Structure of Pollen Apertures in the Detarieae *sensu stricto* (Leguminosae: Caesalpinioideae), with Particular Reference to Underlying Structures (Zwischenkörper)

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This study presents the pollen aperture morphology of 148 out of approx. 200 species in 16 genera of the Detarieae *s.s.* and 13 related genera, investigated with light microscopy, scanning electron microscopy and transmission electron microscopy, using various staining techniques. Features of detarioid legume pollen apertures are described, illustrated and discussed in relation to function and phylogeny. Protruding apertures in the mature pollen grains of taxa in the Detarieae *s.s* are associated with underlying structures composed of pectic substances called 'Zwischenkörper'. This is the first report of Zwischenkörper in legume pollen. A review of previous literature on Zwischenkörper, evidence of how they differ from onci, and a discussion of developmental origins, terminology and function, is given. Zwischenkörper occur in pollen of *Daniellia*, *Eurypetalum*, *Eperua*, *Augouardia*, *Stemonocoleus*, *Baikiaea*, *Copaifera*, *Pseudosindora*, *Detarium*, *Sindora*, *Sindoropsis*, *Tessmannia*, *Gilletiodendron*, *Hylodendron*, *Hymenaea*, *Peltogyne* and *Guibourtia* of the Detarieae *s.s.* clade of recent molecular analyses. Exinous projections and/or bridges are present over the centre of apertures in the pollen of *Sindora*, *Copaifera*, *Detarium*, *Pseudosindora*, *Hylodendron* and *Sindoropsis*, and may cover the Zwischenkörper of live pollen. Zwischenkörper also occur in the closely related genus *Barnebydendron*, the sister genus to the Detarieae *s.l.* clade *Goniorrhachis*, and also *Cercis* of the sister clade to the Detarieae *s.l.* plus *Goniorrhachis*. A modified form of Zwischenkörper occurs in the pollen of *Schotia*. Zwischenkörper were not detected in the genera *Colophospermum*, *Prioria*, *Gossweilerodendron*, *Oxystigma*, *Kingiodendron* and *Hardwickia* of the *Prioria* clade, or in *Endertia*, *Lysidice* and *Saraca* of the Amherstieae clade. None of these taxa have protruding apertures.   © 2003 Annals of Botany Company

Key words: Pollen aperture morphology, Zwischenkörper, Leguminosae, Caesalpinioideae, Detarieae, Alcian blue stain, systematics.

INTRODUCTION

Of the three subfamilies in the Leguminosae, the pollen of subfamily Caesalpinioideae is the most morphologically diverse when compared with the Papilionoideae and Mimosoideae, and the Detarieae *s.l.* has the highest floral and pollen morphological diversity within the legume family (Graham and Barker, 1981; Muller, 1981, 1984; Ferguson, 1987; Guinet and Ferguson, 1989; Banks and Klitgaard, 2000). There are perforate, rugulate, striate, reticulate, gemmate, verrucate and psilate ornamentation types; however, systematically informative characters have only been delimited from aperture morphology and wall structure (colporate or porate apertures; aperture membrane type; apocolpium present, syncolporate or parasyncolporate; presence or absence of aperture membrane or exinous bridges; presence or absence of prominent aperture margins; presence or absence of a foot layer, additional infratectal layer or supratectal structures) (Banks and Klitgaard, 2000). This paper examines the pollen aperture structures present in the Detarieae *s.s.* with particular reference to underlying structures that are usually removed by standard preparation techniques (termed 'Zwischenkörper'), and assesses these data in the context of the systematics of the group.

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Designation of the Detarieae s.s.

Of the three subfamilies that are widely recognized within the Leguminosae, recent molecular analyses have demonstrated that the Caesalpinioideae is paraphyletic, with subfamilies Papilionoideae and Mimosoideae nested within it (Doyle *et al*., 1997, 2000, based on *rbcL* data; Bruneau *et al*., 2000, 2001, based on *trnL* data). Taxonomy in subfamily Caesalpinioideae has remained problematical due to difficulties in finding clear characters to differentiate the taxa. A summary of taxonomic history is given by Bruneau *et al*. (2001). The Detarieae *s.l.* [Detarieae and Amherstieae (Cowan and Polhill, 1981) or Detarieae and Macrolobieae (Breteler, 1995)] form a well-supported monophyletic clade in the chloroplast *trnL* analysis of Bruneau *et al*. (2001). This clade can be recognized by a combination of morphological characters, and the presence of a large insertion in the *trnL* intron is a synapomorphy for the Detarieae *s.l.* clade (Bruneau *et al*., 2001). It comprises approx. 84 genera (Bruneau *et al*., 2000), which is just over half the 161 genera of subfamily Caesalpinioideae (Lewis *et al*., 2003), and is both economically and ecologically important. Within the Detarieae *s.l.*, there are three well-supported clades: the Detarieae *s.s.*, the *Prioria* clade and the 'Amherstieae' clade; however, relationships among these three clades are not resolved. The *Prioria* clade share many morphological characteristics with members of the Detarieae *s.s.* (gland-

dotted leaves, early caducous stipules, tendency towards apetaly, ten free stamens), but are distinguished by having an ovary with one ovule (Bruneau *et al.*, 2001). The Amherstieae clade includes all members of the tribe Macrolobieae (Breteler, 1995) as well as some of the Amherstieae tribe of Cowan and Polhill (1981). Morphological characters include leaves that are not gland-dotted, persistent intrapetiolar stipules, some apeta-

lous species, and a reduction in stamen number in several species (Bruneau *et al.*, 2001). A group of ten genera was circumscribed as the *Detarium* group in tribe Detarieae (Table 1) by Cowan and Polhill (1981). In the *trnL* analysis of Bruneau *et al.* (2001), eight of these ten *Detarium* group genera form a clade together with eight other genera, and this is designated the Detarieae *s.s.* (Fig. 1; Table 1). Of the two genera present in the *Detarium* group *sensu* Cowan and

TABLE 1. *Aperture structures of the Detarieae* s.s.

Genus	Total no. of species	No. of species with acetolysed pollen examined	No. of species with pollen stained with Alcian blue	Figures	Exinous bridge across meso-aperture	Exinous projections over centre of aperture	Apertures protrude, especially when pollen is dehydrated	Presence of fastigium	Response to Alcian blue stain
Detarieae *s.s.*									
Daniellia Benn.	9	3	1	5B	x	x	+	?	+
Eurypetalum Harms	3	2	1	–	x	x	+	?	+
Eperua Aubl.	14	14	4	–	x	x	+12 of 14 species, x in 2	+/?	+
Augouardia Pellegr.	1	1	1	–	x	x	x	?	+
Stemonocoleus Harms	1	1	1	–	x	x	+	+	+
Baikiaea Benth.	4	4	2	3D, 4B and D	x	x	+	+	+
Copaifera L.	25–30	11	3	2E, 4C, 5D	+/x	+	+	+	+
Pseudosindora Symington	1	1	3	3B	x	+	+	?	+
Detarium Juss.	3	3	1	5A and C	x	?	?	?	+
Sindora Miq.	18–20	10	2	2A–D, and F, 3F	+/x	+	+	+	+
Sindoropsis J. Léonard	1	1	1	3E	x	+	+	?	+
Tessmannia Harms	11	7	2	–	x	x	+	+	+
Gilletiodendron Vermoesen	5	5	2	–	x	x	?	?	+
Hylodendron Taub.	1	1	3	–	x	+	+	+	+
Hymenaea L.	15	10	5	4A	x	x	+	+	+
Peltogyne Vogel	23	7	1	3A and C	x	x	+	?	+
Guibourtia Benn.	16–17	8	2	–	x	x	+	+	+
Schotia Jacq.	6	6	1	5E	x	x	+	?	+
Prioria clade									
Colophospermum J. Léonard	1	1	1	5F	x	x	x	x	x
Prioria Griseb.	1	1	1	–	x	x	x	x	x
Gossweilerodendron Harms	2	2	1	–	x	x	x	x	x
Oxystigma Harms	5	4	1	–	x	x	x	x	x
Kingiodendron Harms	6	4	1	–	x	x	x	x	x
Barnebydendron J.H. Kirkbr.	2	2	2	–	x	x	+	x	+
Goniorrhachis Taub.	1	1	2	–	x	x	x/+	?	+
Amherstieae clade									
Endertia Steenis & deWit	1	1	1	–	x	x	x	x	x
Lysidice Hance	1	1	1	–	x	x	x	x	x
Saraca L.	8	3	1	–	x	x	x	x	x
Cercideae									
Cercis L.	6	1	1	–	x	+	x	?	+

Layout follows the phylogeny of Bruneau *et al.* (2001) (Fig. 1). Genera in bold indicate that they are members of the *Detarium* Group of Cowan and Polhill (1981).

x, absent; +, present; ?, data are missing or inconclusive.

Polhill (1981) but not in the Detarieae *s.s.* of Bruneau *et al.* (2000, 2001), the Brazilian monotypic *Goniorrhachis* is sister to the Detarieae *s.l.* clade (Fig. 1), and *Pseudosindora* was not sampled by Bruneau *et al.* The other eight members of the Detarieae *s.s.* comprise three genera from the *Crudia* group (*Augouardia*, *Stemonocoleus* and *Guibourtia*), two from the *Hymenostegia* group (*Daniellia* and *Eurypetalum*), one from the *Brownea* group (*Eperua*) and two from the *Hymenaea* group (*Hymenaea* and *Peltogyne*). Six further genera of the *Crudia* group form the *Prioria* clade
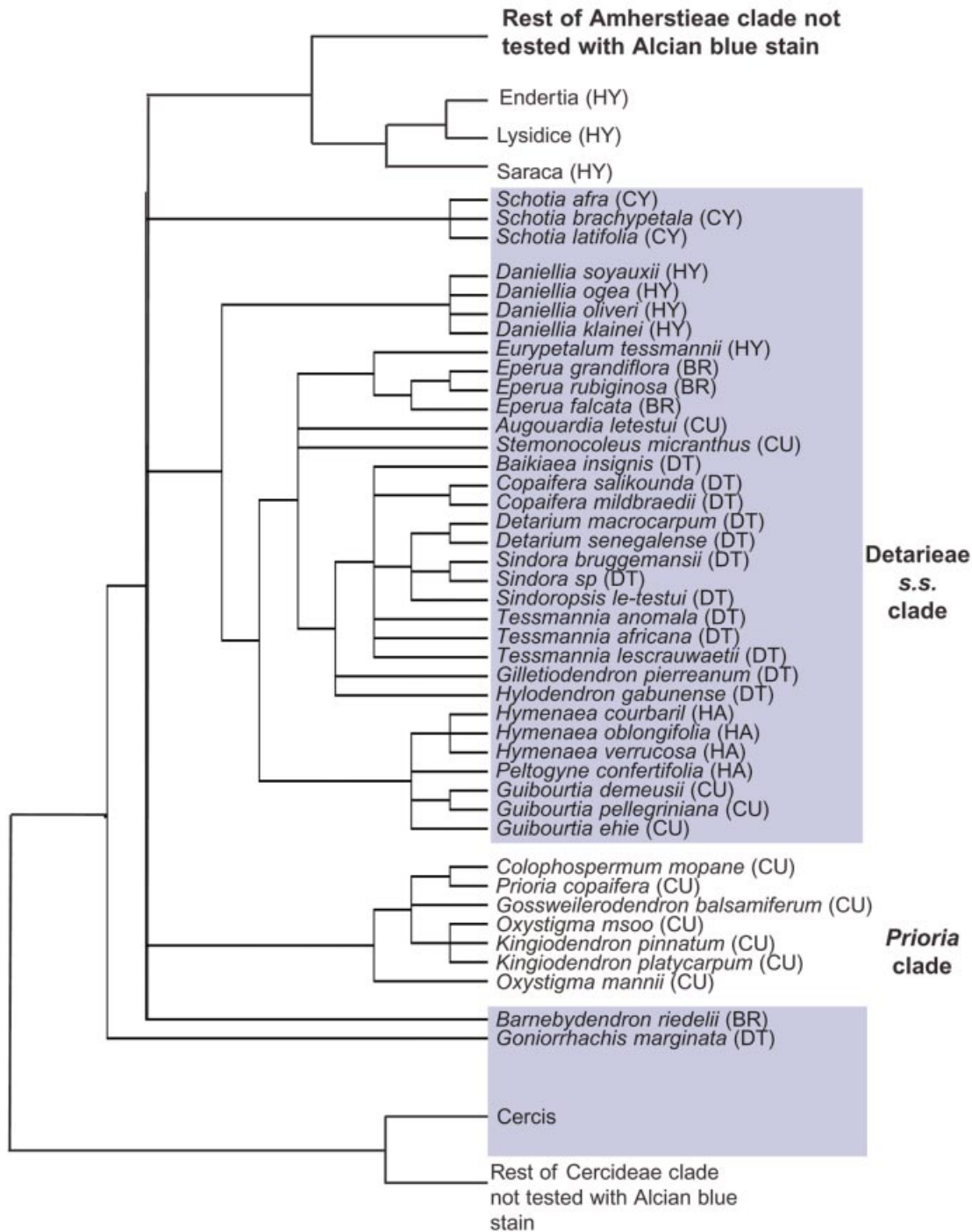
FIG. 1. Phylogeny of Detarieae *s.l.* inferred from *trnL* intron sequences (based on analyses of Bruneau *et al.*, 2001, with permission). The distribution of a positive response to Alcian blue stain (presence of Zwischenkörper) is shown shaded. In parentheses following species names are the generic groups of Cowan and Polhill (1981). CY, *Cynometra* group; HY, *Hymenostegia* group; BR, *Brownea* group; CU, *Crudia* group; DT, *Detarium* group; HA, *Hymenaea* group.

(*Colophospermum*, *Prioria*, *Gossweilerodendron*, *Oxystigma*, *Kingiodendron* and *Hardwickia*).

Pollen morphology of the Detarieae s.s.

Although pollen of taxa in the *Detarium* group has been described by many authors, relatively little attention has been paid previously to aperture structure. For example, Fasbender (1959) carried out a detailed survey of caesalpinioid genera using light microscopy (LM), but only described 'some consticticolpi' in *Detarium*, and *Tessmannia* pollen as being crassimarginate. Although pollen samples of *Copaifera* and *Sindora* were examined, the survey did not include species with exinous bridges. A survey of the Caesalpinioideae using scanning electron microscopy (SEM) (Graham and Barker, 1981) concen-
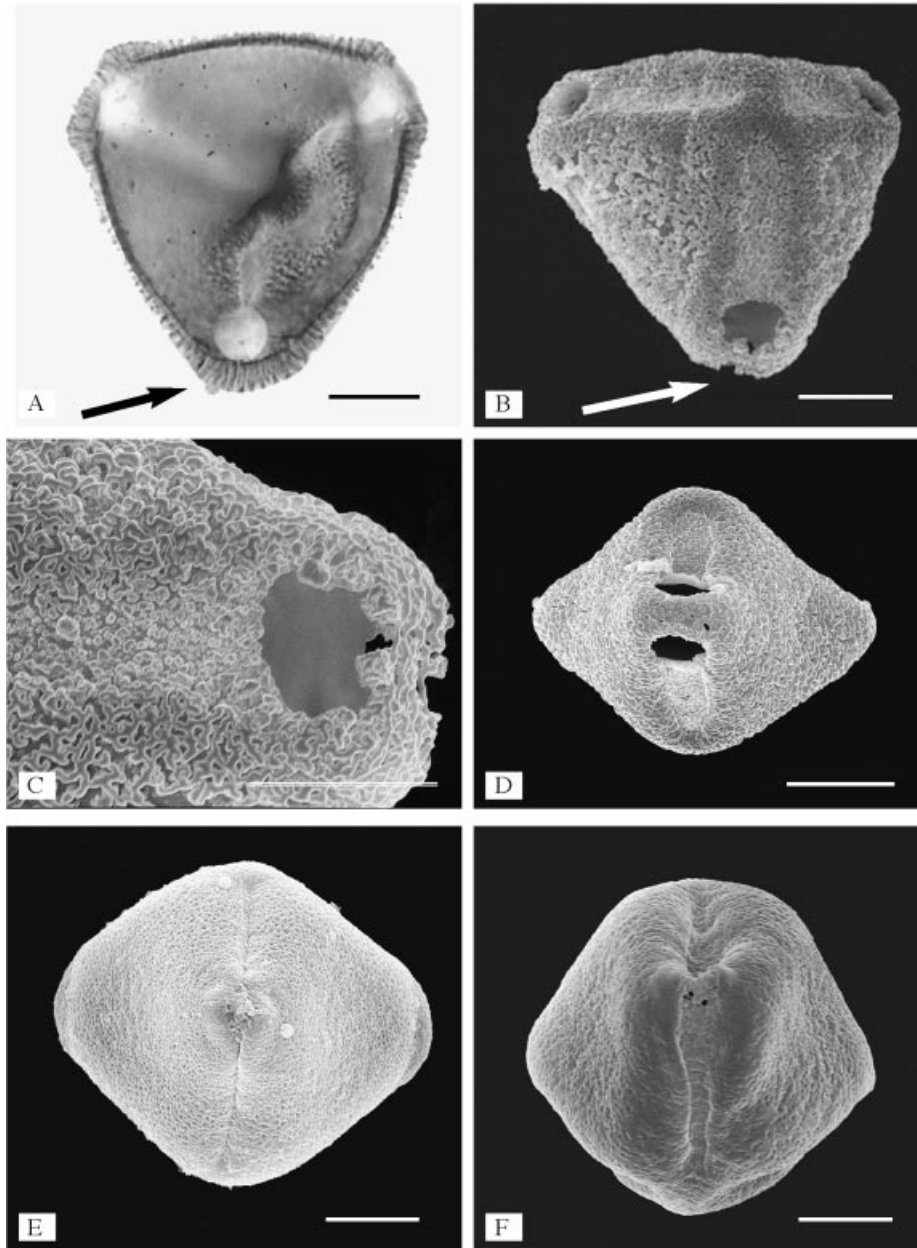

FIG. 2. Aperture structure of *Sindora* and *Copaifera* illustrated using acetolysed pollen. A, *Sindora maritima*: polar view showing columellate exinous bridges across the centre of the apertures (arrow, LM) (compare with B showing a grain in similar orientation illustrated using SEM, and Fig. 5A where no exinous bridge is present). B, *Sindora coriacea*: polar view showing a grain with exinous bridges across the centre of the colpori (arrow) in oblique polar view (SEM). C, *Sindora coriacea*: detail of exinous bridge (SEM). D, *Sindora maritima*: equatorial view showing exinous bridges across centre of the aperture (SEM). E, *Copaifera multijuga*: equatorial view showing exinous projections, rather than exinous bridges, across centre of the apertures (SEM). F, *Sindora klaineana*: equatorial view showing exinous projections, rather than exinous bridges, across the centre of the apertures (SEM). Scale bars = 10 μm in A–C, E and F, 20 μm in D.

trated mainly on surface ornamentation of the group; and another using transmission electron microscopy (TEM) (Ferguson, 1987) concentrated on exine stratification. However, apertures are often one of the most important features of pollen with regard to providing informative characters for systematic analysis (e.g. Blackmore and Crane, 1998). A *Sindora* pollen type, distinct due to paired endoapertures in the colpi (Fig. 2A–D), is probably the earliest record of Leguminosae pollen from the Maastrichtian of Siberia, Canada and Colombia (Muller,

1981; Herendeen *et al.*, 1992). Despite such an early appearance (around 70 million years BP) of this pollen type in the fossil record, apertures separated by a broad bridge of exine across the centre of the aperture at the equator, creating two distinct endoapertures in the apertures (Fig. 2C and D), have only been reported previously to occur in the pollen of the genus *Sindora*. Within the Caesalpinioideae, the exinous bridge structure has only been reported in taxa of the Detarieae *s.s.* Within the Leguminosae, *Dumasia villosa* DC. in subfamily Papilionoideae (Ferguson and
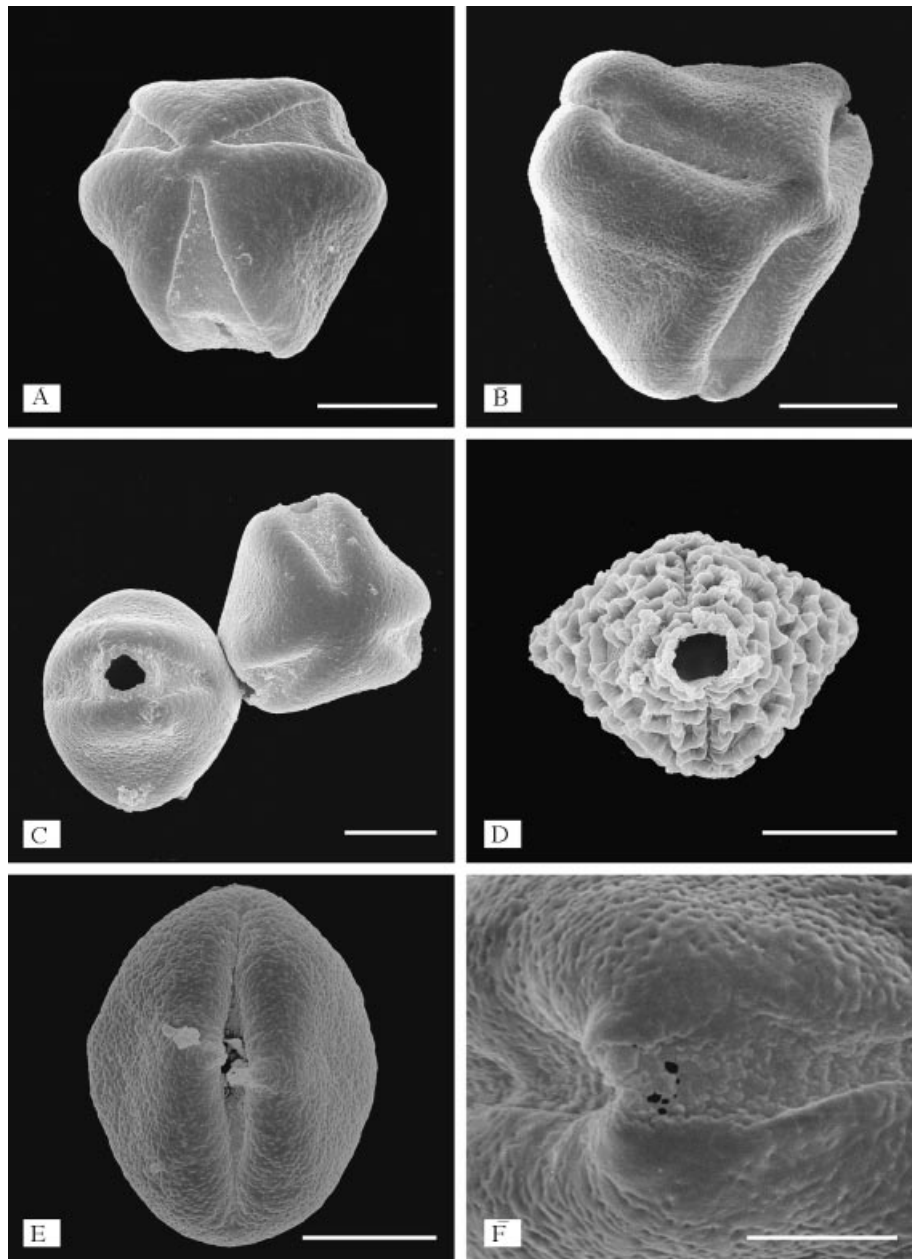

FIG. 3. Protruding apertures and exinous projections. A, *Peltogyne paniculata*: polar view showing protruding apertures compared with mesocolpial areas (SEM). B, *Pseudosindora palustris*: oblique view showing protruding apertures (SEM). C, Two grains of *Peltogyne lecointei* showing protruding apertures (SEM). D, *Baikiaea suzannae*: equatorial view showing protruding apertures (SEM). E, *Sindoropsis letestui*: equatorial view showing protruding apertures and exinous projections over the centre of the apertures (SEM). F, *Sindora klaineana*: close-up of aperture showing exinous projections (SEM). Scale bars = 10 μm in A–E, 5 μm in F.
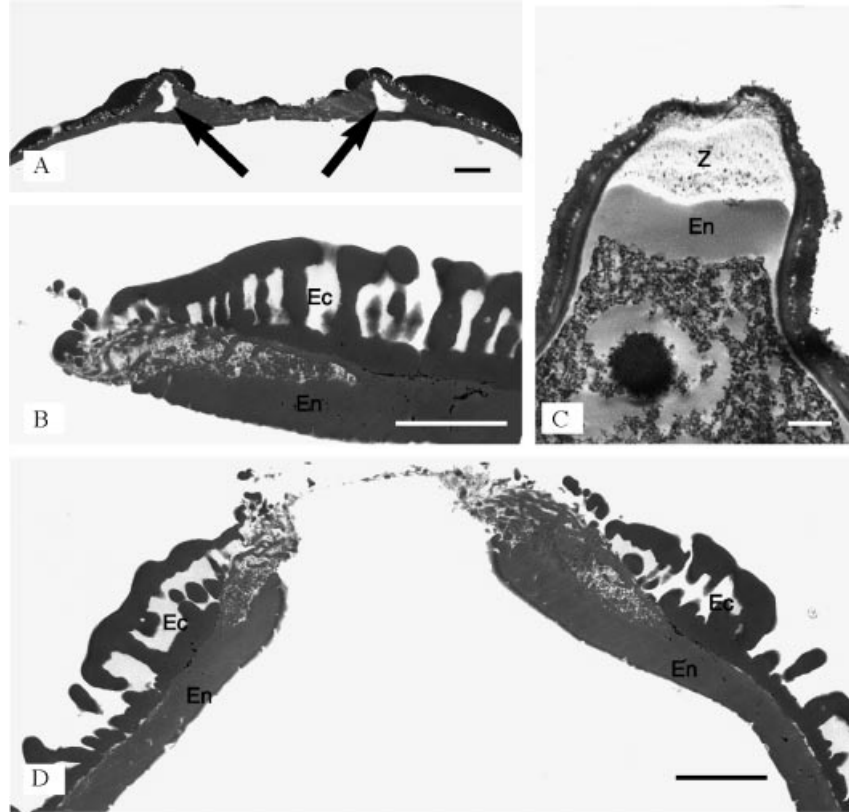
FIG. 4. Zwischenkörper in TEM. A, *Hymenaea velutina*: transverse section through edge of endoaperture area showing fastigium; arrows show separation of ectexine from endexine (TEM). B, *Baikiaea plurijuga*: transverse section of the pollen wall one side of the endoaperture area showing a wedge-shaped separation of the ectexine (Ec) and endexine (En) (TEM). C, *Copaifera baumiana*: section through the endoaperture of non-acetolysed pollen showing Zwischenkörper (Z) beneath the endoaperture above the thickened endexine (En) (TEM). D, *Baikiaea plurijuga*: section through the endoaperture area showing fastigium and some separation of endexine (En) and ectexine (Ec). All parts show that the ectexine becomes thinner at the aperture margins. The endexine is slightly thickened at the aperture margins, but not enough to account for the prominence of the endoapertures. Scale bars = 2 µm.

Skvarla, 1981) is the only other species so far found to have an exinous bridge across the centre of the aperture, in this case separating two pores.

In some genera of the former *Crudia* group (Table 1; Fig. 1), Banks and Gasson (2000) showed that the apertures do not buckle inwards in dehydrated pollen, as in most other legume pollen (Heslop-Harrison, 1976; Banks and Gasson, 2000; Banks and Klitgaard, 2000), but appear to be protruding and fixed open. Consequently the mesocolpial wall buckles inwards (Figs 2F and 3A–C) (see also Crane, 1986, p. 186; Banks and Klitgaard, 2000, Figs 47 and 48). This could be significant to the wall structure. Instead of a convex bend to the wall associated with tensile forces exerted on the tectum and infratectum, created by apertural areas buckling inwards, a convex bend caused by meso-colpial wall buckling inwards will be associated with compressive forces on the tectum and infratectum (Crane, 1986).

MATERIALS AND METHODS

Pollen material was obtained from the herbarium of the Royal Botanic Gardens, Kew (K) (Table 1). A full list of samples examined can be obtained from the author. Anthers

were taken from mature, unopened buds. Standard acetolysis techniques were employed to initially survey 360 samples of 83 detarioid genera (Banks and Klitgaard, 2000). This study presents pollen aperture morphology of 148 species out of approx. 200 in 29 genera representing the Detarieae *s.s.* and relatives (Table 1). Mature, unopened buds were dissected in a 1 % solution of Libsorb using a Leica Wild M8 micro-scope. Pollen was acetolysed using the method of Erdtman (1960), and prepared for light microscopy (LM) by mount-ing in glycerol jelly. Measurements were recorded using a Nikon Labophot LM. Acetolysed pollen exines in 95 % ethanol were allowed to air-dry onto specimen stubs, sputter-coated with platinum, and examined in a Hitachi S-2400 SEM. All acetolysed samples were examined using LM and scanning electron microscopy (SEM).

Selected samples of acetolysed material were fixed with a 2 % solution of osmium tetroxide in cacodylate buffer, pre-stained with 0·5 % uranyl acetate, embedded in Epon–Araldite resin following the method of Skvarla (1966), thin-sectioned, post-stained with uranyl acetate and lead citrate, then examined using a Hitachi H-300 transmission electron microscope (TEM).

For examination of the protruding apertures, pollen samples of *Baikiaea plurijuga* Harms and *Detarium*
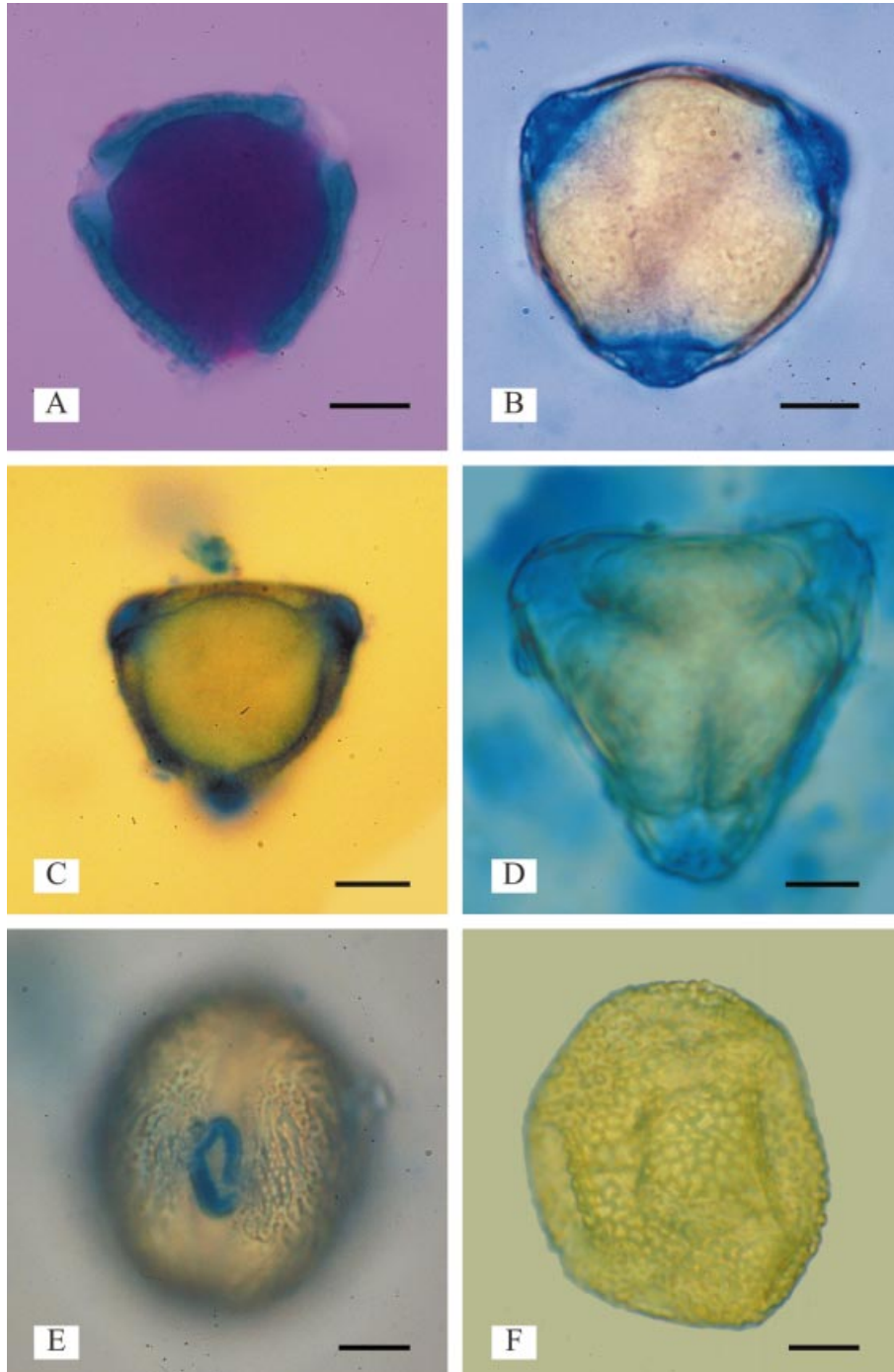
FIG. 5. Zwischenkörper below endoapertures (LM). A, *Detarium senegalense* pollen showing dark red cytoplasm and green-blue exine stained with Alexander's stain. The area below the endoapertures (Zwischenkörper) has not stained, showing that it is not composed of exine or cytoplasm (LM). B, *Daniellia oliveri*: Zwischenkörper stained with Alcian blue (LM). C, *Detarium senegalense*: Zwischenkörper stained with Alcian blue (LM). D, *Copaifera cnearensis*. E, *Schotia brachypetala*: an area encircling the endoaperture is stained with Alcian blue (LM). F, *Colophospermum mopane* showing no staining with Alcian blue. Scale bars = 10 µm.

senegalense J.F. Gmel. were taken from mature anthers and stained with Alexander's stain (Alexander, 1969). After warming gently for about 30 s, the pollen was examined and photographed using a Nikon Labophot LM.

Forty-six samples of pollen from 29 detarioid genera were taken from mature anthers and stained with Alcian blue (Table 1). Where possible, samples were selected for this pollen study to match the species used for the molecular

analysis of Bruneau *et al*. (2001) (Fig. 1). It should be noted, however, that the staining response in pollen taken from some older specimens was not as good as that of younger specimens. After 5–10 min, the pollen was examined and photographed using a Nikon Labophot LM.

Unacetolysed pollen of *Copaifera baumiana* Harms from spirit material was embedded in Epon–Araldite resin following the method of Skvarla (1966) and thin-sectioned using a diamond knife. Some sections were stained with Alcian blue and examined using a Nikon Labophot LM, other sections were post-stained with uranyl acetate and lead citrate in an LKB 2168 Ultrostainer. Sections were examined using a Hitachi H-300 TEM.

Terminology follows Punt *et al*. (1994).

RESULTS

This study found apertures with exinous bridges over the endoaperture region at the equator in the pollen of eight out of the 18–20 species in *Sindora* (of 13 species examined), and one species out of the 25–30 in *Copaifera* (of 16 species examined) (Fig. 2A–D). Pollen of the other species within these two genera has apertures with exinous projections over the centre of the aperture (Fig. 2E and F). This type of aperture structure is also found in the pollen of *Detarium, Pseudosindora* (Fig. 3B)*, Hylodendron* and *Sindoropsis* (Fig. 3E). The pollen of *Baikiaea* (Fig. 3D), *Copaifera* (Fig. 2E), *Sindora* (Fig. 2B and D), and *Tessmannia* have greatly protruding apertures that form diamond-shaped pollen (Figs 2D and E and 3D), and the pollen of other taxa in the Detarieae *s.s.* have protruding apertures that are more pronounced in dehydrated pollen. There were no structures present in acetolysed pollen to account for the protruding apertures illustrated in Figs 2 and 3. No thickening of the exine was observed around the apertures, in fact thin section images of acetolysed pollen show that a cavity or fastigium is present in the endoaperture area in some taxa (Table 1). On either side of the endoaperture, a separation of the ectexine from the endexine occurs in the wall structure (Fig. 4). In unacetolysed pollen stained with Alexander's stain (Fig. 5A), exine is stained turquoise and cytoplasm magenta. A lens-shaped structure in the endoapertural area can be seen, although it is not stained. TEM sections of unacetolysed pollen also show a structure present overlying the endexine (Fig. 4C). Alcian blue was found to stain these structures preferentially (Fig. 5B–E). The structures observed fit the definition of a Zwischenkörper described by Rowley (1964), Heslop-Harrison and Heslop-Harrison (1980, 1981), Heslop-Harrison *et al*. (1986), El-Ghazaly and Jensen (1987), Heslop-Harrison and Heslop-Harrison (1991) and El-Ghazaly (2000). The presence of this structure causes the apertures to protrude. Removal of the structure by acetolysis leaves a fastigium in the endoaperture area, and wedge-shaped separation of the ectexine from the endexine in the endoaperture margins (Figs 4A–D; Table 1). Fastigia are present in all pollen that stain positively with Alcian blue except for *Barnebydendron*. However, sections have to be cut precisely across the endoaperture area in order to see whether fastigia are present, and it is possible that in this case the sectioning was not in exactly the right region.

In pollen from the closely related *Prioria* clade, the apertures are not protruding (Fig. 1), and Alcian blue did not preferentially stain the aperture areas (e.g. *Colophospermum*, Fig. 5F). In the one sample of *Schotia* examined, a protruding ring is present around the endoaperture (Banks and Klitgaard, 2000, Fig. 30) and only this area stains positively with Alcian blue (Fig. 5E). It has the appearance of an annulus, but the exine is not thickened. This suggests that the protrusion is created by the underlying structure that stains in response to Alcian blue.

DISCUSSION

Distribution of Zwischenkörper

This is the first report of Zwischenkörper in legume pollen. Projecting apertures with underlying Zwischenkörper are present in all genera that form the Detarieae *s.s.* clade plus *Schotia, Barnebydendron* and *Goniorrhachis* (Table 1; Fig. 1), and *Cercis* which occurs in a basal position in the sister group (tribe Cercideae) in the molecular phylogeny of Bruneau *et al*. (2001). Alcian blue stain tests indicate that Zwischenkörper are also present in putative sister families Quillajaceae and Surianaceae (F. Claxton, pers. comm., 2002). Zwischenkörper are absent from the *Prioria* clade and three genera in the Amherstieae clade that form the sister group to the rest of the Amherstieae (*Endertia, Lysidice* and *Saraca*) in the molecular phylogeny of Bruneau *et al*. (2001). Zwischenkörper may have been either lost twice from the Amherstieae and *Prioria* groups, or there could have been one loss for both clades; however, this remains ambivalent, since there is currently lack of resolution at this node, resulting in a polytomy (Fig. 1). Zwischenkörper were not detected in pollen that does not have protruding apertures or exinous projections, although a more extensive survey would be required to confirm a correlation. However, results so far suggest that exinous projections are present where the apertures protrude due to the presence of Zwischenkörper (Table 1; Fig. 3F). Exine that projects over the centre of the apertures is present in the pollen of other groups of the Leguminosae (Banks and Klitgaard, 2000; Banks *et al*., 2003) and in putative sister families Quillajaceae and Surianaceae (F. Claxton, pers. comm., 2002).

Definition of Zwischenkörper

The term 'Zwischenkörper' was first used by Fritzche (1837), and translated by Beer (1906) as 'interstitial body'. Zwischenkörper and onci have been described previously in grass and *Corylus* pollen (Rowley, 1964; Heslop-Harrison and Heslop-Harrison, 1980, 1981, 1991; Heslop-Harrison *et al*., 1986; El-Ghazaly and Jensen, 1986*a*, *b*, 1987). There has been ongoing confusion concerning terminology; both 'oncus' and 'Zwischenkörper' have been used interchangeably to describe structures present under the apertures. Some literature (e.g. El-Ghazaly, 1999*b*) suggests that

Zwischenkörper are always present in microspores, but by maturation of the pollen grains they are replaced by an intinous oncus.

Blackmore and Crane (1988) described endoapertures as being formed during development where the presence of onci disrupts endexine formation. Onci determine pollen size and shape during dehydration and rehydration (Frenguelli *et al.*, 1997). Punt *et al.* (1994) defined an oncus as 'a lens-shaped structure that is not resistant to acetolysis and occurs beneath the apertures of many kinds of pollen grains'. Zwischenkörper had the same description, but with the comment that, although it resembles an oncus it is 'treated as distinct because some pollen grains have both features'. Zwischenkörper comprise a layer of well-developed pectic substances, and physically overlie and seal off the middle part of the intine (Heslop-Harrison and Heslop-Harrison, 1991).

A review and advice on the confused terminology applied to structures underlying pollen apertures are given by Rodríguez-García and Fernández (1988). These authors state that both onci and Zwischenkörper have different origins, and that it is yet to be determined whether both have similar or different roles in development and germination. They suggest that it is appropriate to use the term 'oncus' for both, 'as long as the origin is clearly stated as intinous or exinous'. However, the structures are not only different in origin (Rodríguez-García and Fernández, 1988), but are also different histochemically (Heslop-Harrison and Heslop-Harrison, 1980; El-Ghazaly and Jensen, 1987). Intine stains with Aniline blue-black, Zwischenkörper do not stain with Aniline blue-black (El-Ghazaly and Jensen, 1987). Zwischenkörper stain with Alcian blue, intine does not stain with Alcian blue (Heslop-Harrison and Heslop-Harrison, 1980). Rowley (1975) compared aperture formation in *Silene*, *Tradescantia*, *Nelumbo* and *Epilobium*, and found several possibly distinct apertural induction processes.

Because the first use of the term 'Zwischenkörper' was applied to the structure that lies above the intine and stains with Alcian blue (Fritzche, 1837; Beer, 1906; Rowley, 1964; Heslop-Harrison and Heslop-Harrison, 1980), it is continued to be used it here to apply to those analogous structures in detarioid legumes. The term 'onci' is used for those structures that are intinous in origin and do not stain with Alcian blue.

Zwischenkörper function

Blackmore and Barnes (1986) pointed out that similar needs for harmomegathy and germination are met through extremely diverse mechanisms in different species. It is probable that much of this diversity has arisen through compromises with selection for other functions, such as mechanical efficiency, economy of materials, adaptations related to storage and release of exine and intine held substances, or adaptations for dispersal, all constrained by ontogenetic inheritance.

Although reported to be present during early development, Zwischenkörper are not always present in mature pollen grains (El-Ghazaly, 1999*b*). In the pollen of *Olea* the Zwischenkörper are greatly reduced at grain maturity and replaced by an oncus developed from intinous material (Rodríguez-García and Fernández, 1988). Zwischenkörper have been described as structures that assist germination of the pollen tube. Heslop-Harrison and Heslop-Harrison (1991) and Heslop-Harrison *et al.* (1986) related Zwischenkörper function to hydration, activation, germination and early pollen tube growth. Of the layers present in the structure of the endoapertures, both papers described a 'central protein-bearing layer', which at germination discharges its proteins into the stigma during early hydration. It is suggested that these proteins could be associated with recognition processes, and also present are enzymes that assist with pollen tube growth. Secondarily, gelatinizing pectins hydrate, expand and push out of the aperture along with any operculum or encapsulating sporopollenin. The hydration of Zwischenkörper functions even in dead pollen, the germination process ceases at the point where the pollen tube tip should differentiate. Other instances are found of intine functions in osmoregulation, e.g. Cresti and Tiezzi (1990) describe the same pollen hydration and germination process as described by Heslop-Harrison and Heslop-Harrison (1980, 1981), although in this case the structure comprises 'the outer layer of the intine'. Zwischenkörper in the pollen of *Betula* are described and illustrated, using chemical fixation and rapid-freeze fixation techniques, in El-Ghazaly (2000). This study suggests that the helical units within the Zwischenkörper observed by freeze-substitution fixation support the hypothesis that the Zwischenkörper has a transportation function. A good review of the development of Zwischenkörper in *Betula* is given in El-Ghazaly (1999*b*), and of *Olea* in Rodríguez-García and Fernández (1988). El-Ghazaly (1999*a*) suggest that *Betula* allergen studies provide histochemical evidence that the intine differs from the Zwischenkörper, that the timing of formation and protein within the structures is different, and that the two layers have different functions.

Systematic information from pollen morphology

In the Detarieae *s.l.*, varied pollen surface ornamentation types can provide useful taxonomic information, but the presence of mixtures of patterns, intermediate forms, and similar surfaces with differing underlying structure, prevent surface ornamentation from being defined for use as characters for cladistic analysis (Banks and Klitgaard, 2000). Aperture morphology and wall structure provide a better source for additional characters to further resolve the phylogeny. The presence of Zwischenkörper in the Detarieae *s.s.* clade, plus *Schotia*, *Barnebydendron*, *Goniorrhachis* and *Cercis*, suggests a closer relationship between the *Prioria* clade and Amherstieae clade than between the Detarieae *s.s.* and Amherstieae clade. Other pollen characters that could provide further systematic information towards resolving relationships include the extra infratectal layer found in the wall structure of *Augouardia*, *Stemonocoleus* and most species of *Eperua*; a similar but more granular structure is also seen in *Prioria* and *Gossweilerodendron*. Some species of *Sindora* and *Copaifera* have exinous bridges across the centre

of the apertures; additionally, *Detarium*, *Pseudosindora*, *Hylodendron* and *Sindoropsis* have exinous projections over the centre of the aperture. Psilate aperture membranes occur in 13 genera of the Detarieae *s.l.* including all genera of the *Prioria* clade. Aperture number may be a useful character in higher level systematic studies. It is intended that the pollen characters discussed here (and in Banks *et al.*, 2003) will be added to other morphological and molecular datasets for future phylogenetic analyses.

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