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Morphology, Anatomy and Ontogeny of Female Cones in *Acmopyle pancheri* (Brongn. & Gris) Pilg. (Podocarpaceae)

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The developmental morphology and anatomy of the female cones of *Acmopyle pancheri* (Brongn. & Gris) Pilg. (Podocarpaceae) are described and illustrated, based on observations, histology, scanning electron microscopy (SEM) and nuclear magnetic resonance (NMR) imaging. Ovulate development is typically podocarpaceous. Ovules are unitegmic, and horizontal or inclined upwards throughout ontogeny; the pollination drop is inverted because of the declinate micropyle. Ontogeny of the epimatium-ovule complex is acropetal, the epimatium developing first. A terminal, distal sterile bract creates a pollen-scavenging area. During development, the whole cone re-orientates through some 270°, and the seed realigns approx. 60° with respect to the receptacle axis. The 'receptacle' or podocarpium supporting the seed is formed by gradual fusion of initially free bracts. The structures adnate to these bracts represent homologues of ovuliferous scales; they bear vestigial epimatia which may develop into supernumerary ovules or non-functional epimatia. Thus, female cones of *A. pancheri* are vestigially multi-ovulate. NMR imaging effectively and non-invasively revealed the three-dimensional arrangement of vascular bundles and resin canals in the cones.

Key words: *Acmopyle pancheri* (Brongn. & Gris) Pilg., anatomy, developmental morphology, gymnosperms, histology, nuclear magnetic resonance (NMR) imaging, ontogeny, ovules, Podocarpaceae, scanning electron microscopy (SEM), seed cones.

INTRODUCTION

Acmopyle Pilg. is one of the most interesting genera in the Podocarpaceae because of its intriguing palaeohistory and evolution (Hill and Carpenter, 1991; Hill and Brodribb, 1999), unusual vegetative morphology (Sahni, 1920) and conservation status (WCMC, 2000). It comprises two living species: *A. pancheri* (Brongn. & Gris) Pilg. on New Caledonia and *A. sahniana* J. Buchholz & N. E. Gray on Fiji (Farjon, 1998). Both are threatened taxa; *A. pancheri* is cultivated at the Royal Botanic Garden Edinburgh (RBGE), UK as part of the RBGE International Conifer Conservation Programme.

Both species of *Acmopyle* are dioecious. Little was known about the reproductive biology of either species until recently, when that of *A. pancheri* was outlined briefly (Möller *et al.*, 1999). Indeed, the first full morphological description of *A. sahniana* appeared very recently (Bush and Doyle, 1997) but contained no details of its reproductive biology. Most of the information available for *A. pancheri* is morphological or floristic (de Laubenfels, 1969, 1972; Gaussen, 1974). Sahni (1920) investigated its gross morphology and anatomy but his paper, which is still the standard reference, contained no information on female cone development. Tomlinson (1992) presented the first scanning electron microscopy (SEM) survey of ovulate cone development in the Podocarpaceae, covering most genera but not *Acmopyle*.

The pollination mechanism of *A. pancheri* was documented by Möller *et al.* (2000). Here we present the first documentation of the morphology and development of the female cones of *A. pancheri*, both before and after pollination, and compare them with those of other members of the Podocarpaceae.

Terminology

Female cones at stages up to and including receptivity are here referred to as ovulate cones, while post-receptivity cones are termed post-pollination cones. 'Female cone' is used in statements applicable to more than one developmental stage, or where (e.g. referring to literature) the developmental stage is imprecisely known. Cones consist of a 'receptacle' (Gibbs, 1912; Page, 1990; more accurately a podocarpium); because the term 'receptacle' is firmly entrenched in Podocarpaceae literature, it is retained here. This receptacle is formed by the metamorphosis of several bracts. Previous authors have distinguished between sterile bracts (most of the receptacular bracts) and the usually single fertile bract that bears the seed. This historical distinction is retained here for convenience, although results presented here indicate that, in *Acmopyle*, it reflects neither the true morphology of the cone nor its potential reproductive capability. The whole structure (peduncle, bracts/receptacle, ovule(s)/seed, regardless of developmental stage)

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is termed the seed cone complex. Terminology of the principal tissue layers of the seed (sarcotesta, sclerotesta, endotesta, prothallus, embryo) follows Sporne (1974), except that endotesta is used for the inner fleshy layer termed 'inner sarcotesta' by Sporne. The epimatium is a specialized structure, unique to the Podocarpaceae, that supports the ovule (Tomlinson, 1992). The terms bilateral and bifacial leaf flattening are standard for the Coniferales (de Laubenfels, 1953) and have been used in all relevant literature on the Podocarpaceae (e.g. Wells and Hill, 1989; Tomlinson, 1992). By far the most common type of flattening, as found, for example, in yew (*Taxus* L.) and hemlock (*Tsuga* Carr.), is bifacial, in the horizontal plane; bilaterally flattened leaves are flattened in the vertical plane.

MATERIALS AND METHODS

Plant material

Living material of *A. pancheri* was used, belonging to RBGE accession 19842681 collected on New Caledonia in 1984 (Province Sud, Mont Mou, *C. N. Page* 22172; voucher, **E**) and cultivated under warm-temperate glasshouse conditions. This accession comprises several plants of both sexes from different wild trees and consequently belonging to different genotypes, and allows natural pollination of female plants. Observations began in January 1999, when pollination drops of the 1998/99 reproductive season were present. Individual cones were tagged to follow their development. Maturing post-pollination cones from the 1997/98 season were also used.

Early stages of ovulate development were followed using conventional observations, histology and SEM. Temporal internal changes in cones at various post-pollination developmental stages were studied non-invasively using nuclear magnetic resonance (NMR) imaging. This has the potential to build up a three-dimensional picture of the cone's internal structure in a way that is impossible, in *A. pancheri*, using conventional histology alone.

Histological studies

Free-hand sections (L.S. and T.S.) were made through cones at post-pollination developmental stages from receptivity to maturation. Thin sections were made using a freezing microtome (Reichert 'Om P', Vienna, Austria) and stained with safranin and fast green, or with Toluidine blue. The contents of 'resin' canals were stained with Sudan III. These techniques were used to validate NMR images (see below). Hand-sectioning and microtoming of whole cones for histological studies was possible during early stages of cone development. As the cone matured, conventional microtoming of the seed portion became impossible due to the development of an extremely hard sclerotesta. Older seeds (>6 months) could only be cut (with difficulty) using a Proxxon Minimod 40E miniature circular saw.

Photography

Tagged cones were photographed *in situ* and *ex situ* using materials and methods previously reported (Möller *et al.*, 2000). Histological sections were photographed using a Zeiss Axiophot microscope.

Scanning electron microscopy

Young shoot tips were fixed overnight in a standard solution of FAA (formalin : acetic acid : ethanol) and dehydrated through an ethanol series to 100 % acetone prior to critical point drying (Emitech K850). They were mounted on aluminium stubs using adhesive carbon discs, sputter coated with a gold/palladium target (Emscope SC500), and examined under a Zeiss DSM 962 scanning electron microscope at 15 kV. For examination of the youngest stages, it was necessary to remove some of the apical sterile bracts to reveal the ovule.

NMR imaging

NMR imaging was carried out in a Bruker AMX300 SWB spectrometer. Intact specimens were placed in capped glass tubes to retain moisture and positioned in coils ranging in diameter from 10 mm for the horizontal phase of cone development to 25 mm for fully ripe cones. Voxel sizes ranged from $(62{\cdot}5\ \mu m)^3$ to $(390\ \mu m)^3$. Three-dimensional spin echo images were acquired with echo times of 6·56 ms and repeat times of 500 ms and thus display some T_1- and T_2-weighting. For further explanation of NMR imaging and its utility in plant biological research see Callaghan (1991), Chudek and Hunter (1997), Glidewell *et al.* (1997), Ishida *et al.* (2000), Möller *et al.* (2000) and Masson *et al.* (2001).

RESULTS

Phenology

Initiation of ovulate cone primordia was observed in September, epimatium and ovule initiation from mid-November. Cones became receptive, with pollination drops, 4–6 weeks after ovule initiation. The receptive period extended from mid-December to mid-February; cone development was not synchronous, several stages being present at any given time. Cones from the 1997/98 season ripened in May 1999, about 16 months after receptivity. The receptacles of some cones from the 1998/99 season ripened after about 8 months, beginning in August 1999; most did not ripen until spring 2000.

Position and number of female cones

Seed cone complexes in *A. pancheri* develop subterminally or terminally on lateral foliage shoots. Cones are also occasionally axillary, either on foliage shoots, or to scale leaves on the axis (Fig. 1C). Though usually solitary, cones may arise in groups of up to three (Fig. 1A). The same foliage shoot increment may bear cones in successive years (Fig. 1B, current year's receptive cone arrowed).

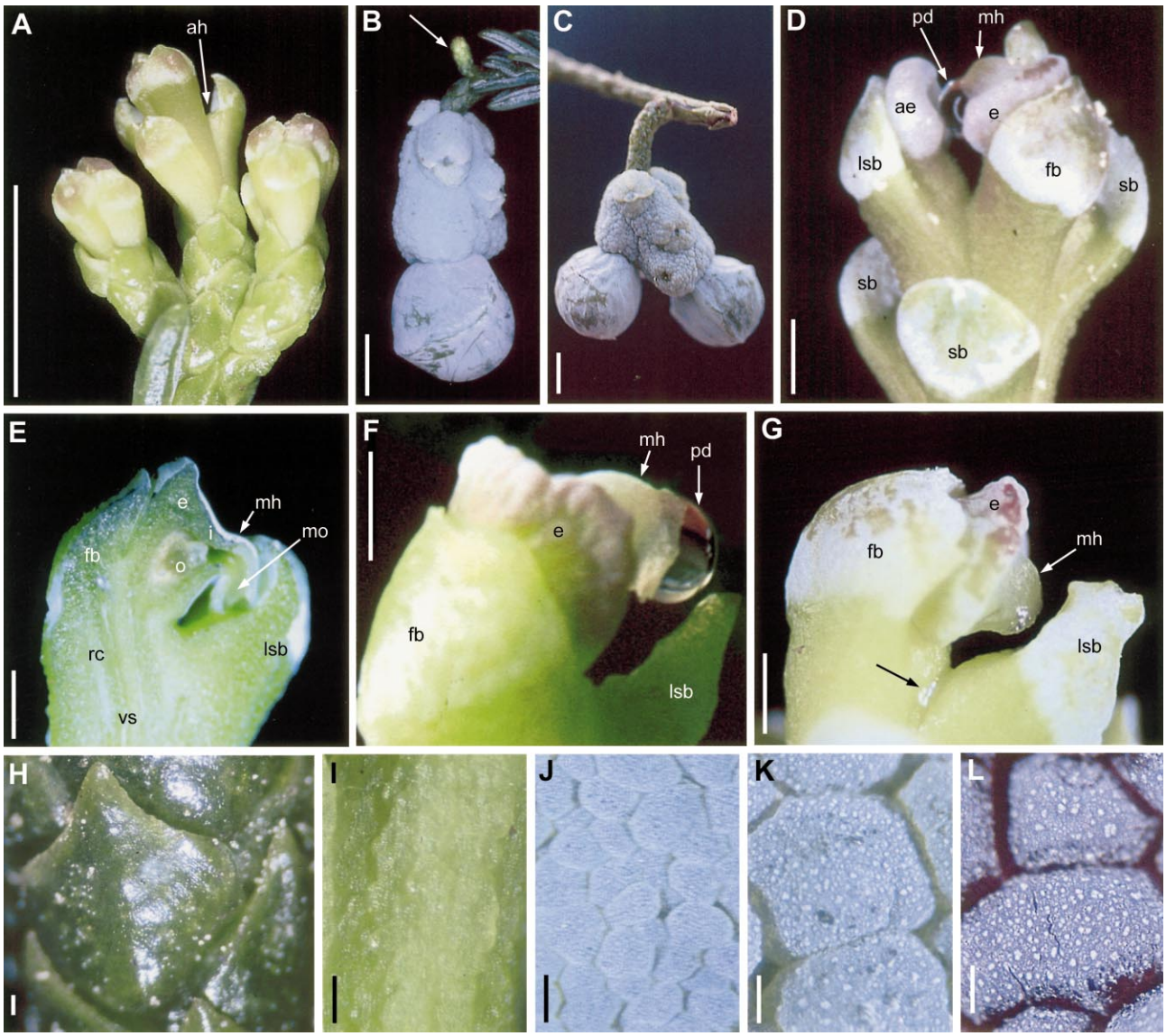


FIG. 1. *Acmopyle pancheri*, cone morphology. A, Shoot with one pseudoterminal cone complex and two lateral cone complexes immediately below. B, Resting-phase cone of previous year accompanied by pre-receptive cone of current year (arrow). C, Two-seeded resting-phase cone. D, Cone with one normal epimatium-ovule complex (right) and one in which the epimatium has developed more or less normally but no ovule has differentiated (left). E, Longitudinal section of pre-receptive ovule. F, Ovule showing micropylar hook and pollination drop. G, Ovule at receptivity, showing an extended pollen scavenging area (black arrow). H, Peduncle bracts (abaxial surface). I, Papillate surface of receptacle of cone 2 weeks after receptivity. J, Surface of receptacle of 6-week-old horizontal-phase cone. K, Surface of receptacle of resting-phase cone. L, Surface of receptacle of ripe cone. ae, Abnormal epimatium; ah, axillary hump; e, epimatium; fb, fertile bract; i, integument; lsb, last (=uppermost) sterile bract; mh, micropylar hook; mo, micropylar orifice; o, ovule; pd, pollination drop; rc, 'resin' canal; sb, sterile bract; vs, vascular supply. Bars = 5 mm (A–C), 1 mm (D–G), 100 μm (H) and 200 μm (I–L).

Seed cone complexes: phyllotaxis, peduncle and bracts

Ovulate cones (Fig. 2A) and vegetative shoot tips (Fig. 2D) both have 3/8 phyllotaxis. In ovulate cones, the lowest three sterile bracts arise from virtually the same point at the cone base, the others arise spirally on its axis.

The cone peduncle only shows a slight increase in size during maturation, the main change being in its orientation. It is subtended by one or two transitional foliage leaves and is clad with spirally arranged bracts, each having a rhombic, 'fingernail-like' tip (Fig. 1H).

Sterile bracts differ from the peduncle bracts, being, except for the uppermost, paler, swollen and more yellow-green (Fig. 1A). The smaller, awl-shaped, uppermost sterile bract is the terminal organ of the reproductive shoot (Fig. 2A and C), being distal to the fertile bract. The change from peduncle bracts to sterile bracts is abrupt, with none being morphologically intermediate. Unlike the peduncle bracts, the sterile bracts are differentiated into a decurrent lower and a free upper part. The latter is further differentiated into proximal and distal regions whose boundary is marked by a stomatiferous zone. Unlike the remainder of

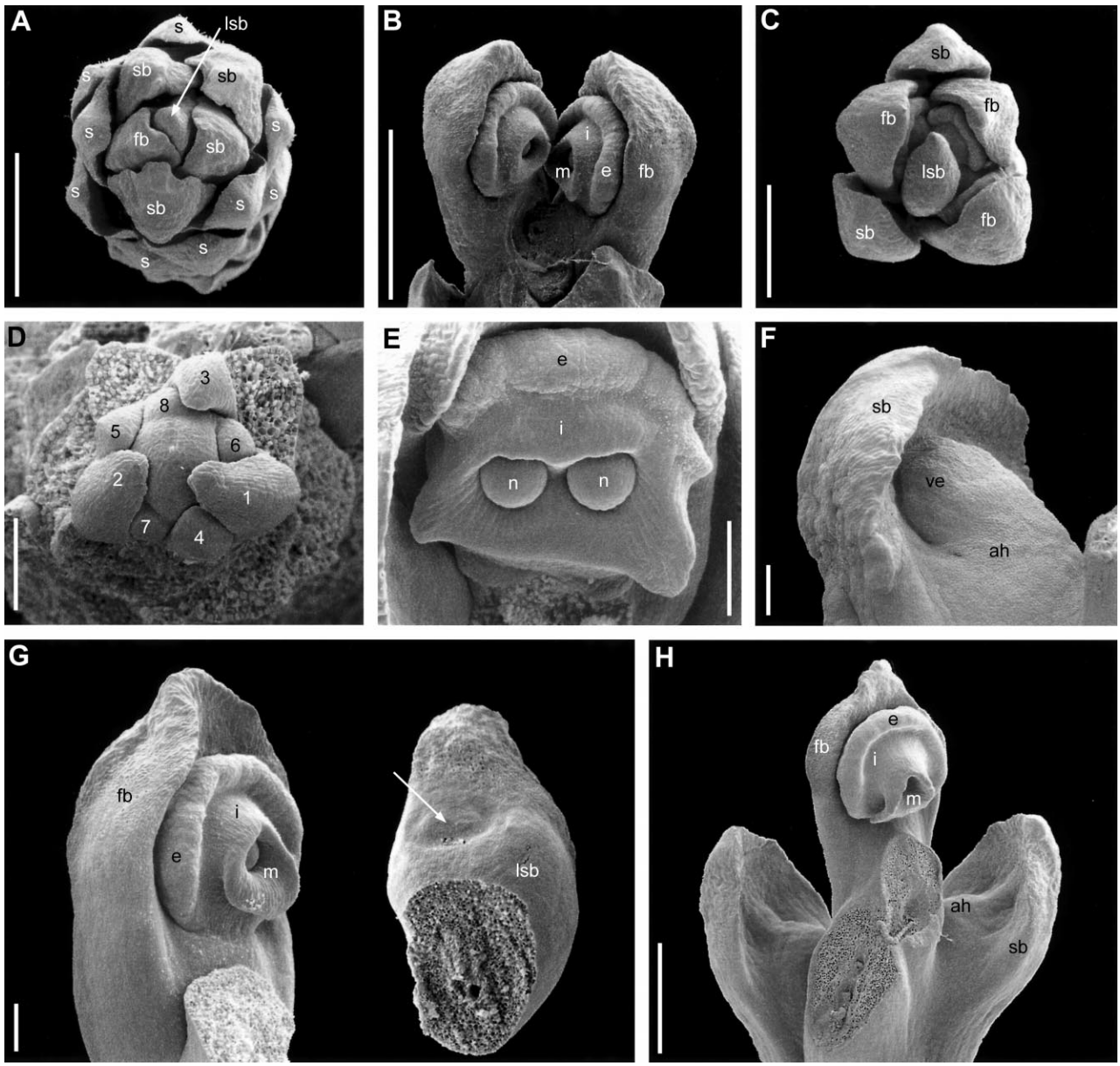


FIG. 2. Scanning electron micrographs of *Acmopyle pancheri*. A, Typical one-ovulate young cone viewed from above. B, Two-ovulate cone, lateral view, last sterile bract removed. C, Three-ovulate cone viewed from above. D, Vegetative shoot tip viewed from above (leaves numbered sequentially). E, Cone with two nucelli developing on one epimatium. F, 'Sterile' bract of cone showing associated axillary hump and vestigial epimatial primordium. G, Ovule and fertile bract (left) with associated sterile bract (removed, right) showing impression of micropylar prongs on sterile bract (arrow). H, Part of cone complex showing relationships of axillary humps, fertile and sterile bracts. ah, Axillary hump; e, epimatium; fb, fertile bract; i, integument; lsb, last sterile bract; m, micropyle; n, nucellus; s, scale-like peduncle bract; sb, sterile bract; ve, vestigial epimatium. Bars = 1 mm (A–C, H) and 200 μm (D–G).

the bract, the 'fingernail' tip of the distal region does not significantly enlarge during development (Fig. 1B). The bracts of the ovulate cone are initially adpressed to the cone axis, concealing the ovule, but spread to expose the epimatium and ovule as the latter reaches receptivity (Fig. 1D). Initially, they are tinged red whereas vegetative buds are not.

Multi-ovulate cones

Each sterile bract bears, in the same position as the fertile bract's epimatium, a small swelling, connected to the cone axis by a hump of tissue but free from the bract except at its base (Fig. 2F and H). Typically, this swelling is transversely elliptic, smaller than a fertile bract's epimatium, and bluish-green. In some cones, at least one of these swellings develops into either a complete epimatium and ovule (Fig. 2B and C; Fig. 2C shows three ovules spirally arranged) or (rarely) an epimatium-like structure only (Fig. 1D). Two ovules (on separate bracts) develop on about 10 % of cones (Fig. 2B). Both may mature (Fig. 1C); usually, one aborts. On one occasion, two ovules developed, twin-like, on the same epimatium (Fig. 2E). Multi-ovulate cones and epimatia were exceptional, though highly significant (see Discussion).

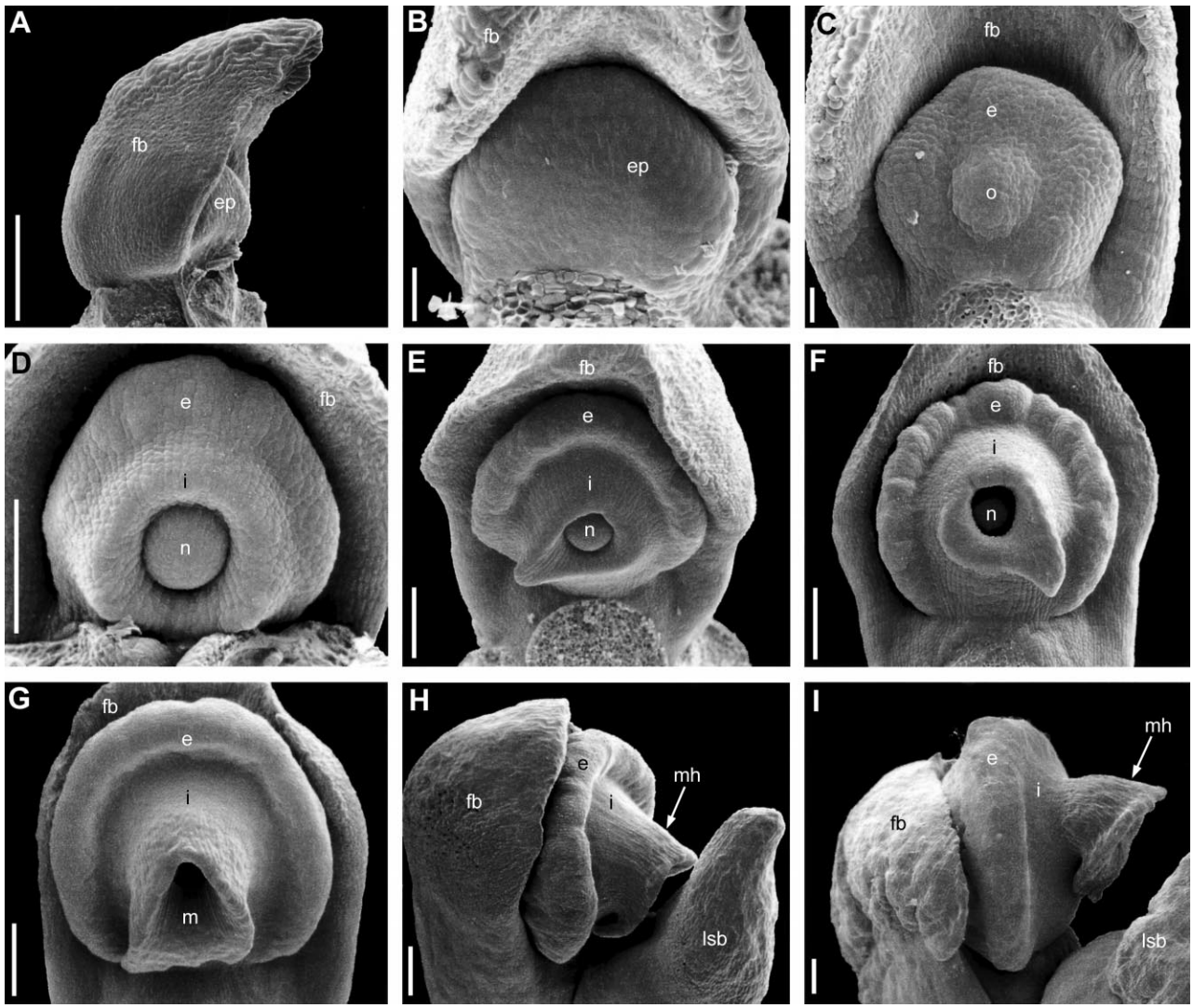
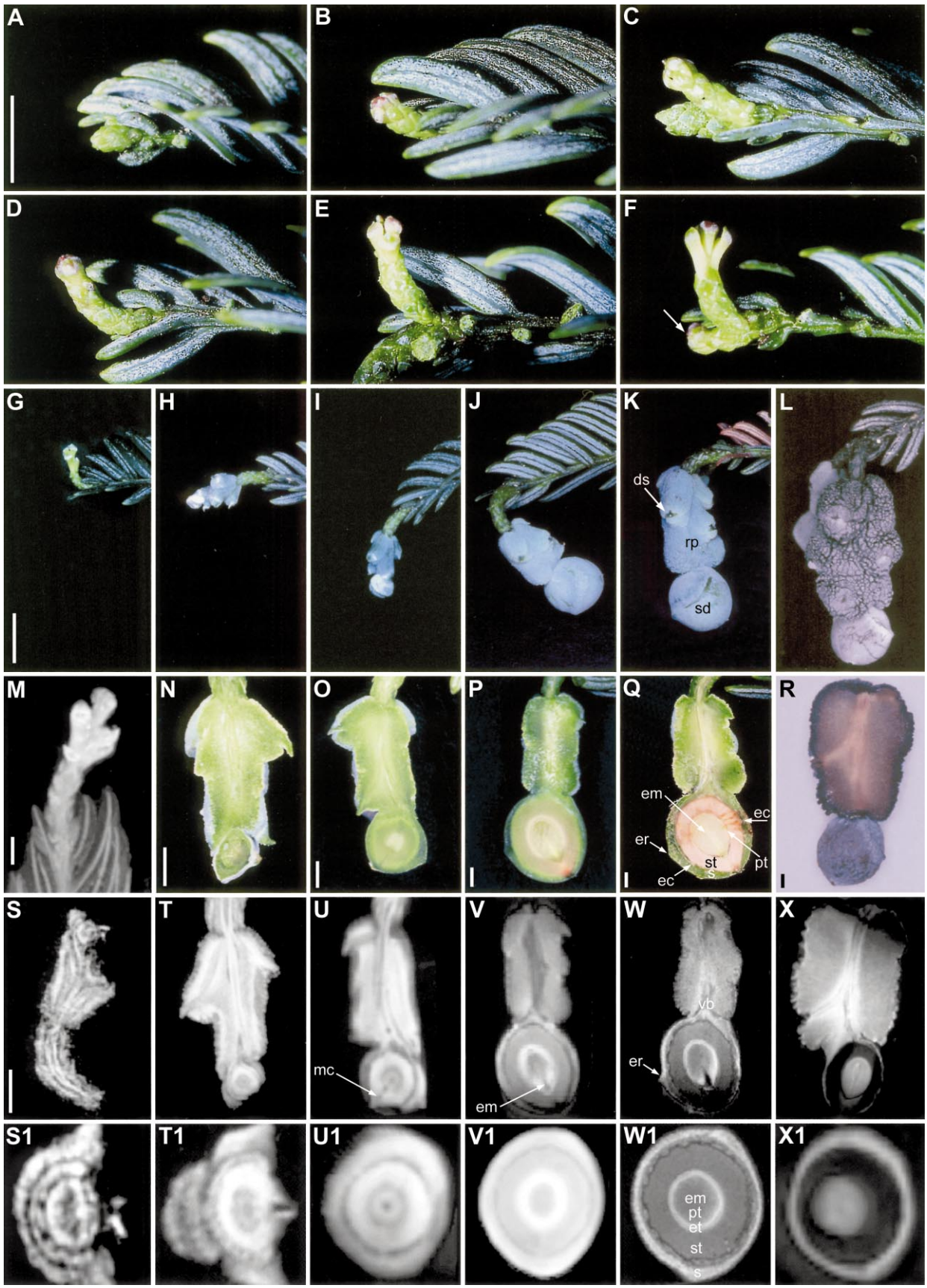


FIG. 3. Scanning electron micrographs of female cones of *Acmopyle pancheri* in initial stages of development. A and B, Fertile bract at earliest observed stage in lateral (A) and frontal (B) views. C, Differentiation of epimatium and ovule. D, Differentiation of single integument and nucellus. E–G, Differentiation of micropyle with engulfment of nucellus. H, Pre-receptive ovule. I, Ovule at receptivity. Sterile bracts removed except for H and I. e, Epimatium; ep, epimatial primordium; fb, fertile bract; i, integument; lsb, last sterile bract; m, micropyle; mh, micropylar hook; n, nucellus; o, ovule. Bars = 50 μm (B, C) and 200 μm (A, D–I).

Ovule ontogeny

Stages of ovulate cone development are illustrated in Fig. 3. At ovule initiation (Fig. 3A and B), the fertile bract is erect, with a hood projecting beyond the 'top' (which is not the morphological apex) of the still undifferentiated epimatium-ovule complex, which fills the adaxial surface of the bract except the hood. Overall lengthening, with differential apical growth, of the fertile bract causes it to enfold the developing epimatium-ovule complex like a spathe. The epimatium differentiates as a broad annular structure, topographically at 90° to the fertile bract, free from the latter except at its base, and facing inwards; the developing ovule appears as a shallow central protuberance (Fig. 3C). Next, the single integument becomes indistinctly differentiated from the epimatium as a ring-like mound; simultaneously, the nucellus is differentiated, free from the integument (Fig. 3D). Further differentiation (Fig. 3E–G) results in the epimatium forming a raised, shallowly lobed or crimped, collar-like structure around the integument, which it encloses laterally and distally but not proximally (Fig. 3F; L.S., Fig. 1E). Concurrently, the nucellus is gradually engulfed within the integument (Fig. 3E–G), which becomes funnel-like and slightly declinate. Continued differential growth of the ovule results in the almost completedis appearance of the epimatium as a recognizably distinct structure. Finally, its presence is indicated only by a ridge, marking its distal edge, which abaxially encircles and closely invests the proximal part of the developing seed (Fig. 3H and I).

When the integument begins to differentiate, the micropyle begins developing from its proximal rim, appearing first as two projections on its topographically lower side (Fig. 3D). These frequently develop asynchronously, so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that in earlier stages (Fig. 3E and F) only one may be visible. The micropyle develops into a tube with two distal prongs forming a fork-like structure (Fig. 3G). Before pollination drop secretion, this micropylar fork is strongly bent backwards and closely adpressed to the sterile bract (Fig. 3H), on which its prongs can leave an impression (Fig. 2G, arrowed). The integumental outgrowth surrounding the micropylar orifice is hook-like, expanded in a $\pm 90^{\circ}$ curvature and finally points more-or-less downwards (Fig. 1E). The micropylar tube disengages from the sterile bract at receptivity. Its tip becomes inclined obliquely outwards and downwards towards, but not touching, the uppermost sterile bract which is always opposite it (Fig. 1F and G). Due to the downwards inclination of the micropylar hook, the exuded pollination drop is inverted, supported by the micropylar fork. Different ovules vary in the morphology of the micropylar hook and uppermost sterile bract; because of this, the drop often becomes attached to the sterile bract in front of the micropyle. After receptivity, the micropylar end of the ovule becomes more remote from the sterile bract and the micropylar tube lengthens and straightens slightly.

Developmental changes in cone orientation

Throughout its development, the ovule is morphologically erect (never inverted) but has no preferred topographical orientation with respect to the shoot axis or peduncle. The cone spatially re-orientates through some 270° (Fig. 4A–L). All developmental stages are portrayed in their correct orientation (except Fig. 4N, which is vertical for comparison with Fig. 4O–R).

Initially, the peduncle is in the same plane as the subtending foliage shoot increment (Fig. 4A–C). It bends upwards during pre-receptive cone expansion (Fig. 4D–F), so that the cone is topographically erect at receptivity (Fig. 4F and G), with the ovule more-or-less horizontal or inclined slightly upwards with respect to the fertile bract. About 1 month after pollination, the cone begins to change position, becoming horizontal again about 2 weeks later (Fig. 4H), finally becoming pendulous, often inclined inwards, after a further 7 to 8 weeks (Fig. 4I and J). The cone remains in its final orientation until ripe (Fig. 4K and L).

Metamorphosis of sterile bracts into receptacle

Until shortly after receptivity, the sterile bracts are discrete structures (Fig. 1A), which (unlike the peduncle bracts) are minutely papillate (Fig. 1I). As the post-pollination cone matures, the sterile bracts and interstitial axis tissues enlarge, become verrucose (Fig. 1J and K), increasingly lose their discrete identities externally, and metamorphose into the fleshy receptacle (Fig. 1L) whose verrucosity results from the division and enlargement of cells in the papillae (Fig. 5B and F).

The bases of the three lowest sterile bracts extend downwards during metamorphosis, concealing the uppermost three peduncle bracts and pushing them outwards and downwards so that they become spreading, but impressed to and seemingly (though not in fact) part of the receptacle. The receptacle enlarges only slightly during reorientation, but considerably once pendulous. A long 'resting' phase (approx. 10 months) begins shortly after the cone has assumed its final position, lasting until shortly before ripening. Throughout this phase, the receptacle is grey-green and firm. During the month before ripening, its girth increases, accompanied by a change in colour to dark purple (Fig. 4K and L), tissue softening, and production of a raspberry-like scent.

Wax development

Many parts of the cone, except the peduncle bracts, become coated with epicuticular wax, particularly post-pollination. Concomitant with the upturning of the peduncle just before receptivity, wax develops on the exterior surfaces of the receptacular bracts, giving them a bloom that increasingly masks their yellow-green colour. Initially, wax develops externally at the base of the distal region of the free part of the bracts (Fig. 1A), whence it develops at first acropetally until receptivity (Fig. 1D). Wax also develops on the distal region of the inner surface of all sterile bracts; however, the proximal region of their free parts is not waxy, and is thus wettable. At receptivity, the epimatium has developed a thin wax coating (Fig. 1D and F).

After receptivity, the wax coating on external bract surfaces extends downwards, so that their previously non-waxy proximal regions are also covered. The interstitial areas on the cone axis remain non-waxy until the second horizontal phase of cone orientation, when they also become coated (Fig. 4H). After receptivity, both the epimatium and micropylar funnel develop heavier wax coatings (Fig. 4H). Wax development on the receptacle and seed continues until they are almost completely covered (Fig. 4I–L).

FIG. 4. Aspects of seed cone development and orientation in *Acmopyle pancheri*. A–F, Sequence of development of pre-receptive seed-cone complexes (from different shoots). A, Close to cone-complex emergence. B, Peduncle beginning to lengthen; note red tips to sterile bracts. C and D, Stages in upturning of peduncle. E and F, Opening of sterile bracts prior to receptivity (arrow in F, terminal foliage shoot). G–L, Changes in orientation: G, Erect phase (receptive). H, Horizontal phase, 6 weeks after pollination. I, Pendulous, 3 months after pollination. J, Pendulous and inclined inwards, 4·5 months after pollination. K, Pendulous during resting phase, 6–14 months after pollination. L, Ripe cone, 16 months after pollination. M, Maximum intensity projection of 3-D NMR image of receptive cone complex and subtending shoot. N–R, Longitudinal sections of receptacle and seed at stages corresponding to H–L. R, Ripe receptacle in L.S. and unsectioned seed. S–X, Longitudinal NMR 'slices' corresponding to stages G–L. S1–X1, Transverse 'slices' of the seed of cones corresponding to stages G–L. In M–X1, the images have been rescaled so that in each one the subject occupies the image area. ds, Distal region of sterile bract; ec, escarpment; em, embryo; er, epimatial ridge; et, endotesta; mc, micropylar canal; pt, prothallus; rp, receptacle; s, sarcotesta; sd, seed; st, sclerotesta; vb, vascular bundle. Bars = 2 mm (M–S), 5 mm (A), 10 mm (G), bar in A applies to A–F, bar in G to G–L; for T–X see N–R.

Seed development

Significant morphological changes occur in the ovule after pollination (Fig. 4G–L). The same cones in longitudinal section are shown in Fig. 4N–R (in Fig. 4R only the receptacle is in L.S.: see below). As well as the spatial re-orientation of the whole cone, the ovule itself re-aligns after receptivity, becoming more erect with respect to the receptacle; the total change in angle amounts to some 60°, from 95–105° at receptivity to approx. 155° at maturity. At receptivity, the ovule, which is approx. 2 mm in diameter, is proximally deeply immersed in the receptacle; immersion decreases during ovular re-alignment, until finally only the extreme base of the ovule is immersed. After pollination, the diameter of the ovule increases. About 6 weeks after pollination, the embryo and prothallus have begun to differentiate, although the sclerotesta is not visible (Fig. 4H and N). At 3 months (Fig. 4I and O), the cone has scarcely enlarged; the seed is approx. 5 mm and more spherical, and differentiation, but not sclerification, of the tissue which will later form the sclerotesta has begun. Approximate final seed size (approx. 11 mm) is reached about 4·5 months after pollination (Fig. 4J and P). During the 'resting phase', little outwardly-visible changes in seed morphology occur; developmental changes are internal (see 'NMR Imaging').

By 14 months after pollination (Fig. 4Q), the inner part of the integument is a well-differentiated, extremely hard, cinnamon-coloured sclerotesta, rendering conventional sectioning impossible. Figure 4Q clearly shows the so-called 'escarpment' (Sahni, 1920), which marks a boundary between the distal and proximal regions of the 'stone' (i.e. sclerotesta plus endotesta, prothallus and embryo); its line bears no relation to the ridge on the seed surface marking the distal edge of the epimatium. Both dorsal and ventral parts of the vascular supply to the seed terminate at this 'escarpment' (Figs. 4Q, 5E).

NMR imaging in relation to developmental morphology

Figure 5 relates transverse conventional sections (Fig. 5D and F) and NMR electronic 'slices' (Fig. 5A and C) through the receptacle (Fig. 5A, D) and peduncle (Fig. 5C, F), and longitudinal and transverse NMR 'slices' through ripe seeds (Fig. 5G and H), to a correctly orientated version of Sahni's schematic anatomical diagram (Sahni, 1920; Fig. 5E). NMR imaging was applied to seed cone complexes from the stage of receptivity onwards (Fig. 4M, S–X, S1–X1). Figure 4M illustrates a maximum intensity projection of a 3-D NMR image of a seed cone complex at receptivity. The lack of planes of symmetry in the seed cone complex makes the understanding of planar sections difficult and the structure is more easily visualized by the use of three-dimensional maximum intensity projections. The outer layers of the seed cone complex, in particular the bract tips, have high intensity, which has the effect of masking any internal structure in the cone. By contrast, the central vascular bundles of the foliage leaves are bright and 'shine through' the darker parenchymatous tissue.

The development of the post-pollination ovule is displayed as median longitudinal orthogonal electronic 'slices' of whole post-pollination cones (Fig. 4S–X) and as transverse 'slices' through the seed (Fig. 4S1–X1). Wherever the 'slice' cuts through the central vascular bundles or those associated with bracts in the receptacle, bright structures are visible at all stages of development. Their position matches that of vascular bundles visible in hand-sectioned receptacles (Fig. 4N–R). The darker areas abaxial to the vascular tissues, which are surrounded by bright rings in NMR images, originate from 'resin' canals. This anatomical interpretation has been corroborated in transverse NMR and histological sections (Fig. 5C and F respectively), which show that the central cylinder of the receptacle is composed of five or six vascular bundles, each with an associated abaxial 'resin' canal (Fig. 5F). The association between 'resin' canal and vascular supply is also valid for each sterile bract; a similar arrangement is apparent in the peduncle (Fig. 5A and D). Sectioning of young cones showed that the 'resin' canals contain an opaque white liquid (Fig. 1E). Microscopical studies revealed a 'granular' consistency of the 'resin' canal contents (Fig. 5I), which are miscible with water but which contain numerous non-polar droplets. These fuse in a polar liquid but dissociate in a non-polar liquid, and stain intensely in Sudan III, indicating their hydrophobic nature (Fig. 5I). The NMR signal from the resin canals showed a very small signal consistent with the presence of terpenes, which are known resin components.

In the developing seed, fine structures such as the micropylar canal (Fig. 4U) can be visualized by NMR imaging, which further reveals structures such as the sarcotesta (including the epimatium; the distinction between these tissues is not apparent), sclerotesta, endotesta and developing prothallus in seeds up to 3 months old (Fig. 4U); the embryo is visible in seeds aged 4·5 months or older (Figs 4V, 5G and H). In most L.S. NMR images, the epimatial 'ridge' (Sahni, 1920) can be seen demarcating the epimatium from the expanding integument (Fig. 4U–X). From 4·5 months onwards, the NMR signal intensity of the seed shows a conspicuous reduction (Fig. 4W and W1), caused by lignification of the sclerotesta during maturation, to the extent that the NMR signal is completely absent in this region of the mature seed (Fig. 4X and X1).

NMR images of seeds of cones whose receptacles began maturing after 8 months, within the same year of pollination, showed that although a prothallus and embryo had begun to form, these did not fill the whole interior of the seed; air spaces existed between the outer edges of the prothallus and the inner edge of the sclerotesta (data not shown).

DISCUSSION

This study of the ovulate cones of *Acmopyle pancheri* has revealed some novel features, most importantly their vestigially multi-ovulate nature with a terminal, distal sterile bract. It has also clarified several previously mis-interpreted aspects of their morphology, the most obvious being ovule orientation, previously described as inverted (e.g. de Laubenfels, 1969), and the orientation of the mature cone, which is pendulous long before maturity. In

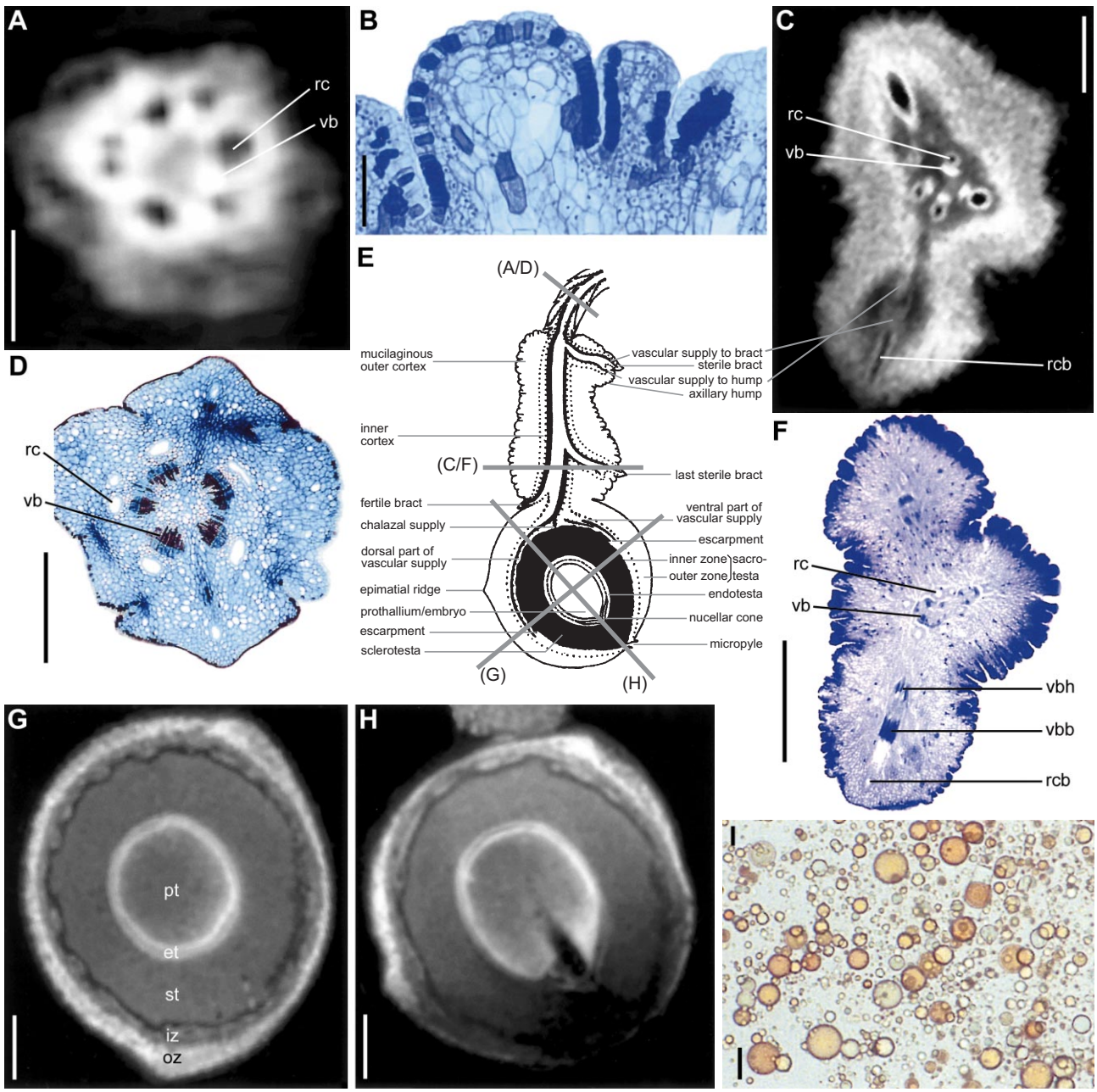

FIG. 5. NMR images and supporting histological micrographs of *Acmopyle pancheri*. A, C, G, H, Single NMR 'slices' through peduncle (A), receptacle (C) and in transverse (G) and longitudinal (H) direction through seed. B, Histological section through receptacle illustrating anatomy of verrucose surface. D and F, Histological sections through peduncle and receptacle respectively. E, Schematic diagram of cone (modified and re-orientated from Sahni, 1920) (positions of sections illustrated in A, C, D, F–H indicated in parentheses). I, 'Resin' canal content stained with Sudan III. et, endotesta; iz, inner zone of outer flesh; oz, outer zone of outer flesh; pt, prothallus; rc, 'resin' canal; rcb, 'resin' canal to sterile bract; st, sclerotesta; vb, vascular bundle; vbb, vascular bundle to sterile bract; vbh, vascular bundle to hump. Bars = 10 μm (I), 100 μm (B), 500 μm (A and D), 2 mm (G and H), 3 mm (C) and 5 mm (F).

the schematic illustration by Sahni (1920; re-published with modified legend by Gaussen, 1973), the cone was misleadingly drawn upright.

Cone and ovule orientation

The degree of spatial reorientation during cone development in *A. pancheri* is remarkable, though perhaps not unique in the Podocarpaceae as it is similar in *Prumnopitys andina* (Poepp. ex Endl.) de Laub. According to Doyle (1945), the mechanism is geotropic. This has not yet been tested in *A. pancheri*.

De Laubenfels (1969) described the ovule of *Acmopyle* as being 'at first inverted and partially covered by the epimatium, eventually becoming nearly erect, fused with the epimatium . . .'. Inverted ovules were not found at any

developmental stage. Initially, the ovule is always horizontal or inclined upwards, and remains so until after pollination. *Acmopyle* thus differs from *Dacrycarpus* (Endl.) de Laub. and *Podocarpus* L'Hérit. ex Pers., which do have inverted ovules (Tomlinson, 1992). In *Acmopyle*, only the pollination drop, not the ovule, is inverted, via the declinate micropylar tube, similar to *Lepidothamnus* Phil. (Tomlinson *et al*., 1991); there is insufficient differential growth at the base of the epimatium to tilt or invert the ovule, as happens in *Dacrycarpus* and *Podocarpus*. The so-called 'fusion' of epimatium and ovule (de Laubenfels, 1969) is a misinterpretation of the differential growth of the ovule, which, from receptivity onwards, causes the previously topographically erect epimatium to become closely adpressed to the proximal part of the ovule. SEM micrographs demonstrate that the epimatium and ovule are organically fused from initiation.

Cone morphology as a function of the pollination mechanism

In most Podocarpaceae, ovulate cones are adapted to 'scavenge' pollen that has previously fallen near the ovule, by means of a pollination drop that extends from the micropyle over an adjacent surface (Tomlinson *et al*., 1991). In *A. pancheri*, the pollen-scavenging area is created by the wettable, non-waxy proximal free parts of the fertile bract(s) and uppermost sterile bract. The latter appears to have a specialized function associated with the pollination mechanism. The pollination drop often becomes attached to it and then spreads downwards by gravity to the non-waxy area, from which the pollen can potentially be scavenged (Möller *et al*., 2000). As in *Dacrycarpus* (Tomlinson *et al*., 1991), drop spread may be facilitated by the papillae of the wettable parts of the receptacular bracts. The shape and position of the scavenging area in *A. pancheri* show most similarity to that of *Dacrycarpus dacrydioides* (A. Rich.) de Laub. as depicted by Tomlinson *et al*. (1991) and, despite apparent similarities in micropylar morphology, is very different in form from that of *Lepidothamnus*, which the same authors described as a 'shallow open receptacle'. However, in *Acmopyle* (Möller *et al*., 2000) and *Lepidothamnus* (Tomlinson *et al*., 1991), which both have ovules inclined upwards (not inverted as in *Dacrycarpus*), pollen initially travels upwards into the micropylar hook, and thence into the pollen chamber.

Our results have confirmed the hypothesis of Sahni (1920) that the uppermost sterile bract, not the fertile bract, is the most distal structure on the receptacular axis; Sahni himself lacked the necessary material to test this. The topographical arrangement of the fertile and uppermost sterile bracts in *A. pancheri* resembles that found in *Lepidothamnus* and *Dacrycarpus* (Tomlinson *et al*., 1991; Tomlinson, 1992), the two bracts being opposed. However, the uppermost sterile bract of *A. pancheri* is not homologous to that of *Dacrycarpus* or *Lepidothamnus*, which is proximal to the fertile bract (Tomlinson, 1992), although the function of the sterile bract (to increase the scavenging area of the drop) is analogous in both genera. The terminal, distal position of the uppermost sterile bract in *Acmopyle* is highly significant, and is correlated with its vestigially multi-ovulate cones. One or more whorls of distal sterile bracts are found in all extant genera of the Podocarpaceae possessing compound ovulate cones (*Microcachrys* Hook. f., *Microstrobos* J. Garden & L. A. S. Johnson, *Saxegothaea* Lindl.), or cones composed of several helically arranged fertile bracts (*Halocarpus* Quinn, *Lagarostrobos* Quinn, *Manoao* Molloy). Distal sterile bracts are absent in genera with a single fertile bract, such as *Dacrycarpus* and *Falcatifolium* de Laub. (Tomlinson, 1992). Thus, *A. pancheri* conforms to the pattern of other multi-ovulate genera.

A study of ovulate cone ontogeny in *A. sahniana* is urgently needed, to establish its reproductive mechanism and compare it with that of *A. pancheri*. Bush and Doyle (1997), whose promised study of this aspect remains unpublished, stated that the female cone of *A. sahniana* was composed of two bracts: the proximal one sterile, and the distal one fertile and arching spathe-like over the ovule (Fig. 33C in Smith, 1979), similar to the situation observed in minature in the earliest developmental stages of *A. pancheri*.

Nature of the receptacle and 'axillary humps'

The early developmental stages of *A. pancheri* were unavailable to Sahni (1920), who was thus unable to determine the arrangement of the sterile bracts of the receptacle, which he presumed were in serial continuation with the peduncle bracts. Our results clearly show a very marked transition, with no real serial continuation, between the unspecialized peduncle bracts and the more specialized receptacular bracts. Sahni (1920) also stated that sterile bracts occurred at the receptacle base that were 'transitional in size and form to the leaves on the distal part of the peduncle'. Our observations demonstrate that these are actually the uppermost three peduncle bracts.

The most enigmatic features of the receptacle of *Acmopyle* have been the structures which Sahni (1920) called 'axillary humps'. Sahni (*op. cit*.: 262, 267) postulated that these represented vestigial axillary shoots. However, he also noted (*op. cit*.: 293) that the vascular supply of *Acmopyle* 'shows the fundamental similarity between the supply to the ovuliferous scale (or its vestige) and the vegetative axillary shoot'. Why he did not therefore consider that the 'humps' might represent vestigial ovuliferous scales, rather than vestigial vegetative axes as he hypothesized, is unclear. The ovulate cone of *A. pancheri* is vestigially multi-ovulate, with most 'sterile' bracts actually being potentially ovuliferous. This represents a highly significant discovery of probable phylogenetic importance.

Differentiation and nature of the epimatium

The epimatium is a structure which is allegedly unique to the Podocarpaceae (though absent in a few genera). Our results demonstrate that an epimatium is well-developed in *A. pancheri*, persistent as a ridge encircling the ripe seed. The removal of *Acmopyle* from Podocarpaceae to Acmopyleaceae in the order Cephalotaxales, on the basis of 'bitegmic ovules' and absence of epimatium (proposed by Melikian and Bobrov, 1997; 2000), represents a misinterpretation of the morphology. Ovulate development in *Acmopyle* is

typically podocarpaceous. Moreover, the ovules are not bitegmic, as supposed by Melikian and Bobrov (2000); they are unitegmic, like those of other members of the Podocarpaceae. These authors may have confused the epimatial ridge with an extra integument. Among the Podocarpaceae, *Acmopyle* is particularly close ontogenetically to *Dacrycarpus* and *Falcatifolium*; these three genera also possess bilaterally flattened foliage leaves (de Laubenfels, 1953, 1969), another character not found in any other family of living conifers (the leaves of *Podocarpus*, *Taxus* etc. are bifacially flattened). This further strengthens the argument for retaining *Acmopyle* in Podocarpaceae.

The epimatium of the Podocarpaceae is generally considered homologous to the ovuliferous scale of the Pinaceae (Sinnott, 1913; Aase, 1915; Florin, 1954) although alternative theories have been proposed. One of these was that the ovule is composed of two completely fused integuments, the epimatium being the outer (Worsdell, 1900); another was that the epimatium was an organ *sui generis*, variously regarded as an 'ovular excrescence' fused with the integument (Pilger, 1903), an outgrowth of the supporting bract (Gibbs, 1912), or a false aril (Page, 1990). From an histological study of *Podocarpus neriifolius* D. Don, Jain (1978) concluded that the epimatium in that species was homologous to the ovuliferous scale and represented an exclusively axial axillary branch. This is supported by our results. In most conifers, the ovuliferous scale comprises two distinct parts: one, relatively similar morphologically to the bract scale, connects it to the cone axis, while the other, more specialized, bears the ovule(s). In *Acmopyle*, these two parts of the ovuliferous scale are represented respectively by the unspecialized axillary hump tissue and the bluish-green swelling at its topographical apex. From the observations reported here, these swellings clearly represent vestigial epimatia, all of which are capable of developing into fertile ones.

Stoffberg (1991) and Tomlinson (1992) proposed diametrically opposed hypotheses concerning the timing and sequence of epimatial differentiation. Stoffberg (1991) believed that the sequence of events in ovulate development was basipetal, the epimatium being initiated after the nucellus and integument. Tomlinson (1992) proposed the alternative hypothesis that initiation was acropetal, the epimatium being initiated first, and serving to support the ovule. Support for the latter, rather than the former, hypothesis is provided by twin ovules developing from a single epimatium (Fig. 2E). This is a rare, previously unreported phenomenon which has proved crucial in understanding the relationship between the epimatium and ovule (for two ovules to develop on one epimatium, the latter must be formed first). The epimatium in podocarps may be compared to the placenta of angiosperms, functionally if not ontogenetically, since both are supporting structures on which the ovule(s) develop.

Waxes in relation to pollination mechanism and cone development

Before and during receptivity, the location of wax deposits is strongly correlated functionally with the pollination mechanism. Only non-waxy areas are wettable, allowing the pollination drop to spread over their surface (Tomlinson *et al.*, 1991; Möller *et al.*, 2000). After pollination, wax gradually covers the whole cone, including the seed and its investing epimatium. In resting and ripe stages, *A. pancheri* has one of the most waxy cones of any podocarp; it is water-impermeable. In post-pollination stages, the wax coating probably protects the cone and its exposed seed against ingress of water and pathogen attack.

NMR imaging and seed cone development

In this study, NMR imaging was applied to (1) analyse internal structures of maturing post-pollination cones which are difficult or impossible to section by conventional means such as hand-sectioning or microtoming, and (2) to document internal changes in the seed during maturation at times where externally little change was observed. Our results for the vasculature in the receptacle confirm those obtained by Sahni (1920). NMR imaging was particularly effective in revealing the three-dimensional arrangement and connectivity of the vascular bundles and resin canals, even those in sterile bracts and leading into the 'axillary humps'. The NMR signal from the resin canals is of low intensity relative to the surrounding ring of cells and the vascular bundles, partly as a consequence of the lower density of protons in terpenes and other probable components of resin than in the predominantly aqueous contents of other tissue, and also probably as a result of the higher viscosity of the resin. A very small signal consistent with resin components (data not shown) was observed in the canals but not in the adjacent vascular traces. 'Resins' are widely distributed in podocarps (see lists of terpenoid compounds in Brooker *et al.* 1966; Cambie, 1976, 1988, 1996; and review by San Feliciano and Lopez, 1991), but the 'resinous' substance found in *Acmopyle* is unusual as it appeared to consist of an emulsion of non-polar 'resin' droplets in a polar matrix. Neither species of *Acmopyle* has yet been chemically analysed for any class of compound.

NMR images of the developing seed non-invasively reveal the great diversity of internal structures identified by Sahni (1920), including the endotesta and, in older seeds (6–14 months), differences between the inner and outer zones of the sarcotesta (Fig. 5G)—the latter not discernible in our hand-sectioned samples. However, NMR imaging did not reveal differences between tissue originating from the epimatium and from the integument comprising most of the sarcotesta.

The marked reduction in NMR signal from the sclerotesta of seeds over 6 months old is due to gradual sclerification, which not only reduces the water content (and hence the number of water protons), but also shortens the T_2 of the protons remaining in the tissue, both of which lead to decreased signal intensity (Callaghan, 1991).

The small kernels (which incompletely filled the interior of the seed) revealed by NMR imaging in 8-month-old cones with ripening receptacles, indicate that these cones were ripening prematurely. This demonstrates the usefulness of NMR in non-invasively determining normal prothallus and embryo formation. This is particularly

important in species such as *A. pancheri*, which are a conservation concern.

CONCLUSIONS

This study has revealed several novel features of female cone ontogeny and morphology of *Acmopyle pancheri*: (1) The cone is vestigially multi-ovulate; nearly all 'sterile' bracts bear vestigial epimatia capable of developing into epimatia which may or may not bear ovules. (2) Ovules are horizontal or inclined upwards at all stages; they are never inverted. (3) Two ovules can develop from a single epimatium, indicating that ovulate development is acropetal, not basipetal. (4) The uppermost sterile bract is terminal and distal, conforming to the pattern of podocarps that have retained the multi-ovulate state. (5) NMR imaging revealed internal 3-D structures from single specimens non-destructively and differentiated non-invasively between viable and non-viable seeds.

Several of these findings have important phylogenetic implications, which will be discussed elsewhere in relation to other genera of the family.

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LITERATURE CITED

Aase HC. 1915. Vascular anatomy of the megasporophylls of Conifers. *Botanical Gazette* **60**: 277–313.

Brooker SG, Cambie RC, James MA. 1966. A New Zealand phytochemical register—Part II. *Transactions of the Royal Society of New Zealand, General* **1**: 205–231.

Bush EW, Doyle MF. 1997. Taxonomic description of *Acmopyle sahniana* (Podocarpaceae): additions, revisions, discussion. *Harvard Papers in Botany* **2**: 229–233.

Callaghan PT. 1991. *Principles of nuclear magnetic resonance microscopy*. Oxford: Oxford University Press.

Cambie RC. 1976. A New Zealand phytochemical register—Part III. *Journal of the Royal Society of New Zealand* **6**: 307–379.

Cambie RC. 1988. A New Zealand phytochemical register—Part IV. *Journal of the Royal Society of New Zealand* **18**: 137–184.

Cambie RC. 1996. A New Zealand phytochemical register—Part V. *Journal of the Royal Society of New Zealand* **26**: 483–527.

Chudek JA, Hunter G. 1997. Magnetic resonance imaging of plants. *Progress in Nuclear Magnetic Resonance Spectroscopy* **31**: 43–62.

de Laubenfels DJ. 1953. The external morphology of coniferous leaves. *Phytomorphology* **3**: 1–20.

de Laubenfels DJ. 1969. A revision of the Malesian and Pacific rainforest conifers, I. Podocarpaceae, in part. *Journal of the Arnold Arboretum* **50**: 274–369.

de Laubenfels DJ. 1972. Gymnospermes. In: Aubreville A, Leroy J-F, eds. *Flore de la Nouvelle-Calédonie et Dépendances*, **4**. Paris: Muséum National d'Histoire Naturelle.

Doyle J. 1945. Developmental lines in pollination mechanisms in the Coniferales. *Scientific Proceedings of the Royal Dublin Society* **24**: 43–62.

Farjon A. 1998. *World checklist and bibliography of conifers*. Kew: Royal Botanic Gardens.

Florin R. 1954. The female reproductive organs of Conifers and Taxads. *Biological Reviews of the Cambridge Philosophical Society* **29**: 367–389.

Gaussen H. 1973. Les Gymnospermes actuelles et fossiles. Fascicule XII. Les Podocarpines. Étude générale, *Travaux du Laboratoire Forestier du Toulouse*, tome 2, vol 1, fasc. XII. Toulouse: Faculté des Sciences.

Gaussen H. 1974. *Acmopyle*. In: Les Gymnospermes actuelles et fossiles. Les Podocarpines sauf les *Podocarpus*, *Travaux du Laboratoire Forestier du Toulouse*, tome 2, vol 1, fasc. XIII. Toulouse: Faculté des Sciences, Ch. XX, 169–173.

Gibbs LS. 1912. On the development of the female strobilus in *Podocarpus*. *Annals of Botany* **26**: 515–571.

Glidewell SM, Williamson B, Goodman BA, Chudek JA, Hunter G. 1997. An NMR microscopic study of grape (*Vitis vinifera* L.). *Protoplasma* **198**: 27–35.

Hill RS, Brodribb TJ. 1999. Southern conifers in time and space. *Australian Journal of Botany* **47**: 639–696.

Hill RS, Carpenter RJ. 1991. Evolution of *Acmopyle* and *Dacrycarpus* (Podocarpaceae) foliage as inferred from macrofossils in south-eastern Australia. *Australian Systematic Botany* **4**: 449–479.

Ishida N, Koizumi M, Kano H. 2000. The NMR microscope: a unique and promising tool for plant science. *Annals of Botany* **86**: 259–278.

Jain KK '1977' publ. 1978. Morphology of female strobilus in *Podocarpus neriifolius*. *Phytomorphology* **27**: 215–233.

Masson D, Glidewell SM, Möller M, Mill RR, Williamson B, Bateman RM. 2001. Non-destructive utilization of herbarium material for taxonomic studies using NMR imaging. *Edinburgh Journal of Botany* **58**: 1–14.

Melikian AP, Bobrov AVFCh. 1997. Sistematicheskoe polozhenie roda *Acmopyle* Pilg. (Podocarpaceae s.l.) po dannym sravnitel'noj morphologii, anatomii i ul'trastruktury semyan. *Proceedings of the International Conference on Plant Anatomy and Morphology, St Petersburg, June 1993*: 92–93.

Melikian AP, Bobrov AVFCh. 2000. Morfologiya shchenskikh reproduktivi'kh obganov i op't rostroenniya filogeneticheskoj sistemy poryadkov *Podocarpales*, *Cephalotaxales* i *Taxales*. [Morphology of female reproductive structures and an attempt of the construction of phylogenetic system of orders *Podocarpales*, *Cephalotaxales* and *Taxales*]. *Botanicheskij Zhurnal (Moscow & Leningrad)* **85**: 50–67.

Möller M, Mill RR, Bateman RM, Glidewell SM, Williamson B, Masson D. 1999. Pollination drop mechanism and cone development of *Acmopyle pancheri* (Podocarpaceae)—present state of knowledge. In: Farjon A, ed. *International Conifer Conference 1999, 23–26 August 1999, Wye College, England—Programme & Abstracts*, 35–36 (Poster Abstract).

Möller M, Mill RR, Glidewell SM, Masson D, Williamson B. 2000. Comparative biology of the pollination mechanisms in *Acmopyle pancheri* and *Phyllocladus hypophyllus* (Podocarpaceae s.l.) and their taxonomic significance. *Annals of Botany* **86**: 149–158.

Page CN. 1990. Podocarpaceae. In: Kubitzki K, ed. *The families and genera of vascular plants, vol. 1. Pteridophytes and Gymnosperms*. Heidelberg & New York: Springer-Verlag, 332–346.

Pilger R. 1903. *Taxaceae*. In: Engler A, ed. *Das Pflanzenreich* IV. 5 (Heft 18). Leipzig: Wilhelm Engelmann.

Sahni B. 1920. On the structure and affinities of *Acmopyle pancheri*, Pilger. *Philosophical Transactions of the Royal Society of London, Series B* **210**: 253–310.

San Feliciano A, Lopez J-L. 1991. Recent chemistry of conifer terpenoids. In: Harborne JB, Tomas-Barbaran FA, eds. *Ecological chemistry and biochemistry of plant terpenoids*. Oxford: Oxford Science Publications, 1–27.

Sinnott EW. 1913. The morphology of the reproductive structures in the *Podocarpineae*. *Annals of Botany* **27**: 39–82.

Smith AC. 1979. *Flora Vitiensis Nova. A new flora of Fiji (spermatophytes only). Vol. 1.* Lawai, Kauai, Hawaii: Pacific Tropical Botanical Garden.

Sporne KR. 1974. *The morphology of gymnosperms. 2nd edn.* London: Hutchinson & Co. Ltd.

Stoffberg E. 1991. Morphological and ontogenetic studies on southern African podocarps. Initiation of the seed scale complex and early development of integument, nucellus and epimatium. *Botanical Journal of the Linnean Society* **105**: 21–35.

Tomlinson PB. 1992. Aspects of cone morphology and development *in Podocarpaceae (Coniferales). International Journal of Plant Science* **153**: 572–588.

Tomlinson PB, Braggins JE, Rattenbury JA. 1991. Pollination drop in relation to cone morphology in Podocarpaceae: a novel reproductive mechanism. *American Journal of Botany* **78**: 1289–1303.

WCMC (World Conservation Monitioring Centre). 2000. Tree conservation database. Website (*http://www.wcmc.org.uk/cgi-bin/SaCGI.cgi/trees.exe?FNC=database__Aindex_html*), maintained by WCMC, Cambridge, U.K.

Wells PM, Hill RS. 1989. Leaf morphology of the imbricate-leaved Podocarpaceae. *Australian Systematic Botany* **2**: 369–386.

Worsdell WC. 1900. The structure of the female 'flower' in Coniferae. *Annals of Botany* **14**: 39–82.