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WOLLEMIA NOBILIS (ARAUCARIACEAE): BRANCHING, VASCULATURE AND HISTOLOGY IN JUVENILE STAGES[1]

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We present a preliminary description of the morphology and anatomy of contrasted axes in the recently discovered conifer *Wollemia nobilis*, based on clonally propagated material. The novelty of this discovery and the tree's size and rarity has led to global interest and a comprehensive and successful conservation program. Our results should serve as a model on which future studies of this tree and other members of the Araucariaceae can be based. The specimens studied are mimics of the architecture of the mature tree, with precise distinction between orthotropic (= trunk) axes, which have radial symmetry (spiral phyllotaxy) and plagiotropic (= branch) axes with dorsiventral symmetry (as a modified decussate phyllotaxy). Trunk axes develop irregular pseudowhorls of branches that originate within the terminal bud by syllepsis, their extension coincident with that of the parent axis. The two kinds of axes show considerable anatomical differences, but are still based on the common feature of a single stelar-derived trace to each leaf that becomes subdivided in the cortex, a feature of the whole family. Trunk axes include extended cortical leaf and branch traces associated with abundant sclerenchyma, but branches have short cortical leaf traces, no branch traces, and limited sclerenchyma. Reiteration is limited and largely involves the formation of basal suckers on the trunk. Branches normally remain unbranched, but can do so most often when damaged. This study thus emphasizes the phenomenon of axis differentiation in conifers, which has been little investigated anatomically, but could be very important in the identification of fossils.

Key words: Araucariaceae; Massart's model; orthotropy; plagiotropy; shoot morphology; vascular anatomy; *Wollemia nobilis*.

Conservation strategies for the rare and threatened conifer *Wollemia nobilis* (Jones et al., 1995) have resulted in the wide distribution of clonal material that has been made freely available. Further work can now be added to the extensive studies that have been carried out (e.g., Burrows, 1999, Burrows and Bullock, 1999; Burrows et al., 2003, 2007; Heady et al., 2002; Hill, 1997; Offord et al., 1999). Much of this effort has concentrated on the establishment of the systematic position of *Wollemia.* Here we report on the contrast between its orthotropic and plagiotropic axes in terms of vasculature, histology, and branching such that a comparison can eventually be made to equivalent material of *Agathis* (21 spp.) and *Araucaria* (19 spp.), which together with *Wollemia,* constitute the distinctive conifer family Araucariaceae. However, we are aware of the caution needed in applying information derived from ex situ material of small plants, which simulate juvenile stages, to an interpretation of the structure and development of mature in situ specimens, a sentiment already voiced by Burrows et al. (2003). They made this proviso with reference to the plants they studied that were 2 m tall specimens grown from seed in a shade house. Our study is even more restricted in the absence of seed-derived material because it is based on similar-sized plants propagated from stem cuttings. We refer to them as "juvenile" although they are not seed-grown. Nevertheless, we expect our findings to have relevance to taller plants that increasingly can be studied as they approach maturity in cultivation. Our study shows how development and histological features differ in the two contrasted kinds of vegetative axis produced by *Wollemia*.

For architectural comparison, we use the set of architectural models, initially defined by Hallé and Oldeman (1970) and elaborated in Hallé et al. (1978) as convenient points of reference in the tree architectural continuum. *Wollemia* conforms well to Massart's model, which shows rhythmic growth of a radially symmetrical orthotropic axis (vertical trunk) that produces tiers of first-order plagiotropic (branch) axes, each with dorsiventral symmetry and horizontal orientation. The different genera of Araucariaceae express this basic model in different ways, especially in terms of the timing of branch initiation in relation to trunk extension and the extent of further branching in the plagiotropic axes. This variation together with variation in the extent to which the architecture can be augmented by its repetition (reiteration) can produce trees with a highly contrasted crown structure, which can further be interpreted in terms of ecological differences (Tomlinson, 2004). However, the immediate need is to provide information about this rare species that can be used for further comparative study.

MATERIALS AND METHODS

Small rooted cuttings of trunk axes with several branches were propagated at the Department of Primary Industries forestry facility in Queensland, Australia and transported to North America in a certified, soil-less shipping medium in compliance with the appropriate import requirements.

A sample specimen is illustrated in Fig. 1. Comparable material from specimens cultivated in the Royal Botanic Gardens, Sydney is shown in Fig. 2A (epicormic sprouts) and Fig. 2B (young saplings). Cuttings were randomized

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Fig. 1. *Wollemia nobilis*. Clonally propagated specimen with basal reiteration and some branching of basal plagiotropic axes.

from maternal source tissue representing the entire genetic diversity of the wild population, which is still low (Peakall et al., 2003), but the parentage of each individual cutting was not identified. A collection of six small plants were donated for our research by Wollemi Pine North America Plants up to 1 m tall with frequent basal reiterations (Fig. 1) were disassembled in various ways, and the resulting material was fixed in FAA (5% formalin : 10% acetic acid : 85% ethanol). Eight terminal buds of trunk axes were examined, including five provided by C. J. Quinn as fixed material from the Royal Botanic Gardens, Sydney. The latter were dissected to a stage comparable to that shown in Fig. 2H to confirm the precocious origin of lateral axes. We made no precise count of the numerous branch axes that were either dissected or sectioned.

Sections—Trunk and branch axes were initially sectioned on a sliding microtome as unembedded material (freehand sections). Terminal buds from both kinds of axis (e.g., Fig. 2D, G) were trimmed of outer enveloping scale leaves (Fig. 2H), dehydrated, and embedded in wax and sectioned serially at 10 μm on a rotary microtome in the usual way. Ribbons were mounted and dewaxed, using standard procedures and stained in safranin and alcian blue. Serial sections of both kinds of axes were helpful in understanding three-dimensional features, notably leaf trace proliferation, branch origin and attachment, and the rather complex interconnection among resin canals.

Freehand sections up to 60 μm thick were either stained using histochemical protocols for starch, lipids, and lignin or in 0.1% aqueous toluidine blue and mounted in glycerine : water (1 : 1) as temporary wet preparations. Because material was extensively tanniniferous, permanent preparations were made of sections bleached for 20 min in commercial household bleach (sodium hypochlorite) diluted 1 : 1 with tap water, washed well, and stained also in safranin and alcian blue.

Maceration—Slivers of stem material were macerated by boiling for 3 min in 10% aqueous potassium hydroxide, washed well in tap water, and completed in 20% chromic acid (chromium trioxide) until sufficiently softened (15–30 min). After further washing in water samples were teased apart and mounted in 50% glycerine. Such preparations gave details of cell shapes and dimensions difficult to observe in sections (e.g., Fig. 3F, G).

Photography—Photography involved a Nikon Coolpix 4500 camera (Nikon, Tokyo, Japan) with a Leitz Periplan 10 eyepiece (Leitz, Strasburg, Germany) and Olympus microscopes (Olympus, Tokyo, Japan) SZH for low power and BHZ for high power. For general separation of tissue types, some sections were photographed under part-polarized light (e.g., Figs. 3A and 4A). Images were processed in Adobe (San Jose, California, USA) Photoshop and plates assembled in Adobe Illustrator.

RESULTS

Wollemia corresponds to Massart's model (Hallé et al., 1978) because it shows rhythmic growth of all axes, but with a precise distinction between orthotropic (trunk) axes with radial symmetry producing contrasted plagiotropic (branch) axes that have dorsiventral symmetry, the branches forming tiers or pseudowhorls corresponding to each unit of extension (Fig. 1). In our material, there is irregularity of spacing between branches, as shown in the dissected buds. The architecture is repeated in basal epicormic sprouts (Fig. 2A) and in material propagated clonally, both of which show juvenile features and are less clearly model-conforming (Fig. 2B). However, the two kinds of axis are well represented in the material we studied and may be described separately to emphasize their differences in symmetry, branching and histology.

Morphology of orthotropic shoots—Rhythmic growth is expressed in the formation of massive resting terminal buds enclosed by numerous scale leaves (Fig. 2G). Phyllotaxis is spiral but irregular, the angle of divergence measured by ruler and

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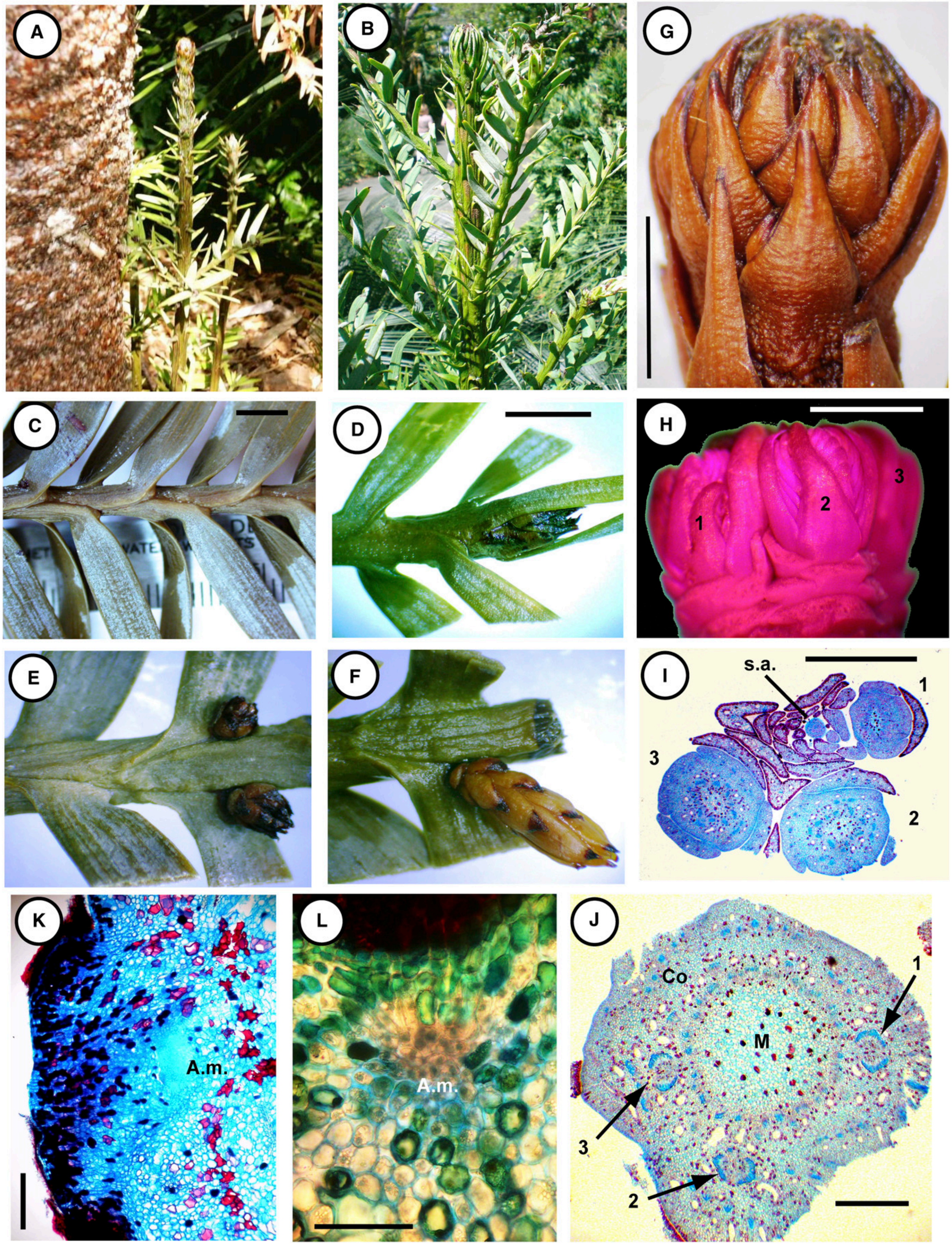
Fig. 2. *Wollemia nobilis*. Juvenile habit, bud morphology, and axillary meristems. (A) Basal suckers from a 12-yr-old tree, trunk with distinctive bark to left. (B) Trunk axis of a seedling with suberect branch axes. (C) Segment of a plagiotropic branch with regularly repositioned leaves with twisted bases. (D) Resting terminal bud of a branch. (E) Axillary buds induced by decapitation of a branch. (F) Extending bud of a decapitated branch developing by prolepsis, with decussately arranged scale leaves. (G) Intact terminal bud of a trunk with numerous spirally arranged bud scales. (H) Terminal bud of a trunk with bud scales removed to show three branch initials (1–3) enclosing the shoot; stained in acid fuchsin. (I–L) Light micrographs of transverse sections. (I) Terminal bud (paraffin section) at the level of its shoot apex (s.a.) and the base of three enclosed branches (1–3) above their attachment level. The inner scale leaves of the bud are much distorted by their confinement within the branch bases. (J) Base of same orthotropic bud (paraffin section), with scale leaves removed, the developing stele between the wide medulla (M) and cortex (Co). Branch traces (1–3) conspicuous in the cortex. (K) Outer cortex of trunk axis; leaf buttress to left with buried axillary meristem (A.m.) developed as an incipient bud; freehand section: safranin and alcian blue. (L) Outer cortex of trunk axis, putative axillary meristem (A.m.) within collapsed black bark patch tissue above; freehand section, wet preparation stained in toluidine blue. Bars: (C–F) = 4 mm; (G) = 5 mm; (H) = 2 mm; (I) = 1 mm; (J, K) = 500 μm; (L) = 100 μm.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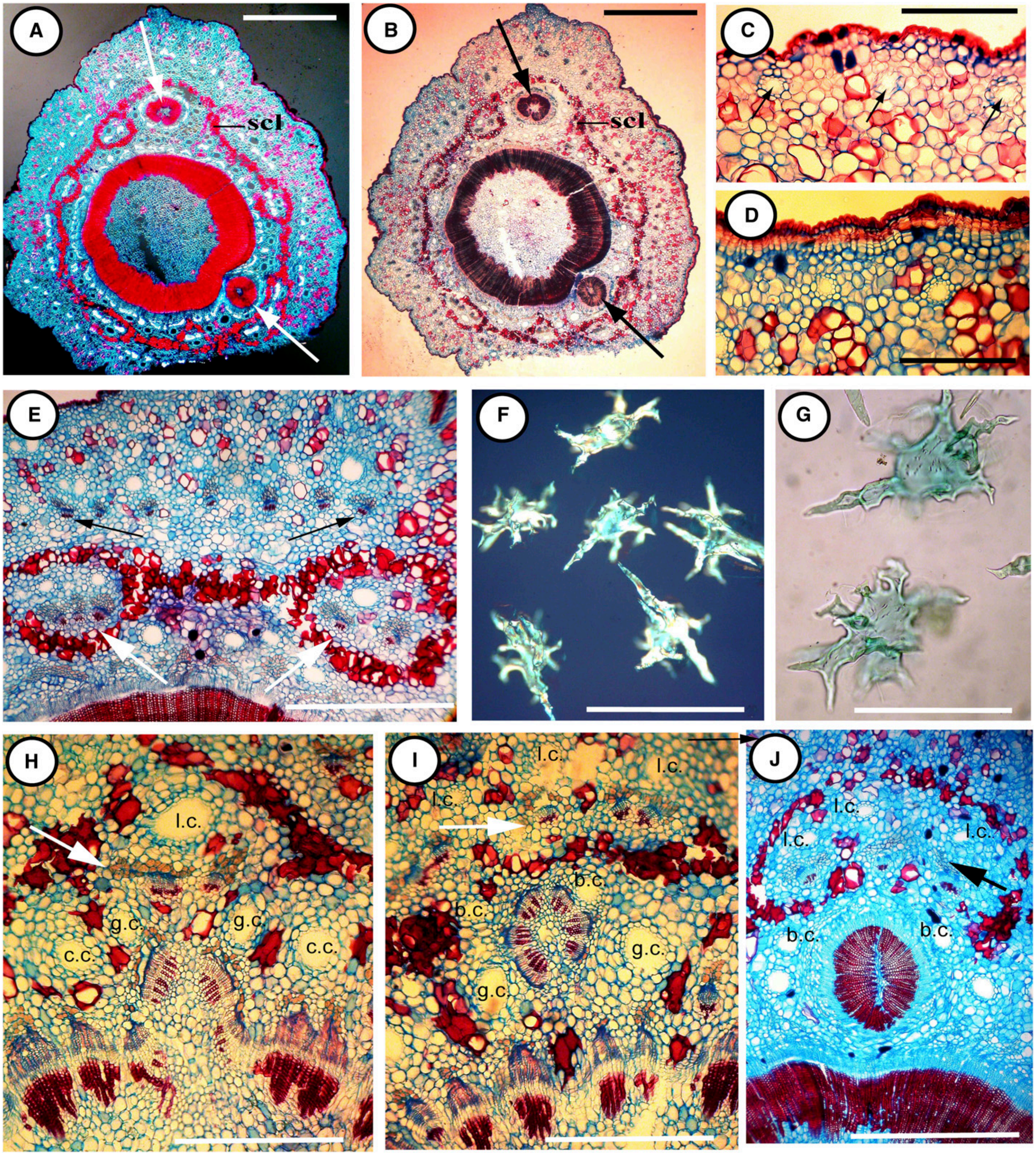

Fig. 3. *Wollemia nobilis*. Light micrographs of anatomy of orthotropic (trunk) axis; all sections stained in safranin and alcian blue. The resin canals are indicated in H–J according to the nomenclature of Suzuki (1979), i.e., b.c. = branch canal, c.c. = central canal, g.c. = guard canal, l.c. = leaf canal. (A) Transverse section (TS) of stem with part-polarized light; red-stained tissue is lignified (central xylem and sclerenchyma ring = scl), blue-stained tissue is ground parenchyma of medulla and cortex, white tissue represents birefringent caps to vascular bundles; arrows = branch traces in cortex. (B) Same section as (A), freehand section; irregular outline of (A) and (B) reflects confluent basal buttresses of scale leaves. (C) TS of surface tissues with narrow resin canals (small arrows). (D) The same in an older stem with phellogen initiated in hypodermal layers. (E) TS of stem cortex with several leaf trace complexes, the innermost (white arrows) enclosed by sclerenchyma; black arrows delimit outer leaf trace complex (seven vascular bundles) with attendant resin canals. (F) Inner cortical astrosclereids from macerated material in part-polarized light selected from macerated tissue and mounted in glycerin. (G) Astrosclereids in

protractor from drawings of transverse sections varying over a range from 50° to 140°, although the Fibonacci angle (137.5°) is approached in many successive leaves. Even so, there may be apparent reversals of the genetic spiral. Leaves are best described as scale leaves, with a base extended down the stem, producing a distinct fluting, the stem in transverse section with a corresponding irregular outline (Fig. 3A, B). The parastichies of a normal pattern are not well expressed, a further consequence of the irregular phyllotaxis.

Branching of the trunk is distinctive because the plagiotropic axes are produced within its bud and are therefore contemporaneous in their development with the parent axis, thus expressing some of the features of a sylleptic branch. This precocious development is revealed when the outer scales of the bud are removed to show the neoformed branches, each in the axil of a scale leaf and with numerous leaf primordia (Fig. 2H). The branches thus overtop the vegetative apex of the parent trunk and because of their rapid development distort the scale leaves inserted above them, a possible factor in the irregularity of the spiral phyllotaxis of the stem (Fig. 2I). An important consequence of syllepsis is the well-established branch vascular connection such that branch traces have an extended path within the trunk cortex; they are conspicuous in most transverse sections (Fig. 2J). This pattern can be revealed by decorticating the axis, as used extensively by Burrows et al. (2007) to demonstrate the resulting potential hydraulic constriction of the branch base.

Branch extension is initiated by bud elongation that is synchronous with that of the trunk at the onset of bud break. Because of their relatively basal position, each pseudowhorl of branches occurs toward the base of its unit of extension, although in our material this is expressed very imprecisely.

Reiteration of orthotropic shoots—Sylleptic branching of this kind reflects the expression of Massart's model, but the axis produces no dormant buds of a conventional kind. However, the model can be repeated by reiteration that results in adventitious shoots (Fig. 2A). These originate from the minute meristems in conifers described initially by Fink (1984; as "detached meristems") and subsequently for Araucariaceae (Burrows et al., 1988: Burrows, 1989, 1990, as "axillary meristems") and especially *Wollemia* (Burrows, 1999). We prefer the latter terminology. A distinction has been made by these authors between "preventitious" vs. "adventitious" meristems, but we are here concerned with the result of their activity upon release. Each meristem originates in the axil of a scale leaf as a group of undifferentiated cells (A.m. in Fig. 2L), which can persist in the outer cortex but is capable of differentiating a shoot apex, resulting in a bud, which in turn can form an epicormic shoot. Although Burrows (1999) suggests that every scale leaf can subtend such a meristem that has the potential to produce a trunk axis, in nature such shoots normally develop as basal sprouts (Fig. 2A). To what extent they can develop on distal stem parts requires examination of adult trees.

Morphology of plagiotropic axes—Branch axes contrast strongly with the trunk axis (Fig. 1), but also show rhythmic growth with terminal buds protected by numerous scale leaves (Fig. 2D). They bear flattened foliage leaves up to 4 cm long in a strict decussate phyllotaxis (Fig. 4C), i.e., leaves in pairs successively at right angles, although the first few pairs are somewhat reduced (not a condition found normally with syllepsis). This phyllotaxis results in four ranks of leaves (orthostichies), which are retained in the bud scales. Otherwise dorsiventrality is largely achieved by twisting of each foliage leaf at its insertion during shoot extension (Fig. 2C–E). As a result, the leaf insertion becomes oblique, in contrast to the transverse basal buttress of the scale leaves on the orthotropic shoot. There is no twisting of the axis itself so that the original orthostichies, although largely eliminated, can still be recognized (Fig. 2C). This leaf reorientation does not occur in bud scales so that their four ranks remain clear. This tetrastichous arrangement is expressed on branches of adult leaves where it is claimed that the leaf becomes inverted, the adaxial surface presented below (Jones et al., 1995), but this clearly does not occur in juvenile leaves. Branching of the plagiotropic axes in principle does not occur, (e.g., Jones et al., 1995; Hill, 1997), but it is evident at least in juvenile material (Fig. 1, 2E, F) in both manipulated and unmanipulated shoots, a process that will require more careful study.

Reiteration of plagiotropic shoots—In normal circumstances, the rhythmic growth of branches is expressed most visibly in the fluctuations of leaf length along a shoot, representing the initial transition from tetrastichous bud scales to flattened foliage leaves, but does not involve branching. However, either in decapitated shoots or when an apex aborts, a bud may appear within the axil of one or both of the leaves of the distal leaf pair of the cut shoot (Fig. 2E, F). These presumably arise from the same kind of axillary meristem that occurs on trunk axes, said to also be located in plagiotropic branches by Burrows (1999). This phenomenon requires further investigation. When such a bud expands, it shows all the features of a plagiotropic shoot, i.e., it repeats the morphology of its parent axis (Fig. 1).

Anatomy of orthotropic shoots—The stem includes three distinct regions (Fig. 3A, B): a central medulla, the vascular system including well-developed secondary xylem, and a broad cortex. The surface layers are very irregular (Fig. 3C), and a phellogen is established in hypodermal layers (Fig. 3D), initiating the future surface "bubble-bark," as it is frequently described (Jones et al., 1995). The medulla is uniformly parenchymatous but with occasional sclereids and lacks resin canals. The primary vascular system is a conventional eustele (Fig. 2J), and as in all conifers, each leaf is supplied initially by a single leaf trace that diverges, either left or right, from a single axial bundle, leaving at its point of departure a leaf gap. The regular relation between phyllotaxis and primary vasculature in conifers, as described in the pioneering work of Barthelmess (1935), is expected in *Wollemia*, but here obscured by the phyllotactic irregularity of these stems. Each trace in its passage across the cortex divides dichotomously several times to produce a leaf trace complex of up to 11 vascular bundles that

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normal transmitted light, as in (F), to show slit-like pits. (H) TS of inner cortex and outer stele including stelar insertion of branch trace below and subtending leaf trace complex above (white arrow, two vascular bundles), with single attendant resin canal (l.c.) outside. (I) TS similar to (H) with beginning of derived resin canal system to left and right from "guard canal" (g.c.); the subtending leaf trace complex above now with three vascular bundles (white arrow). (J) TS of inner cortex, branch base with well-developed secondary xylem and associated leaf complex of three leaf traces (black arrow) enclosed by discontinuous sheath of sclereids. Bars: (A, B) = 2 mm; (C–D) = 200 μm; (E) = 1 mm; (F) = 500 μm; (G) = 250 μm; (H–J) = 400 μm.

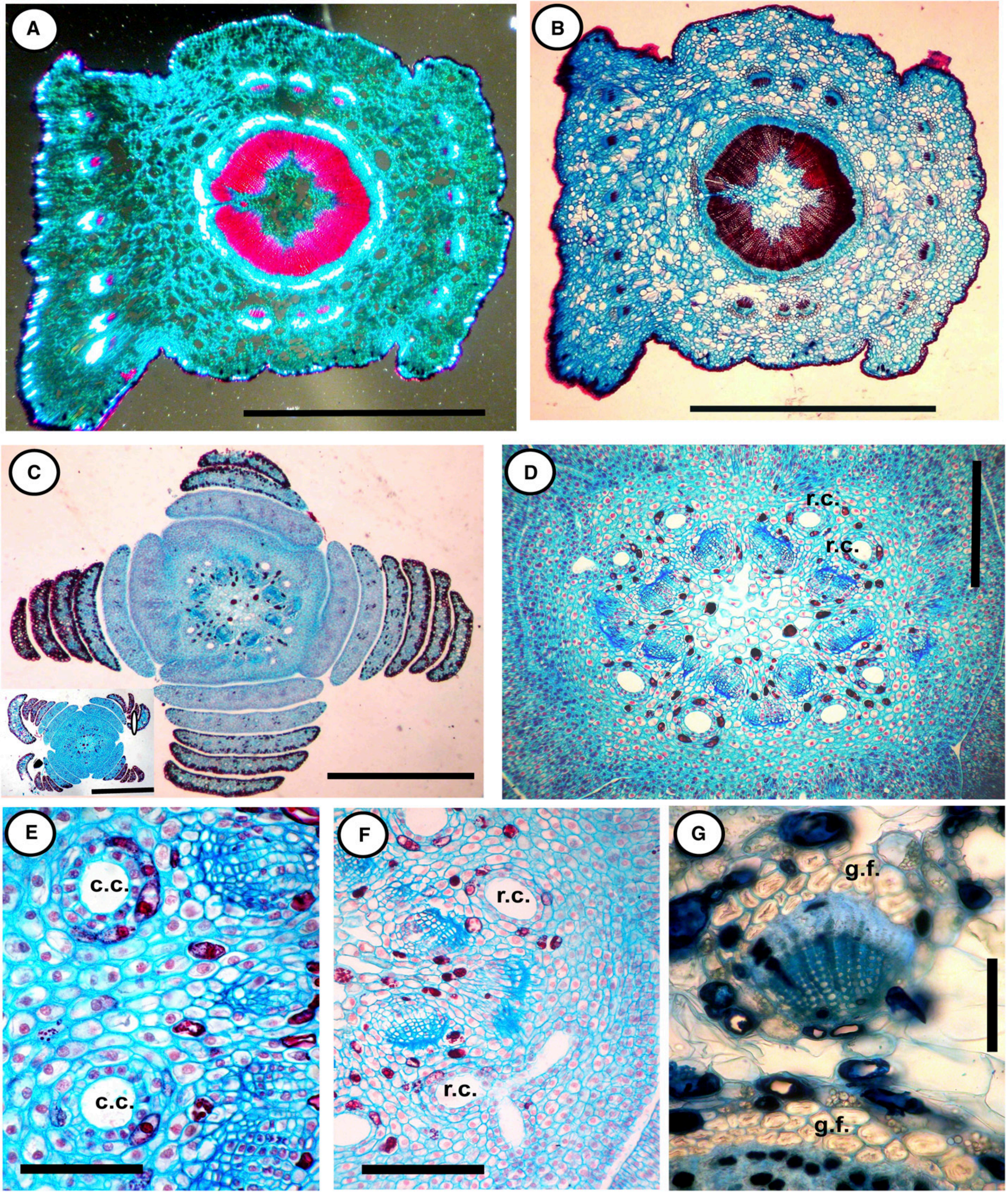

Fig. 4. *Wollemia nobilis*. Light micrographs of anatomy of plagiotropic (branch) axis; all sections except (G) stained in safranin and alcian blue. (A) Transverse section (TS) of branch with well-developed secondary xylem, photographed in part-polarized light; red-stained tissue is lignified (xylem) but sclereids absent, ground tissue blue, white represents birefringent unlignified fibers of vascular system and hypodermis. (B) TS of similar branch in normal light, with only two pairs of leaf trace complexes in the cortex representing the four orthostiches of leaf placement; sclereids absent, medulla with charac-

become the leaf veins. This complexity is a unifying feature of all Araucariaceae. Because of this large number of multiplying traces, each referrable to a single original trace, the cortex is occupied by a large number of vascular bundles (cortical traces), but discretely separated from each other as the supply to a single leaf (Fig. 3B). The outermost, having completed their divisions, can be recognized as a tangentially extended series (small black arrows in Fig. 3E), the innermost are segregated by their association with sclerenchyma, as described later (large white arrows in Fig. 3E). Leaf traces have an extended course in the cortex because of their oblique, rather than radial, passage into the scale-leaf base.

Mechanical tissues include the xylem of the stele, xylem of the included branches (Fig. 3I), numerous sclereids, and unlignified vascular fibers, readily contrasted in Fig. 3A by the use of polarizing optics. The sclerenchyma includes a broad but discontinuous band of astrosclereids (scl. in Fig. 3A, B). The individual sclereids are very irregular (Fig. 3F), interlocking with each other, and have extensively pitted walls (Fig. 3G). Outer sclereids are less branched. All sclereids originate from enlarged ground tissue cells, those toward the periphery differentiating last. In the passage of a leaf trace complex through the inner sclerotic ring, the complex becomes temporarily enclosed by sclereids forming a boundary layer easily recognizable in transverse sections (Fig. 3A, B; large white arrows in Fig. 3E).

Fibers are associated exclusively with vascular bundles; they are unlignified and are seen well in polarized light because of their birefringence (white dots in Fig. 3A). Each fiber strand forms a "cap" to a stelar vascular bundle and leaf trace, the fiber strand dividing as the leaf trace itself divides. Individual fibers are very refractive in wet preparations and resemble somewhat gelatinous fibers of angiosperm wood because the inner wall layers collapse and the middle lamella is sometimes lignified (cf. Fig. 4G, g.f.). However, because they are not distributed asymmetrically, they may not function as tension fibers. Fibers are very long, upward of several mm, and originate in the cell layers immediately outside the protophloem; clearly their development involves intrusive growth. Resin canals complete the histological specialization of the cortical tissues and are described in detail later.

Insertion of plagiotropic branches—Branch development on trunk axes can be followed in sequential sections of different shoots. A branch trace always originates distal to an initial leaf trace as a pair of vascular bundles diverging left and right from a pair of axial bundles (Fig. 3H), resulting in a wide branch gap (Fig. 3H). The branch trace remains inside and opposite its subtending leaf trace complex in its passage across the cortex (white arrows in Fig. 3H, I).

The branch trace assumes an elliptical outline in transverse section (Fig. 3I) and becomes associated with resin canals, as described later. Its medulla is extended in a radial plane (Fig. 3J), and secondary xylem becomes well developed. The original broad parenchymatous connection with the medulla of the trunk axis persists, a feature of sylleptic branches. A transverse asymmetry defines the initial morphology of the branch axis because its prophyll pair is inserted perpendicular to the radial plane of the branch. In the early development of the branch, in its passage across the cortex, separate stelar bundles are retained (Fig. 3I), but this is later obscured by the development of secondary xylem (Fig. 3J). However, a fluted effect may be achieved distally, resulting in the "stranded" appearance described by Burrows et al. (2007) in the abscission area, where the branch xylem bends through 45° and exits the stem into the morphological branch base. The vascular continuity from branch insertion into the branch base thus largely involves a considerable change in stem anatomy that is eventually obscured as secondary xylem is formed.

Anatomy of plagiotropic shoots—The anatomy of branch axes differs appreciably from that of the trunk, in part related to their contrasted phyllotaxis and possibly their short lifespan. A conspicuous difference is the infrequent development of astrosclereids in the cortex so that the only extensive mechanical tissue is that of the secondary xylem and fibers of the vascular bundle caps (Fig. 4A). However, there is an additional series of unlignified hypodermal fibers, continuous into the leaf (peripheral white dots in Fig. 4A). Furthermore, the cortex is relatively uncomplicated because it lacks the more extensive series of leaf traces that characterizes the trunk axis (cf. Figs. 3B, 4B). Leaf traces are still initiated as single vascular bundles diverging from an axial bundle and, as in the trunk axis, immediately divide sequentially to produce a tangential complex of usually seven bundles, which become the veins of the leaf. At any one level, the series for each of the four orthostichies is clear (Fig. 4B) because each complex eventually represents the supply to the leaf two nodes above its level of origin as a single vascular bundle; each original trace and its derivatives thus remain in the cortex for only two internodes. Extension of cortical leaf traces over several internodes, as occurs in the trunk axis, is thus just not topographically possible.

The regularity and symmetry of the vascular system based on the decussate phyllotaxis (Fig. 4C) is revealed most clearly in its primary expression close beneath the shoot apex. The stele includes eight prominent axial bundles (Fig. 4D), with occasional minor derivative bundles. Each pair of axial bundles gives rise to leaf traces in the plane of insertion of each of the four orthostichies (cf. Fig. 5A), the traces diverging from the axial bundle either left or right, in an apparently random sequence. Each leaf trace is then opposite a consistently maintained gap. The medulla in transverse view is consequently cruciately eight-pointed, as seen in Fig. 4B, where it is outlined by the secondary xylem. The symmetry is further characterized by the eight resin canals (Fig. 4D), each corresponding to an axial bundle. A similar symmetry might exist in the trunk axis, if it were not obscured by the irregular phyllotaxis. Because the plagiotropic branch normally produces no branches

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teristic regular stellate outline. (C) TS of extending plagiotropic shoot with precise decussate phyllotaxis and four leaf orthostichies; inset = shoot with older leaves on each orthostichy bending toward a single plane with mirror-image symmetry. (D) Detail of stele as in (C) with regular pattern of axial bundles, leaf traces and eight central resin canals. (E) Details of developing "central" resin canals (c.c.) outside stele, the second, outer epithelial layer developing abundant tannin. (F) Leaf trace departing stele with attendant "guard" resin canals (= r.c.), the lowest branching to provide part of the system to the leaf. (G) Wet preparation of single leaf trace from older axis stained in toluidine blue, the unlignified fibers (= g.f.) of the trace fiber cap and outermost part of the stele with collapsed inner cell wall layer reminiscent of tension fibers. Bars: (A, B) = 2 mm; (C) = 1 mm; (D) = 500 µm; (E) = 80 µm; (F) = 400 µm, (G) = 100 µm.

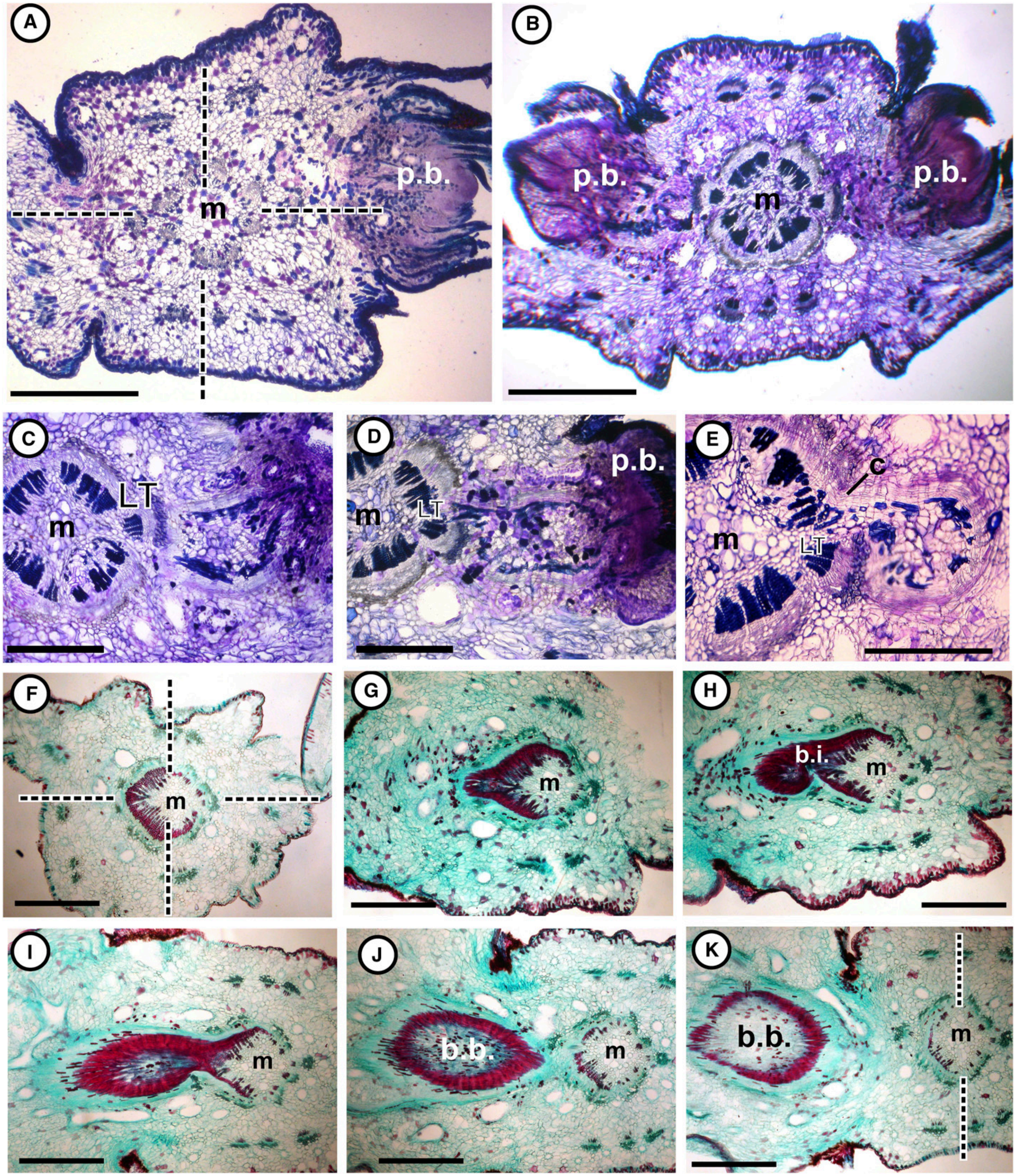

Fig. 5. *Wollemia nobilis.* Light micrographs of attachment of reiterated plagiotropic branch axes, all transverse sections. (A) Section below level of branch insertion; dotted lines = plane of insertion of the four leaf orthostichies in decussate phyllotaxis. (B) Axis with a pair of opposite buds, corresponding to Fig. 2E. (C–E) Three successive sections in basipetal sequence including the right-hand bud in (B); LT = initial leaf trace; c in (E) = cambial union between bud trace and stele of parent axis. (F–K) Sequential sections in acropetal succession at intervals of ~300 μm with asymmetric branch attachment, see text. Bars: (A–D, F–K) = 1 mm; (E) = 0.5 mm. b.b = branch base; b.i. = branch insertion; m = medulla of parent axis; p.b. = plagiotropic bud.

(except as described next), its cortex includes no complicating branch traces.

Reiteration in plagiotropic axes—The availability of branched plagiotropic shoots (e.g., Fig. 1) provided the opportunity to study branch attachment where buds had developed behind decapitated or aborted terminal buds. Because this process is by definition prolepsis, as distinct from the sylleptic branching of trunk axes, a major difference in branch connection was to be expected. We had no material to show the earliest stages of development of a reiterated branch and so could not study the origin of such branches from presumed axillary meristems. We have not located such meristems in a survey of unbranched nodes, so our results deal only with visible buds or branches, as shown in Fig. 2E, F, i.e., either two buds (Figs. 2E, 5B) or one bud (Fig. 2F, 5A). Buds have a series of enveloping bud scales (Fig. 2D, p.b. in Fig. 5A, B) and are attached only by a preliminary branch trace (Fig. 5C–E); established branches had a well-developed axis with abundant secondary xylem (Fig. 5F–K).

Although the buds arise at a leaf base, because of the long oblique insertion of the subtending foliage leaf, there is no precisely defined axillary midpoint to the leaf. However, the bud can be seen to arise consistently in an approximate axillary position (Fig. 2E, F), corresponding to one of the leaf orthostichies, but there is still appreciable asymmetry, and the actual stelar attachment is itself often also considerably asymmetric. Despite this, the branch has a precise attachment to a leaf gap belonging to a leaf on the same orthostichy as the subtending leaf (identified as LT in Fig. 5C–E), but belonging to a leaf inserted two nodes above,

In development, this attachment originates centripetally and involves the dedifferentiation of cortical cells, including those of the adjacent resin canals so that the canal system of parent axis and branch become continuous. Vascular differentiation involves the formation of a procambial and later cambial cylinder (Fig. 5C, D) that is continuous with the vascular cylinder of the parent axis (c in Fig. 5E). This attachment is always to the axial bundles bordering the leaf gap of the leaf at the lower node. The asymmetry is reflected in the attachment to the axial bundle at two different levels. Even in early stages, there is visible distal differentiation of some tracheary elements in the branch trace before any appear in proximal regions, supporting the basipetal course of maturation.

The attachment of a mature branch, shown in the sequence of images from below upward in Fig. 5F–K, is characterized by the well-developed xylem cylinder at its base (Fig. 5H–K), whereas limited development of secondary xylem occurs in the parent axis (Fig. 5F), with none at higher levels (Fig. 5J, K). The branch attachment is made to the axial bundles defining a leaf gap, as described earlier, although the leaf trace itself is obscured by the medullary connection between branch and parent axis (Fig. 5G, H). Asymmetry of the branch connection is now very clear (Fig. 5G–I), and the contrast in the amount of xylem in distal (Fig. 5K) and proximal (Fig. 5G) levels is striking. The lack of further secondary xylem in the parent axis (Fig. 5F, G) reflects either a dormant terminal bud or its disappearance. The overall result is a branch attachment that is essentially radial within the cortex and in complete contrast to the extended cortical branch traces of the orthotropic axes (Fig. 3A, B).

Resin canals—A conspicuous feature of the stem cortex of both kinds of axis is the anastomosing system of resin canals that are interconnected in a consistent way so that there is direct continuity for the pathway of resin from the stem into the leaf. Following the terminology of Suzuki (1979) in describing then known Araucariaceae, we can recognize in the orthotropic axis a series of *central canals* immediately outside the axial bundles (as in Figs. 3H and 4E, c.c.), the series of *leaf canals*, which are associated with the vascular bundles of each leaf trace complex in the cortex (l.c. in Fig. 3H–J), which in turn become the resin canals alternating with the veins in the lamina, and the *peripheral canals*, a more obscure group of narrow canals in the subsurface layers (arrows in Fig. 3C). To this should be added *branch canals,* at least for *Wollemia*, which are the canals that become associated with the branch traces (as in Fig. 3I, J, b.c.). The system of interconnection is most complete in trunk axes because branch axes lack the peripheral and branch canals. Individual resin canals originate schizogenously by separation of cells and become surrounded by a two-layered epithelium, the inner, presumed secretory, layer remains thin-walled, whereas the outer layer of large cells become conspicuously tanniniferous (Fig. 4E).

The system of canals is simplest in the plagiotropic shoots because there are no branch or peripheral canals and "guard" canals and "central" canals are the same.

The interconnection between the different topographic systems can be described from below upward, the reverse of the direction used by Suzuki (1979). This approach need not imply a developmental direction, except that it is clear in the branch axes that central canals differentiate earlier than other kinds of canals. The leaf canals are derived by the branching of either one of the two canals that are situated on each side of the outgoing leaf trace. These are the *guard canals* of Suzuki (1979), and they obviously correspond to the eight canals of the central system in the branch axes (Fig. 4D), whereas the system in trunk axes is much less regular and any canal can function as a guard canal. The derivate canal (the first leaf canal) takes up a position immediately exterior to the developing leaf trace complex (e.g., above the white arrow in Fig. 3H). This canal produces further canals by continued branching corresponding to that of the increasing number of leaf traces in a complex so that about equal numbers of leaf traces and resin canals result (e.g., the outer leaf complex in Fig. 3E). All these canals pass into the leaf. The peripheral stem system is derived from that of the leaf trace system by branching of its marginal bundles. The canals that supply the canals to the plagiotropic branch are derived directly from the pair of guard canals adjacent to the branch gap (Fig. 3H). This pair of derivative canals ultimately divides first into four (Fig. 3I) and then into the eight axial canals of the branch (Fig. 4D). Early branching of the axial canals may obscure the regularity as seen in a single section (e.g., r.c. in Fig. 4F). The overall result is an extensive system of canals that accumulates white resin under pressure so that it oozes from any cut surface. This accounts for the white "cap" of resin that may enclose terminal buds and is the result of resin secretion, even though there seems to be no direct pathway for resin to reach the exterior.

DISCUSSION

A comparison of *Wollemia* with *Agathis* and *Araucaria* is limited by the lack of information about the latter two genera and the fact that our study is restricted to that of clonal material equivalent to that of juvenile stages of seedling trees. Nevertheless, earlier studies provide information that demonstrates several common features for the family, e.g., the leaf-trace complex, sclereids, and resin canals (Seward and Ford, 1906). Thomson (1914) examined features that could be seen in wood of fossil seed plants in attempts to understand the relation between modern conifers and their putative antecedents, especially drawing attention to the configuration of persistent leaf traces buried within secondary xylem. This configuration has been reported by Heady et al. (2002) for *Wollemia*. Seward and Ford (1906) described a number of anatomical features in *Araucaria* and *Agathis*, but did not identify which particular vegetative axis was examined, even though we have now shown that anatomy and development can be strikingly different between trunk and branch. *Wollemia* in some ways is architecturally simple because the first-order branches remain unbranched in architectural terms, whereas in *Araucaria* there may be up to four further branch orders, e.g., *A. cunninghamii* (Tomlinson, 2004). Each branch order can have a different physiognomy and development, as in *A. heterophylla* and *A. columnaris* (Massart, 1924; Veillon, 1978, 1980). Each would need separate study in a more extended comparative account. *Agathis* is much more plastic in its crown structure, although as a sapling it conforms well to Massart's model with clear distinction between orthotropic and plagiotropic axes, albeit with some lesser stability in phyllotactic pattern. As an adult, it develops a broad crown, unusual in the family, because distal portions of old branches can lose their plagiotropic expression and become orthotropic, thence persisting as the major limbs of the canopy (Tomlinson, 2004). In this sense, the statement of Hill (1997) that *Wollemia* possesses a "unique combination of model and reiteration" might apply to any tree. The definition of Massart's model obviously admits of much variation even when applied to as natural group as the Araucariaceae.

A closer examination of developmental features shows important differences not indicated in the simple model. For example, the branch tiers that partly define Massart's model have different developmental origins in the three genera (Tomlinson, 2004). In *Agathis* and *Wollemia*, they originate at the base of the future shoot unit but within the terminal bud well before the shoot apex goes into the dormant phase (Fig. 2H) and extend with later bud extension, in the manner of a sylleptic branch. The resulting branch tier is at the *base* of the new leader although in *Agathis* and juvenile *Wollemia* the tier may not be well expressed because internodes between successive branches can be long. This similarity accords well with the suggestion that *Agathis* and *Wollemia* are sister taxa (Gilmore and Hill, 1997). In *Araucaria*, it appears that the branch tier is initiated at the apex of the leader (still by syllepsis), just before the shoot goes into dormancy, and appears at the apex of the shoot so that the presence of branches is visible externally. This configuration may account for the contrasted methods of branch insertion in the two genera (Burrows et al., 2007). Furthermore, the branch tier in *Araucaria* can continue to extend as the trunk axis remains dormant, producing a characteristic flat top to the crown. *Wollemia*, as studied by us in juvenile material, clearly belongs to the contrasted *Agathis* model as seen in the flush of terminal trunk growth evident in photographs of mature *Wollemia* trees. The resin canal system described by Suzuki (1979) for *Agathis* also seems very close to the pattern described here for *Wollemia*.

Perhaps the most distinctive feature we have described for *Wollemia* is the complexity of the vasculature of the primary cortex of the trunk axis because it includes an extended series of cortical leaf and branch traces that may be unique for conifers, especially in the axial orientation of the woody cylinder of the branch trace before it exits the stem (cf. Burrows et al., 2007). An analogous configuration occurs in the dicotyledonous *Schefflera*, in which branch traces have an extended cortical passage because they are largely derived from cortical leaf traces, which are numerous because of the encircling leaf base (Tomlinson et al., 2005). Here leaf traces become the template for the branch traces, a condition not found in *Wollemia*.

Within Araucariaceae, different methods of reiteration can be contrasted in different species. The basis for this process seems to be the existence of axillary meristems in many taxa, and how their expression determines crown form may have important ecological consequences (Tomlinson, 2004). Grosveld et al. (1999) show in *Araucaria araucana* that reiteration takes many forms, including the formation of root suckers, a rare condition in conifers but also present in the Australian *A. cunninghamii* and *Agathis robusta* (G. E. Burrows, personal communication). Of interest in *Araucaria araucana* is the substitution of terminal seed cones on plagiotropic axes from a reiterated branch, a situation that leads to sympodial growth, virtually unknown in conifers. The distinctive method of branch substitution in juvenile branches of *Wollemia* that we have described raises the possibility that adult axes may also have similar sympodial growth by substitution of terminal seed cones. Here we assume that the origin of these plagiotropic reiterated axes is from the same kind of axillary meristems that produce trunk axes on orthotropic axes, as recorded by Burrows et al. (2003) in *Wollemia.* As we have described, this development is proleptic and involves a basipetal branch attachment from the developing bud to the vascular system of the parent plagiotropic axis, in complete contrast to the syllepsis of the initial branch to the parent trunk. With this knowledge, it should now be possible to establish the contrast to be expected in orthotropic shoots between branch connections of sylleptic origin and those of basal reiterated or epicormic sprouts, as illustrated in Fig. 2A, once larger specimens become available for experiment and destructive sampling. The contrast in *Wollemia* is remarkable because it sustains the generalization that where there exists axis differentiation in woody plants (Tomlinson 1978), reiterated shoots repeat the morphology only of the parent axis, i.e., orthotropic begets orthotropic, plagiotropic begets plagiotropic. This repetition is common in conifers and distinctive because the reiterated axes develop from unspecialized axillary meristems and not preformed reserve buds. Our description of *Wollemia* is still preliminary because of restricted access to mature trees, but is intended to draw attention to features that need to be studied in more detail, which should be possible as trees are widely grown to maturity.

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