

Detailed seed cone morpho-anatomy of the Prumnopityoid clade: an insight into the origin and evolution of Podocarpaceae seed cones

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- **Background and Aims** Seed cone traits are significant for understanding the evolutionary history of conifers. Podocarpaceae has fleshy cones with a distinct morphology compared with other conifers. However, we have a poor understanding of the seed cone morphology of the Prumnopityoid clade and within Podocarpaceae. This study presents detailed seed cone morpho-anatomy and the evolution of fleshy structures traits in the Prumnopityoid clade.
- **Methods** We investigated the detailed seed cone morpho-anatomy of selected species from the nine genera using the histological method. The evolution of morpho-anatomical traits was assessed using ancestral state reconstruction methods.
- **Key Results** The Prumnopityoid clade has evolved fleshy seed cones using different functional structures (e.g. aril, epimatium, bracts or receptaculum) and fleshiness is an ancestral trait in the clade. An epimatium is present in all genera except *Phyllocladus*, but with different structural morphologies (e.g. a fleshy asymmetrical cup-like epimatium or an epimatium that is fused with the integument, forming a fleshy sarcotesta-like seed coat). In all species with fleshy sarcotesta-like seed coats, the endotesta is hard and woody, forming a sclerotesta-like structure and the epimatium and exotesta are fused, forming a fleshy sarcotesta-like structure.
- **Conclusions** This study highlights that the Prumnopityoid clade has an amazing diversity of structures and complex evolutionary patterns. Fleshiness is an ancestral trait of the clade and has been achieved via diverse evolutionary pathways and structures. This clade has four distinct seed cone types, i.e. drupe-like, receptaculate, ariloid and dacrydioid cones, based on morpho-anatomical structures and traits. The macrofossil record also demonstrates the presence of several structures and traits.

Key Words: Australasia, conifers, fleshy structures, seed dispersal, palaeobotany, reproductive morphology, trait evolution.

INTRODUCTION

Gymnosperm evolution has a key significance in understanding the origin and the evolution of seed plants. The evolution and mechanism of fleshy seed cones in lineages of surviving gymnosperms is an open topic of discussion (Contreras *et al.*, 2017; Leslie *et al.*, 2017; Khan and Hill, 2021; Nigris *et al.*, 2021).

The Podocarpaceae have an amazing diversity of seed cone morphology and possess different morphotypes across all major clades. Several studies have investigated seed cone evolution in conifers (Tomlinson, 1992; Mundry, 2000; Restemeyer, 2002; Mill *et al.*, 2004; Herting *et al.*, 2020; Klaus and Matzke, 2020). Klaus and Matzke (2020) reported that the ancestral seed cones in Podocarpaceae were non-fleshy, and fleshy cone structures appeared seven times independently in podocarps. Herting *et al.* (2020) used a modern trait-evolution approach to investigate the ancestral conifer cone and suggested that the ancestral conifer seed cones were non-fleshy, but in podocarps fleshiness is an ancestral trait. Most of the recent studies are based on published data regarding species' functional traits, but due to few detailed investigations on the morpho-anatomy and functional traits of the Prumnopityoid clade there has

been misidentification of traits in several genera (e.g. seed cones of *Manoao*, *Sundacarpus*, *Prumnopitys*, *Pectinopitys*, *Phyllocladus* and *Retrophyllyum* have been reported as non-fleshy, but they do in fact have fleshy structures) in these ancestral reconstruction studies (Klaus and Matzke, 2020; Herting *et al.*, 2020). The living podocarps display diversity in functional structures (epimatium, bracts, aril and receptacle) and use these structures to achieve fleshiness (Khan and Hill, 2021). Some previous recent studies discussing the evolution of the coniferous seed scale in podocarps and conifers suggests that the epimatium is a homologous structure to the seed scale in other conifers (Herting and Stützel, 2020; Khan and Hill, 2021). Nigris *et al.* (2021) stated that these fleshy structures in gymnosperms are associated with ovule protection and seed dispersal.

The Podocarpaceae is a morphologically diverse family with 20 extant genera (several monotypic) and >200 species (Khan and Hill, 2021). Molecular studies divide the family into three major clades, i.e. Prumnopityoid, Podocarpoideae, Dacrydioid, and one distinct grade (Biffin *et al.*, 2011; Knopf *et al.*, 2012). The Prumnopityoid clade contains the largest number of genera (nine). These genera can be placed into two broad subclades

(Halocarpioid and Pectinopityoid) based on both morphological (the former typically scale-leaved and the latter broad-leaved) and molecular studies (although some genera are paraphyletic) (Leslie *et al.*, 2017, 2018). The Halocarpioid subclade is also called the scale-leaved clade as most of the genera (*Lepidothamnus*, *Manoao*, *Lagarostrobos* and *Parasitaxus*) bear scale leaves (Andruchow-Colombo *et al.*, 2019). However, *Phyllocladus* has developed specialized photosynthetic phylloclades and *Halocarpus* species show leaf dimorphism (scale leaves and flattened leaves). *Phyllocladus* species are morphologically unique among podocarps, having lost their true leaves (except in seedlings), and flattened the lateral short shoots into a two-dimensional flattened photosynthetic structure called a phylloclade (Keng, 1978; Dörken *et al.*, 2021). Phylloclades are variable in shape and can be classified as simple (*P. trichomanoides* and *P. aspleniifolius*) and pinnately compound (*P. trichomanoides*, *P. hypophyllus* and *P. toatoa*). Some researchers have placed *Phyllocladus* in the separate family Phyllocladaceae (Keng, 1973; Tomlinson *et al.*, 1997; Melikian and Bobrov, 2000), but molecular studies place them in the Halocarpioid subclade. The Halocarpioid subclade consists of six genera (12 species) predominantly in New Zealand, Australia and New Caledonia, but *Lepidothamnus fonkii* is found in Argentina and Chile and *Phyllocladus hypophyllus* is distributed in Brunei, Papua New Guinea, Malaysia, Indonesia and the Philippines.

The Pectinopityoid subclade was recently revised by Page (2019) and now includes three genera (*Prumnopitys*, *Pectinopitys* and *Sundacarpus*) and consists of ten species distributed in South America, Southeast Asia, New Caledonia, Papua New Guinea, New Zealand and Australia (Farjon, 2018). All species of the Pectinopityoid subclade have bifacially flattened leaves, which are hypostomatic, with a single resin canal. Both a hypodermis and accessory transfusion tissue are absent in *Prumnopitys* and *Pectinopitys* but are present in *Sundacarpus* (Salter, 2004; Knopf *et al.*, 2012). *Pectinopitys* consists of six dioecious tree species, distributed in South America, New Zealand, New Caledonia and Australia (Page, 2019). *Prumnopitys* consist of three dioecious tree species distributed in South America and New Zealand. *Sundacarpus* is a monotypic genus (containing *S. amarus*) distributed in Australia, Papua New Guinea and into Southeast Asia. *Pectinopitys* and *Sundacarpus* are segregated from *Prumnopitys* based on both morphological and molecular data (Kelch, 2002; Page, 2019). Melikian and Bobrov (2000) proposed a new family, Prumnopityaceae, for this subclade, but this is not supported by molecular data.

The Prumnopityoid clade has diverse seed cone morphology and evolved fleshiness using several functional structures. However, comprehensive studies on the seed cone morphology and functional traits of this clade are lacking. This resulted in miss-identifying both traits in several genera and ancestral reconstruction of the evolution of fleshy seed cones. This study describes the detailed seed cone morphology and combines it with available fossil seed cone records and evaluates the diversity and evolution of functional traits among the genera of the Prumnopityoid clade. This study will contribute to the complete understanding of the complicated evolutionary history of fleshiness and other functional traits of Podocarpaceae.

MATERIALS AND METHODS

Seed cone collections

Seed cones at different developmental stages were collected from the Royal Botanic Gardens Melbourne, Victoria; Mount Lofty Botanical Garden, South Australia; National Botanic Gardens, Canberra, ACT; The Tasmanian Arboretum, Devonport; and the Cairns Botanic Gardens, North Queensland, Australia (Table S1). For this study, we used living seed cones (ten seed cone replicates for each species) and we have stored four specimens of each species in the plant spirit collection with voucher numbers from UOA-61 to UOA-120 at the Department of Ecology and Evolution, University of Adelaide, Australia.

Morphology and distribution of the investigated taxa

Members of the Halocarpioid subclade are usually shrubs or sometimes trees with dimorphic leaves, while members of the Pectinopityoid subclade are usually large trees with flattened leaves and fleshy seed cones. In this study, we examined the following: *Manoao colensoi* (New Zealand); *Lagarostrobos franklinii* (Tasmania); *Lepidothamnus fonkii*, *L. intermedius* and *L. laxifolius* (Argentina, Chile and New Zealand); *Parasitaxus usta* (the only parasitic gymnosperm, New Caledonia); *Falcatifolium taxoides* (New Caledonia); *Halocarpus bidwillii*, *H. biformis* and *H. kirkii* (New Zealand); *Phyllocladus aspleniifolius* (Tasmania), *P. toatoa* and *P. trichomanoides* (both New Zealand); *Phyllocladus hypophyllus* (Brunei, Papua New Guinea, Malaysia, Indonesia and the Philippines); *Prumnopitys andina* (Chile and Argentina); *Pectinopitys ferruginea* (New Zealand); and *Sundacarpus amarus* (northeastern Australia through Papua New Guinea, the Philippines, to Brunei).

Taxon processing and sectioning

Specimens of 14 taxa (Table 1) were fixed in 200 mL of FAA (100 ml 95 % ethanol + 80 mL dH₂O + 20 mL 37 % formaldehyde solution) immediately after collection. Whole seed cones, plus their longitudinal and cross sections were photographed with a Nikon-SMZ25 stereomicroscope. For histology, seed cones were fixed for 48–72 h and processed for a 48-h cycle using a Sakura Tissue-Tek VIP6 Vacuum Infiltration Tissue Processor. They were then embedded in paraffin wax (Sakura Tissue Tek Embedding Centre) and then longitudinal and cross sections of 8–10 µm thickness, were produced using a Leica RM 2235 Rotary Microtome and stained with H and E (Dako CoverStainer) and toluidine blue stains.

Measurements and character mapping

Many morpho-anatomical and embryological characters were recorded (Table 1). The terminology used in this study is based on Khan and Hill (2021). The anatomical layers were measured from mature seed cones using ImageJ 1.8.0_112 software. Data on seed cone size and other traits were

TABLE 1. Seed cone morphological and anatomical qualitative and quantitative characters of the *Prumnopioid* clade

Characters	<i>Halocarpus bidwillii</i>	<i>Halocarpus biformis</i>	<i>Phyllocladus trichomanoides</i>	<i>P. asplenifolius</i>	<i>P. ioatua</i>	<i>P. hypophyllus</i>	<i>Lepidothamnium intermedium</i>	<i>L. laxifolius</i>	<i>Manoao colensoi</i>	<i>Lagarostrobos franklinii</i>	<i>Parasitaxus usta</i>	<i>Prumnopioides andina</i>	<i>Pectinopyxis ferruginea</i>	<i>Sundaecarpus amarus</i>
Reproductive cycle	2 years	2 years	1 year	1 year	1 year	1 year	2 years	2 years	2 years	2 years	(1 year)	2 years	2 years	2 years
Cone shape	Ellipsoid	Ellipsoid	Ellipsoid	Ellipsoid	Ellipsoid	Ellipsoid	Ellipsoid	Ellipsoid	Ovoid	Narrow cylindrical	Globose	Ellipsoid	Ellipsoid	Ellipsoid
Cone size (mm)	3–5 × 2–2.5	2.5–4.5 × 1.5–2	5–8 × 1–2	5–8 × 2–2.5	6–8 × 1.2–2.6	7–10 × 1.6–2.5	5–8 × 2–3	6–7 × 2–3	4–6 × 1.5–2.5	4–5.5 × 2–3	3–5 × 3–4	18–25 × 16–20	14–20 × 10–16	25–35 × 22–31
Colour	White brownish	Yellow brownish	Reddish brown	Red	Reddish brown	Red purplish	Reddish brown	Reddish brown	Yellowish brown	Yellowish brown	Reddish brown	Yellowish	Reddish	Reddish
Number of seeds per cone	1 or 2	1 or 2	2–4	2–5	2–8	1–3	1 or 2	1 or 2	1–3	5–8	1	4–12	1 or 2	1 or 2
Seed size (mm)	2.5–4.5 × 1.5–2	1–3 × 1.5–2	4–5 × 3	4–5 × 3.4	3.5–4.5 × 1.2–2.6	6–8 × 1.6–2.5	4–5 × 2–2.5	4–5 × 2.5	3–4 × 1.5–2.5	2.5–3 × 1.5–2	2.6–4.6 × 2.5–3.8	12–18 × 10–15	10–15 × 8–12	20–30 × 8–27
Seed shape	Ellipsoid	Ellipsoid	Ellipsoid	Ellipsoid	Ellipsoid	Ellipsoid	Ellipsoid	Ellipsoid	Ovoid	Ovoid	Globose	Ellipsoid	Ellipsoid	Ellipsoid
Seed surface	Undulate	Undulate	Undulate	Undulate	Smooth	plicate	Undulate	Undulate	Undulate	Undulate	Rugose	Rugose	Rugose	Rugose
Seed colour	Greenish black	Greenish black	Greenish black	Greenish black	Dark brown	Brown	Purplish black	Purplish black	Black	Brown	Brown	Brownish	Brownish	Brownish
Ovule orientation	Inverted	Inverted	Erect	Erect	Erect	Erect	Erect	Erect	Inverted	Inverted	Inverted	Inverted	Inverted	Inverted
Aril	Present	Present	Present	Present	Present	Present	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Aril shape	Cup-shaped	Cup-shaped	Cup-shaped	Cup-shaped	Cup-shaped	Cup-shaped	-	-	-	-	-	-	-	-
Aril colour	White	Orange	White	White	White	White	-	-	-	-	-	-	-	-
Aril structure	Fleshy	Fleshy	Fleshy	Fleshy	Fleshy	Fleshy	-	-	-	-	-	-	-	-
Aril cells	Isodiametric	Isodiametric	Rectangular	Isodiametric	Elongated oval	Elongated oval	-	-	-	-	-	-	-	-
Cuticle	thick	thick	Thin	Thin	Thin	Thin	Thin	Thin	Thin	Thin	Thin	Thin	Thin	Thin
Epidermal layers	1	1	1	1–2	1 or 2	1	1	1	1	1	1	1	1	1
Epidermal cell shape	Round	Round	Isodiametric	Isodiametric	Isodiametric	Isodiametric	Elongated triangular	Elongated triangular	Round	Elongated triangular	Isodiametric	Isodiametric	Isodiametric	Isodiametric
Exostea	4–6	3–5	2	1–2	3–4	3–4	3–5	2–5	4–6	2–4	10–16	16–22	12–16	14–26
Mesostea	-	-	1–2	5–8	2–5	5–7	-	-	-	-	-	6–10	3–6	4–6
Endostea	4–8	3–6	8–12	4–8	6–8	3–4	4–7	3–6	2–7	4–10	6–10	12–20	6–12	3–6
Nucellus	3–6	4–8	10–16	4–10	10–12	5–9	3–6	3–6	2–5	1–3	3–7	6–12	13–18	8–12
Embryo shape	Straight	Straight	Straight	Straight	Straight	Straight	Straight	Straight	Straight	Straight	Straight	Straight	Straight	Straight
Embryo size (mm)	0.36–0.8 × 0.25–0.45	0.52–0.95 × 0.29–0.55	0.6–0.7 × 0.2–0.3	0.35–0.62 × 0.15–0.25	0.3–0.7 × 0.2–0.25	0.5–0.6 × 0.2–0.3	0.42–0.85 × 0.38–0.62	0.4–0.82 × 0.33–0.58	0.4–0.6 × 0.2–0.3	0.3–0.5 × 0.15–0.2	0.2–0.5 × 0.1–0.2	0.6–0.9 × 0.2–0.4	0.6–1.0 × 0.3–0.5	0.5–1.1 × 0.2–0.4
Bracts	4–5	4–5	6–8	6–8	8–20	12–15	3–5	3–4	4–7	5–10	3–6	1	1–2	1–2
Stomata on bracts	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present
Epimatium	Present	Present	Absent	Absent	Absent	Absent	Present	Present	Present	Present	Present	Present	Present	Present
Epimatium colour	dark-Brown	dark-Brown	Absent	Absent	Absent	Absent	Red	Red	Yellowish	Brown	Brown	Yellowish	Red	Red

TABLE 1. Continued

Characters	<i>Holocarpus bidwillii</i>	<i>Holocarpus bififormis</i>	<i>Phyllocladus trichomanoides</i>	<i>P. asplenifolius</i>	<i>P. toatoa</i>	<i>P. hypophyllum</i>	<i>Lepidothamnus intermedius</i>	<i>L. laxifolius</i>	<i>Manoao colensoi</i>	<i>Lagarostrobos franklinii</i>	<i>Parasitaxus usta</i>	<i>Prumnopitys andina</i>	<i>Pectinopitys ferruginea</i>	<i>Sandacarpus amarus</i>
Epimatium morphology	coriaceous and not fused with testa	coriaceous and not fused with testa	Absent	Absent	Absent	Absent	Fleshy asymmetrical cup-like shape	Fleshy asymmetrical cup-like shape	Fleshy asymmetrical cup-like shape	Fleshy or dry, papery asymmetric	Fleshy and fused with testa	Fleshy and fused with testa	Fleshy and fused with testa	Fleshy and fused with testa
Receptaculum	Absent	Absent	Absent	Absent	Absent	Absent	Present	Present	Absent	Absent	Absent	Absent	Absent	Absent
Sclereids in testa	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present
Resin canals	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present
Resin ducts														
Dispersal	Zoochory (birds)	Zoochory (birds)	Zoochory (birds)	Zoochory (birds)	Zoochory (birds)	Zoochory (birds)	Zoochory (birds)	Zoochory (birds)	Zoochory (birds)	Hydrochory/zoochory	Zoochory	Zoochory (birds)	Zoochory (birds)	Zoochory (birds)

obtained from herbarium specimens and Eckenwalder (2009), Farjon (2010, 2017, 2018) and Farjon and Filer (2013). For ancestral reconstruction, we used the dated phylogeny (Table S2) of Khan and Hill (2021) based on three chloroplast genes (*rbcL*, *matK* and *trnL-trnF*) and three nuclear genes (*NEEDLY*, *PHYP* and *ITS2*). The characters were mapped for their evolution using RASP 4.2-BayesTraits (Yu *et al.*, 2020) and Mesquite 3.6 (Maddison and Maddison, 2019) with maximum likelihood and the Markov chain Monte Carlo reconstruction method.

RESULTS

Lepidothamnus seed cone morpho-anatomy

The *Lepidothamnus intermedius* seed cone is oblong (Fig. 1A–C), 5–8 mm long and 2–3 mm wide, consisting of one or two ovules each surrounded by a fertile bract and three to five sterile bracts. The ovules are obliquely erect with a recurved micropyle. The seed cone longitudinal and cross sections show six anatomical regions (Fig. 2A–D).

(1) Epimatium: 8–14 layers of elongate and rounded cells produce an epimatium that is free, fleshy, asymmetrical, and cup-shaped, covering one-third of the seed (Fig. 1A–C). ii. Exotesta: 3–5 layers of elongated and rounded cells. (3). Endotesta: 5–10 compact layers of sclerified cells. (4). Nucellus: 4–7 layers of cells arranged in rows above the megagametophyte. (5). Megagametophyte: straight; the embryo is 0.4–0.9 × 0.4–0.6 mm. vi. Receptacle: bright reddish and fleshy.

Manoao seed cone morpho-anatomy

Mature seed cones of *Manoao colensoi* are oblong, erect, glaucous purple-black and surrounded by five to seven spoon-shaped spirally arranged bracts (Fig. 1D–F). There are two to four fertile bracts per cone. Seed cones are oblong, about 4–6 mm long and 1.5–2.5 mm wide. The inverted ovules are surrounded by a fleshy, asymmetrical, cup-like, free epimatium and five to seven bracts. The seed cone longitudinal and cross sections show five major anatomical regions (Fig. 2E–H).

(1). Epimatium: 12–18 layers of large and small isodiametric cells produce a fleshy asymmetrical, cup-shaped epimatium that surrounds the seed (Fig. 1D–F). An enlarged vascular bundle and resin canal are present. (2). Exotesta: 4–6 layers of long and isodiametric cells. (3). Endotesta: 2–7 layers of compact, dense, sclerified cells. (4). Nucellus: 4–8 layers of cells arranged in rows around the megagametophyte. (5). Megagametophyte: about 10–16 layers of cells; the mature embryo is 0.4–0.6 × 0.2–0.3 mm.

Lagarostrobos seed cone morpho-anatomy

Lagarostrobos franklinii has a brown cylindrical (subglobose), strictly pendulous seed cone, about 4–5.5 mm long and 2–3 mm wide, containing five to eight seeds, each surrounded by a fertile bract. Each seed conelet contains a light

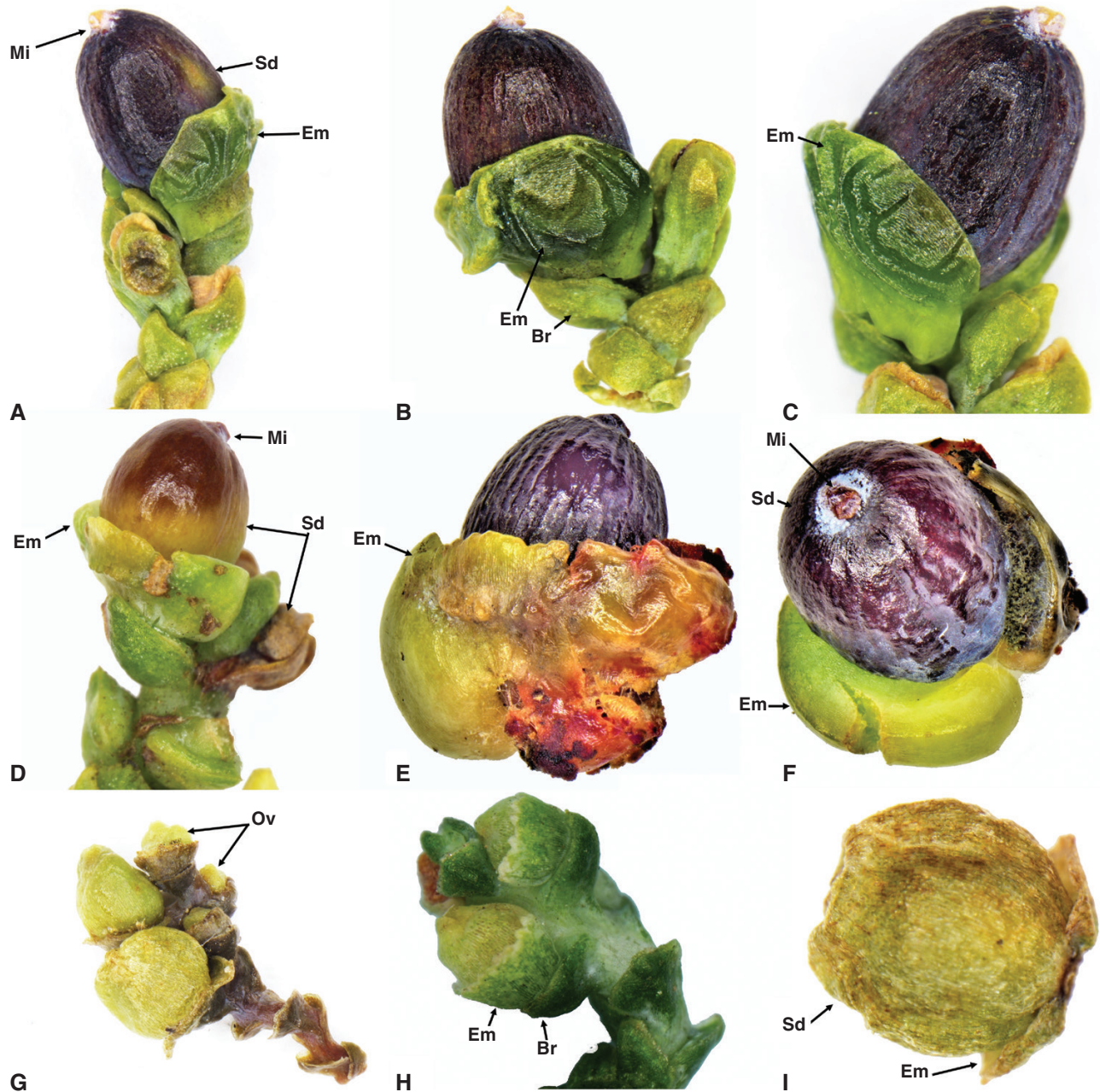


FIG. 1. Seed cone showing morphological features of *Lepidothamnus intermedius* (A–C), *Manoa colensoi* (D–F) and *Lagarostrobos franklinii* (G–I). Sd, seed; Em, epimatium; Ov, ovule; Mi, micropyle; Br, bract.

brown, fleshy (initially) or dry, papery asymmetrical epimatium in the basal part (Fig. 1G–I). The ovule is inverted. The seed cone cross section shows five anatomical regions.

(1). Epimatium: 6–10 layers of large and small isodiametric cells produce an asymmetrical epimatium covering about half of one side of the seed. A vascular bundle and resin canal are present. (2). Exotesta: 2–4 layers of long, isodiametric cells. (3). Endotesta: 4–10 layers of compact, dense, sclerified cells. (4). Nucellus: 3–7 layers of cells arranged in rows above the megagametophyte. (5). Megagametophyte: straight; the embryo is 0.3–0.5 × 0.15–0.2 mm.

Parasitaxus seed cone morpho-anatomy

Parasitaxus usta has globose, red-purplish seed cones, 3–5 mm long and 3–4 mm wide. Each cone has a single seed surrounded by a fertile bract. The outer testa is fleshy. The ovule is inverted. The seed cone cross sections show five major zones.

(1). Epimatium: 3–6 layers of rectangular and rounded cells produce an epimatium that surrounds the testa and entirely fuses with it. (2). Exotesta: 10–16 layers of small and large round and elongated cells, with several vascular traces and resin canals, along with sclereids. (3). Endotesta: 6–10 compact layers of small dense cells. (4). Nucellus: 3–7 layers of dense

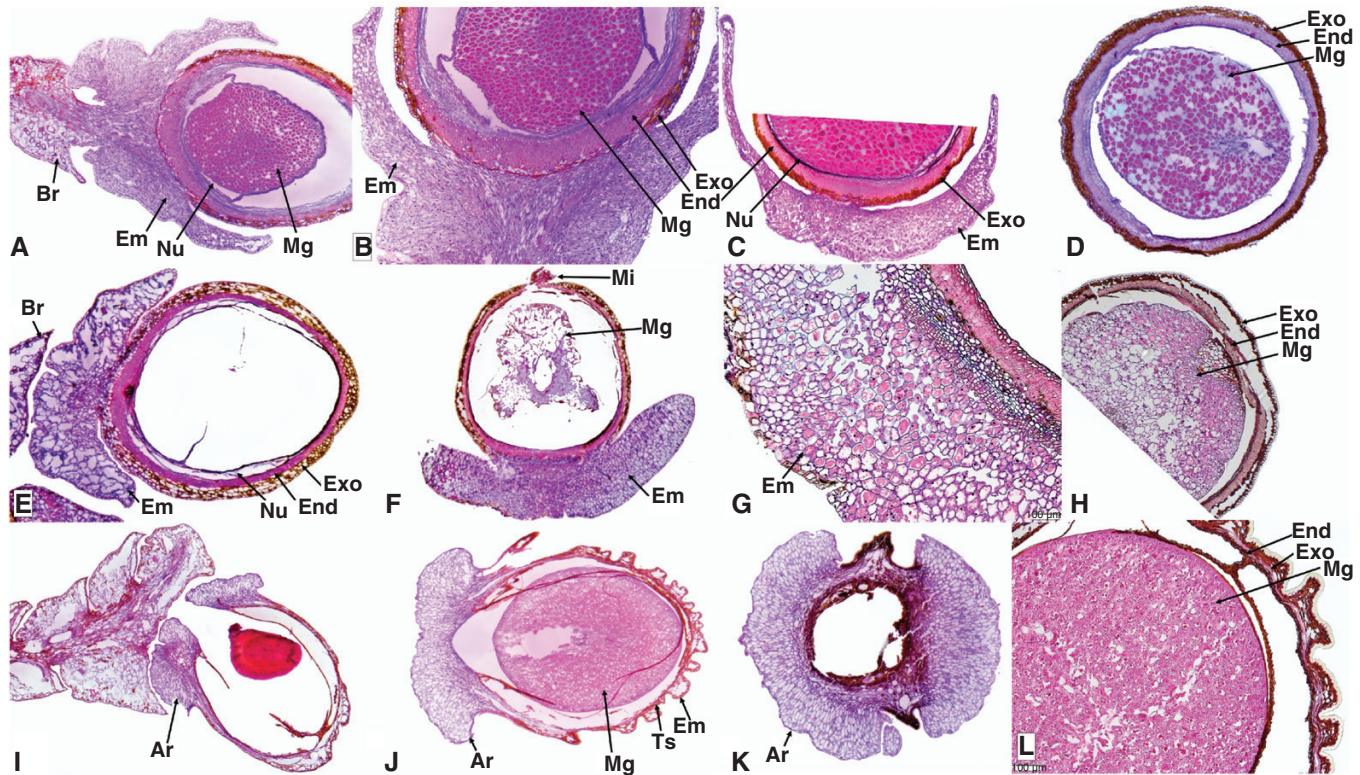


FIG. 2. Seed cone longitudinal and cross sections showing morpho-anatomical features of *Lepidothamnus intermedius* (A–D), *Manoa colensoi* (E–H) and *Halocarpus bidwillii* (I–L). Br, bract; Ar, aril; Em, epimatium; Ts, testa; Exo, exotesta; End, endotesta; Nu, nucellus; Mg, megagametophyte; Mi, micropyle; Vb, vascular bundles. Scale bars (G, L) = 100 μ m.

and smaller cells. (5). Megagametophyte: straight; the embryo is 0.2–0.5 \times 0.1–0.2 mm.

Halocarpus seed cone morpho-anatomy

The *Halocarpus bidwillii* seed cone is oblong, 3–5 mm long and 2–2.5 mm wide, with one or two inverted ovules surrounded by a fertile bract, four or five sterile bracts and one or two carpidia (a carpidium is a sub-terminal bract that is larger than the associated bract). A dark brown fleshy bract (also cited as the epimatium in the literature) is adnate to the carpidium. A white to yellowish v-shaped, fleshy free aril surrounds the seed at the proximal end. The seed cone cross section can be divided into six anatomical zones (Fig. 2I–L)

(1) Aril: free, 8–14 layers of small and elongated cells. (2) Epimatium: 2–4 layers of round-shaped cells. (3) Exotesta: 4–8 layers of small and long isodiametric cells with vascular bundles present. (4) Endotesta: 4–8 layers of compact dense cells. (5) Nucellus: 3–6 layers of cells around the megagametophyte. (6) Megagametophyte: straight; the mature embryo is 0.4–0.8 \times 0.3–0.5 mm.

Phyllocladus seed cone morpho-anatomy

Phyllocladus trichomanoides seed cones occur on the phylloclades, either singly or in a pair. The seed cone is reduced and

usually consists of one or two ovules in a cup-like elongated bract. The erect ovule is surrounded by a white, free arillus and two fused green fleshy bracts (one fertile and one sterile) (Fig. 3A–D). The seed cross section shows six anatomical regions (Fig. 4A–D).

(1) Aril: free, 3–8 layers of elongated-oval cells surround the ovule but do not fully enclose it. (2) Exotesta: 2 layers of irregular cells. (3) Mesotesta: 1 or 2 compact layers of sclerified cells. (4) Endotesta: 8–12 layers of isodiametric cells. (5) Nucellus: 10–16 layers of cells arranged in rows above the megagametophyte. (6) Megagametophyte: the mature embryo is about 0.6–0.7 \times 0.2–0.3 mm.

Phyllocladus hypophyllus produces red-purple ovoid-oblong (7–10 \times 1.6–2.5 mm) seed cones on the phylloclade. The ovule is erect, and the seeds are half covered by the aril (Fig. 3E–H). The seed cross section shows six anatomical regions (Fig. 4E–H).

(1) Aril: free, 10–16 layers of elongated-oval cells surround the ovule but do not fully enclose it. (2) Exotesta: 3 or 4 layers of irregular cells. (3) Mesotesta: 5–7 compact layers of sclerified cells. (4) Endotesta: 3 or 4 layers of isodiametric cells. (5) Nucellus: 6–12 layers of cells, arranged in rows above the megagametophyte. (6) Megagametophyte: the mature embryo is 0.5–0.6 \times 0.2–0.3 mm.

Phyllocladus aspleniifolius produces pink-red fleshy multiovulate cones on the phylloclade. The cones are ovoid-oblong, 5–8 mm long and 2–2.5 mm wide with two to five greenish-black semi-ovoid seeds. Each seed is half

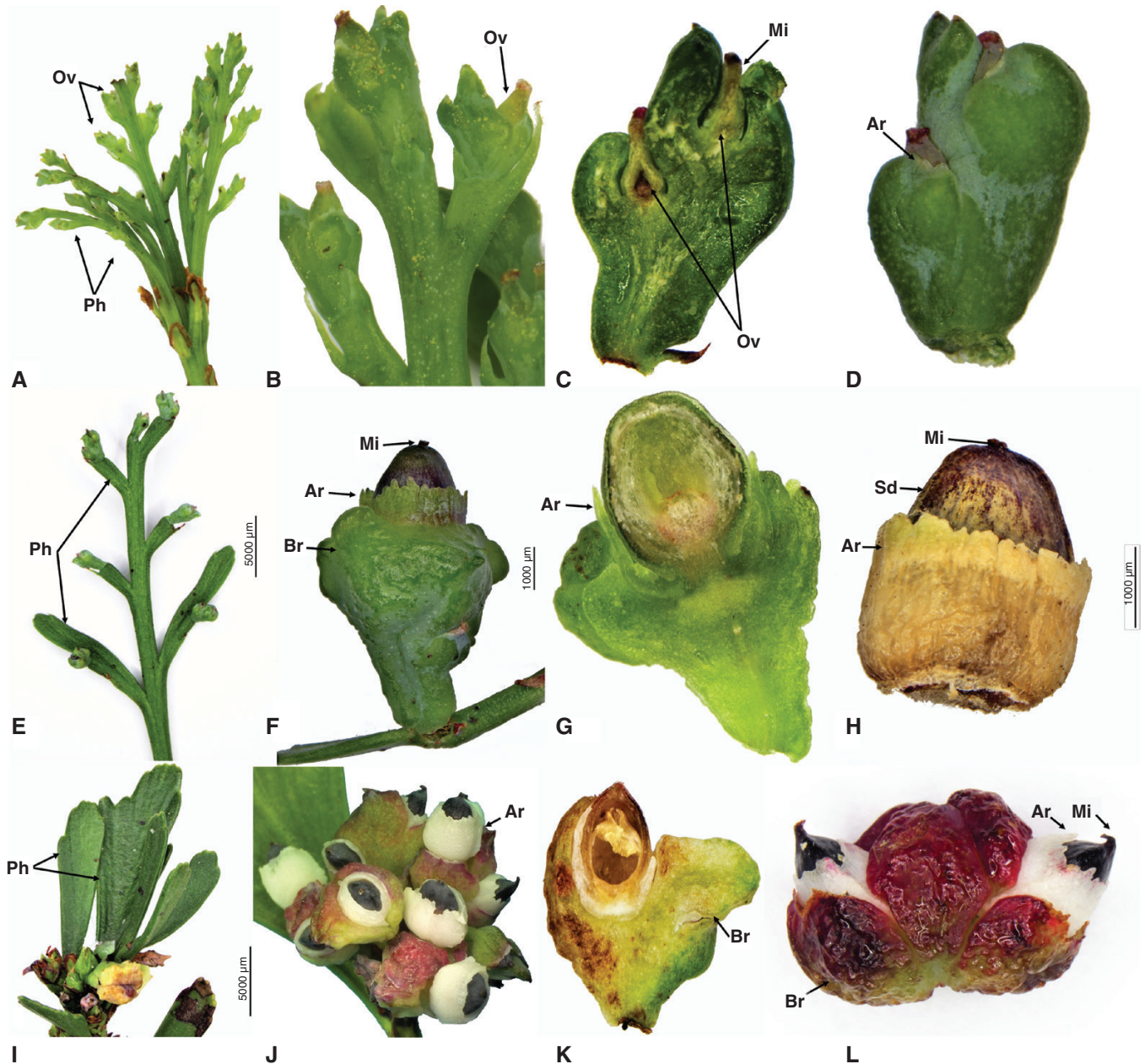


FIG. 3. Seed cone morpho-anatomical features of *Phyllocladus trichomanoides* (A–D), *P. hypophyllus* (E–H) and *P. aspleniifolius* (I–L). Ph, phylloclades; Sc, seed cones; Ov, ovule; Ar, aril; Mi, micropyle; Br, bract. Scale bars (F, J) = 5000 μm ; (G, H) = 1000 μm .

enclosed by a white aril and fleshy bracts, which are intensely coloured (varying from pink to dark red) (Fig. 3I–L). The seed cross section shows six anatomical regions (Fig. 4I–L).

(1) Aril: free, 4–8 layers of rounded cells subtend the bracts but do not fully enclose the seed. (2) Exotesta: 1 or 2 layers of rounded cells. (3) Mesotesta: 5–8 compact layers of sclerified cells. (4) Endotesta: 3 or 4 layers of fibre cells. (5) Nucellus: 4–10 layers of cells surrounding the megagametophyte. (6) Megagametophyte: the mature embryo is 0.4–0.6 mm \times 0.2–0.3 mm.

Prumnopitys andina seed cone morpho-anatomy

Prumnopitys andina produces yellow, oblong-subglobose seed cones with 4–12 inverted ovules. Each ovule is half surrounded by a fertile bract (Fig. 5A–C). The seed cone cross sections show six major zones (Fig. 5D–F).

(1) Epimatium: fused, 4–12 layers of small isodiametric cells. (2) Exotesta: 16–22 layers of small and large round and elongated cells. The exotesta is fleshy and a connate part of the epimatium forms a fleshy sarcotesta-like seed coat. The outer layers have larger cells than the inner layer, which has smaller

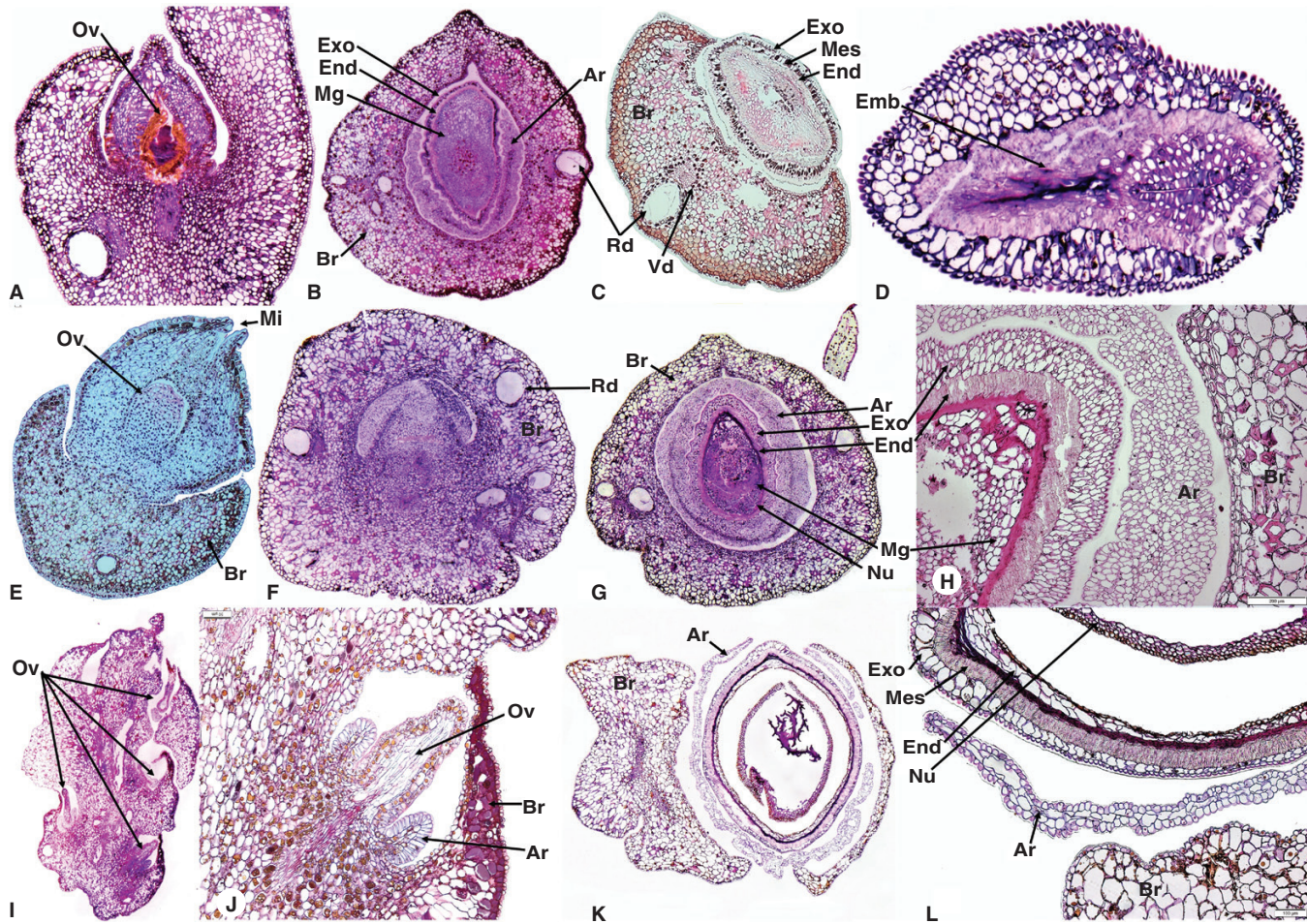


FIG. 4. Seed cone longitudinal and cross-sections showing morpho-anatomical features of *Phyllocladus trichomanoides* longitudinal sections (A, B, D) and cross section (C), *P. hypophyllus* (E–H) and *P. asplenifolius* (I–L). Br, bract; Sd= Seed; Ov, ovule; Ar, aril; Mi, micropyle; Exo, exotesta; End, endotesta; Emb, embryo; Rd, resin duct; Vb, vascular bundles; Nu, nucellus (Nu); Mg, megagametophyte. Scale bars (H) = 200 μm ; (L) = 100 μm .

and dense cells. Vascular traces and resin canals are present in the inner layer. (3) Mesotesta: 6–10 layers of denser and compact isodiametric, parenchymatic cells. (4) Endotesta: 12–20 compact layers of small, dense, sclerified cells allow the woody part of the seed cone to form a sclerified sclerotesta-like structure. (5) Nucellus: 6–12 layers of dense and smaller cells constitute the protective cover of the embryo. (6) Gametophyte: 10–16 layers of cells; the mature embryo is 0.6–0.9 \times 0.2–0.4 mm.

Pectinopitys ferruginea seed cone morpho-anatomy

Pectinopitys ferruginea produces red, ellipsoid seed cones. The seed cones have one or two inverted ovules each surrounded by a fertile bract (Fig. 5G–I). The seed cone cross sections show six major zones (Fig. 5J–L).

(1) Epimatium: fused, 4–6 layers of rectangular and rounded cells, including sclereids. (2) Exotesta: 12–16 layers of large and small elongated cells constitute the fleshy layers of the epimatium forming a fleshy sarcotesta-like seed coat, containing several vascular traces and resin canals, along with several enlarged resin ducts. (3) Mesotesta: 3–6 layers of dense

and compact isodiametric, parenchymatic cells. (4) Endotesta: 6–12 compact layers of dense sclerified cells constitute the woody part of the seed cone, forming a sclerified sclerotesta-like structure. (5) Nucellus: 13–18 layers of smaller, dense cells constitute the protective cover of the embryo. (6) Gametophyte: straight; the mature embryo size is 0.6–1.0 \times 0.3–0.5 mm.

Sundacarpus amarus seed cone morpho-anatomy

Sundacarpus amarus produces reddish, oblong-subglobose seed cones with an inverted ovule (Fig. 5M–O). The seed cone cross sections show six major zones (Fig. 5P–R).

(1) Epimatium: fused, 3–6 layers of rectangular and rounded cells. (2) Exotesta: 14–26 layers of small and large round and elongated cells. The exotesta is the fleshy part of the epimatium, forming a fleshy sarcotesta-like seed coat, with many vascular bundles and small and elongated resin canals. (3) Mesotesta: 4–6 layers of dense and compact round to elongated irregular, parenchymatic cells. Sclereids are not present. (4) Endotesta: 3–6 compact layers of small, dense, sclerified cells make the woody part of the seed cone, forming a sclerified sclerotesta-like

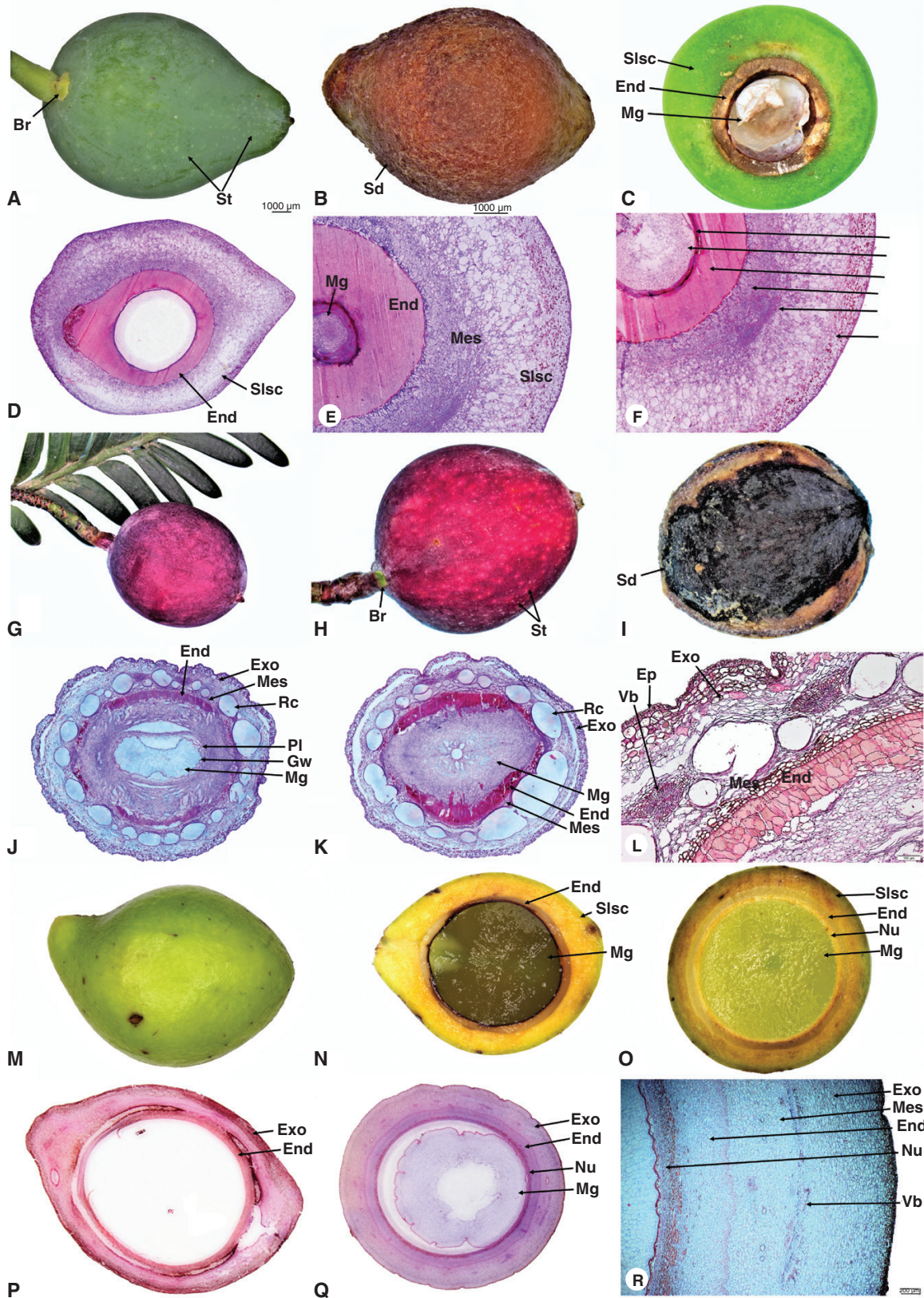


FIG. 5. Seed cone photos (A, B, G, H, I and M), longitudinal and cross-sections showing morpho-anatomical features of *Prumnopitys andina* longitudinal (D) and cross sections (C, E, F); *Pectinopitys ferruginea* longitudinal (J) and cross sections (K, L); *Sundacarpus amarus* longitudinal (N, P) and cross sections (O, Q, R); Br, bract; St, stomata; Slsc, fleshy-sarcotesta-like seed coat; Sd, seed; Exo, exotesta; End, endotesta; Mg, megagametophyte; Nu, nucellus; Vb, vascular bundles; Mes, mesotesta; Rc, resin canal; Gw, gynospore wall; PI, Parietal layers. Scale bars (A, B) = 1000 μ m; (L) = 100 μ m; (R) = 200 μ m.

structure. (5) Nucellus: 13–18 layers of dense and smaller cells constitute the protective cover of the embryo. (6) Gametophyte: straight; the mature embryo size is $0.7\text{--}1.3 \times 0.3\text{--}0.5$ mm.

Comparison of seed cone morpho-anatomical traits

Species of the Prumnopityoid clade show both morpho-anatomical affinities and variation of functional traits and structures within the clade and with other Podocarpaceae genera. The shape of the seed cone varies from ovoid (e.g. *Manoao colensoi*), narrow cylindrical (e.g. *Lagarostrobos franklinii*), globose (e.g. *Parasitaxus usta*) to ellipsoid. Seed cone sizes are the smallest in the scale-leaved Halocarpioid subclade species, while species of the broad-leaved Pectinopityoid subclade have the largest cone size (Supplementary Data Fig. 1). *Manoao*, *Lagarostrobos* and *Lepidothamnus* have a free, asymmetrical, cup-shaped epimatium, while in *Halocarpus* a coriaceous epimatium surrounds the ovule but is not fused with the testa (Figs 1 and 2). In *Phyllocladus*, the epimatium is absent but there is a well-developed free fleshy aril (Figs 3 and 4). *Halocarpus* has both an aril and an epimatium. The mesotesta is not evident in *Lepidothamnus*, *Manoao* or *Lagarostrobos* and the endotesta has sclerified cells but is not woody. The exotesta of mature seed cones of *Pectinopitys* is vascularized and has several enlarged resin ducts. Stomata are present on the seed cone surface of these genera. The seed cone arrangement varies from solitary (single-seeded) (e.g. *Parasitaxus*), both solitary and multiovulate (e.g. *Manoao*, *Lepidothamnus*, *Halocarpus*, *Pectinopitys*, *Lagarostrobos* and *Phyllocladus*) to multiovulate (e.g. *Prumnopitys andina*). Species of both subclades have differently coloured seed cones (Table 1). The morphology of the epimatium is thinly fleshy in *Lepidothamnus*, *Manoao* and *Lagarostrobos*, while in *Parasitaxus*, *Prumnopitys*, *Pectinopitys* and *Sundacarpus* the epimatium is fleshy and fused with the testa, forming a fleshy sarcotesta-like structure, with an endotesta that is hard and woody (sclerotesta) (Fig. 5B, I). Seed dispersal is mainly zoochorous except in *Lagarostrobos*, which also exhibits hydrochory.

DISCUSSION

Seed cone morpho-anatomical traits and structures

Both subclades of the Prumnopityoid clade have evolved diverse structures and traits in their seed cones.

Seed cone arrangement. In the Halocarpioid subclade, *Parasitaxus* has a single ovule per cone, *Halocarpus* and *Lepidothamnus* produce solitary cones (1 or 2 ovules per cone), and *Phyllocladus*, *Manoao* and *Lagarostrobos* have multiovulate cones. In the Pectinopityoid subclade, *Prumnopitys* subgenus *Botryopitys* (e.g. *P. montana*) and related genera (*Pectinopitys* and *Sundacarpus*) have simple multiovulate cones (2 seeds per cone, Page, 2019). *Prumnopitys* subgenus *Prumnopitys* has multiovulate cones (*P. andina* has about 4–12 seeds and *P. taxifolia* about 5–10 seeds; see also Mill et al., 2004).

Fleshy seed cones. All genera of the Pectinopityoid and Halocarpioid subclades produce colourful fleshy seed cones (Figs 1, 3 and 5), although the mechanism and fleshy structures (e.g. epimatium, aril, receptaculum and bracts) differ between and within these subclades. Fleshiness is an ancestral trait in podocarps, and *Pherosphaera* and *Saxegothaea* are the only two extant genera with non-fleshy seed cones (Herting et al., 2020; Khan and Hill, 2021). *Halocarpus* produces fleshy cones using the aril, *Phyllocladus* uses the aril plus bracts, *Lepidothamnus* uses the receptaculum plus epimatium, and *Manoao* and *Lagarostrobos* use the epimatium, while in *Prumnopitys*, *Pectinopitys*, *Sundacarpus* and *Parasitaxus* fleshy seed cones are produced by the fusion of the epimatium and exotesta.

Prumnopitys, *Pectinopitys*, *Sundacarpus* and *Parasitaxus* produce drupe-like seed cones. The other two genera with similar drupe-like cones are *Retrophyllum* and *Afrocarpus*. *Lepidothamnus* produces Dacrydioid-type cones and has a fleshy receptaculum and asymmetrical epimatium. The other two genera with similar cone morphology are *Falcatifolium* and *Dacrydium* (Khan and Hill, 2021). *Phyllocladus* and *Halocarpus* have special ariloid-type cones with an aril and fleshy bracts. *Manoao* and *Lagarostrobos* have seed cones with fleshy, asymmetrical epimatium.

Epimatium morphology and occurrence. An epimatium is present in all genera except *Phyllocladus* and *Pherosphaera* (Tomlinson et al., 1991; Khan and Hill, 2021). The epimatium is either free or fused with the outer testa in this clade. In the taxa studied here, the epimatium is free and fleshy in *Lepidothamnus*, *Manoao* and *Lagarostrobos*. *Dacrydium* and *Falcatifolium* are the other two Podocarpaceae genera in the Dacrydioid clade with a similar free and fleshy epimatium (Khan and Hill, 2021). When the epimatium is fused with the testa it is either fleshy or papery, forming a sarcotesta-like seed coat (Fig. 6), such as in *Parasitaxus*, *Pectinopitys*, *Prumnopitys* and *Sundacarpus*, and this also occurs in *Retrophyllum*, *Afrocarpus* and some species of *Podocarpus* (i.e. *P. smithii*, *P. henkelii*, *P. madagascariensis* and *P. capuronii*) in the Podocarpioid clade (Mill et al., 2001, 2004; Khan and Hill, 2021).

In *Halocarpus*, the epimatium is also fused with the outer testa but is papery, similar to *Dacrycarpus*, *Acropyle* and most species of *Nageia* and *Podocarpus* (Khan and Hill, 2021). An epimatium is considered equivalent to an ovuliferous scale and is possibly eliminated in *Phyllocladus* by a reduction similar to that observed in *Pherosphaera* (Sinnott, 1913; Wilde, 1944; Tomlinson, 1992; Khan and Hill, 2021).

Aril morphology and occurrence. A fleshy, cup-shaped aril is only present in *Phyllocladus* (Fig. 3) and *Halocarpus*. The ancestral state reconstruction shows that an aril is a derived structure and evolved twice independently (Fig. 7). *Halocarpus* has an inverted ovule with a fused papery epimatium and a fleshy aril that develops at the proximal end of the bract (carpidium) and hence is the only genus with an aril, an epimatium and a distinct carpidium (Molloy, 1995). The only other conifer family with an aril is the Taxaceae and there the aril is interpreted as a fused pair of strongly swollen leaves (e.g. *Pseudotaxus chienii*) rather than a modified integument (Dörken et al., 2019). Hooker (1852) placed *Phyllocladus* in Taxaceae due to the similarity of their arils.

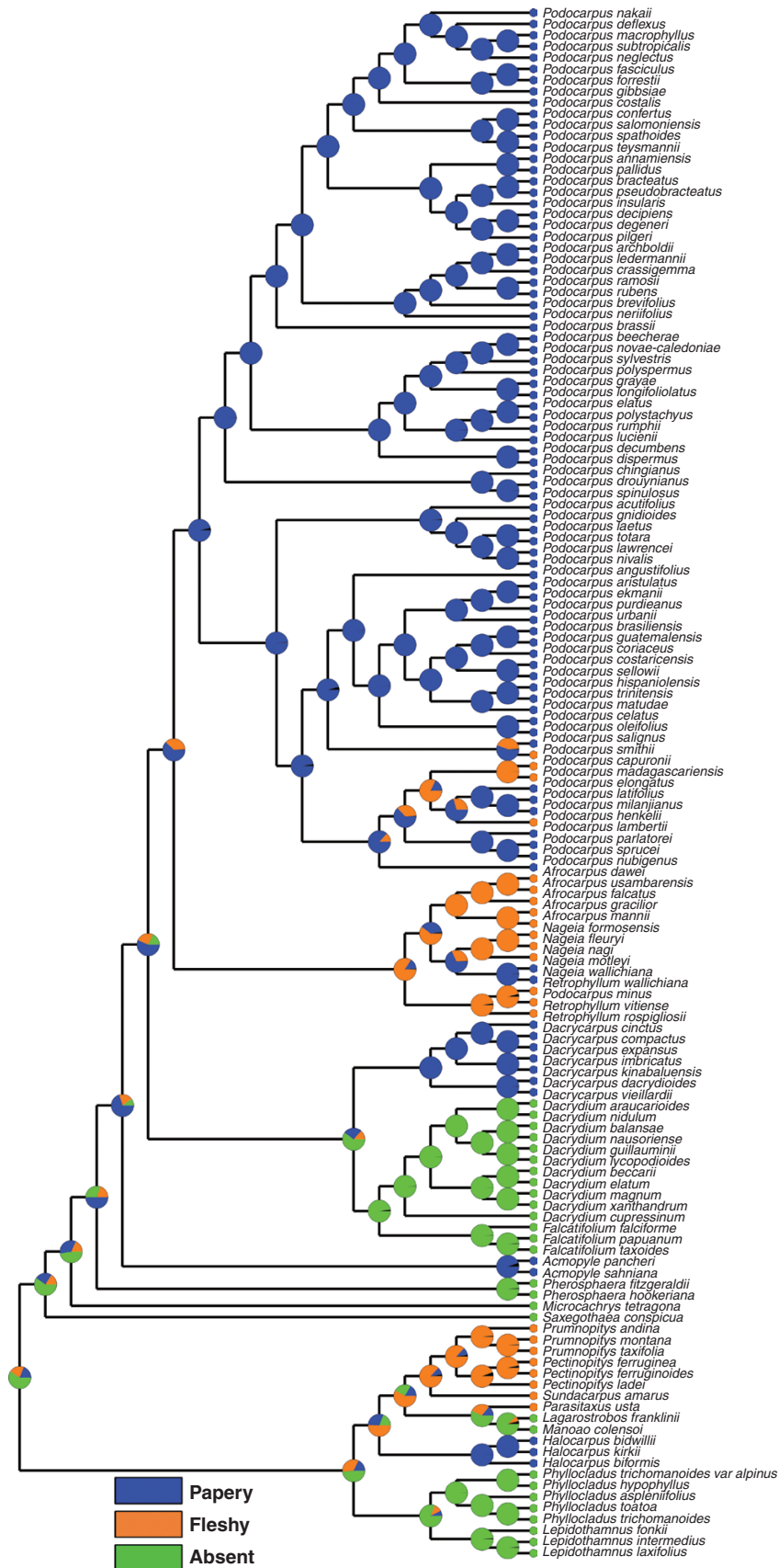


FIG. 6. Character mapping of the fused epitium morphology in different genera of Podocarpaceae using maximum likelihood.

Testa morpho-anatomy. In *Lepidothamnus*, *Halocarpus*, *Phyllocladus*, *Manoao* and *Lagarostrobos* the testa is non-fleshy and has a papery structure (Supplementary Data Fig. 3). The endotesta in these genera is also non-woody. Other podocarp genera, e.g. *Dacrydium*, *Dacrycarpus*, *Falcatifolium*, *Nageia* (except *N. wallichiana* and *N. motleyi*) and *Podocarpus* (except *P. smithii*, *P. henkelii*, *P. madagascariensis* and *P. capuronii*), have a similar testa morphology (Khan and Hill, 2021), but in *Acmopyle* the epimatium and exotesta are papery and the endotesta is woody.

In *Parasitaxus*, *Pectinopitys*, *Prumnopitys* and *Sundacarpus* the outer testa is fused with the epimatium, forming a fleshy sarcotesta-like seed coat (Fig. 6). The endotesta is stony and woody and forms a sclerotesta-like structure. The ancestral state reconstruction shows that this sclerotesta-like structure has evolved multiple times in podocarps (Fig. 8) (e.g. *Retrophyllum*, *Afrocarpus*, *Nageia* and some species of *Podocarpus*) (Khan and Hill, 2021). The testa is resinous and vascularized in the Pectinopityoid subclade. Mill *et al.* (2004) also reported the presence of a vascularized testa (sarcotesta) in *Pectinopitys*, *Prumnopitys* and *Afrocarpus*. In all three genera, the endotesta is woody and stony (sclerotesta). Sclereids are present in the exotesta of *Pectinopitys ferruginea*. In both *Prumnopitys andina* and *P. taxifolia* the mesotesta is more or less sclerified and the seed cone anatomy shows the presence of simple vascular traces and few unbranched resin canals. In contrast, the seed cone of *Pectinopitys ferruginea* has an interconnected network of resin ducts (Mill *et al.*, 2004).

Ovule morphology and traits. All of the Prumnopityoid clade genera have inverted ovules except *Lepidothamnus* and *Phyllocladus*, where ovules are erect (i.e. ovules are vertical to the bract) (Tomlinson *et al.*, 1991; Herting *et al.*, 2020). Within the Podocarpaceae, *Pherosphaera* and *Acmopyle* are the only other genera with erect ovules. All species of *Phyllocladus* examined here have erect ovules (see also Möller *et al.*, 2000, who reported erect ovules in *P. hypophyllum*). This pendulous position of the ovules in conifers is important for success in pollen capture and the pollination drop mechanism takes advantage of the saccate pollen grains in Podocarpaceae (Tomlinson *et al.*, 1991; Owens *et al.*, 1998; Dörken and Jagel, 2014). Ovule inversion occurs in the rest of the Prumnopityoid clade. Some early studies (Sahni, 1921; De Laubenfels, 1969) reported the presence of an inverted ovule in *Acmopyle* in the early developmental stages but Mill *et al.* (2001) reported that no developmental stage showed an inverted ovule in *Acmopyle*.

The micropyle orientation in the mature seed differs among the genera of the Prumnopityoid clade and both inverted and erect ovules are present in the early developmental stages (Fig. 9). The mature seed micropyle of *Manoao*, *Lagarostrobos*, *Phyllocladus*, *Prumnopitys*, *Pectinopitys* and *Lepidothamnus* is oriented towards the apex of the cone axis (Fig. 1). *Acmopyle*, *Pherosphaera*, *Dacrydium* and *Falcatifolium* share this orientation (Herting *et al.*, 2020). *Lepidothamnus* and *Manoao* seeds have a hook-like micropyle, a character shared with *Acmopyle* (Tomlinson *et al.*, 1997; Möller *et al.*, 2000).

Comparative reproductive biology. The reproductive cycle of the Halocarpioid subclade species is usually completed in

1–2 years, while in the Pectinopityoid subclade it is usually completed in ≥ 2 years (e.g. Tomlinson, 1992). The number of prosuspensor cells varies among the genera: *Saxegothea* has 3 or 4, *Phyllocladus* has 4–6, *Prumnopitys* has 7–9, *Dacrydium* has 7–11 and *Nageia* has 18–23 (Doyle and Looby, 1939; Buchholz, 1941). The embryological development stages of *Phyllocladus trichomanoides* are similar to those of *P. aspleniifolius* (Kildahl, 1908; Quinn, 1986). The process of archegonia formation in *P. trichomanoides* is also similar to that in *Prumnopitys andina* (Looby and Doyle, 1944) and a megaspore (gynospore) membrane similar to that of *Phyllocladus* is also reported in *Lepidothamnus laxifolius* and *Pectinopitys ferruginea* (Quinn, 1965). The pattern of seed cone development of *Manoao colensoi* is closely related to that of *Lepidothamnus laxifolius* (Quinn, 1966), although the megaspore membrane of *L. laxifolius* is thinner than in *M. colensoi* (Quinn, 1965). In *Sundacarpus*, two embryo systems have been reported (Buchholz, 1941).

Diversity in functional traits of living podocarps and fossil evidence

All species in the Prumnopityoid clade produce fleshy seed cones, although the fleshy structures differ among genera. Podocarps in general produce fleshy seed cones using different structures and they have evolved multiple times (Khan and Hill, 2021). Klaus and Matzke (2020) reported that the earliest transition from non-fleshy to fleshy seed cones in the Podocarpaceae occurred in the Cretaceous (~82.4 Ma), but the fossil record contains fleshy seed cones much earlier than this. The fleshy structure in the fossilized seed cones of *Lepidothamnus* (Fig. 10) from the Middle Cretaceous of Winton, Queensland, Australia, also supports the presence of fleshy seed cones early in the fossil record (Peters, 1985). Similarly, a fleshy epimatium has been reported in the fossil seed cones of *Harrisiocarpus gucikii* and *H. cracoviensis* from the Middle Jurassic of Poland (Reymanówna, 1987). Similarly, fleshy seed cones of *Podocarpospermum* (Rajmahal Basin, India) are reported from the Lower Cretaceous (Banerji and Ghosh, 2006).

The presence of an epimatium is an ancestral trait in the Prumnopityoid clade and in Podocarpaceae and it is present in all genera of the clade except *Phyllocladus*. The ovuliferous scales of some extinct podocarps (e.g. *Mataia* and *Nipaniostrobus*) are folded over, covering half of the seed and are interpreted as an early evolutionary stage of epimatium evolution (Rao, 1943; Townrow, 1967; Miller, 1977; Stewart *et al.*, 1993). *Stalagma* is another extinct genus from the Late Triassic in which a thin layer of tissue partially covers the seed and is considered the first evidence of an epimatium (Miller, 1977; Taylor *et al.*, 2009; Contreras *et al.*, 2017). The seed cones from the Middle Jurassic of Poland of two extinct fossil species (*Harrisiocarpus gucikii* and *H. cracoviensis*) resemble those of *Microcachrys* and show the presence of a fleshy epimatium (Reymanówna, 1987). Similarly, seed cones of an undescribed extinct species of *Lepidothamnus* from the middle Cretaceous of Winton, Queensland (Peters, 1985), show the presence of an asymmetrical cup-shaped epimatium (Fig. 10).

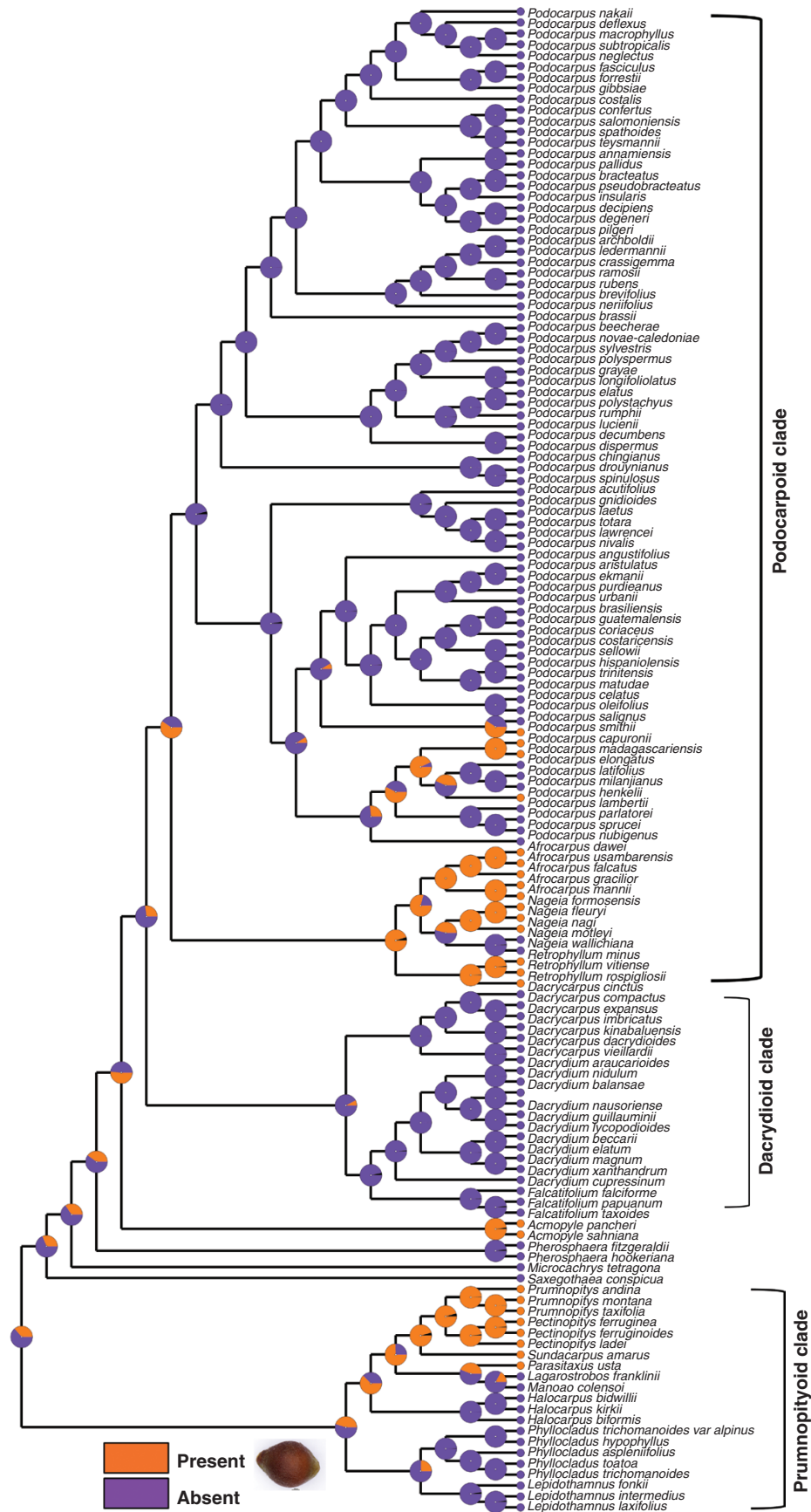
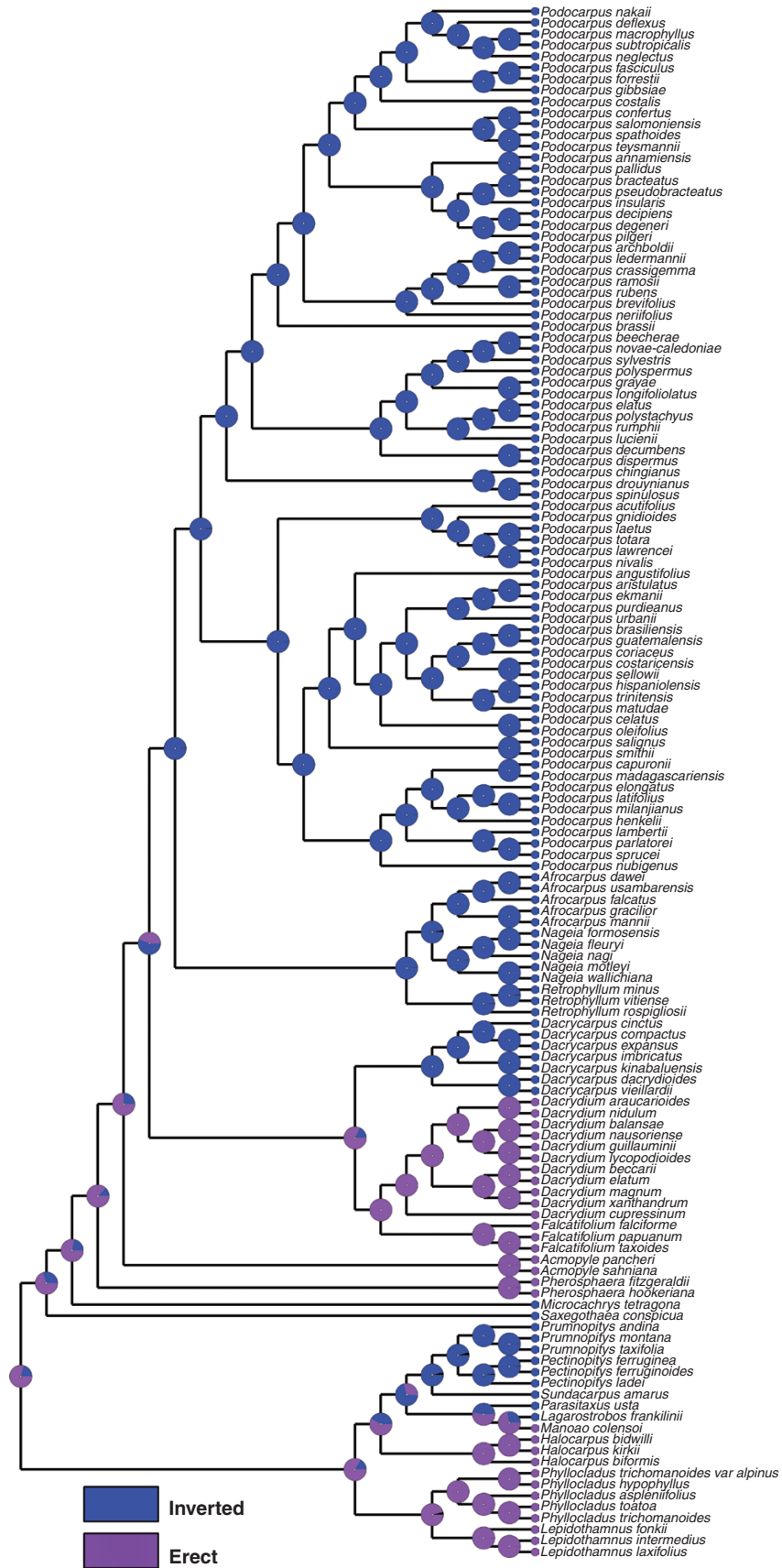


FIG. 8. Maximum likelihood character mapping of the presence of sclerotesta-like structures in Podocarpaceae.



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FIG. 9. Character mapping of micropyle orientation in the mature seed in different genera of Podocarpaceae using RASP 4.2 and maximum likelihood.

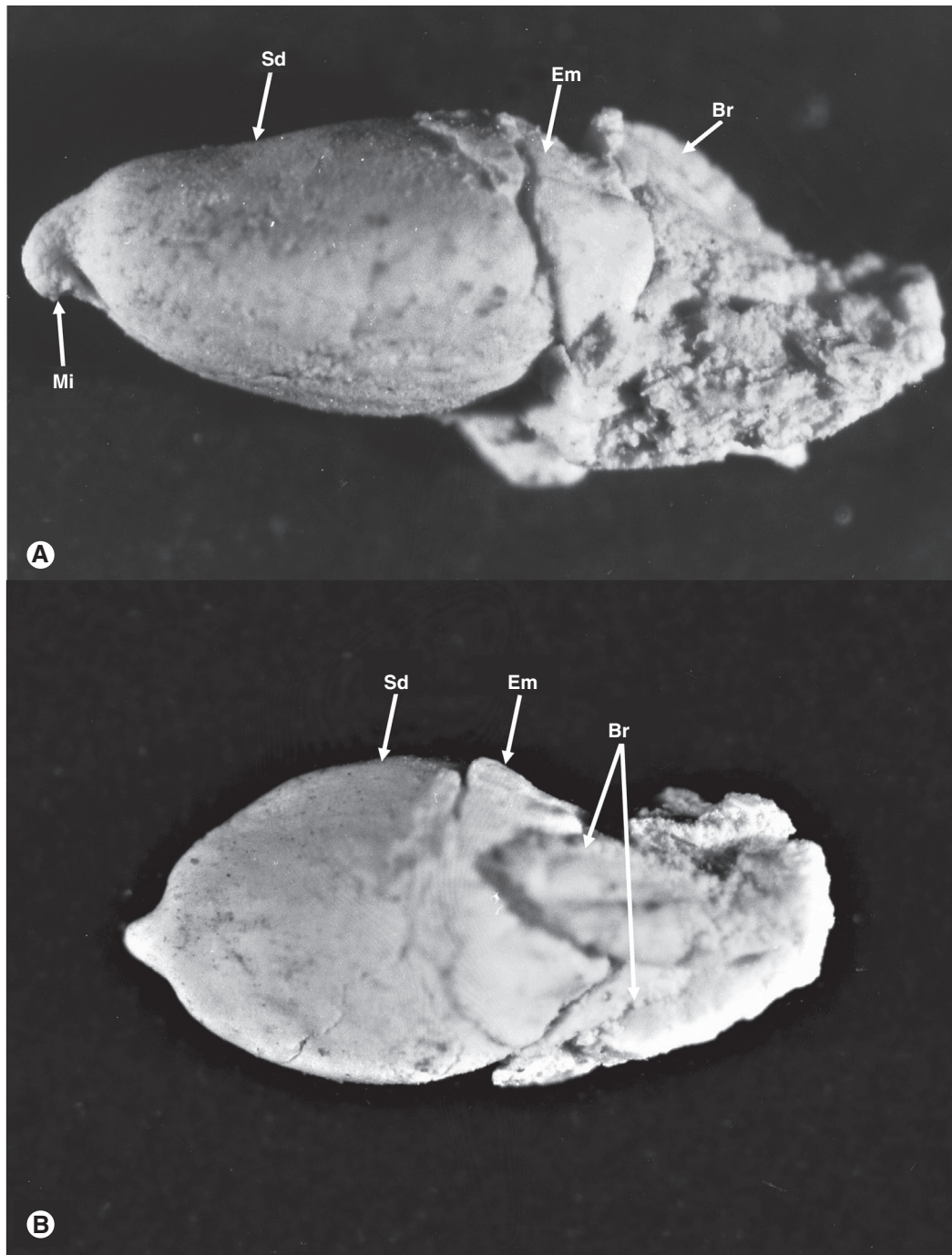


FIG. 10. The seed cones (A, B) of an undescribed *Lepidothamnus* species from the mid-Cretaceous Winton, Queensland, shows morphological similarities, e.g. of the bract (Br), seed (Sd) and epimatium (Em), to those of living *Lepidothamnus* species. The photograph was taken by Dr M. D. Peters and is from the collection held in the David T. Blackburn Palaeobotany Collection at the University of Adelaide.

The Triassic and Jurassic fossil record shows the presence of seed cones with inverted ovules (e.g. *Mataia*, *Harrisiocarpus* and *Nipaniostrobus*) (Rao, 1943; Townrow, 1967; Reymanówna, 1987). In the undescribed species of *Lepidothamnus* from the mid-Cretaceous of Queensland, the micropyle of the seed is towards the seed cone apex (Peters, 1985).

Dispersal of seed cones

The gain or loss of traits within lineages influences their ecological dispersal through evolutionary time (Klaus and Matzke, 2020). Seed cones from the Prumnopityoid clade have predominantly zoochorous dispersal and the fleshy structures, e.g. aril, epimatium, receptaculum and sarcotesta-like seed coat,

complement zoochory in extant species (Khan and Hill, 2021, 2022). *Lagarostrobos* is the only genus in the clade that shows both zoochory and hydrochory. *Lagarostrobos franklinii* is usually dispersed by water due to the flotation of seeds. However, green rosellas (*Platycercus caledonicus*) have also been observed to feed on *Lagarostrobos* and can potentially disperse the seeds (Shapcott, 1991). *Lagarostrobos* produces many seed cones in cycles of 5–6 years. *Retrophyllum comptonii* is another podocarp that shows both zoochory and hydrochory as a dispersal mechanism. *Manoao* has zoochorous seed dispersal and the *Lepidothamnus* seed cone morphology also favours zoochory (Molloy, 1995). *Phyllocladus* seeds are bird-dispersed and the birds digest the fleshy bracts and aril and pass the hard seeds in droppings (Tomlinson et al., 1989; Barker, 1995; Wagstaff, 2004; Jennings and Neyland, 2011).

The Pectinopityoid subclade also exhibits zoochory. *Prumnopitys andina* grows in mid-altitude forests in Argentina and Chile, where birds eat the seed cones, and the seeds are dispersed in their droppings (Beveridge, 1964). In the central North Island of New Zealand, *Pectinopitys ferruginea* exhibits frugivory (Beveridge, 1964). *Prumnopitys taxifolia* also exhibits zoochory in the Central North Island of New Zealand (Beveridge, 1964). The fleshy seed cones of the Prumnopityoid clade encourages zoochory, especially bird dispersal, and this is important for dispersal over long distances. Westcott et al. (2005) reported that *Sundacarpus* is probably dispersed by the flightless cassowary (*Casuarius*).

Evolutionary perspective

The Prumnopityoid clade has evolved considerable diversity in the fleshy and coloured seed cones. Four distinct types of fleshy seed cones (e.g. drupe-like, receptaculate, arilloid and dacrydioid-type cones) were recognized based on functional structures in the Prumnopityoid clade. Both free and fused epimatia have evolved in the clade (Fig. 6). An aril is a unique feature of this clade (only present in *Halocarpus* and *Phyllocladus*) in the Podocarpaceae. Similarly, both imbricate and flattened leaves are present in this clade and phylloclades are a unique trait present only in this clade. The presence of phylloclades could be a possible adaptation to cope with the competition and pressure (e.g. low light intensity) following the arrival of angiosperms, especially in the Palaeocene–Eocene (Dörken et al., 2021).

Fleshy and coloured seed cones seem likely to be an ancestral trait in this clade. The fact that the Prumnopityoid clade uses different pathways to produce fleshy seed cones demonstrates the ecological and evolutionary significance of this strategy. Klaus and Matzke (2020) stated that podocarps had probably evolved fleshy structures by the Late Cretaceous (~82.4 Ma), correlating with the early diversification of birds (~70–80 Ma) (Viseshakul et al., 2011; Klaus and Matzke, 2020). This evolutionary trait helps to attract birds and other animals for the dispersal of the seeds and could have played a role in long-distance dispersal in the Late Cretaceous (~70–80 Ma) (Klaus and Matzke, 2020). Birds are considered to have evolved from theropod dinosaurs during the Jurassic (around 165–150 Ma) (Brusatte et al., 2015). The living genera of the clade are predominantly

zoochorous and the production of fleshy seed cones is ancestral in the Prumnopityoid clade. However, most of these lineages demonstrate the presence of fleshy structures by the Triassic–Jurassic.

CONCLUSIONS

The reconstruction of the origin and drivers of the evolution of fleshy seed cones in podocarps is complex. The current study of the Prumnopityoid clade shows that fleshiness of seed cones is an ancestral trait that has been achieved using diverse mechanisms and functional structures. The Prumnopityoid clade now contains four main types of distinctive seed cones. The extant species of this clade are predominantly adapted to zoochorous dispersal. The evolution of different functional traits (e.g. fleshiness, aril, epimatium and receptaculum) shows multiple evolutions of fleshiness among these taxa. The seed cone morphology of the Pectinopityoid subclade is quite distinctive from that of the species of the Halocarpoid subclade.

SUPPLEMENTARY DATA

Supplementary data are available online at [https://academic.oup.com/aob](https://academic.oup.com/aob/article/130/5/637/6652287) and consist of the following. **Figure S1**: seed cone size of both subclades shows that the Pectinopityoid subclade has larger cone size than the Halocarpoid subclade. **Figure S2**: character mapping of the presence of a receptaculum in different genera of Podocarpaceae using RASP 4.2 and maximum likelihood. **Figure S3**: character mapping of testa morphology in different genera of Podocarpaceae using maximum likelihood. **Table S1**: fossil taxa used for calibration of the phylogeny. **Table S2**: seed cone specimens collected from the Botanical Gardens.

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