

Pollen morphology of *Dioscorea* (Dioscoreaceae) and its relation to systematics

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This paper adds new data on the pollen and orbicule morphology of 61 *Dioscorea* L. (Dioscoreaceae) species to the survey of Schols *et al.* (2001). The results indicate that pollen characters may be significant in infrageneric systematics in *Dioscorea*. Pollen and orbicule characters are described based on observations with light microscopy, and scanning and transmission electron microscopy, and are critically evaluated and discussed in the context of existing hypotheses of systematic relationships within the genus. Pollen is mostly disulcate (sometimes monosulcate) with a perforate, microreticulate, striate, gemmate, rugulate, or cerebriform perforate sexine. The basal section *Stenophora* is one of the few sections with monosulcate pollen. *Brachyandra*, *Cardiocrapsa*, and *Seriflorae*, three Malagasy sections, are characterized by striate pollen. Pollen morphology strongly supports section *Enantiophyllum* as a monophyletic group. The correlation between pollen size and tuber type, as suggested previously by P. Su (1987), is confirmed by our data. As found in our earlier survey, orbicules in *Dioscorea* are mostly spherical with a smooth or spinulose surface. © 2003 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2003, 143, 375–390.

ADDITIONAL KEYWORDS: orbicules – pollen aperture – pollen ultrastructure – sectional classification – sexine ornamentation.

INTRODUCTION

Pollen and orbicule morphology of 35 species of *Dioscorea* L. (Dioscoreaceae), a large tropical and subtropical genus of some 400 species, were examined by Schols *et al.* (2001) which includes an overview of previous work. They concluded, based on this limited sample, that pollen morphological characters provided useful systematic data within the genus. The results from this study indicated that pollen of *Dioscorea* is disulcate (see discussion on p. 380) or sometimes monosulcate, with a perforate, microreticulate or striate sexine, and orbicules are spherical with a smooth or spinulose surface (Schols *et al.*, 2001). The close relationship between sections *Asterotricha* and *Enantiophyllum* (for authors of all taxa, see Appendix) originally proposed by Burkill (1960) was supported by

pollen morphological characters, as were the macromorphological differences between sections *Botryosicyos* and *Lasiophyton*. The results also supported the hypothesis of Su (1987) that pollen of taxa with annual tubers is smaller than that of sections with persistent tubers. Schols *et al.* (2001) were the first to focus on the relevance of pollen morphology in the infrageneric systematics of *Dioscorea*. This was a preliminary study and, in the present paper, the data set of Schols *et al.* (2001) is expanded to include a further 61 species, in order to cover all lineages suggested to exist by macromorphology and to investigate more fully the systematic importance of pollen morphology within *Dioscorea*.

MATERIAL AND METHODS

MATERIAL

Dried material came from the Herbarium of the Royal Botanic Gardens, Kew (K: followed by collector's name

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and number) and the National Botanic Garden of Belgium (BR: followed by collector's name and number). Species are listed alphabetically by section in the Appendix, and those specimens examined using transmission electron microscopy are indicated with an asterisk.

METHODS

Light microscopy (LM)

Pollen was acetolysed for 10 min in a heating block at 90°C using the method of Reitsma (1969) and embedded in Kaiser's glycerine jelly. The stratification of the pollen exine was observed using LM in all 61 species.

Scanning electron microscopy (SEM)

Every specimen was subjected to two treatments: acetolysis as for LM and critical point drying (CPD). Methods are as in Schols *et al.* (2001).

Transmission electron microscopy (TEM)

Anthers of *D. bridgesii* were placed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2), de-aerated under vacuum for 1 h and fixed for 16–20 h at 4°C. They were washed in cacodylate buffer, post-fixed in 1% buffered osmium tetroxide for 3 h at room temperature and washed again. Tissues were dehydrated through an ethanol series followed by three changes of 100% ethanol and embedded in LR White resin (London Resin Co.) in gelatin capsules. Semithin sections were stained with thionin (0.1%) and methylene blue (1%) and examined using a Leica light microscope with a Nikon camera attachment. Ultrathin sections on copper grids were stained with uranyl acetate and lead citrate. Electron micrographs were taken using a Zeiss EM906 transmission electron microscope at 80 kV.

Pollen measurements

For each species, the longest axis (LA) and the shortest axis (SEA) were measured from ten grains, using LM slides of acetolysed pollen. Measurements of perforation size, perforation density, width of muri, murus density, and orbicule diameter were carried out using Carnoy 2.1 for Mac OS X (Schols *et al.*, 2002) on digital SEM images (Table 1). Terminology follows the international glossary (Punt *et al.*, 1998). To test the correlation between pollen size and tuber type (Su, 1987) we checked the normality (Kurtosis Normality test) and equality of variances (Variance-Ratio Equal-Variance test and Modified-Levene Equal-Variance test) of both groups (annual vs. persistent tubers). These latter tests confirmed equal variances and the former confirmed a normal distribution of data in both groups using a 0.05 confidence interval. Subsequently,

a two-sample equal-variance *t*-test (one-tailed distribution) was carried out to test whether the difference in pollen size between both groups is significant (Sokal & Rohlf, 1981).

RESULTS AND DISCUSSION

POLLEN AND ORBICULE CHARACTERS

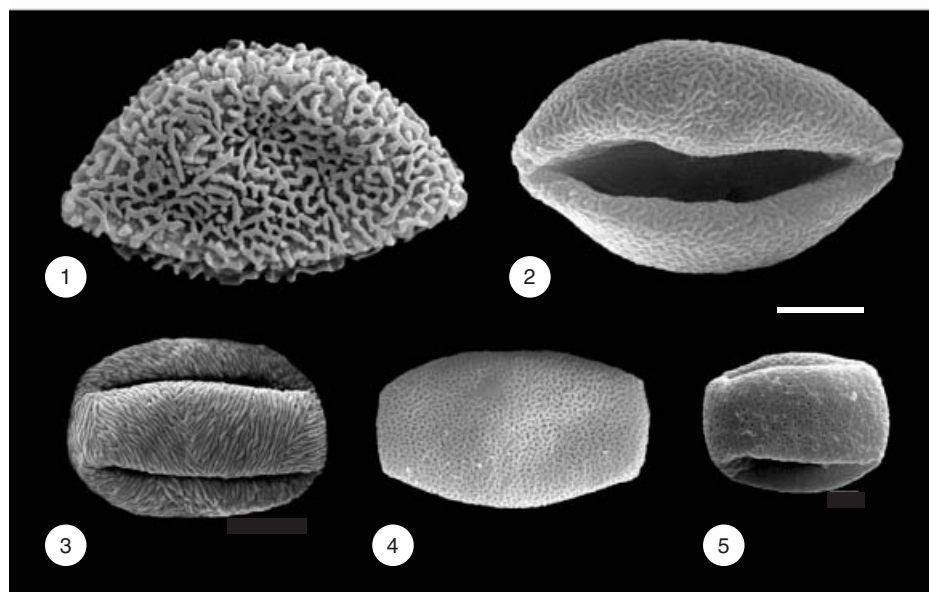
Dioscorea pollen is mostly disulcate (sometimes monosulcate) with a perforate, microreticulate, striate, gemmate, rugulate, or cerebroid perforate sexine. The average LA ranges from 20 to 58 µm. Orbicules are mostly spherical with a smooth or spinulose surface (microechinate is the term preferred by Punt *et al.* (1998) but we have retained the term spinulose because it was used in our earlier paper (Schols *et al.*, 2001)). These characters are discussed below and summarized in Table 1. Percentages given in the following paragraphs refer to all 96 species examined in this survey and that of Schols *et al.* (2001). All other data are given for the 61 species observed in this paper.

Pollen size

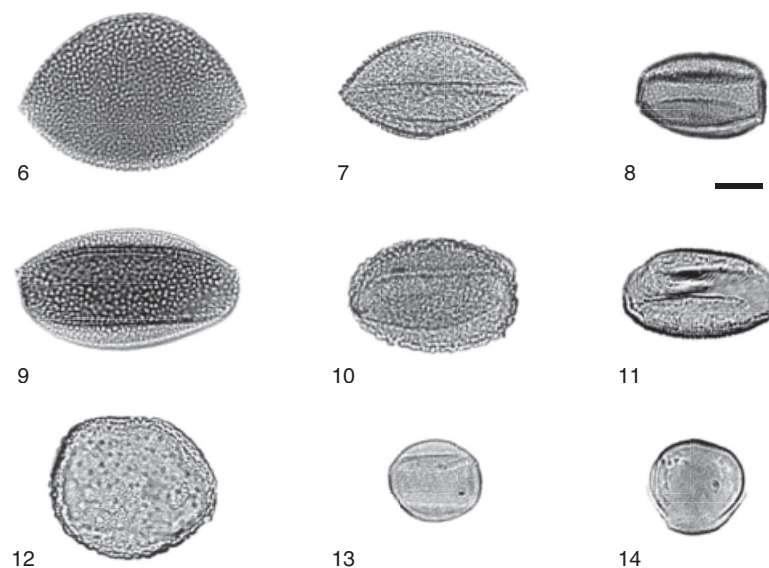
Dioscorea exhibits a wide infrageneric variation in pollen size (Table 1, Figs 1–14). The mean value of LA for all species investigated is 35 µm (Table 1). The smallest pollen grains were found in *D. sinuata* (section *Brachystigma*) and *D. alatipes* (section *Brachyandra*). These species have an average LA of 20 µm and 22.5 µm, respectively. Species from sections *Brachyandra*, *Brachystigma*, and *Enantiophyllum* have pollen grains that are mostly smaller than 32 µm. By contrast, rather large pollen grains can be found within sections *Borderea*, *Parallelostemon*, *Rhacodophyllum*, *Shannicorea*, *Stenophora*, and *Testudinaria*, with mean LA values ranging from 35 to 40 µm. *D. pyrenaica* has the largest average LA in this survey, with a value of 58 µm. The smallest average in Schols *et al.* (2001) was for *D. bulbifera*, with an LA of only 16 µm, whereas the largest LA was found in *D. buchananii* (45 µm).

Measurements of the shortest equatorial axis (SEA) range from 13 µm (*D. nako*, *D. ovinala*, *D. sinuata*) to 45 µm (*D. rupicola*), compared with values of between 10 and 34 µm found by Schols *et al.* (2001). SEA values are correlated with LA values to a great extent (Table 1). Note that SEA values might not be as reliable as LA values owing to harmomegathic accommodation because the grains collapse inwards along the longest axis (see 'Apertures' on p. 380).

From comparing pollen size (Table 1, Figs 1–14) we found that the largest pollen grains occur in sections *Stenophora* and *Shannicorea*. Somewhat smaller grains occur in *Apodostemon*, *Borderea*, *Dematostemon*, *Rhacodophyllum*, and *Trigonobasis* and the smallest grains are found in *Brachyandra*,



Figures 1–5. Comparison of pollen size (SEM). All micrographs are at the same magnification. Pollen size in parentheses. Fig. 1. *D. nipponica* (sect. *Stenophora*) equatorial view, monosulcate, rugulate (45 µm). Fig. 2. *D. caucasica* (sect. *Stenophora*) distal polar view, monosulcate, perforate (45 µm). Fig. 3. *D. olfersiana* (sect. *Cryptantha*) distal polar view, disulcate, striate (30 µm). Fig. 4. *D. cirrhosa* (sect. *Enantiophyllum*) proximal polar view, disulcate, perforate (30 µm). Fig. 5. *D. sinuata* (sect. *Brachystigma*) distal polar view, disulcate, perforate (20 µm). Scale bar = 5 µm.



Figures 6–14. Overview of pollen size, shape and ornamentation (LM). All micrographs are at the same magnification. Fig. 6. *D. caucasica* (sect. *Stenophora*) equatorial view, monosulcate, perforate. Fig. 7. *D. nipponica* (sect. *Stenophora*) distal polar view, monosulcate, rugulate. Fig. 8. *D. densiflora* (sect. *Oxypetalum*) distal polar view, disulcate, perforate. Fig. 9. *D. minima* (sect. *Polyneuron*) distal polar view, disulcate, perforate–microreticulate. Fig. 10. *D. bridgesii* (sect. *Parallellostemon*) distal polar view, disulcate, cerebriform perforate. Fig. 11. *D. namorokensis* (sect. *Brachyandra*) distal polar view, disulcate, striate. Fig. 12. *D. ridleyi* (sect. *Stenophora*) distal polar view, disulcate, cerebriform perforate. Fig. 13. *D. nummularia* (sect. *Enantiophyllum*) distal polar view, disulcate, perforate. Fig. 14. *D. nummularia* (sect. *Enantiophyllum*) equatorial view, disulcate, perforate. Scale bar = 10 µm.

Table 1. Summary of pollen and orbicle characters for all species studied – x = minimum; y = mean; z = maximum; Ap. = number of apertures; Ornam. = ornamentation; Cer.Perf. = cerebroid perforate; Perf.-mret. = perforate to microreticulate; Perf.-rug. = perforate to rugulate; LA = longest equatorial axis; SEA = shortest equatorial axis; P = polar axis; Perfsiz = perforation size; Perfm² = mean number of perforations per square micrometre; Wmuri = width of muri in striate pollen; Muri/ μ m = muri per micrometre; L = diameter of orbicules; Con = connections between orbicules; Spi = spines on the orbicle surface; / = no observations; * = not applicable; + = present; – = absent

Section	Species	Ap.	Ornam.	Pollen			Orbicules						
				LA (μm)	SEA (μm)	P (μm)	Perfsize (μm)	Perf μm^{-2}	Wmuri (μm)	Muri (μm^{-1})	L (μm)	Con Spi	
Madagascar <i>Brachyandra</i>	<i>D. alatipes</i>	2	Gemmate	18-(22)-28	18-(22)-25	/	*	*	*	*	/	/	/
	<i>D. fandra</i>	2(1)	Striate	38-(42)-48	20-(27)-38	/	*	*	0.29	3.1	0.31-(0.47)-0.62	—	—
	<i>D. nako</i>	2	Striate	28-(31)-43	13-(18)-23	28-(30)-33	*	*	0.23	4.9	0.14-(0.32)-0.39	—	—
	<i>D. sambiranensis</i>	2	Striate	25-(28)-33	15-(17)-20	25-(28)-30	*	*	0.26	3.1	0.16-(0.46)-0.87	—	—
	<i>ssp. ambrensis</i>												
<i>Cardiocrapsa</i>	<i>D. soso</i>	2	Striate	33-(36)-40	20-(23)-28	28-(33)-35	*	*	0.31	2.9	0.35-(0.41)-0.48	—	—
	<i>D. namorokensis</i>	2	Striate	25-(30)-35	18-(21)-25	/	*	*	/	/	/	/	/
	<i>D. proteiformis</i>	2	Striate	33-(35)-38	23-(26)-30	/	*	*	0.23	4.1	/	/	/
	<i>D. ovinala</i>	2	Perforate	28-(30)-35	13-(18)-23	/	/	/	*	*	0.23-(0.27)-0.29	—	—
	<i>D. tsaratananensis</i>	2	Striate	23-(27)-30	15-(19)-23	/	*	*	0.22	3.4	0.21-(0.27)-0.32	—	—
<i>Xylinoapsa</i>	<i>D. antaly</i>	2	Perf.-mret.	23-(30)-38	18-(20)-25	25-(27)-30	0.20-(0.35)-0.55	1.4	*	*	0.46-(0.57)-0.69	—	—
Africa <i>Borderea</i>	<i>D. gillettii</i>	2	Striate	35-(46)-53	23-(29)-38	/	*	*	0.37	2.6	0.16-(0.35)-0.47	+	+
	<i>D. brounii</i>	2	Striate	35-(42)-47	22-(27)-35	37-(46)-50	*	*	0.41	2.0	0.53-(0.64)-0.76	—	—
	<i>D. burchellii</i>	2	Striate	35-(37)-40	/	/	*	*	0.39	3.2	—	*	*
	<i>D. cotinifolia</i>	2	Perforate	/	/	/	0.04-(0.12)-0.26	6.8	*	*	0.21-(0.43)-0.61	+	—
<i>Macrourea</i> <i>Rhacodophyllum</i>	<i>D. sansibarensis</i>	2	Perforate	37-(39)-42	25-(27)-30	35-(36)-37	0.12-(0.19)-0.44	2.2	*	*	0.31-(0.54)-1.02	+	+
	<i>D. rupicola</i>	2	Perforate	30-(42)-55	25-(30)-45	/	0.05-(0.13)-0.25	3.6	*	*	0.32-(0.49)-0.77	+	—
	<i>D. elephantipes</i>	2	Perforate	48-(51)-55	27-(30)-32	32-(44)-52	0.09-(0.18)-0.26	3.5	*	*	0.33-(0.56)-0.81	—	+
	<i>Testudinaria</i>												
Eurasia & Australia <i>Borderea</i>	<i>D. pyrenaica</i>	1	Gemmate	55-(58)-62	27-(32)-40	/	0.60-(1.17)-1.90	*	*	*	0.26-(0.40)-0.62	—	—
	<i>D. cirrhosa</i>	2	Perforate	23-(24)-28	18-(21)-23	22-(23)-25	0.05-(0.10)-0.22	10.2	*	*	0.40-(0.60)-0.90	—	—
	<i>D. hastifolia</i>	2	Perforate	17-(20)-22	15-(17)-20	/	0.04-(0.13)-0.20	9.6	*	*	0.50-(0.65)-0.89	—	—
	<i>D. laurifolia</i>	2	Perforate	20-(23)-25	15-(17)-20	/	0.06-(0.09)-0.16	10.6	*	*	0.26-(0.48)-0.80	—	—
	<i>D. nummularia</i>	2	Perforate	25-(27)-30	20-(21)-23	18-(20)-23	0.06-(0.11)-0.15	11.8	*	*	0.20-(0.31)-0.37	—	—
<i>Paramecocarpa</i> <i>Shannicorea</i>	<i>D. wallichii</i>	2	Perforate	23-(25)-28	15-(16)-20	/	0.06-(0.09)-0.13	14.2	*	*	0.42-(0.59)-0.70	—	—
	<i>D. flabellifolia</i>	1?	Perforate	40-(42)-45	22-(25)-27	/	0.08-(0.15)-0.21	3.0	*	*	0.48-(0.69)-0.87	+	+
	<i>D. pseudo-nitens</i>	2	Perforate	47-(48)-50	33-(35)-36	/	0.21-(0.44)-0.83	1.2	*	*	0.37-(0.50)-0.60	+	—
	<i>D. velutipes</i>	2	Perforate	32-(38)-40	22-(24)-27	/	0.10-(0.14)-0.18	2.6	*	*	0.51-(0.68)-1.18	+	+

Brachystigma, *Cardiocrapsa*, *Cotinifoliae*, and *Enantiophyllum*. More striking, however, is the correlation between pollen size of a given section and tuber type, first described by Su (1987). He noticed that pollen of sections with persistent tubers (*Apodostemon*, *Borderea*, *Dematostemon*, *Rhacodophyllum*, and *Trigonobasis*) is larger than that of sections with annual tubers (*Brachyandra*, *Enantiophyllum*, and *Cardiocrapsa*). Section *Stenophora*, with the largest pollen grains, is characterized by a rhizome. Our results seem to confirm this hypothesis: in this survey and in Schols *et al.* (2001), we examined 25 species with annual tubers and 23 species with persistent tubers or rhizomes. The *t*-test on the LA measurements confirms that the difference between both groups is significant ($P = 0.00011$). If we remove the species possessing a rhizome from the latter group, the difference is still significant below the 1% level ($P = 0.00440$). This confirms the results of our previous *t*-test with a *P* value of 0.013 (Schols *et al.*, 2001). The correlation between pollen size and tuber type seems accidental: the rhizome of sect. *Stenophora* is hypothesized to be homologous with that of *Trichopus* Gaertn. and therefore plesiomorphic within *Dioscorea* (Caddick *et al.*, 2002a). Large pollen grains may also represent the plesiomorphic state within *Dioscorea*. This is supported by the limited sequence data available. Perennial and annual tubers have a patchy distribution across the genus, appearing more derived.

Apertures

The appearance of the apertures is affected by the hydration state of the pollen grain. Aperture margins tend to fold along the direction of the sulcus/sulculi, and therefore the infolded apertures look smaller and closer to each other and are difficult to observe in SEM (Fig. 4). Therefore, aperture number was established from LM observations (Figs 6–14).

Disulcate apertures (see below) are most common in *Dioscorea*, with about 75% of all species examined possessing two apertures (Table 1, Figs 1–14). In most cases, the number of apertures is consistent within taxa sampled from a section, and all species sampled from sections such as *Apodostemon*, *Enantiophyllum*, and *Polyneuron* are disulcate. Generally, disulcate apertures are rather uncommon, being restricted to some monocotyledons and basal angiosperms. They occur in Amaryllidaceae (Snijman & Linder, 1996), some Arecaceae (Harley, 1998), and Pontederiaceae (Simpson, 1987; Ressayre, 2001) in monocots, and in Trimeniaceae, some Annonaceae, Eupomatiaceae, Calycanthaceae, and Hydnoraceae (usually placed among magnoliids) in basal angiosperms (Furness, Rudall & Sampson, 2002; Watson & Dallwitz, 2002).

In contrast to Schols *et al.* (2001), we are using disulcate as proposed by Punt *et al.* (1998) to indicate that the pollen grains have two elongated latitudinal apertures, not situated at the poles. The position of the apertures was established from TEM observations of the pollen ontogeny of *Dioscorea communis* (P. Schols, C. A. Furness, P. Wilkin & E. Smets, unpubl. data).

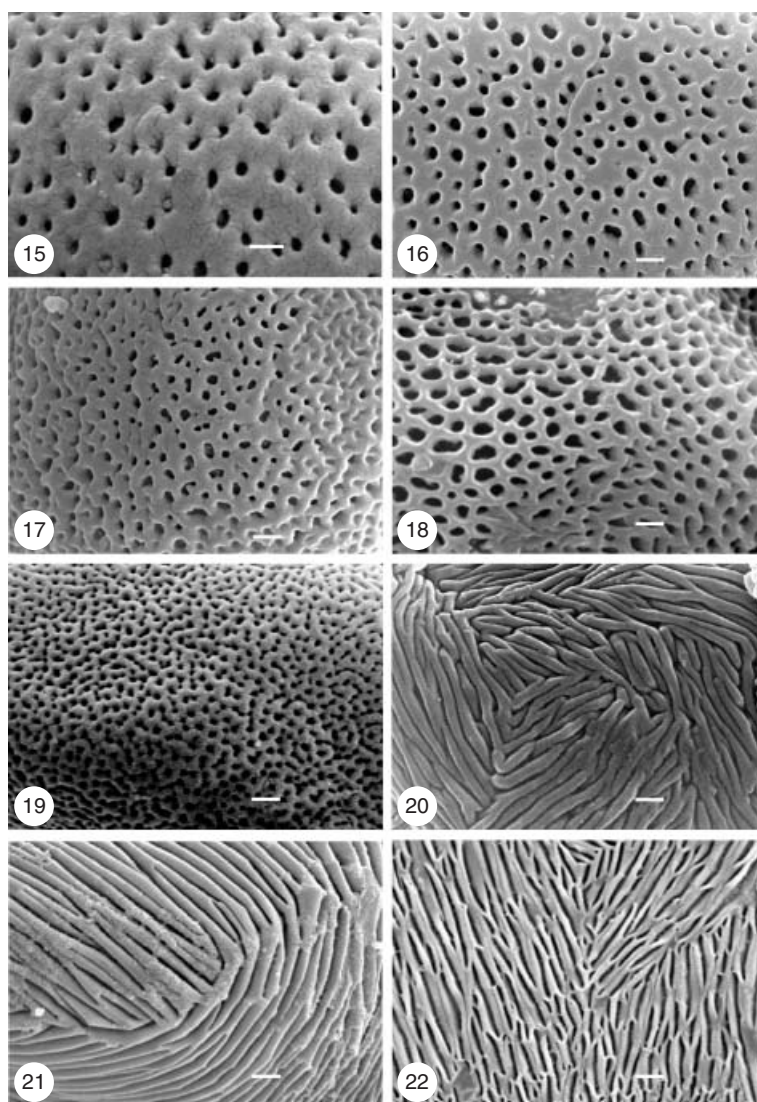
About 10% of the total number of species we have examined are monosulcate (Table 1, Figs 1–14), i.e. with a single elongated latitudinal aperture situated at the distal pole (Punt *et al.*, 1998). The aperture position at the distal pole was observed in tetrads in *Tacca* J.R. Forst. & G. Forst. (P. Schols, C. A. Furness, P. Wilkin & E. Smets, unpubl. data), a close relative of *Dioscorea* (Caddick *et al.*, 2002a). Whereas monosulcate is the most common aperture type in monocots (e.g. Furness & Rudall, 1999b), in *Dioscorea* it seems to be confined to a few sections, such as *Stenophora* (Table 1). Examination of a further 61 taxa supports the hypothesis of Schols *et al.* (2001) that monosulcate pollen is plesiomorphic in *Dioscorea*, as in monocots in general, because it is mainly confined to the basal section *Stenophora*, which could be sister to the rest of the genus, although it also occurs in section *Borderea* and (possibly) section *Paramecocarpa* (Table 1), which are embedded well within *Dioscorea*. Crown groups, such as sections *Enantiophyllum* and *Lasiophyton*, are characterized by disulcate pollen.

The remaining 15% of the species examined have both disulcate and monosulcate pollen, even within one anther (Table 1). When disulcate and monosulcate pollen occur in one specimen, one type prevails (95% or more per sample). In most taxa the disulcate type predominates; *D. caucasica* is the only exception with mainly monosulcate pollen grains.

Trisulcate pollen, reported in Dioscoreaceae by Erdtman (1969) in *D. densiflora* (Bourgeau 7687) and *Rajania cordata* L., was not found in the specimen of *D. densiflora* (Bourgeau 1487) examined by us. We did not examine any pollen of *Rajania* L.

Sexine ornamentation

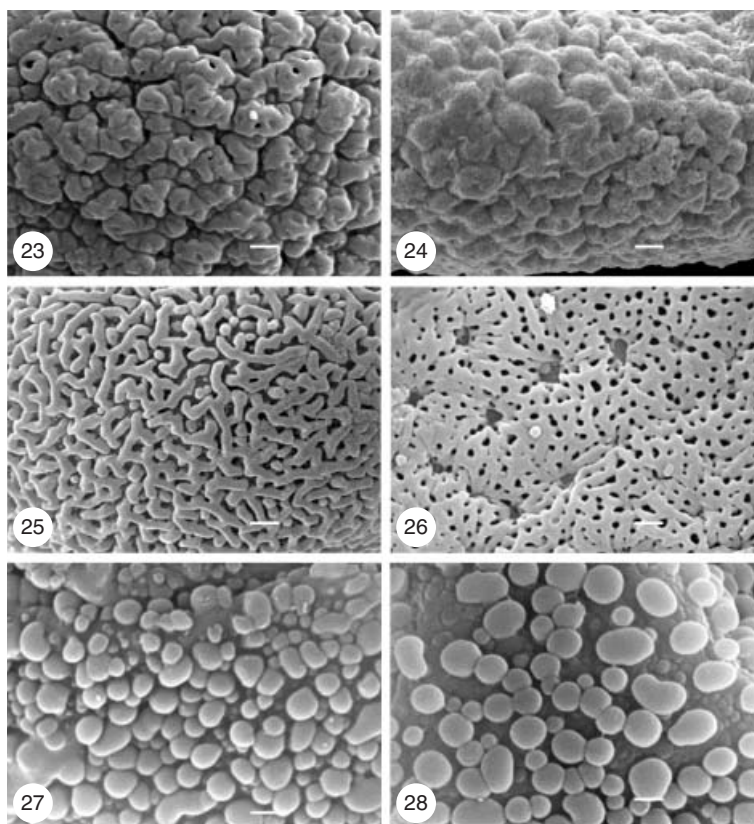
There is considerable variation in sexine ornamentation (Table 1, Figs 15–28). Schols *et al.* (2001) reported perforate (Figs 15–19), striate (Figs 20–22), and microreticulate sexine patterns. In the additional 61 species examined, rugulate (Fig. 25), cerebroid perforate (Figs 23, 24, 26), and gemmate ornamentation patterns (Figs 27, 28) were also observed (Table 1). About 54% of the total number of species examined are perforate, 28% are striate, 6% are perforate to microreticulate (features of both ornamentation types are observed in the same grain), and 6% are cerebroid perforate. Only 3% of species have a rugulate sexine pattern, 2% are gemmate, and 1% are perforate to rugulate.



Figures 15–22. Sexine ornamentation (SEM). Fig. 15. *D. anomala* (sect. *Dematostemon*), perforate sexine with a large perforations and a low perforation density. Fig. 16. *D. minima* (sect. *Polyneuron*), perforate sexine with large perforations and a low perforation density. Fig. 17. *D. caucasica* (sect. *Stenophora*), perforate sexine. Fig. 18. *D. orientalis*, perforate to microreticulate sexine. Fig. 19. *D. nummularia* (sect. *Enantiophyllum*), perforate sexine with small perforations and a high perforation density. Fig. 20. *D. gillettii* (sect. *Borderea*), striate sexine, striations are arranged in concentric polygons. Fig. 21. *D. microbotrya* (sect. *Trigonocarpa*), striate sexine. Fig. 22. *D. proteiformis* (sect. *Cardicapsa*), striate sexine, note the connections between the muri. Scale bars = 1 μm .

Perforate ornamentation (Figs 15–19). As reported previously by Schols *et al.* (2001), perforate sexine ornamentation is most common in *Dioscorea*, except for the Malagasy species, which are mostly striate. This is especially true for the mainly Eurasian sections, such as *Enantiophyllum* and *Paramecocarpa*, some African sections (e.g. *Macrourea*, *Rhacodophyllum*), and a majority of the New World sections (e.g. *Lasiogyne*, *Oxypetalum*, *Trigonobasis*), although this is based on limited sampling. Much variation was found in this survey in perforation size and perfora-

tion density (Table 1, Figs 15–19). Average perforation size ranges from 0.08 μm (in *D. multinervis*) to 0.50 μm (in *D. orientalis*) (Fig. 18); the average value is 0.23 μm . Small perforations are common in section *Enantiophyllum* (e.g. *D. nummularia*) (Fig. 19). Perforation density (number of perforations per μm^2) seems to characterize some sections, especially when combined with perforation size. The perforations are evenly distributed on the pollen surface in most species, which makes perforation density an accessible character. Both characters are partially dependent:



Figures 23–28. Sexine ornamentation (SEM). Fig. 23. *D. bridgesii* (sect. *Parallelostemon*), cerebriform perforate sexine. Fig. 24. *D. pallens* (sect. *Cincinnorachis*), cerebriform perforate sexine. Fig. 25. *D. nipponica* (sect. *Stenophora*), rugulate sexine. Fig. 26. *D. ridleyi* (sect. *Stenophora*), cerebriform perforate sexine, note the two perforation types. Fig. 27. *D. pyrenaica* (sect. *Borderea*), gemmate sexine. Fig. 28. *D. pyrenaica* (sect. *Borderea*), detail of gemmate sexine. Scale bars = 1 μm .

large perforations result in a low perforation density. Most perforate species have a perforation density below $6 \mu\text{m}^{-2}$ (Table 1). Sect. *Enantiophyllum*, however, is characterized by a high perforation density (more than $10 \mu\text{m}^{-2}$), for example *D. wallichii*.

Striate ornamentation (Figs 20–22). Striate pollen is present in most Malagasy sections (*Brachyandra*, *Cardiocrapsa*, *Seriflorae*), in some African sections (e.g. *Cotinifoliae*), in some species of section *Stenophora*, and in a few New World sections (e.g. *Apodostemon*, *Cryptantha*, *Heterostemon*, *Trigonocarpa*) (Figs 20–22).

Striate pollen is an informative taxonomic character at the sectional level. The almost exclusive occurrence of a striate sexine in the Malagasy species, for example, might support the hypothesis of Burkill (1960) that most Malagasy sections are closely related, although this requires further testing using molecular sequence data. By contrast, it is very unlikely that the striate pattern links the Malagasy species to other groups such as the former genus *Rajania* (sunk by Caddick *et al.*, 2002b), which contains at least one stri-

ate species. Preliminary results based on *rbcL* data (Raz *et al.*, 2001; Caddick *et al.*, 2002a) suggest that *Rajania* is a monophyletic group embedded in *Dioscorea s.l.*, implying that striate pollen originated independently in this group because it only occurs in one *Rajania* species. Moreover, the striate sexine of section *Stenophora* seems to have arisen independently, given the isolated position of the section in recent molecular and micromorphological analyses (Caddick *et al.*, 2002a). A striate sexine has thus possibly evolved at least three times in *Dioscorea*: in the Malagasy sections, in section *Stenophora*, in *Rajania*, and probably a fourth time in the New World sections. A combined molecular–morphological analysis is needed to investigate this hypothesis.

One of the characters that could indicate an independent origin for the striate pollen in the above sections is the width of the muri (the ridges of the striae). The muri tend to be wider in the African species (about $0.40 \mu\text{m}$) than in the Malagasy and New World species (about $0.25 \mu\text{m}$), indicating a possible independent origin of the African species. Striate pollen is rather uncommon in monocots (van der Ham, Hetter-

scheid & van Heuven, 1998). The evolution of a striate sexine in Madagascar could be linked to a specific Malagasy pollinator but little pollination data exist for *Dioscorea*. Barroso *et al.* (1974) reported that some South American species are pollinated by *Meliponini*, a tribe of stingless bees. More pollination data, especially for the African and Malagasy species, are required to investigate the influence of pollinators on the pollen morphology of the genus.

Cerebroid perforate ornamentation (Figs 23, 24, 26). This unique ornamentation found in *D. ridleyi*, *D. palawana* (section *Stenophora*), and in some New World taxa such as *D. glandulosa* and *D. pallens* was first described by Su (1987) as 'cerebroid perforate'. Both this ornamentation and rugulate patterns are called 'rugulose perforate' by Xifreda (2000). To distinguish between the clearly distinct rugulate and cerebroid perforate ornamentation, we adopt Su's terminology.

Cerebroid perforate ornamentation in *Stenophora* species is superficially similar to the pattern in New World species. More detailed examination reveals an important difference: in *D. palawana* and *D. ridleyi* two perforation types occur, small perforations of about 0.1–0.2 µm distributed on the entire surface and larger perforations (about 0.5 µm) found in between the bulges (Fig. 26). All cerebroid perforate species from the New World lack this perforation dimorphism (Figs 23, 24). Although both perforation types are called 'cerebroid perforate' in this paper, they are most likely not homologous. This unusual perforation type isolates *D. palawana* and *D. ridleyi* from other *Stenophora* species, as discussed by Wilkin *et al.* (2002).

Gemmate ornamentation (Figs 27, 28). Gemmate ornamentation was not found in the species examined by Schols *et al.* (2001). However, it was reported by Garcia, Antor & Espadaler (1995) and Caddick *et al.* (1998). Two species in the present survey, *D. pyrenaica* (sect. *Borderea*) and *D. alatipes* (sect. *Brachyandra*), are characterized by this unusual ornamentation. However, it is unlikely that gemmate pollen indicates a close relationship between them because it could be an adaptation to another pollinator. *D. pyrenaica* is pollinated by ants (Garcia *et al.*, 1995) and *D. alatipes* species have inflorescences close to the ground, possibly indicating a similar pollination syndrome. Pollen size, however, is very different in both species (Table 1).

Rugulate ornamentation (Fig. 25). *D. nipponica* (sect. *Stenophora*) and *D. remotiflora* (sect. *Macrogynodium*) show a somewhat rugulate sexine pattern. The sexine of both species does not look alike and given the dis-

tant relationship between both species, it is very unlikely that both occurrences of a rugulate sexine are homologous.

Exine stratification and ultrastructure

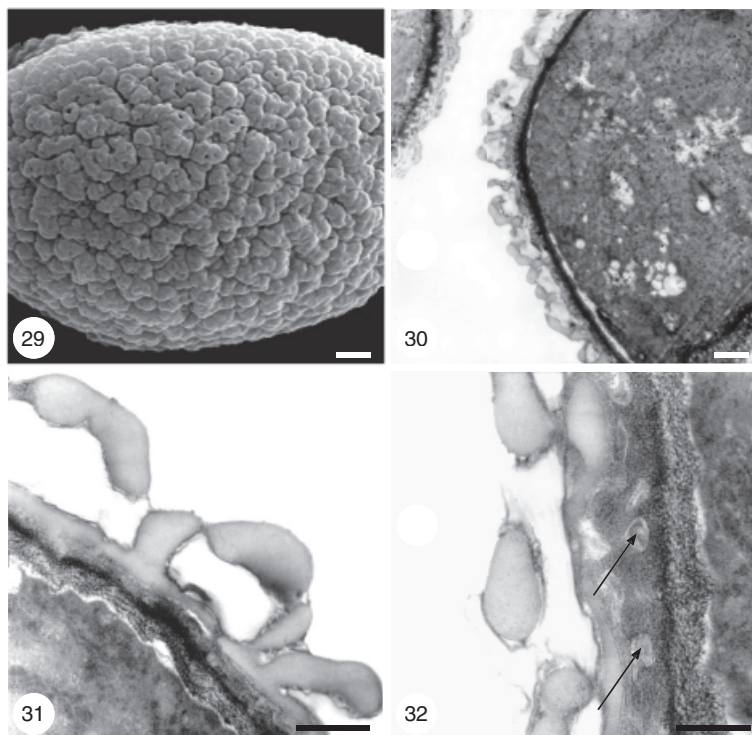
The wall structure of *Dioscorea* pollen is always tectate–columellate and there is little variation in exine thickness (0.6–1.5 µm) for the species examined in this survey, which agrees with that found by Schols *et al.* (2001). Cerebroid perforate pollen has longer columellae (c. 0.6 µm) than perforate pollen (c. 0.2 µm). In *D. bridgesii*, the cerebroid ornamentation is composed of undulate exine. The pollen is tectate, and the raised areas of tectum form the bulges of the cerebroid pattern; the depressions between these bulges are where the columellae are located (Figs 29–32). Whether *Dioscorea* has an endexine remains unclear. White lines are visible at the bottom of the foot layer in mature stages, and this could indicate the presence of a weakly developed endexine (Figs 31, 32).

Intine stratification and ultrastructure

An intine thickening beneath the sulculi was observed in the species examined using TEM, *D. bridgesii*, ranging from 0.2 µm in the non-apertural regions to 1.8 µm below the sulculi (Fig. 32). This confirms previous observations (Schols *et al.*, 2001). Intine channels of c. 0.1 µm diameter are embedded in the entire intine, but concentrated beneath the apertures. In transverse sections, these channels appear in a honeycomb pattern. Similar intine patterns are encountered in some other monocots (Liliaceae, Iridaceae, Zingiberales and Poaceae; see discussion in Suárez-Cervera *et al.*, 2000). Some of these monocots have inaperturate (omniaperturate) pollen, e.g. *Crocus* (Iridaceae) and many Zingiberales, with a thick, channelled intine over the whole grain (Furness & Rudall, 1999a). This is different to *Dioscorea*, where the thick, channelled intine is concentrated at the apertures (Schols *et al.*, 2001). The development of intine channels has been studied in *Aristea major* (Iridaceae: Suárez-Cervera *et al.*, 2000) who observed three distinct intine layers: a pectic outer layer (I1), a pectic–proteinic channelled layer (I2), and a fibrillar cellulosic inner layer (I3), based on cytochemical tests. This is similar to the pattern we found in several *Dioscorea* species. Channelled intine appears to be widespread in monocots, and therefore it is not a useful character at the infrageneric level in *Dioscorea*, although it could potentially be useful at higher taxonomic levels.

Orbicules

Fifty-eight of 61 species examined have orbicules on the inner locule wall, ranging in size from 0.12 µm



Figures 29–32. Sexine ultrastructure of *D. bridgesii* (sect. *Parallelostemon*) (SEM/TEM). Fig. 29. Cerebroid perforate sexine (SEM). Fig. 30. Section of cerebroid perforate sexine (TEM). Fig. 31. Section of cerebroid perforate sexine with curved tectum, columellae and foot layer (TEM). Fig. 32. Detail of oblique section through exine and intine, note the intine channels (arrows) (TEM). Scale bars = 1 μ m.

(*D. multinervis*) to 1.25 μ m (*D. glandulosa*) (Table 1), whereas Schols *et al.* (2001) have reported a range of 0.12–1.90 μ m. Most species have either spherical orbicules (Figs 33–35, 38), or rarely elliptical orbicules (*D. antaly*, Fig. 36). Few species have spherical to elliptical orbicules (*D. cotinifolia*, Fig. 37). Some, but not all, striate species have small spines on the orbicule surface, as found by Schols *et al.* (2001). Thin threads between orbicules occur in *D. gillettii*, *D. cotinifolia*, *D. rupicola*, and *D. sinuata*.

Overall, orbicule data seem of little importance in the systematics of *Dioscorea*. An exception might be the section *Stenocorea*, which seems to be characterized by large orbicules (see Systematic discussion).

SYSTEMATIC DISCUSSION

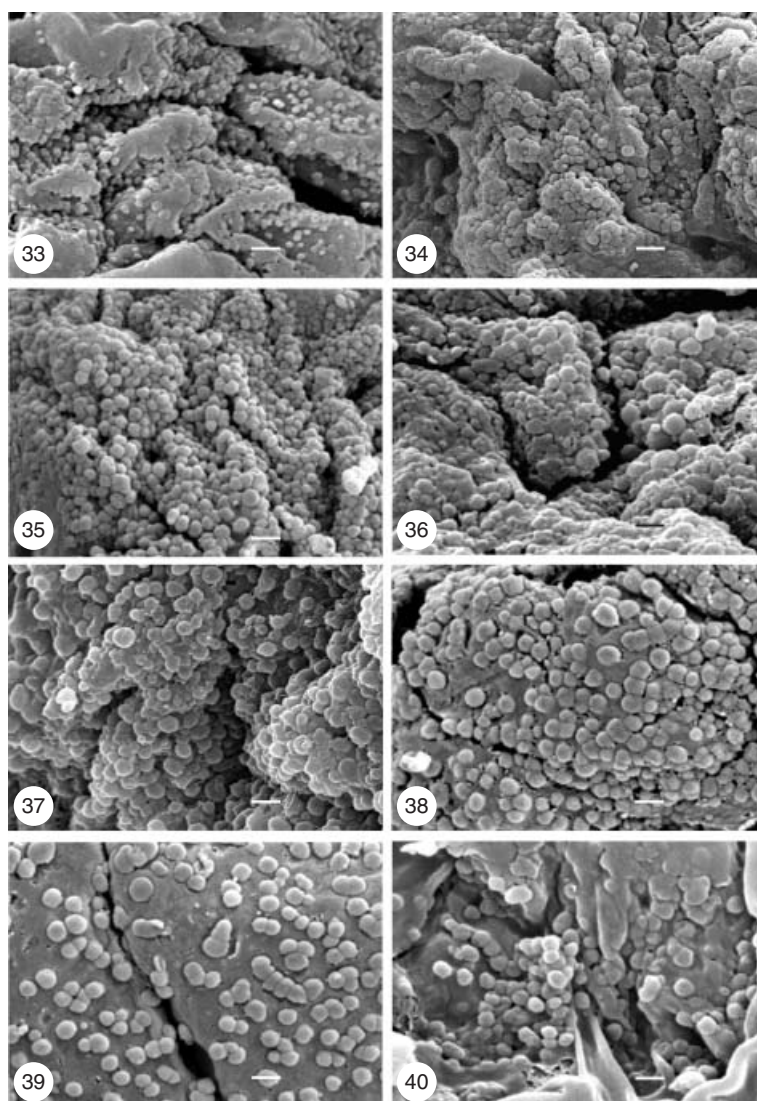
Several current taxonomic hypotheses are supported by the pollen data, and the increasing amount of molecular and palynological data makes it possible to propose some hypotheses about the relationships among the sections of *Dioscorea*. A combined analysis of pollen and molecular data is planned to test these further. Below, selected sections are discussed under the geographical area in which they occur.

MADAGASCAR

All Malagasy species have a very similar pollen morphology. Except for *D. ovinala* (sect. *Pachycapsa*) and *D. antaly* (sect. *Xylinocapsa*) which are perforate, and *D. alatipes* (sect. *Brachyandra*) which is gemmate, all species in this survey possess a striate sexine. The LA of most species is about 30 μ m, and all species are disulculate. The width of the striae is also very similar: all are around 0.25 μ m wide.

As discussed above, the striate pattern could be an adaptation to an endemic Malagasy pollinator. In that case, it is possible that the striate pattern originated more than once in several Malagasy sections. A more parsimonious and more likely explanation, however, is that most Malagasy sections (*Brachyandra*, *Cardiocapsa*, *Madagascarienses* (*D. arcuatineris* Hochr., Schols *et al.*, 2001), and *Seriflorae*) are closely related (Burkill, 1960), which would suggest that the striate pattern originated only once in their common ancestor, but this requires further testing.

D. alatipes, with a gemmate sexine, is the only species of section *Brachyandra* lacking a striate sexine. A gemmate sexine is also found in the ant-pollinated *D. pyrenaica* from southern Europe (Garcia *et al.*, 1995). The inflorescence of *D. alatipes* grows very low



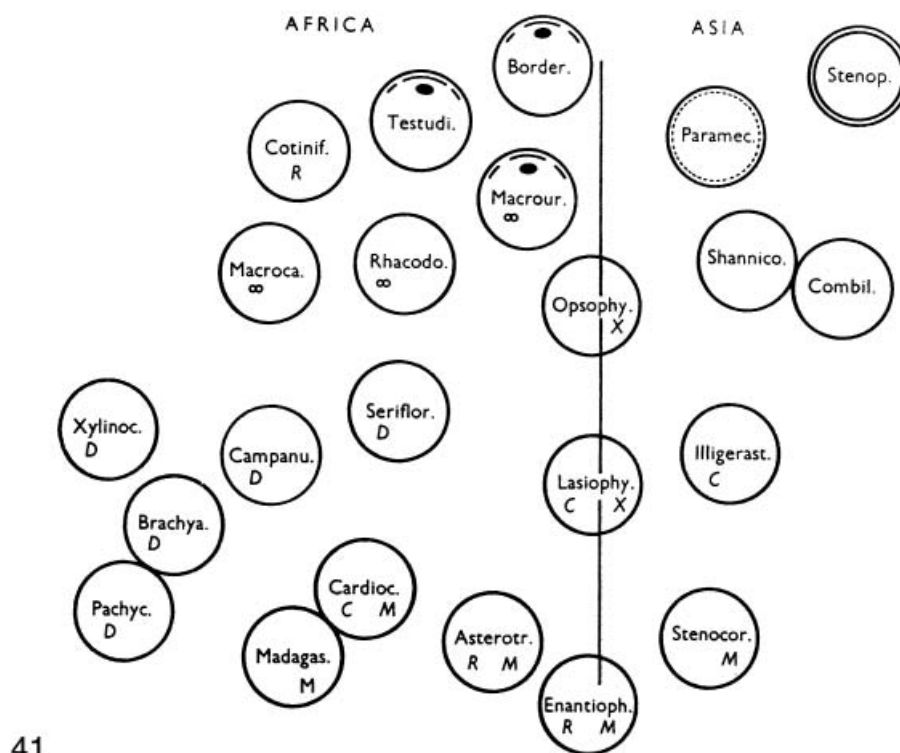
Figures 33–40. Orbicules on the locule wall (SEM). Fig. 33. *D. ovinata* (sect. *Pachycapsa*), small spherical orbicules. Fig. 34. *D. anomala* (sect. *Dematostemon*), small spherical orbicules. Fig. 35. *D. birmanica* (sect. *Stenophora*), spherical orbicules. Fig. 36. *D. antaly* (sect. *Xylinocapsa*), elliptical orbicules. Fig. 37. *D. cotinifolia* (sect. *Cotinifoliae*), spherical to elliptical orbicules. Fig. 38. *D. cirrhosa* (sect. *Enantiophyllum*), large spherical orbicules. Fig. 39. *D. wallichii* (sect. *Enantiophyllum*), large elliptical orbicules. Fig. 40. *D. paradoxa* (sect. *Stenocorea*), large elliptical orbicules. Scale bars = 1 μ m.

to the ground on leafless lower stems, unlike most other members of its section, which might be associated with ant pollination (see discussion on gemmate sexine patterns).

D. ovinata (sect. *Pachycapsa*) and *D. antaly* (sect. *Xylinocapsa*) were placed in two separate sections by Burkill (1960) because their fruit is different from the other Malagasy species. Both species have fruits with a fleshy pericarp in the early stages of development. This is supported by our pollen data because they are the only perforate Malagasy species. However, molecular data suggest that *D. ovinata* and *D. antaly* are not related (P. Wilkin, P. Schols, M. W.

Chase, K. Chamayarit, C. A. Furness, R. Geeta, S. Huysmans, F. Rakotobasalo, L. Raz, E. Smets & C. Thapayai, unpubl. data), implying that fleshy fruits have arisen at least three times in *Dioscorea*: independently in the latter two species and a third time in the former genus *Tamus*.

Some of the ideas proposed by Burkill (1960) in his diagram of relationships (Fig. 41) are reflected in the pollen morphology, such as the close relationship between the sections *Cardiocapsa* and *Madagascarienses*. By contrast, Burkill suspected the sections *Brachyandra* and *Pachycapsa* to be closely related, which is not entirely supported by our pollen data,



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Figure 41. Burkill's diagram of sectional relationships (Burkill, 1960).

caused by a reversal to a perforate sexine pattern (P. Schols, C. A. Furness, P. Wilkins & E. Smets, unpubl. data).

AFRICA

Section *Cotinifoliae*. This section seems to be very heterogeneous in both its macro- and its pollen morphology: *D. cotinifolia* and *D. burchellii* are macromorphologically very similar, except that *D. cotinifolia* is a right-hand climber with perforate pollen grains, whereas *D. burchellii* is left-twining and has striate pollen grains. Moreover, *D. brownii*, another member of this section, does not climb at all, but it does share striate pollen grains with *D. burchellii*.

Section *Borderea*. *D. gillettii* was placed in section *Borderea* by Milne-Redhead (1975), but palynologically it shares very little with *D. pyrenaica*, the other *Borderea* representative in this survey. Both species have rather large pollen grains, but *D. gillettii* is disulcate and striate, whereas *D. pyrenaica* is monosulcate and gemmate.

Section *Rhacodophyllum*. This African section with perennial tubers is strongly supported by our pollen data: *D. buchananii* (Schols *et al.*, 2001) and *D. rupicola* both have rather large (LA of about 44 μm)

disulcate pollen with similar low perforation densities of about 3.4 μm^{-2} .

Section *Testudinaria*. *D. elephantipes* has large, perforate, disulcate pollen grains, similar to those of *D. sylvatica* (Schols *et al.*, 2001). There is little doubt that this section is monophyletic, as suggested by macromorphological synapomorphies such as the thick cork layer that covers the tuber, and by molecular data (Caddick *et al.*, 2000a).

EURASIA

Section *Stenophora*. This section is remarkably diverse in its pollen morphology. Its species have a rhizome instead of a tuber, and could be the sister group of the remainder of *Dioscorea* (Caddick *et al.*, 2002b). The pollen grains are mainly monosulcate, in contrast to the other *Dioscorea* sections in which the number of apertures is mostly two. The ornamentation patterns are very diverse: *D. ridleyi* is characterized by a unique type of cerebroid perforate sexine, and other ornamentation types found in sect. *Stenophora* include perforate and striate plus rugulate in *D. nipponica*.

The perforate species *D. birmanica* is possibly misplaced in this section (P. Wilkin, pers. comm.) and this is supported by our pollen data: *D. birmanica* has dia-

perturate grains. This species appears to show a stronger affinity with *D. esculenta* (sect. *Combilium*). The occurrence of yet another ornamentation type in section *Stenophora* underscores the eurypalynous character of this section.

Of all groups directly related to *Dioscorea*, only *Tacca* is monosulcate. *Tacca* was recently placed in the Dioscoreaceae by Caddick *et al.* (2002b) and an analysis by the same authors suggests a position near the base of the Dioscoreaceae.

Section *Enantiophyllum*. The four *Enantiophyllum* species we have examined for this paper are all disulcate and have a high perforation density, ranging from 10 to 14 μm^{-2} . The mean LA varies from 23 to 27 μm and the perforation size from 0.09 to 0.11 μm . Moreover, these data and ranges are very similar to those found by Schols *et al.* (2001), showing that this section has a very homogeneous pollen morphology. *Enantiophyllum* can be delimited by its rather small pollen grains with small perforations and high perforation density (see discussion in Schols *et al.*, 2001).

Section *Shannicorea*. Both representatives of section *Shannicorea* share perforate and rather large, disulcate pollen grains and a low perforation density between 1.2 and 2.6 μm^{-2} . *D. pseudo-nitens* has significantly larger perforations than *D. velutipes*, but overall this section seems well supported by pollen morphology. Burkill (1960) placed *Shannicorea* very close to section *Combilium* (Fig. 41). This seems to be confirmed by pollen data as well, because *D. esculenta* also has rather large, disulcate pollen and 2.6 perforations μm^{-2} (Schols *et al.*, 2001).

Section *Stenocorea*. As most Eurasian sections, *Stenocorea* is also characterized by disulcate and perforate pollen grains. Orbicule size seems to be a striking characteristic for this section: *D. paradoxa* has the second largest orbicules of the species included in this survey (0.70 μm on average) whereas *D. daunea*, another member of this section, had the largest orbicules of the species treated by Schols *et al.* (2001) (0.77 μm on average).

The former genus *Tamus* L. *Tamus* was recognized as a separate genus within Dioscoreaceae, placed close to *Dioscorea* and *Rajania* (Dahlgren, Clifford & Yeo, 1985), mainly based on its fruit type (a berry) that differs from that of *Dioscorea* (a capsule). Cladistic analyses based on morphological and molecular data suggest that *Tamus* is nested within *Dioscorea* (Caddick *et al.*, 2000) and recently *Tamus* was included in *Dioscorea* (Caddick *et al.*, 2002b). This is supported by palynological data (Clarke & Jones, 1981; Caddick *et al.*, 1998; Schols *et al.*, 2001).

The pollen morphology of *Dioscorea communis* (L.) Caddick & Wilkin (*Tamus communis* L.) was observed in the former three papers cited above and that of *Dioscorea orientalis* (J. Thieb.) Caddick & Wilkin (*Tamus orientalis* J. Thieb.) is examined in the present paper (Fig. 18). *D. orientalis* differs clearly from *D. communis* in its flexuous male inflorescence. Their pollen morphology is, however, similar and is characterized by an LA of about 40 μm and large perforations, suggesting a close relationship between both former *Tamus* species.

NEW WORLD

Although the paper of Burkill (1960) has given us much insight into the morphology and evolution of Old World Dioscoreaceae, a similar overview of the New World taxa is lacking, although about 50% of all *Dioscorea* species occur in the New World. One of the most valuable contributions is the paper of Matuda (1954), monographing 60 Mexican species. He presented a subgeneric regrouping of five unnamed groups, based on seed wing morphology. All other subdivisions of New World taxa find their origin in the classification of Knuth (1924), which has a narrow sectional delimitation.

The poorly known taxonomy and the large number of sections makes it very difficult to assess the value of our palynological data for the delimitation of sections. Moreover, except for the paper by Xifreda (2000), no significant work has been done on the palynology of New World taxa. Nevertheless, we will try to discuss our pollen data in the light of current hypotheses.

Huber (1998) proposed a relationship between the sections *Hyperocarpa*, *Trigonocarpa*, *Stenocarpa*, and *Trifoliatae*. The pollen data in this paper do not support this although both species are disulcate and have a mean LA of about 30 μm : *D. microbotrya* (*Trigonocarpa*) has a striate sexine, whereas *D. trifoliata* (*Trifoliatae*) is perforate.

In the same paper (Huber, 1998), a relationship between sections *Centrostemon*, *Cycladenium*, *Monadelphina*, and *Trigonobasis* is postulated. Our data seem to add weight to this hypothesis: *D. galeottiana* (*Trigonobasis*), *D. glandulosa* and *D. piperifolia* (*Centrostemon*) have a similar mean LA value of about 30 μm , a perforation density of about 3 μm^{-2} and a mean perforation size of about 0.2 μm . The only difference is that *D. galeottiana* is perforate whereas *D. glandulosa* and *D. piperifolia* are cerebroid perforate. This is confirmed by the results of Xifreda (2000): she studied four species of section *Centrostemon* which are all cerebroid perforate and one species of *Trigonobasis* which is perforate. She also looked at six species of section *Cycladenium* which were all perforate and six

species of section *Monadelpha*, in which both ornamentation types occur.

CONCLUSIONS

Palynological characters have been demonstrated to be useful in investigating relationships within and between sections of *Dioscorea*. Hypotheses previously proposed by Knuth (1924), Burkill (1960) and Ayensu (1972), for example, are supported by our pollen data. Major conclusions include:

- Most Malagasy species are characterized by a striate sexine, supporting their close relationship. Only two Malagasy species in this survey are perforate (*D. antaly* and *D. ovinala*), possibly owing to character state reversals. Burkill (1960) placed these two species in the monotypic sections *Xylinocapsa* and *Pachycapsa*, respectively, because they have different fruit morphologies.
- The sexine ornamentation is mostly consistent within a section, except for section *Stenophora* which has an extreme variation in exine patterns. It is one of the very few sections with large, monosulcate pollen grains.
- Pollen morphology strongly supports section *Enantiophyllum* as a monophyletic group.

The next step in our research is to test the hypotheses presented in this paper by combined phylogenetic analyses. Therefore, we are compiling a dataset for up to 110 *Dioscorea* species with other collaborators, comprising pollen data, macromorphological characters together with *rbcL*, *matK* and other gene sequences, although not all characters will be available for all taxa. A cladistic analysis of these data will enable us to draw firmer conclusions about the evolution of palynological characters within *Dioscorea*.

Observations of the earliest stages of pollen ontogeny, which could clarify questions concerning aperture configuration, are currently in progress and could also shed light on the origin of diaperturate pollen grains within the monocots.

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APPENDIX

Material examined, listed alphabetically by section for each major geographical region (* = specimen investigated with TEM)

Section	Species	Herbarium and collection	Country
Madagascar			
<i>Brachyandra</i> Uline	<i>D. alatipes</i> Burkill & H. Perrier	K: Phillipson 3208	Madagascar
	<i>D. fandra</i> H. Perrier	K: Caddick <i>et al.</i> 324	Madagascar
	<i>D. nako</i> H. Perrier	K: Phillipson 1703	Madagascar
	<i>D. sambiranensis</i> ssp. <i>Ambrensis</i> Pax	K: Perrier de la Bathie 17551	Madagascar
	<i>D. soso</i> Jumelle & Perrier	K: Wilkin <i>et al.</i> 1105	Madagascar
	<i>D. namorokensis</i> Wilkin	K: Wilkin <i>et al.</i> 1118	Madagascar
<i>Cardiopsis</i> Uline	<i>D. proteiformis</i> H. Perrier	K: McPherson <i>et al.</i> 14203	Madagascar
<i>Pachycapsa</i> Burkill & H. Perr.	<i>D. ovinata</i> Baker	K: Wilkin <i>et al.</i> 1115	Madagascar
<i>Seriflorae</i> Burkill & H. Perr.	<i>D. tsaratananensis</i> H. Perrier	K: Perrier de la Bathie 15248	Madagascar
<i>Xylinocapsa</i> Burkill & H. Perr.	<i>D. antaly</i> Jumelle & H. Perrier	K: Wilkin <i>et al.</i> 1103	Madagascar
Africa			
<i>Borderea</i> Benth. & Hook.	<i>D. gillettii</i> Milne-Redh.	BR: Friis, Gilbert & Rasmussen 943	Ethiopia
<i>Cotinifoliae</i> Burkill	<i>D. brownii</i> Schinz	K: Rudatis 1247	S. Africa
	<i>D. burchellii</i> Baker	K: Archibald 7592	S. Africa
	<i>D. cotinifolia</i> Kunth	BR: Ward 1918	S. Africa
<i>Macrourea</i> (R. Knuth) Burkill	<i>D. sansibarensis</i> Pax	K: Faden <i>et al.</i> 96/12	Tanzania
<i>Rhacodophyllum</i> Uline	<i>D. rupicola</i> Kunth	BR: Bamps 7240	S. Africa

APPENDIX *Continued*

Section	Species	Herbarium and collection	Country
<i>Testudinaria</i> (Salisb.) Burkill	<i>D. elephantipes</i> Engl.	K: Archibald 8014	S. Africa
Eurasia & Australia			
<i>Borderea</i> Benth. & Hook.	<i>D. pyrenaica</i> Bub. & Bordere ex Gren.	K: Sandwith 4745	Spain
<i>Enantiophyllum</i> Uline	<i>D. cirrhosa</i> Lour.	K: Shiu Ying Hu 9970	China
	<i>D. hastifolia</i> Nees	K: Melville 4138	Australia
	<i>D. laurifolia</i> Wall.	K: Nur SFN 33964	Malaysia
	<i>D. nummularia</i> Lam.	BR: Wenzel 3135	Philippines
	<i>D. wallichii</i> Hook.f.	K: Wilkin 1078	Thailand
<i>Paramecocarpa</i> Prain & Burkill	<i>D. flabellifolia</i> Prain & Burkill	K: Wong WKM1613	Brunei
<i>Shannicorea</i> Prain & Burkill	<i>D. pseudo-nitens</i> Prain & Burkill	K: Garrett 781	Thailand
	<i>D. velutipes</i> Prain & Burkill	K: Robertson 354	Burma
<i>Stenocorea</i> Prain & Burkill	<i>D. paradoxa</i> Prain & Burkill	K: Kerr 20463	Thailand
<i>Stenophora</i> Uline	<i>D. balcanica</i> Kosanin	K: Dorfler 959	Albania
	<i>D. birmanica</i> Prain & Burkill	K: Lace 6184	Burma
	<i>D. caucasica</i> Lipsky	BR: Unknown 346	—
	<i>D. deltoidea</i> Wall.	BR: Buisus 7300	—
	<i>D. nipponica</i> Makino	BR: Togasi 1214	—
	<i>D. palawana</i> Prain & Burkill	K: Dransfield in SMHI 1250	Philippines
	<i>D. ridleyi</i> Prain & Burkill	K: Anderson 25529	Malaysia
	<i>D. villosa</i> L.	K: Melville 7233	USA
	<i>D. zingiberensis</i> C.H.Wright	K: Wilson 2921	China
Unknown	<i>D. orientalis</i> (J. Thieb.) Caddick & Wilkin	K: Maitland 2	Lebanon
New World			
<i>Apodostemon</i> Uline	<i>D. macrostachya</i> Benth.	BR: Kunkel 140	Guatemala
	<i>D. mexicana</i> Scheidw.	BR: von Wedel 1760	Panama
<i>Brachystigma</i> Uline	<i>D. sinuata</i> Lam.	BR: Frazao 7558	Brazil
<i>Centrostemon</i> Griseb.	<i>D. glandulosa</i> Klotzsch ex Kunth	BR: Schott 0530	Brazil
	<i>D. piperifolia</i> Klotzsch ex Kunth	K: Sucre 7023	Brazil
<i>Cincinnorachis</i> Uline	<i>D. pallens</i> Schlecht.	BR: Rosas 819	Mexico
<i>Cryptantha</i> Uline	<i>D. olfersiana</i> Klotzsch ex Griseb.	BR: Unknown 0503	Brazil
<i>Cycladenium</i> Uline	<i>D. coriacea</i> Wight ex Wall.	K: Fleming 143	Ecuador
<i>Dematostemon</i> Griseb.	<i>D. anomala</i> Griseb.	BR: Hatschbach <i>et al.</i> 53875	Brazil
<i>Hemidematostemon</i> Griseb.	<i>D. debilis</i> Uline ex Knuth	K: Harley <i>et al.</i> 20871	Brazil
<i>Heterostemon</i> Uline	<i>D. floribunda</i> Mart. & Gal.	BR: Nee 24743	Mexico
<i>Lasiogyne</i> Uline	<i>D. dodecaneura</i> Vell.	K: Hatschbach <i>et al.</i> 56578	Brazil
<i>Lychnostemon</i> Uline	<i>D. polygonoides</i> Humb. Ponbl ex Willd.	BR: Smith 4970	Columbia
<i>Macrogynodium</i> Uline	<i>D. remotiflora</i> Kunth	BR: Pringle 4527	Mexico
<i>Microdioscorea</i> Uline	<i>D. saxatilis</i> Poepp.	K: Mahu 9633	Chile
<i>Oxypetalum</i> Uline	<i>D. densiflora</i> Hemsl.	BR: Bourgeau 1487	Mexico
<i>Parallelostemon</i> Uline	<i>D. bridgesii</i> Griseb. ex Kunth	BR: Luming 1666*	Chile
<i>Polyneuron</i> Uline	<i>D. minima</i> Robins. & Seaton	BR: Pringle 4157	Mexico
	<i>D. multinervis</i> Benth.	BR: Pringle 1270	Mexico
<i>Pygmaeophyton</i> Uline	<i>D. ancashensis</i> Knuth	K: Stafford 1246	Peru
<i>Siphonantha</i> Uline	<i>D. longituba</i> Uline	K: Hinton 2703	Mexico
<i>Trifoliatae</i> R. Knuth	<i>D. trifoliata</i> H.B. & K.	BR: Rimachi	Peru
<i>Trigonobasis</i> Uline	<i>D. galeottiana</i> Kunth	BR: Diaz Vilchis 1228	Mexico
<i>Trigonocarpa</i> Uline	<i>D. microbotrya</i> Griseb.	BR: ACE 1205	Argentina