



## Systematic palynology in Ebenaceae with focus on Ebenoideae: Morphological diversity and character evolution

A. Geeraerts<sup>a,\*</sup>, J.A.M. Raeymaekers<sup>b</sup>, S. Vinckier<sup>c</sup>, A. Pletsers<sup>d</sup>, E. Smets<sup>a,e</sup>, S. Huysmans<sup>a</sup>

<sup>a</sup> Laboratory of Plant Systematics, Kasteelpark Arenberg 31, P.O. Box 3427, BE-3001 Leuven, Belgium

<sup>b</sup> Laboratory of Animal Diversity and Systematics, Charles Deberiotstraat 32, P.O. Box 2439, BE-3000 Leuven, Belgium

<sup>c</sup> Center for Transgene Technology and Gene Therapy, Flanders Institute for Biotechnology (VIB-3), K.U.Leuven, campus Gasthuisberg, Herestraat 49, BE-3000 Leuven, Belgium

<sup>d</sup> Department of Botany and Centre for the Environment, School of Natural Sciences, Trinity College Dublin, Dublin 2, Ireland

<sup>e</sup> National Herbarium of The Netherlands, Leiden University Branch, P.O. Box 9514, 2300 RA Leiden, The Netherlands

### ARTICLE INFO

#### Article history:

Received 7 July 2008

Received in revised form 26 September 2008

Accepted 3 October 2008

Available online 25 October 2008

#### Keywords:

Ebenaceae

phylogeny

palynology

Bayesian inference

principle component analysis

### ABSTRACT

This study examines the diversity and character transformations in pollen and orbicule morphology of Ebenaceae, with a focus on subfamily Ebenoideae (ca. 600 sp.). 62 specimens comprising all three genera of Ebenoideae (*Diospyros*, *Euclea*, *Royena*), were studied using LM and SEM. Bayesian phylogenetic analysis was performed on molecular sequence data to establish an evolutionary hypothesis that was then used as an evolutionary framework to identify synapomorphies and trace evolutionary trends of palynological data with Bayesian posterior mapping and principle component analyses (PCA). Ebenoideae pollen is generally shed as monads (permanent tetrads in two species), medium-sized, prolate-spheroidal to subprolate and tricolporate. A substantial amount of variation is found in pollen size, equatorial outline (lobate, subtriangular, circular and hexagonal) and sexine ornamentation type ((micro)rugulate, striate, granulate and gemmate). Moreover, orbicules were present on the inner locule wall in all specimens examined. Their abundance, degree of fusion with tapetal membrane and aggregation vary considerably. We can conclude that Ebenaceae pollen is more heterogeneous than previously assumed. We traced palynological synapomorphies for groups at different taxonomic levels: subfamily level (pollen size, pollen wall stratification and aperture morphology), generic level (size, equatorial outline and sexine ornamentation types) and subgeneric clades (size, ectocolpus morphology, equatorial outline and sexine ornamentation subtypes) respectively. The granular infratectum and the unique sculpturing pattern on the orbicule walls are the most discriminating pollen features for subfamily Ebenoideae.

© 2008 Elsevier B.V. All rights reserved.

### 1. Introduction

Ebenaceae (Ventenat, 1799) are woody plants, mostly confined to tropical latitudes, with sympetalous flowers, biovulate carpels, pendulous ovules and fleshy fruits. Characteristic features are the common, simple leaves with scattered extrafloral nectaries, often used for fossil identification (Contreras and Lersten, 1984; Wallnöfer, 2001, 2004a). The ebony family is of considerable economic importance: many species have edible fruits, e.g. *Diospyros virginiana* L. (persimmon) and *D. kaki* L. (kaki), and about 30 African and Asian Ebenaceae, such as *D. ebenum* Hiern, yield most of the ebony. Descriptions of Ebenaceae representatives are widely available in flora treatments of the neotropics (e.g., White, 1978; Sothers and Berry, 1998; Ribeiro, 1999; Berry, 2001), Asia and the Pacific (e.g., Shu-Kang, 1987) and Africa (e.g., De Winter, 1963; Letouzey and White, 1970; White and Letouzey, 1970; Phiri, 2005). The Pacific species were revised by Bakhuizen-van den Brink (1936–1955), Kostermans (1977) and Singh (2005).

Until now, there is no agreement on the circumscription of genera, subgenera, sections and even species (for instance the *Diospyros ferrea*-complex, Bakhuizen-van den Brink, 1936–1955; St. John, 1986). Recently two molecular studies including a worldwide sampling became available (Duangjai et al., 2006) providing a first framework for the discussion of intrafamilial relationships and morphological evolutionary trends. Ebenaceae comprise two subfamilies (Berry et al., 2001; Wallnöfer, 2001, 2004a,b,c; APG II, 2003; Duangjai et al., 2006): the small neotropical Lissocarpoideae (*Lissocarpa*, 9 species) and the pantropical Ebenoideae (*Diospyros*, *Euclea*, *Royena*, 500–600 species). Both subfamilies can be distinguished mainly by differences in floral morphology, wood anatomy and pollen morphology (Wallnöfer, 2004a). It is argued that *Royena* – previously an African section under *Diospyros* (White, 1980) – should again be recognized at generic level (Duangjai et al., 2006) and it will be treated as such in the present study. *Royena* has strong affinities to the genus *Euclea*. The other *Diospyros* species are subdivided in several subclades, each corresponding to a certain geographical area. The African *Diospyros* species with the largest morphological variation are found in at least four different clades throughout the phylogeny (Duangjai et al., 2006).

\* Corresponding author. Tel.: +32 16 32 86 36; fax: +32 16 32 19 55.

E-mail address: [Anke.Geeraerts@bio.kuleuven.be](mailto:Anke.Geeraerts@bio.kuleuven.be) (A. Geeraerts).

This evolutionary framework is poorly supported by morphological data, especially at intrageneric level. The micromorphological variation in *Lissocarpa*, *Diospyros* and *Euclea* remains poorly documented and could contribute to a better understanding of the intrafamilial relationships inferred from molecular data. Pollen morphology already proved to be a valuable source of systematically useful characters in Ebenaceae (Morton, 1994). Lissocarpoideae generally have suboblate to oblate-spheroidal pollen grains with three pores (Erdtman, 1971; Ng, 1971; Morton and Dickison, 1992; Franceschi, 1993). Ebenoideae mainly have tricolporate monads with a triangular polar outline and a predominantly (sub)prolate to prolate-spheroidal shape in equatorial view (e.g., Franceschi, 1993; Morton, 1994; Sharma and Gupta, 1979). The length of the polar axis and equatorial diameter varies between 20 and 55  $\mu\text{m}$ . Endoapertures are lalongate and the long ectocolpi have a granular membrane. Franceschi (1993) and Morton (1994) described sculptural differences between apocolpium and mesocolpium in a single grain.

Previous palynological studies were mainly based on a limited number of LM observations (see Morton (1994) for a summary of previous studies). Two papers combine SEM and TEM techniques with a relatively large sampling. Franceschi (1993) studied 21 Indian Ebenaceae species and provided suggestions for phylogenetic relationships based on a cladistic analysis. Morton (1994) included 92 species in her study based on LM, SEM and TEM observations and was the first to apply multivariate analysis to assess the morphological variation. Orbicules (Erdtman et al., 1961) or Ubisch bodies (Kosmath, 1927) – tiny sporopollenin granules which might occur in the anthers on the radial and innermost tangential walls of secretory tapetal cells – were completely overlooked (for a review on orbicules see Huysmans et al., 1998, 2000).

Our study aims (1) to give a detailed overview of the palynology of Ebenaceae using LM and SEM, (2) to explore this palynological variation with multivariate analyses and look for synapomorphies at family, subfamily, generic and subgeneric level and (3) to infer palynological evolutionary trends in Ebenaceae pollen by comparing our morphological results with an original phylogeny based on molecular sequence data using Bayesian posterior mapping.

## 2. Materials and methods

### 2.1. Material

#### 2.1.1. Pollen morphological study

Flowers and flower buds were collected from the herbarium and spirit collection of the National Herbarium of the Netherlands (L, WAG) and the herbarium of the National Botanic Garden of Belgium (BR). Additional living material was collected during a field trip to South Africa (2005). In total, 62 specimens (53 species) covering all three genera of Ebenoideae and 11 out of 18 sections of *Diospyros* (White, 1980) were investigated (see Appendix A). The type specimens of the three genera are all represented in the study (*Diospyros lotus*, *Euclea racemosa*, *Diospyros whyteana*). Multiple specimens of four widespread or morphologically diverse species (*D. abyssinica*, *D. austro-africana*, *D. lotus* and *E. natalensis*) and two well-defined species (*D. iturensis* and *D. zenkeri*) were included to assess possible intraspecific variation. In this paper *Royena* is considered as a separate genus as suggested by Duangjai et al. (2006).

#### 2.1.2. Molecular study

DNA sequences for 50 Ebenaceae species were analyzed: 44 species were complementary to the pollen study. For three other species namely *Diospyros lotus*, *D. cuneata* and *Diospyros* sp., closely related species were included due to lack of leaf material. For 43 species, material was obtained from KEW DNA bank and BR, WAG, K, MO, BRLU and L herbaria. Sequences for seven additional species were retrieved from Genbank. As outgroup, species from the genera *Argania*, *Madhuca* and *Manilkara* were included following Duangjai et al. (2006). Voucher information and Genbank Accession Numbers are listed in Appendix B.

### 2.2. Methods

All flowers and buds from herbarium material were rehydrated in agepon<sup>®</sup> wetting agent (Agfa Gevaert, Leverkusen, Germany) for a minimum of two days. Anthers were removed from the flowers and for each specimen split in two parts. One part was acetolysed (ace) according to Reitsma (1969) – mainly to prepare LM slides for the observation of endoapertures – and the other part critical-point dried (cpd). Special attention is paid to possible artifacts caused by acetolysis.

Pollen was acetolysed for 9 min. in a heating block at 95 °C. One part of the acetolysed pollen was mounted on specimen stubs, dried down from 70% ethanol. From the remaining part of the acetolysed pollen, permanent LM slides were made by embedding pollen in Kaiser's glycerine jelly. For CPD, the stamens were dehydrated through a graded ethanol-DMM series, critical point dried in DMM (CPD 030, Balzers) and mounted on stubs with double adhesive tape. Prior to sputter coating with gold (SPI-MODULE TM Sputter Coater, SPI Supplies, West Chester, PA, USA), the locules of the stamens were carefully opened to expose pollen and orbicules. Observations were made by a Leica LM DB microscope (LM) and a Jeol JSM-6360 microscope at 15 kV (SEM).

For each species the following characters were measured: length polar axis (*P*), length equatorial diameter (*E-D*), length of the ectoaperture (*L*), width of the ectocolpus at the equator (*W*), distance between the apertures at the poles (*d*), size of the granules on the colpus membrane (*G*), size of the sexine elements, diameter and abundance of the orbicules and the thickness of the exine and its sublayers. Measurements were made using Carnoy v. 2.0 (Schols et al., 2002) based on digital SEM graphs. Values for length polar axis (*P*), length equatorial axis (*E*), length colpus (*L*), width colpus (*W*), length equatorial axis measured in polar view (*D*), distance between the apices of the two ectocolpi (*d*), endocolpus size, thickness of exine sublayers and orbicule abundance are indicative and based on a single measurement due to a very low number of fully expanded grains in the samples. Size of the granules on the colpus membrane (*G*), size of the exine elements and orbicule diameter represent averages based on 10 measurements. Categorical characters studied include equatorial outline class, shape of ecto- and endoaperture and exine sculpturing pattern of the pollen grains, and shape and wall ornamentation of the orbicules. All were observed on SEM graphs except the endoaperture shape (LM).

Terminology follows the online edition of the *Glossary of Pollen and Spore Terminology* (Punt et al., 2007). For nomenclature of categorical characters (equatorial outline and ornamentation type), Buchner and Weber (2000, onwards) was used as a reference.

### 2.3. Bayesian phylogenetic analysis

Bayesian analysis of a combined dataset including five chloroplast-regions (*matK*, *ndhF*, *trnL-trnF*, *trnH-psbA*, *trnC-petN*) was performed with MrBayes 3.1.2 (Huelsenbeck et al., 2001). A GTR+G+I model (as chosen by MrModeltest v2.2, Posada and Crandall, 1998) was applied and the data was divided in two partitions: non-coding and coding (Bayesian analysis under mixed models, Nylander et al., 2004). The analysis was run for 5 million generations (nchains 4, nrns 2), sampling every 1000 generations. 50% of the trees were discarded as burning. Tracer 1.4 (Rambaut and Drummond, 2007) was used to assess convergence.

### 2.4. Multivariate analyses

Principle component analysis (PCA) was carried out in Statistica (Statsoft, Tulsa, USA) software. Six continuous characters were analyzed: length of the polar axis (*P*), length of the equatorial axis (*E*), apocolpium index (A.I.), relative colpus width (*W/E*), length exine elements and orbicule size. Afterwards, different labels were used to see whether pollen clustered according to phylogenetic relationships or biogeographical distribution. Scatterplots and analysis of variance were applied afterwards to confirm PCA observations.

Table 1

Continuous and categorical pollen character states related to pollen size, shape and aperture configuration

Species	Size				Shape				Ectoaperture						Endoaperture				Figs.	
	P (μm)		E (μm)		P/E	Amb	Colpus length (μm)		Colpus width (μm)		Diameter granules (μm)		Granules striated	A.I.	Length (μm, SEM)	Width (μm, SEM)	Type (LM)			
DIOSPYROS																				
<i>D. abyssinica</i> (1)	cpd	ace	cpd	ace	cpd	ace	C/L	cpd	ace	cpd	ace	cpd	ace	cpd	ace					
<i>D. abyssinica</i> (2)	24.0	–	20.1	–	1.19	–	S	19.1	29.5	1.8	2.0	–	0.2	(+)	0.3	0.3	9.4	3.6	Short, obtuse	
<i>D. barteri</i>	23.1	22.9	24.4	19.4	0.95	1.18	S	16.6	17.6	0.7	1.2	0.2	0.2	+	0.3	0.3	–	–	Short, obtuse	
<i>D. batocana</i>	–	30.9	–	27.3	–	1.13	H	–	25.2	–	4.1	–	0.3	(+)	–	0.2	5.2	1.8	Short, obtuse (more narrow)	1I
<i>D. bipindensis</i>	–	34.1	–	31.5	–	1.01	H	–	29.4	–	2.0	–	0.3	+	–	0.3	–	–	Short, obtuse (more narrow)	
<i>D. chamaethamnus</i>	20.9	–	21.6	–	1.00	–	C	14.5	–	0.4	–	–	–	NA	0.5	–	–	–	Connected into endocingulum	1H,K
<i>D. cooperi</i>	–	35.3	–	30.6	–	1.15	H	–	31.1	–	3.7	–	0.2	+	–	0.2	–	–	Short, obtuse	1M,O
<i>D. crassiflora</i>	–	39.8	–	36.7	–	1.08	S	–	32.3	–	1.6	–	0.4	o	–	0.3	–	–	–	
<i>D. curranii</i>	39.9	–	36.5	–	1.09	–	S	29.0	–	1.3	–	0.3	–	o	0.1	–	–	–	–	
<i>D. deltoidea</i>	27.8	32.3	26.6	30.4	1.03	1.06	C	24.6	28.0	3.4	4.2	0.3	0.3	+	0.3	0.3	–	–	Short, obtuse	
<i>D. dendo</i>	29.2	–	31.5	–	0.93	–	C	15.9	–	2.2	–	0.3	–	o	0.7	–	–	–	Endocingulum /colpus, acute	
<i>D. iturensis</i> (1)	24.3	30.9	24.1	27.1	1.01	1.14	S	20.3	23.1	1.3	2.0	–	0.3	o	0.3	0.3	8.7	1.2	Short colpus, acute	
<i>D. iturensis</i> (2)	16.3	–	16.2	–	1.01	–	L	13.3	–	1.3	–	–	–	(+)	0.2	–	–	–	Short, wide truncate	
<i>D. kirkii</i>	27.5	–	21.1	–	1.30	–	L	22.4	–	1.5	–	0.2	–	(+)	0.3	–	–	–	–	
<i>D. longiflora</i> (1)	31.6	31.6	25.8	30.2	1.22	1.05	H	22.2	24.2	2.2	3.0	0.2	0.3	(+)	0.4	0.3	–	–	Very short, obtuse	
<i>D. longiflora</i> (2)	28.1	–	NA	–	NA	–	C	NA	–	NA	–	NA	–	NA	NA	–	–	–	–	1B
<i>D. lotus</i> (1)	29.0	–	NA	–	NA	–	C	NA	–	NA	–	NA	–	NA	NA	–	–	–	–	
<i>D. lotus</i> (2)	28.9	–	26.7	–	1.08	–	S	23.9	–	1.5	–	0.5	–	+	0.3	–	–	–	Short, obtuse, wide	
<i>D. macrocalyx</i>	50.6	–	51.5	–	0.98	–	S	44.7	–	8.2	–	0.5	–	+	0.3	–	–	–	Short, acute	1L
<i>D. malabarica</i>	27.2	–	27.2	–	1.00	–	S	24.2	–	4.1	–	0.4	–	o	0.4	–	–	–	–	
<i>D. mannii</i> (1)	32.6	40.1	32.6	39.0	1.00	1.03	C	27.1	43.3	1.9	3.3	0.2	0.3	o	0.3	0.3	8.8	2.5	Short, obtuse	2C
<i>D. mannii</i> (2)	29.1	–	NA	–	NA	–	C	NA	–	0.8	–	NA	–	NA	NA	–	–	–	–	
<i>D. mespiliformis</i>	30.3	–	NA	–	NA	–	C	NA	–	0.6	–	NA	–	NA	NA	–	–	–	–	1A,C
<i>D. monbutensis</i>	–	32.3	–	24.8	–	1.30	H	–	26.6	–	1.4	–	0.2	(+)	–	0.3	9.0	1.5	Tapering colpus (endocracks)	
<i>D. natalensis</i>	28.6	–	26.1	–	1.10	–	S	11.8	–	2.6	–	0.3	–	+	0.3	–	–	–	Variable	
<i>D. oubatchensis</i>	22.6	–	24.1	–	0.94	–	S	19.7	–	2.3	–	0.3	–	+	0.3	–	–	–	Variable	
<i>D. polystemon</i>	25.6	–	25.5	–	1.00	–	C	20.7	–	4.3	–	–	–	o	0.3	–	–	–	Short, obtuse	
<i>D. pseudomespilus</i>	44.6	–	39.9	–	1.12	–	S	37.6	–	2.3	–	0.7	–	+	0.3	–	10.0	2.6	Obtuse (endocracks)	
<i>D. rabiensis</i>	29.6	–	27.6	–	1.07	–	S	25.9	–	3.0	–	0.4	–	o	0.1	–	–	–	–	
<i>D. rotundifolia</i>	–	–	22.9	–	1.03	–	S	–	–	2.0	–	0.3	–	(+)	0.2	–	–	–	Short, obtuse	
<i>D. sanzaminika</i>	25.1	–	25.4	–	0.99	–	S	21.4	–	1.8	–	0.3	–	(+)	0.2	–	–	–	–	
<i>D. soubreana</i>	33.8	–	33.1	–	1.02	–	C	31.6	–	3.1	–	0.3	–	+	0.3	–	–	–	Endocingulum /colpus	1N
<i>D. squarrosa</i>	27.3	–	24.7	–	1.11	–	S	21.7	–	1.2	–	0.2	–	o	0.4	–	–	–	–	
<i>D. texana</i>	25.4	–	22.8	–	1.12	–	C	19.0	–	1.0	–	0.2	–	o	0.4	–	–	–	Short colpus, acute	
<i>D. thwaitesii</i>	28.1	37.4	24.1	25.3	1.17	1.48	S-L	22.7	30.5	2.8	1.3	0.3	0.3	o	0.2	0.3	–	–	Short colpus	
<i>D. virginiana</i>	31.5	32.5	30.4	27.4	1.04	1.19	L	24.3	28.3	2.9	2.3	0.3	0.2	+	0.3	0.3	–	–	–	
<i>D. viridicans</i>	–	30.7	–	29.7	–	1.03	S	–	27.1	–	2.6	–	0.2	(+)	–	0.3	–	–	Short, obtuse	
<i>D. zenkeri</i> (1)	41.4	–	31.2	–	1.33	–	S	33.4	–	2.8	–	0.3	–	o	0.2	–	–	–	Colpus (endocracks)	
<i>D. zenkeri</i> (2)	28.0	28.0	26.6	27.1	1.05	1.03	L	22.0	21.0	1.8	2.0	0.2	0.2	o	0.3	0.3	6.2	1.6	Short colpus, acute	2A
<i>Diospyros</i> sp.	25.3	–	23.6	–	1.07	–	S	21.2	–	2.6	–	0.2	–	o	0.2	–	8.0	1.8	Colpus, acute or obtuse	
	23.0	–	20.1	–	1.14	–	S	19.5	–	1.2	–	0.2	–	o	0.3	–	–	–	–	
	27.5	–	26.8	–	1.03	–	C	21.1	–	2.8	–	0.3	–	o	0.3	–	12.0	3.3	Colpus, acute	1J
EUCLEA																				
<i>E. balfouri</i>	21.8	21.5	19.3	19.0	1.13	1.13	L	17.1	18.4	1.2	1.7	0.3	0.4	o	0.3	0.3	9.8	1.8	Short, obtuse	2B
<i>E. crispa</i>	–	20.3	–	16.4	–	1.24	L	–	15.8	–	1.4	–	0.2	o	–	0.3	7.5	1.5	Short, obtuse	
<i>E. divinatorum</i>	–	23.3	–	22.5	–	1.04	L	–	18.3	–	2.2	–	0.2	o	–	0.3	–	–	Short, obtuse	
<i>E. natalensis</i> (1)	21.2	–	18.3	–	1.16	–	S	17.0	–	1.3	–	0.2	–	o	0.3	–	–	–	–	
<i>E. natalensis</i> (2)	20.3	19.6	19.4	17.3	1.04	1.13	S	15.8	15.9	1.1	1.1	0.2	0.2	o	0.3	0.3	–	–	Short, obtuse	
<i>E. pseudobenus</i>	25.6	24.9	19.6	21.8	1.31	1.14	L	18.6	19.3	1.3	2.0	0.2	0.2	o	0.3	0.2	2.6	0.9	Very short, obtuse	
<i>E. racemosa</i>	–	22.4	–	19.0	–	1.18	L	–	17.6	–	0.8	–	0.2	o	–	0.3	–	–	–	
<i>E. schimperii</i>	16.9	–	17.5	–	0.97	–	C	14.0	–	1.8	–	0.1	–	o	0.4	–	–	–	–	
<i>E. tomentosa</i>	–	30.0	–	23.0	–	1.30	L	–	25.6	–	1.3	–	0.2	o	–	0.3	5.0	2.1	Ring on inside pollenwall?	
<i>E. undulata</i>	17.1	22.4	15.9	18.2	1.08	1.23	L	14.9	17.4	1.2	1.4	–	0.2	o	0.3	0.3	–	–	Longer, obtuse	
ROYENA																				
<i>D. austro-africana</i> (1)	25.9	–	24.6	–	1.05	–	L	22.4	–	1.3	–	0.3	–	o	0.2	–	–	–	–	1D
<i>D. austro-africana</i> (2)	27.9	–	24.0	–	1.16	–	S	22.4	–	0.8	–	–	–	o	0.3	–	–	–	–	
<i>D. austro-africana</i> (3)	–	29.7	–	25.9	–	1.15	C	–	24.4	–	2.1	–	–	o	–	–	–	–	–	
<i>D. dichrophylla</i>	25.3	–	23.8	–	1.06	–	S	22.0	–	2.8	–	0.4	–	o	0.4	–	–	–	Short, obtuse	
<i>D. fischeri</i>	28.3	23.8	26.8	26.9	1.06	0.88	S	23.3	24.3	1.9	1.9	0.2	0.3	o	0.4	0.3	–	–	Short, obtuse	
<i>D. glabra</i>	29.5	–	26.7	–	1.10	–	S	23.2	–	2.1	–	0.1	–	o	0.3	–	–	–	Short, obtuse	
<i>D. lycioides</i>	–	29.1	–	27.4	–	1.06	S	–	23.0	–	1.5	–	0.3	(+)	–	0.4	8.0	2.5	Short, obtuse	
<i>D. scabrida</i>	33.6	–	31.4	–	1.07	–	S	24.6	–	2.4	–	0.3	–	o	0.2	–	–	–	Short, obtuse	1G
<i>D. loureiriana</i>	30.6	–	27.5	–	1.11	–	L	24.1	–	2.0	–	0.0	–	o	0.2	–	–	–	Short, colpus	
<i>D. whyteana</i>	34.9	37.9	28.8	32.5	1.21	1.17	S	29.4	28.4	2.2	2.0	–	0.3	o	0.3	0.4	–	–	Short, colpus	
<i>D. zombensis</i>	–	26.3	–	22.8	–	1.15	S	–	16.7	–	0.8	0.3	0.3	o	–	0.3	–	–	Short, colpus	



## 2.5. Bayesian posterior mapping

Two categorical characters (equatorial outline and ornamentation type) and two continuous characters (pollen and orbicule size) were mapped onto the molecular phylogeny using the Bayesian approach of posterior mapping (SIMMAP v1.0, Bollback, 2006) to account for uncertainty in phylogeny inference. The two continuous characters were coded using Thiele's method allowing seven character states per character (Thiele, 1993) as implemented in Morphocod (Schols et al., 2004). A single stochastic mapping was done per tree (using the final 500 trees of the Bayesian analysis) and the ancestral states for the subfamilies, genera and subclades were inferred for the consensus tree.

## 3. Results

### 3.1. Pollen morphology

All pollen investigated is isopolar, radially symmetric and 3-colporate. Variation can be observed in dispersal unit, size, P/E ratio, equatorial outline, ecto- and endoaperture configuration, and pollen wall sculpturing (Tables 1 and 2).

#### 3.1.1. Dispersal unit

Ebenoideae pollen is generally shed as monads. In two species, however, *Diospyros mannii* and *D. longiflora*, permanent calymmate tetrahedral tetrads were observed (Fig. 1A–C). This observation was verified in two specimens. Surprisingly, the two specimens investigated of *D. mannii* show a different exine ornamentation (see Section 3.1.5.1).

#### 3.1.2. Size

Measured on SEM graphs (Table 1), critical point dried pollen has an average size of  $28.1 \pm 6.6 \mu\text{m} \times 25.9 \pm 6.4 \mu\text{m}$ . The pollen size of genus *Royena* ( $Px = 29.5 \pm 3.4 \times 26.7 \pm 2.6 \mu\text{m}$ , Fig. 1D) and genus *Diospyros* ( $Px = 29.1 \pm 6.7 \times 27.1 \pm 6.7 \mu\text{m}$ ) is comparable but a larger size variation can be seen in *Diospyros*. In the latter, the smallest grains are  $20.9 \times 21.6 \mu\text{m}$  and belong to *D. bipindensis*. The largest grains are found in *D. lotus* (Vasak s.n.,  $50.6 \times 51.1 \mu\text{m}$ ). *Royena* exhibits a size range from  $26.3 \times 22.5 \mu\text{m}$  (*D. zombensis*) to  $48.1 \times 36.7 \mu\text{m}$  (*D. fischeri*). *Euclea* clearly has the smallest pollen ( $Px = 20.5 \pm 3.2 \times 18.3 \pm 1.4 \mu\text{m}$ , Fig. 1E).

#### 3.1.3. Shape

The P/E ratio is predominantly prolate-spheroidal (58%, Fig. 1D) or subprolate (20%, Fig. 1E). Exactly 50% of *Euclea* pollen is prolate-spheroidal, whereas 75% of the *Royena* pollen investigated is prolate-spheroidal and 25% subprolate. In *Diospyros* also oblate-spheroidal (13%) and spheroidal (four species, *D. bipindensis*, *D. macrocalyx*, *D. malabarica* and *D. natalensis*) pollen is observed. Most species of which several specimens were studied showed intraspecific shape variation. Of these eight species, *D. abyssinica*, *D. austro-africana* and *E. natalensis*, are morphologically diverse and subdivided in sub-species or varieties.

Four different equatorial outlines are represented (Fig. 1F): lobate (27% of the specimens investigated), subtriangular (25%), circular (14%) and hexagonal (10%). *Euclea* and *Royena* have respectively a lobate and subtriangular equatorial outline as the dominant type, while 45% of the *Diospyros* species investigated has subtriangular pollen and 31% circular (Table 1). Intraspecific variation occurs in three species. Two specimens of *Diospyros abyssinica* were studied. Tisserant 1898 has pollen grains with a circular and lobate equatorial outline. Pollen of De Wilde 4837 has a

subtriangular equatorial outline. Three specimens of *Diospyros austro-africana* were studied, including two varieties. *D. austro-africana* var. *rugosa* has subtriangular and circular pollen. *D. austro-africana* var. *austro-africana*'s pollen is more lobate in outline. In *Diospyros squarrosa* we observed variation in one specimen ranging from subtriangular to lobate.

#### 3.1.4. Apertures

Ebenoideae pollen grains are zono-3-colporate. In one specimen of *Diospyros austro-africana* var. *rugosa* 4-aperturate pollen was observed occasionally.

**Ectocolpi**—The ectoapertures are always colpi and pollen is angulaperturate in case of (sub)triangular or hexagonal pollen (Fig. 1F). We observed a considerable variation in ectocolpus width and length, the distinctness of the margins and the shape of the ectocolpus ends.

The ectocolpus length (*L*) ranges from  $11.8 \mu\text{m}$  (*D. monbutensis*) to  $44.7 \mu\text{m}$  (*D. lotus*) with an average of  $22.3 \pm 6.3 \mu\text{m}$ . *Diospyros* comprises a large variation in colpus lengths ( $L = 23.0 \pm 6.9 \mu\text{m}$ , Fig. 1G–H) while *Euclea* has the smallest colpi ( $L = 16.2 \pm 1.7 \mu\text{m}$ ). The colpi of *Royena* species measure  $23.9 \pm 2.4 \mu\text{m}$ . All genera have an average apocolpium index (A.I.) of 0.3. This index is highest (shortest ectocolpus) in *D. deltoidea* (A.I. = 0.7) and lowest (longest ectocolpus) in *D. crassiflora* and *D. polystemon* (A.I. = 0.1).

The maximum ectocolpus width is  $8.2 \mu\text{m}$  (*D. lotus*, Fig. 1I). *D. bipindensis* and both specimens of *D. mannii* have slit-like apertures (W between 0.4 and  $0.8 \mu\text{m}$ , Fig. 1J). The colpus membrane can be clearly distinct from (e.g., *D. austro-africana*, Fig. 1D) or fade gradually into the surrounding exine (e.g., *D. barteri*, Fig. 1F lower right). Finally, the ectocolpus ends can be obtuse or acute. Acute ectocolpi have very sharp pointed ends like in *D. scabrida* (Fig. 1K) and *D. virginiana*. Obtuse ones have blunt, rounded ends, for example the colpi of *D. barteri* (Fig. 1L) and *D. cooperi*. Between these two extreme character states, we can find occasionally intermediate states (Fig. 1M).

The ectocolpus membrane is (mostly densely) covered with irregularly shaped granules (average  $G = 0.3 \pm 0.1 \mu\text{m}$ ), of which the surface ornamentation often reflects the ornamentation pattern of the pollen wall (Fig. 1M). In acetolysed pollen the colpus membrane is always sheared at the equator. Sometimes these cracks extend to the poles. In four species the mesocolpial area is differentiated, thereby resembling hexacolpate pollen (Fig. 1N, see also Section 3.1.5.2). Parasyncolpate pollen was occasionally observed in *D. longiflora*: the three ectocolpi fuse at the apocolpia (Fig. 1B). In some grains the fusion of apertures was incomplete (Fig. 1O).

**Endocolpi**—LM observations (not shown) reveal a rather continuous variation between short, obtuse endocolpi (e.g., *D. malabarica*, all *Euclea* specimens) to a distinct endocingulum (e.g., *D. deltoidea*, *D. rotundifolia* and *D. texana*), occasionally with short triangular extensions on their margins as in *D. virginiana*. The length measured on SEM graphs ranges from  $2.6 \mu\text{m}$  (*E. pseudebenus*) to  $12.0 \mu\text{m}$  (*Diospyros* undet.) and width from  $0.9 \mu\text{m}$  (*E. pseudebenus*) to  $3.6 \mu\text{m}$  (*D. abyssinica*, Tisserant 1898). Some of the variation is shown in Fig. 2A–C.

#### 3.1.5. Pollen wall

**3.1.5.1. Sexine ornamentation.** Four sexine ornamentation types could be discerned based on size, shape and organization of the sculpturing elements: (micro)rugulate, striate, granulate and microgemmate (see Table 2).

1. The dominant ornamentation pattern is (micro)rugulate: elongated exine elements are irregularly arranged. Between species there is a

#### Notes to Table 1:

Measurements of CPD pollen are indicated in white, of ACE pollen in grey. Missing data is listed as ‘-’. Amb types: C (circular), L (lobate), S (subtriangular) and H (hexacolpate). Striation of granules: large striae (+), fine inconspicuous striae ((+)) or no striae (o). E, P/E, L, W, G and A.I. were only measured on monads. In case of tetrads, these characters are listed as not applicable (NA).

**Table 2**

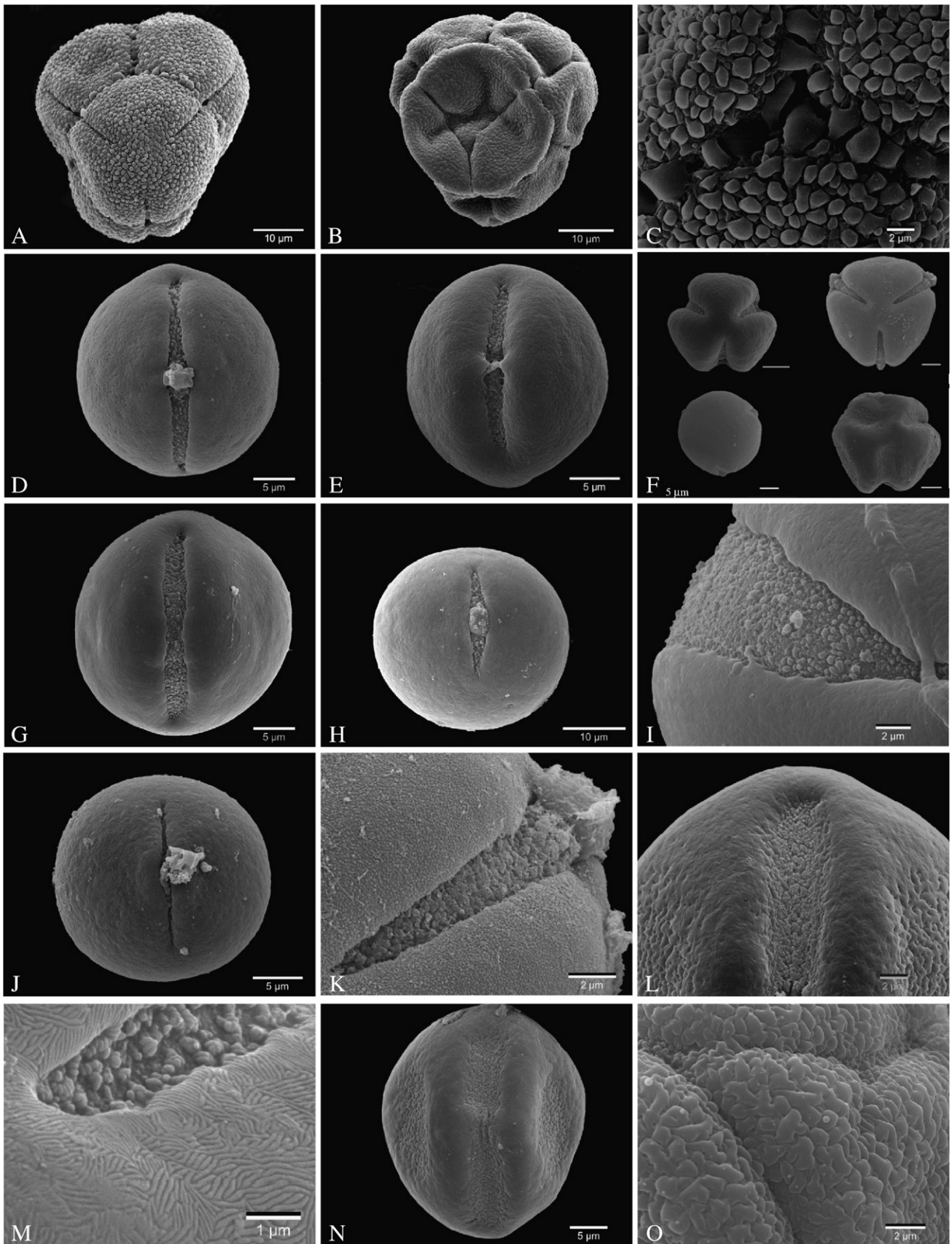
Continuous and categorical pollen character states related to pollen wall characters

Species	Ornamentation		Tectum					Stratification					Figs.
	Type	Length exine elements (µm)	Apocolpia perforate	Apocolpia fossulate	Mesocolpia perforate	Mesocolpia fossulate	Margo perforate	Thickness exine (µm)	Thickness sexine (µm)	Thickness nexine (µm)	Sexine/nexine	Thickness tectum (µm)	
DIOSPYROS													
<i>D. abyssinica</i> (1)	1a	0.2–(0.3)–0.4	o	+	o	+	o	1.2	0.8	0.4	2.0	0.4	3D, 2F 6A
<i>D. abyssinica</i> (2)	1a	0.3–(0.6)–0.8	o	+	o	+	o	–	–	–	–	–	
<i>D. barteri</i>	1b	0.5–(1.1)–2.4	o	o	o	+	+	0.8	0.5	0.4	1.3	0.3	3B
<i>D. batocana</i>	1b	0.6–(0.9)–1.2	o	o	+	o	+	0.7	0.3	0.4	0.8	0.2	2G
<i>D. bipindensis</i>	1a	0.07–(0.1)–1.2	+	o	+	o	o	0.7	0.4	0.3	1.3	0.3	2E
<i>D. chamaethamnus</i>	1b	0.7–(1.1)–1.9	o	o	o	+	+	–	–	–	–	–	
<i>D. cooperi</i>	1c	0.6–(0.9)–1.3	o	o	o	o	o	1.0	0.6	0.4	1.5	0.4	2I
<i>D. crassiflora</i>	1c	0.9–(1.2)–1.6	o	o	o	o	o	–	–	–	–	–	2J
<i>D. curranii</i>	1b	0.5–(1.3)–2.1	+	o	+	o	o	0.8	0.5	0.3	1.7	0.4	
<i>D. deltoidea</i>	1b	0.4–(1.5)–3.5	+	o	+	o	o	–	–	–	–	–	2H
<i>D. dendo</i>	1a	0.4–(0.5)–0.6	+	o	+	o	+	0.8	0.6	0.2	3.0	0.3	
<i>D. iturensis</i> (1)	1b	0.2–(0.5)–0.7	o	o	+	o	+	–	–	–	–	–	2N
<i>D. iturensis</i> (2)	1b	0.4–(0.7)–1.1	o	o	+	o	+	–	–	–	–	–	
<i>D. kirkii</i>	1a	0.2–(0.5)–1.2	+	o	+	+	+	1.1	0.6	0.5	1.2	0.3	3C
<i>D. longiflora</i> (1)	1c	–	–	–	–	–	–	–	–	–	–	–	
<i>D. longiflora</i> (2)	1c	–	–	–	–	–	–	–	–	–	–	–	
<i>D. lotus</i> (1)	1b	0.5–(1.1)–1.9	+	o	+	o	o	–	–	–	–	–	
<i>D. lotus</i> (2)	1b	1.0–(1.0)–1.0	+	o	+	o	o	–	–	–	–	–	
<i>D. macrocalyx</i>	2	0.4–(2.5)–5.7	o	o	o	o	o	–	–	–	–	–	
<i>D. malabarica</i>	1b	0.6–(1.4)–2.9	+	o	+	o	o	–	–	–	–	–	
<i>D. mannii</i> (1)	1c	–	o	o	o	o	o	–	–	–	–	–	6C–F
<i>D. mannii</i> (2)	4	0.7–(0.9)–1.2	o	o	o	o	o	1.8	1.3	0.5	2.6	–	20, 3L
<i>D. mespiliformis</i>	1b	0.2–(0.6)–1.0	+	o	+	+	+	–	–	–	–	–	
<i>D. monbuttensis</i>	1	–	+	(+)	+	(+)	o	–	–	–	–	–	
<i>D. monbuttensis</i>	1a	0.2–(0.3)–0.6	+	o	+	o	o	–	–	–	–	–	
<i>D. natalensis</i>	1a	0.1–(0.2)–0.3	+	o	+	o	o	–	–	–	–	–	
<i>D. oubatchensis</i>	2	0.8–(2.9)–5.5	o	o	o	o	o	1.7	0.9	0.8	1.1	0.7	
<i>D. polystemon</i>	1a	0.3–(0.5)–0.7	+	o	+	o	o	–	–	–	–	–	
<i>D. pseudomespilus</i>	1a	0.3–(0.5)–0.7	+	o	+	o	o	–	–	–	–	–	
<i>D. rabiensis</i>	1a	0.3–(0.5)–0.7	+	(+)	+	(+)	o	–	–	–	–	–	
<i>D. rotundifolia</i>	1b	0.4–(1.6)–3.9	+	o	o	o	o	–	–	–	–	–	
<i>D. sanza–minika</i>	1b	1.0–(1.4)–1.9	o	o	+	o	+	–	–	–	–	–	3F–G
<i>D. soubreana</i>	1a	0.6–(1.0)–1.0	o	o	+	o	+	–	–	–	–	–	
<i>D. squarrosa</i>	1a	0.4–(0.5)–0.5	+	(+)	+	(+)	o	0.9	0.6	0.3	2.0	0.4	
<i>D. texana</i>	1b	0.5–(1.0)–1.2	+	o	+	o	o	–	–	–	–	–	
<i>D. thwaitesii</i>	1b	0.5–(0.9)–1.3	MF	o	o	o	o	0.9	0.5	0.4	1.3	0.4	
<i>D. virginiana</i>	1b	0.7–(1.1)–1.5	+	o	+	o	o	–	–	–	–	–	
<i>D. viridicans</i>	1a	0.3–(0.4)–0.5	MF	(+)	MF	(+)	o	1.0	0.6	0.4	1.5	0.4	3J, 6B–E
<i>D. zenkeri</i> (1)	1b	0.6–(1.3)–2.0	+	o	+	+	+	1.0	0.6	0.4	1.5	0.3	
<i>D. zenkeri</i> (2)	1b	–	+	o	+	+	+	–	–	–	–	–	
<i>Diospyros</i> sp.	1b	1.0–(1.0)–2.4	+	o	+	o	o	1.3	0.8	0.5	1.6	0.5	3D
EUCLEA													
<i>E. balfouri</i>	1b	0.5–(0.7)–0.9	+	o	+	o	o	1.3	0.8	0.5	1.6	0.4	3E
<i>E. crispa</i>	1b	–	+	o	+	o	o	1.2	0.8	0.4	2.0	0.4	
<i>E. divinatorum</i>	1b	–	+	o	+	o	o	–	–	–	–	–	
<i>E. natalensis</i> (1)	2	1.5–(2.0)–2.6	+	o	+	o	o	–	–	–	–	–	
<i>E. natalensis</i> (2)	1b	0.6–(1.3)–2.9	+	o	+	o	o	–	–	–	–	–	
<i>E. pseudebenus</i>	1b	0.4–(1.6)–3.6	+	o	+	o	+	1.4	0.8	0.6	1.3	0.5	
<i>E. racemosa</i>	1b	–	+	o	+	o	o	1.2	0.8	0.4	2.0	0.5	
<i>E. schimperii</i>	1b	–	+	o	+	o	o	–	–	–	–	–	
<i>E. tomentosa</i>	1a	0.1–(0.2)–0.4	++	o	++	o	o	1.2	0.8	0.4	2.0	0.4	3H–I
<i>E. undulata</i>	1b	0.4–(0.8)–1.2	MF	o	MF	o	o	0.8	0.5	0.2	2.6	0.2	
ROYENA													
<i>D. austro–africana</i> (1)	1a	0.2–(0.3)–0.4	+	o	+	o	o	–	–	–	–	–	
<i>D. austro–africana</i> (2)	1	0.5–(1.5)–2.3	o	o	o	o	o	–	–	–	–	–	
<i>D. austro–africana</i> (3)	1b	–	o	o	o	o	o	–	–	–	–	–	
<i>D. dichrophylla</i>	1a	0.2–(0.3)–0.5	+	o	+	o	o	–	–	–	–	–	2D
<i>D. fischeri</i>	2	0.4–(2.2)–5.5	+	o	+	o	o	1.0	0.7	0.3	2.3	0.3	2L
<i>D. glabra</i>	1a	0.2–(0.3)–0.6	o	o	+	o	+	–	–	–	–	–	
<i>D. lycioides</i>	1a	0.2–(0.2)–0.3	+	o	+	o	o	–	–	–	–	–	
<i>D. scabrida</i>	3	0.1	+	o	+	o	o	–	–	–	–	–	
<i>D. loureiriana</i>	2	0.4–(2.8)–4.8	o	o	o	o	o	–	–	–	–	–	2K
<i>D. whyteana</i>	3	0.2	+	o	+	o	o	1.1	0.7	0.3	2.3	0.5	2M, 3K
<i>D. zombensis</i>	2	0.9–(2.0)–3.4	+	o	(+)	o	o	–	–	–	–	–	

Length exine elements is denoted as minimum value–(average)–maximum value. – = missing data, (+), ++ = presence of character, 0 = absence of character, MF = micro-foveolae.

variation in length, width, shape and organization of the rugulae. Several subtypes are defined for descriptive purposes but variations and transitional conditions occur.

Subtype a (Fig. 2D–F) shows very short, irregularly shaped and randomly arranged rugulae, mostly of average length between 0.1 and 0.5 µm. The elements are more elongated than they are in the



**Fig. 1.** Pollen morphology. A–C. Dispersal Unit. Permanent tetrads in (A) *D. mannii* and (B) *D. longiflora*, (C) calymmate tetrad of *D. mannii*. D–F. Pollen shape. (D) Prolate-spheroidal: *D. austro-africana*, (E) subprolate: *E. racemosa*, (F) (upper left) lobate pollen: *E. racemosa*, (upper right) subtriangular: *D. scabrida*, (lower left) circular: *D. bipindensis*, (lower right) hexagonal: *D. barteri*. G–O. Ectocolpus shape. (G) long in *Diospyros* sp., (H) short in *D. deltoidea*, (I) wide in *D. lotus*, (J) slit-like in *D. bipindensis*, (K) acute ends (*D. scabrida*), (L) obtuse ends (*D. chamaethamnus*), (M) striated granules on ectocolpus membrane (*D. rotundifolia*), (N) pseudo-hexacolpate pollen (*D. chamaethamnus*) and (O) parasyncolporate pollen in *D. longiflora*.



granulate pattern (type 3) but shorter and thinner than the microrugulate types 1b and 1c. This subtype is often correlated with fissures (fossulae) between the rugulae in the intercolpia resulting in an undulate pollen surface (Fig. 2F, Table 2).

Subtype b, finely (micro)rugulate (Fig. 2G–H), is characterized by straight to sinuate, mostly superficial rugulae, densely arranged per 2–10. The average length of the elements usually varies between 0.6 and 1.6  $\mu\text{m}$ , but can exceed 2.0  $\mu\text{m}$  as in *D. macrocalyx*, *D. oubatchensis*, *D. loureiriana* and *D. zombensis*. Their width is generally smaller than or equals 0.1  $\mu\text{m}$ , but can be larger as in *D. malabarica*, *D. sanza-minika*, *D. virginiana* and *E. natalensis*. Sometimes, the mesocolpia of these species are deeply fossulated. In subtype c the coarse rugulae exceed the width of 0.1  $\mu\text{m}$  as is the case in all other rugulate and striate patterns observed (Fig. 1I–J). The average width is 0.4  $\mu\text{m}$  and the average length 1.0  $\mu\text{m}$ . The rugulae are loosely arranged and might be interwoven. Only three species belong to this subtype, namely *D. cooperi*, *D. crassiflora* and *D. longiflora*. One specimen of *D. mannii* (Adam 27725) also shows a coarsely rugulate sexine ornamentation, in contrast to specimen Zenker 3439.

2. Two species, *D. loureiriana* and *D. fischeri*, have elongated exine elements with an average length longer than 2  $\mu\text{m}$  (Fig. 2K–L). In *D. loureiriana* the striae are arranged in parallel, without grooves between individual striae. In *D. fischeri* the elements have a variable width and a random organization.
3. The tectum of *D. scabrida* and *D. whyteana* consists of spherical exine elements with a diameter of 0.1 – 0.2  $\mu\text{m}$ . They are irregularly and densely arranged. This granular ornamentation type (see also Morton, 1994) is clearly distinguishable and found in only two *Royena* species, *D. scabrida* and *D. whyteana* (Fig. 2M). One species, *D. iturensis*, has granules on top of the rugulate ornamentation pattern in both specimens investigated (Fig. 2N).
4. The globular exine elements, more or less constricted at the base, of *D. mannii* have a mean height of 0.7  $\mu\text{m}$  and width of 0.9  $\mu\text{m}$  (Fig. 2O). The distance between two exine elements more or less equals the diameter of one gemma.

**3.1.5.2. Secondary ornamentations and differentiation of mesocolpium.** Three types of secondary ornamentations could be observed: fossulate, perforate and microfoveolate.

- (1) In three species (*D. abyssinica*, *D. barteri* and *D. chamaethamnus*) is the entire pollen wall (margo, apocolpium and mesocolpium) covered with fine, shallow rugulae, but in the mesocolpia additional elongated, irregular grooves or fossulae are present (see Table 2 and Fig. 3A). In nine other species these fossulae are observed on the entire pollen wall (Fig. 3B–C). The fossulae can be deep, like in *D. abyssinica* (denoted + in Table 2, Fig. 3B). In other species with an undulate pollen surface, the fossulae are often shallow (denoted (+) in Table 2, Fig. 3C).
- (2) The tectum has small rounded intrusions or perforations in at least 45 species. There is, however, a substantial variation in size, abundance and distribution of these perforations. Many species like *D. austro-africana*, *D. fischeri* and *D. rabiensis* have a limited number of inconspicuous perforations in the intercolpia (Fig. 3D). In *D. batocana*, *D. iturensis*, *D. sanza-minika* and *D. glabra*, for example, the perforations are distinctly limited to the mesocolpium and margo and are clearly visible in the finely rugulate pattern (Fig. 3E–F).
- (3) In *D. thwaitesii*, *D. viridicans* and *E. tomentosa*, larger perforations or micro-foveolae are found over the entire pollen wall (diameter ranging from 0.1 to 0.5  $\mu\text{m}$ , Fig. 3G–I).

**3.1.5.3. Pollen wall stratification.** Exine thickness varies between 0.7  $\mu\text{m}$  (*D. batocana*) and 1.8  $\mu\text{m}$  (*D. mannii*) with an average of  $1.0 \pm 0.2 \mu\text{m}$  (Table 2). Ebenoideae pollen is tectate–perforate (Fig. 3J–L). Further-

more, the wall stratification is characterized by a thin, granular infratectum (average thickness =  $0.2 \pm 0.1 \mu\text{m}$ ).

### 3.2. Orbicule morphology

In each specimen examined, orbicules were present. Variation can be found in their size, shape, orbicule wall characteristics, abundance and degree of fusion with tapetal membrane (Table 3 and Fig. 4). Ebenoideae orbicules (Fig. 4A–B) have an average diameter of  $0.5 \pm 0.2 \mu\text{m}$ . *Royena* has the largest orbicules ( $0.6 \pm 0.2 \mu\text{m}$ ), followed by *Diospyros* ( $0.4 \pm 0.2 \mu\text{m}$ ) and *Euclea* ( $0.3 \pm 0.05 \mu\text{m}$ ). Their shape is spherical (S, Fig. 4C) in for instance *D. monbuttensis*, *D. polystemon* and *E. crispa*, or irregular (I, Fig. 4D) such as in e.g., *D. sanza-minika*, *D. squarrosa*, *E. tomentosa*. In *D. barteri*, *D. deltoidea* and *D. texana* flattened orbicules with a central perforation occur (donut-shaped, D, Fig. 4E). The orbicule wall is often beset with one (e.g., *D. loureiriana*, Fig. 4F) or more (e.g., *D. fischeri*, *D. hallieri*, *D. lycioides*, *D. oubatchensis*, *D. polystemon*, *D. rotundifolia*, Fig. 4G) striae. Orbicules can be very abundant (Fig. 4C–E–H) or only sparsely present (Fig. 4A). All specimens of genus *Royena* have a low abundance (denoted o or (+)). Surprisingly, the abundance sometimes varies within one locule (Fig. 4I). Sometimes the orbicules are aggregated (Fig. 4E). They can be partly or entirely fused with the tapetal membrane (Fig. 4A–D). The degree of fusion and aggregation can also vary within one specimen.

### 3.3. Bayesian phylogenetic analysis

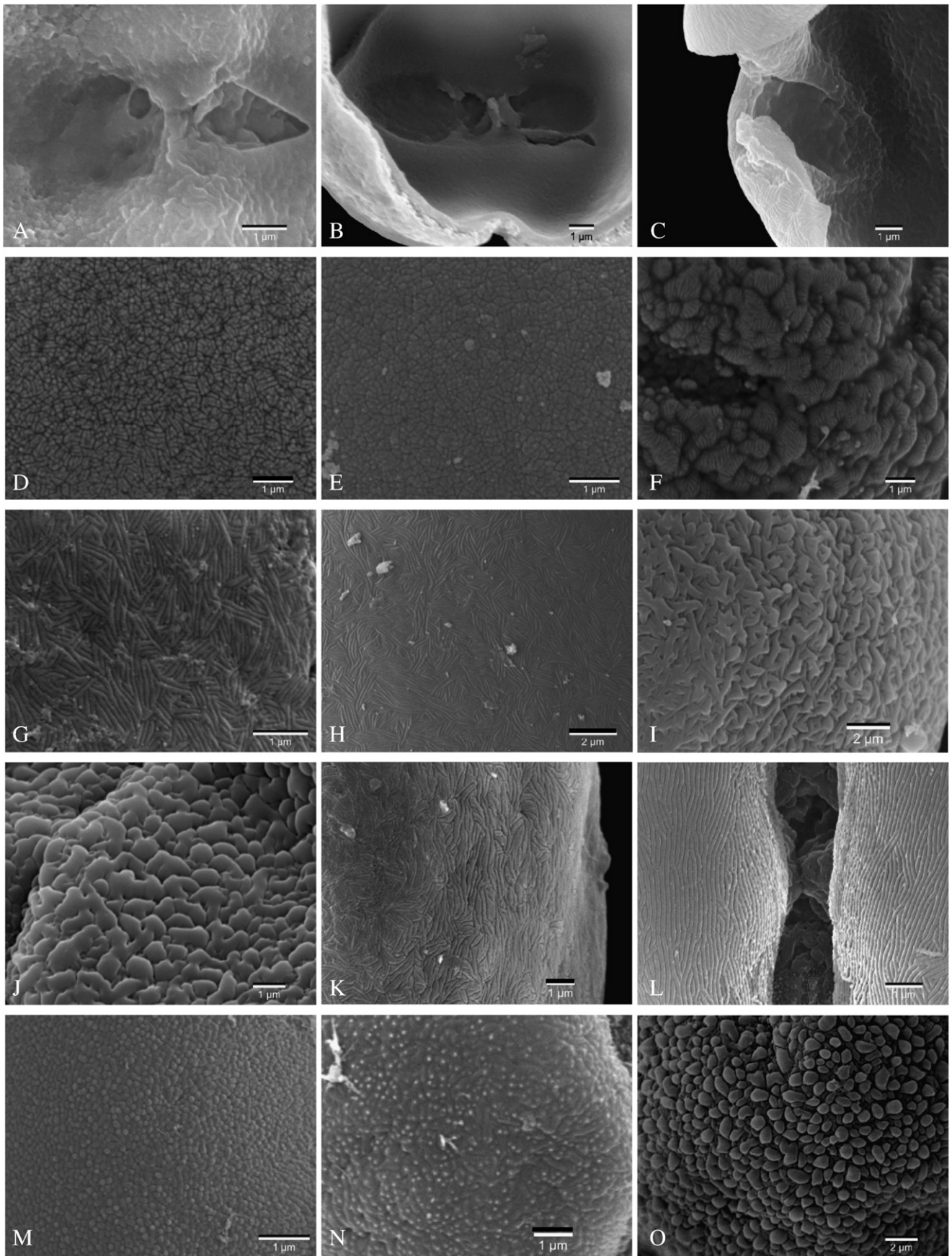
The Bayesian analysis of molecular data resulted in a majority-rule consensus tree in which seven major clades are recognized: *Euclea* (A), *Royena* (B) and *Diospyros* with five subgroups (C to G). *Euclea* and *Royena* are sistergroups, as expected, with high support (BPP 100). In *Royena*, two subgroups can be identified: *D. austro-africana*–*D. fischeri*–*D. loureiriana* are subgroup 1, the remaining *Royena* species subgroup 2. The five *Diospyros* subgroups are well supported. Clade C is composed of two African species (*D. natalensis* and *D. rotundifolia*) and one unidentified specimen from Madagascar. Clade D is the first large African subgroup which is again divided in two sistergroups. Clade E is another small clade consisting of two African (*D. kirkii* and *D. mespiliformis*) and one tropical Asian (*D. curranii*) specimen. Clades F1 and F2 are two non-African clades uniting a mix of neotropical, Pacific and Asian species. Sister to clade F2, we find the second clade with African endemics (clade G).

### 3.4. Multivariate analyses

The first two components of the PCA analysis explained 62% of the total variation in continuous traits. PC1 correlated with pollen and orbicule size, PC2 with A.I., exine length and to a lesser extent W/E. PC3 and PC4 correlated with A.I. and exine length (Fig. 6A–C).

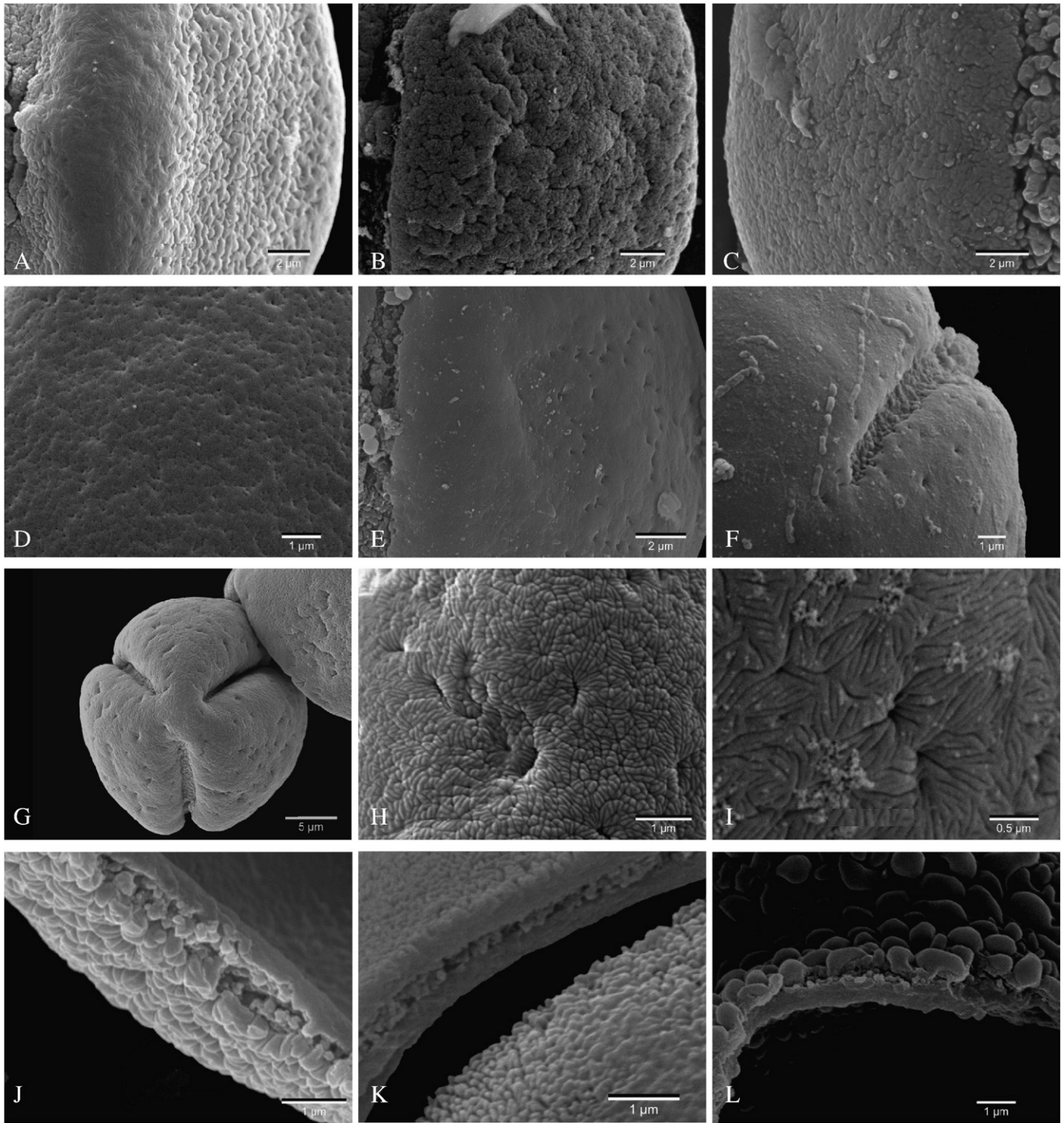
Clades A (*Euclea*), G (Africa II) and F (non-African species) of the Bayesian analysis (Fig. 5) are relatively well defined in the PCA analyses, both in pc1 vs pc2 and pc3 vs pc4. Pollen and orbicules of *Euclea* and the Africa II clade are all quite small. Furthermore, pollen of *Euclea* species has long, narrow ectocolpi and relatively long exine elements. These characters vary more extensively in the Africa II clade. The non-African species (clade F) are characterized by relatively large orbicules and pollen with long ecto-colpi and rather short exine elements.

Representatives of clades B (*Royena*) and D (Africa I) do not cluster well (Fig. 6A–B). The variation in these clades can be attributed to the length of exine elements and the apocolpium index. In *Royena* two groups can be distinguished based on the scatterplots: pollen of *D. austro-africana* (Goldblatt 4320), *D. fischeri* and *D. loureiriana* is characterized by long exine elements, while *D. austro-africana* (Schlieben and Ellis 12370), *D. dichrophylla*, *D. glabra*, *D. scabrida* and *D. whyteana* have short exine elements. The Africa I clade unites



**Fig. 2.** Pollen morphology. A. Endocolpus of *D. viridicans*. B. endocolpus of *E. balfouri*. C. endocolpus of *D. malabarica* (C). D–O. Exine ornamentation types. (D–F) Finely microrugulate type 1a in *D. dichrophylla* (D), *D. bipindensis* (E) and *D. monbutensis* (F). (G–H) Finely (micro)rugulate type 1b in *D. batocana* (G) and *D. deltoidea* (H). (I–J) Coarsely rugulate Type 1c in *D. cooperi* (I) and *D. crassiflora* (J). (K–L) Striate: *D. usambarensis* (K) and *D. fischeri* (L). (M) Granulate in *D. whyteana*. (N) Supratectal granules of *D. iturensis*. (O) Gemmate ornamentation in *D. manni*.





**Fig. 3.** Pollen morphology. Local differentiation of exine. A–C. Fossulae in mesocolpia of *D. barteri* (A), *D. abyssinica* (B) and *D. polystemon* (C). D–F. Perforations in the pollen wall. (D) *E. balfouri* with perforations in the entire pollen wall. (E) Perforate mesocolpia and (F) margo in *D. sanza-minika*. G–I. Microfoveolae in *D. tomentosa* (G, H) and *D. thwaitesii* (I). J–L. Pollen wall stratification of *D. viridicans* (J), *D. whyteana* (K) and *D. mannii* (L).

species with small grains and orbicules, with the exception of *D. crassiflora*.

Clades C (represented by *D. rotundifolia*, *D. natalensis* and *Diospyros* sp.) and E (represented by *D. curranii*, *D. kirkii* and *D. mespiliformis*) were characterized by a large variation in length of the exine elements and apocolpium index.

There seems to be a correlation between geographical distribution and size of the pollen grains and orbicules. The PCA scatter plots indicate

that the non-African species (b and c) have relatively large pollen and large orbicules while the African species (a) have smaller pollen and orbicules (Fig. 6C). The analyses of variance confirmed these results for pollen size but not for orbicule size ( $F_{2,45}$  pollen=8.32,  $p=0.008$ ;  $F_{2,54}$  orbicules=1.41;  $p=0.253$ ). Besides these non-African species, six African species have exceptionally large pollen: *D. austro-africana*, *D. fischeri*, *D. glabra*, *D. loureiiriana*, *D. crassiflora* and *D. rotundifolia*. The four species in the beginning of this list belong to genus *Royena*. If we performed the

**Table 3**  
Continuous and categorical orbicule character states

Species	Size (µm)	Shape	Striae	Fusion	Aggr.	Figs.
DIOSPYROS						
<i>D. abyssinica</i> (1)	0.3–(0.5)–0.6	I	+	o	+	4E
<i>D. abyssinica</i> (2)	0.4–(0.5)–0.7	I	+	o	+	
<i>D. barteri</i>	0.4–(0.5)–0.7	D	+	o	+	
<i>D. batocana</i>	0.3–(0.4)–0.5	I	+	o	o	
<i>D. bipindensis</i>	0.2–(0.3)–0.4	I	+	+	+	
<i>D. chamaethamnus</i>	0.2–(0.3)–0.4	I	o	+	+	
<i>D. cooperi</i>	–	–	–	–	–	
<i>D. crassiflora</i>	0.6–(0.9)–1.3	I	o	o	o	
<i>D. curranii</i>	0.2–(0.3)–0.4	S	+	o	(+)	
<i>D. deltoidea</i>	0.3–(0.4)–0.5	D, S	+	o	o	
<i>D. dendo</i> (1)	0.4–(0.5)–0.6	S	+	o	o	
<i>D. iturensis</i> (1)	0.2–(0.3)–0.4	I	o	o	++	4H
<i>D. iturensis</i> (2)	0.3–(0.3)–0.4	I	+	o	+	
<i>D. kirkii</i>	0.2–(0.3)–0.4	I	o	+	+	
<i>D. longiflora</i> (1)	0.4–(0.5)–0.9	I	o	+	o	
<i>D. longiflora</i> (2)	0.3–(0.4)–0.6	I	o	+	o	
<i>D. lotus</i> (1)	0.3–(0.3)–0.4	S	+	o	o	
<i>D. lotus</i> (2)	0.6–(0.8)–1.1	S	+	o	o	4B
<i>D. macrocalyx</i>	0.5–(0.6)–0.7	S	+	+	+	
<i>D. malabarica</i>	0.3–(0.5)–0.6	I	o	+	+	
<i>D. mannii</i> (1)	0.4–(0.5)–0.6	I	+	(+)	(+)	
<i>D. mannii</i> (2)	0.5–(0.8)–1.2	I	+	(+)	(+)	
<i>D. mespiliformis</i>	0.2–(0.2)–0.3	S	+	o	(+)	
<i>D. monbutensis</i>	0.2–(0.3)–0.4	S	+	o	o	
<i>D. natalensis</i>	0.2–(0.4)–0.5	S	+	o	o	4C
<i>D. oubatchensis</i>	0.5–(0.7)–0.9	S	+	o	+	4F
<i>D. polystemon</i>	0.6–(0.7)–1.1	S	+	+	++	
<i>D. pseudomespilus</i>	–	–	–	–	–	
<i>D. rabiensis</i>	0.3–(0.4)–0.6	S	o	o	o	
<i>D. rotundifolia</i>	0.4–(0.5)–1.0	S	+	+	+	
<i>D. sanza–minika</i>	0.3–(0.4)–0.6	I	o	o	o	
<i>D. soubreana</i>	0.3–(0.4)–0.4	I	o	+	o	
<i>D. squarrosa</i>	0.2–(0.3)–0.4	I	o	+	(+)	
<i>D. texana</i>	0.3–(0.4)–0.5	S, D	+	o	(+)	
<i>D. thwaitesii</i>	0.5–(0.6)–0.6	I	+	++	+	4D
<i>D. virginiana</i>	0.3–(0.5)–0.7	S	+	+	+	
<i>D. viridicans</i>	0.2–(0.3)–0.4	I	o	++	o	
<i>D. zenkeri</i> (1)	0.2–(0.4)–0.5	I	o	++	++	
<i>D. zenkeri</i> (2)	0.3–(0.3)–0.3	I	o	+	+	
<i>Diospyros</i> sp.	0.3–(0.4)–0.5	S	o	(+)	(+)	
EUCLEA						
<i>E. balfouri</i>	0.3–(0.3)–0.4	S	+	o	(+)	
<i>E. crispa</i>	–	–	–	–	–	
<i>E. divinatorum</i>	0.2–(0.3)–0.3	S	+	o	(+)	
<i>E. natalensis</i> (1)	0.3–(0.4)–0.4	S	o	o	o	
<i>E. natalensis</i> (2)	0.3–(0.3)–0.4	S	o	o	o	
<i>E. pseudebenus</i>	0.3–(0.4)–0.5	S	+	o	(+)	
<i>E. racemosa</i>	0.2–(0.3)–0.3	S	+	o	(+)	
<i>E. schimperii</i>	0.2–(0.3)–0.4	S	o	o	(+)	
<i>E. tomentosa</i>	0.3–(0.4)–0.5	I	o	+	o	
<i>E. undulata</i>	0.1–(0.2)–0.3	S	o	+	o	4A
ROYENA						
<i>D. austro–africana</i> (1)	0.4–(0.6)–0.8	S	+	o	o	
<i>D. austro–africana</i> (2)	0.5–(0.8)–0.9	S	+	(+)	o	
<i>D. austro–africana</i> (3)	–	–	–	–	–	
<i>D. dichrophylla</i>	0.6–(0.7)–0.9	S	+	o	(+)	
<i>D. fischeri</i>	0.5–(0.6)–0.7	S	+	o	(+)	4I
<i>D. glabra</i>	0.5–(0.7)–0.8	S	+	o	o	
<i>D. lycioides</i>	0.5–(0.5)–0.6	S	+	(+)	(+)	
<i>D. scabrida</i>	0.3–(0.3)–0.5	I	+	+	(+)	
<i>D. loureiriana</i>	0.3–(0.7)–1.0	S	+	o	o	4G
<i>D. whyteana</i>	0.2–(0.3)–0.5	I	+	(+)	(+)	
<i>D. zombensis</i>	–	–	–	–	–	

Size is denoted as minimum value–(average)–maximum value. – = missing data. Shape states are spherical (S), irregular (I) or donut-shaped (D). Presence of striae on orbicule wall (striae), fusion of orbicules with inner locule wall (fusion) and aggregation of orbicules (aggr.) is denoted as ‘++’, ‘+’ in case of all or most orbicules, ‘(+)’ some orbicules and ‘o’ no orbicules.

analysis of variance again, excluding these *Royena* species, the results remained significant for pollen size but not for orbicule size ( $F_{2,37}$  pollen=8.68,  $p=0.008$ ;  $F_{2,45}$  orbicules=2.54;  $p=0.0902$ ).

### 3.5. Character evolution and ancestral states

The characters and their states are indicated on Fig. 7. Stochastic mapping of morphological characters was only performed on the categorical characters (equatorial outline and ornamentation type) and the two most variable continuous characters (P and orbicule size). The most significant Bayesian posterior probabilities estimated using SIMMAP for the occurrence of the character states of these characters are listed in Table 4. Simulated character states and ancestral states are plotted on the Bayesian phylogeny (Fig. 7A–D). Only the states with highest probability are shown.

The most common recent ancestor of Ebenaceae was probably characterized by medium-sized pollen (state 3, Fig. 7A). *Euclea* gradually developed smaller grains, while *Royena* developed larger pollen (states 3–4). The subclades in *Diospyros* have respectively medium-sized (C, E and G) or large (F1 and F2) pollen. Clade D exhibits a large size variation with many state transformations.

Concerning the equatorial outline (Fig. 7B), the ancestral state for Ebenaceae, Ebenoideae and *Royena* is subtriangular. *Euclea* pollen developed from a lobate ancestor and *Diospyros* from a circular one. A circular equatorial outline was preserved in clade C, while the African clades (D and G) developed multiple equatorial outlines from a subtriangular ancestor. The African representatives of clade E both have a hexagonal equatorial outline, which distinguishes them from the third species in this clade: *D. curranii*. The two non-African clades F1 and F2 developed from respectively a subtriangular and circular ancestor. The subtriangular ancestral state remained preserved in the species of clade F1. In clade F2, however, the South-American species all developed lobate equatorial outlines in contrast to the circular equatorial outlines of the Asian species of this clade.

The ancestral state for ornamentation type (Fig. 7C) is a micro-rugulate ornamentation of subtype 1b. *Euclea* and *Diospyros* preserved this state, while *Royena* knows a high variety of ornamentation types. In subclade 1 the exine elements become smaller resulting in a granulate exine, while the size of the elements increased in subgroup 2 becoming a striate pattern. In *Diospyros*, only rugulate types can be seen. Few character transformations between subtypes are noticed, except in group D in which all three subtypes are present.

The evolutionary pattern of orbicule size is quite similar to that of pollen size. The ancestral state is most probably a medium-sized orbicule that during evolution gradually became larger (*Royena*) or smaller (*Euclea*).

## 4. Discussion

### 4.1. Form and function in Ebenoideae pollen

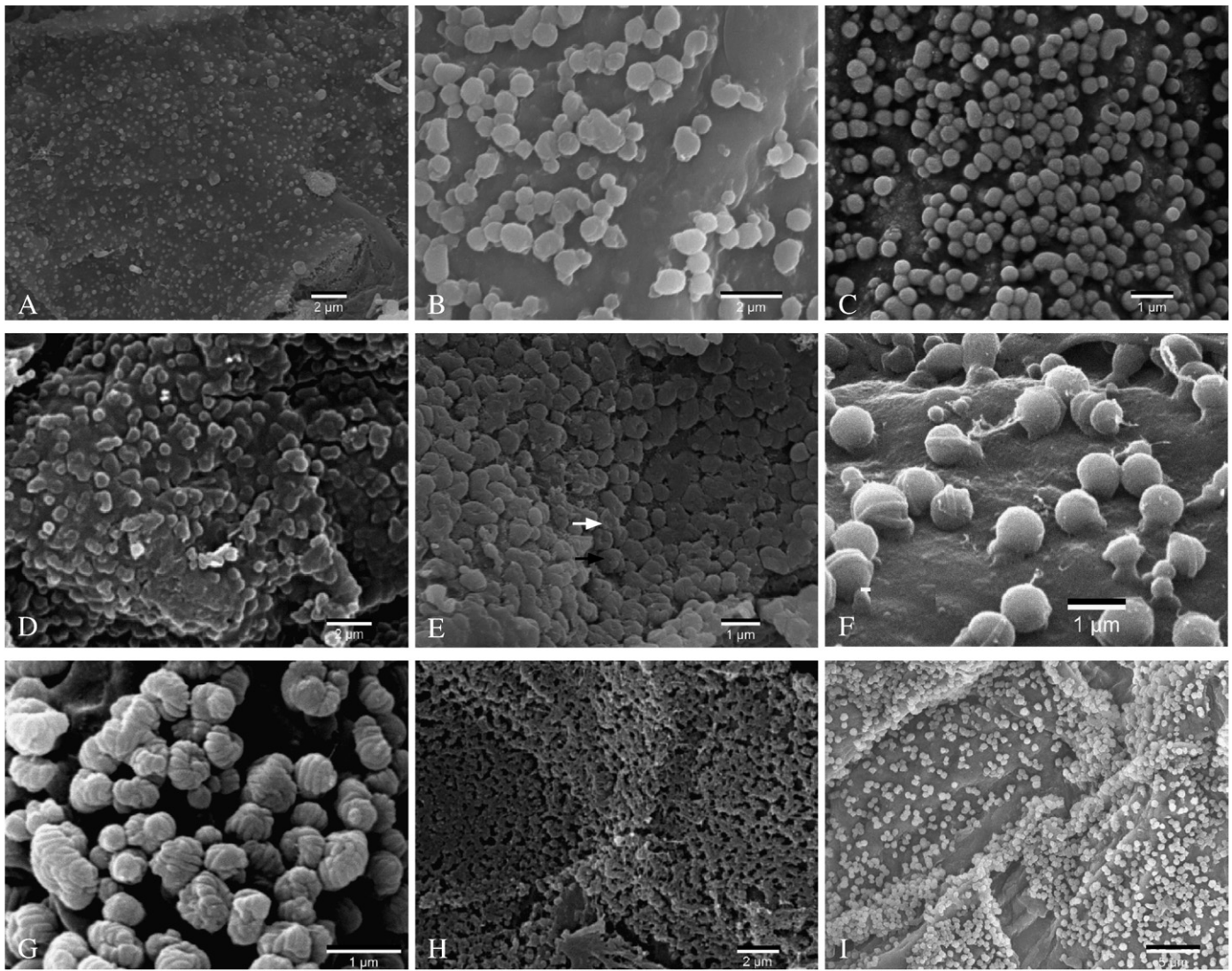
Ebenaceae pollen shows a substantial amount of variation in dispersal unit, size, equatorial outline, colpus length and shape, endocolpus shape and pollen wall ornamentation and structure. Additional to the pollen, orbicules were present in all specimens studied and also vary in size, shape and wall sculpturing. In the next paragraph we will highlight some remarkable pollen features in Ebenaceae.

#### 4.1.1. Tetrads

This study is the first to report on the presence of permanent tetrads in Ebenaceae. *D. mannii* and *D. longiflora* have permanent, tetrahedral and probably calymmate tetrads. Plants with compound pollen are assumed to have a high number of ovules per ovary (Walker, 1971). The landing of one compound pollen on a stigma could thus lead to the fertilization of many ovules. The female flowers of *D. longiflora* are unknown. *D. mannii* has female flowers with 10–12 ovules and 5 stigmas, a number that is rather high for African Ebenaceae which rarely have more than 5–6 ovules and 3–4 stigmas.

The relationship of *D. mannii* with *D. longiflora* remains unclear in analysis based on DNA sequences but both are classified in a different section following White (1980). About 52–55 families of angiosperms have members that shed pollen in an aggregated or compound form





**Fig. 4.** Orbicule morphology. A. *E. undulata*: overview of inner locule wall with orbicules. B. *D. lotus*: orbicules on tapetal membrane. C. *D. natalensis*: spherical orbicules. D. *D. thwaitesii*: irregular shaped orbicules. E. Donut-shaped orbicules in *D. barteri*. F–G. Single and multiple striae on the orbicule walls of respectively *D. oubatchensis* (F) and *D. usambarensis* (G). H. *D. iturensis*, inner locule wall with a high density of orbicules. I. variability in density in one locule of *D. fischeri*.

(Shukla et al., 1998, see also Table 1 in Copenhagen (2005) for a list of species producing tetrads). Tetrads represent a derived evolutionary state in angiosperms relative to monads and seem to have independently originated many times (Walker and Doyle, 1975; Walker, 1976). In Ericales at least three other families have members that shed pollen as tetrads: Ericaceae (Oldfield, 1959; Ueno, 1962; see also Copenhagen (2005) for a detailed survey), Actinidiaceae (Dickson et al., 1982) and Marcgraviaceae (Erdtman, 1969). These are not closely related to each other or Ebenaceae.

#### 4.1.2. Harmomegathy

The mesocolpial area of four species (*D. barteri*, *D. chamaethamnus*, *D. kirkii* and *D. mespiliformis*) is highly fossulated between the shallow microrugulae. Another five species show a high amount of perforations, distinctly restricted to mesocolpia and/or margo's (*D. batocana*, *D. iturensis*, *D. sanza-minika*, *D. soubreana* and *D. glabra*). As these fossulae and perforations weaken the pollen wall, it will deform more easily due to (de)hydration. Especially the fossulated species are often correlated with a hexagonal equatorial outline: the mesocolpial areas are sunken just like the colpi. Moreover, the endexine in these areas is thinner in comparison with the poles or margo (Morton, 1994). The mesocolpia of these grains could be considered as additional apertures which would make the pollen pseudohecolpate.

In Ebenoideae four equatorial outline types are recognized: lobate, subtriangular, circular and hexagonal. In Ebenoideae, aperture morphology seems to attribute to pollen shape. Pollen with a hexagonal or circular equatorial outline has relatively large and wide ectocolpi. Small and narrow colpi are here linked to a more lobate equatorial outline.

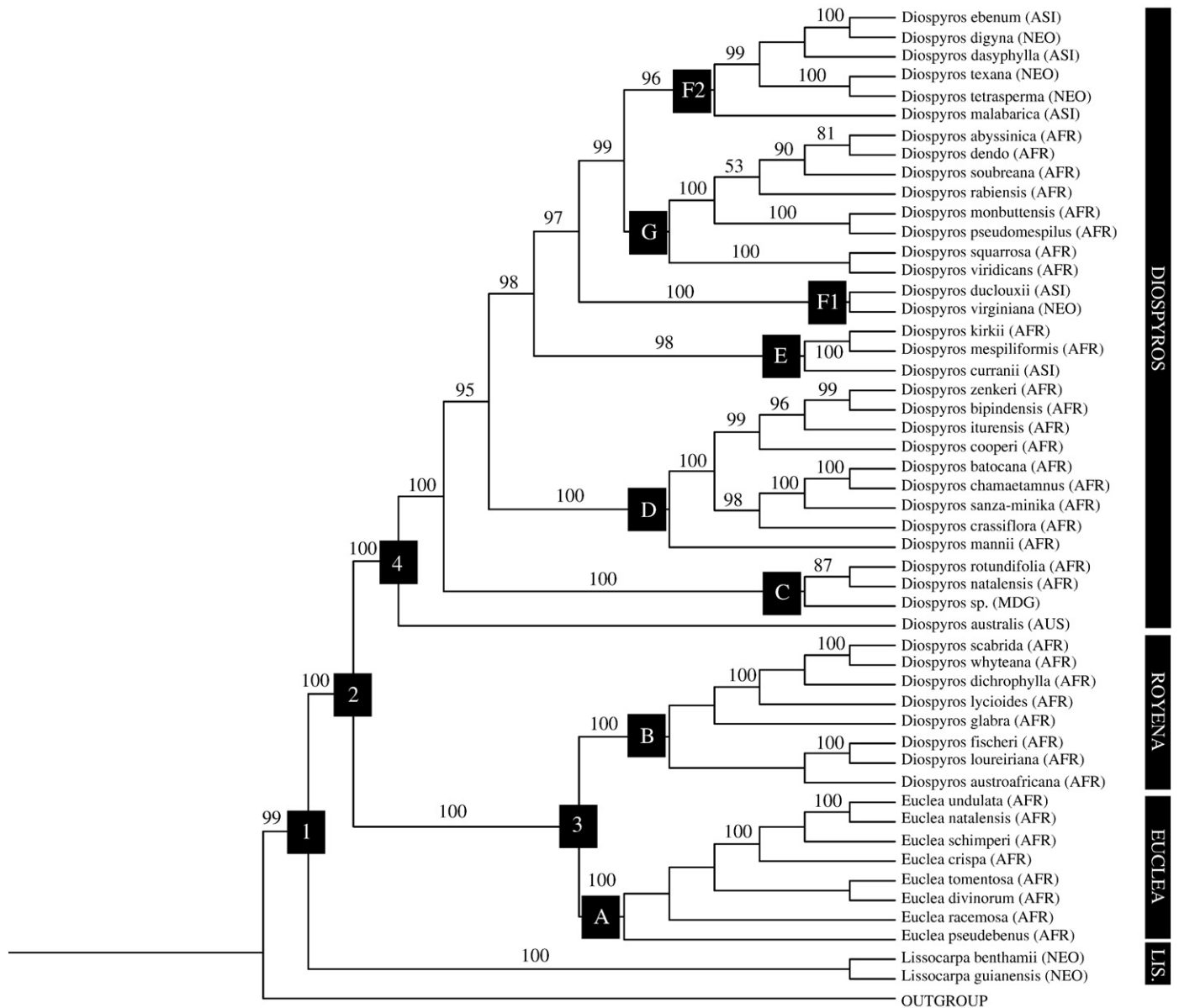
Besides the length and width of the ectocolpus, the rigidity of the colpus membrane is also an important factor when looking at the mode of infolding of a pollen grain. The colpus membrane is beset with granules of different sizes which influence the rigidity. Membranes with small granules experience less support, are less rigid and thus have a larger tendency to deform. This is confirmed by our results in which lobate and pseudohecolpate grains have smaller granules on the colpus membranes (average diameter 0.20 µm) compared to circular (0.25 µm) and subtriangular (0.30 µm) pollen.

#### 4.2. Taxonomic significance of pollen and orbicule morphology in Ebenoideae

##### 4.2.1. Subfamily level

Ebenoideae and Lissocarpoideae can be separated based on pollen characteristics. Ebenoideae pollen is shed as monads or tetrads, is medium-sized ( $28.1 \pm 6.6 \mu\text{m} \times 25.9 \pm 6.4 \mu\text{m}$ ) and 3-colporate. Pollen





**Fig. 5.** Majority-rule consensus tree based on a Bayesian analysis of the combined dataset of 5 chloroplast regions. BPP are indicated below branches. Clade names (A–G and 1–4) are indicated on the nodes (black squares). Letters refer to clades used in the PCA analysis.

grains of the small, neotropical subfamily Lissocarpoideae is shed as monads, is 3-porate and 40–70 µm in diameter (Erdtman, 1971; Ng, 1971; Wallnöfer, 2004a). Ebenoideae pollen can be best distinguished by the presence of a granular infratectal layer. Sapotaceae pollen shares most features with Ebenoideae: it is tricolporate, with usually narrow ectocolpi, a tectate–perforate exine structure with a thickness ranging from 1.0–8.0 µm, an infratectum with granules or reduced columellae and largely overlapping exine sculpturing patterns (Harley, 1986; Morton, 1994). Sapotaceae pollen, however, is slightly larger than Ebenoideae pollen (16–92 µm).

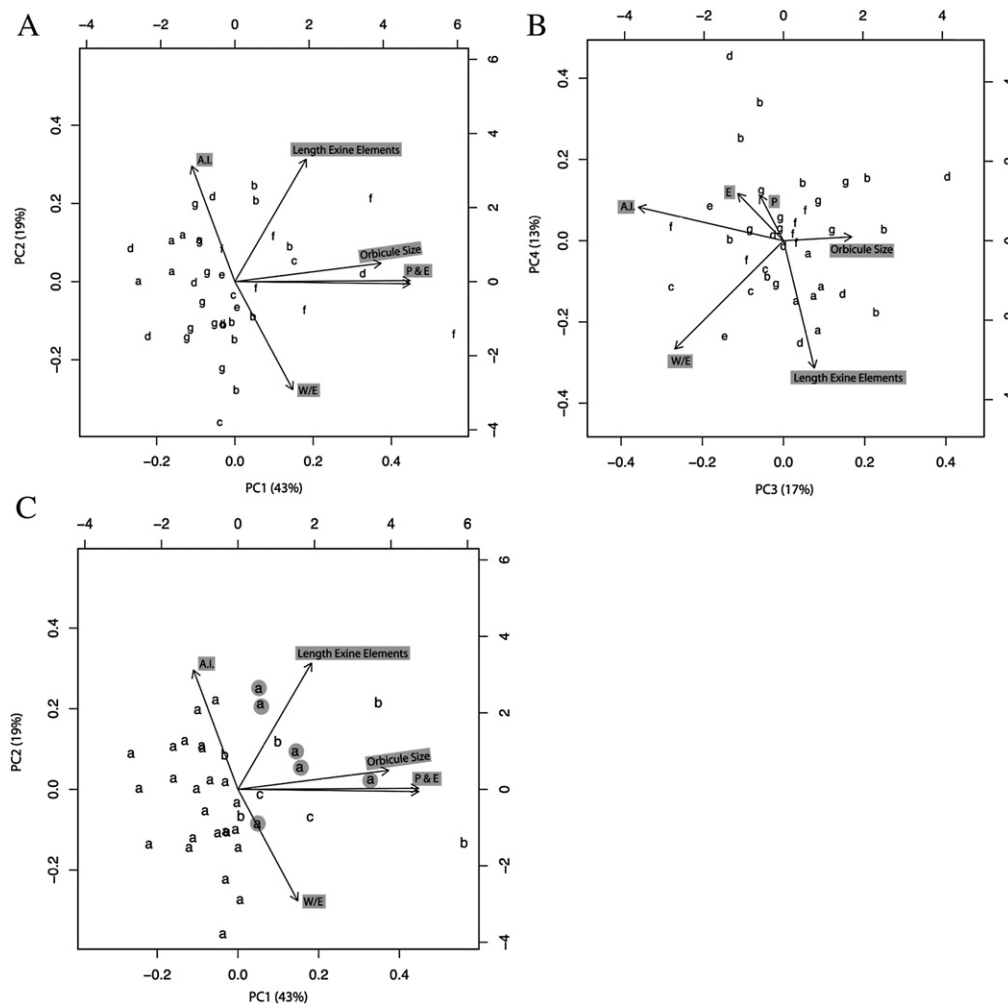
#### 4.2.2. Generic level

The three genera of Ebenoideae, *Euclea*, *Royena* and *Diospyros*, can be distinguished based on pollen and orbicule size, equatorial outline and exine ornamentation.

*Euclea* has solitary flowers or branched racemes with unisexual flowers and one-seeded fruits without persistent calyx that are considerably smaller than *Diospyros* fruits (Wallnöfer, 2001). The embryo of the seed is curved in a perpendicular plane and the radicle is

surrounded by the testa. The wood of *Euclea* has silica bodies in the ray cells, a character shared with *Royena* (Lens et al., 2005; Pletsers, 2005). Our palynological data provide additional evidence that *Euclea* is clearly distinct from the other genera. It has the smallest pollen and orbicules in Ebenaceae (see also Morton, 1994). The equatorial outline of *Euclea* pollen is predominantly lobate and the dominant ornamentation pattern is microrugulate subtype b. Although perforations occur in the exine, these are not limited to the mesocolpia as is the case in the African representatives of *Diospyros* and in *Royena*.

*Royena*, the second, relatively small African genus of Ebenaceae has not yet formally been given generic rank again, but molecular data show convincingly its monophyly (Morton et al., 1996; Duangjai et al., 2006). Although its general morphology closely resembles that of *Diospyros*, *Royena* shares several characteristics with *Euclea* such as the presence of club-shaped, multicellular glandular hairs, a deep invagination of the testa around the radicle and the presence of silica bodies in the ray cells of the wood (De Winter, 1963; Wallnöfer, 2001; Lens et al., 2005). *Royena* can, however, be distinguished from *Euclea* by some macromorphological characters: its structurally



**Fig. 6.** Principle component analysis: 2D-plots based on variation in six continuous characters (P, E, A.I., W/E, length exine elements and orbicule size). A. PC1 versus PC2. Letters correspond to the clades in Fig. 5. B. PC3 versus PC4. Letters correspond to clades in Fig. 5. C. PC1 versus PC2. Labels correspond to biogeographical distribution (see also Fig. 5): a = Africa, b = Asia, c = Neotropics.

hermaphroditic flowers, usually enlarged accrescent calyx and many seeded fruits (Duangjai et al., 2006). Palynologically, its subtriangular pollen and orbicules are quite large and comparable with the large grains of non-African species.

While size and equatorial outline are good characters to discriminate between *Euclea* and *Royena*, *Diospyros* species display a larger variety in equatorial outlines and sizes. Due to this large variation, these characters can be used to characterize subgeneric clades (see below). However, all *Diospyros* species share a rugulate exine ornamentation.

#### 4.2.3. Infrageneric level

In *Royena*, exine ornamentation varies considerably. Two subgroups can be distinguished in which there is an evolutionary trend towards smaller (rugulate subtype 1a, granular) or larger (striate) exine patterns. This tendency is also observed in *Euclea*: exine elements gradually become smaller.

Size, ectocolpus morphology, equatorial outline and exine ornamentation subtypes are the most valuable characters to distinguish between *Diospyros* subclades thereby confirming the conclusions of Morton (1994).

The African *Diospyros* species display the largest morphological variation within the entire family. For palynological characters, this variation seems to be restricted to clade D. Generally, the pollen and orbicule morphology of representatives of this clade resemble *Euclea* pollen and orbicules. Excluding *D. crassiflora* and *D. cooperi* as outliers, the pollen is small with long colpi but with a considerable amount of variation in the lengths of the exine elements and equatorial outline

type. In the two subgroups, the sexine elements evolved simultaneously from wide, long rugulae to fine elements smaller than 1 µm. Orbicule size can be used to distinguish between subgroups of this clade.

The second African clade (clade G) has rather uniform pollen and orbicule features: it has medium-sized pollen with mainly a subtriangular equatorial outline and microrugulate ornamentation of subtype 1a. The macromorphology of these forest species is also quite similar but some of the differences between species, e.g. the degree of tapering and shape of the leaf-apices, are easy to recognize and are of diagnostic value (White, 1956).

The Neotropical, Pacific and Asian Ebenoideae show relatively uniform palynological features, in line with a lower diversity at the macromorphological level. Species of temperate, non-African areas like North America, New Caledonia and temperate Asia are characterized by pollen with long, narrow colpi (with the exception of *D. lotus* and *D. texana*). The pollen and orbicules are variable in shape and size, but are generally very large. We could not confirm the observation that species from the New world have larger pollen than species of the Old World (Morton, 1994) due to insufficient sampling of the Neotropics.

## 5. Conclusions

The most recent ancestor of Ebenaceae and Ebenoideae most probably had subtriangular, medium-sized pollen with finely rugulate ornamentation (subtype b) in combination with medium-sized

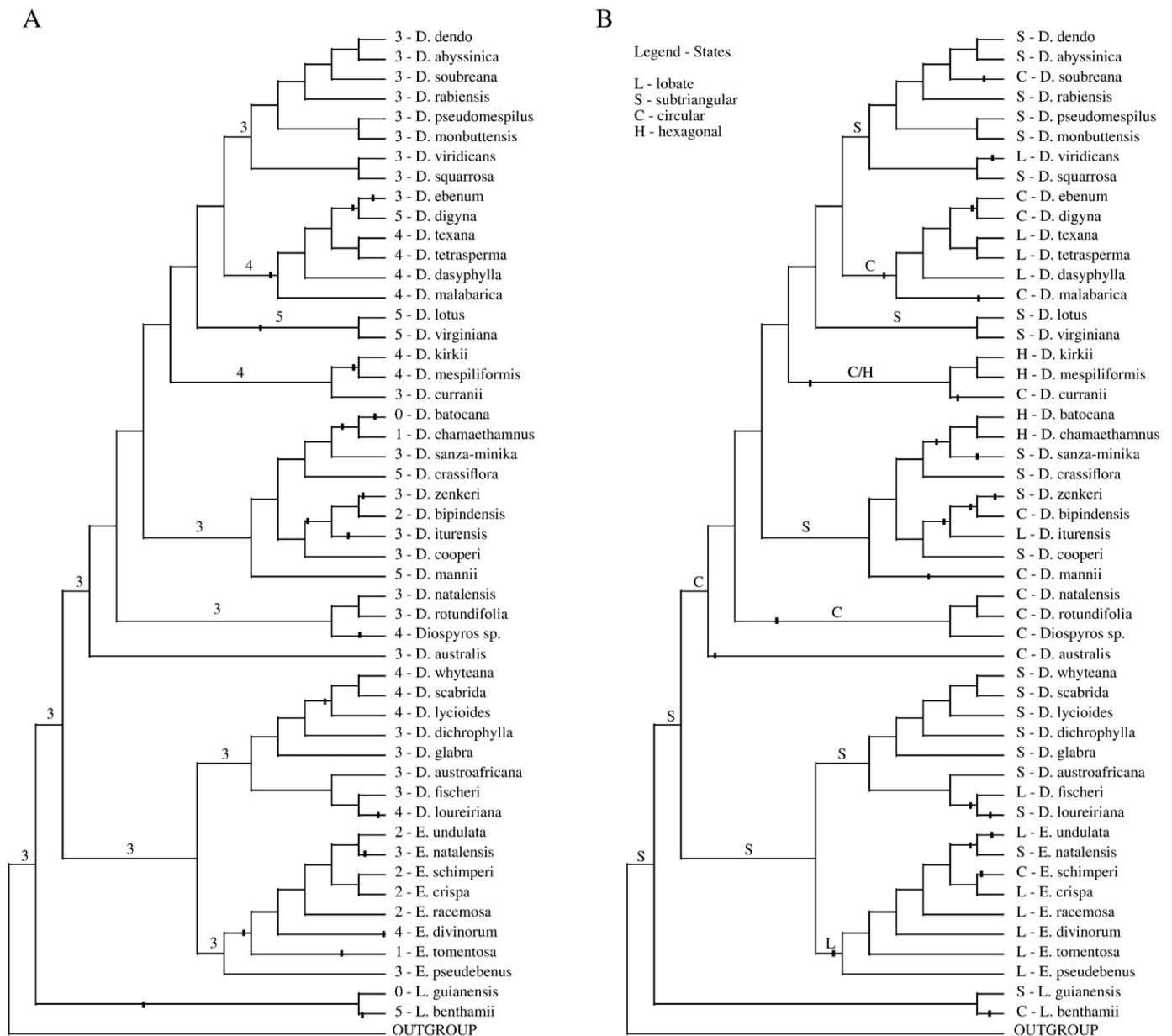
orbicules. The fossil record of Ebenaceae indicates that for the last 45 million years the same floral and foliar patterns exist (Basinger and Christophel, 1985). However, palynological characters vary considerably and provide synapomorphies for subfamilies (size, stratification of the pollen wall and ectocolpus shape and structure), genera (pollen and orbicule size, equatorial outline and sexine ornamentation) and infrageneric clades (e.g., pollen and orbicule size, ectocolpus morphology, equatorial outline and ornamentation (sub)type). Ebenaceae pollen and orbicules are thus more variable than previously assumed, but the variation is often found in continuous characters. Principle component analysis, scatterplots and analysis of variance are ideal tools to fully explore this kind of variation. In addition, Bayesian phylogenetic inference in combination with Bayesian posterior mapping are valuable alternatives for parsimonious approaches to gain more insight in ancestral states and character transformation.

The assessment of character homology remains the challenge for the future. Ornamentation types, endoaperture structure and pollen

wall stratification should be studied using TEM to obtain a clear picture of the ultrastructure. In addition, studies of sterile pollen in non-functional stamens of female flowers might also contribute to the understanding of the pollen morphological diversity in Ebenaceae.

### Acknowledgments

We thank the National Botanic Garden of Belgium (BR), the herbarium of the Royal Botanic Gardens Kew (K), the National Herbarium of the Netherlands, Leiden University Branch (L) and Wageningen University Branch (WAG). This project was financed by research grants of the K.U. Leuven (OT/05/35) and the Fund for Scientific Research-Flanders (FWO, Belgium) (G.0268.04). Material was collected in South Africa with financial support of the American Society of Plant Taxonomists (Graduate Student Research Grant) and the Leopold III Fund for Nature Research and Conservation (Belgium). Collecting of flowers in WAG was



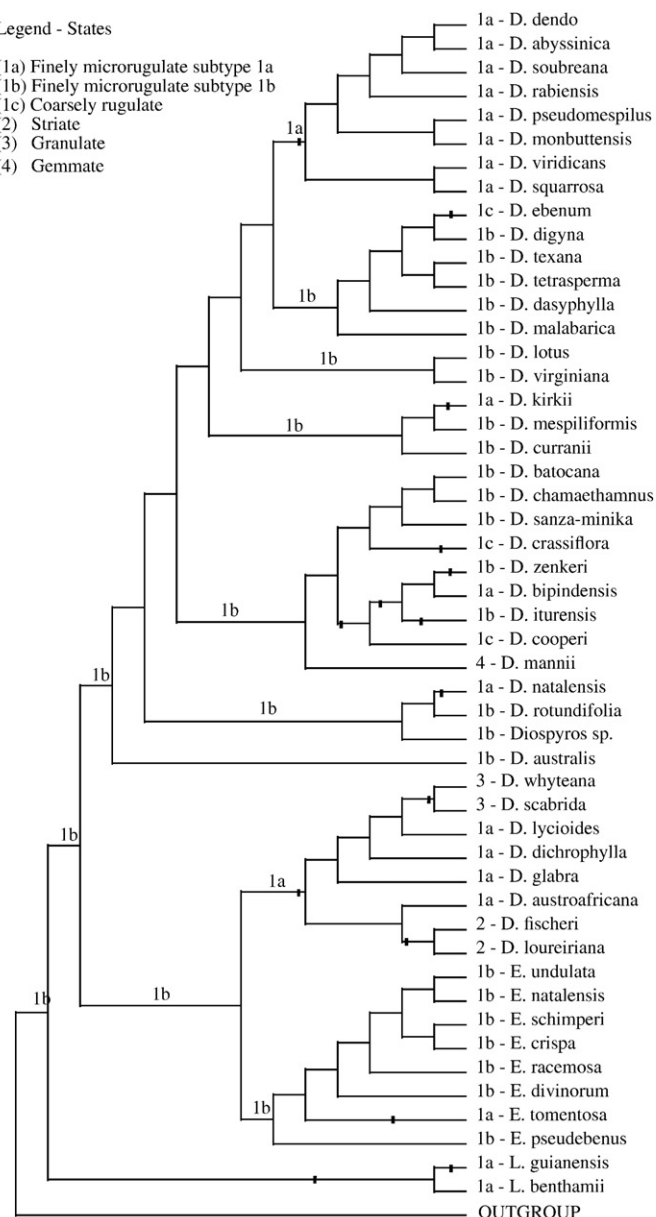
**Fig. 7.** Bayesian posterior mapping and inference of ancestral states for pollen and orbicule morphological characters. Ancestral states are indicated above branches. Rectangles on branches correspond to character state changes. Numbers in front of species names correspond to (simulated) character states. A. Pollen size. B. Amb. C. Sexine ornamentation type. D. Orbicule size.



C

## Legend - States

- (1a) Finely microrugulate subtype 1a  
 (1b) Finely microrugulate subtype 1b  
 (1c) Coarsely rugulate  
 (2) Striate  
 (3) Granulate  
 (4) Gemmate



D

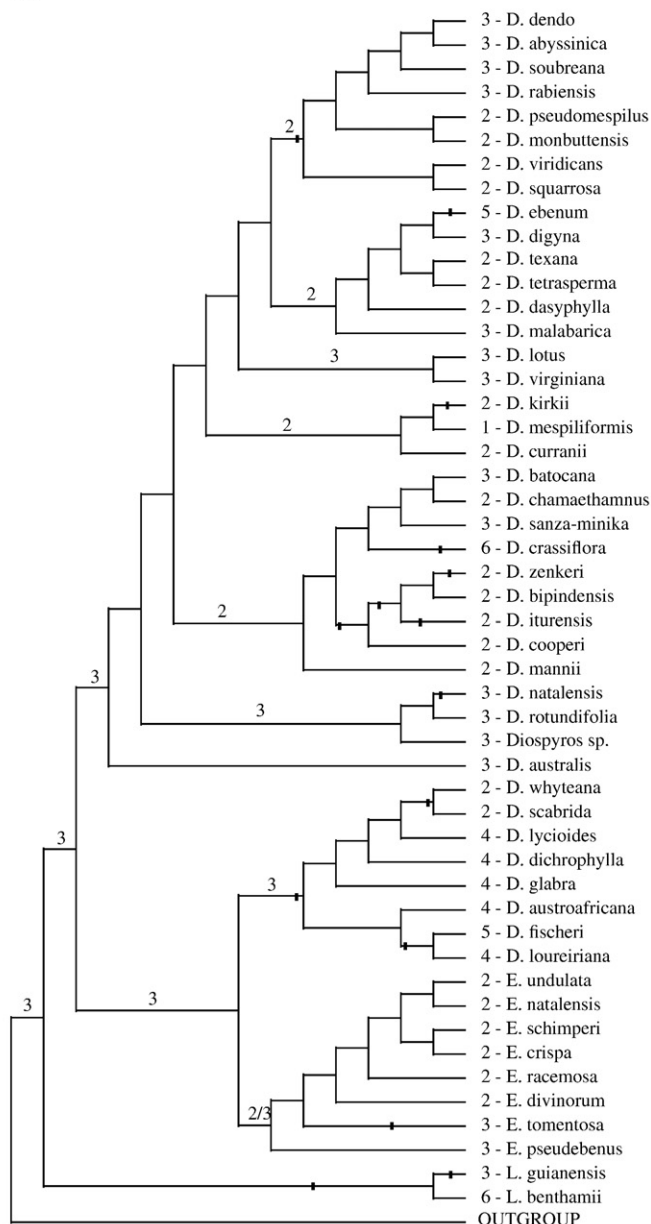


Fig. 7 (continued).

**Table 4**  
 Characters and their ancestral states

Characters	Pollen size Fig. 7A	Amb Fig. 7B	Ornamentation type Fig. 7C	Orbicule size Fig. 7D
Groups (Node)				
Ebenaceae (1)	3 (0.92)	Subtriangular (0.57)	1b (0.89)	3 (0.61)
Ebenoideae (2)	3 (1.00)	Subtriangular (0.72)	1b (0.98)	3 (0.68)
Euclea-Royena (3)	3 (1.00)	Subtriangular (0.60)	1b (0.90)	3 (0.54)
Euclea (A)	3 (0.98)	Lobate (1.00)	1b (0.95)	2 (0.53)/3 (0.47)
Royena (B)	3 (1.00)	Subtriangular (1.00)	1a (1.00)	3 (1.00)
Diospyros (4)	3 (1.00)	Circular (0.66)	1b (1.00)	3 (0.73)
Diospyros, clade C	3 (0.99)	Circular (1.00)	1b (1.00)	3 (1.00)
Diospyros, clade D	3 (1.00)	Subtriangular (0.97)	1b (1.00)	2 (0.96)
Diospyros, clade E	4 (0.80)	Circular (0.43)/ hexagonal (0.46)	1b (1.00)	2 (1.00)
Diospyros, clade F1	5 (0.77)	Subtriangular (1.00)	1b (1.00)	3 (0.99)
Diospyros, clade F2	4 (0.99)	Circular (0.59)	1b (1.00)	2 (0.61)
Diospyros, clade G	3 (1.00)	Subtriangular (1.00)	1a (1.00)	2 (0.74)

Posterior probabilities are given between brackets. None significant posterior probabilities are indicated in italic.

made possible by a Synthesys grant (NL-TAF-3633). Anke Geeraerts holds a personal PhD research grant from the IWT-Flanders.

#### Appendix A. List of specimens examined in this study and voucher information (country, collector, collection number and location). Spirit material is denoted with (S)

##### Diospyros

- D. abyssinica* (Hiern) F. White (1). Central African Republic. Tisserant 1898 BR.  
*D. abyssinica* (Hiern) F. White (2). Ethiopia. De Wilde 4837 BR.  
*D. barteri* Hiern. Gabon. Zenker 4524 BR.  
*D. batocana* Hiern. Zambia. Pope, Smith, Goydec 2196 BR.  
*D. bipindensis* Gürke. Cameroon. Bos 6775 WAG (S).  
*D. chamaethamnus* Dinter ex Mildbr. No country. Austaller, Klaassen, Mannheimer, Curtis SA24 WAG.  
*D. cooperi* (Hutch. & Dalz.) F. White. Ivory Coast. Beentje 885 WAG.  
*D. crassiflora* Hiern. Cameroon. van der Burgt 679 WAG.  
*D. curranii* Merr. Malaysia. Sumbing & Askin 96348 BR (S).  
*D. deltoidea* F. White. Gabon. Breteler et al. 10637 WAG.  
*D. dendo* Welw. ex Hiern (1). Gabon. McPherson 16271 BR.  
*D. dendo* Welw. ex Hiern (2). Cameroon. Leeuwenberg 5692 WAG.  
*D. iturensis* (Gürke) Letouzey & F.White (1). Cameroon. Leeuwenberg 9733 BR.  
*D. iturensis* (Gürke) Letouzey & F.White (2). s.l. Wieringa et al. 2795 WAG.  
*D. kirkii* Hiern. Malawi. Jackson 1407 BR.  
*D. longiflora* Hiern (1). Cameroon. Bos 3200 WAG.  
*D. longiflora* Hiern (2). Cameroon. De Wilde 8074 WAG.  
*D. lotus* L. (1). Pamir mountains. Vasak s.n. BR.  
*D. lotus* L. (2). Maroc. Thomas s.n. BR.  
*D. macrocalyx* Klotzsch. Mozambique. De Carvalho 1293 BR.  
*D. malabarica* Kostel. Sri Lanka. Collector unknown. BR.  
*D. mannii* Hiern (1). Liberia. Adam 27725 BR.  
*D. mannii* Hiern (2). Cameroon. Zenker 3439 L.  
*D. mespiliformis* Hochst. ex A.DC. D.R. Congo. Lisowski 23431 BR.  
*D. monbuttensis* Gürke. Ghana. Jongkind 2709 WAG (S).  
*D. natalensis* (Harv.) Brenan. Zimbabwe. Chase 7352 BR.  
*D. oubatchensis* Kosterm. New Caledonia. MacKee 32774 BR.  
*D. polystemon* Gürke. s.l. De Wilde 8090 WAG (S).  
*D. pseudomespilus* Mildbraed. Africa. Breyne 3210 WAG.

- D. rabiensis* Bret. Gabon. Schoenmaker 170 WAG (S).  
*D. rotundifolia* Hiern. South Africa. De Winter & Vahrmeijer 8513 BR.  
*D. sanza-minika* A. Chev. Ivory Coast. De Koning s.n. WAG (S).  
*D. soubreana* F. White. Ivory Coast. Leeuwenberg 3695 WAG.  
*D. squarrosa* Klotzsch. s.l. Robertson 4144 WAG.  
*D. texana* Scheele. USA. Daniel & Butterwick 8434 BR.  
*D. thwaitesii* Bedd. Sri Lanka. Bernardi 15741 BR.  
*D. virginiana* L. USA. Bell 12107 WAG.  
*D. viridicans* Hiern. Cameroon. Thomas 5714 BR/WAG.  
*D. zenkeri* (Gürke) F. White (1). Gabon. Breteler et al. 10750 WAG.  
*D. zenkeri* (Gürke) F. White (2). Gabon. Wieringa et al. 3010 WAG (S).  
*Diospyros* sp. Madagascar. Gautier & Totozafy 3169 WAG.

##### Euclea

- E. balfouri* Hiern ex Balf.f. South Africa. Ash 394 BR.  
*E. crispa* (Thunb) Gürke. South Africa. Bayliss BRI-728 BR.  
*E. divinorum* Hiern. South Africa. Geeraerts, Willaert & Ellis 89 BR.  
*E. natalensis* A. DC. (1). Zimbabwe. Norrgran 168 BR.  
*E. natalensis* A. DC. (2). Tanzania. Magogo 1995 BR.  
*E. pseudebenus* E. Mey. South Africa. Seydel 353 BR.  
*E. racemosa* Murr. South Africa. Breyne 5409 BR.  
*E. schimperi* (DC.) Dandy. Ethiopia. Bos 9787 WAG.  
*E. tomentosa* E. Mey. South Africa. Martens 2268 BR.  
*E. undulata* Thunb. Botswana. De Beer 679 BR.

##### Royena

- D. austro-africana* De Winter (1). South Africa. Schlieben & Ellis 12370 WAG.  
*D. austro-africana* De Winter (2). South Africa. Goldblatt 4320 BR.  
*D. austro-africana* De Winter (3). South Africa. Schlechter 5139 BR.  
*D. dichrophylla* (Gand.) De Winter. Mozambique. Zunguze, Beane & Dungo 581 BR.  
*D. fischeri* Gürke. Tanzania. Richards & Arasululu 25945 WAG.  
*D. glabra* (L.) De Winter. South Africa. Goldblatt 3077 BR.  
*D. lycioides* Desf. Namibia. Merxmüller & Gies 32446 BR.  
*D. scabrida* (Harv. Ex Hiern) De Winter. South Africa. Bayliss 6664 WAG.  
*D. loureiriana* G. Don. Mozambique. Torre & Correia 16658 BR.  
*D. whyteana* (Hiern) De Winter. Zimbabwe. Bamps, Symoens & Van den Bergen 362 BR.  
*D. zombensis* (B.L. Burtt) F. White. Tanzania. Mayers 288 WAG.

#### Appendix B

Species	Voucher information	Accession numbers				
		MatK	NdhF	trnH-psbA	trnL-trnF	trnC-petN
Outgroup						
<b>Argania</b>	<b>Sequences from Genbank</b>	DQ924090	DQ924198	DQ923981	DQ924307	AM179647
<b>Madhuca</b>	<b>Sequences from Genbank</b>	DQ924091	DQ924199	AM179727	DQ924308	AM179661
<b>Manilkara</b>	<b>Sequences from Genbank</b>	DQ924092	DQ924200			
<i>Diospyros</i>						
<i>Diospyros abyssinica</i> (Hiern) F. White	Luke WRQ & PA 11664 BR	FJ238143	FJ238307	FJ238228	FJ238271	FJ238185
<i>Diospyros australis</i> (R.Br.) Hiern	Provance 46 s.l.	FJ238131		FJ238214	FJ238257	
<i>Diospyros batocana</i> Hiern	Steyl 88 K			FJ238219	FJ238262	FJ238176
<b><i>Diospyros bipindensis</i> Gürke</b>	<b>Sequences from Genbank</b>	DQ923996	DQ924106		DQ924213	
<i>Diospyros chamaethamnus</i> Dinter ex Mildbr.	Horn 23 K			FJ238220	FJ238263	FJ238177
<b><i>Diospyros cooperi</i> (Hutch. &amp; Dalz.) F. White</b>	<b>Sequences from Genbank</b>	DQ924007	DQ924117		DQ9241224	
<i>Diospyros crassiflora</i> Hiern	Senterre & Ngomo 658 BRLU	FJ238126	FJ238301	FJ238221	FJ238264	FJ238178
<b><i>Diospyros curranii</i> Merr.</b>	<b>Sequences from Genbank</b>	DQ924009	DQ924119		DQ924226	
<b><i>Diospyros dasyphylla</i> Kurz</b>	<b>Sequences from Genbank</b>	DQ924019	DQ924120		DQ924227	
<i>Diospyros dendo</i> Welw. ex Hiern	Heuertz 0825 BRLU	FJ238144	FJ238308	FJ238229	FJ238272	FJ238186
<i>Diospyros digyna</i> Jacq.	Billiet S3693 BR	FJ238153	FJ238314	FJ238238	FJ238281	FJ238195
<i>Diospyros duclouxii</i> Dode in Pourtet	Coombes 1976/8047 L	FJ238141	FJ238305	FJ238226	FJ238269	FJ238183
<i>Diospyros ebenum</i> Hiern	Chase 1146 K	FJ238154	FJ238315	FJ238239	FJ238282	FJ238196
<i>Diospyros iturensis</i> (Gürke) Letouzey & F.White	Senterre & Obiang 4061 BRLU	FJ238137	FJ238302	FJ238222	FJ238265	FJ238179
<i>Diospyros kirkii</i> Hiern	Luke WRQ & PA 11577 BR	FJ238132	FJ238297	FJ238215	FJ238258	FJ238172
<i>Diospyros malabarica</i> Kostel	Chase 1247 K	FJ238151	FJ238312	FJ238236	FJ238279	FJ238193

(continued on next page)

## Appendix B (continued)

Species	Voucher information	Accession numbers				
		MatK	NdhF	trnH-psbA	trnL-trnF	trnC-petN
<i>Diospyros mannii</i> Hiern	Senterre & Obiang 3964 BRLU	FJ238138	FJ238303	FJ238223	FJ238266	FJ238180
<i>Diospyros mespiliformis</i> Hochst. ex A.DC.	Luke 11657 BR	FJ238133	FJ238298	FJ238216	FJ238259	FJ238173
<i>Diospyros monbutensis</i> Gürke	Senterre & Kouop 1240 BRLU	FJ238145		FJ238230	FJ238273	FJ238187
<i>Diospyros natalensis</i> (Harv.) Brenan	Ellis et al. 118 BR	FJ238134	FJ238299	FJ238217	FJ238260	FJ238174
<i>Diospyros pseudomespilus</i> Mildbraed	Walters et al. 956 MO	FJ238146	FJ238309	FJ238231	FJ238274	FJ238188
<i>Diospyros rabiensis</i> Bret.	Haegens 37 WAG	FJ238147		FJ238232	FJ238275	FJ238189
<i>Diospyros rotundifolia</i> Hiern	Ellis et al. 112 BR	FJ238135	FJ238300	FJ238218	FJ238261	FJ238175
<i>Diospyros sanza-minika</i> A. Chev.	Senterre & Obiang 4242 BRLU	FJ238139	FJ238304	FJ238224	FJ238267	FJ238181
<i>Diospyros soubreana</i> F. White	Billiet S3698 BR	FJ238148	FJ238310	FJ238233	FJ238276	FJ238190
<i>Diospyros squarrosa</i> Klotzsch	Luke WRQ & PA 11683 BR	FJ238149	FJ238311	FJ238234	FJ238277	FJ238191
<i>Diospyros tetrasperma</i> Sw.	Chase 14254 K	FJ238152	FJ238313	FJ238237	FJ238280	FJ238194
<b><i>Diospyros texana</i> Scheele</b>	<b>Sequences from Genbank</b>	DQ924060	DQ924170		DQ924277	
<i>Diospyros virginiana</i> L.	De Meyere 95/0647 BR	FJ238142	FJ238306	FJ238227	FJ238270	FJ238184
<i>Diospyros viridicans</i> Hiern	Wieringa 4266 WAG	FJ238150		FJ238235	FJ238278	FJ238192
<i>Diospyros zenkeri</i> (Gürke) F. White	Breteler 14886 WAG	FJ238140		FJ238225	FJ238268	FJ238182
<b><i>Tetractis cf. clusiaefolia</i> Hiern</b>	<b>Sequences from Genbank</b>	DQ924089	DQ924197		DQ924306	
<i>Euclea</i>						
<i>Euclea crispa</i> (Thunb) Gürke	Ellis et al. ZA42 BR	FJ238116	FJ238284	FJ238198	FJ238241	FJ238156
<i>Euclea divinorum</i> Hiern	Ellis et al. ZA89 BR	FJ238117	FJ238285	FJ238199	FJ238242	FJ238157
<i>Euclea natalensis</i> A. DC.	Ellis et al. ZA66 BR	FJ238118	FJ238286	FJ238200	FJ238243	FJ238158
<i>Euclea pseudebenus</i> E. Mey.	Ellis et al. ZA152 BR	FJ238119		FJ238201	FJ238244	FJ238159
<i>Euclea racemosa</i> Murr.	Ellis et al. ZA1 BR	FJ238120	FJ238287	FJ238202	FJ238245	FJ238160
<i>Euclea schimperi</i> (DC.) Dandy	Ellis et al. ZA52 BR	FJ238121	FJ238288	FJ238203	FJ238246	FJ238161
<i>Euclea tomentosa</i> E. Mey.	Joubert ZA1 L		FJ238289	FJ238204	FJ238247	FJ238162
<i>Euclea undulata</i> Thunb.	Ellis et al. ZA98 BR	FJ238122	FJ238290	FJ238205	FJ238248	FJ238163
<i>Lissocarpa</i>						
<i>Lissocarpa guianensis</i> Gleason	Arets s.n. U	FJ238115	FJ238283	FJ238197	FJ238240	FJ238155
<b><i>Lissocarpa benthamii</i> Gürke</b>	<b>Berry et al. 7217 K</b>	DQ924077	DQ924187		DQ924294	
<i>Royena</i>						
<i>Diospyros austro-africana</i> De Winter	Daniel 9316 CAS	FJ238123		FJ238206	FJ238249	FJ238164
<i>Diospyros dichrophylla</i> (Gand.) De Winter	Ellis et al. ZA63 BR	FJ238124	FJ238291	FJ238207	FJ238250	FJ238165
<i>Diospyros fischeri</i> Gürke	Luke WRQ & PA 11576 BR	FJ238125	FJ238292	FJ238208	FJ238251	FJ238166
<i>Diospyros glabra</i> (L.) De Winter	Ellis et al. ZA25 BR	FJ238126	FJ238293	FJ238209	FJ238252	FJ238167
<i>Diospyros loureiriana</i> G.Don	Luke WRQ & PA 11649 BR	FJ238127	FJ238294	FJ238210	FJ238253	FJ238168
<i>Diospyros lycioides</i> Desf.	Ellis et al. ZA54 BR	FJ238128	FJ238295	FJ238211	FJ238254	FJ238169
<i>Diospyros scabrida</i> (Harv. Ex Hiern) De Winter	Ellis et al. ZA50 BR	FJ238129		FJ238212	FJ238255	FJ238170
<i>Diospyros whyteana</i> (Hiern) De Winter	Ellis et al. ZA20 BR	FJ238130	FJ238296	FJ238213	FJ238256	FJ238171

## References

- Angiosperm Phylogeny Group, 2003. An update of the angiosperm phylogeny group classification for the orders and families of flowering plants: APGII. Bot. J. Linn. Soc. 141, 399–436.
- Bakhuizen-van den Brink, R.C., 1936–1955. Revisio Ebenacearum Malayensium. Bull. Jard. Bot. Buitenzorg 3, 1–515.
- Basing, J.F., Christophel, D.C., 1985. Fossil flowers and leaves of the Ebenaceae from the Eocene of southern Australia. Can. J. Bot. 63, 1825–1843.
- Berry, P.E., 2001. Lissocarpaceae. Flora of the Venezuelan Guayana, vol. 6.
- Berry, P.E., Savolainen, V., Sytsma, K.J., Hall, J.C., Chase, M.W., 2001. *Lissocarpa* is sister to *Diospyros* (Ebenaceae). Kew Bull. 56, 725–729.
- Bollback, J.P., 2006. Stochastic character mapping of discrete traits on phylogenies. BMC Bioinformatics 7, 88.
- Buchner, R., Weber, M., 2000 onwardss. PalDat—A Palynological Database: Descriptions, Illustrations, Identification, and Information Retrieval. (<http://www.paldat.org/>).
- Contreras, L.S., Lersten, N.R., 1984. Extrafloral nectaries in Ebenaceae: anatomy, morphology and distribution. Am. J. Bot. 71, 865–872.
- Copenhagen, G.P., 2005. A compendium of plant species producing pollen tetrads. J. N.C. Acad. Sci. 121, 17–35.
- De Winter, B., 1963. Ebenaceae. Flora S. Afr. Flower. plants 26, 54–99.
- Dickson, W.C., Nowicke, J.W., Skvarla, J.J., 1982. Pollen morphology of the Dilleniaceae and Actinidiaceae. Am. J. Bot. 69, 1055–1073.
- Duangjai, S., Wallnöfer, B., Samuel, R., Munzinger, J., Chase, M.W., 2006. Generic delimitations and relationships in Ebenaceae sensu lato: evidence from six plastid DNA regions. Am. J. Bot. 93, 1808–1827.
- Erdtman, G., 1969. Handbook of Palynology: Morphology–Taxonomy–Ecology: An Introduction to the Study of Pollen Grains. Munksgaard, Copenhagen.
- Erdtman, G., 1971. Pollen Morphology and Plant Taxonomy: Angiosperms, (3rd edition). Hafner Publishing Co., New York.
- Erdtman, G., Berglund, B., Pragłowski, J., 1961. An Introduction to Scandinavian Pollen Flora. Almqvist & Wiksell, Uppsala.
- Franceschi, D. de, 1993. Phylogénie des Ebénales: analyse de l'ordre et origin biogéographique des espèces indienne. Publ. Dép. Écol., Inst. Fr. ponichéry 33, 1–153.
- Harley, M.M., 1986. Distinguishing pollen characters for the Sapotaceae. Can. J. Bot. 64, 3091–3100.
- Huelsenbeck, J.P., Ronquist, F., Nielsen, R., Bollback, J.P., 2001. MrBayes: Bayesian inference of phylogeny. Bioinformatics 17, 754–755.
- Huysmans, S., El-Ghazaly, G., Smets, E., 1998. Orbicules in angiosperms: morphology, function, distribution and relation with tapetum types. Bot. Rev. 64, 240–272.
- Huysmans, S., El-Ghazaly, G., Smets, E., 2000. Orbicules: still a well hidden secret of the anther. Plant Systematics for the 21st century. Wenner-Gren Int. Ser. 77, 201–212.
- Kosmath, L. von, 1927. Studien über das Antherentapetum. Österr. Bot. Z. 76, 235–241.
- Kostermans, A.J.G.H., 1977. Notes on Asiatic, Pacific and Australian *Diospyros*. Blumea 23, 449–474.
- Lens, F., Dressler, S., Vinckier, S., Janssens, S., Dessein, S., Van Evelghem, L., Smets, E., 2005. Palynological variation in Balsaminoid Ericales. I. Margraviaceae. Ann. Bot. 96, 1047–1060.
- Letouzey, R., White, F., 1970. Ebenaceae. Flore Cameroun 11, 3–181.
- Morton, C., 1994. The use of pollen morphology and wood anatomy in the study of the phylogeny of Ebenaceae and Rutaceae subtribe Cuspariinae. PhD dissertation, The City University of New York.
- Morton, C.M., Dickson, W.C., 1992. Comparative pollen morphology of Styracaceae. Grana 31, 1–15.
- Morton, C.M., Chase, M.W., Kron, K.A., Swensen, S.M., 1996. A molecular evaluation of the monophyly of the order Ebenales based upon *rbcL* sequence data. Syst. Bot. 21, 567–586.
- Ng, F.S.P., 1971. A taxonomic study of the Ebenaceae with special reference to Malesia. Thesis at the University of Oxford, 221 pp. (microfilmed by British lending library division: no. D 183904).
- Nylander, J.A.A., Ronquist, F., Huelsenbeck, J.P., Nieves-Aldrey, J.L., 2004. Bayesian phylogenetic analysis of combined data. Syst. Biol. 53, 47–67.
- Oldfield, F., 1959. The pollen morphology of some of the West European Ericales. Pollen Spores 1, 19–48.
- Phiri, P.S.M., 2005. A checklist of Zambian vascular plants. S. Afr. Bot. Div. Network Report 32, 32.



- Pletsers, A., 2005. Houtanatomie en pollenmorphologie van Ebenaceae. Masters Thesis. Institute of Botany and Microbiology, K.U.Leuven.
- Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14, 817–818.
- Punt, W., Blackmore, S., Nilsson, S., Le Thomas, A., 2007. Online Glossary of Pollen and Spore Terminology. (<http://www.bio.uu.nl/%7epalaeo/glossary/glos-int.htm>).
- Rambaut, A., Drummond, A.K., 2007. Tracer v1.4 (<http://beast.bio.ed.ac.uk/Tracer>).
- Reitsma, T., 1969. Size modifications of recent pollen under treatments. *Rev. Palaeobot. Palyn.* 9, 175–202.
- Ribeiro, J.E.L. da S., 1999. Flora da Reserva Ducke. INPA. DFID.
- Schols, P., Dessein, S., D'hondt, C., Huysmans, S., Smets, E., 2002. Carnoy: a new digital measurement tool for palynology. *Grana* 41, 124–126.
- Schols, P., D'Hondt, C., Geuten, K., Merckx, V., Jansen, S., Smets, E., 2004. Morphocode: coding quantitative data for phylogenetic analysis. *Phyloinformatics* 4, 1–4.
- Sharma, C., Gupta, H.P., 1979. Pollen morphology and phylogeny of Indian Ebenaceae. *Geophytology* 8, 209–214.
- Shu-Kang, L., 1987. Ebenaceae. *Fl. Reipubl. Popularis Sin.* 60, 84–154.
- Shukla, A.K., Vijayaraghavan, M.R., Chaudry, B., 1998. *Biology of Pollen*. APH Publishing Corporation, New Delhi.
- Singh, V., 2005. Monograph on Indian *Diospyros* L. (persimmon, ebony) Ebenaceae. Botanical Survey of India, Kolkata, India.
- Sothers, C., Berry, P.E., 1998. Ebenaceae. In: Steyermark, J.A., Berry, P.E., Holst, B.K. (Eds.), *Flora of the Venezuelan Guayana. Caesalpinaceae–Ericaceae*, vol. 4. MBG press, USA.
- St. John, H., 1986. Revision of the Hawaiian *Diospyros* (Ebenaceae). *Phytologia* 59, 389–405.
- Thiele, K., 1993. The holy grail of the perfect character: the cladistic treatment of morphometric data. *Cladistics* 9, 275–304.
- Ueno, J., 1962. Palynological notes on Ericaceae and Pyrolaceae from Japan and its neighbors. *Acta Phytotaxon. Geobot.* 20, 101–112.
- Ventenat, E.P., 1799. *Tableau du regne végétal, Selon la méthode de Jussieu. II. Del'impr. De J. Drisonnier*, Paris.
- Walker, J.W., 1971. Pollen morphology, phytogeography, and phylogeny of Annonaceae. *Contrib. Gray Herb. Harvard Univ.* 202, 1–132.
- Walker, J.W., 1976. Evolutionary significance of the exine in pollen of primitive angiosperms. In: Ferguson, I., Muller, J. (Eds.), *The Evolutionary Significance of the Exine*. Academic Press, London, pp. 251–308.
- Walker, J.W., Doyle, J.A., 1975. The bases of angiosperm phylogeny: palynology. *Ann. Mo. Bot. Gard.* 62, 664–723.
- Wallnöfer, B., 2001. The biology and systematics of Ebenaceae: a review. *Ann. Naturhist. Mus. Wien* 103B, 485–512.
- Wallnöfer, B., 2004a. A revision of *Lissocarpa* Benth. (Ebenaceae subfam. Lissocarpoideae (Gilg. In Engler) B. Walln.). *Ann. Naturhist. Mus. Wien* 105B, 515–564.
- Wallnöfer, B., 2004b. Ebenaceae. In: Kubitzki, K. (Ed.), *The families and genera of vascular plants*, Vol. 6. Springer Verlag, Berlin, Heidelberg, pp. 125–130.
- Wallnöfer, B., 2004c. Lissocarpaceae. In: Kubitzki, K. (Ed.), *The Families and Genera of Flowering Plants*, vol. 6. Springer Verlag, Berlin, Heidelberg, pp. 236–238.
- White, F., 1956. Notes on the Ebenaceae II. *Diospyros piscatoria* and its allies. *Bull. Jard. Bot. Etat Brux.* 26, 277–307.
- White, F., 1978. Flora of Panama Part VIII. Family 155. Ebenaceae. *Ann. Mo. Bot. Gard.* 65, 145–154.
- White, F., 1980. Notes on Ebenaceae. VIII. The African sections of *Diospyros*. *Bull. Jard. Bot. Natl. Belg.* 50, 445–460.
- White, F., Letouzey, R., 1970. Ebenacées. *Flore Gabon* 18, 1–189.