

2016

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Sherwin Carlquist

Santa Barbara Botanic Garden, Santa Barbara, California

Mare Nazaire

Rancho Santa Ana Botanic Garden, Claremont, California

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Recommended Citation

Carlquist, Sherwin and Nazaire, Mare (2016) "SEM Studies of Two Riparian New-Caledonian Conifers Reveal Air Channels in Stem Wood; Field Observations," *Aliso: A Journal of Systematic and Floristic Botany*. Vol. 34: Iss. 1, Article 2.

Available at: <https://scholarship.claremont.edu/aliso/vol34/iss1/2>

SEM STUDIES OF TWO RIPARIAN NEW-CALEDONIAN CONIFERS REVEAL AIR CHANNELS IN STEM WOOD; FIELD OBSERVATIONS

SHERWIN CARLQUIST^{1,2} AND MARE NAZAIRE³

¹*Santa Barbara Botanic Garden, 1212 Mission Canyon Road, Santa Barbara, California 93105*

²*Corresponding author (s.carlquist@verizon.net)*

³*Rancho Santa Ana Botanic Garden, 1500 North College Avenue, Claremont, California 91711*

ABSTRACT

Two New Caledonian conifers, *Dacrydium guillauminii* and *Retrophyllum minor*, both of Podocarpaceae, occupy distinctive habitats in which roots are characteristically inundated. SEM studies of stem wood anatomy of both reveal characteristic vertical intercellular channels, not apparent in light microscope examination. These channels are regular in spacing as seen in radial sections and indefinite in vertical extent; they were observed only adjacent to rays. These channels, not reported in other conifers, are a potential source of aeration for roots and submersed stem portions. Other wood details are reported. In order to provide a more comprehensive picture of adaptation to these habitats, observations based on field work are provided: these conifers are not only adapted to little-varying inundation of stems and lower roots, they are subjected to much greater sunlight than are upland conifers of New Caledonia.

Key words: aeration in wood, aquatic ecology, ecological anatomy, Podocarpaceae, wood anatomy.

INTRODUCTION

The occurrence of aeration systems in aquatic plants is a familiar phenomenon, but the anatomical solutions to the problems of gas exchanged in submersed plant parts vary according to organography and morphology. Some tissue aeration adaptations have been known for a long time, as evidenced by reports in Haberlandt's *Physiologische Pflanzen-anatomie* in 1884 (reprint edition 1965). Other instances have remained unstudied. Among these are the only two species of aquatic Podocarpaceae, *Dacrydium guillauminii* J. Buchholz and *Retrophyllum minor* (Carrière) C.N. Page (= *Podocarpus palustris*, *P. minor*). By coincidence, these two species occur in the Rivière des Lacs system on the Plaine des Lacs in southernmost New Caledonia (Laubenfels 1972), an archipelago known for its metal-rich soils (Jaffré et al. 2013). They do not occur in the same ecological niche, however. *Dacrydium guillauminii* occurs along the margins of streams, its roots in water-saturated soil that supports other streamside vegetation (Fig. 1A). *Retrophyllum minor* typically occurs in the lakes formed in wider stream portions, where plants are inundated and rarely occur close to the shore (Fig. 3A). The base of the tree of *R. minor* in Fig. 3B is exposed, perhaps because the ordinarily inundated lake margin is at a lower level than usual because of seasonal drying or human activity.

Both conifers are confined to a relatively small area. The population of *R. minor* was estimated at 2500 individuals and declining (Jaffré et al. 1998; Herbert et al. 2002), whereas *D. guillauminii* is less common (Jaffré et al. 2010). Individuals of both species occur within a natural area created as the Chute de la Madeleine Reserve (Jaffré et al. 2010).

Neither of these species have pneumatophores, the root-borne aeration adaptations of lacustrine taxodioids (e.g., *Glyptostrobus*, *Metasequoia*, and species of *Taxodium* such as *T. distichum* (L.) Rich.). Therefore, one presumes that air might enter roots by way of stems. Air channels in bark would be subject to deformation as stem circumference increases. More importantly, liquid-preserved material of bark, in which cellular structure would be intact, was not available. Search for possible air channels in wood seemed a feasible endeavor because the rigidity of wood cells should preserve any passageways. To be sure, there is no precedent for occurrence of air channels in conifer wood, judging from the monograph of Greguss (1955). The availability of scanning electron microscopy (SEM) was important, because SEM can image three-dimensional structures well. The range of magnifications of SEM images is well suited to resolve wall layers, and to differentiate, therefore, between cellular and intercellular structures. In fact, the images provided by SEM here are sufficiently decisive that no light microscopy was undertaken.

Much more than wood anatomy is related to the habit and structure of the two New Caledonian riparian conifers. Therefore, field observations and associated illustrations are presented in order to provide a more comprehensive picture of the distinctive adaptations of these two species.

MATERIALS AND METHODS

The collections of both species were made close to the bridge over the Rivière des Lacs, the locality featured in most of the collections cited by Laubenfels (1972). The collections employed here are cited in the captions for the figures. Wood samples were preserved by drying in air. Wood portions for study were cut from these, boiled in water, and then stored in 50% aqueous ethanol.

Sections were cut by hand using single-edged razor blades. These sections were then subjected to three changes of warm distilled water, and dried between clean glass slides under

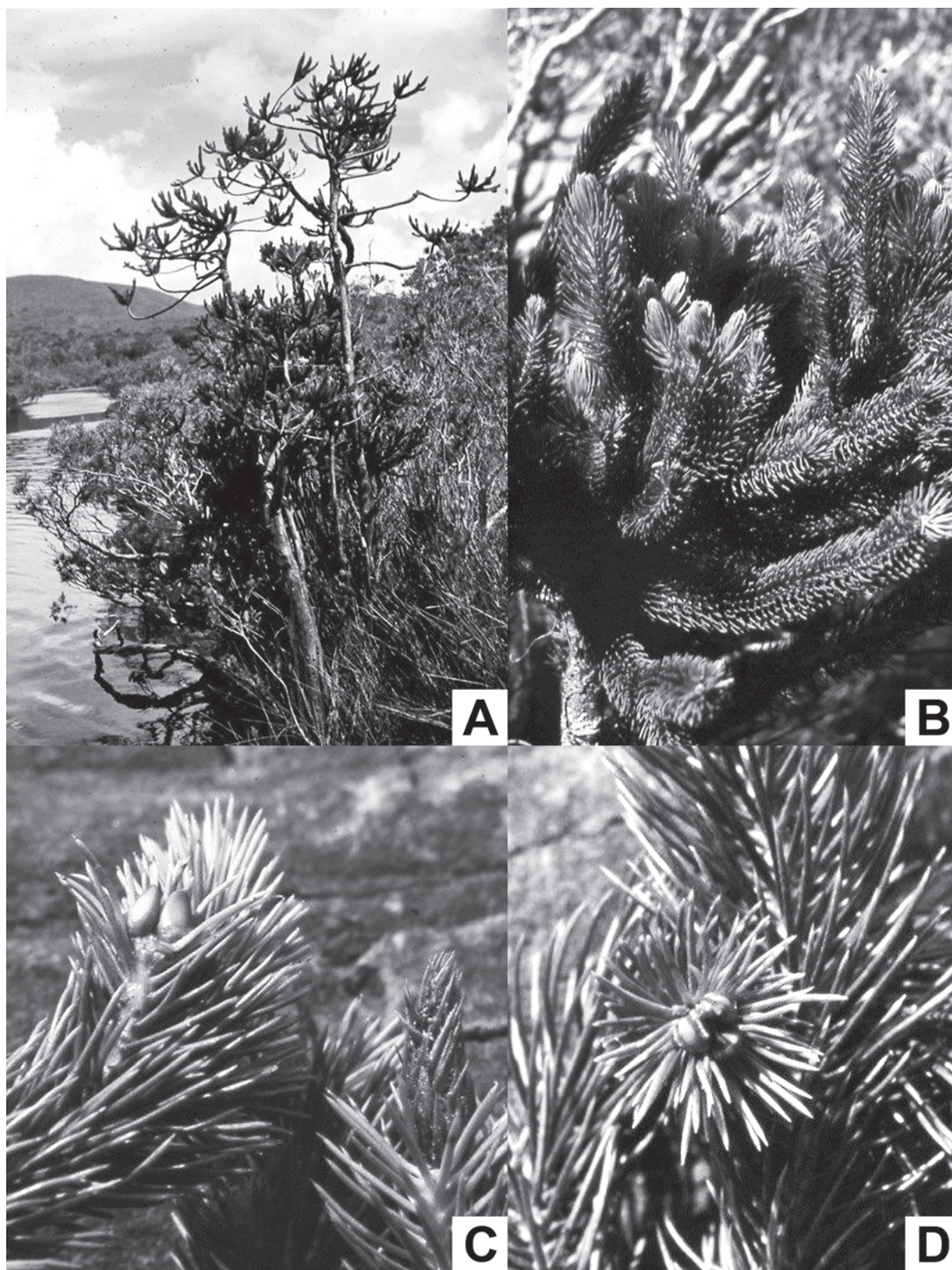


Fig. 1. *Dacrydium guillauminii*, Carlquist 4319 (RSA). A. Habit (two small trees), Rivière des Lacs. B. End of a branch, showing characteristic grouping of stems. C–D. Tips of fertile branches. C. Lateral view; at left, two female cones, several leaves removed to expose them; at right, male cone (sporophylls have acicular tips similar to foliage leaves). D. Head-on view, showing three female cones.

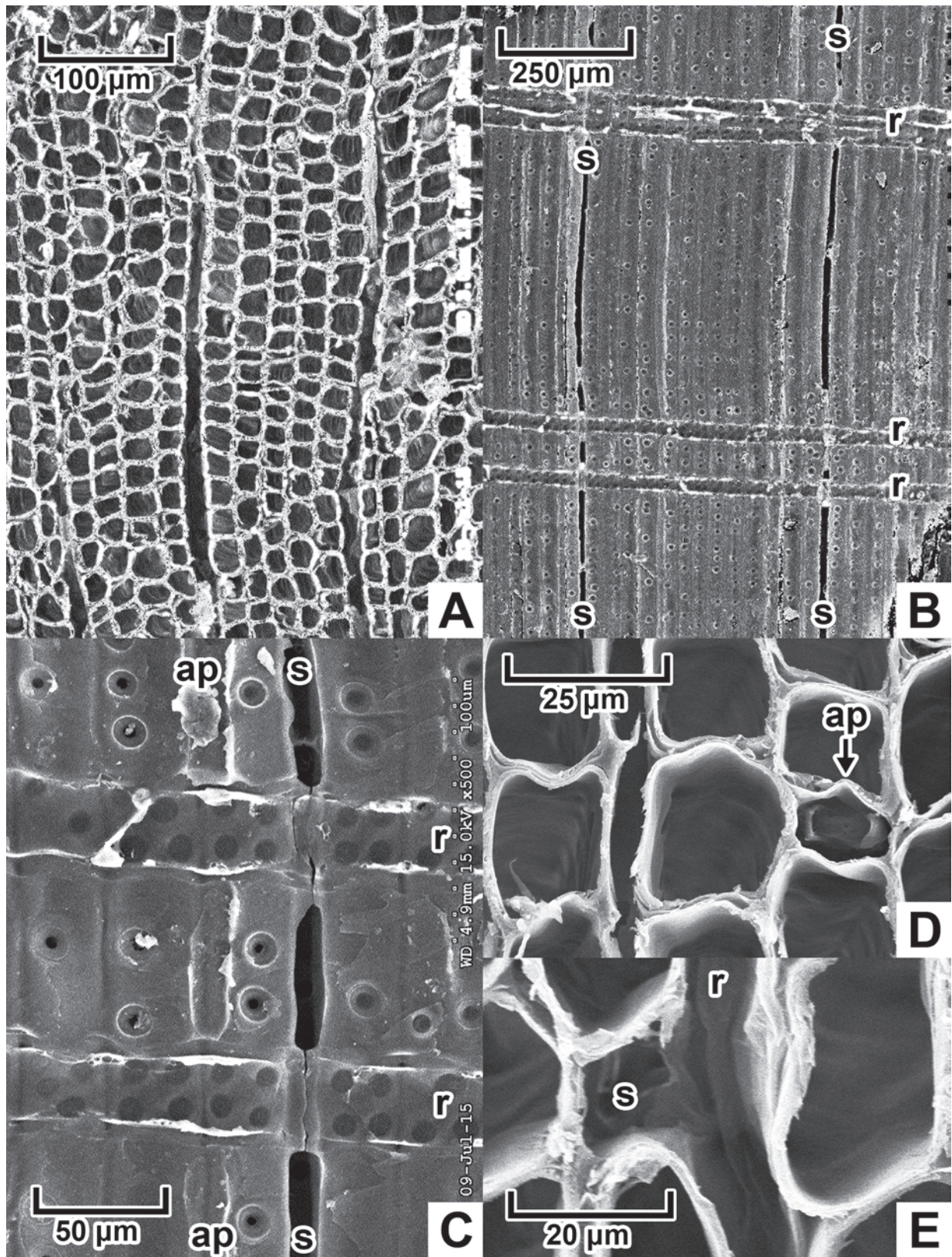


Fig. 2. *Dacrydium guillauminii*, Carlquist 4319 (RSAw). SEM micrographs of wood sections from stem. A. Transverse section; radial dimensions of tracheids vary only slightly. B–C. Radial sections. B. Lower-power magnification to show two air spaces (s) and three rays (r). C. Enlarged portion (corresponding to lower left in B) to show an axial parenchyma strand (ap), an air space (s), and two rays. D, E. Transverse section portions. D. An axial parenchyma cell containing resin. E. An air space adjacent to a ray.

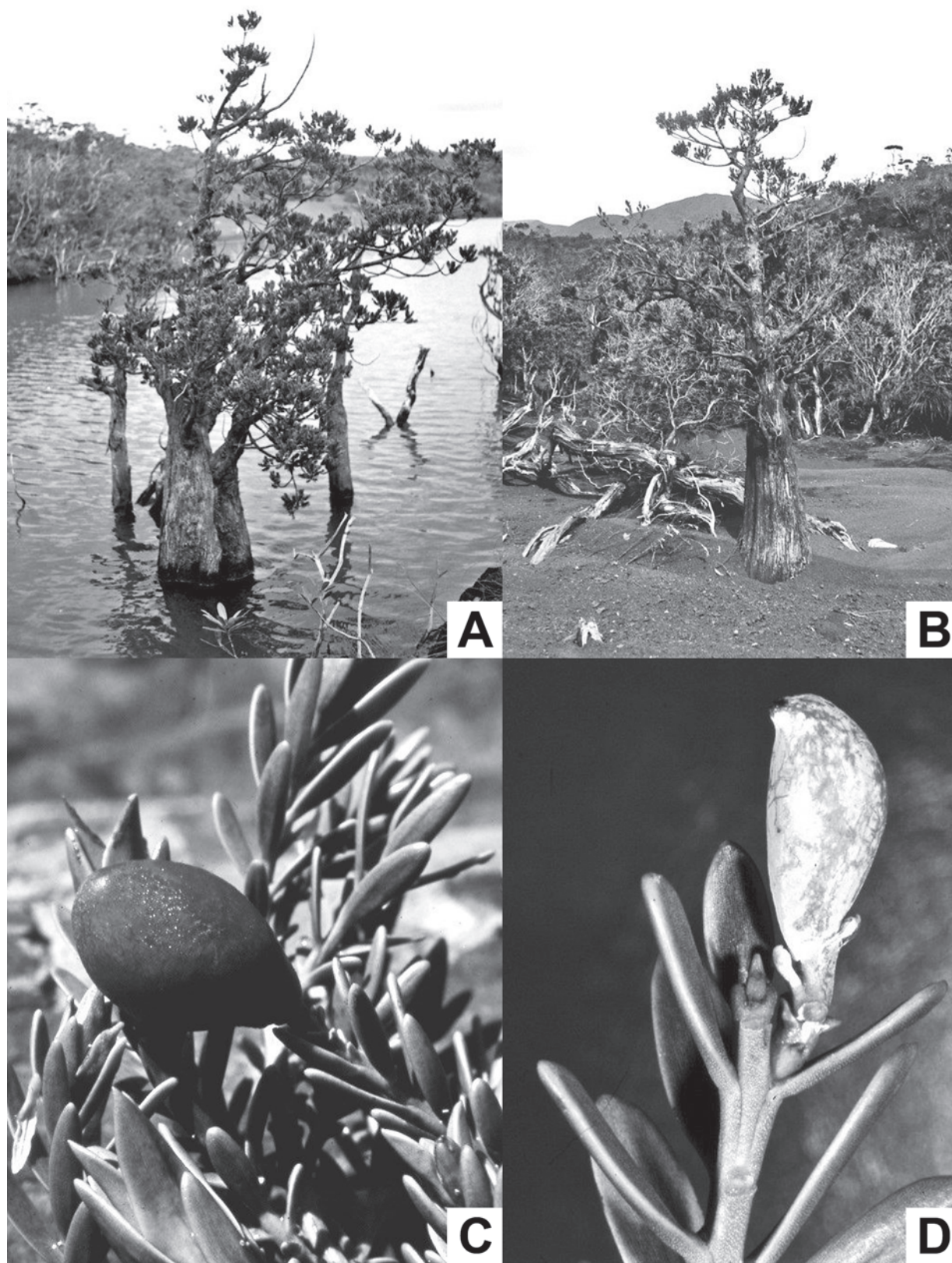


Fig. 3. *Retrophyllum minor*, Carlquist 4307 (RSA). A. Habit of trees in wider pond-like portion of Rivière des Lacs. B. A single tree in an area not currently inundated. C. Habit of branches, to show diagonal leaf positioning and a mature seed. D. An axillary glaucous cone, plus characteristically thick, blunt leaves.

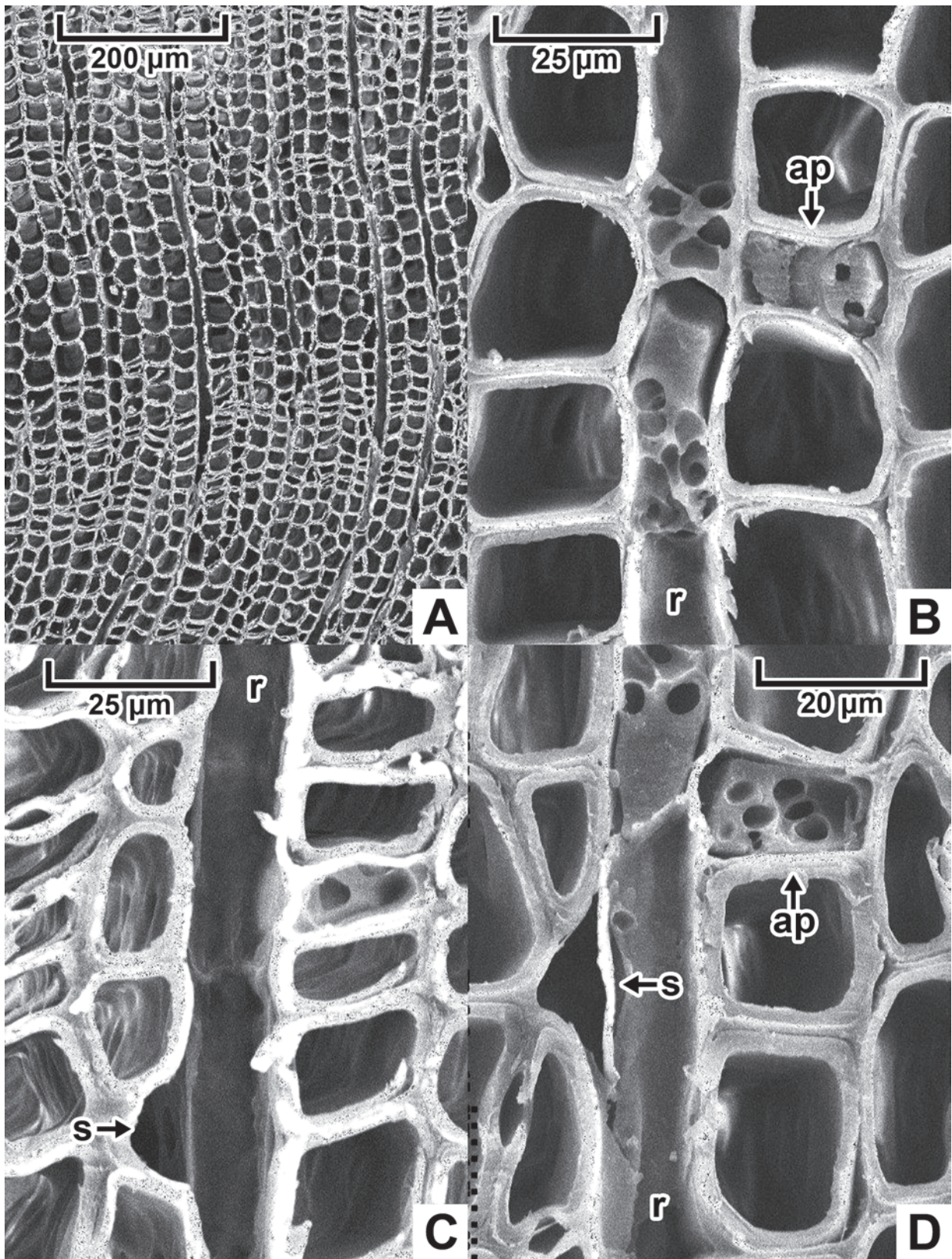


Fig. 4. *Retrophyllum minor*, C. T. White 2261 (A, RSAw), transverse sections of stem wood. A. Fluctuation of radial tracheid diameters reflects seasonal changes. B. Ray (r) and axial parenchyma (ap); resin deposits occur in both. C, D. Section portions showing triangular air spaces (s) adjacent to ray (ray cell wall delimiting the air space can be seen in both, more prominent in D).

pressure on a warming table (even moderate pressure prevents curling of the sections as they dry). These simple methods have several advantages over traditional wood sectioning techniques. The greater thickness of the sections permits one to gain three-dimensional understanding of wood structures, and three-dimensionality, by visualizing greater context and range in structures, is one of the key advantages of SEM imaging. Intercellular space systems, their shapes and extents, are more readily demonstrated. Thicker sections are less subject to tears and other artifacts than are the relatively thin sections cut on a sliding microtome, especially where tracheids and other cells are thin-walled, as they are in these two species. Because the thick hand-cut sections are not made into permanent slides, they are not subjected to organic solvents such as xylene, and thus resins, characteristic of conifers, are retained and their presence can be noted, a help in identifying cell types (ray cells, axial parenchyma). After the drying procedure, sections were mounted on aluminum stubs, sputter coated with gold, and examined with a Hitachi S2600N SEM (Hitachi High Technologies America, Inc., Schaumburg, IL).

Following the International Plant Names Index, we use the name *R. minor* (Page 1988) rather than *R. minus*.

WOOD ANATOMY

Dacrydium guillauminii (Fig. 2)

Transverse sections (Fig. 2A) showed little fluctuation in radial diameters of tracheids on a seasonal basis, so that growth ring activity is not evident. Tracheids and ray cells have thin walls which can collapse to a minimal extent during the sectioning process (Fig. 2A, D). Axial parenchyma is sparse; one strand is present, Fig. 2B, at left edge. In Fig. 2C, a strand (ap) runs behind the rays. Resin deposits may be present in axial parenchyma (Fig. 2D). Rays are one to three cells high (Fig. 2B) and thin-walled. Vertical air spaces occur at rather regular intervals (s, Fig. 2B). They occur adjacent to rays (Fig. 2B, C, E). Other air spaces in the wood are by comparison very small and not in contact with the rays, so the vertical air system is distinctive.

Retrophyllum minor (Fig. 4)

Tracheids fluctuate in radial diameter, so that growth ring activity is evident, although not markedly defined. Narrow tracheids form latewood bands (Fig. 4A, C). Tracheids are thin-walled (Fig. 4B, D). Axial parenchyma (ap, Fig. 4B, D) cells are moderately common, and often contain resin deposits. The resin deposits, which also occur in rays, appear as solid blocks containing globular spaces that represent evaporation of volatile resin components (Fig. 4B, D). Rays (r) are one to many cells high. Ray cell walls are well shown facing the vertical air spaces (Fig. 4C, D). Air spaces are all adjacent to rays (Fig. 4C, D; note the prominent ray cell wall between the air space, indicated by s and arrow, and the ray cell). Any other air spaces are extremely small by comparison.

There has been no previous description of the wood of *Dacrydium guillauminii*. The description for *Retrophyllum minor* agrees with the figures of Greguss (1955). His illustrations (as *Podocarpus minor* (Carrière) Parl.) show a

prominent growth ring. Greguss gives no verbal descriptions, only illustrations, for this species. His light microscope micrographs do not show any air spaces with certainty, and none is expected to be readily visible at the level of magnification he provides.

FIELD OBSERVATIONS

Although the two endemic conifers are restricted to the same stream and lake systems on the Plaine des Lacs in southernmost New Caledonia (Laubenfels 1972), they occupy different microclimates. *Dacrydium guillauminii* (Fig. 1A) occupies streambanks that fluctuate little in water level, and are water-saturated. The roots are probably almost constantly submersed. *Dacrydium guillauminii* is in crowded shrubby vegetation, which may correlate with the fact that branches do not occur lower on the trunk, but start to emerge above the level of other shrubs. The branches are tightly grouped (Fig. 1B). Leaves are narrow, needle-like, and straight (Fig. 1C, D), and dispersed spirally around stems rather than in flat sprays. Some other species of *Dacrydium* native to shadier habitats have distichous distribution of bifacial leaves (as in the branchlets of *Sequoia* and *Taxodium*). *Dacrydium araucarioides* Brongn. & Gris., which grows near *D. guillauminii* but away from streambanks, has wedge-shaped curved leaves that face the stem, so that the adaxial faces are not exposed to direct sunlight. Both species are clearly conifers the foliage of which is adapted to high light intensity.

The female cones of *D. guillauminii* consist of a single seed subtended by fused scales (Fig. 1C, D). Each of these cones is in the axil of a foliage leaf, as claimed by Laubenfels (1972). The one-seeded cones are dry at maturity, and, when shed, float in water (Carlquist 1974).

Retrophyllum minor does not characteristically grow along the margins of streams, but rather is more numerous in inundated areas (Fig. 3A). The occurrence of a few individuals on exposed lake or pond margins (Fig. 3B) may be an unusual occurrence; roots probably still reach the water table, which is a short distance below the surface, as digging reveals. Individuals of *R. minor* are grouped (Fig. 3A) or solitary (Fig. 3B), but do not co-occur with other species in the lake areas where they occur. The heavy-metal content of the water is evident from its color, and probably also accounts for the lack of algal mats.

The bases of *R. minor* trunks are markedly flared and covered by thick rough bark quite unlike the smooth trunks of *D. guillauminii*. Upper branches are crowded with leaves (Fig. 3C, D), which point generally upward or diagonally and are very thick compared to leaves of, say, *Sequoia*, which are essentially all shade leaves except for the scale-leaves on the main branches. This comparison is pertinent here because Laubenfels (1972) considers *R. comptonii* (J. Buchholz) C.N. Page, also of New Caledonia, to be the species closest to *R. minor*. Leaves of *R. comptonii* are thin and in flat sprays, like those of *Sequoia*, and are thus shade leaves; *R. comptonii* occurs in shady forests and is one of the most common conifers of the island (Laubenfels 1972).

The seeds of *R. minor*, although glaucous when young (Fig. 3D), change to red-purple when mature (Fig. 3C). The mature seeds float (Carlquist 1974). Whether they continue to float if the fleshy covering is removed is not known.

CONCLUSIONS

There are a number of reasons why the air spaces in the two riparian New Caledonian podocarps are not likely to be artifacts: (1) the air spaces are of uniform width, about the width of a tracheid; if these spaces represented fractures, they should be of various widths. (2) The air spaces are indefinite in axial length; if they were artifacts, one would expect spaces of various lengths. (3) The air spaces are always flanked by ray cells; as seen in transverse sections, the ray cell walls are always intact; if the spaces were artifacts, the ray cell wall flanking a space should be broken. (4) If the spaces were artifacts, one would expect that, as seen in transverse section, cell separation would be present among tracheids adjacent to the air space; a fracture that is always localized to be one cell in width without separation of neighboring tracheids is highly unlikely. (5) The spaces are always adjacent to rays; one would expect fractures to be scattered throughout a wood. (6) If the spaces represented fractures, the tracheid walls facing the spaces should bear large circular bordered pits typical of conifer tracheid lateral walls; such pits do not face the spaces. (7) As seen in radial sections, the spaces occur at rather regular intervals; if the spaces were fractures, they would be expected to be randomly scattered throughout a radial section.

The thin-walled tracheids of both *D. guillauminii* and *R. minor* are distinctive compared to those of other Podocarpaceae and may relate to lower values for mechanical strength in stems. One might think that the wide tracheid lumina could serve as channels for aeration, but there are reasons to believe that this may not be true. The pits of both *D. guillauminii* and *R. minor* have well-defined torus-margo structure and, once tracheids are no longer conductive, the pits aspirate, so that the torus effectively closes the pit to entry of air. Pit tori can be seen in older portions of wood in both species, so this sealing presumably continues indefinitely. The longitudinal air spaces adjacent to rays in both species seem an operative aeration device, but experimental work is now needed to see if that can be demonstrated. Breakdown of ray cell walls could add rays to the aeration system, but the degree of ray cell breakdown is not known. There is no known association of axial parenchyma with ray cells in these conifers: in both species, axial parenchyma seems scattered randomly. The occurrence of resin deposits in both ray cells and in axial parenchyma would seem to disqualify either of these cell

types as contributing extensively to an aeration system. The facts that this is the first known report of prominent vertical air spaces in conifers, and that this feature occurs in both of the riparian conifers of New Caledonia, seem compelling at present.

The aeration systems within stem wood are not the only features of *D. guillauminii* and *R. minor* that seem to be adaptations to the riparian habitat. Seed flotation capability is certainly pertinent in this regard. The placement of branches, direction of leaves, and thickness of leaves are all features of a sunny habitat, and the comparison of *R. comptonii*, a shady forest species, with *R. minor*, is compelling. Riparian habitats are sunny, at least for the taller species such as these conifers. Experimental evidence to demonstrate how the physiology of these two conifers is alike, and how, in turn, it may differ from physiology of forest Podocarpaceae, would be of considerable interest.

LITERATURE CITED

- CARLQUIST, S. 1974. Island biology. Columbia University Press, New York. 660 p.
- GREGUSS, P. 1955. Identification of living gymnosperms on the basis of xylotomy. Akadémiai Kiadó, Budapest, Hungary. 257 p. + 350 plates.
- HABERLANDT, G. 1965. Physiological plant anatomy, reprint edition (translated by M. Drummond). Today and Tomorrow's Book Agency, New Delhi, India. 775 p.
- HERBERT, J., P. M. HOLLINGSWORTH, M. E. GARDINER, R. R. MILL, AND R. THOMAS. 2002. Conservation genetics and phylogenetics of New Caledonian *Retrophyllum* species. *New Zealand J. Bot.* **40**: 175–188.
- JAFFRÉ, T., P. BOUCHET, AND J.-M. VEILLON. 1998. Threatened plants of New Caledonia: is the system of protected areas adequate? *Biodivers. & Conservation* **7**: 36.
- , J. MUNZINGER, AND P. P. LOWRIE. 2010. Threatened conifer species found on ultramafic massifs and proposals to improve their protection. *Biodivers. & Conservation* **19**: 1485–1502.
- , Y. PILLON, S. THOMINE, AND S. MERLOT. 2013. The metal hyperaccumulators from New Caledonia can broaden our understanding of nickel accumulation in plants. *Frontiers in Plant Science* **4**: 279.
- LAUBENFELS, D. J., DE. 1972. Flore de la Nouvelle Calédonie et dépendances. 4. Gymnospermes. Muséum National d'Histoire Naturelle, Paris, France. 168 p.
- PAGE, C. N. 1988 (1989). New and maintained genera in the conifer families Podocarpaceae and Pinaceae. *Notes Roy. Bot. Gard. Edinburgh* **45**: 377–395.