Cross-sectioning to the core of conifers: pith anatomy of living Araucariaceae and Podocarpaceae, with comparisons to fossil pith

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Summary – Pith in woody species fulfills essential roles, from functioning as the first vascular tissue in shoots, to serving as starch storage and facilitating heartwood formation. While the spongy cells of pith may die and be reabsorbed at maturity by some species, the pith persists throughout the lifespan of conifer trees. Pith features and functions of extant conifers have been documented in contemporary studies, and pith anatomy has been described for extinct progymnosperms and coniferous ancestors through fossils. However, up to now, few studies have described the wood anatomy of pith in living conifers and covered only 24 species in four families. Here we describe the pith of 7 genera and 16 species from the previously unstudied conifer families of Araucariaceae and Podocarpaceae, based on stained and unstained cross-sections. Comparisons between pith sections of the same tree in successive years yielded insights into maturation of the conifer pith. Conservative pith characteristics were identified among genera and families. Araucariaceae pith is dissimilar on a familial level, but the genus Araucaria is unified by pith shape and heterocellularity. In contrast, all Podocarpaceae piths develop secondary cracks, and most species have irregularly shaped piths. Beyond our study of similarities and differences of pith in Araucariaceae and Podocarpaceae, a look at pith patterns in the paleobotanical record and further examples in living conifers could increase the understanding of conserved characteristics and pith evolution.

Keywords – Afrocarpus, Agathis, Araucaria, conifer pith, Dacrydium, Nageia, Podocarpus, Wollemia.

Introduction

With the first flush of young green shoots in spring, pith becomes the primary vascular system in woody plants. The initial parenchyma cells in the pith are large and rapidly followed by smaller primary xylem cells which increase the girth of the shoot. Growing from the basal axial part of the apical meristem, pith continues to lengthen the tree trunk and branches and serves as the initial vasculature for upward and outward growth (Panshin & de Zeeuw 1980; Longuetaud & Caraglio 2009). Once the secondary xylem begins to develop, the function of the pith often changes to starch storage. Cell death in the pith occurs as the wood matures, leading to heartwood formation (Nakaba et al. 2012) or to the loss and reassimilation of cells (Kwon et al. 2001). The final fate of pith differs widely among the gymnosperms, monocotyledons, and dicotyledons, but there are nonetheless major evolutionary trends that can be observed among vascular plants.

Classifications of pith, which is also called the medulla, and the broader topic of the stele have been studied since 1886 by van Tieghem (Beck et al. 1982; Schmid 1982). Interest in the topic of pith has waxed and waned throughout scientific history, decreasing with pseudoscience theories and increasing with the advent of new technologies. Modern studies often look for the role of pith in an individual species, under certain conditions, or as growth markers...
in future studies (Kwon et al. 2001; Eisner et al. 2002; Scofield 2006; Burrows et al. 2007; Longuetaud & Caraglio 2009; Nakaba et al. 2012). For example, Burrows et al. (2007) described the unique morphology of *Wollemia nobilis* branch pith and abscission properties; similarly, Eisner et al. (2002) studied the compartmentalization and discoloration of pruning branches in hardwoods. However, relatively few studies have focused on describing the wood anatomy of pith. Older studies are difficult to access or lack information that has been made available through modern methods (Shimakura 1937; Doyle & Doyle 1948), and newer studies are rarely conducted (exceptions include Carlquist 1992; Crivellaro & Schweingruber 2013). This gap in knowledge affects not only botanical science, but also proves to be detrimental in paleobotany. As discussed later, due to lack of pith studies among living plants, many fossils with various degrees of pith preservation have been described but cannot be compared to extant relatives.

The present study proposes to fill this gap by focusing on conifers, the dominant trees of the Mesozoic forests that have left behind an abundance of fossils today. Until now, three studies have described the pith of living conifers. The first study was carried out eight decades ago by Shimakura (1937) which included a full anatomical description of the pith of *Taiwania cryptomerioides*. Next, Doyle & Doyle (1948) described 13 species which, in the end, resulted in a net gain of only 12 new descriptions, as they had not realized that *T. cryptomerioides* had been worked on earlier by Shimakura. Third and lastly, Crivellaro & Schweingruber (2013) produced the only modern study in which the pith descriptions of 11 additional conifer species are enhanced by biologically stained, high-resolution, colored micrographs and provide much new information on pith anatomy. In total, these three studies describe the pith of 24 species: 17 Cupressaceae, five Pinaceae, one Sciadopityaceae, and one Taxaceae.

In addition to a general paucity in pith descriptions, there is virtually nothing known about the pith of the surviving conifer families that once dominated the global forests of the Jurassic, the Araucariaceae and Podocarpaceae. For this reason, we focus here on the pith of 16 conifer species in these two families, as well as comparing pith anatomy in regard to branch age, genus, and family. Furthermore, comparisons are made to fossil conifer pith to elucidate common features shared by living conifers and their ancient relatives.

**Materials and methods**

**Species sampled**

Sixteen species of Araucariaceae and Podocarpaceae were sampled in total (Table 1), specifically, nine species from the former family and seven from the latter. Specimens were sourced from the living collections of The Huntington Botanical Garden in San Marino, California, United States of America, and the Bonn Botanical Garden of the University of Bonn, North Rhine–Westphalia, Germany. This study aimed to collect as many representative specimens from the Araucariaceae and Podocarpaceae as possible. For four of the species, two branch samples were collected from the same individual tree, as noted in Table 1.

**Sample preparation**

Branches were collected that ranged from 1.5 to 3 cm in diameter to ensure multiple years of growth and pith maturity, because pith is only considered mature after three years of age (Doyle & Doyle 1948). Branch samples and cut blocks of wood were stored in paper bags to hinder fungal growth.

Branch lengths measuring 2 cm were roughly sawn from each wood sample to fit the sample holder of the Microtome GSL1 (cf. Gärtner & Schweingruber 2013). The rough blocks were then refined with the microtome by cutting the wood blocks laterally to ensure secure placement in the sample holder. Then the cross-sectional surface was trimmed to remove saw marks. Water was applied with a fine paintbrush to the cutting surface during all stages of refining and final sectioning (cf. Gärtner & Schweingruber 2013; Yeung et al. 2015). Disposable NT-Cutter blades (A-
### Table 1. Species studied, inventory numbers, sources of material, accession numbers and dates collected.

<table>
<thead>
<tr>
<th>Family</th>
<th>Genus and species</th>
<th>Inventory No.</th>
<th>Source of material</th>
<th>Accession No. (IPEN)</th>
<th>Date collected</th>
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Four species, listed here with different collection dates, were sampled multiple times from the same individual. Abbreviations: HBG, The Huntington Botanical Garden; BBG, Bonn Botanical Garden.
type, 0.38 mm) were used in accordance with the Microtome GSL1 instructions (Fujii 2003; Gärtner & Schweingruber 2013; Gärtner et al. 2014) during all stages of microtome use.

After initial sample preparation was completed, optimal soaking times in a softening solution consisting of equal parts of water, ethanol, and glycerol were worked out for each type of wood (cf. Hoadley 1990; Gärtner & Schweingruber 2013; Yeung et al. 2015). Blocks were soaked in covered glass beakers of solution ranging from 24 hours up to 10 to 15 days.

Softened samples were mounted into the microtome with the pith axis perpendicular to the blade, and cross-sections were cut. By trial and error, it was found that sections of 50 to 80 μm in thickness ensured that the larger pith cells were visible and intact, in contrast to other studies that suggest a thickness of only 30 μm (Yeung et al. 2015). Multiple section depths were made for each species to ensure all structures could be described.

During sectioning, care was taken to cut across the pith diameter in a single movement. Contrary to other methods reported in literature (Gärtner & Schweingruber 2013; Yeung et al. 2015), it was found that it was best to allow sections to curl slightly because the force of flattening often caused cracks in the pith. Furthermore, the friction commonly detached the pith from the heartwood. If the pith remained intact, it was immediately guided onto a glass slide with a wet paintbrush (cf. Gärtner & Schweingruber 2013). Alternatively, this study found that a coverslip held by water adhesion to the blade mount could also transport the section to the glass slide. The coverslip also aided in reducing the friction of the delicate pith cells against the blade mount to keep them intact during sectioning. Subsequently, curled sections could be lifted from the mount with the use of the coverslip technique that otherwise would have been difficult to handle.

Thin sections on the glass slides were then mounted temporarily in water and loosely covered with a coverslip to allow for the cells to rehydrate and for the pressure of a coverslip to gently flatten the section (cf. Brown 1919). If a curl in the heartwood proved to be too severe to uncurl without damage to the pith, a blade was used to remove part of the secondary xylem.

**Preparation of unstained slides**

After flattening, sections were moved to a clean glass slide and rehydrated with drops of 87% glycerol solution to allow glycerol to flow into the large pith cells and displace air bubbles, then set aside in a lidded container to protect them from dust. A dissecting light microscope and dissection tools were used to manipulate air out of the cells whenever necessary. Glass coverslips then covered the temporarily mounted slides during microscope photography.

**Preparation of stained slides**

A set of flattened cross-sections, one depth per species, were selected for the staining process. The staining method follows previously established methods (Kraus et al. 1998; Gärtner & Schweingruber 2013). Slides were worked in batches, with a series of bleach and water washes. Initial tests on the material confirmed that a 50% diluted solution of bleach dripped across the section and allowed to oxidize for 3 to 5 minutes was sufficient in lightening the color without destroying the cells. Next, the slides were stained with the biological stains of Safranin O and AstraBlue (both dyes from Carl Roth GmbH, Karlsruhe, Germany) to differentiate between lignified and un lignified cells. Temporary mounting of the stained cross-sections followed procedures similar to those of the unstained slides.

**Imaging**

Images of unstained and stained samples were made with a DFC400 camera attached to a Leica DM 2500 compound light microscope or a Leica S APO dissection microscope (Leica Microsystems, Wetzlar, Germany). The microscope-integrated software ImageAccess easyLab (version 7.08.1; iMagic, Glattburg, Switzerland) was also used for measurement and cataloguing.
Section mounting and repository

Sections previously mounted in glycerol and water were washed in successive baths of increasing ethanol concentrations. After the 90% ethanol bath, cross-sections were placed onto cleaned glass slides to allow for evaporation. After a few seconds, Euparal mounting medium (Carl Roth, Karlsruhe, Germany) was applied by pipette, and a coverslip was placed on top. Slide weights were used during the first 24 hours whenever necessary. Slides were placed into a preparation oven at 50°C for 48 hours to decrease curing times (cf. Berlin & Miller 1980; Yeung et al. 2015). Once slides could be safely handled, they were cleaned with a solvent, or excess dried Euparal was removed with a scalpel.

All thin sections are deposited at the Herbarium of the Huntington Botanical Gardens, San Marino, CA, USA, labeled with a CPX inventory number.

Results

The following descriptions are based on cross-sections, and the anatomical structures are viewed here in two dimensions. A hypothetical pith showing special features, such as stellate pulls, isolated sclereids, clusters of sclereids, thick-walled parenchyma, and astral sclereids, is presented in Figure 1.

Araucariaceae Henkel & W. Hochst.

Agathis Salisb.

Agathis australis (D. Don) Lindl.

Agathis australis (Fig. 2A, B) has an oblong pith with stellate pulls. Parenchyma and sclerenchyma are both present. The majority of the pith is composed of parenchyma cells, especially at the pith periphery, while the cells in the pith center have interspersed cells of both types (Fig. 2C). Cells are densely packed with little interstitial space, and cell sizes are variable. Sclerenchyma cells in the form of sclereids are generally larger than parenchyma, and can be arranged in clusters. There is a trend for cell sizes to decrease towards the periphery of the pith. Unstained cell contents appear translucent. Nonlignified cells are present and readily distinguished from red lignified cells in the stained section (Fig. 2B).

Agathis lanceolata Warb.

The two specimens of Agathis lanceolata under study each show slight variation in some details (Fig. 3). The branch of Agathis lanceolata 1 is three years old, while Agathis lanceolata 2 is two years old. The overall shape of A. lanceolata 1 is circular (Fig. 3A), while that of A. lanceolata 2 has a very widely ovate shape (Fig. 3C). Both specimens contain only parenchyma in the pith, but they differ in their cell density. The pith of A. lanceolata 1 is less dense and has increased interstitial space towards its periphery (Fig. 3B), while A. lanceolata 2 is denser and does not show any changes in interstitial space (Fig. 3D). Both specimens show uniform cell sizes. Another key distinction is that A. lanceolata 1 contains medium brown-colored cells throughout the unstained pith (Fig. 3A), whereas the pith of A. lanceolata 2 consists of mostly translucent cells with a few scattered light brown cells (Fig. 3C). The majority of the stained section of A. lanceolata 2 is made up of red lignified cells; blue nonlignified cells occur at the pith periphery or are scattered in the center of the pith (Fig. 3E, F). As previously mentioned, the density of cells at the pith–xylem transition is different in the two specimens, and the less dense, more delicate structure of the A. lanceolata 1 pith caused it to fracture during sectioning.
The two specimens of *Agathis robusta* studied differ by showing some variation in pith anatomy (Fig. 4). *Agathis robusta* 1 is four years old and *Agathis robusta* 2 is three years old. Both specimens have circular piths, with parenchyma cells surrounding clusters of sclerenchyma cells (Fig. 4A–D). However, *A. robusta* 1 exhibits a stronger development of this pattern, and cell type abundance reflects this difference between the branches. *A. robusta* 1 has a higher ratio of sclerenchyma to parenchyma, while *A. robusta* 2 is more abundant in parenchyma. Both specimens show a distinctive circular pattern of dense sclerenchyma clusters surrounded by parenchyma which can be easily observed in *A. robusta* 1, but is less apparent in *A. robusta* 2. Both specimens have parenchyma cells uniform in size and shape, and the sclerenchyma cells are larger and irregularly shaped. *A. robusta* 1 displays light brown-colored parenchyma, while *A.
Fig. 2. Pith cross-sections of *Agathis australis*, inventory number CPX-001. (A) Unstained section, 60 μm thickness. (B) Section stained with Safranin O and Astra blue, 70 μm thickness. (C) Close-up of a second unstained section, 80 μm thickness.
Fig. 3. Pith cross-sections of *Agathis lanceolata*. (A, B) *A. lanceolata* 1, inventory number CPX-002. (A) Unstained section, 80 μm thickness. (B) Close-up of unstained section, 80 μm thickness. (C–F) *A. lanceolata* 2, inventory number CPX-003. (C) Unstained section, 60 μm thickness. (D) Close-up of a second unstained section, 70 μm thickness. (E) Section stained with Safranin O and Astra blue, 70 μm thickness. (F) Close-up of stained section in E, 70 μm thickness.
Fig. 4. Pith cross-sections of Agathis robusta. (A, B) A. robusta 1, inventory number CPX-004. (A) Unstained section, 80 μm thickness. (B) Close-up of unstained section in A, 80 μm thickness. (C–F) A. robusta 2, inventory number CPX-005. (C) Unstained section, 80 μm thickness. (D) Close-up of unstained section in C, 80 μm thickness. (E) Section stained with Safranin O and Astra blue, 70 μm thickness. (F) Close-up of stained section in E, 70 μm thickness.
Robusta 2 has translucent parenchyma. Both specimens have colorless but slightly opaque sclerenchyma. The stained section of *A. robusta* 2 is entirely colored with lignified cells (Fig. 4E, F). *A. robusta* 1 has more interstitial space at the pith–xylem transition compared to the dense cells of *A. robusta* 2 which decrease in size toward the pith periphery (Fig. 4B, D).

**Araucaria** Juss.

*Araucaria angustifolia* (Bertol.) Kuntze

*Araucaria angustifolia* has an oblate pith with very shallow stellate pulls (Fig. 5A, B). The majority of the cells are composed of sclerenchyma, including astral sclereids, with parenchyma cells interspersed between the groups of sclerenchyma and in the pith periphery. There is little interstitial space, especially in sclerenchyma bundles, resulting in a dense pith. Sclerenchyma cells are large and irregularly shaped, especially the astral sclereids, and the parenchyma cells are small and regularly shaped. Colored cell contents are mostly transparent, with some scattered cells with medium brown coloring which does not appear to be linked with cell type. The stained section shows predominately red lignified cells and very few small cells without lignification in blue (Fig. 5C). The pith–xylem transition shows a dense line of cells without a gradual change in cell size.

*Araucaria araucana* (Molina) K. Koch

*Araucaria araucana* pith shape is oblate with shallow stellate pulls (Fig. 5D–F). Parenchyma and sclerenchyma are both abundant, with astral sclereids present. Both cell types are dispersed throughout the pith, with a trend toward a greater number of parenchyma cells near the periphery. Cells are dense with little interstitial space. Sclerenchyma and astral sclereids are large and irregularly shaped, while parenchyma cells are small and regularly shaped. Unstained sections show that sclerenchyma cells are translucent and some parenchyma contain light brown colored cell contents. The stained section exhibits a mixed distribution of blue nonlignified and red lignified cells. In the stellate pulls, there is an increase in interstitial space and a decrease in cell sizes from the stem center to the pith periphery.

*Araucaria bidwillii* Hook.

Two specimens of *Araucaria bidwillii* were sampled due to the difficulty in obtaining intact pith in the sections. Both branches have been determined to be three years old (Fig. 6A, B). They have widely elliptic piths with shallow and widely spaced stellate pulls. Both piths contain predominately parenchyma cells with few sclerenchyma cells, specifically astral sclereids, interspersed among the parenchyma. There is a large amount of interstitial space in both specimens, which results in a spongy, delicate structure that was difficult to keep intact after sectioning. The parenchyma cells are small and regular in size and shape, while the sclerenchyma cells are large and irregular in shape. Both unstained sections contain transparent cells. When stained, the section of *A. bidwillii* 2 displays red lignified cells with blue nonlignified cells only at the border of the entire pith (Fig. 6C). There is an abrupt cell size change between pith and xylem.

*Araucaria columnaris* (G. Forst.) Hook.

*Araucaria columnaris* displays a widely elliptic pith with shallow stellate pulls (Fig. 6D, E). The majority of cells are sclerenchyma in sclereid masses, with few parenchyma cells found between the masses and around the pith periphery. The parenchyma cells are small, the sclerenchyma cells are large, and both cell types are regular in shape. The sclereid masses are densely packed, but there is interstitial space around parenchyma cells and within the stellate pulls. The parenchyma at the periphery is a medium to dark brown color, while the sclereid masses are colorless but slightly opaque. The stained section shows predominately red lignified cells, with a small amount of blue nonlignified cells in between the sclerenchyma cells and around the periphery of the pith (Fig. 6F).
Fig. 5. Pith cross-sections of *Araucaria angustifolia* and *Araucaria araucana*. (A–C) *A. angustifolia* inventory number CPX-006. (A) Unstained section, 70 μm thickness. (B) Close-up of section in A, 70 μm thickness. (C) Section stained with Safranin O and Astra blue 60 μm thickness. (D–F) *A. araucana*, inventory number CPX-007. (D) Unstained section, 60 μm thickness. (E) Close-up of a second unstained section, 70 μm thickness. (F) Section stained with Safranin O and Astra blue, 70 μm thickness.
Fig. 6. Pith cross-sections of Araucaria bidwillii and Araucaria columnaris. (A) A. bidwillii 1, inventory number CPX-008, unstained section, 80 μm thickness. (B, C) A. bidwillii 2, inventory number CPX-009. (B) Unstained section, 70 μm thickness. (C) Section stained with Safranin O and Astra blue, 80 μm thickness. (D–F) A. columnaris inventory number CPX-010 (D) Unstained section, 70 μm thickness. (E) Close-up of a second unstained section, 50 μm thickness. (F) Stained section, 70 μm thickness.
**Araucaria muelleri** (Carrière) Brongn. et Gris

*Araucaria muelleri* has a circular pith with short and wide stellate pulls (Fig. 7A, B). Parenchyma is the dominant cell type found around the pith periphery and surrounds clusters of sclerenchyma cells, including astral sclereids. The central parenchyma and sclerenchyma tissues both consist of large cells, with a slight decrease in size towards the periphery. Parenchyma cells are regular in shape, while sclerenchyma cells and astral sclereids are irregular. This pith is dense with an increase in interstitial space at the pith–xylem margin. In unstained sections, a majority of cells are translucent with some peripheral and scattered central cells showing a light brown coloration. In stained slides, most cells are blue nonlignified cells interspersed with groups of red lignified cells (Fig. 7C). At the pith–xylem transition, parenchyma cells somewhat decrease in size but still remain large compared to xylem cell sizes.

**Wollemia** W.G. Jones, K.D. Hill et J.M. Allen

**Wollemia nobilis** W.G. Jones, K.D. Hill et J.M. Allen

The pith of *Wollemia nobilis* is very widely ovate with shallow stellate pulls (Fig. 7D–F). Parenchyma appears to be absent, while sclerenchyma, including some larger, dispersed astral sclereids, is present. Sclerenchyma cells are regularly shaped and uniformly sized, and astral sclereids are larger and irregularly shaped. Interstitial space is found between the cells and in the stellate pulls. The sclerenchyma is a uniformly light brown color, while astral sclereids remain translucent. In a stained section, the entire pith is red, confirming that all pith cells are lignified. Cell sizes decrease at the pith–xylem transition boundary.

**Podocarpaceae Endl.**

**Afrocarpus** (J. Buchholz et N.E. Gray) C.N. Page

**Afrocarpus mannii** (Hook. f.) C.N. Page

*Afrocarpus mannii* has an irregularly shaped outline with stellate pulls of varying widths and lengths (Fig. 8A, B). Parenchyma cells line the periphery and are dispersed among some scattered, large sclerenchyma cells. Another cell type is present, one with granule inclusions, which may represent starch grains. The pith is not dense and contains large amounts of interstitial space. Sclerenchyma cells are the largest in cell size, then the granule cells, while the parenchyma cells are the smallest. In unbleached sections, parenchyma cells are a medium brown color, sclerenchyma is a light brown to a transparent tan color, and the unidentified granule cells are translucent. Stained sections show predominately red lignified cells, with a few small cells that are nonlignified and dyed blue.

**Dacrydium** Sol. ex G. Forst.

**Dacrydium cupressinum** Sol. ex G. Forst.

The pith of *Dacrydium cupressinum* is oblate in shape, and any stellate pulls are extremely shallow (Fig. 8C). Parenchyma is the primary cell type, although there is some scattered sclerenchyma. Cells are moderately dense with little interstitial space except for a secondary crack that persists through the length of the branch. Cells are round and uniform in shape, with a decrease in size towards the pith margins. Most cells are translucent, with a few displaying light brown color contents. The stained section shows most pith cells are nonlignified (Fig. 8D).

**Nageia** Gaertn.

**Nageia nagi** (Thunb.) Kuntze

*Nageia nagi* displays an irregularly shaped pith with rounded and narrow stellate pulls (Fig. 9A, C). Sections include a branching pith, which is visible in the lower left corners of each image. The pith is comprised mostly of parenchyma cells, with solitary thick-walled parenchyma cells in the transitional space of the branching pith (Fig. 9C, D, arrowheads). The cells have a moderate density, with some interstitial space and with secondary cracks that run
Fig. 7. Pith cross-sections of *Araucaria muelleri* and *Wollemia nobilis*. (A–C) A. muelleri, inventory number CPX-011. (A) Unstained section, 80 μm thickness. (B) Close-up of a second unstained section, 80 μm thickness. (C) Section stained with Safranin O and Astra blue, 80 μm thickness. (D–F) W. nobilis, inventory number CPX-012. (D) Unstained section, 70 μm thickness. (E) Close-up of unstained section in D, 70 μm thickness. (F) Stained section, 70 μm thickness.
Fig. 8. Pith cross-sections of *Afrocarpus mannii* and *Dacrydium cupressinum*. (A, B) *A. mannii*, inventory number CPX-013. (A) Unstained section, 70 μm thickness. (B) Section stained with Safranin O and Astra blue, 60 μm thickness. (C, D) *D. cupressinum*, inventory number CPX-014. (C) Unstained section, 70 μm thickness. (D) Stained section, 60 μm thickness.

the length of the specimen. Cell sizes are not uniform, but cell shape remains regular. Unstained cells vary from light brown to transparent in color. In stained sections, the majority of cells are colored red, with a few blue, nonlignified cells located mostly on the edges of the pith cracks (Fig. 9B, D). Cell size decreases into the primary xylem with no interstitial space.

Remarks

The thick-walled cells in Fig. 9C and D (arrowheads) are identified here as thick-walled parenchyma based on the images and descriptions published by Crivellaro & Schweingruber (2013).

*Podocarpus* L'Hér. ex Pers.

*Podocarpus gracilior* Pilg.

The pith of *Podocarpus gracilior* is irregularly shaped with short and wide stellate pulls (Fig. 10A). Both parenchyma and sclerenchyma are abundant, with parenchyma surrounding the sclerenchyma. Cell structure is dense with no interstitial space. However, there are secondary cracks that run through the length of the specimen. The parenchyma
Fig. 9. Pith cross-sections of Nageia nagi, inventory number CPX-015. (A) Unstained section, 70 μm thickness. (B) Section stained with Safranin O and Astra blue, 70 μm thickness. (C) Close-up of a second unstained section, 70 μm thickness. (D) Close-up of a second stained section, 70 μm thickness. (C, D) White arrowheads indicate thick-walled parenchyma cells.

cells are smaller in size than those of the sclerenchyma, but both types of cells are regular in size and shape. Colored cell contents in unstained sections are a medium to light brown color in the parenchyma with the color darkening towards the pith periphery. Stained sections distinguish between the larger lignified cells in red and smaller nonlignified cells in blue (Fig. 10B). Cells do not decrease in size at the pith–xylem transition.

Podocarpus latifolius (Thunb.) R. Br. ex Mirb.

Podocarpus latifolius has a pith with an irregular outline, with stellate pulls of varying widths and lengths (Fig. 10C). Both parenchyma and sclerenchyma are present, with the majority of cells as parenchyma and a few dispersed cells as sclerenchyma. There are scattered thick-walled parenchyma cells in the pith that are not limited to any one part of the pith. The pith cells are densely packed, with the exception of secondary cracks that persist through the length of
Fig. 10. Pith cross-sections of *Podocarpus gracilior* and *Podocarpus latifolius*. (A, B) *P. gracilior*, inventory number CPX-016. (A) Unstained section, 70 μm thickness. (B) Section stained with Safranin O and Astra blue, 60 μm thickness. (C, D) *P. latifolius*, inventory number CPX-017. (C) Unstained section, 80 μm thickness. (D) Stained section, 70 μm thickness. (C, D) White arrowheads indicate thick-walled parenchyma cells.
the branch. Sclerenchyma cells are large and circular, while parenchyma cells are smaller and regular in shape. Most parenchyma cells are light to medium brown, and sclerenchyma is light brown. In stained sections, all cells are red and lignified (Fig. 10D). Cell size decreases towards the pith periphery, and there is a clear distinction between the pith–xylem transition with no interstitial space.

**Remarks**

The thick-walled cells in Fig. 10C and D (arrowheads) are identified here as thick-walled parenchyma based on the images and descriptions published by Crivellaro & Schweingruber (2013).

*Podocarpus macrophyllus* (Thunb.) Sweet var. maki Siebold et Zucc.

The two specimens of *Podocarpus macrophyllus* var. *maki* collected show some anatomical variation (Fig. 11A, B). *Podocarpus macrophyllus* var. *maki* 1 is four years old, while *Podocarpus macrophyllus* var. *maki* 2 is three years old. Both piths have an irregular outline with stellate pulls of different lengths and widths. The cell types present are similar, consisting primarily of parenchyma cells with a few sclerenchyma cells limited to the stem center. Notably, the pith of *Podocarpus macrophyllus* var. *maki* 2 has additional grain-filled cells that may represent starch. Both piths have a dense cellular structure with little interstitial space, except for secondary cracks that persist through the entirety of the branches. Parenchyma cell size is smaller than sclerenchyma cell size, and there is a decrease in size with distance from the center. Another difference between branches is *Podocarpus macrophyllus* var. *maki* 1 is comprised primarily of translucent cells with a few light brown colored cells, while *Podocarpus macrophyllus* var. *maki* 2 has cells of medium brown color in the center and darker ones at the pith periphery. The stained section of *Podocarpus macrophyllus* var. *maki* 2 displays mostly blue nonlignified cells with some red lignified cells (Fig. 11C).

*Podocarpus neriifolius* D.Don

*Podocarpus neriifolius* displays an oblate pith with irregular protrusions, the longest of these which may have extended into a branch (Fig. 11D, E). The majority of the pith consists of thin-walled parenchyma, with a few cells of thick-walled parenchyma (Fig. 11E, arrowheads) and sclerenchyma cells interspersed in the stem center. The cells are densely packed with no interstitial space except for secondary cracks in the pith. Cell size varies among all tissue types, but generally decreases towards the pith periphery. Unstained cells are translucent with very few cells containing light brown-colored contents. The stained sections show a mixed distribution of red lignified cells and blue nonlignified cells (Fig. 11F). The cell size transition in the pith to the secondary xylem is abrupt, with no interstitial space between the tissue types.

**Remarks**

The thick-walled cells in Fig. 11E (arrowheads) are identified here as thick-walled parenchyma based on the images and descriptions published by Crivellaro & Schweingruber (2013).

**Discussion**

**Comparisons of pith within the same tree**

Pith morphology can vary between woody axes within one tree, between trees of the same species, and as a result of branch age (Doyle & Doyle 1948; Kwon et al. 2001; Longuetaud & Caraglio 2009). The sampling of two branches from the same tree for four conifer species in our study offers an opportunity to compare branches at different points of pith maturity. The first comparison made here is between *Agathis lanceolata* 1 and 2, which are only one year apart in age, yet show broad changes in pith shape, colored cell contents, and cell density. The more mature pith of *A. lanceolata* 1 is circular, darker in color, and contains more interstitial space, leading to a mature pith that is more
Fig. 11. Pith cross-sections of *Podocarpus macrophyllus* var. *maki* and *Podocarpus neriifolius*. (A) *P. macrophyllus* var. *maki* 1, inventory number CPX-018, unstained section with 80 μm thickness. (B, C) *Podocarpus macrophyllus* var. *maki* 2, inventory number CPX-019. (B) Unstained section, 80 μm thickness. (C) Section stained with Safranin O and Astra blue, 60 μm thickness. (D–F) *Podocarpus neriifolius*, inventory number CPX-020. (D) Unstained section, 60 μm thickness. (E) Close-up of a second unstained section, 70 μm thickness. White arrowheads indicate thick-walled parenchyma cells. (F) Stained section, 60 μm thickness.

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delicate in structure. *A. lanceolata* 2 has a very wide ovate pith, with translucent cells, and its cells are densely spaced throughout the center of the stem. This pith of *A. lanceolata* 2 is thus more robust in structure, which readily resulted in intact sections.

In the second example of pith maturity, the differences between *Agathis robusta* 1 and 2 are also clearly visible even at only one year apart in age. The pith of this species differs in cell type and abundance, cell density, and number of cells with colored contents. The more mature pith of *A. robusta* 1 has a recurring pattern of dense sclerenchyma bundles surrounded by parenchyma cells, more interstitial space towards the pith periphery, and a darker color of its parenchyma cells. *A. robusta* 2 displays a less developed version of this pattern, with smaller and less densely packed sclerenchyma bundles, uniform spacing between cells, and translucent parenchyma. It is interesting to note that, if pith is indeed mature at three years (Doyle & Doyle 1948), then *Agathis robusta* 2 should be fully mature. While this threshold for pith maturity would be appropriate for *Agathis lanceolata* 1, the comparison of these two species here suggests that one single rule may not be enough to define pith maturity across all species.

The third species of which two specimens were collected is *Araucaria bidwillii*. Both branches collected are three years old and do not differ in their pith anatomy. Their similarity in pith structure illustrates one case in which pith characteristics are conserved and do not differ within a single tree.

The last and fourth comparison is between *Podocarpus macrophyllus* var. *maki* 1 and 2, which are one year apart in age. The key difference of these branches is the presence of grain-filled cells that occur only in *P. macrophyllus* var. *maki* 2; further testing should be done to identify the grains. It is also interesting to note that the colored cell contents of *P. macrophyllus* var. *maki* may lighten with maturity, because they are a medium brown color in *P. macrophyllus* var. *maki* 2, but translucent in the older pith of *P. macrophyllus* var. *maki* 1. This is the inverse branch age and color pattern observed in the piths of *Agathis lanceolata* and *Agathis robusta*.

### Comparisons within and among genera

Further comparisons in pith morphology can be made within genera in which multiple species were sampled. In this study, *Agathis*, *Araucaria*, and *Podocarpus* can be used for this type of analysis.

There are a few characteristics that are shared by the pith of all three *Agathis* species. These include a circular to near-circular pith shape, as well as a lack of astral sclereids. However, the presence of cell types, colored cell contents, and density of cells vary across all species, as well as in relation to maturity within a single tree, as discussed above. A comparison of *Agathis australis* and *A. robusta* pith, for example, shows that while both species have sclerenchyma in the form of sclereids and can be found in clusters, they do not occur in the same abundance. This suggests that *Agathis* exhibits few conserved traits in its pith.

In contrast, the genus *Araucaria* appears to share two characteristics across all species. All *Araucaria* species sampled have circular to near-circular pith shapes and display heterocellularity with both parenchyma and sclerenchyma cell types present. There are additional characteristics that are shared by most *Araucaria* species, such as dense cell structure (except *A. bidwillii*), astral sclereids (except *A. columnaris*), or a decrease in parenchyma cell size at the pith periphery (except *A. angustifolia* and *A. bidwillii*). It is noteworthy that the type species of the genus, *A. araucana*, exhibits all shared characteristics.

In the genus *Podocarpus*, there are a few characteristics that occur in all species sampled in this study. All but one species (*P. neriifolius*) has an irregular outline. However, *P. neriifolius* does have irregular stellate pulls around an oblate pith, making it visually similar to the other members of the genus. A second, shared characteristic is the dense packing of cells throughout the pith, with exception of the area with secondary cracks. Unstained colored cell contents also follow a color pattern of light brown in the center and darker towards the pith periphery in all species. Furthermore, the transition to xylem is marked by abrupt cell size changes and a lack of interstitial space. These four shared characteristics are not uniformly shared by other genera in this study and thus sets the pith of
*Podocarpus* species apart. Lastly, half of the species sampled, such as *P. latifolius* and *P. neriifolius*, contain thick-walled parenchyma cells, which may prove to be a conserved trait based on evidence discussed in the next section.

**Comparisons within families**

In the family Araucariaceae, the pith anatomy observed in the nine species studied here is quite variable, although all three genera show a circular to near-circular overall pith shape. As discussed above, the three species of *Agathis* are generally dissimilar to one another, with the exception of the overall pith shape. The genus *Araucaria* contains a higher degree of similarities in regard to pith shape and heterocellularity, with the exception of one or two species. The pith morphologies of *Araucaria* spp. and *Wollemia nobilis* are similar in having a near-circular pith shape and containing astral sclereids, while *Agathis* spp. and *Wollemia* share the feature of high levels of similarly sized parenchyma.

Piths in the Podocarpaceae may show shared characteristics, but it is unclear if this is enough to justify one type of pith for the entire family. The pith of all podocarpaceous species studied here have an irregular outline except for *Dacrydium cupressinum* and *Podocarpus neriifolius*. However, as mentioned previously, the pith of *P. neriifolius* with its irregular stellate pulls is visually similar to the irregular outlines in the genus and, in this way, is characteristic of the family. This makes *D. cupressinum* and its oblate pith shape an outlier in regard to Podocarpaceae pith shape. One unifying feature found in all podocarpaceous piths sampled so far is the occurrence of secondary cracks in the branches. These are probably not artifacts due to sampling because these species were collected in different locations, at different times of the year, with different branch ages, and with different intervals of time between collection to sectioning. It is most likely a unifying feature due to structural similarity in anatomy. The feature of the thick-walled parenchyma was found in three of the species sampled, that is, *Nageia nagi*, *Podocarpus latifolius*, and *P. neriifolius*. As the presence of this characteristic was not found in Araucariaceae pith, this may prove to be a conserved trait of Podocarpaceae.

**Comparisons to previously described conifer pith**

As previously noted, there has been very little research carried out on the pith anatomy of living conifers. Up to now, some conifer families have even been neglected entirely. There are, however, three published studies that do describe living conifer pith (Shimakura 1937; Doyle & Doyle 1948; Crivellaro & Schweingruber 2013) and encompass the following families: Cupressaceae, Pinaceae, Sciadopityaceae and Taxaceae.

The first study, by Shimakura (1937), describes the wood anatomy of *Taiwania cryptomerioides* and includes one paragraph on pith, as well as two micrographs. The pith of *T. cryptomerioides* is irregularly shaped and contains both parenchyma and sclereids. Notably, there is a rough description of the difference in cell abundance between pith maturity in a “yearling” sample and in a mature pith. The younger branch only has parenchyma cells, while the mixed cell types are found in the mature sample. This is similar to the pattern found in *Agathis lanceolata* and *A. robusta* of the present study.

In 1948 Doyle and Doyle described the pith of 13 species as the members of the Taxodiaceae, 12 of which would be classified today as Cupressaceae and one as Sciadopityaceae. The cupressaceous genera would be grouped into several subfamilies: the Athrotaxidoideae, Cunninghamhamioideae, Sequoioidae, Taiwanioidae, and Taxodioidae. Overall, Doyle & Doyle (1948) note a distinct lack of unifying characteristics among the genera, which would mean that the Cupressaceae as recognized today also lack unifying characteristics. *Sciadopitys* and the cupressaceous subfamilies Cunninghamhamioideae, Sequoioidae, and Taiwanioidae are described with only one species each, and comparisons among these taxa are therefore not possible. It seems that Doyle & Doyle (1948) were not aware of Shimakura’s (1937) anatomical description of *Taiwania cryptomerioides* wood; however, both studies describe an irregularly shaped, mixed cell type pith for *T. cryptomerioides*. 
The Athrotaxidoideae is represented in the work of Doyle & Doyle (1948) by three species (*Athrotaxis cupressoides*, *A. laxifolia* and *A. selanginoides*) and described with the unifying characteristics of similar pith shape, cell density, and cell types, with both parenchyma and sclereids present. The largest number of taxa sampled by Doyle & Doyle (1948) came from the subfamily Taxodioidae (*Cryptomeria japonica*, *C. japonica* var. *elegans*, *Glyptostrobus pensilis*, *Taxodium ascendens*, *T. distichum* and *T. mucronatum*), totaling six of the 13 species in their study. However, these three genera have different pith characteristics and do not show united pith characteristics for the subfamily. Hence, Doyle & Doyle (1948) may not have found conserved characteristics for each family or subfamily, but they did find unifying characteristics on the genus level. The only genus sampled by Doyle & Doyle (1948) that does not possess similar pith characteristics is *Taxodium*. While *Taxodium ascendens* and *T. distichum* share many key identifiable features, such as cells with “cross-bars,” the pith of *T. mucronatum* is distinctly different by having regularly shaped cells with a gradual decrease in cell size towards the pith periphery.

The most recent observations on conifer pith were made by Crivellaro & Schweingruber (2013) which include 11 species altogether. Five species pertain to each the Cupressaceae and Pinaceae, and one species to the Taxaceae. The Cupressaceae wood sampled (*Cupressus sempervirens*, *Juniperus excelsa*, *J. foetidissima*, *J. oxycedrus* and *J. phoenicea*) all belong to the subfamily of Cupressoideae and are only united in having pith-containing parenchyma with thick cell walls. In contrast, the pine family is represented by the two subfamilies of Abietoideae (*Cedrus brevifolia* and *C. libani*) and Pinoideae (*Pinus brutia, P. nigra* subsp. *pallasiana* and *P. pinea*), but they all share a polygonally shaped pith. Additionally, the two Abietoideae wood species sampled both have thick-walled parenchyma present, unifying the subfamily. Thick-walled parenchyma is absent from the Pinoideae. Lastly, because *Taxus baccata* is the only species that was sampled of the family Taxaceae, no conclusions can be drawn due to the lack of other closely related woods.

As observed so far, pith characteristics are not necessarily conserved consistently in phylogenetic lineages, but there are some certain cell types or distributions found across conifers, such as thick-walled parenchyma. Crivellaro & Schweingruber (2013) describe thick-walled parenchyma in six of 11 species (*Cedrus brevifolia*, *C. libani*, *Cupressus sempervirens*, *Juniperus excelsa*, *J. oxycedrus* and *J. phoenicea*). A similar cell type may have been noted by Doyle & Doyle (1948) because four of 13 species (*Cryptomeria japonica*, *C. japonica* var. *elegans*, *Glyptostrobus pensilis* and *Taxodium ascendens*) have thick-walled cells in addition to sclereids. Additionally, *Glyptostrobus pensilis* is described with branching sclereids, which, based on the accompanying illustration, could refer to astral sclereids.

**Comparisons to Fossil Pith of Progymnosperms and Conifers**

Although pith has been relatively little studied among living conifer species, there are a number of anatomical descriptions of pith in progymnosperms and conifers from the fossil record. The oldest, anatomically preserved piths in both herbaceous and woody plants come from the Devonian (e.g., Beck 1960; Xu et al. 2017; Tanrattana et al. 2019; Tomescu & McQueen 2022). In general, fossil piths vary in their quality of preservation, but those with the best cellular preservation are silicified. For the following overview of studies on fossil pith, we searched for as many descriptions of pith in progymnosperms and conifers in the paleobotanical record as we could find, especially in the conifer families Araucariaceae and Podocarpaceae. This survey will likely prove incomplete, however, because pith descriptions are difficult to locate, as they are usually very brief and mentioned within a more comprehensive work on fossil stems and wood. Hence, pith descriptions do not often show up in titles, abstracts, or keywords.

Among the studies that we consulted on fossil pith, a few key common characteristics stand out. Twelve taxa described in ten studies are parenchymatous (Walton 1927; Beck 1960; Rothwell 1982; Meyer-Berthaud & Taylor 1991; Tidwell & Medlyn 1992; Decombeix et al. 2005; Cornet et al. 2012; Feng 2012; Feng et al. 2012; Faria et al. 2018), while 13 taxa in 11 studies have mixed cell types (Galtier et al. 1992; Ohsawa et al. 1995; Rothwell et al. 2005; Gnaedinger 2007; Jiang et al. 2012; Falcon-Lang et al. 2014; Shi et al. 2017; Wan et al. 2017; Faria et al. 2018; Tanrattana et al. 2019; Santos et al. 2021). Of those piths composed of only parenchyma, three taxa (*Cedroxyylon greenlandicum*, *Behuninia*, and *Lebachia*) contain a few cells with thickened cell walls (Walton 1927; Rothwell 1982; Tidwell & Medlyn 1992), which resemble...
the “thick-walled cells” described by Crivellaro & Schweingruber (2013), as well as in the present study in *Nageia nagi* (Fig. 9C, D), *Podocarpus latifolius* (Fig. 10C, D), and *P. nerifolius* (Fig. 11E).

Most fossil piths described with multiple cell types contain sclerenchymatous cells (e.g., *Giblingodendron* sp.; Falcon-Lang et al. 2014) which are often scattered throughout the pith (Galtier et al. 1992; Gnaedinger 2007; Jiang et al. 2012; Falcon-Lang et al. 2014). These patterns of sclerenchyma distribution resemble those found in *Agathis australis* and *A. robusta* (Figs 2 and 4), *Araucaria araucana* and *A. muelleri* (Figs 5D–F and 7A–C), and *Podocarpus gracilior* (Fig. 10 A, B) described here. Similarly, clusters or dense bundles of sclereids appear in five fossil taxa (*Atlanticoxyylon ibiratinum*, *Hanskerpia hamiltonensis*, *Macdonaldodendron giganticus*, *Callixylon wendtii*, *Agathoxylon santanensis*) (Rothwell et al. 2005; Falcon-Lang et al. 2014; Faria et al. 2018; Tanrattana et al. 2019; Santos et al. 2021) and also occur in the extant species of *Agathis australis*, *Agathis robusta*, *Araucaria angustifolia*, and *Araucaria muelleri* (Figs 2, 4, 5D–F and 7A–C). However, it should be noted that the features present in conifer pith, whether fossil or recent, are not exclusive to conifers, but can occur in other groups of woody plants. Abundant sclerenchyma in the pith, such as observed in extant *Araucaria muelleri* (Fig. 7A–C), for instance, can be found in the pith of extinct seed ferns and in at least one progymnosperm (e.g., Tanrattana et al. 2019). Thus, some seemingly characteristic pith features may be plesiomorphic and not taxonomically diagnostic.

Another recurring characteristic described or identified through images is what our study refers to as stellate pulls (see Fig. 1). Variously referred to as lobes, wedges of primary xylem, or woody wedges, this feature appears in at least four fossil taxa (*Abietopitys* sp., *Cedroxylon greelandicum*, *Medulloprotaxodioxylon triassicum*, *Plyophyllioxylon hulstaiense*), but may appear unidentifiable in photos (Walton 1927; Feng 2012; Jiang et al. 2012; Wan et al. 2017; Faria et al. 2018).

From a taxonomic viewpoint, there are only a few studies of pith in fossil woods attributed specifically to the Araucariaceae and Podocarpaceae. One well-preserved araucariaceous fossil pith with clusters of sclerenchyma was described in *Agathoxylon santanensis* (Santos et al. 2021). Preserved pith has also been noted as occurring in the wood of *Agathoxylon malaminbandense* (Gnaedinger & Herbst 2009; Crisafulli & Herbst 2011), but neither an anatomical description nor an image was available to characterize the pith. Fossil pith in a small-diameter branch trace in a woody axis of *Agathoxylon cozzoi* was described as parenchymatous (Gnaedinger & Zavattieri 2020), but it is unclear if this branch had reached a maturity of three years, as was recommended by Doyle & Doyle (1948) for pith studies.

Fossil woods pertaining to Podocarpaceae that were formerly assigned to the genus *Circoporoxylon* but show preserved pith and primary features are now assigned to a specially established genus, *Circoporopitys*. Two species of *Circoporopitys*, *C. argentinum* and *C. shanense*, have both been described with heterogeneous pith of parenchyma and sclerenchyma (Gnaedinger 2007).

A more extensive look at pith anatomy in individual fossil specimens in Araucariaceae and Podocarpaceae could better discern whether anatomical pith patterns are correlated with taxonomy or perhaps show evolutionary changes through time. Another approach to understanding the evolution of pith is to analyze xylem ontogeny in well-preserved fossil plants. In an Early Devonian land plant, for example, serial sections in a permineralized, 400-million-year-old early land plant were recently studied to show the earliest stages of pith development in terrestrial plants, offering a window into pith evolution (Tomescu & McQueen 2022).

In any case, in addition to more in-depth research on fossil pith, future work on extant conifer pith should concentrate on the sampling and anatomical description of taxa beyond the total of 40 species studied here and in previous work to further our understanding of the biological affinities of pith anatomy.

**Conclusions**

The 16 species of Araucariaceae and Podocarpaceae studied here differ widely in pith shape, cell types, the abundance and distribution of cell types, density of cells, uniformity in cell size and shape, colored cell contents
in unstained cells, distribution of stain, and the transition into xylem at the pith periphery. The four examples of branches sampled from the same tree more than once show variation in accordance with age, and suggest that pith maturity is an important factor in anatomical descriptions. Not all genera or families exhibit conserved characteristics in pith anatomy, although generalizations can be made for some genera, for example, that *Araucaria* has circular to near-circular pith shape or that the Podocarpaceae commonly show secondary cracks in their pith. The 16 new descriptions of conifer pith provided here supplement the few, readily accessible studies describing pith (Shimakura 1937; Doyle & Doyle 1948; Crivellaro & Schweingruber 2013), bringing the total number of conifer taxa to 40, and offering a much broader basis for understanding the evolutionary trends in pith anatomy and function through the plant kingdom.

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**References**


Pith of Araucariaceae and Podocarpaceae


