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Morphology of pollen and orbicules in some *Dioscorea* species and its systematic implications

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Pollen and orbicule morphology of 35 *Dioscorea* L. species is described based on observations with light microscopy, and scanning and transmission electron microscopy. Pollen and orbicule characters are critically evaluated and discussed in the context of existing hypotheses of systematic relationships within the genus. Pollen is mostly bisulcate (sometimes monosulcate) with a perforate, microreticulate or striate sexine. Our results indicate that pollen data may be significant at sectional rank. The close relationship between sections *Asterotricha* and *Enantiophyllum* proposed by Burkill and Ayensu is supported by pollen morphology as all species investigated share bisulcate, perforate pollen with small perforations and a high perforation density. Macromorphological differences between the two compound-leaved sections *Botryosicyos* and *Lasiophyton* are also supported by pollen morphology; pollens of these two sections have very different perforation patterns. Orbicules in Dioscorea are mostly spherical and possess a smooth or spinulose surface. The latter is often correlated with a striate sexine.

ADDITIONAL KEY WORDS: pollen aperture – pollen ultrastructure – sectional classification – sexine ornamentation.

INTRODUCTION

Pollen morphological data have proved crucial to the resolution of relationships within Dioscoreales (Caddick *et al.*, 1998). The present paper considers the systematic importance of pollen morphology within *Dioscorea* L., the core genus of the Dioscoreaceae.

Dioscorea consists of approximately 400 species (Wilkin, in press), growing in humid tropical and subtropical areas. The genus was divided into four subgenera, based on seed morphology, and about 60 sections (Knuth, 1924) mainly based on a few characters from floral morphology, such as the number of anthers, while micromorphological data remained poorly known. Revised hypotheses about relationships between the old and new world sections were published by Matuda (1954) and Burkill (1960), while the majority of sections recognized by Knuth were unchanged.

The need for re-evaluation of the infrageneric classification of *Dioscorea* using molecular and micromorphological data is indicated by recent cladistic analyses, for example by Wilkin & Caddick (2000). Palynological data in particular are rather scarce for *Dioscorea*; existing publications mostly survey only a few *Dioscorea* species using light microscopy (LM) (for example, Selling, 1947; Kuprianova, 1948; Sharma, 1967; Erdtman, 1969; Heusser, 1971; Huang, 1972; Chávez, Ludlow-Wiechers & Villanueva, 1991). According to these reports, pollen grains of Dioscorea are 1-, 2- or 3-sulcate with the longest axis ranging from 18 to 45 μm.

A survey using scanning electron microscopy (SEM) (Su, 1987), described the pollen morphology of 33 Chinese *Dioscorea* species from five sections. Pollen grains are all bisulcate, except for those of section *Stenophora* (see Appendix A for authors of taxa), which are monosulcate. Su also suggested a correlation between pollen size and tuber type.

Pollen of ten Dioscorea species was described by

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Caddick *et al.* (1998): pollen is usually bisulcate, sometimes monosulcate, and striate to perforate or finely reticulate. The mainly bisulcate pollen and a similarity in sexine sculpturing support a close relationship between *Dioscorea* and the other dioecious taxa *Epipetrum*, *Rajania* and *Tamus* (Caddick *et al.*, 1998). Among the three hermaphrodite genera formerly included in Dioscoreaceae (*Avetra*, *Stenomeris* and *Trichopus*), *Avetra* and *Trichopus* have very similar pollen morphology with spinulate sculpturing (Caddick *et al.*, 1998). Pollen grains of the closely related family Taccaceae are monosulcate (Caddick *et al.*, 1998), as is the case in some Dioscoreaceae.

Orbicules are sporopollenin bodies in the anther locule usually produced by secretory tapeta; their function is unknown although there have been various suggestions (see Huysmans, El-Ghazaly & Smets, 1998, 2000). Although most Dioscoreales are known to have a secretory tapetum (Wunderlich, 1954; Furness & Rudall, 1998), orbicules have never been examined in this group.

As part of an ongoing study tracing the diversity and evolutionary relationships of members of the Dioscoreaceae, this paper aims to present a detailed description of the pollen and orbicules of 35 *Dioscorea* species from 26 sections, focusing particularly on pollen aperture number and sexine ornamentation (Schols, 1999). The implications of these data for the systematics of the genus are then discussed.

MATERIAL AND METHODS

MATERIAL

Fresh material was obtained from the Living Collections of the Royal Botanic Gardens, Kew (HK: followed by Gardens' accession number). Dried material came from the Herbaria of the Royal Botanic Gardens, Kew (K: followed by collector's name and number) and the National Botanic Garden of Belgium (BR: followed by collector's name and number). Species are listed alphabetically by section, and specimens examined with TEM are indicated by an asterisk (see Appendix).

METHODS

LM. Pollen was acetolyzed for 10 min in a heating block at 90°C using the method of Reitsma (1969) and embedded in Kaiser's glycerine jelly.

SEM. Because Dioscorea pollen is relatively thin-walled, tends to collapse and is quite difficult to prepare, each specimen was subjected to two treatments: acetolysis as for LM and critical point drying (CPD). Acetolyzed pollen was mounted on specimen stubs, dried down from 70% ethanol and micrographs were taken using digital imaging on a JEOL JSM 5800 scanning electron microscope. Before CPD, mature flowers were rehydrated in Agepon wetting agent and dehydrated through an acetone series. They were critical point dried in a Balzers CPD 030 apparatus. Following CPD, anthers were removed and pollen grains were mounted on a stub with carbon strip tape. Micrographs were taken as for acetolyzed pollen. LM and SEM were carried out on dried material except for the species examined for TEM.

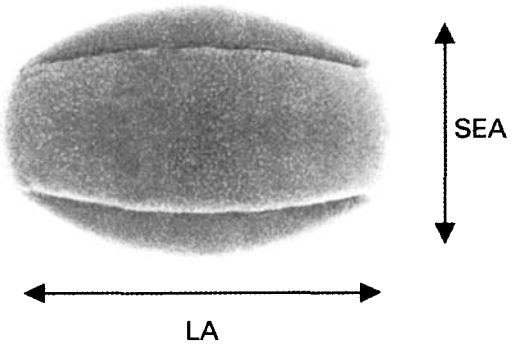


Figure 1. Indication of the axes used in pollen grain measurements. LA = longest axis; SEA = shortest equatorial axis.

TEM. Fresh whole anthers were fixed in 2% glutaraldehyde in 0.05 M sodium cacodylate buffer (pH 7.4) for 24 h, and post-fixed in 2% OsO_4 for 1 h. Anthers were bloc stained with uranyl acetate (1%) for 10 min, dehydrated through an acetone series followed by propylene oxide treatment and embedded in araldite. Semithin sections were stained with thionin (0.1%) and methylene blue (1%) and examined using a Labophot light microscope and a Nikon AFX-II camera attachment. Ultrathin sections on copper grids were stained with uranyl acetate and lead citrate. Anthers of *D. schimperiana* 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., Reading, UK) in gelatin capsules. Electron micrographs were taken using a Zeiss EM906 transmission electron microscope at 80 kV.

For each species, the longest axis (LA) and the shortest equatorial axis (SEA) were measured from ten grains, using LM slides of acetolyzed pollen (Fig. 1). The polar and equatorial axes could only be determined by examination of the orientation of the grains at the tetrad stage, which has not been carried

out to date (Clarke & Jones, 1981). Measurements of perforation size, perforation density, striation thickness and orbicules were carried out using NIH Image 1.62 (Rasband, 1996) on digital SEM images (Table 1). Terminology follows the international glossary (Punt *et al.*, 1998), unless stated otherwise.

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 tests confirmed equal variances and 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.

RESULTS AND DISCUSSION

POLLEN AND ORBICULE CHARACTERS

The 35 species examined show considerable variation in pollen and orbicule morphology. Pollen and orbicule characters are discussed below and summarized in Table 1.

Pollen size

The range of the longest axis (LA) varies from 15 μm in *D. bulbifera* to 51 μm in *D. buchananii*. The average mean value for all species investigated is 27 μm (Table 1). The smallest pollen grains were found in *D. bulbifera* (section *Opsophyton*) and *D. bemarivensis* (section *Cardiocapsa*). These species have an average LA of 16.2 μm and 18.4 μm respectively. Almost all species examined from sections *Opsophyton*, *Asterotricha*, *Brachyandra* and *Enantiophyllum* have pollen grains that are smaller than 32 μm. In contrast, rather large pollen grains can be found within sections *Rhacodophyllum*, *Sarcocapsa* and *Testudinaria*, with mean LA values ranging from 35–40 μm. However, since the number of species examined per section is small (sometimes only one), these results need to be treated with caution and require further testing.

Measurements of the shortest equatorial axis (SEA) range from 10 μm (*D. bulbifera*) to 34 μm (*D. sylvatica*). SEA values are well correlated with LA values (Table 1). Note that SEA values might not be as reliable as LA values due to harmomegathic accommodation because the grains collapse inwards along the shortest axis. Su (1987) suggested that pollen of sections with annual tubers (namely *Brachyandra*, *Enantiophyllum*, *Lasiophyton* and *Opsophyton*) is smaller than that of almost all the sections with persistent tubers (*Apodostemon*, *Dematostemon*, *Rhacodophyllum* and *Testudinaria*). Our results seem to confirm this hypothesis. However, we examined only 16 species with annual tubers and four species with persistent tubers, of which *D. buchananii* (sect. *Rhacodophyllum*) and *D. sylvatica* (sect. *Testudinaria*) clearly comply with this rule, both having grains that are *c.* 40 μm. The *t*-test on the LA measurements confirms that the difference between both groups is significant ($P=0.013$), although the sample sizes are low.

Apertures

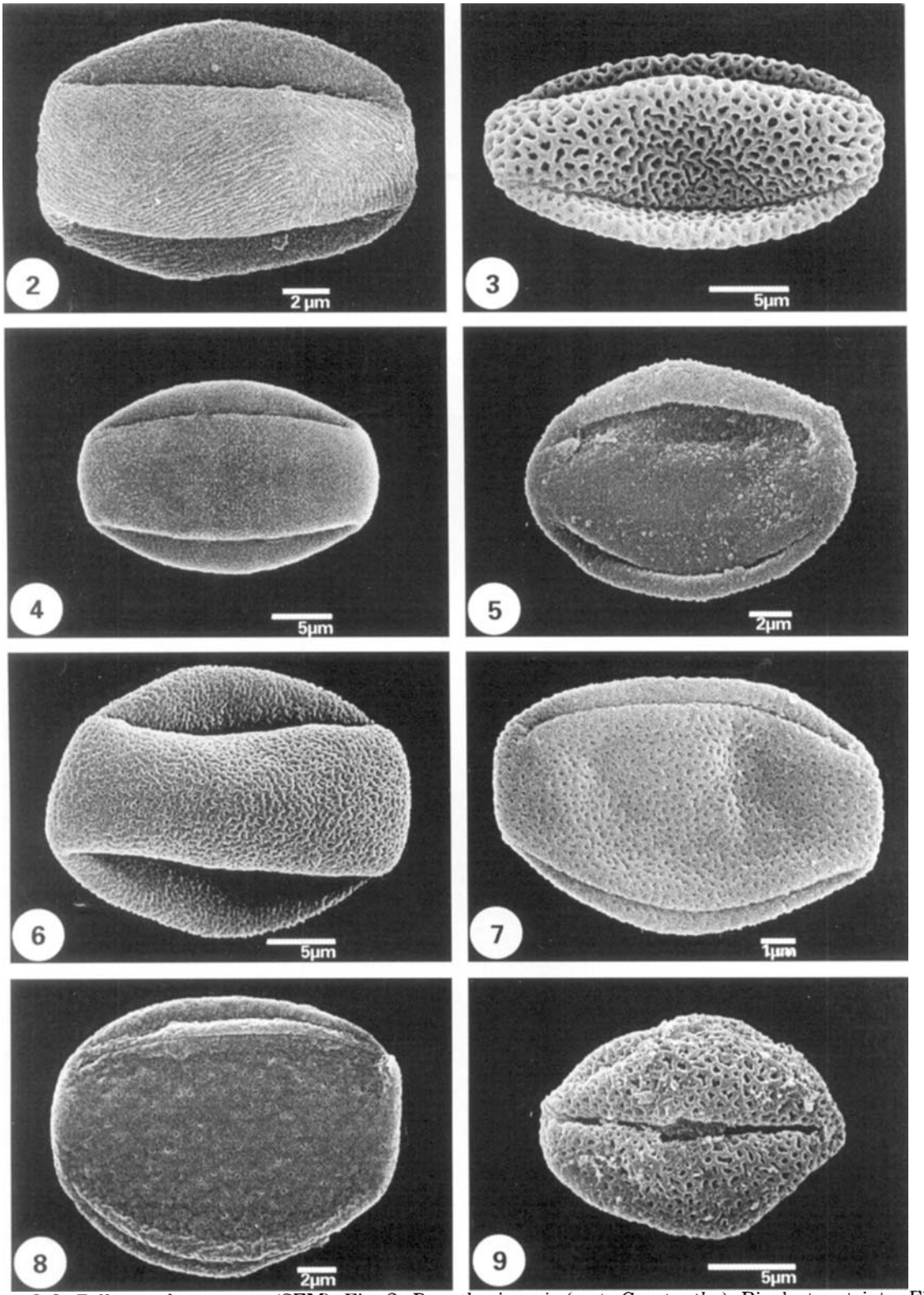
Although this term has a morphogenetic origin, apertures of *Dioscorea* pollen grains are usually called 'sulci'. As in Clarke & Jones (1981), no observations of tetrads were made and consequently no definite term can be assigned to the apertures of *Dioscorea* until their development in the tetrad stage is examined. In this paper we will use 'sulci' for the sake of simplicity and because of the occurrence of monoaperturate pollen grains (e.g. *D. membranacea*), which are called monosulcate in most monocots.

Aperture orientation and aperture width are highly dependent on the hydration state of the grain. Dehydrated pollen grains tend to fold along their shortest axis, leading to more infolded apertures that look smaller and closer to each other.

Dioscorea pollen grains are relatively thin-walled, as is pollen of many monocots, and tend to collapse, especially when prepared from herbarium material for SEM. Thus observation of the apertures may be difficult with SEM, and the number of apertures was established from LM observations. Twenty-two species in this survey have bisulcate pollen, while 11 species have both monosulcate and bisulcate pollen (Table 1; Figs 2–9). When mono- and bisulcate pollen occur in one specimen, one type prevails (95% or more per sample). Nine species are predominantly bisulcate with a low percentage (5% or less per sample) of monosulcate pollen and two species are predominantly monosulcate with a low percentage (5% or less per sample) of bisulcate pollen. Only two species, *Dioscorea lagoasanta* (sect. *Monadelpha*) and *D. membranacea* (sect. *Macropoda*) have exclusively monosulcate pollen (Fig. 9). Bisulcate pollen predominates in the *Dioscorea* species examined, although monosulcate predominates in sect. *Stenophora*, and *Monadelpha* (Table 1), but sample sizes are low. Aperture number could potentially be a useful character if more taxa were examined. Studies of aperture development are needed to investigate aperture variation within and between species. The variability in aperture number encountered in this survey (both within one specimen and in a single anther) is rather uncommon for seed plants with a small number of apertures. Aperture orientation is related to the tetrad and differences in aperture number and position might be correlated with different tetrad morphologies or with other ontogenetic

Table 1. Summary of pollen and orbicule characters for all species studied. All measurements in μm. Abbreviations: LA = longest axis; SEA = shortest equatorial axis. W str. = width of striations; conn. = connections between orbicules; spine = spines on the orbicule surface; / = no observations; * = not applicable

Section	Species	LA	SEA	Ornam.	No. apertures	Perf. size (μm)	Perf./μm²	W str. (μm)	Orbicule diam.	Conn.	Spine
Apodostemon	*D. megacarpa*	25–(26.0)–27	13–(16.0)–19	striate	2	*	*	0.2	*	*	*
Asterotricha	*D. hirtiflora*	22–(24.2)–26	16–(23.6)–23	perforate	2	0.04–(0.06)–0.07	5.5	*	0.22–(0.45)–0.64	–	–
	D. schimperiana	18–(19.5)–21	12–(15.0–17	perforate	2	0.08–(0.11)–0.16	6.0	*	0.38–(0.57)–0.99	+	–
Botryosicyos	*D. pentaphylla*	22–(23.2)–26	14–(15.9)–18	perforate	2	0.06–(0.10)–0.16	2.3	*	/	–	–
	D. quartiniana	24 – 24.8)–25	15–(15.8)–17	perforate	2	0.09–(0.16)–0.32	2.6	*	0.56–(0.93)–1.87	–	–
Brachyandra	*D. hexagona*	20–(21.6)–23	17–(17.6)–18	striate	2 (1)	*	*	0.21	0.19–(0.38)–0.54	–	–
Campanuliflorae	*D. karatana*	22–(24.2)–26	20–(20.2)–24	striate	2	*	*	0.22	0.25–(0.37)–0.47	+	+
	D. maciba	22–(23.0)–24	12–(14.5)–16	striate	2 (1)	*	*	0.27	0.18–(0.26)–0.31	–	+
Cardiocapsa	*D. bemarivensis*	17–(18.4)–20	16–(18.6)–20	striate	2 (1)	*	*	*	0.16–(0.30)–46	–	+
Chirophyllum	*D. brachybotrya*	28–(31.8)–36	20–(22.0)–24	perforate	2	0.06–(0.21)–0.38	3.4	*	0.22–(0.34)–0.45	/	/
Chondrocarpa	*D. riparia*	25–(26.4)–29	15–(17.4)–19	perforate	2	0.07–(0.13)–0.21	5.2	*	0.28–(0.43)–.68	–	–
Combilium	*D. esculenta*	30–(31.6)–33	16–(17.3)–19	perforate	2	0.09–(0.18)–0.32	2.6	*	*	*	*
Cryptantha	*D. catharinensis*	22–(24.1)–26	17–(20.0)–28	striate	2 (1)	*	*	0.22	0.22–(0.38)–0.55	+	–
Dematostemon	*D. adenocarpa*	26–(29.0)–33	19–(19.8)–21	mic. ret.	2	0.14–(0.49)–0.67	2.4	*	0.32–(0.46)–0.65	+	–
Enantiophyllum	*D. decipiens*	20–(21.0)–22	15–(15.0)–22	perforate	2	0.12–(0.16)–0.22	3.2	*	0.35–(0.46)–0.63	–	+
	D. glabra	19–(20.6)–28	14–(18.3)–21	perforate	2	0.05–(0.07)–0.10	12.0	*	0.41–(0.69)–0/89	–	–
	D. hamiltonii	25–(26.2)–27	19–(19.7)–21	perforate	2	0.06–(0.14)–0.29	8.7	*	0.28–(0.33)–0.51	–	–
	D. praehensilis	25–(28.7)–32	14–(19.0)–21	perforate	2	0.08–(0.14)–0.27	10.2	*	0.32–(0.59)–0.91	+	–
Lasiogyne	*D. dodecaneura*	30–(33.0)–39	15–(17.6)–21	perforate	2 (1)	0.09–(0.20)–0.34	4.6	*	0.12–(0.27)–0.40	–	–
Lasiophyton	*D. dumetorum*	25–(26.2)–27	20–(21.7)–23	perforate	2	0.06–(0.14)–0.23	8.2	*	0.44–(0.68)–1.10	–	–
	D. dregeana	18–(19.4)–21	16–(17.0)–18	perforate	2	0.05–(0.13)–0.24	9.2	*	*	*	*
Macrocarpaea	*D. preussii*	27–(31.2)–34	19–(22.8)–25	perforate	2	0.08–(0.20)–0.32	4.5	*	0.35–(0.57)–0.76	+	–
Macrogynodium	*D. trifida*	33–(34.7)–36	21–(22.1)–25	perforate	2	0.09–(0.18)–0.41	3.6	*	*	*	*
Madagascarienses	*D. acruatinervis*	20–(20.5)–21	12–(13.7)–15	striate	2 (1)	*	*	0.25	0.27–(0.37)–0.52	–	+
Monadelpha	*D. lagoa-santa*	25–(28.6)–30	14–(16.8)–20	perforate	1	0.10–(0.31)–0.49	3.0	*	0.23–(0.38)–0.58	–	–
Opsophyton	*D. bulbifera*	15–(16.2)–17	10–(10.2)–11	perforate	2 (1)	0.05–(0.08)–0.12	8.8	*	0.39–(0.48)–0.62	+	–
Rhacodophyllum	*D. buchananii*	42–(45.8)–51	22–(27.8)–33	perforate	2 (1)	0.10–(0.23)–0.42	3.2	*	*	*	*
Sarcantha	*D. amazonum*	27–(29.7)–31	16–(17.7)–19	striate	2	*	*	0.25	0.31–(0.38)–0.50	–	+
Sarcocapsa	*D. oaxacensis*	32–(35.0)–38	18–(21.8)-25	perforate	2 (1)	0.09–(0.13)–0.19	4.7	*	*	*	*
Spaerantha	*D. multiflora*	26–(27.5)–30	13–(17.5)–21	perforate	2	0.11–(0.24)–0.42	3.7	*	0.25–(0.35)–0.57	–	–
Stenocorea	*D. daunea*	32–(36.4)–40	19–(25.1)–27	perforate	2	0.14–(0.22)–0.30	3.2	*	0.49–(0.77)–1.15	–	–
Stenophora	*D. althaeoides*	25–(25.3)–26	16–(16.6)–17	striate	1 (2)	*	*	0.28	0.20–(0.37)–0.56	+	–
	D. collettii	35–(36.0)–39	21–(22.5)–25	perforate	1 (2)	0.11–(0.22)–0.37	4.4	*	*	*	*
	D. membranacea	20–(21.8)–24	13–(17.5)–20	perforate	1	0.10–(0.23)–0.36	3.2	*	0.19–(0.33)–0.47	–	–
Testudinaria	*D. sylvatica*	37–(40.4)–44	27–(30.1)–34	perforate	2	0.10–(0.20)–0.35	2.8	*	0.30–(0.52)–0.72	–	–



Figures 2–9. Pollen and apertures (SEM). Fig. 2. *D. catharinensis* (sect. *Cryptantha*). Bisulcate, striate. Fig. 3. *D. adenocarpa* (sect. *Dematostemon*). Bisulcate, microticulate. Fig. 4. *D. hamiltonii* (sect. *Enantiophyllum*). Bisulcate, perforate. Fig. 5. *D. decipiens* (sect. *Enantiophyllum*). Bisulcate, perforate. Fig. 6. *D. preussii* (sect. *Macrocarpaea*). Bisulcate, perforate to microreticulate. Fig. 7. *D. bulbifera* (sect. *Opsophyton*). Bisulcate, perforate. Fig. 8. *D. hirtiflora* (sect. *Asterotricha*). Bisulcate, perforate. Fig. 9. *D. membranecea* (sect. *Stenophora*). Monosulcate, perforate to microreticulate.

events, such as the position of rER strands at the tetrad stage (Furness & Rudall, 1999a,b). TEM observations of the earliest stages of pollen ontogeny, which could clarify questions concerning aperture configuration, are currently under investigation and could also shed light on the origin of biaperturate pollen grains within the monocots.

Microsporogenesis in *Dioscorea* is of the simultaneous type with tetrahedral and decussate tetrads, as in other members of Dioscoreaceae examined, and in Stenomeridaceae and Taccaceae in Dioscoreales (Caddick *et al.*, 1998; Furness & Rudall, 1999b, 2000). Simultaneous microsporogenesis is less common in monocots than the successive type, with predominantly tetragonal tetrads, although it characterizes some groups (Furness & Rudall, 1999b, 2000).

Monosulcate pollen is generally accepted to be the plesiomorphic character state within the monocots (Dahlgren, Clifford & Yeo, 1985; Furness & Rudall, 1997, 1999a). The transition from a single distal sulcus to multiaperturate pollen grains has occurred in numerous groups within the monocots such as Alismatales (Zavada, 1983; Blackmore & Crane, 1998). In *Dioscorea*, monosulcate pollen predominates in the basal section *Stenophora*, while less basal sections, such as *Enantiophyllum* and *Lasiophyton*, are characterized by bisulcate pollen grains. Trisulcate pollen, as reported by Erdtman (1969), was not found in the species examined in this survey.

Sexine ornamentation

All *Dioscorea* species examined have either perforate, striate or microreticulate ornamentation. Of the 35 species, 25 have a perforate sexine, nine are striate and only one species, *D. adenocarpa*, has a microreticulate sexine. The two major ornamentation patterns were compared using SEM and TEM (Figs 16, 17, 27, 28) The striate pattern is formed by supratectal striae in *D. karatana* (Figs 16, 17).

Striate ornamentation

Striate pollen is present in the following sections: *Apodostemon, Brachyandra, Campanuliflorae, Cardiocapsa, Cryptantha, Stenophora, Madagascarienses* and *Sarcantha* (Figs 10, 17). All five Malagasy endemic species and only three of the 11 New World species in this survey are characterized by a striate sexine. (Appendix; Table 1). The width of the striae ranges from 0.20 to 0.28 μm. In *D. bemarivensis* (sect. *Cardiocapsa*) and *D. arcuatinervis* (sect. *Madagascarienses*), the striations are arranged in concentric circles (Figs 12, 13).

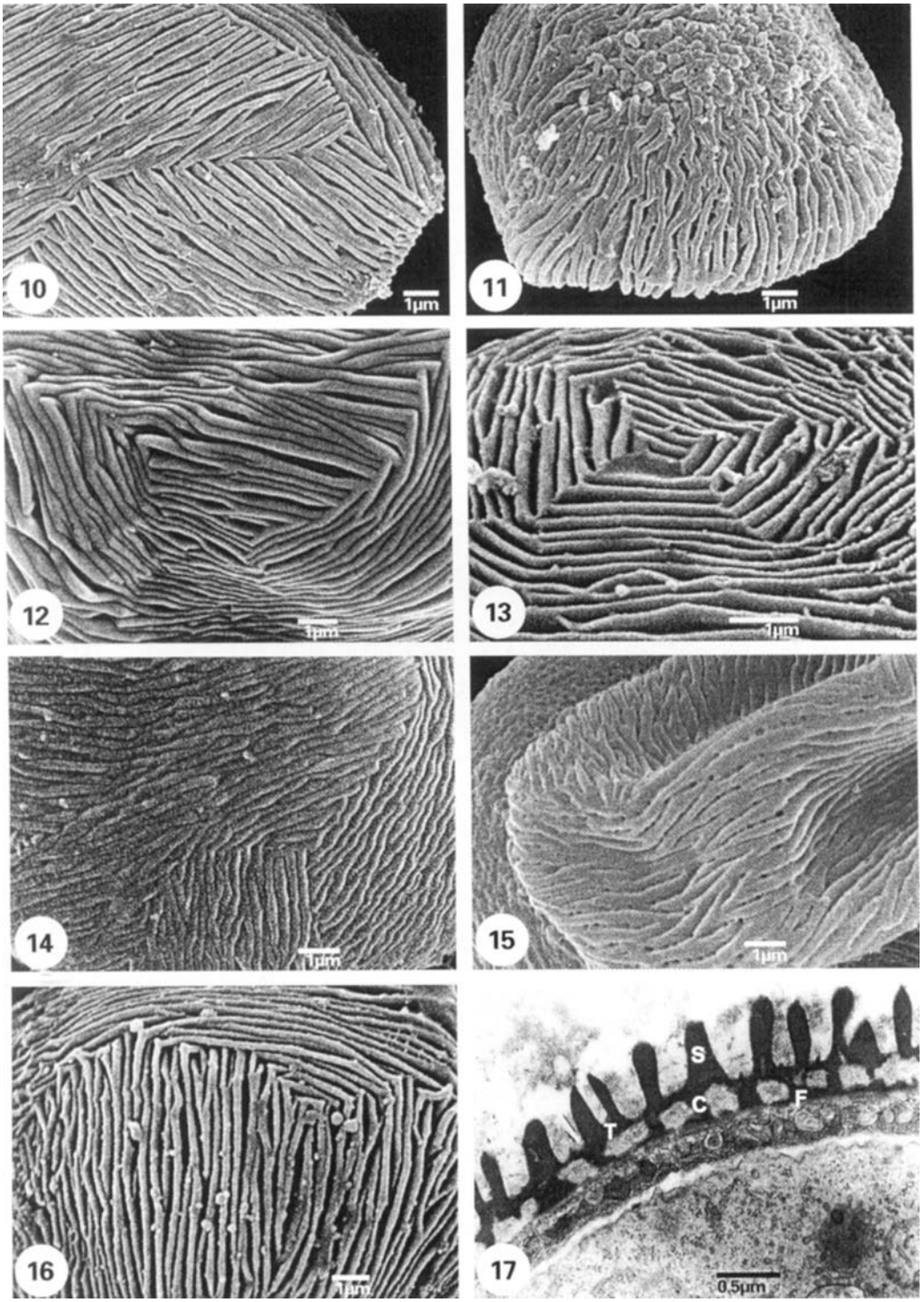
The striate ornamentation of *D. althaeoides* (sect. *Stenophora*) (Fig. 15) is somewhat surprising, because the other two members of *Stenophora* in this survey (*D. collettii* and *D. membranacea*) both have a perforate sexine (Fig. 26), however, *D. althaeoides* has perforations between the striae. These also occur in *D. catharinensis* (sect. *Cryptantha*) (Fig. 14) (see pp. 310). A comparison of pollen grain size (length in (μm) of striate and perforate pollen is presented (Fig. 18). The LA of striate pollen grains clearly shows less variation than the LA of the perforate species: the striate pollen is more uniform in size. The smaller number of striate species ($N=9$) cannot be the sole explanation for this fact.

Striate pollen is considered to be rare within the monocots (van der Ham, Hetterscheid & van Heuven, 1998). The striate sexine in all Malagasy species examined could be an adaptation to a specific Malagasy pollinator. Unfortunately, little is known about pollination in *Dioscorea*. Barroso *et al.* (1974) gave an overview of the pollination of about 12 *Dioscorea* species in South America, of which some are pollinated by *Meliponini*, a tribe of stingless bees, perhaps a species of *Hypotrigona*. More pollination data, especially for the African and Malagasy *Dioscorea* species, are required to help interpret pollen morphology within the genus.

Variation in perforate sexine patterns

Much variation was found in both perforation size and perforation density (Figs 19–26). Perforation sizes range from 0.04 μm (in *D. hirtiflora*) (Fig. 23) to 0.67 μm (in *D. adenocarpa*) (Fig. 24); the average value is 0.18 μm. Small perforations are common in section *Enantiophyllum* and in some species (e.g. *D. decipiens*) (Fig. 5) the sexine ornamentation is rather punctate. *D. hamiltonii* (sect. *Enantiophyllum*) has a specific 'barnacle like' perforation pattern that was not found elsewhere (Fig. 25).

Perforation density (number of perforations μm^{-2}) in particular, seems to characterize some sections, especially when combined with perforation size. In most species, the perforations are evenly distributed on the pollen surface, which makes perforation density an accessible character. Note that both characters are partially dependent: large perforations result in a low perforation density. Most perforate species have a perforation density below 5 μm^{-2} (Table 1). Sect. *Enantiophyllum* however, is characterized by a high perforation density (more than 8 μm^{-2}), for example, *D. hamiltonii* (Fig. 25). *Dioscorea decipiens* is the only exception in this section (3 μm^{-2}). Sect. *Asterotricha* also has a fairly high perforation density (5–6 μm^{-2}) (e.g. *D. hirtiflora*, Fig. 23). *D. dumetorum* (Fig. 20) and *D. dregeana*, both members of sect. *Lasiophyton*, a compound-leaved section, are characterized by a perforation density of more than 8 μm^{-2}, compared with *D. quartiniana* (Fig. 19) and *D. pentaphylla* of the



Figures 10–17. Striate sexine patterns. All SEM, except Fig. 17 TEM. Fig. 10. *D. megacrapa* (sect. *Apodostemon*) Striate sexine with striae running in two directions. Fig. 11. *D. maciba* (sect. *Campanuliflorae*). Striate sexine. Fig. 12. *D. arcuatinveris* (sect. *Madagascarienses*). Striations are arranged in concentric polygons. Fig. 13. *D. bemarivensis* (sect. *Cardiocapsa*). Striations are arranged in concentric polygons. Fig. 14. *D. catharinensis* (sect. *Cryptantha*). Striate sexine with tightly connected striations. Fig. 15. *D. althaeoides* (sect. *Stenophora*). Striate sexine with tightly connected striations. Small perforations are visible between the striae. Figs 16, 17. *D. karatana* (sect. *Campanuliflorae*). Fig. 16. Striate sexine with striae running in different directions. Fig. 17. Section through striate sexine. C = columellae, F = footlayer, S = striae, T = tectum.

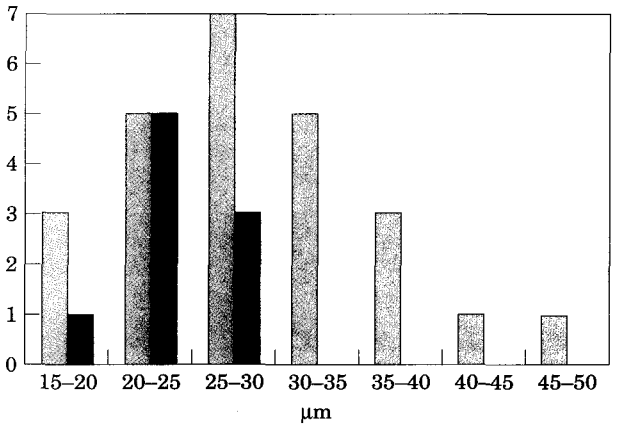


Figure 18. Comparison between pollen size and ornamentation. For each pollen size class (LA in μm) the number of species are indicated per ornamentation type (■, striate: $N=9$; ▦, perforate: $N=26$).

compound-leaved section *Botryosicyos*, which have a value of *c.* 3 μm^{-2} for this character.

Wall stratification and ultrastructure

The ultrastructure of the pollen exine was observed with LM in all 35 species and with TEM in four species (*D. dumetorum*, *D. karatana*, *D. schimperiana* and *D. sylvatica*) (Figs 17, 27–31). The wall structure of *Dioscorea* pollen is always tectate-columellate. There is only minor variation in exine thickness (1–2 μm). In *D. sylvatica*, the exine consists of a nexine of *c.* 0.3 μm, collumellae of 0.4–0.5 μm and a perforate tectum of 0.6 μm. As a consequence, the exine is *c.* 1.4 μm thick (Fig. 28). *D. karatana*, a species with a striate sexine, has an exine *c.* 1 μm thick. It consists of a nexine of 0.2 μm, columellae of 0.3 μm and a tectum of 0.1 μm, supporting supratectal striae *c.* 0.4 μm thick (Fig. 17).

White lines are visible at the bottom of the footlayer, which could indicate there is some endexine in this layer (Fig. 28). Endexine is often reduced or absent in monocots (Zavada, 1983; El-Ghazaly, 1993; Furness & Rudall, 1997). The endexine of *D. polygonoides* was described as thin and granular, similar to the endexine of *Zea mays* L. (Poaceae) (Zavada, 1983).

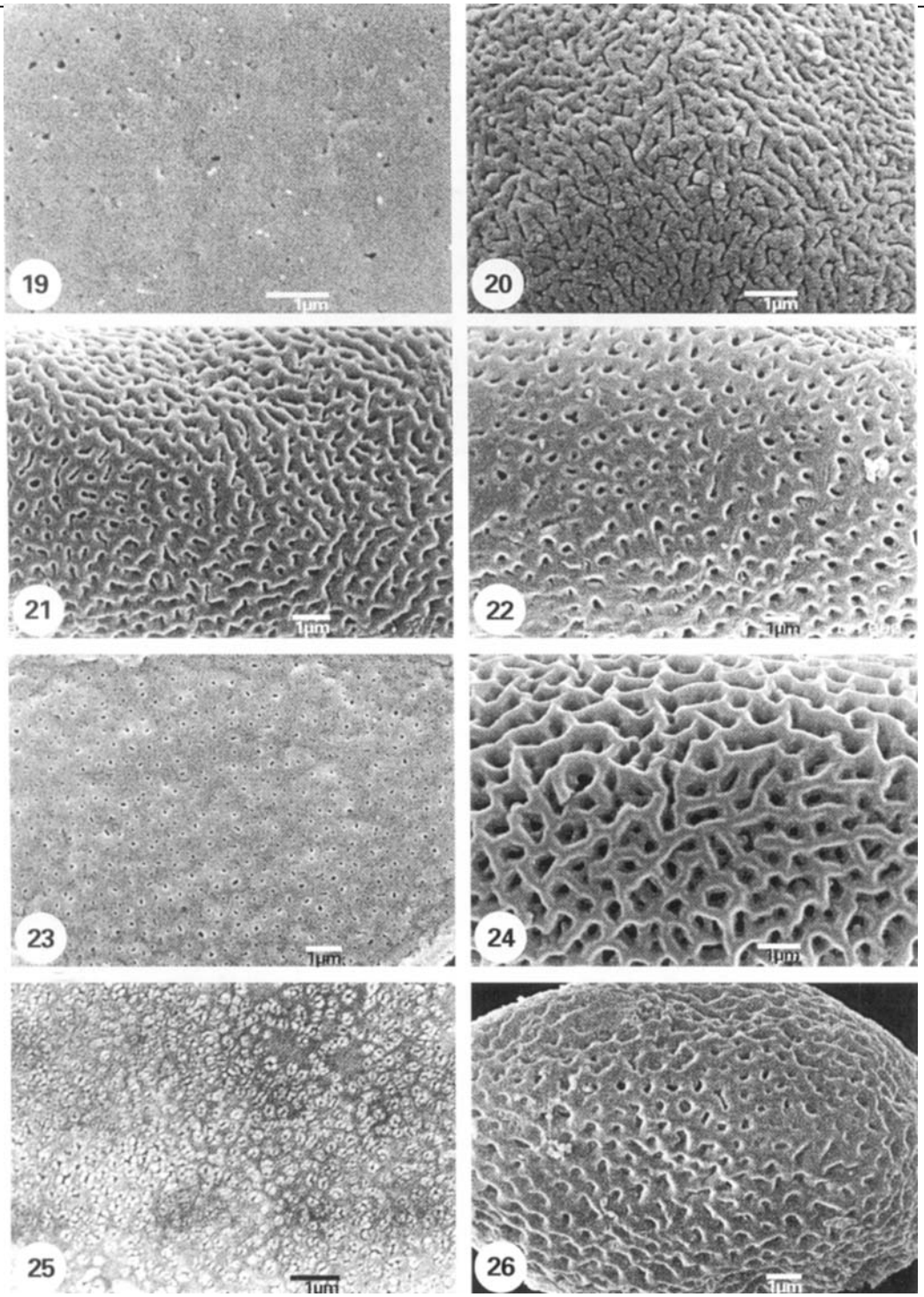
Intine was observed which thickens beneath the sulci in all four species examined using TEM, ranging from 0.2 μm in the non-apertural regions to 1.8 μm below the sulci (Figs 29, 30). Intine channels of about 0.1 μm diameter are embedded in the entire intine, but concentrated beneath the apertures, of all four species. In TEM sections, these channels appear in a honeycomb pattern, especially in *D. dumetorum* (Fig. 34). A thick intine below the apertures serves as a barrier against dehydration and infection by microorganisms (Thanikaimoni, 1986) and provides the necessary enzymes for pollen tube growth. These enzymes are stored together with antigens in radial channels as seen in TEM sections (Figs 30, 34). The intine inclusions occur in the form of radially orientated vesicles and may arise from protoplasmic protrusions, according to studies on the development of *Triticum aestivum* L. pollen (El-Ghazaly & Jensen, 1986).

Pollen cytoplasm

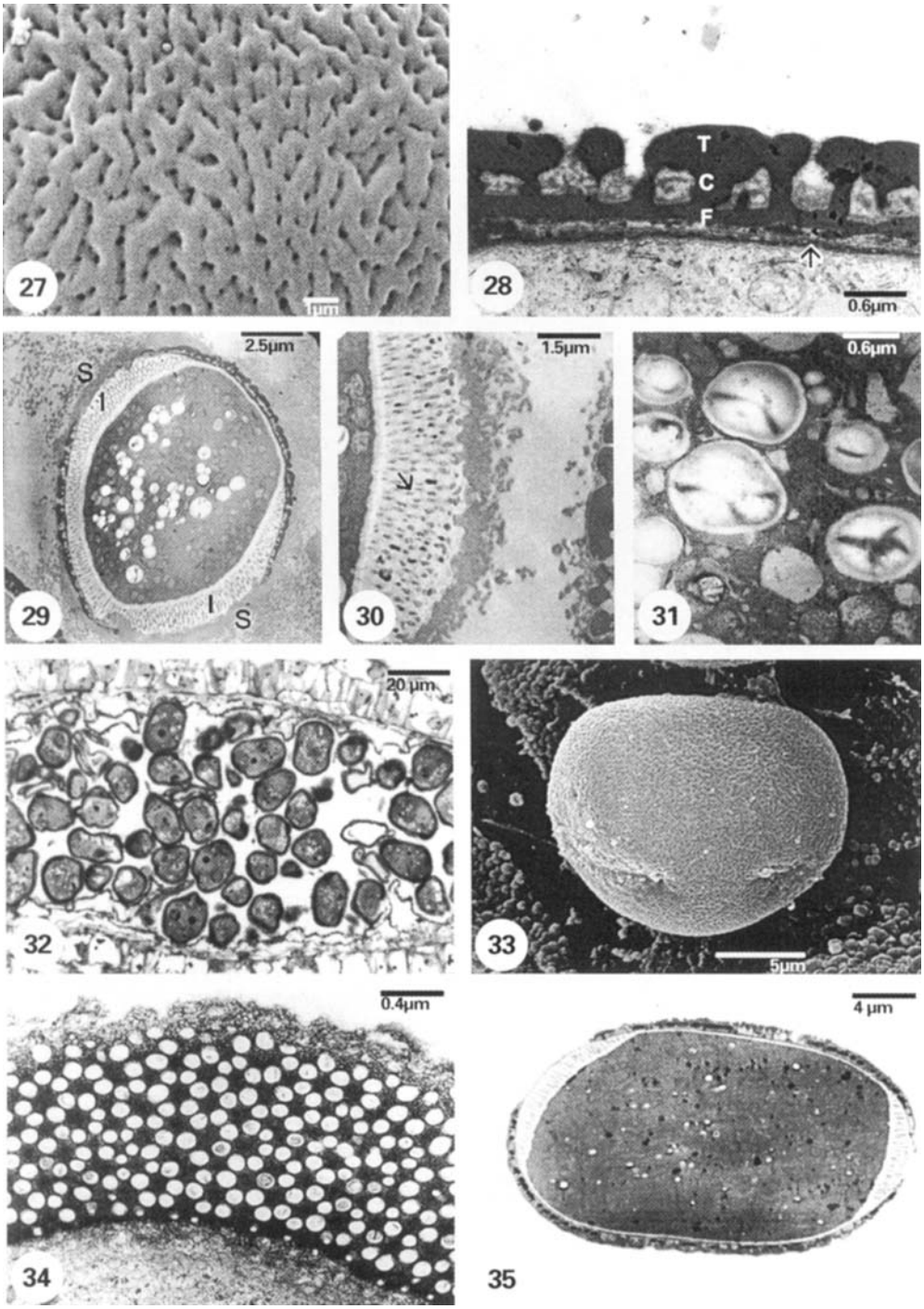
Pollen is dispersed at the binucleate stage (Fig. 32), which is common in monocots, except for Alismatiflorae and Commeliniflorae which are trinucleate and Ariflorae which have both types (Dahlgren & Clifford, 1982; Grayum, 1986). Starch grains are abundant in *D. sylvatica* (Fig. 31), while mature pollen grains of *D. dumetorum* possess mainly lipid droplets, both in the cytoplasm and on the pollen surface (Fig. 35). Broader sampling for starch or lipid could possibly be useful for *Dioscorea* at the infrageneric level. Among aroids, 73% of genera studied contained starch in the pollen, the rest being starch-free; thus starch was found to be a useful character within the Araceae (Grayum, 1985). Pollen of some monocot families contains starch, although there are some conflicting results and further work is required (see discussion in Rudall & Furness, 1997).

Orbicules

Twenty-seven out of 34 species examined have orbicules in the anther locule, ranging in size from 0.12 to 1.90 μm. Seven species lack orbicules, thus orbicule presence/absence may be a useful systematic character. Orbicule size (diameter in μm) was compared between striate and perforate pollen (Fig. 36). The mean orbicule size of all striate species ranges from 0.26 to 0.38 μm, while in perforate species the orbicule size ranges from 0.27 to 0.93 μm. The size range of orbicules in striate and perforate species overlaps, but perforate species can have orbicules which are larger than those of striate species: the size range is larger for perforate species. However, this observation may not be significant due to the small sample size. Most species have spherical orbicules (Figs 37, 41–46), rarely they are elliptical (*D. daunea*) or irregular shaped (*D. quartiniana*, *D. schimperiana*) (Figs 38–40). Some species have small spines on the orbicule surface (*D. amazonum*, *D. arcuatinervis*, *D. catharinensis*, *D. decipiens*, *D. karatana* and *D. maciba*), which is frequently correlated with a striate pollen sexine (Figs 45, 50, 51). Thin threads between orbicules occur in *D. adenocarpa*, *D. althaeoides*, *D. bemarivensis*, *D. bulbifera*, *D. catharinensis*, *D. karatana*, *D. praehensilis*, *D. preussii* and *D. schimperiana* (Fig. 43). Small granules occur together with orbicules in *D. dumetorum* (Fig. 42).



Figures 19–26. Perforate sexine patterns. All SEM. Fig. 19. *D. quartiniana* (sect. *Botryosicyos*). Perforate sexine with tiny perforations and a very low perforation density. Perforations not evenly scattered. Fig. 20. *D. dumetorum* (sect. *Lasiophyton*). Perforate to microregulate sexine with small elongated perforations and a high perforation density. Fig. 21. *D. preussii* (sect. *Macrocarpaea*). Perforate sexine. Fig. 22. *D. esculenta* (sect. *Combilium*). Perforate sexine with evenly spaced perforations. Fig. 23. *D. hirtiflora* (sect. *Asterotricha*). Perforate sexine with small perforations. Fig. 14. *D. adenocarpa* (sect. *Dematostemon*). Microreticulate sexine. Fig. 25. *D. hamiltonii* (sect. *Enantiophyllum*). Perforate sexine with small perforations and a high perforation density. Perforations are surrounded by an elevated, irregular rim, resembling barnacles. Fig. 26. *D. membranecea*. (sect. *Stenophora*). Perforate sexine.



Figures 27–35. *D. sylvatica* (sect. *Testudinaria*). Fig. 27. Perforate sexine, uneven tectum with small, round perforations often situated in grooves (SEM). Fig. 28. Section of perforate sexine with footlayer, collumellae, and tectum. Note the white lines below the footlayer (arrow), possibly indicating the presence of the endexine (TEM). C = columellae, F = footlayer, T = tectum. Fig. 29. Oblique section through a bisulcate pollen grain, filled with vesicles and starch grains. Thick channeled intine (I) below the sulci (S) (TEM). Fig. 30. Detail of 29. Radial intine channels (arrowed). Fig. 31. Starch grains in the pollen cytoplasm. Fig. 32. *D. schimperiana* (sect. *Asterotricha*). Longitudinal section through the anther. Binucleate, biaperturate pollen grains surrounded by tapetal remnants (LM). Figs 33–35. *D. dumetorum* (sect. *Lasiophyton*). Fig. 33. Bisulcate pollen grain against the locule wall, which is covered with orbicules (SEM). Fig. 34. Radial intine channels are arranged in a 'honeycomb' pattern (TEM). Fig. 35. Ultrastructure of a bisulcate pollen grain. Thick intine below the sulci, and many lipid droplets in the cytoplasm (TEM).

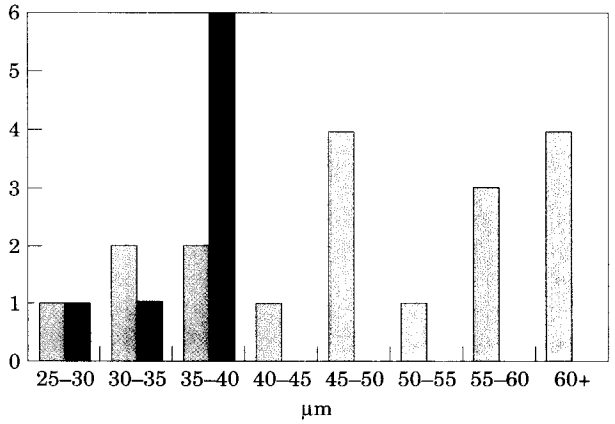


Figure 36. Comparison between orbicule size and pollen ornamentation. For each orbicule size class (diameter in 10^{-2} μm) the number of species are indicated per pollen ornamentation type (■, striate: $N=8$; ▦, perforate: $N=18$).

TEM observations revealed that orbicules are present on the inner and outer tangential walls and on the radial walls of the tapetum (Fig. 48). Considerable variation was found in orbicule ultrastructure. Most orbicules possess a core that is slightly more electron-lucent. In *D. sylvatica* one small depression (and sometimes more) occurs in almost every orbicule causing the latter to be somewhat irregularly shaped (Figs 47, 49, 50). The ultrastructure of the orbicules of *D. sylvatica* somewhat resembles that of *Lilium* (Clement & Audran, 1993) sharing an electron-lucent core and a more electron-dense orbicule wall with depressions or notches that reach to the orbicule core. The walls of *Lilium* orbicules, however, shows more bulges and *Lilium* orbicules are bigger (*c.* 2.5 μm) than those of *D. sylvatica* (*c.* 0.6 μm). The orbicules of *D. dumetorum* are similar to those of *D. sylvatica* but lack a notch. In *Dioscorea karatana*, the only species with striate pollen where orbicules were examined using TEM, the orbicules have a more heterogeneous ultrastructure, containing electron dense parts, and small spines on the orbicule surface (Figs 51, 52).

SYSTEMATIC DISCUSSION

At present, evolutionary relationships within *Dioscorea* are unclear and much new data are required, both molecular and morphological (for example, Wilkin, 2000). A complete picture of the pollen evolution within the genus is some way off, although several current taxonomic hypotheses are supported by the pollen data. Some of the sections (*sensu* Knuth, 1924 and Burkill, 1960) are discussed below. Note that, for most sections, pollen of only one or a few species was observed. Further sampling is needed to strengthen conclusions about taxonomic relationships.

Sections *Cardiocapsa, Madagascarienses, Campanuliflorae* and *Brachyandra*

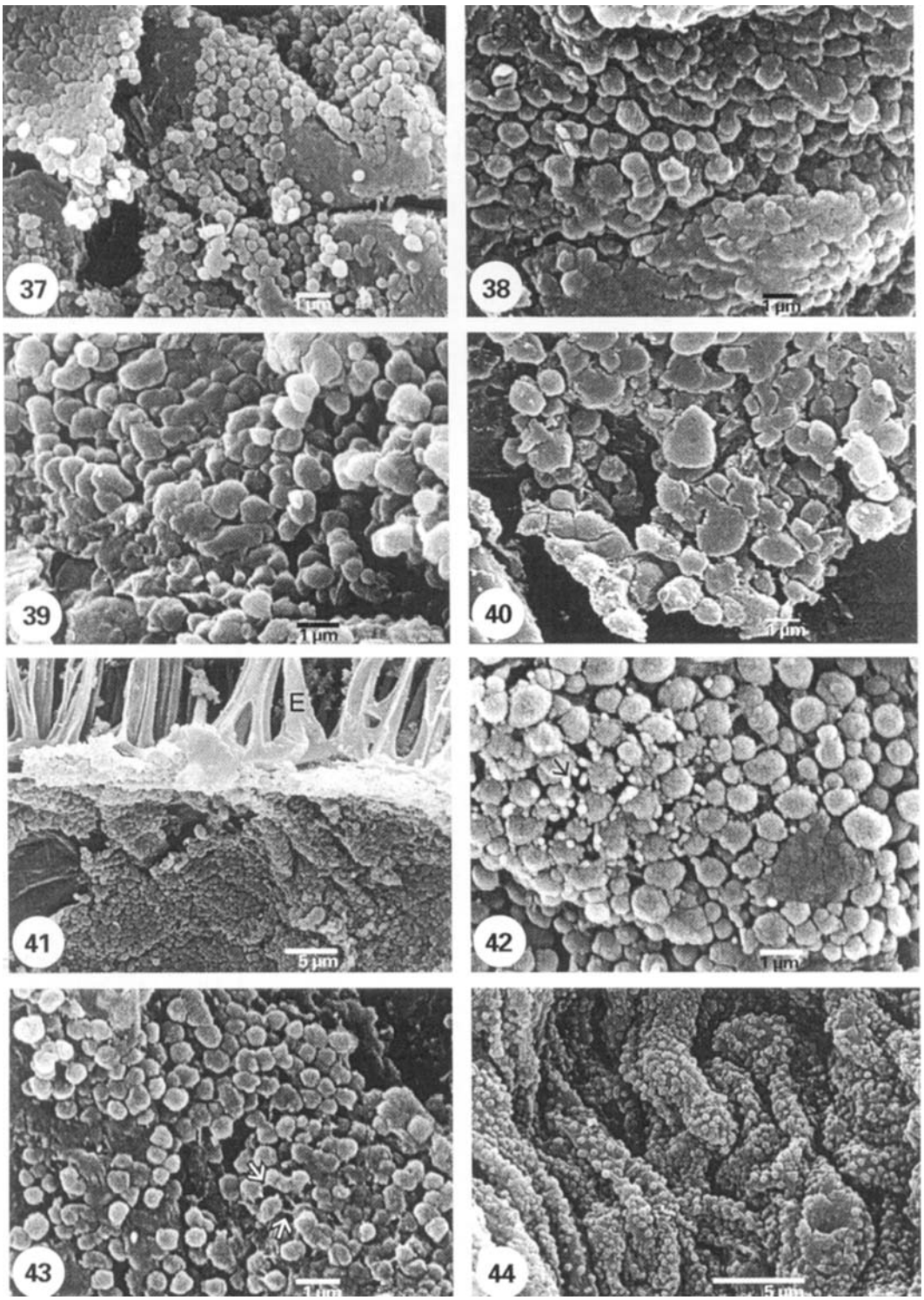
These four Malagasy sections are represented by five species in this survey, all characterized by a striate sexine, with average values for LA 18 μm–24 μm. The width of the striae is also very similar: all are *c.* 0.2 μm. Moreover, all five species have orbicules of almost identical size (*c.* 0.35 μm) and a spinulose orbicule surface (Figs 45, 50), except for *D. hexagona*, which has a smooth orbicule surface. As mentioned 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 explanation, however, is that all four Malagasy sections 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. bemarivensis* and *D. arcuatinervis* from sections *Cardiocapsa* and *Madagascarienses* respectively, have striate pollen with the striations arranged in distinct concentric circles (Figs 12, 13). Both these Malagasy sections were considered to be closely related by Burkill (1960), who placed them close to each other in his diagram of relationships.

Sections *Botryosicyos* and *Lasiophyton*

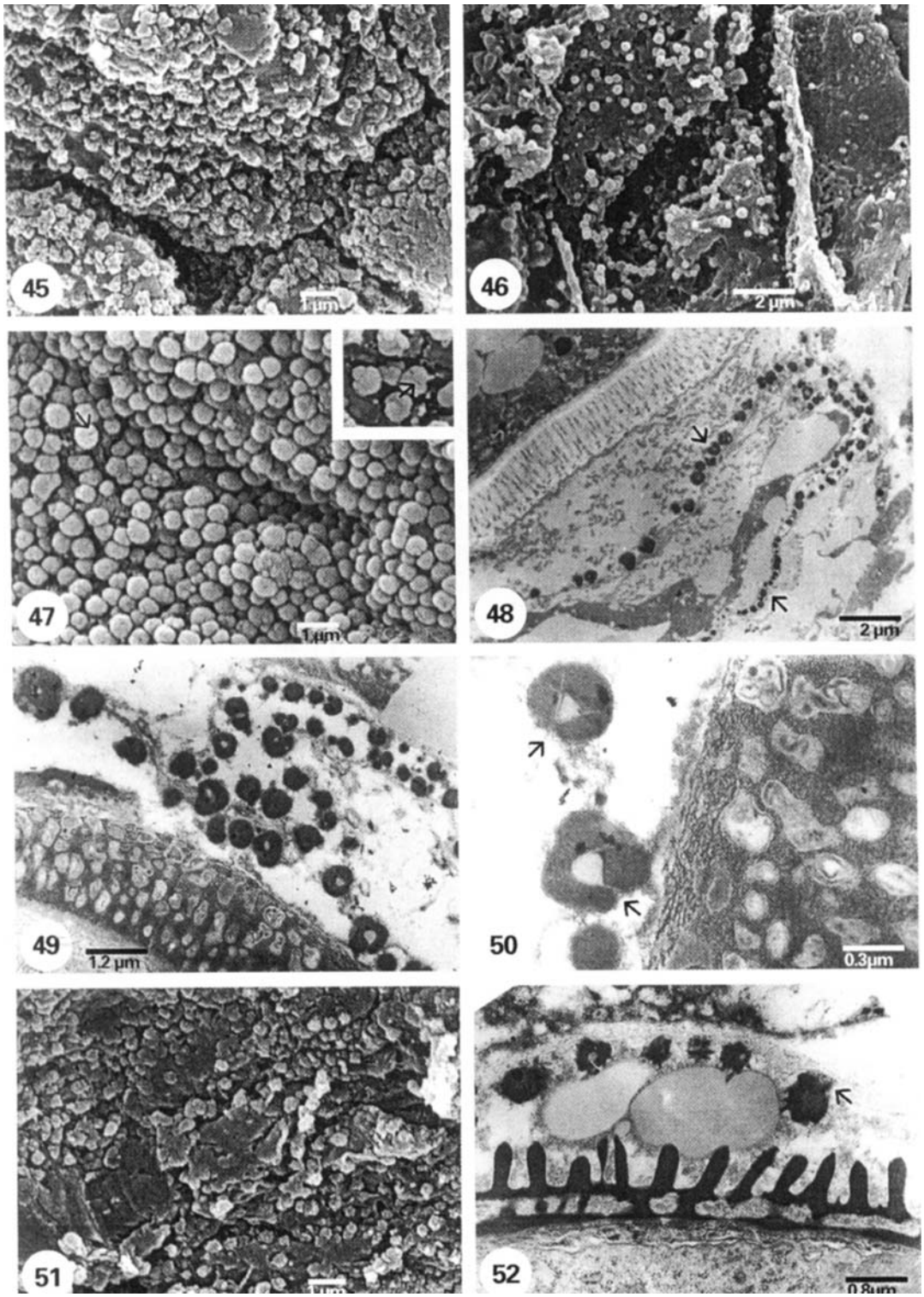
Both sections *Botryosicyos* and *Lasiophyton* contain compound-leaved species and for that reason the two sections were thought to be closely related (Uline, 1897). Pollen of both sections is bisulcate and perforate with small perforations. Their perforation density, however, is clearly different: pollen of section *Lasiophyton* is characterized by a high perforation density (*c.* 8 μm^{-2}), while pollen of section *Botryosicyos* has a very low perforation density (*c.* 3 μm^{-2}) (Figs 19, 20). This difference in pollen morphology supports the observed differences in *rbc*L sequence data and vegetative morphology (Wilkin, 1999; Wilkin & Caddick, 2000). The sections can be easily distinguished by their leaf morphology: section *Lasiophyton* is characterized by possessing three leaflets, each with three to seven main veins and section *Botryosicyos* has three to five or more leaflets, each with a single main vein (Wilkin, 1999).

Sections *Enantiophyllum* and *Asterotricha*

A close relationship between sections *Enantiophyllum* and *Asterotricha* based on macromorphological characters was proposed by Burkill (1960) and supported



Figures 37–44. Orbicules on the locule wall (SEM). Fig. 37. *D. althaeoides* (sect. *Stenophora*). Spherical orbicules. Fig. 38. *D. glabra* (sect. *Enantiophyllum*). Elliptical orbicules. Fig. 39. *D. daunea* (sect. *Stenocorea*). Elliptical orbicules. Fig. 40. *D. quartiniana* (sect. *Botryosicyos*). Irregularly shaped orbicules. Fig. 41. *D. dumetorum* (sect. *Lasiophyton*). Anther wall with endothecium (E), and tapetal remnants covered with orbicules. Fig. 42. *D. dumetorum* (sect. *Lasiophyton*). Detail of 41. Note the small granules (arrow) between the spherical orbicules. Fig. 43. *D. bulbifera* (sect. *Opsophyton*). Spherical orbicules, sometimes connected by threads (arrows). Fig. 44. *D. catharinensis* (sect. *Cryptantha*). Spherical orbicules.



Figures 45–52. Orbicules. Fig. 45. *D. amazonum* (sect. *Sarcantha*). Spiny orbicules on the locule wall (SEM). Fig. 46. *D. dodecaneura* (sect. *Lasiogyne*). Small spherical orbicules on the locule wall (SEM). Figs 47–50. *D. sylvatica* (sect. *Testudinaria*). Fig. 47. Orbicules with one or two notches (arrows) (SEM). Detail of 47 (inset) (SEM). Fig. 48. Section showing part of the intine, beneath an aperture and orbicules with one or two notches (TEM). Fig. 50. Detail of 49. Orbicules with one or two notches (arrows). Figs 51, 52. *D. karatana* (sect. *Campanuliflorae*). Fig. 51. Spiny orbicules (SEM). Fig. 52. Section through spiny orbicules (arrows) (TEM).

by N'Kounkou (1994). Both sections (which are right-twining) have exclusively bisulcate and perforate pollen with small perforations and a high perforation density (5–8 μm^{-2} in sect. *Asterotricha* and more than 8 perforations μm^{-2} in sect. *Enantiophyllum*). Orbicules always have a smooth surface.

D. decipiens, traditionally accepted as a member of section *Enantiophyllum*, deviates by having a low perforation density and spinulose orbicules. Recent molecular data also suggest that *D. decipiens* may not be a member of section *Enantiophyllum* (P. Wilkin, pers. comm.).

Sections *Stenophora* and *Cryptantha*

The three species studied from section *Stenophora* have very different pollen ornamentation: *D. membranacea* and *D. collettii* have perforate sexines with large perforations, while *D. althaeoides* has a striate sexine (with perforations between the striae). This is rather surprising because it is the only Asian species in this survey with striate pollen, while its relatives in section *Stenophora* (*D. membranacea* and *D. collettii*) are perforate. *D. tenuipes* and *D. poilanei*, two section *Stenophora* species surveyed by Su (1987) are also perforate.

All three species, however, are mainly monosulcate; in *D. althaeoides* and *D. collettii* a low percentage of bisulcate pollen grains was found. *D. tenuipes* and *D. poilanei* have been described as exclusively monosulcate (Su, 1987).

Pollen and orbicules of *D. althaeoides* and *D. catharinensis* (section *Cryptantha*) are very similar (Figs 14, 15). Both species have a perforations between the striae, which may indicate some degree of plasticity in the development of the sexine ornamentation; this requires further work. These observations support the hypothesis of Ayensu (1972) that sections *Cryptantha* and *Macropoda* (which was merged in *Stenophora* by Burkill, 1960) are closely related based on their vascular anatomy. Perforate pollen was reported in *D. regnelli* of section *Cryptantha* (Barroso *et al.*, 1974). Both sections obviously possess striate and perforate pollen. The striate pattern in *D. althaeoides* could be an example of convergent evolution implying that a striate sexine has independent Asian, Malagasy and South American origins. Further research on the distribution and ultrastructure of striate pollen is needed to establish the homology of this character state.

Sections *Combilium* and *Macrocarpaea*

A relationship between these sections was proposed by Ayensu (1972). They both have bisulcate pollen with small perforations (*c.* 20 μm diameter) and a rather low perforation density (less than 5 μm^{-2}) (Figs 21, 22). Both sections have T-shaped hairs (Knuth, 1924).

Genus *Tamus*

Tamus has been recognized as a separate genus within *Dioscoreaceae*, placed close to *Dioscorea* and *Rajania* (Dahlgren *et al.*, 1985). Recent cladistic analyses based on morphological and molecular data suggest that *Tamus* is nested within *Dioscorea* making the latter genus paraphyletic (Caddick *et al.*, 2000). This is supported by palynological data (Clarke & Jones, 1981; Caddick *et al.*, 1998; this paper). The pollen morphology of *Tamus communis* does not differ significantly from the *Dioscorea* species sampled in this survey. We have, however, no observations from *Tamus orientalis*, another member of this small genus.

CONCLUSIONS

Palynological characters are 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 limited pollen data, which also provide some support for more recent hypotheses of relationship based on cladistic analysis of morphological and molecular data:

(1) The macromorphological distinction between the compound leaved sections *Lasiophyton* and *Botryosicyos* is supported by differences in pollen sexine ornamentation.
(2) Pollen morphology indicates support for the close affinity between sections *Asterotricha* and *Enantiophyllum*, and sections *Combilium* and *Macrocarpaea*.
(3) The pollen of *Tamus communis* falls within the range of pollen morphological variation of *Dioscorea*.

The pollen characters examined here will be of value in future combined analyses with other morphological and molecular data, which will also shed light on pollen evolution within *Dioscorea*. A pollen morphological study of more than 60 additional *Dioscorea* species is in progress. Developmental studies of pollen apertures, for example, are also in progress to investigate the homologies of character states discussed in this paper. Additionally, both these morphological and developmental studies are being expanded to include Taccaceae, a family which is closely related to Dioscoreaceae (Caddick *et al.*, 1998, 2000).

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APPENDIX

Section	Species	Collector and number	Country
Apodostemon Uline	*D. megacarpa* Gleason	K: Forest Dept. of Brit. Guyana 4284	Guyana
Asterotricha Uline	*D. schimperiana* Hochst.	K: Wilkin & Tawakai 763	Malawi
	D. schimperiana Hochst.*	HK: 1995-1458	
	D. hirtiflora Pax	K: White 6302	Zambia
Botryosicyos (Hochst.) Uline	*D. pentaphylla* L.	K: Boulanger 1109	Thailand
	D. quartiniana Rich.	K: Archbold 1087	Tanzania
Brachyandra Uline	*D. hexagona* Baker	K: Wilkin *et al.* M960	Madagascar
Campanuliflorae Burkill & H. Perr.	*D. karatana* Wilkin	K: Wilkin *et al.* M947	Madagascar
	D. karatana Wilkin*	HK: 1998-0515	
	D. maciba Jum. & H. Perr.	K: Wilkin *et al.* M964	Madagascar
Cardiocapsa Uline	*D. bemarivensis* Jum. & H. Perr.	K: Phillipson 3027	Madagascar
Chirophyllum Uline	*D. brachybotrya* Poepp.	K: Comber 462	Chile
Chondrocarpa Uline	*D. riparia* Knuth & Schomb.	K: Smith 3485	Guyana
Combilium Prain & Burkill	*D. esculenta* Burkill	K: Beguin 2093	Mollucas
Cryptantha Uline	*D. catharinesis* R. Knuth	K: Dusen 7856	Brazil
Dematostemon Griseb.	*D. adenocarpa* Mart.	K: Heringer *et al.* 5101	Brazil
Enantiophyllum Uline	*D. decipiens* Hook. f.	K: Wilkin 860	Thailand
	D. glabra Roxb.	K: Wilkin 892	Thailand
	D. hamiltonii Hook. f.	K. Wilkin *et al.* 886	Thailand
	D. praehensilis Benth.	K: Pawek 7980	Malawi
Lasiogyne Uline	*D. dodecaneura* Vell.	K: Tessmann 6113	Brazil
Lasiophyton Uline	*D. dumetorum* (Kunth) Pax	K: Wilkin & Tawakali 786	Malawi
	D. dumetorum (Kunth) Pax*	HK: 1995-1455	
	D. dregeana (Kunth) Pax	BR: Schlieben 7601	S. Africa
Macrocarpaea Uline	*D. preussii* Pax	K: Pilz 1801	Nigeria
Macrogynodium Uline	*D. trifida* L. f.	K: Philcox & Freeman 4668	Brazil
Madagascarienses Burkill & H. Perr.	*D. arcuatinervis* Hochr.	K: Caddick *et al.* 309	Madagascar
Monadelpha Uline	*D. lagoa-santa* Uline ex R. Knuth	K: Seidel & Carrizole 7825	Bolivia
Opsophyton Uline	*D. bulbifera* L.	K: Wilkin 864	Thailand
Rhacodophyllum Uline ex R. Knuth	*D. buchananii* Benth.	K: Biegel 2893	Zimbabwe
Sarcantha Uline	*D. amazonum* Mart. ex Griseb.	K: Maas *et al.* 2679	Guyana
Sarcocapsa Uline	*D. oaxacensis* Uline	K: Solinas 6182	Mexico
Spaerantha Uline	*D. multiflora* Mart.	K: Krapovickas 35458	Brazil
Stenocorea Prain & Burkill	*D. daunea* Prain & Burkill	K: Middleton & Parnell 1468	Thailand
Stenophora R. Knuth	*D. althaeoides* R. Knuth	K: ACE 493	China
	D. collettii Hook. f.	K: ACE 382	China
	D. membranacea Pierre	K: Middleton & Parnell 1468	Thailand
Testudinaria Uline	*D. sylvatica* Eckl.	K: Chase 7939	Zimbabwe
	D. sylvatica Eckl.*	HK: 1994-796	

* Specimens examined with TEM.