiaceae. *American Journal of Botany* **89**: 145–159.
- Schols P, Dessein S, D'hondt C, Huysmans S, Smets E. 2002.** Carnoy: a new digital measurement tool for palynology. *Grana* **41**: 124–126.
- Vinckier S, Huysmans S, Smets E. 2000.** Morphology and ultrastructure of orbicules in the subfamily Ixoroideae (Rubiaceae). *Review of Palaeobotany and Palynology* **108**: 151–174.
- Vinckier S, Smets E. 2002.** Systematic importance of orbicule diversity in Gentianales. *Grana* **41**: 158–182.