



Evolution of pollen, stigmas and ovule numbers at the caesalpinioïd–mimosoid interface (Fabaceae)

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In this study we examine the pollen, stigmas and ovaries from 62 collections of herbarium material representing 16 genera, using light and scanning electron microscopy. The caesalpinioïd *Dimorphandra* group (*Burkea*, *Dimorphandra*, *Erythrophleum*, *Mora*, *Pachyelasma*, *Stachyothyrsus* and *Sympetalandra*) pollen grains are small, tricolporate monads, with perforate or psilate ornamentation. *Dinizia*, *Pentaclethra* and *Aubrevillea* have morphological characters that have suggested either a mimosoid or caesalpinioïd placement. *Dinizia* pollen is in permanent tetrads with clavate ornamentation. *Pentaclethra* pollen grains are monads, two species have tricolporate pollen and the third is porate. *Aubrevillea* has tricolporate, finely reticulate monads. All ten genera have variable, non-predictable stigma type and ovule number. The mimosoid *Adenanthera* group (*Adenanthera*, *Tetrapleura*, *Amblygonocarpus*, *Pseudoprosopis*, *Calpocalyx* and *Xylia*) pollen grains are in 8- to 16-grain polyads. In all *Adenanthera* group species, the stigmatic cavity is only large enough to accommodate one polyad. In addition, the number of ovules present matches the number of pollen units in one polyad. Polyads have porate, operculate apertures that differ in layout, aperture morphology and development when compared with caesalpinioïd and other eudicot pollen. © 2010 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2010, **162**, 594–615.

ADDITIONAL KEYWORDS: apertures – Caesalpinioideae – Leguminosae – Mimosoideae – monad – phylogeny – pollen development – polyad – reproductive syndrome.

INTRODUCTION

In Fabaceae, at the caesalpinioïd–mimosoid boundary, relationships that include subfamily Mimosoideae and most Cassieae and Caesalpinieae subgroups are not fully resolved in the molecular phylogenetic analyses published to date (Bruneau *et al.*, 2008). Phylogenetic studies so far indicate that some members of the *Dimorphandra* group (based on *Dimorphandra* Schott) of Caesalpinieae (e.g. *Pachyelasma* Harms, *Erythrophleum* Afzel. ex G. Don; Bruneau *et al.*, 2008) comprise the sister group to Mimosoideae, although relationships of lineages near the ‘base’ of the mimosoid clade are still poorly resolved and the *Dimorphandra* group resolves into two clades in the molecular phylogeny of Bruneau *et al.* (2008). Pollen is of

great interest in this group, as it is mostly released in monads (or rarely tetrads, Ferguson & Banks, 1994) in caesalpinioïds (Graham & Barker, 1981; Banks & Gasson, 2000; Banks & Klitgaard, 2000; Banks *et al.*, 2003), whereas mostly polyads are found in mimosoid taxa (Guinet, 1981; Guinet & Ferguson, 1989). This study was carried out to investigate the pollen, stigmas and number of ovules per ovary of taxa in the *Dimorphandra* group and first branching members of Mimosoideae, both to provide characters for phylogenetic analyses and to investigate the putative evolution of compound pollen from monads.

MATERIAL AND METHODS

Sixty-two samples from 42 collections of the 99 species in 16 genera (Table 1) were examined using light microscopy (LM) and scanning electron microscopy (SEM). The taxonomy used is that of Lewis (2005) based on the earlier studies of Polhill & Vidal (1981) and Polhill (1994). Although there is a more

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GPL and HB usually publish under the family name Leguminosae, but are here following journal style in following APG III (2009) family names.

Table 1. Samples examined

<i>Adenanthera abrosperma</i> F.Muell.	Australia	Kenneally 9362
<i>Adenanthera aglaosperma</i> Alston	Sri Lanka	Meijer 457
<i>Adenanthera novo-guineensis</i> Baker f.	New Guinea	Darbyshire 665
<i>Adenanthera pavonina</i> L. Examined for pollen only	China	Liang 62942
<i>Adenanthera pavonina</i> L.	Brazil	Sapam & Hage 2183
<i>Amblygonocarpus andongensis</i> (Welw. ex Oliv.) Exell & Torre Examined for pollen only	Central African Republic	Linder 3463
<i>Amblygonocarpus andongensis</i> (Welw. ex Oliv.) Exell & Torre	Tanzania	Vollesen MRC 2913
<i>Aubrevillea kerstingii</i> (Harms) Pellegr.	Ghana	Enti FE 1271
<i>Burkea africana</i> Hook.	Zimbabwe	Eyles 859
<i>Burkea africana</i> Hook. Examined for pollen only	Tanzania	Greenway & Kanuri 14628
<i>Calpocalyx brevibracteatus</i> Harms	Liberia	Baldwin 10255
<i>Calpocalyx dinklagei</i> Harms	Cameroon	Thomas 393
<i>Dimorphandra conjugata</i> Sandwith Examined for pollen only	Guyana	Breteler 13800
<i>Dimorphandra cuprea</i> Sprague & Sandwith Examined for pollen only	Guyana	Sprague & Sandwith 1431
<i>Dimorphandra cuprea</i> subsp. <i>cuprea</i>	Guyana	Maguire & Fanshawe 32223
<i>Dimorphandra davisii</i> Sprague & Sandwith	Guyana	Redden 3202
<i>Dimorphandra exaltata</i> Schott	Brazil	Ducke 1869
<i>Dimorphandra gardneriana</i> Tul. Examined for pollen only	Brazil	da Silva <i>et al.</i> 3515
<i>Dimorphandra jorgei</i> M.Freitas da Silva Examined for pollen only	Brazil	Hage 2179
<i>Dimorphandra pennigera</i> Tul. Examined for pollen only	Brazil	Calderon <i>et al.</i> 2712
<i>Dimorphandra mollis</i> Benth.	Brazil	Heringer <i>et al.</i> 2675
<i>Dimorphandra unijuga</i> Tul.	Brazil	Ducke 23967
<i>Dimorphandra vernicosa</i> Spruce ex Benth. & Hook. f.	Brazil	Philcox <i>et al.</i> 4839
<i>Dinizia excelsa</i> Ducke Examined for pollen only	Brazil	Ducke 975
<i>Dinizia excelsa</i> Ducke	Guyana	Jansen-Jacobs <i>et al.</i> 1900
<i>Dinizia excelsa</i> Ducke Examined for pollen only	Guyana	Barneby 1990
<i>Erythrophleum africanum</i> (Benth.) Harms Examined for pollen only	Tanzania	Gillman 1091
<i>Erythrophleum africanum</i> (Benth.) Harms	Angola	Barbosa 10789
<i>Erythrophleum chlorostachys</i> (F.Muell.) Baill. Examined for pollen only	Australia	Evans 3421
<i>Erythrophleum chlorostachys</i> (F.Muell.) Baill. Examined for pollen only	Australia	Must 768
<i>Erythrophleum couminga</i> Baill.	Madagascar	Capuron 24230 SF
<i>Erythrophleum fordii</i> Oliv.	Indo-China	Tsang 29048
<i>Erythrophleum suaveolens</i> (Guill. & Perr.) Brenan	Guinea	Langdale-Brown 2614

Table 1. *Continued*

<i>Erythrophleum suaveolens</i> (Guill. & Perr.) Brenan Examined for pollen only	Central African Republic	Fay <i>et al.</i> <i>s.n.</i>
<i>Mora ekmanii</i> Britton & Rose	Dominican Republic	Ekman H11201
<i>Mora excelsa</i> Benth. Examined for pollen only	Trinidad	Broadway 5042
<i>Mora excelsa</i> Benth. Examined for pollen only	Guyana	Jenman 2006
<i>Mora gonggripii</i> Sandwith	Guyana	<i>s.n.</i> D570/2611
<i>Mora paraensis</i> Ducke Examined for pollen only	Brazil	Ducke 23965
<i>Pachyelasma tessmannii</i> (Harms) Harms	Zaire	Kole 1464
<i>Pachyelasma tessmannii</i> (Harms) Harms Examined for pollen only	Zaire	Hart 361
<i>Pachyelasma tessmannii</i> (Harms) Harms Examined for pollen only	Zaire	Hart 1464
<i>Pentaclethra eetveldeana</i> De Wild. & T.Durand Examined for pollen only	Cameroon	Letouzey 11747
<i>Pentaclethra macrophylla</i> Benth. Examined for pollen only	Congo	Léonard 993
<i>Pentaclethra macroloba</i> Kuntze	Brazil	Pires & da Silva 1782
<i>Pseudoprosopis bampsiana</i> Lisowski	Sierra Leone	Morton SL317
<i>Pseudoprosopis euryphylla</i> Harms	Tanzania	Lock & Fison 88/83
<i>Stachyothyrsus staudtii</i> Harms Examined for pollen only	Equatorial Guinea	Tessmann 270
<i>Stachyothyrsus staudtii</i> Harms	Cameroon	Zenker 4500
<i>Stachyothyrsus stapfiana</i> (A.Chev.) J.Léonard & Voorh. Examined for pollen only	Sierra Leone	Jordan 2072
<i>Sympetalandra borneensis</i> Stapf Examined for pollen only	Borneo	Sam 36730(a)
<i>Sympetalandra borneensis</i> Stapf Examined for pollen only	Sarawak	Ilias <i>et al.</i> S.34154
<i>Sympetalandra densiflora</i> (Elm.) Steenis Examined for pollen only	Philippines	Sulit <i>s.n.</i> Philippines Nat. Herb. 22896
<i>Sympetalandra densiflora</i> (Elm.) Steenis Examined for pollen only	Philippines	Sulit <i>s.n.</i> Philippines Nat. Herb 14499
<i>Sympetalandra unijuga</i> (Shaw) Steenis	Malaysia, North Borneo	Muliadi A808
<i>Tetrapleura tetraptera</i> Taub. Examined for pollen only	Belgium Congo	Donis 2809
<i>Tetrapleura tetraptera</i> Taub.	Cameroon	Leeuwenberg 8976
<i>Xylia hoffmannii</i> Drake Examined for pollen only	Madagascar	Antilaminena <i>et al.</i> 326
<i>Xylia hoffmannii</i> Drake	Madagascar	Capuron 24438 SF
<i>Xylia xylocarpa</i> var. <i>kerrii</i> (Craib & Hutch.) I.C.Nielsen	Myanmar	Lace 6110
<i>Xylia torreana</i> Brenan	Zimbabwe	Eccles <i>s.n.</i>

recent molecular phylogenetic analysis that includes a number of additional genera in an expanded, but not monophyletic, *Dimorphandra* group (Bruneau *et al.*, 2008), not all the genera in the more traditional *Dimorphandra* group were sampled in that study.

Pollen material was obtained from the herbarium of the Royal Botanic Gardens, Kew (K).

For pollen studies, mature, unopened buds from herbarium specimens were used. For the study of unprocessed pollen, anthers were dissected out of the dry buds and mounted onto SEM stubs using double-sided sticky tape. These were then opened using fine needles, sputter coated with platinum and examined using a Hitachi S4700 cold field emission SEM at 2 kV. To prepare pollen using the acetolysis method, mature unopened buds were taken from herbarium specimens and dissected in a 1% solution of Libsorb wetting agent. Pollen was acetolysed according to Erdtman (1960) and prepared for LM by mounting in glycerol jelly. At least 10 grains of each sample were measured using a Nikon Labophot light microscope with a $\times 100$ oil immersion lens. Light micrographs were taken using a Leica DMLB microscope with an Axiocam digital camera. For SEM, acetolysed pollen exines in 95% ethanol were pipetted onto specimen stubs and allowed to air dry. Specimens were sputter coated with platinum and examined using a Hitachi S-2400 SEM at 18 kv or a Hitachi S4700 cold field emission SEM at 2 kv.

Pollen samples are databased and LM slides are available for reference at the Jodrell Laboratory, Royal Botanic Gardens, Kew. Palynological terminology follows Punt *et al.* (1994).

Floral morphology was examined using LM and SEM. For genera containing one, two or three species, sample selection was based mainly on the quality of material available. For more species-rich genera, sampling was either based on geography, with the aim to sample these genera across their entire range or, in the case of *Dimorphandra*, sampling followed the division of the genus into three subgenera in a revision by Silva (1986); two species from each subgenus were sampled.

Herbarium samples were rehydrated in a 1% solution of Libsorb for 16–24 h. They were then examined under a light microscope. Flowers were dissected and ovaries were dissected to determine the number of ovules. For the spirit collection sample, the process was identical except that the rehydration in Libsorb was omitted. Following dissection, samples were taken through an ethanol series and critical point dried in an Autosamdri-815B CPD (Tousimis Research Corporation, USA). Specimens were then sputter coated with platinum and examined using a Hitachi S4700 cold field emission SEM at 2 kv. Floral terminology follows Lewis & Elias (1981).

RESULTS

DIMORPHANDRA (11 COLLECTIONS EXAMINED FROM EIGHT OUT OF 26 SPECIES)

Stigma size (sessile on ovary, style lacking) 2 mm long (Fig. 1). Number of ovules (4–) 13.2 (–24) (from examining 110 ovaries) (Fig. 2). Stigmatic pore 50–130 μm in diameter (Fig. 3). Pollen released as tricolporate zonocolporate monads with psilate to psilate–perforate surface ornamentation (Fig. 4). $P \times E = (21-)$ 23.3 (–32) \times (21–) 29.8 (–38) μm . (For further pollen descriptions, measurements and images, see Banks & Lewis, 2009.)

ERYTHROPHLEUM (EIGHT COLLECTIONS EXAMINED FROM FIVE OUT OF 10 SPECIES)

Stigmatic cylinder/pore 50–100 μm in diameter (Fig. 6). Number of ovules (6–) 8.0 (–11) (from examining 83 ovaries) (Fig. 5). Pollen released as isopolar, tricolporate zonocolporate monads with psilate–finely perforate or perforate surface ornamentation (Fig. 6). $P \times E = (18-)$ 25.0 (–36) \times (18–) 24.8 (–32) μm . (For further pollen descriptions and images, see Banks & Lewis, 2009.)

MORA SCHOMB. EX BENTH. (FIVE COLLECTIONS EXAMINED FROM FOUR OUT OF SIX SPECIES)

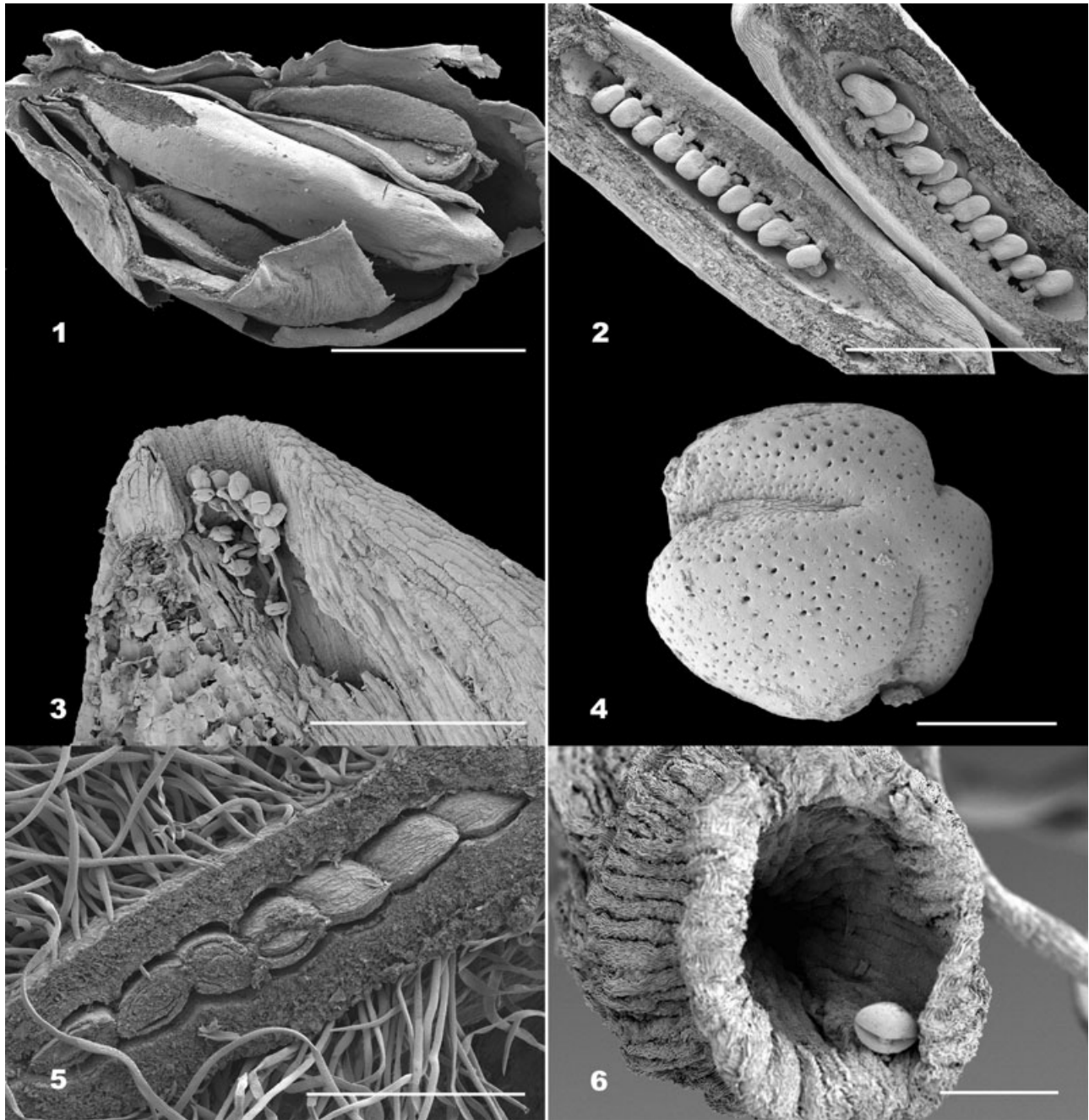
Stigmatic pore ~ 20 μm in diameter (Fig. 7). Number of ovules (2–) 3 (–4) (from examining 24 ovaries). Pollen released as isopolar, tricolporate zonocolporate monads with psilate to psilate–perforate surface ornamentation. $P \times E = (39-)$ 43.3 (–48) \times (31–) 44.7 (–59) μm . (For further pollen descriptions, measurements and images, see Banks & Lewis, 2009.)

PACHYELASMA (THREE COLLECTIONS EXAMINED OF THE SINGLE SPECIES)

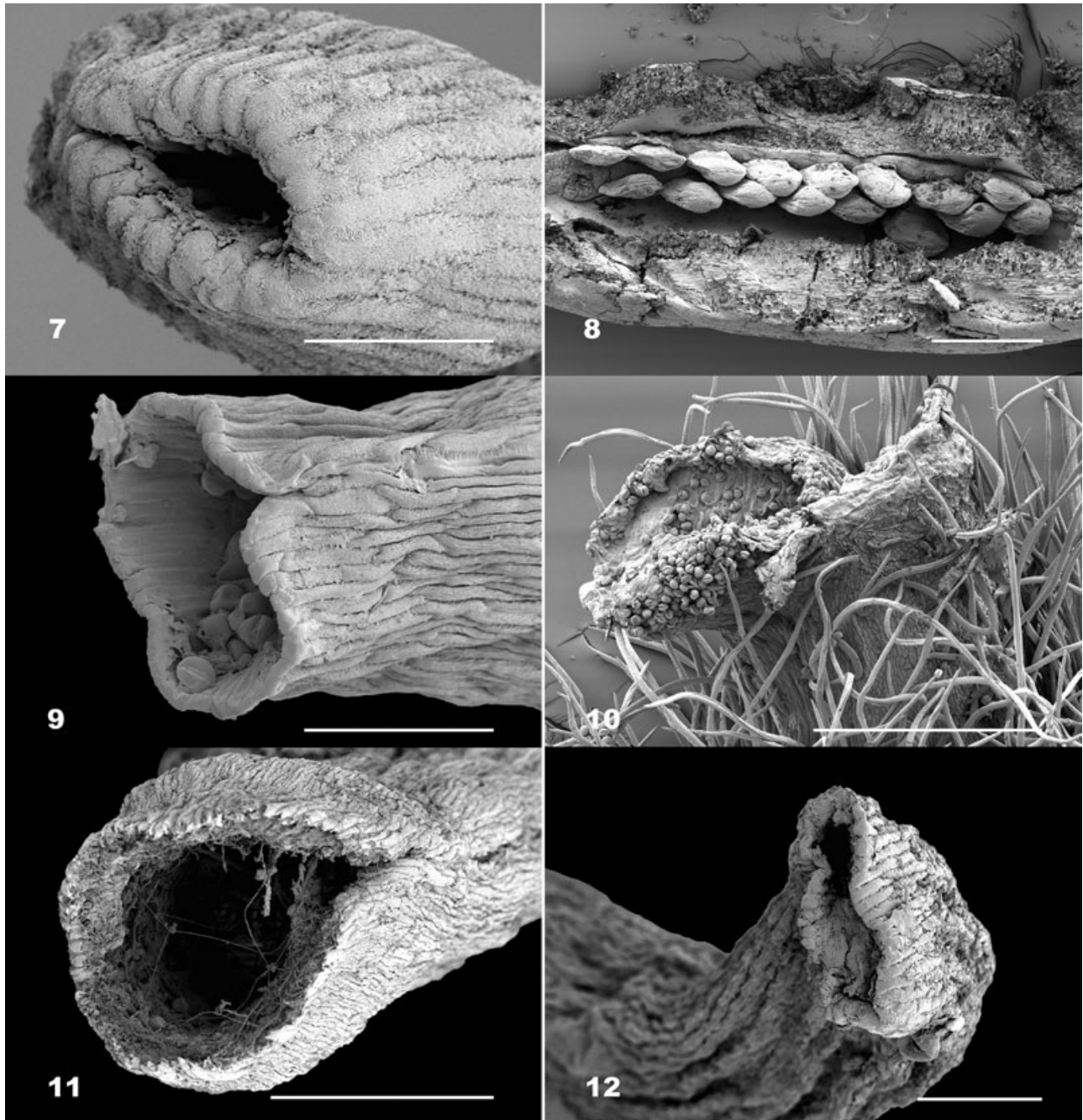
Stigmatic cylinder ~ 100 μm in diameter (Fig. 9). Number of ovules (18–) 18.5 (–19) (from examining 13 ovaries) (Fig. 8). Pollen released as isopolar, tricolporate zonocolporate monads with perforate–finely rugulate surface ornamentation. $P \times E = (26-)$ 27.2 (–29) \times (24–) 26.3 (–28) μm . (For further pollen descriptions, measurements and images, see Banks & Lewis, 2009.)

BURKEA BENTH. (TWO COLLECTIONS EXAMINED OF THE SINGLE SPECIES)

Stigmatic funnel ≥ 500 μm in diameter (Fig. 10). Number of ovules (1–) 1.6 (–2) (from examining 15 ovaries). Pollen released as isopolar, tricolporate zonocolporate monads with psilate to finely granular surface ornamentation. $P \times E = (19-)$ 21.5 (–23) \times (19–)



Figures 1–6. *Dimorphandra* and *Erythrophleum*. Fig. 1. *Dimorphandra mollis*, half flower. Scale bar, 1 mm. Fig. 2. *Dimorphandra mollis*, ovary cut into two, with 21 ovules clearly visible. Scale bar, 1 mm. Fig. 3. *Dimorphandra mollis* stigma bisected, showing germinated pollen with pollen tubes within the stigmatic area. Scale bar, 200 μ m. Fig. 4. *Dimorphandra davisii* pollen. Scale bar, 10 μ m. Fig. 5. *Erythrophleum couminga*, ovary bisected to show seven ovules. Scale bar, 400 μ m. Fig. 6. *Erythrophleum africanum*, close-up of stigma showing one pollen grain within the stigmatic cavity. Scale bar, 50 μ m.



Figures 7–12. *Mora*, *Pachyelasma*, *Burkea*. Fig. 7. *Mora gonggrijpii*, close-up of stigma. Scale bar, 50 μm . Fig. 8. *Pachyelasma tessmannii*, half an ovary with 16 ovules visible. Scale bar, 500 μm . Fig. 9. *Pachyelasma tessmannii*, stigma with at least 11 pollen grains visible within the stigmatic cavity. Scale bar, 100 μm . Fig. 10. *Burkea africana*, stigma with numerous pollen grains visible in contact with stigmatic surface. Scale bar, 400 μm . Fig. 11. *Stachyothyrsus staudtii*, close-up of stigma with a pollen grain visible within the stigmatic cavity. Scale bar, 300 μm . Fig. 12. *Sympetalandra unijuga*, close-up of stigma with a pollen grain visible on the ‘underside’ edge of the stigmatic opening. Scale bar, 100 μm .

23.3 (–25) μm . (For complete pollen description, measurements and images, see Banks & Lewis, 2009.)

STACHYOTHRYSUS HARMS (THREE COLLECTIONS EXAMINED FROM TWO OUT OF TWO SPECIES)

Stigmatic cylinder ~400 μm in diameter (Fig. 11). Number of ovules 2 (from examining 13 ovaries). Pollen released as isopolar, tricolporate zonocolporate monads with perforate to reticulate surface ornamentation. $P \times E = (22\text{--}) 26.7 (–34) \times (19\text{--}) 26.6 (–30) \mu\text{m}$. (For complete pollen description and images, see Banks & Lewis, 2009.)

SYMPETALANDRA STAPP (FIVE COLLECTIONS EXAMINED FROM THREE OUT OF FIVE SPECIES)

Stigmatic cylinder 50–100 μm in diameter (Fig. 12). Number of ovules (4–) 4.1 (5) (from examining 14 ovaries). Pollen released as isopolar, tricolporate zonocolporate monads with psilate to psilate–finely perforate surface ornamentation. $P \times E = (31\text{--}) 41.6 (–52) \times (36\text{--}) 42.5 (–49) \mu\text{m}$. (For further pollen descriptions, measurements and images, see Banks & Lewis, 2009.)

DINIZIA DUCKE (THREE COLLECTIONS EXAMINED OF THE SINGLE SPECIES)

Stigmatic funnel 200–250 μm in diameter (Fig. 13). Number of ovules (7–) 9.3 (–10) (from examining 20 ovaries). Pollen released as acalymmate tetrads with semitectate, clavate surface ornamentation, with larger clavae in mesocolpial areas and smaller, more densely packed clavae around aperture margins and polar areas (Fig. 14). Pollen diameter (63–) 73.9 (–86) μm .

PENTACLETHRA BENTH. (THREE COLLECTIONS EXAMINED, ONE OF EACH SPECIES)

Stigmatic cylinder 150–200 μm in diameter (Fig. 15). Number of ovules 10 (from examining 10 ovaries). Pollen released as isopolar, tricolporate or triporate (*P. macrophylla*) zonocolporate monads with psilate to psilate–finely perforate surface ornamentation. $P \times E = (39\text{--}) 41.6 (–45) \times (51\text{--}) 55.6 (–58) \mu\text{m}$ (Figs 15, 16).

AUBREVILLEA PELLEGR. (ONE COLLECTION EXAMINED OF ONE OUT OF TWO SPECIES)

Stigmatic funnel/cylinder 150 μm in diameter (Figs 17, 19). Number of ovules (2–) 2.5 (–4) (from examining 22 ovaries). Pollen released as isopolar, tricolporate zonocolporate monads with perforate

surface ornamentation. $P \times E = (26\text{--}) 27.3 (–30) \times (28\text{--}) 29.3 (–31) \mu\text{m}$ (Fig. 18).

ADENANTHERA L. (FIVE COLLECTIONS EXAMINED OF FOUR OUT OF 13 SPECIES)

Stigmatic cylinder/pore ~30–50 μm in diameter (Fig. 20). Number of ovules (11–) 13.8 (–17) (from examining 49 ovaries). Pollen released as polyads with (14–)16 pollen grain units per polyad. Polyads vary in shape and size. Individual pollen units within the polyad vary in shape and size (Fig. 21). Pollen diameter (25–) 40.9 (–70) μm . Apertures (3–) 4 porate, operculate, with thickened margins of adjacent exine. Surface ornamentation psilate–microperforate to finely rugulate.

TETRAPLEURA BENTH. (TWO COLLECTIONS EXAMINED, ONE OF EACH SPECIES)

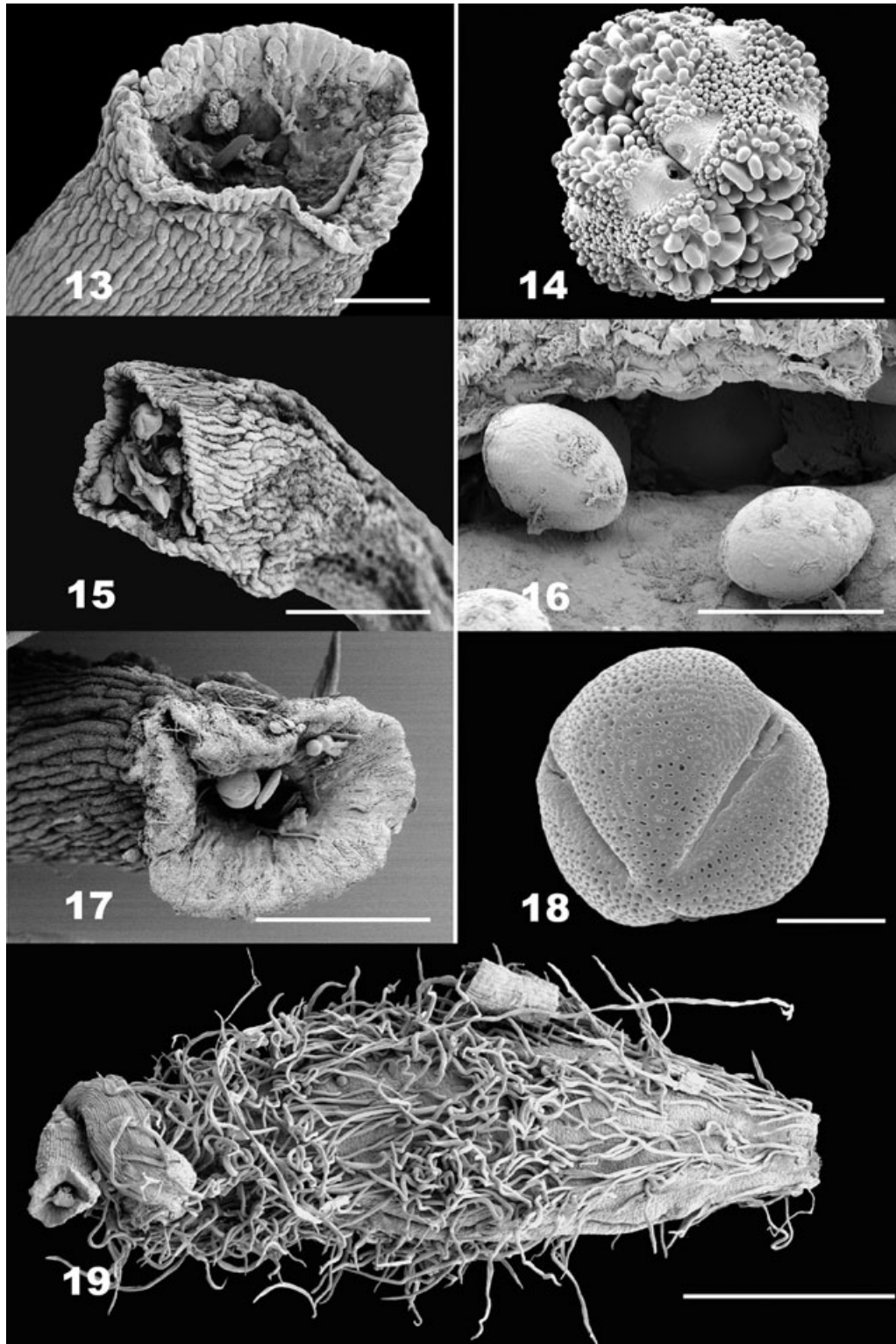
Stigmatic cylinder/pore ~40–60 μm in diameter (Fig. 22). Number of ovules (14–)15.5 (–17) (from examining 18 ovaries) (Fig. 24). Pollen released as calymmate polyads with (\pm) 16 pollen grain units per polyad. Polyads vary in shape and size (45–) 53.4 (–60) μm . Individual pollen units within the polyad vary in shape and size (Fig. 23). Apertures (3–) 4 porate, operculate, with thickened margins of adjacent exine (Figs 23, 25). Surface ornamentation psilate–microperforate to finely rugulate (Fig. 25).

AMBLYGONOCARPUS HARMS (TWO COLLECTIONS EXAMINED OF THE SINGLE SPECIES)

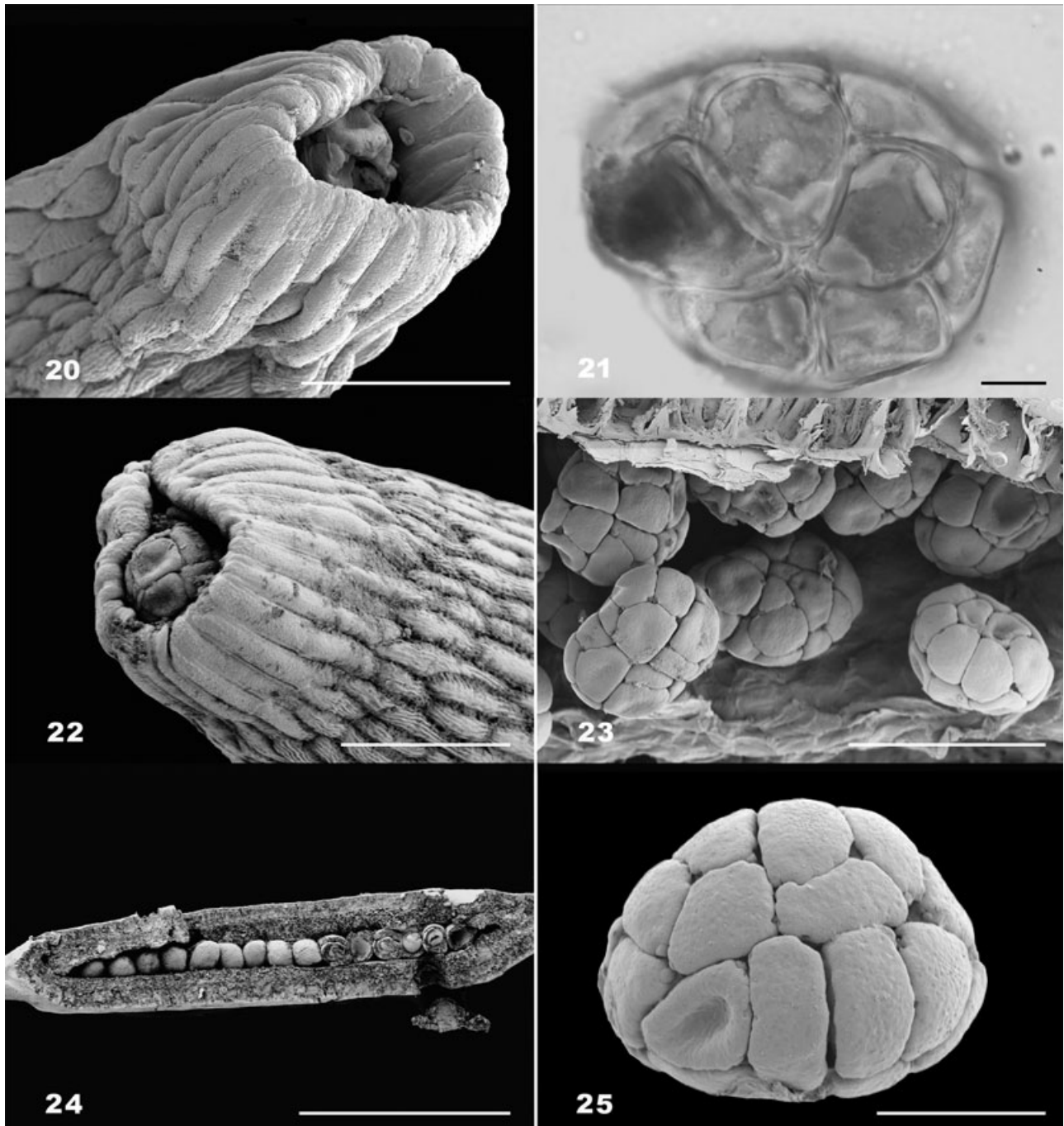
Stigmatic cylinder ~80–100 μm in diameter (Figs 26, 28). Number of ovules (11–) 12.4 (–13) (from examining 15 ovaries). Pollen released as calymmate polyads with (11–) 12 (–16) pollen grain units per polyad (Fig. 27). Polyads vary in shape and size (59–) 62.8 (–70) μm (Fig. 27). Individual pollen units within the polyad vary in shape and size (Fig. 27). Apertures (3–) 4 porate, operculate (Fig. 29). Surface ornamentation finely rugulate to rugulate (Figs 27, 29).

PSEUDOPROSOPIS HARMS (TWO COLLECTIONS EXAMINED FROM TWO OUT OF SEVEN SPECIES)

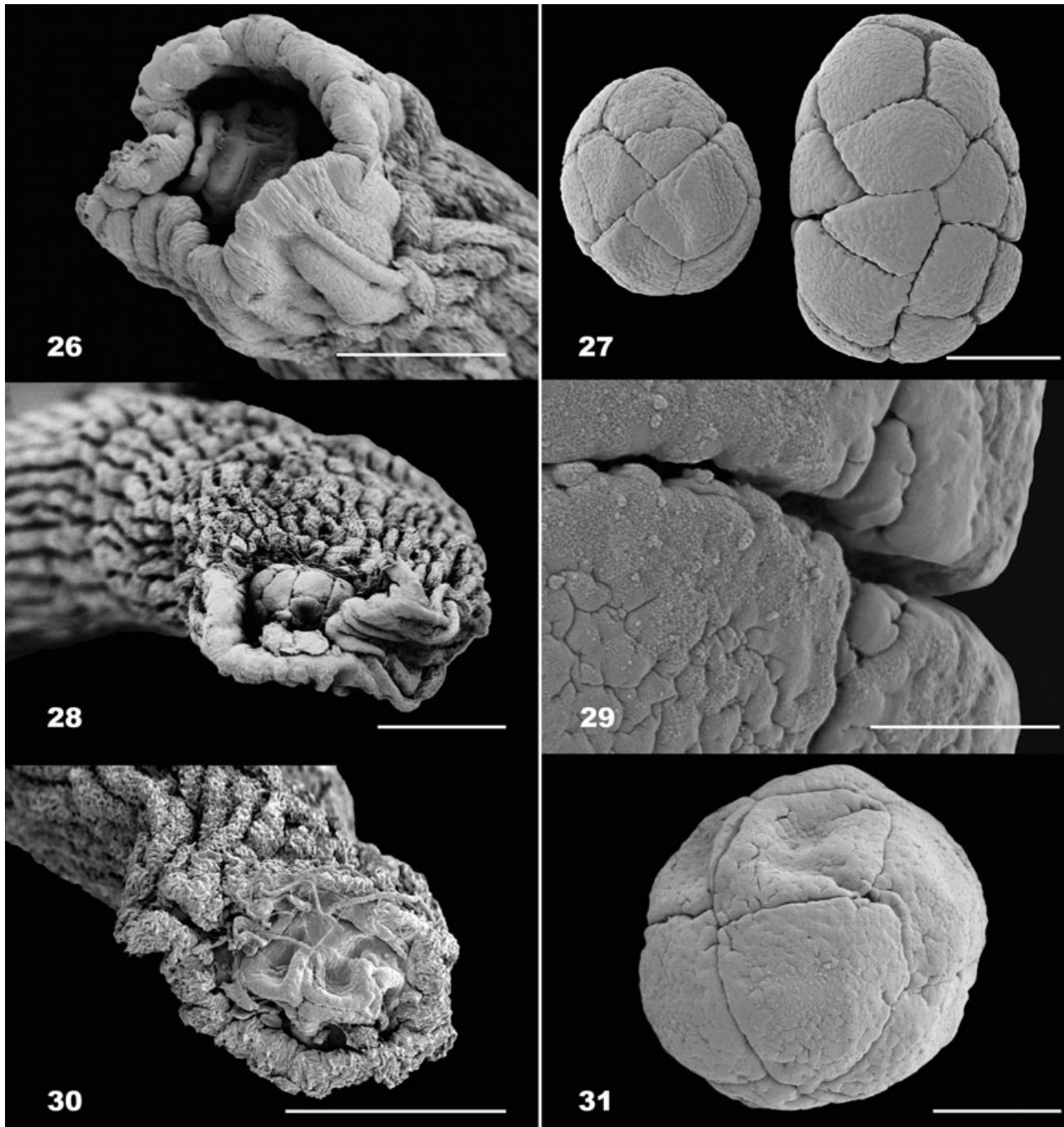
Stigmatic cylinder/pore ~30–50 μm in diameter (Fig. 30). Number of ovules (6–) 7.1 (–8) (*P. euryphylla* Harms, from examining 14 ovaries) or (8–) 9.9 (–11) (*P. bampsiana* Lisowski, from examining 24 ovaries). Pollen released as calymmate polyads with eight (*P. euryphylla*) or (8–) 12 (–16) (*P. bampsiana*) pollen grain units per polyad (Fig. 31). In *P. bampsiana*, most of the polyads in one sample had 12 pollen units, but one polyad of each of the following was seen;



Figures 13–19. *Dinizia*, *Pentaclethra* and *Aubrevillea*. Fig. 13. *Dinizia excelsa*, stigma with one pollen tetrad visible within the stigmatic cavity. Scale bar, 100 μ m. Fig. 14. *Dinizia excelsa*, pollen tetrad. Scale bar, 30 μ m. Fig. 15. *Pentaclethra macroloba*, stigma with pollen grains visible within the stigmatic cavity. Scale bar, 200 μ m. Fig. 16. *Pentaclethra macrophylla*, close-up of porate pollen grains within an anther. Scale bar, 40 μ m. Fig. 17. *Aubrevillea kerstingii*, close-up of stigma with a pollen grain visible within the stigmatic cavity. Scale bar, 100 μ m. Fig. 18. *Aubrevillea kerstingii*, pollen grain. Scale bar, 40 μ m. Fig. 19. *Aubrevillea kerstingii*, ovary, unfurled style and stigma. Scale bar, 500 μ m.



Figures 20–25. *Adenanthera* and *Tetrapleura*. Fig. 20. *Adenanthera novo-guineensis*, stigma with polyad present that entirely fills the stigmatic cavity. Scale bar, 50 μ m. Fig. 21. *Adenanthera pavonina*, stained polyad showing thin and thick areas on the surface of each pollen unit. Scale bar, 10 μ m. Fig. 22. *Tetrapleura tetraptera*, close-up of stigma with polyad within, filling the stigmatic cavity. Scale bar, 50 μ m. Fig. 23. *Tetrapleura tetraptera*, polyads within anther, showing variable size and shape of the polyads and also showing the variation in individual pollen units that make up each of the polyads. Scale bar, 50 μ m. Fig. 24. *Tetrapleura tetraptera*, dissected ovary showing 15 ovules. Scale bar, 1 mm. Fig. 25. *Tetrapleura tetraptera* single polyad, showing irregular shape of each pollen unit making up the polyad and also showing operculate pores at the ‘corners’ of each pollen unit. Scale bar, 20 μ m.



Figures 26–31. *Amblygonocarpus* and *Pseudoprosopis*. Fig. 26. *Amblygonocarpus andongensis*, stigma with polyad within stigmatic cavity. Scale bar, 50 μm . Fig. 27. *Amblygonocarpus andongensis*, two polyads showing variation in size and shape and also variation of size and shape of individual pollen units within each polyad. Scale bar, 40 μm . Fig. 28. *Amblygonocarpus andongensis*, stigma with polyad within stigmatic cavity. Scale bar, 50 μm . Fig. 29. *Amblygonocarpus andongensis*, close-up of polyad showing apertures covered by opercula. Scale bar, 5 μm . Fig. 30. *Pseudoprosopis bampsiana*, stigma showing polyad within the stigmatic cavity. Scale bar, 40 μm . Fig. 31. *Pseudoprosopis bampsiana*, polyad. Scale bar, 10 μm .

eight, 10, 11, 12, 13 and 16 pollen units per polyad. In one sample of *P. fischeri* Harms taken from the Kew LM slide reference collection (Burtts 384, Tanzania), 60% of polyads had 16 pollen units per polyad and 30% had 12 pollen units per polyad.

Polyads irregular in shape and variable in size. Diameter = (19–) 29.6 (–43) μm . Individual pollen units within the polyad vary in shape and size. Apertures (3–) 4 porate, operculate (Fig. 31). Surface ornamentation finely rugulate to rugulate (Fig. 31).

CALPOCALYX HARMS (TWO COLLECTIONS EXAMINED FROM TWO OUT OF 11 SPECIES)

Stigmatic pore ~30–50 μm in diameter (Fig. 32). Number of ovules (5–) 6.7 (–8) (from examining 19 ovaries). Pollen released as calymmate polyads with eight pollen grain units per polyad (Fig. 33). Polyads regular in shape with two sets of four grains joined together, not in the same orientation but ‘twisted’ so that each of the grains of one set of four grains overlap two adjacent grains. Polyad diameter (32–) 42.7 (–58) μm . Apertures 3-porate, operculate (Fig. 34). Surface ornamentation rugulate (Figs 33, 34).

XYLIA BENTH. (FOUR COLLECTIONS EXAMINED FROM THREE OUT OF NINE SPECIES)

Stigmatic pore ~20–100 μm in diameter (Fig. 36). Number of ovules (5–) 5.8 (–6) (*X. hoffmanni* Drake, from examining five ovaries) (Fig. 35). Number of ovules (10–) 10.9 (–12) (*X. xylocarpa* (Roxb.) W.Theob. var. *kerrii* (Craib & Hutch.) I.C.Nielsen, from examining 22 ovaries). Pollen released as calymmate polyads with eight pollen grain units per polyad (*X. hoffmannii*, Fig. 37) or 16 pollen grain units per polyad (*X. xylocarpa* var. *kerrii*). Polyads bisymmetrical in shape. In *X. hoffmannii*, two hemispherical sets of four grains are joined together to form a cylinder. The individual grains of one half of the polyad are never in the same orientation as the other half, but ‘twisted’ so that each of the grains of one set of four do not line up with the adjacent grains (Fig. 37). In *X. xylocarpa* var. *kerrii* the polyads comprise 16 grains and are arranged in a disk shape with four central grains and the others arranged around them. Polyad size: *X. hoffmannii*; long axis \times short axis = (45–) 47.8 (–50) \times (35–) 38.3 (–40) μm . *X. xylocarpa* var. *kerrii*; long axis = (75–) 90 (–100), short axis ~30 μm . Apertures 3-porate, operculate. Surface ornamentation finely rugulate (Fig. 37).

DISCUSSION

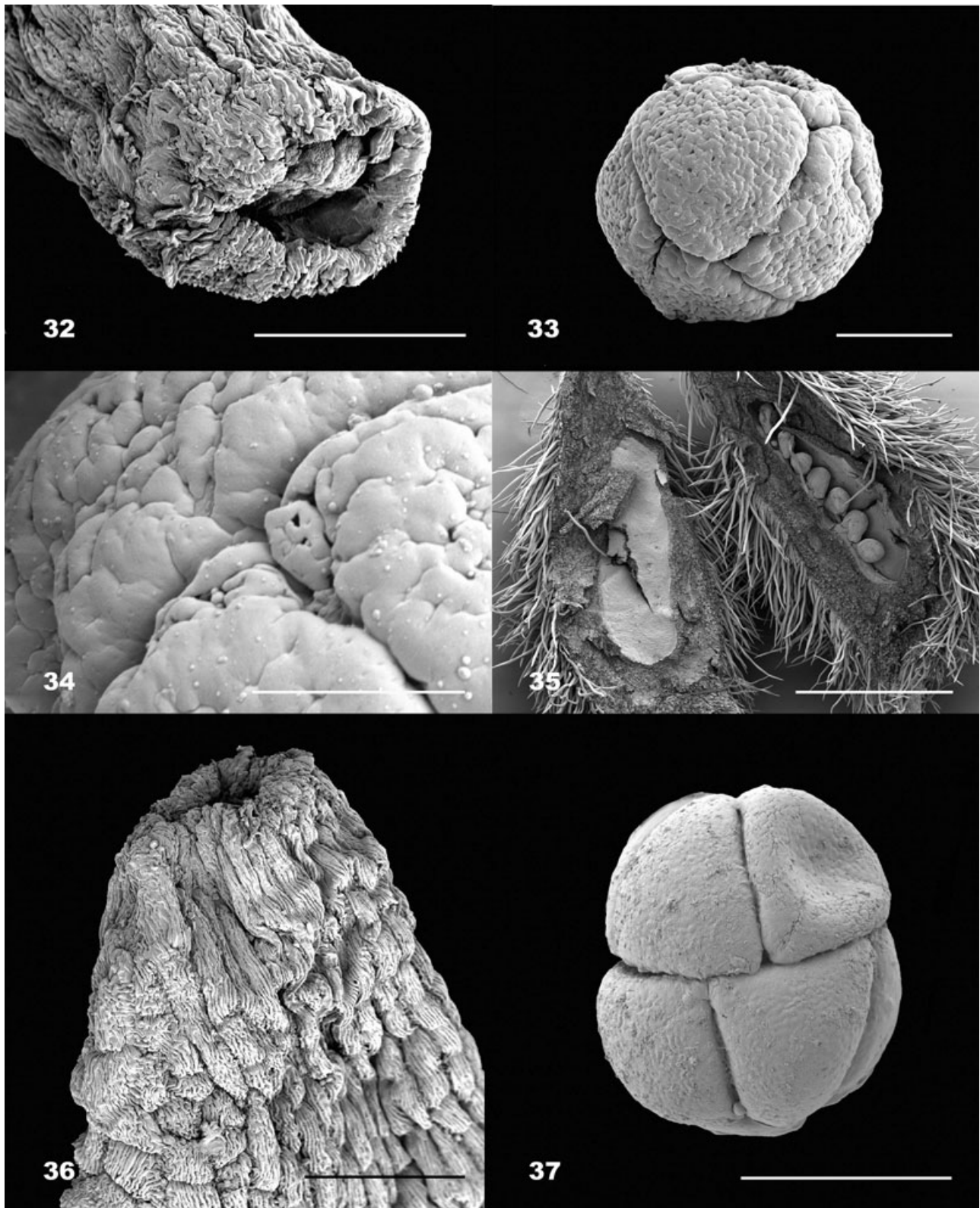
POLLEN

Pollen grains of *Burkea*, *Dimorphandra*, *Erythrophleum*, *Mora*, *Pachyelasma*, *Stachyothyrsus*, *Sym-*

petalandra, *Pentaclethra* and *Aubrevillea* are tricolporate (apart from those of *Pentaclethra macrophylla* Benth., which are porate, Guinet & Ferguson, 1989 and Fig. 16), small and generalized (Banks & Lewis, 2009). Banks *et al.* (2003) found that pollen of caesalpinoids is relatively more fixed and homogeneous in the more derived clades when compared with the great diversity of pollen types found in the basally branching lineages. The *Dimorphandra* group is a relatively derived caesalpinoid element and the homogeneous pollen that occurs in the group corresponds with this pattern (Banks & Lewis, 2009). The clavate acalymmate tetrahedral tetrads of *Dinizia* are unlike any other pollen found in Fabaceae. Tetrads occur in three other caesalpinoid genera (*Bauhinia* L., *Afzelia* Sm. and *Diptychandra* Tul., Ferguson & Banks, 1994; Banks *et al.*, 2003), but are more common in Mimosoideae (Guinet, 1981; Guinet & Ferguson, 1989) and are absent from Papilionoideae (Guinet & Ferguson, 1989). However, the highly ornate ornamentation of *Dinizia* (Fig. 14) is unusual; previous studies describe it as intectate (Guinet & Ferguson, 1989) and as having non-supracteal clavae (Feuer, 1987); transmission electron microscope (TEM) studies should further the understanding of the exine structure. Although all the pollen grains of *Dinizia* seen in this study were in permanent tetrahedral tetrads, it has been reported that monads also occur in the same stamens of some plants (Guinet, 1981).

Polyads are present in all the taxa that make up the *Adenanthera* group. *Adenanthera*, *Tetrapleura*, *Amblygonocarpus* and *Pseudoprosopis* have lumpy asymmetrical calymmate polyads with great variability in size and shape (Figs 21, 23, 25, 27, 31), whereas the polyads found in species of *Calpocalyx* and *Xylia* are relatively more regular in organization and number/size of pollen units making up the polyads (Fig. 33, 37). In previous literature, the number of pollen units per polyad varies in the *Adenanthera* group (Table 2).

The evolution of aggregations of pollen (including tetrads, polyads, pollinia and adhesion by viscin threads) from monads has occurred at least 39 times in angiosperms according to Harder & Johnson (2008) and approximately 50 times according to Walker & Doyle (1975). The evolution of aggregated pollen has usually resulted in the production of tetrads (39 families), with polyads being found in Annonaceae, Celastraceae, Fabaceae and Hydrocharitaceae (Watson & Dallwitz, 2006; Harder & Johnson, 2008). The aggregation of pollen units into polyads should only occur under special circumstances according to Harder & Johnson (2008) and models that demonstrate that diminishing returns select for subdivision rather than aggregation are presented in their paper. Such special circumstances might include infrequent pollinators



Figures 32–37. *Calpocalyx* and *Xylia*. Fig. 32. *Calpocalyx brevibracteatus*, stigma showing polyad within the stigmatic cavity. Scale bar, 50 μm . Fig. 33. *Calpocalyx brevibracteatus*, polyad. Scale bar, 10 μm . Fig. 34. *Calpocalyx brevibracteatus*, close-up of porate operculate apertures. Scale bar, 5 μm . Fig. 35. *Xylia torreana*, ovary. Scale bar, 1 mm. Fig. 36. *Xylia torreana*, stigma showing stigmatic pore. Scale bar, 50 μm . Fig. 37. *Xylia hoffmanii*, polyad. Scale bar, 20 μm .

Table 2. Number of pollen units in polyads in the *Adenanthera* group

Genus	Most common number of pollen units per polyad in this study	Average number of ovules (this study, to nearest whole number)	Previous reports of number of pollen units per polyad
<i>Adenanthera</i>	(14–) 16	14	8 or 12: Guinet & Ferguson, 1989. 8 or 16; Guinet, 1981.
<i>Tetrapleura</i>	16	16	16: Guinet & Ferguson, 1989.
<i>Amblygonocarpus</i>	(11–) 12 (–16)	12	
<i>Pseudoprosopis</i>	8 in <i>P. euryphylla</i> ; (8–) 12 (–16) in <i>P. bampsiana</i>	7 in <i>P. euryphylla</i> ; 10 in <i>P. bampsiana</i>	8: Guinet & Ferguson, 1989.
<i>Calpocalyx</i>	8	7	
<i>Xylia</i>	8 in <i>X. hoffmannii</i> ; 16 in <i>X. xylocarpa</i> var. <i>kerrii</i>	6 in <i>X. hoffmannii</i> ; 11 in <i>X. xylocarpa</i> var. <i>kerrii</i>	4, 16: Guinet, 1981. 8: Guinet & Ferguson, 1989. 4, 8: Hughes, 1997.

(in which case aggregates would increase the chance of dispersal), brief pollen viability (aggregates would increase the chance of enough pollen grains being available while still viable), synchronous ovule availability, low pollen transport efficiency, stigmas susceptible to being overwhelmed by pollen from another species or many ovules per ovary. It has been suggested that tetrads may evolve most commonly when pollinators visit infrequently; in such cases they increase the probability that pollen removed by a pollinator will reach a conspecific stigma (Harder & Johnson, 2008). On the one hand, pollen in compound units is thought to have a reproductive advantage because it ensures that maximum seed set can result from a single pollination event (Kenrick & Knox, 1982). Also, the pollen to ovule ratio is lower, meaning that less pollen has to be produced resulting in a more efficient system (Cruden, 2000). On the other hand, Cruden (2000) also reported that four to six pollen grains per ovule are necessary for maximum seed set, to reduce the likelihood of a low-quality pollen tube reaching an ovule, and reports cases of multipaternity (more than one polyad completes the seed set of one flower) in *Acacia* Mill. and *Calliandra* Benth. However, Muona, Moran & Bell (1991) found that only 8% and 15% of *Acacia melanoxylon* R.Br. pods from two populations contained seeds sired by two donors rather than one. Harder & Johnson (2008) also show data from two *Acacia* spp. that indicate that stigmas receive a single polyad more frequently than would be expected from random (when it is possible for more than one polyad to be received) and they argued that polyads could promote resource-sharing among seeds, enhance total seed production and reduce seed size variance within fruits. In Apocynaceae, Wyatt & Lipow (2007) suggested that, because

of late-acting self-incompatibility, mixed loads of self- and cross-pollen cause abortion of whole fruits; thus, the evolution of polyads represents an adaptation to prevent or compensate for such losses. Polyads limit improper pollen transfer and favour speciation (Guinet, 1986). A higher number of grains per polyad increases reproductive capacity, but hybridization can result in polyads with fewer grains in legumes (Guinet & Ferguson, 1989). Polyads function as a single harmomegathic unit (McGlove, 1978) and the small operculate pores present in mimosoid polyads suggest that reduction of water loss may be an important attribute. Both the largest (*Calliandra*, 320 µm) and smallest (*Mimosa* L., 6 µm) pollen units in angiosperms are found in Mimosoideae (Elias, 1981).

There is still a great deal to be learnt about tetrads and polyads. For example, there is controversy about how mimosoid polyads develop, whether a mimosoid polyad is formed from one pollen mother cell (PMC) or several (e.g. in *Calliandra*, opposing interpretations are given by Chen, 1973; Greissl, 2006; and Teppner, 2007). When compared with monads of caesalpinoids, polyads greatly differ in development and morphology, as opposed to tetrads which are recognizable as developing microspores that have not separated. Tetrads have arisen three times independently in caesalpinoids (in addition to mimosoids such as *Dinizia*) and are restricted to small groups of one or two species (*Bauhinia*, *Diptychandra*, *Azelia*, all caesalpinoids; Sorsa, 1969; Ferguson & Banks, 1994; Banks *et al.*, 2003), whereas polyads are present only in Mimosoideae as circumscribed in the recent phylogenetic analyses of Luckow, White & Bruneau (2000), Luckow *et al.* (2003), Wojciechowski, Lavin & Sanderson (2004), Lewis (2005) and Bruneau *et al.* (2008).

Table 3. Differences between monads/tetrads and polyads

Monads, tetrads	Polyads
Apertures follow Fischer's rule	Apertures do not follow Fischer's rule
Colporate	Porate, operculate
3 apertures per pollen unit	(3–) 4 apertures per pollen unit

POLLEN APERTURES

The polyads in the Adenantha group have porate, operculate apertures and the morphology, distribution and number of pollen apertures on each grain are different to those of caesalpinoid monads and the developing microspores in tetrahedral tetrads of those monads. The pores of the Adenantha group are difficult to see as they are small and often the opercula conceal them, unless removed during processing (acetolysis). The porate pollen type is by far the most frequent aperture form in Mimosoideae (Guinet, 1981), whereas colporate apertures are the most frequent aperture form in caesalpinoids (Graham & Barker, 1981; Banks & Gasson, 2000; Banks & Klitgaard, 2000; Banks *et al.*, 2003). According to the known developmental processes, the position of apertures within the polyads of the Adenantha group suggests a different developmental pathway to the pollen of the Dimorphandra group plus *Aubrevillea*, *Dinizia* and *Pentaclethra* (Table 3).

Evidence available to date suggests that caesalpinoid pollen develops in tetrahedral tetrads following simultaneous cytokineses (Ferguson & Banks, 1994; Banks, Feist-Burkhart & Klitgaard, 2006), as in the majority of eudicots (Erdtman, 1969; Blackmore & Crane, 1988, 1998; Ressayre *et al.*, 2002; Nadot *et al.*, 2008). In Mimosoideae, there are reports of successive microsporogenesis in *Calliandra* (Greissl, 2006), but these are disputed by Teppner (2007). Although Prakash (1987) stated that simultaneous cytokinesis is a consistent feature within Fabaceae, with microspore tetrads being generally tetrahedral, the presence of decussate, isobilateral and T-shaped tetrads (which would imply successive microsporogenesis) are stated as being 'not uncommon'; there are no references to studies that support this statement.

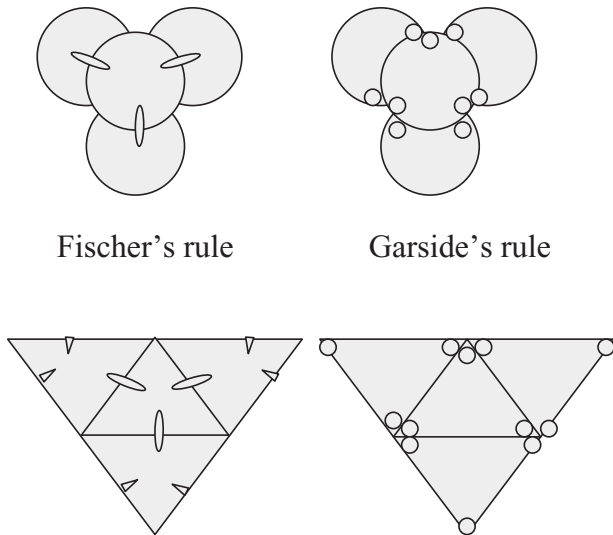
The pores in polyads cannot be equidistantly spaced because of internal constraints, as the pollen units cannot develop as far apart from each other as in tetrahedral tetrads, but are closely packed together in polyads. Porate apertures that follow Garside's rule and Fischer's rule are both reported in mimosoid polyads by Guinet (1981). Because apertures that follow Garside's rule would be unexpected in legumes, this is a significant point that requires

further investigation (see Table 4). In eudicot microspore tetrads, the pores form at the last point of cytoplasmic contact between daughter cells. These occur in pairs at six points on the tetrahedral tetrad surface in Fischer's rule (inter-radial arrangement of pores) as opposed to four groups of three pores in 'Garside's rule' (Garside, 1946) in a radial arrangement (Erdtman, 1969; Blackmore & Crane, 1988, 1998; Ressayre *et al.*, 2002) (Fig. 38). In the eudicot genus *Dryandra* R.Br. (Proteaceae, now included in *Banksia* L.f.; Mast & Thiele, 2007) where apertures develop according to Garside's rule, cytokinesis is successive and the biporate condition is likely to be correlated with the presence of a dyad stage (Blackmore & Barnes, 1995). Furness (2008) reported that there is successive microsporogenesis in Berberidaceae, where it is associated with spiraperturate or inaperturate pollen. In mimosoid polyads, pores do occur in groups of three (and therefore appear to follow Garside's rule). However, Teppner (2007) reported that tetrahedral tetrads develop following simultaneous microsporogenesis in *Calliandra* and *Inga* Scop. (unlike Garside's rule, Table 4). Further studies are required to examine developing microspores in mimosoids to determine the type of cytokinesis, whether the pores are in a radial or inter-radial arrangement and whether they follow Garside's rule, Fischer's rule or an alternative type of development. The position that each microspore takes up is determined by the size and shape of the locule that constrains its development in *Calliandra* and *Inga* (Teppner, 2007). The fact that the polyads of the Adenantha group are not constrained by locule walls within the anther (Fig. 23) may explain why the polyads are so variable in size and shape (Figs 21, 23, 25, 27). Developmental studies are planned in a future project.

In Fabaceae, the widespread unspecialized type of pollen is tricolporate and, in taxa that have pollen with more than three apertures, the number is often variable and has a range rather than a fixed number (Banks & Klitgaard, 2000; Banks *et al.*, 2003). The existence of a range of aperture numbers fits the hypothesis of aperture number heteromorphism in eudicots described by Ressayre *et al.* (2002). This model hypothesizes that simultaneous meiosis allows variation in the nature of interaction between the nuclei of the four developing microsporocytes. The distribution of microtubules (MTs) is affected by the amount of nuclear surface and, if the nucleation begins just at the end of anaphase II, haploid sets of chromosomes would not have recovered a spherical shape. Sister and non-sister nuclei would then vary in shape. MTs are involved in callose transport which forms the cell plates and they are therefore responsible for the formation of cleavage planes. Aperture

Table 4. Comparison of angiosperm pollen development types (successive and simultaneous microsporogenesis and Fischer's rule with Garside's rule)

Simultaneous microsporogenesis	Successive microsporogenesis
Occurs in basal angiosperms, monocots and eudicots No dyad stage, cytokinesis occurs after both nuclear divisions are complete	Occurs mainly in basal angiosperms and monocots Two clear stages of development, callose cell walls are formed after both meiosis I and meiosis II, resulting in a dyad stage
Associated with tetrahedral tetrads	Associated with decussate, tetragonal, linear, rhomboidal and T-shaped tetrads
Eudicot pollen is predominantly tricolpate (and tricolpate-derived): these apertures are formed only by simultaneous microsporogenesis and follow Fischer's rule	In basal angiosperms and monocots pollen is predominantly monosulcate, formed by successive or simultaneous microsporogenesis. Tricolpate pollen formed by successive microsporogenesis follows Garside's rule
Fischer's rule	Garside's rule
Fischer's rule (inter-radial aperture orientation), apertures occur in pairs at six points on the tetrad surface	Garside's rule (radial aperture arrangement), apertures occur in groups of three at four points on the tetrad surface
Can only occur in tetrahedral tetrads formed by simultaneous microsporogenesis	Occurs following successive microsporogenesis
Evolved (or become fixed) only once (characterizes the eudicots)	Evolved multiple times

**Figure 38.** Diagram of differences between Fischer's rule and Garside's rule.

number is correlated with differences in MT distribution and cleavage plane formation. The shape of the secondary spindles formed by the MTs determines the number of apertures the grains will have. The pollen of the caesalpinoid genus *Duparquetia* Baill. is heteromorphic and has two endoapertures and one

ectoaperture, but the development still follows Fischer's rule (Banks *et al.*, 2006), although the pores are in pairs at four points in a tetrahedral tetrad of *Duparquetia* microspores, rather than in pairs at six points as for tricolporate grains. The individual pollen apertures within polyads are small, operculate and difficult to see using standard SEM or LM techniques (Figs 21, 23, 25, 27, 31, 33, 37). However, there are usually four pores which would result in it being impossible for the typical patterns seen in Fischer's rule to be present. More research is planned in this area.

STIGMA TYPE, CORRELATION OF POLLEN UNIT AND OVULE NUMBER

The stigma form and size, and the number of ovules in an ovary, are all variable and unpredictable among the *Dimorphandra* group plus *Dinizia*, *Pentaclethra* and *Aubrevillea*. (Table 5). In contrast, in the *Adenanthera* group, only one polyad will fit into the stigmatic cavity and the number of pollen units per polyad closely match the number of ovules present (Tables 2 and 5).

The stigmas of the *Adenanthera* group are either cylinders or pores. Differences in size and shape of the tip of the style were noted by Lewis & Elias (1981). Funnel-shaped to cupular stigmatic tips are reported

Table 5. Characters of pollen, stigmas and ovules

Genus, species, authority	Average no. of ovules	Stigmatic cavity diameter	Pollen size	Estimate of average no. of pollen units that can occupy a stigma	Aperture type
<i>Adenanthera abrosperma</i> F.Muell.	(14–) 15.1 (–16)	~60–80 µm	Diameter (35–) 42.3 (–50) µm	1 polyad of ±16 pollen units	Individual pollen units (3–) 4 porate–operculate, apertures in groups of 3 or 4 in polyad
<i>Adenanthera aglaosperma</i> Alston	(11–) 13.1 (–15)	~30–50 µm	Diameter (28–) 34.1 (–40) µm	1 polyad of ±16 pollen units	Individual pollen units (3–) 4 porate–operculate, apertures in groups of 3 or 4 in polyad
<i>Adenanthera novo-guineensis</i> Baker f.	(13–) 14.7 (–17)	~30–50 µm	Diameter (25–) 30.4 (–37) µm	1 polyad of ±16 pollen units	Individual pollen units (3–) 4 porate–operculate, apertures in groups of 3 or 4 in polyad
<i>Adenanthera pavonina</i> L.	(12–) 12.1 (–15)	~60–80 µm	Diameter (45–) 56.67 (–70) µm	1 polyad of (14–) 15.4 (–16) pollen units	Individual pollen units (3–) 4 porate–operculate, apertures in groups of 3 or 4 in polyad
<i>Amblygonocarpus andongensis</i> (Welw. ex Oliv.) Exell & Torre	(11–) 12.4 (–13)	~80–100 µm	Diameter (59–) 62.8 (–70) µm	1 polyad of (11–) 12.3 (–16) pollen units	Individual pollen units (3–) 4 porate–operculate, apertures in groups of 3 or 4 in polyad
<i>Aubrevillea kerstingii</i> (Harms) Pellegr.	(2–) 2.5 (–4)	~150 µm	P × E = (26–) 27.3 (–30) × (28–) 29.3 (–31) µm	~25 monads	Tricolporate monads
<i>Burkea africana</i> Hook.	(1–) 1.6 (–2)	≥ 500 µm	P × E = (19–) 21.5 (–23) × (19–) 23.3 (–25) µm	~516 monads	Tricolporate monads
<i>Calpocalyx brevibracteatus</i> Harms	(7–) 7.4 (–8)	~30–50 µm	Diameter (32–) 35.9 (–39) µm	1 polyad of 8 pollen units	3 porate–operculate, in groups of 2 or 4
<i>Calpocalyx dinklagei</i> Harms	(5–) 5.9 (–7)	N/A	Diameter (40–) 49.5 (–58) µm	1 polyad of 8 pollen units	3 porate–operculate, in groups of 2 or 4
<i>Dimorphandra cuprea</i> subsp. <i>cuprea</i>	(9–) 9.2 (–10)	~100–30 µm	P × E = (25–) 29.1 (–30) × (21–) 22.4 (–26) µm	~20 monads	Tricolporate monads
<i>Dimorphandra davisii</i> Sprague & Sandwith	(4–) 4.5 (–5)	~50–70 µm	P × E = (23–) 26.2 (–29) × (29–) 30.4 (–32) µm	~5 monads	Tricolporate monads
<i>Dimorphandra exaltata</i> Schott	(16–) 18 (–20)	~50–70 µm	P × E = (26–) 27.8 (–29) × (29–) 30.6 (–34) µm	~4 monads	Tricolporate monads
<i>Dimorphandra gardneriana</i> Tul. Samples examined for pollen only	N/A	N/A	P × E = (21–) 22.7 (–25) × (30–) 31.4 (–33) µm	N/A	Tricolporate monads
<i>Dimorphandra pennigera</i> Tul. Samples examined for pollen only	N/A	N/A	P × E = (29–) 30.2 (–32) × (30–) 33.2 (–38) µm	N/A	Tricolporate monads
<i>Dimorphandra mollis</i> Benth.	(21–) 22.6 (–24)	~100–120 µm	P × E = (21–) 24.6 (–27) × (14–) 15.5 (–18) µm	~32 monads	Tricolporate monads
<i>Dimorphandra unijuga</i> Tul.	(15–) 15.8 (–17)	~80–100 µm	P × E = (21–) 24.3 (–29) × (22–) 25.3 (–31) µm	~13 monads	Tricolporate monads

Table 5. *Continued*

Genus, species, authority	Average no. of ovules	Stigmatic cavity diameter	Pollen size	Estimate of average no. of pollen units that can occupy a stigma	Aperture type
<i>Dimorphandra vernicosa</i> Spruce ex Benth. & Hook. f.	(8–) 9.3 (–10)	~100–120 µm	P × E = (26–) 29.3 (–32) × (33–) 35.5 (–38) µm	~5 monads	Tricolporate monads
<i>Dinizia excelsa</i> Ducke	(7–) 9.3 (–10)	200–250 µm	Diameter (63–) 73.9 (–86) µm	~9 tetrads, 36 pollen units	Tricolporate tetrads, Fischer's rule
<i>Erythrophleum africanum</i> (Benth.) Harms	(6–) 6.4 (–7)	70–80 µm	P × E = (21–) 23.7 (–28) × (24–) 27.6 (–31) µm	~9 monads	Tricolporate monads
<i>Erythrophleum chlorostachys</i> (F.Muell.) Baill.	(6–) 7.1 (–8)	~100 µm	P × E = (18–) 20.5 (–23) × (18–) 21.5 (–25) µm	~23 monads	Tricolporate monads
<i>Erythrophleum couminga</i> Bail.	(7–) 7.6 (–8)	~50 µm	P × E = (19–) 22.5 (–26) × (20–) 23.5 (–27) µm	~5 monads	Tricolporate monads
<i>Erythrophleum fordii</i> Oliv.	(8–) 8.4 (–10)	< 100 µm	N/A	N/A	Tricolporate monads
<i>Erythrophleum suaveolens</i> (Guill. & Perr.) Brenan	(10–) 10.6 (–11)	~75 µm	P × E = (30–) 33.2 (–36) × (20–) 26.5 (–32) µm	~6 monads	Tricolporate monads
<i>Mora ekmanii</i> Britton & Rose	(3–) 3.9 (–4)	~90–100 µm	~40–45 µm (limited data)	~5 monads	Tricolporate monads
<i>Mora excelsa</i> Benth. Samples examined for pollen only	N/A	N/A	P × E = (40–) 42.5 (–45) × (31–) 35.0 (–37) µm	N/A	Tricolporate monads
<i>Mora gonggrijpii</i> Sandwith	2	~20–50 µm	N/A	N/A	Tricolporate monads
<i>Mora paraensis</i> Ducke Samples examined for pollen only	N/A	N/A	P × E = (39–) 44.0 (–48) × (48–) 54.4 (–59) µm	N/A	Tricolporate monads
<i>Pachyelasma tessmannii</i> (Harms) Harms	(18–) 18.5 (–19)	~100 µm	P × E = (26–) 27.2 (–29) × (24–) 26.3 (–28) µm	13	Tricolporate monads
<i>Pentaclethra eetveldeana</i> De Wild. & T.Durand Samples examined for pollen only	N/A	N/A	P × E = (70–) 75.8 (–82) × (64–) 71.4 (–79) µm	N/A	Tricolporate monads
<i>Pentaclethra macrophylla</i> var. <i>petalifera</i> Benth. Samples examined for pollen only	N/A	N/A	P × E = (39–) 41.6 (–45) × (51–) 55.6 (–58) µm	N/A	Triporate monads
<i>Pentaclethra macroloba</i> Kuntze	10	150–200 µm	~40 µm (limited data)	~18 monads	Tricolporate monads
<i>Pseudoprosopis bampsiana</i> Lisowski	(8–) 9.9 (–11)	~30–50 µm	Diameter = (30–) 35.1 (–43) µm	1 polyad of (8–) 12 (–16) pollen units	Individual pollen units (3–) 4 porate–operculate, apertures in groups of 3 in polyad
<i>Pseudoprosopis euryphylla</i> Harms	(6–) 7.1 (–8)	~30–50 µm	Diameter = (19–) 24 (–31)	1 polyad of 8 units	3–4 porate in groups of 3 or 4
<i>Stachyothyrsus staudtii</i> Harms	2	~400 µm	P × E = (28–) 29.7 (–34) × (28–) 29.1 (–30) µm	~190 monads	Tricolporate monads

Table 5. Continued

Genus, species, authority	Average no. of ovules	Stigmatic cavity diameter	Pollen size	Estimate of average no. of pollen units that can occupy a stigma	Aperture type
<i>Stachyothyrsus stapfiana</i> (A.Chev.) J.Léonard & Voorh. Samples examined for pollen only	N/A	N/A	P × E = (22–) 23.6 (–25) × (19–) 24.0 (–27) µm	N/A	Tricolporate monads
<i>Sympetalandra borneensis</i> Stapf Samples examined for pollen only	N/A	N/A	P × E = (38–) 40.1 (–42) × (39–) 42.5 (–44) µm	N/A	Tricolporate monads
<i>Sympetalandra densiflora</i> (Elm.) Steenis Samples examined for pollen only	N/A	N/A	P × E = (42–) 48.3 (–52) × (41–) 46.1 (–49) µm	N/A	Tricolporate monads
<i>Sympetalandra unijuga</i> (Shaw) Steenis	(4–) 4.1 (–5)	50–100 µm	P × E = (31–) 36.5 (–42) × (36–) 39 (–42) µm	~4 monads	Tricolporate monads
<i>Tetrapleura tetraptera</i> Taub.	(14–) 15.5 (–17)	~40–60 µm	Diameter = (45–) 53.4 (–60) µm	1 polyad of ±16 pollen units	(3–) 4 porate, operculate, in groups of 3 or 4
<i>Xylia hoffmannii</i> Drake	(5–) 5.75 (–6)	N/A	Long axis × short axis = (45–) 47.8 (–50) × (35–) 38.3 (–40) µm	Each polyad has 8 pollen units	3-porate pollen units, pores in groups of 2 and 4
<i>Xylia xylocarpa</i> var. <i>kerrii</i> (Craib & Hutch.) I.C.Nielsen	(10–) 11 (–12)	~80–100 µm	Long axis = (75–) 90 (–100) Short axis – not enough data	1 polyad of 16 pollen units	3–4 porate in groups of 3 or 4
<i>Xylia torreana</i> Brenan	7	~20–30 µm	N/A	N/A	

N/A, not available.

as characteristic of *Dinizia* and *Aubrevillea*, whereas a narrow porate stigma is characteristic of *Adenantha* and *Xylia*.

No living plants were available for study, but observations of a thin cap-like structure over the stigmatic cavity were made in spirit material of *Tetrapleura* Benth. (in which there is better preservation of delicate structures than in herbarium material). The stigmatic area was found to be covered by a cuticle or membrane during pollination research carried out on *Strongylodon* Vogel (Papilionoideae) (Prychid, Owens & Rudall, 1998). In *Trifolium* L. a similar barrier prevents pollination taking place before the membrane is physically removed by a visiting pollinator (Heslop-Harrison & Heslop-Harrison, 1982) and thus it could act as a mechanism to prevent self-pollination. Owens, Prychid & Cox (1995) described how stigmatic secretions build up beneath the intact cuticle of *Caesalpinia* L., so that once ruptured the stigmatic fluid fills the crater. Stigmatic fluid is required to hydrate pollen and act as a medium for

germination (Heslop-Harrison & Heslop-Harrison, 1982, 1983; Prychid *et al.*, 1998). In *Dimorphandra*, the receptive stigmatic surface is described as a crater filled with clear fluid by Owens (1989). There have been more studies on stigma types of caesalpinioids (Owens, 1989; Owens & Lewis, 1989, 1996; Owens & Stirton, 1989) than on Mimosoideae, especially *Acacia* (Kenrick & Knox, 1989). Observations on the stigmatic structure and pollination system of living plants in this group are needed.

PHYLOGENETIC CONSIDERATIONS

In the *matK* and *trnL* molecular analysis of Bruneau *et al.* (2008), nine taxa (*Pachyelasma tessmannii* Harms, *Erythrophleum ivorense* A.Chev., *Erythrophleum suaveolens* (Guill. & Perr.) Brenan, *Dimorphandra conjugata* (Splitg.) Sandwith, *Burkea africana* Hook., *Mora gonggrijpii* (Kleinh.) Sandwith, *Calpocalyx dinklagei* Harms, *Dinizia excelsa* Ducke and *Pentaclethra macrophylla* Benth.), representing

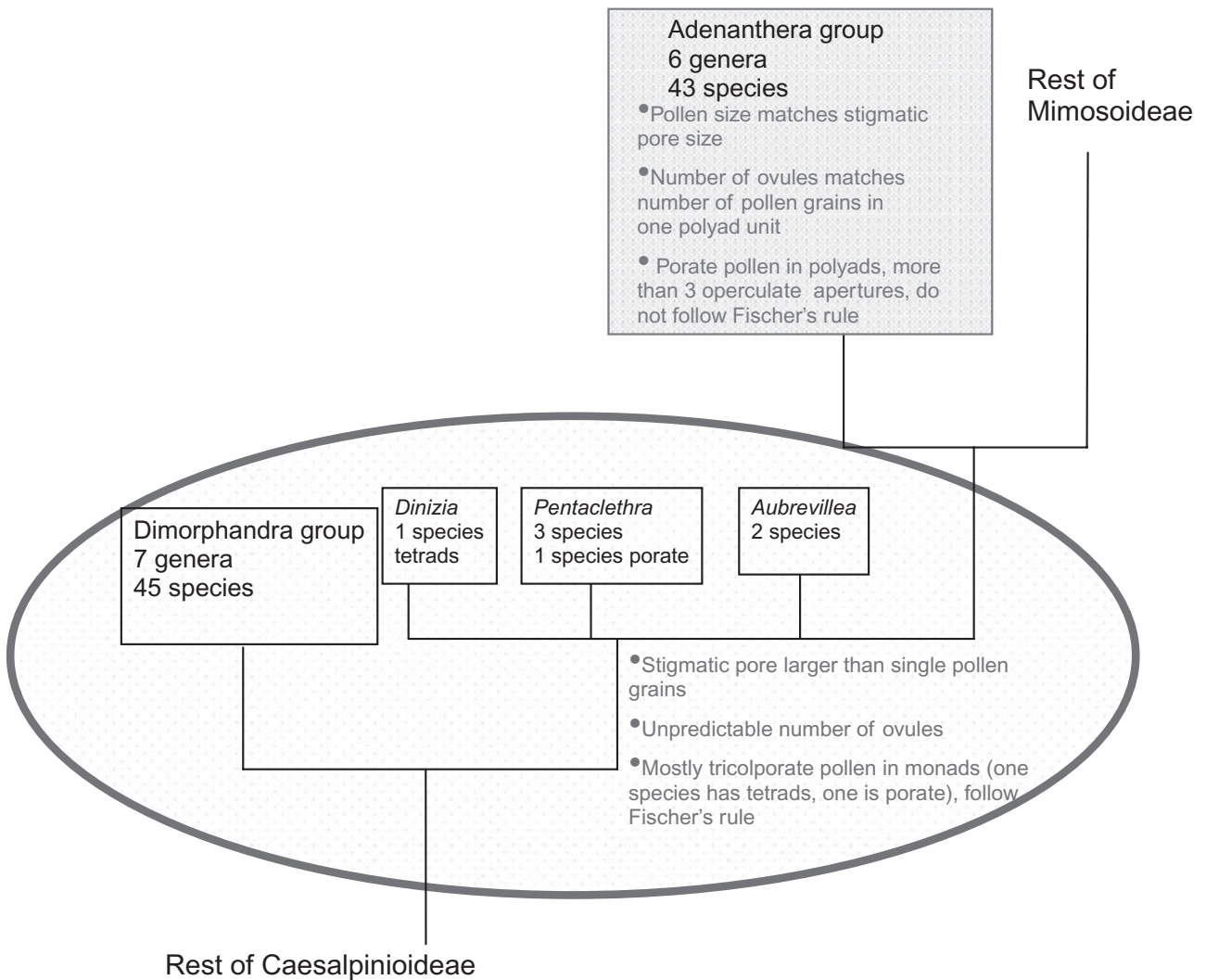


Figure 39. Diagram of the distribution of pollen, stigma and ovule characters at the caesalpinioid–mimosoid interface (phylogeny based on Lewis, 2005; Lewis *et al.*, 2005; Luckow, 2005).

seven out of the 16 genera analysed here, are represented. The *Dimorphandra* group falls in two distinct clades in the molecular analyses with genera not included in our study. The homogeneous pollen types found in the *Dimorphandra* group are separated into these two clades, each of which also include taxa that release pollen in permanent tetrads (*Dinizia* in one clade and *Diptychandra* in the other; Banks & Lewis, 2009). Of the remaining three genera analysed in both studies, two (*Calpocalyx* and *Pentaclethra*) together form a polytomy with the rest of the Mimosoideae clade, with *Erythrophleum* sister to this combined group, and *Pachyelasma* sister to Mimosoideae, *Pentaclethra*, *Calpocalyx* and *Erythrophleum*. The third genus, *Dinizia*, is placed in the other *Dimorphandra* group clade, sister to *Burkea*,

Mora and *Dimorphandra*. Polyads occur only in the Mimosoideae clade.

FOSSILS

There are notable similarities of floral (stigma size, anther number and shape, lack of anther glands, lack of anther dimorphism) and pollen characters (size, exine characters, aperture type) between the genera *Aubrevillea*, *Pachyelasma*, *Stachyothyrsus* and *Erythrophleum* and primitive mimosoid flowers from Palaeocene–Eocene fossils described from western Tennessee (Crepet & Taylor, 1986). The pollen grain images of *Protomimosaidea* (Crepet & Taylor, 1986, Figs 13, 20) compare particularly well with the pollen images of *Pachyelasma* (Banks & Lewis, 2009,

Fig. 1E, F). However, *Aubrevillea*, *Pachyelasma*, *Stachyothyrsus* and *Erythrophleum* are palaeotropical, with the centre of diversity in West Africa, although the two *Dimorphandra* group members *Dimorphandra* and *Mora* are found in South and Central America and several North American fossil legumes appear to be related to genera that are now restricted to tropical Africa (Herendeen, 1992; Herendeen, Crepet & Dilcher, 1992). Pollen ultrastructure would be worth investigating further, as the TEM images of Crepet & Taylor (1986) indicate the possible presence of a fastigium (Fig. 14) and thin extra infratectal layer (Figs 14, 15) as noted by Banks *et al.* (2003) in the few samples of *Dimorphandra* group pollen (*Erythrophleum*, *Stachyothyrsus*, *Dimorphandra* and *Mora*) that have so far been examined using TEM.

CONCLUSION

The *Dimorphandra* group plus *Dinizia*, *Pentaclethra* and *Aubrevillea* have typical eudicot pollen development that occurs with simultaneous cytokinesis and tricolporate apertures that follow Fischer's rule, in accordance with pollen throughout caesalpinoid legumes and the vast majority of eudicots. The monads and tetrads of the group in this study are associated with varying, non-predictable stigma type and ovule number (Fig. 39). The permanent tetrads, such as those found in *Dinizia* (and in caesalpinoid *Bauhinia*, *Afzelia* and *Diptychandra*), develop in the same way as other caesalpinoid microspores that develop into monads and only differ in that they do not separate from each other when mature. Tetrads have arisen independently three times in caesalpinoids and are also present in Mimosoideae, whereas polyads are only present in Mimosoideae. In the *Adeanthera* group, polyads are present and one polyad exactly fits in to the stigmatic cavity of the same species (Fig. 39). Additionally, ovules will predictably not be greater in number than the number of pollen units per polyad of the parent plant. Polyads differ in layout and aperture morphology and therefore in development when compared with monad caesalpinoid and other eudicot pollen.

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