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Tyloses in fossil plants: New data from a Mississippian tree, with a review of previous records

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Abstract: Tyloses are protoplasmic swellings of parenchyma cells into the lumen of adjacent conducting cells. They develop as part of the heartwood formation process, or in response to embolism or pathogen infection. Here, we report the oldest fossil evidence to date of tylosis formation that occurs in permineralized wood of the (pro)gymnosperm *Dameria hueberi* from the Tournaisian (lower Mississippian, ca. 350 Ma) of Australia. Different developmental stages of tylosis formation are recognizable that range from small bubble-like protrusions to dense tyloses entirely filling the lumen of the conducting cells. The trigger for the development of tyloses in *D. hueberi* remains unknown. A survey of the fossil record of tyloses shows their occurrence in most groups of vascular plants since the Carboniferous. Future research in this field will screen even older (Devonian) fossils for evidence of tyloses and aim to understand the roles these structures have had in plant-pathogen interactions and plant hydraulic properties in the past.

Keywords: paleobotany; Carboniferous; plant; anatomy; embolism; xylem; plant-pathogen interactions

Introduction

Petrifaction and permineralization are two modes of fossil preservation in which plant tissues and cells can be faithfully preserved, sometimes down to the finest cellular or even subcellular details (e.g., Schopf 1975). Such fossils offer rare glimpses of the internal organization of extinct plants, and can yield information that can be used to better understand their physiology and date the appearance of significant functional traits. When looking at well-preserved

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permineralized wood, one important structural detail that is relatively easy to spot is tyloses, which are protoplasmic swellings formed by a parenchyma cell into the lumen of an adjacent conducting cell (Molisch 1889; Esau 1965). Tyloses extend into the conducting cell through a pit, and some eventually occlude the entire lumen of the conducting cell (Fig.1). Moreover, deposition of lignin, suberin, and/or phenolic compounds can make the tylosis wall entirely impermeable (Rioux et al. 1995); in rare cases, there may also be a significant secondary thickening of the walls (sclerotic tyloses, IAWA Committee 1989; De Micco et al. 2016). The presence of tyloses in vessels of extant angiosperms was noted in some of the earliest studies of plant anatomy, with one of the oldest mentions and illustrations of these structures by Marcello Malpighi in 1686 (his Figs 21 & 23). The first detailed description of tylosis formation and accurate interpretation of their nature can be traced back to an anonymous paper from 1845, likely authored by Ottone Hermine Schuh, née Freiin von Reichenbach (for details, refer to Zimmerman 1979; Hillbrand-Grill 1998). This author noted that tyloses are typically formed in vessels filled with air.

[Insert Fig. 1 near here].

While tylosis formation has primarily been studied in angiosperm secondary xylem vessels (Zürcher et al. 1985; Bonsen 1991), it appears to be a widespread phenomenon among vascular plants, occurring both in vessels and tracheids, in primary and secondary xylem. Tyloses have been reported in conifer tracheids (Raatz 1892; Chrysler 1908; Gerry 1914), in the vessels of Gnetales (e.g., Carlquist 1996), and in the primary xylem of certain monocotyledons, such as bamboos (e.g., Weiner and Liese 1997) and bananas (VanderMolen et al. 1987). They also occur in ferns, either in conducting cells (Holden 1925; Bierhorst 1960; Miller and Duckett 1979) or within the degraded remnants of the protoxylem ("cavity parenchyma", McNicol, 1908; Holden 1925). Structures comparable to tyloses have also been described in the carinal canals of certain sphenophytes (Holden 1925 and references therein; Schweingruber and Börner 2018).

Tyloses are produced either as part of natural heartwood formation process, or in response to external stimuli, such as wounding or infection in the conductive part of the wood (Gerry 1914; Klein 1923; Chattaway 1949; De Micco et al. 2016 and references therein). While the exact triggers leading to tylosis formation are still debated (e.g. Rioux et al. 1998; Sun et al. 2007), it is clear that these structures play a major role in the ability of the plants to compartmentalize the vascular system. Tyloses are effective in sealing off non-functional conducting cells that are air-filled due to wounding or embolism, and in slowing down or preventing the progression of pathogens through the vascular tissues, and are a key element in the compartmentalization of decay in trees (CODIT; Shigo 1984; see also Morris et al. 2016; Kashyap et al. 2021). As a result, tyloses are instrumental for the survival of vascular plants, and hence investigating these structures in the fossil record can yield critical information in regard to the evolution of (1) plant defense strategies against pathogens, and (2) the hydraulic system, especially strategies to render embolized conducting cells harmless.

Here, we present evidence of tyloses in permineralized wood of the arborescent (pro)gymnosperm *Dameria hueberi* from the Tournaisian (Mississippian; Carboniferous) of Australia. *Dameria hueberi* predates the previously oldest record of tyloses (Scheckler and Galtier 2003) by some 15 million years. Fossil preservation makes it even possible to distinguish developmental stages in tylosis formation in *D. hueberi*. The new data is placed in a broader context by surveying the fossil record of tyloses. We compile the information available for the different lineages of vascular plants, thereby focusing on the oldest records and the occurrence of tyloses in major extinct lineages of plants. Finally, we outline future research directions and highlight the importance of documenting tyloses in fossil plants to advance our understanding of plant paleophysiology.

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Material & methods

Fossil material

Tyloses were observed in two specimens of *Dameria hueberi* from Queensland, Australia. They are part of a group of eight silicified wood specimens collected in 2005 and 2008 in the Teddy Mountain Formation (Bundock Creek Group), at Montgomerry Dam (S 19° 26′ 06.8″; E 144° 38′), from a plant-bearing interval that is middle Tournaisian in age (lower *crenulata* conodont Zone; Mawson and Talent 1997, 1999; ca. 350 Ma). Details of the locality, stratigraphic context, wood anatomy, and possible affinities of *D. hueberi* are provided by Decombeix et al. (2011). The woods are heavily silicified and were, therefore, prepared as thin-sections (Haas and Rowe 1999), rather than acetate peels, in the transverse, tangential, and radial planes. In the absence of reproductive structures, it is not possible to determine whether *D. hueberi* was a progymnosperm or a seed plant (Decombeix et al. 2011), and it will, therefore, henceforth be referred to as a (pro)gymnosperm. The specimens and slides are deposited in the Collections de Paléobotanique, Université de Montpellier, France, under specimen numbers MOD2 and MOD5.

Additional fossil material illustrated in this paper includes specimens of the Pennsylvanian early ferns *Ankyropteris* (Tedelaceae) and *Zygopteris/Etapteris* (Zygopteridales) in which tyloses were first illustrated by Phillips and Galtier (2005, 2011), as well as one specimen of the progymnosperm *Protopitys buchiana* from the Viséan of France, in which tyloses were first illustrated by Scheckler and Galtier (2003). Specimen numbers and repository information are given in the Data Availability section.

Imaging and measurements.

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Digital images were captured using a Keyence VHX 7000 electronic microscope and associated software (Figs 2A-E, 3A, 3B) and a Sony XCD-U100CR camera attached to an Olympus BX51 compound microscope using the image capture software Archimed (Microvision Instruments) (Figs 2F-G, 3C-F; Fig 6). Images in Fig. 5 were obtained from scans of original photographs taken by J. Galtier. Composite figures were prepared with Adobe Photoshop CS5 version 12.0 (Adobe Systems Inc.). Measurements were taken using ImageJ (Schneider et al 2012).

Nomenclature.

Throughout the text, the name and absolute ages of geological divisions follow the 2022/02 ICS International Chronostratigraphic Chart (Cohen et al. 2022). The higher taxonomic divisions for extant and fossil plants (i.e., Sphenophytes, Equisetales) follow Taylor et al. (2009).

Results – Tyloses in *Dameria hueberi*

The eight specimens of *Dameria hueberi* are all pieces of silicified wood with no associated primary tissues preserved. In transverse section, they do not show any curvature of the rays and radial rows of tracheids, and therefore likely represent the outer part of the wood of large axes (i.e., trunks or large branches). None of the specimens, not even the largest one which is 8 cm thick (radial dimension in cross section), show any evidence of growth ring boundaries or local changes in tracheid diameter (Figs 2A, 2B). The wood of *D. hueberi* is composed of tracheids with mean diameters ranging from 40 to 65 µm (maximum 96 µm), and numerous low, uniseriate to partly biseriate parenchymatous rays (Fig. 2, Fig. 3). Radial pitting of the tracheids is mostly uniseriate. Radial pits are circular (Fig. 3F) and show circular apertures whose mean diameter is 5.5–6.5 µm. Cross-field pitting (i.e. ray-tracheid contact) consists of 1–5, typically 2, circular pits that are somewhat smaller than the radial pits and possess an oval to circular aperture, about 4 to 5 µm wide (Fig. 3G)

Tyloses have been detected in specimens MOD2 and MOD5. The smaller of the two, MOD2, is 10 cm long and measures 3.7 (radial diameter) x 2.5 cm (tangential diameter). It contains only small areas of well-preserved wood, which show a few tyloses. On the other hand, MOD5 is 6.3 cm long and measures 6.5 (radial diameter) x 10.3 cm (tangential diameter). It contains many well-preserved areas, some with abundant tyloses (Fig.2C), and others devoid of them (Fig.2B). The remaining six *D. hueberi* specimens, one of which is about three times larger than MOD5, either lack tyloses or are too poorly preserved to reveal these structures.

A typical transverse section of the wood of MOD5 shows areas where tracheids with a dark brown content tend to occur in several nearby radial files (Fig.2C). These areas have no conspicuous pattern of distribution within the wood. At higher magnification, the lumina of many of the tracheids containing brown matter provide evidence of thin cell walls corresponding to tyloses (Fig.2D-G). Most of the tracheids are in fact completely filled with tyloses. In this case the tyloses are crammed together and may have polygonal outlines (Fig.2D-F). Some tyloses show a darker, possibly thicker wall (Fig.2F). There are also examples of earlier stages of tylosis formation, showing clearly one or several small, rounded swellings inside the tracheid lumen (a, b in Fig.2G). Tangential sections of the wood show that rays are abundant, and each tracheid is in contact with a sufficiently large number of parenchyma cells that all could serve as a source for the tyloses (Fig.3A). Tyloses with thicker walls and in different stages of development are also visible in these sections; they range from small swellings to prominent inflations that completely fill the tracheid lumen (Fig.3B-E). Radial sections show circular pits in uni- to biseriate arrangement that are characterized by a rounded aperture typical of *D. hueberi*, as well as some evidence of tyloses filling tracheid lumina (Fig.3F). No evidence has been found of fungal hyphae in the wood.

[Insert Fig. 2 and 3 near here].

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Discussion

Significance of tyloses in Dameria hueberi

The two specimens of *Dameria hueberi* wood containing tyloses from the Tournaisian of Australia represent the oldest evidence to date of these structures in plant fossils. They are approximately 15 million years older than the heretofore oldest fossil tyloses described by Scheckler and Galtier (2003) in *Protopitys buchiana* from the upper Viséan of France. Preservation of the *D. hueberi* wood is not excellent, but still allows a full documentation of the tyloses in all spatial planes (tangential, radial, and transverse), and even provides glimpses of the development of these structures in the form of distinct growth stages. A late stage of development is characterized by tightly packed clusters of small tyloses that completely fill the lumen of the tracheids, suggesting that the tyloses may have undergone divisions (Schmitt and Liese 1994). In addition, the dark outline of some of the tyloses suggests that substances have been added into or onto their walls. Division and deposition of materials, such as lignin or suberin, rendering the tylosis wall impermeable are active processes that occur during tylosis formation in many extant taxa (De Micco et al. 2016). It is not clear whether the brown coloration of the lumen of some of the *D. hueberi* tracheids filled with tyloses is an optical effect caused by superimposed layers of cell walls, or resulted from the production of gel or gum by *D. hueberi*. The latter is known to co-occur with tylosis formation in many extant plants (Rioux et al. 1998).

Tyloses arise from parenchyma cells and expand into the neighboring conducting cells through the pits. In *Dameria hueberi*, each tracheid is in contact with a large number of ray parenchyma cells that could have produced the tyloses, but the apertures of the cross-field pits that constitute the ray-tracheid contacts is small (on average only 4–5µm). This aperture size is, however, still above the threshold of 3 µm established by Bonsen and Kučera (1990) for the formation of tyloses in extant woods.

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Tyloses in *Dameria hueberi* are not evenly distributed throughout the wood, nor do they occur in a characteristic distribution pattern, at least there is none recognizable from the material at hand. They are located in the outer part of the wood, where they appear in irregular patches. Thus, the production of these structures in *D. hueberi* is not comparable to the centrifugal pattern of heartwood formation seen in extant plants. Tylosis formation can also be linked to environmental conditions. The climate of Queensland during the Tournaisian has been reconstructed as relatively warm, tropical to temperate, and relatively wet (e.g. Boucot et al. 2013). All specimens of *D. hueberi*, including the larger ones, are devoid of growth rings or conspicuous false rings, which suggests that the source trees have grown in favorable conditions with no marked seasonality or major periodic ecological disturbances. In addition, the tyloses in *D. hueberi* are not distributed in tangential layers as could be expected if they were produced in response to punctual or temporary environmental stresses. They cannot, therefore, be linked to environmental fluctuations. It is noteworthy in this context that a contemporaneous assemblage of anatomically preserved plants from another north-eastern Queensland locality reported by Galtier and Hueber (2001), Decombeix et al. (2011), and Laloux and Decombeix (accepted) does not contain any specimens displaying tylosis formation. Another possible causative agent of tylosis formation are microbial pathogens. However, we did not find any evidence of fungi or other microbes in *D. hueberi* specimens with tyloses. The preceding considerations render it impossible presently to advance a relevant hypothesis on the cause of tylosis formation in *D. hueberi*.

Previous records of tyloses in fossil plants

The new evidence presented in this study adds to the inventory of fossil plants known to produce tyloses, and expands the geological range of this structural element of the water-conducting system of vascular plants by some 15 million years. The earliest descriptions of tyloses in fossil

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plants come from the second half of the 19th century, when paleobotanists first looked at the anatomy of permineralized plants (e.g., Williamson 1877, 1888). Since that time, tyloses have been reported in fossil representatives of most groups of vascular plants (Fig 4), with the notable exception of lycophytes. There is one mention of cortical cells with septa that "resemble cells with tyloses" in the Pennsylvanian lycopsid genus *Chaloneria* (Pigg and Rothwell 1983, p. 139), but we are not aware of any report of tyloses in conducting cells.

[Insert Fig. 4 near here].

Ferns and early fern-like plants

The oldest evidence of tylosis formation in ferns occurs in Pennsylvanian (=late Carboniferous) taxa belonging to the filicalean ferns and the Zygopteridales. Williamson (1888, p. 315) described "*cells apparently identical with the thylosis found in some living plants*" in the petiole of *Rachiopteris corrugata*. This species, today named *Ankyropteris corrugata*, belongs to the Tedelaceae, which is an extinct family of filicalean ferns (Eggert and Taylor 1966). Tyloses in *A. corrugata* were later also illustrated by other authors, including Weiss (1906), Holden (1930), and more recently Phillips and Galtier (2011). In Early to Middle Pennsylvanian specimens, abundant tyloses can indeed be seen in the metaxylem of the stem (Fig. 5A), as well as in the phyllophore/petiole both in its basalmost part still connected to the stem (Fig. 5B) and in more distal regions (Fig. 5C). Tyloses also occur in large primary xylem tracheids of the stems, petioles, and roots of another species, *Ankyropteris brongniartii* (Mickle 1980; Phillips and Galtier 2011), which is characterized by a climbing stem. In this species, small parenchyma cells are widely networked among the large metaxylem tracheids, which permits tylosis formation in entire xylary sectors.

The Zygopteridales are an extinct order of late Paleozoic ferns that possessed rhizomatous stems with a succulent cortex from which extended erect phyllophores/petioles of the *Etapteris* type in spiral arrangement (Phillips and Galtier 2005). Some of these early ferns produced small amounts of wood, and tyloses occurred in both the primary and secondary xylem (Phillips and Galtier 2005) of stems, petioles, and roots (Fig. 5D-F).

Tyloses have also been reported in fossil representatives of extant fern families. They are, for instance, abundant in the metaxylem tracheids of *Psaronius* sp. (Marattiales) from the Early Pennsylvanian of Lewis Creek, USA (Smoot and Taylor 1981). Among geologically younger taxa, tyloses have been reported in fossil specimens of the genus *Gleichenia* (Gleicheniaceae) from the Eocene of Canada (Mindell et al. 2006).

Tyloses-like contents have also been reported in non-vascular tissues of several fossil ferns, including cortical cells of *Botryopteris multifolia* (Botryopteridaceae,Filicales; He et al. 2020) and *Tiania yunnanense* (Osmundales; Wang et al. 2014) from the Permian of China. In the leaf traces of *Knorripteris taylorii* (Galtier et al. 2018), a probable fern of Triassic age with unresolved affinity, tylose-like structures occur outside the vascular strand; however, the exact nature and mode of formation of these elements remain uncertain.

[Insert Fig. 5 near here].

Sphenophytes

The presence of tyloses has also been reported in several fossil members of the Sphenophyta, which is the group that also includes the extant horsetails (genus *Equisetum*). In fossil sphenophytes, tyloses typically do not occur in conducting cells, but rather are present in the carinal canals, a situation similar to that seen in extant *Equisetum* (Holden 1925). This makes sense because the carinal canals are lacunae that result from the breakdown of the protoxylem and are believed to be effective in water conduction (Leroux et al. 2011). Fossil examples of tyloses in carinal canals include a specimen of *Archaeocalamites* described by Walton (1949) from the Viséan (Scott 1990) of Arran, Scotland, and *Arthropitys bistriata*, a structurally

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preserved calamite from the lower Permian of Chemnitz, Germany (Rößler and Noll 2010, 2012; Rößler et al. 2012). Interestingly, unlike extant *Equisetum*, many Paleozoic sphenophytes produced secondary xylem. This tissue was typically rich in parenchyma (up to 50%), with very large and high rays (e.g., Renault 1893–1896; Cichan 1986a; Rößler et al. 2012), so tyloses could, in theory, have formed in the secondary xylem tracheids. Marguerier (1968) indeed reported the presence of tyloses in secondary xylem tracheids of *Calamodendron punctatum* (= *Calamitea striata*; see Rößler and Noll 2007) from the Upper Pennsylvanian of France. Sphenophyllales are an extinct group of sphenophytes that includes taxa with a peculiar wood anatomy in which all the tracheids are in close contact with a network of parenchyma cells (Cichan 1985). Some Pennsylvanian and Permian members of the genus *Sphenophyllum* are known to have had the largest and longest tracheids in plants (>100 µm in diameter and >25 mm long; Cichan 1985). Combined with numerous radial pits, these features of the tracheids suggest a high degree of conduction but also a high vulnerability to cavitation in case of water stress (Wilson 2013). To our knowledge, tyloses have hitherto not been reported in this group. However, our observation of one early Permian *Sphenophyllum* stem from Autun, France, suggests that tyloses may in fact have formed in these plants, but further investigation is needed to confirm this. We did not find evidence of tyloses in the more typical wood (i.e., smaller tracheids and “regular” rays) of *Sphenophyllum insigne* from the Mississippian of France and Germany (Terreaux de Félice et al. 2018), and of the UK (ALD pers. observation).

Lignophytes

The lignophytes is a clade that includes the extinct progymnosperms and all fossil and extant seed plants (Kenrick and Crane 1997). Tyloses have been documented in fossil lignophytes from the Carboniferous to the present. There are innumerable reports of tyloses in angiosperm and gymnosperm woods from the Mesozoic and Cenozoic, based on both permineralized (e.g.,

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Medlyn and Tidwell 1975; Wheeler and Manchester 2002; Wei et al. 2019; Franco et al. 2020; Oh et al. 2020) and anthracological specimens, including subfossils from archaeological sites (e.g., Dufraisse et al. 2018). Here, we will focus on the oldest documented evidence of tyloses in extant groups and their occurrence in key extinct groups.

Tyloses have been reported in some of the oldest known angiosperm woods dating back to the Cretaceous. This is, for instance, the case in *Carlquistoxylon*, a wood from the Lower Cretaceous of Argentina (Nunes et al. 2018), and in several North American and European Early to Late Cretaceous species of *Paraphyllanthoxylon*, a genus comprising the largest Mesozoic angiosperm trees known to date (e.g., Thayne et al. 1983; Gryc et al. 2009; Jud et al. 2018; Chin et al. 2019).

The oldest documented evidence of tyloses in fossil conifers comes from the Permian of China. Feng and collaborators (2013) have illustrated in detail different stages of tylosis formation in permineralized wood attributed to the genus *Shenoxylon*. To the extent of our knowledge, there are no reports of tyloses in fossil representatives of the other extant gymnosperm groups, i.e., ginkgophytes, cycadales, and gnetales.

Tyloses have also been reported in several extinct groups of seed plants. For example, possible tyloses occur in a Bennettitale from the Cretaceous of New Jersey (Chrysler 1932) and in at least one member of the Corystospermales, namely *Rhexoxylon tetrapteridoides* from the Triassic of South Africa (Walton, 1924). In the latter, Walton (p. 90) noted "*The very close resemblance of the* Rhexoxylon *tracheids, with their included and apparently once living cells, to tracheids with tyloses in certain fossil conifers*". There is, however, only one photograph that does not allow to fully appreciate the structures.

The presence of tyloses in Glossopteridales from the Permian of Antarctica has been suggested following the observation of "thin-walled balloon-shaped structures" inside the rays (Weaver et al. 1997, p. 661) or of tracheids with contents occluding the lumen (Harper et al. 2017), but, as far as we are aware of, there is no clear evidence as yet that these structures in fact represent tyloses. Tyloses have been reported in several woods assigned to the Cordaitales, the Carboniferous-Permian sister-group of the conifers. Recent examples include Pennsylvanian woods from Spain assigned to *Cordaixylon* (Césari et al. 2015) and *Dadoxylon* (Tosal et al. 2021), as well as a Permian cordaitalean root from China (Shi et al. 2020). Abundant tyloses have also been reported in the secondary xylem of *Medullosa olseniae* (Medullosale) from the Permian of Texas (Roberts and Barghoorn 1952). *Medullosa* is a Carboniferous-Permian genus of seed plants characterized by wide tracheids, often > 100 µm in diameter. In *M. olseniae*, the tyloses are "*one of the most conspicuous features in longitudinal sections of the secondary wood*" (Roberts and Barghoorn 1952, p.195). They are also possibly present in *M. noei* from the Pennsylvanian of Illinois (Steidmann 1944, fig. 4). The oldest evidences of tyloses in lignophytes comes from the Mississippian. They occur in woods from the upper Visean (ca. 335 Ma) of France, where tyloses have been described in detail in a specimen of *Protopitys buchiana* (Scheckler and Galtier 2003), an enigmatic taxon assigned to the progymnosperms (Decombeix et al. 2015). In this specimen (Fig. 6), tyloses are abundant in certain parts of the wood where they tend to be organized in a similar fashion as that seen in *Dameria hueberi*, namely in neighboring radial files.

[Insert Fig. 6 near here].

Physiological evidence and interpretation

Tyloses in fossil plants have often been mentioned in passing, out of curiosity, or as a diagnostic feature (especially in angiosperm woods), but rarely have these structures as fossils received much consideration with regard to the possible cause(s) of their formation. Biotic and abiotic stresses that have been shown to trigger tylosis formation in extant plants include freezing, drought, mechanical wounding, insect attack, and pathogen infection (De Micco et al. 2016).

In addition, tyloses may also form as part of the natural formation of heartwood, i.e., wood that "has ceased to contain living cells and in which the reserve materials have been removed or converted into heartwood substances" (IAWA 1964, p.32). Two principal causes have been invoked for the formation of tyloses in fossil plants, namely drought-induced embolism and pathogen infection.

The abundance of tyloses in the tracheids of the Pennsylvanian ferns *Zygopteris/Etapteris* and *Ankyropteris* has been interpreted as a result of drought-induced embolism (Phillips and Galtier 2005, 2011). Both genera are typically found in peat swamp assemblages, indicating growth in a habitat with abundant water, and have large tracheids of typically more than 100 µm (up to 250 µm in some species) wide (Fig. 5). This suggests that the tracheids had a high hydraulic conductivity, but also that they were prone to embolism in case of drier episodes. *Zygopteris* was characterized by succulent rhizomes interpreted as an adaptation to water stress (Phillips and Galtier 2005). *Ankyropteris corrugata*, also characterized by succulent rhizomes, additionally possessed an inner ground tissue in the petiole that may have functioned in water storage (Phillips and Galtier 2011). The plant was a scrambler or climber with a long, slender stem/petiole. Mechanical stresses, such as twisting or bending, of the long/slender stems could readily have led to temporary (partial or complete) interruptions or permanent breakage of the water column (Fisher and Ewers 1991). There is no evidence of fungal infection associated with the tyloses in these taxa. Likewise, the abundance of tyloses in the wood of the Permian conifer *Shenoxylon mirabile* was interpreted by Feng and collaborators (2013) as a response to certain environmental conditions. These authors described the tyloses as "obviously arranged in concentric zones" (p. 1078), but also with no direct correlation in terms of position relative to growth increments. They speculated that the tree was growing in an environment governed by frequent water stress based on the presence of false rings in the wood (Feng et al. 2013). Because there is no evidence of fungi, and the tyloses are found from the inner to the outer part

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of the stem, Feng and collaborators (2013) dismissed the possibility of tylosis formation in response to fungal infection or as part of the natural heartwood formation.

Scheckler and Galtier (2003) noted that, in *Protopitys buchiana* from the Mississippian, tyloses tend to be mostly present in the widest wood tracheids (up to 95 µm) and that they seem to have a distribution pattern that is somehow connected to changes in growth. Although a few fungal hyphae have been noted in the wood, the tyloses were nevertheless interpreted as having most likely formed in response to episodes of water stress and/or dormancy. This interpretation is supported by the presence of faint bands of tracheids with reduced radial diameter and depositional evidence of growth in a disturbed, volcanic area of the subtropical zone.

Tylosis formation in the fossil record has also been discussed in connection with fungal infection, based in part on observations of present-day plant responses to vascular wilts, which are among the most destructive diseases in annual crops and woody perennials (Mace et al. 1981; Yadeta and Thomma 2013). These diseases are caused by soil-borne bacteria, fungi, and oomycetes that enter the plant through the roots and infect the xylem where they proliferate and adversely affect the transportation of water and minerals. The presence of the pathogen activates defense mechanisms in the form of either physical responses, which hinder the pathogen from further spread, or chemical responses that inhibit or kill the pathogen (Beckman 1964; Talboys 1972; Agrios 2005). A common physical defense against vascular wilt pathogens is the formation of tyloses (Kashyap et al. 2021). Harper and collaborators (2012) have described a Jurassic permineralized conifer axis from Antarctica in which tylosis formation co-occurs with abundant fungal remains, suggesting that the tyloses served as mechanical barriers against the advancing hyphae. However, the fact that fungal hyphae are found within and around the tyloses, as well as in the rays and in the phloem, suggests that the fungus was able to surmount this barrier. A similar interpretation has been offered by Khan and collaborators (2018) for Plio-Pleistocene angiosperm wood from Tibet that also contains both tyloses and fungal remains.

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While tyloses are abundant, fungal remains are relatively scarce, and these authors interpreted this as representing an early stage of pathogen invasion. Like the Jurassic conifer, this angiosperm wood also yields evidence of hyphae penetrating or circumventing the tyloses, which indicates that the pathogen may have been able to evade them (Khan et al. 2018). It is indeed known in present-day plants that tyloses can impede the movement of fungi within the xylem, slow down the colonization process, and localize the infection, but also that many fungi can grow readily through these structures (Blanchette 1992; Schwarze and Baum 2000). Thus, tyloses perhaps represent a means for the plant to gain some time to deploy (i.e. synthesize and allocate) another, more effective chemical defense. One of the oldest documented fossils showing the co-occurrence of certain balloon-shaped structures interpreted as tyloses and fungal remains is a Permian cordaitalean root from China (Shi et al. 2020). However, persuasive evidence of parenchyma protruding into the tracheid lumina has not been produced, and thus renders the interpretation of the structures as tyloses doubtful.

Future avenues of research

When did plants first form tyloses, and why?

An interesting insight that has emerged from reviewing the fossil record of tyloses is that there is no documented evidence of these structures older than the Mississippian. However, all the necessary structural and physiological components for the development of tyloses were in place by the Devonian, at least in principle. Secondary xylem with parenchymatous rays is known to have existed since the Early Devonian (Gerrienne et al. 2011; Hoffman and Tomescu 2013), and primary xylem, possibly in contact with parenchyma cells, has also been described in a number of taxa since the evolution of the first tracheids in the Early Devonian (e;g., Edwards 2003; Decombeix et al. 2019 and references therein). Similarly, the types of external stimuli that are known to trigger tylosis formation in plants today could all have affected plant growth also in the Devonian, including intermittent periods of drought, mechanical injuries, and pathogen infection. This raises the question as to why there is no evidence as yet of tyloses in Devonian plants?

From a physiological point of view, it is possible that the mechanisms underlying the production of tyloses simply had not yet evolved. In addition, while tylosis formation is often regarded as a result of the loss of water pressure in vessels, and is characteristically found in vessels that are filled with air (e.g. Beckman 1964), there is also evidence of a hormonal trigger (ethylene, see Sun et al. 2007; jasmonates and ethylene, see Leśniewska et al. 2017), which might have been absent in Devonian plants. From an anatomical point of view, it is possible that tylosis formation in some Devonian plants was limited by the small number of parenchyma cells that actually were in direct contact with the water-conducting cells. There is evidence that some Devonian plants had only small amounts of parenchyma in their secondary xylem that occurred in the form of small and/or low-density rays. This appears to have been the case in, for instance, some of the oldest known woody plants (Gerrienne et al. 2011; Hoffman and Tomescu 2013). However, there are also several taxa with abundant and/or large rays, including some of the Middle to Late Devonian archaeopteridalean progymnosperms (Orlova and Iurina 2011). Finally, as so often in paleontology, it is important to take into account the possible existence of a preservation and/or preparation bias. The number of anatomically preserved Devonian plants is small and their modes of preservation and preparation are not always favorable to the recognizability of tyloses. For example, the pyritized Late Devonian aneurophytalean progymnosperms that we analysed for this study have been prepared as thick wafers, which need to be examined in reflected light. Reflected light, however, limits the possibility to observe the content of the conducting cells. Likewise, acetate peels of stems (for details, refer to Galtier and Phillips 1999) generally are not conducive to the study of tyloses, although it is possible in some instances to see them, as illustrated in Fig.5. Taylor et al. (2011) explained that thicker thin-sections often allow for a more accurate examination and documentation of three-dimensional objects in fossil cells than acetate peels. This also holds good for tyloses in conducting cells. As to whether the lack of tyloses in Devonian woods is a preservation or preparation/documentation artifact, or a biological fact remains to be resolved. Studies of anatomically preserved vascular plants from that period should, therefore, screen carefully also for tyloses whenever possible.

Tyloses and fungi

Plants today employ a wide range of different mechanical and chemical defenses (e.g., tyloses, resins, induced antimicrobial compounds) to combat microorganisms, such as bacteria, oomycetes, and fungi, that invade the vascular system (e.g., Pearce 1996). Fungi frequently occur in the vicinity of anatomical alterations in the wood of present-day plants, and it was likely just the same in the past. However, such co-occurrences have rarely been documented and critically evaluated in the fossil record (Harper et al. 2012; Feng et al. 2013; Boura et al. 2013; Shivanna et al. 2017; Greppi et al. 2018; Khan et al. 2018; Shi et al. 2020), in part because it is generally difficult to determine the nature of the interrelationship, if any, that existed between an anatomical alteration and a co-occurring fungus based on fossils, which do not permit experiments and long-term observations (Harper and Krings 2021). It is, therefore, a challenging task to establish whether tylosis formation in fossils was triggered by the presence of a microbial pathogen, such as a fungus, or developed due to any other cause that had nothing to do with the presence of the pathogen. The few studies that describe direct contacts between fungi and tyloses in fossil wood (Harper et al. 2012; Khan et al. 2018) all hypothesize that the tyloses were a host response that formed a physical barrier to prevent the fungus from spreading. Other studies present fossil examples of fungi and tyloses that co-occur in a wood, but are not in direct contact. These patterns of spatial distribution have been variously regarded as tylosis

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formation probably caused by the fungi (Shivanna et al. 2017; Shi et al. 2020), a relationship that cannot be determined (Boura et al. 2013; Greppi et al. 2018), or the authors state that it is unlikely that the fungi caused tylosis formation (Feng et al. 2013). It should be noted that, while tyloses may form in the absence of fungi or other pathogenic causative agents, they still can later serve as a defense. Thus, even if a fungus and tyloses co-occur in direct contact in a fossil wood, the formation of the tyloses does not necessarily represent a host response to the presence of this fungus.

Taxonomic identification of the fungi that occur in wood fossils with tyloses provide the best chance to infer nutritional mode(s), and hence assess as to whether these fungi could have indeed induced tylosis formation. Knowing the affinities of the fungi associated with fossil plants yields a framework for direct comparisons with present-day relatives, and thus can provide insights into the nature of the interactions between the fungi and their host plants. For example, one of the causative agents of 'vascular wilt' disease today, *Fusarium oxysporum,* induces tyloses in host trees to prevent vertical spread of the pathogen (Yadeta and Thomma 2013; Kashyap et al. 2021). To restrict horizontal movement of *F. oxysporum*, reinforcement via a conspicuously thick coating occurs at the vessel walls, parenchyma cells and pit membranes to impede pathogen growth (Araujo et al. 2014). Morphological characteristics of *F. oxysporum* include oval to kidney-shaped microconidia, sickle-shaped macroconidia, false head formation of microconidia, and terminal chlamydospores (Fourie et al. 2011); some of these reproductive structures (conidia) can be produced within vessels (e.g., Phipps and Stipes 1976). Although it is unlikely that all of these characters of a fungus and infected host plant are found within a single fossil specimen, many of the features, collectively, can help to provide a context regarding the pathogen: Is there evidence of the formation of multiple tyloses in a localized area of the wood that also contains the fungus? In addition, is there evidence of swellings or ergastic substances in the parenchymatous cells or pits? Are there any diagnostic features of the vegetative hyphae, such as septa, clamp connections, sterile projections? Although rare, is there any evidence of fungal reproductive structures, e.g., spores, conidia, spore-bearing and associated structures (e.g., phialides, sterigmata)? Knowing the fungus can help to discriminate pathogenic fungi, such as *Fusarium* spp., which colonized the vascular tissues during the lifetime of the plant, from other, saprotrophic fungi that used the conducting cells as convenient pathways into a dead plant part lying on the forest floor, or within the soil (Boddy 2016). If fungi do not provide information on their reproductive biology that can be used for identification (which is common in fossil wood), other methodologies need to be employed. For example, geochemical techniques that can be directly applied to fossil fungal mycelia (Speranza et al. 2015) or to pieces of fossil wood to trace biochemical transformation processes during wood decomposition (Bechtel et al. 2007).

Tyloses and the hydraulic properties of fossil plants.

Despite having the same xylem cell types as present-day gymnosperms (i.e. tracheids, and ray or axial parenchyma), extinct vascular plants, especially those that thrived during the Paleozoic, had a higher disparity of secondary xylem anatomies (Decombeix et al. 2019 and references therein). This includes taxa with tracheid diameters of well over 100 µm and/or abundant parenchyma, in the form of dense, large (> 4 cells) and/or high (> 100 cells) rays. The fact that these different anatomies lead to a diversity of hydraulic properties is supported by studies that have extrapolated the hydraulic conductivity of fossil plants from the properties of isolated tracheids (i.e., diameter, length, and pitting; Cichan 1986b; Wilson 2013, 2016; Cascales-Miñana et al. 2019). These studies also show that, in certain taxa, the tracheids were the hydraulic equivalent of angiosperm vessels and had a low hydraulic safety, i.e. a high risk of embolism in the event of factual or physiological drought. For example, several members of the Medullosales and Sphenophyllales had slender stems and (very) large tracheids. Tyloses

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are, therefore, one of the reasons why future studies should take into account the parenchymatous portion of the secondary xylem when discussing the hydraulic properties of fossil woods. In addition to being not conducting, and thus changing the properties at the scale of the tissue (Tanrattana et al. 2019), parenchyma could be important for its role in mitigating embolism in taxa with "high-risk" strategies via the formation of tyloses. Interestingly, the examples of the early ferns *Zygopteris* or *Ankyropteris* show that tyloses may have played an important role also in the evolution of high-risk hydraulic strategies in extinct plants with no or little secondary xylem. Such taxa have not been included in previous hydraulic studies, which typically focus on early vascular plants, such as *Psilophyton*, and *Asteroxylon*, and/or plants with secondary growth (Cichan 1986b; Strullu-Derrien et al. 2014; Wilson 2016 and references therein; Cascales-Miñana et al. 2019). Finally, the presence of tyloses is a reminder that the conducting cells in a plant are not always all functional, which is also important when extrapolating hydraulic properties to a whole stem. For example, more than 50% of the tracheids shown in Fig. 2E are occluded by tyloses. To more accurately understand the role of tyloses in the physiology of extinct plants, we need to determine the relationship between the presence of tyloses and the risk of embolism. This relationship can be estimated by using reconstructed paleo-environmental conditions and anatomical characters such as tracheid diameter.

Conclusions

The wood of the (pro)gymnosperm tree *Dameria hueberi* from the Tournaisian of Australia contains well-preserved tyloses showing different stages of development. Combined with previous reports, this new data demonstrates that tyloses have been produced in several groups of vascular plants since at least the early Mississippian, approximately 350 million years ago. This suggests that tyloses may have played important roles in the early evolution of arborescence and in the development of the specific properties and requirements of arborescent plants with regard to conditions of stress and xylem-invading pathogens. Future research will focus on investigating even older occurrences yielding suitable plant fossils in search for evidence of tyloses, and on assessing more precisely how these structures have affected plant hydraulic properties and pathogen interactions in the fossil record.

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ALD: conceptualization; investigation; visualization; writing – original draft, review and editing. CJH: investigation; visualization; writing – review and editing. JG: investigation;

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visualization; writing – review and editing. BMB: investigation; writing – review and editing. MK: investigation; writing – review and editing.

Data availability

Digital data: full resolution original files of the images used in Figs 2 and 3 are available open-access in Figshare at https://doi.org/10.6084/m9.figshare.c.5869925.

Non-digital data: consists of thin-sections and mounted acetate peels of fossil plants. For *Dameria hueberi* the specimens and slides are deposited in the Collections de Paléobotanique, Université de Montpellier, under specimen numbers MOD2 and MOD5. For *Zygopteris/Etapteris* the material is deposited in the Collections of the University of Illinois, Urbana-Champaign, under specimen numbers UI 3457 and UI 22655. For *Ankyropteris corrugata* two specimens are illustrated: one from Bouxharmont, Belgium, collections of the University of Liège, specimen number BX 336, and one from Burnley, England, Collections de Paléobotanique, Université de Montpellier, specimen number B 309. For *Protopitys buchiana* the material is deposited in the Collections de Paléobotanique, Université de Montpellier, under specimen number BOU 1500.

Slide/peel numbers and repositories for all illustrated specimens are available at https://doi.org/10.6084/m9.figshare.c.5869925.

REFERENCES

Agrios GN. 2005. Plant Pathology. Burlington, MA: Elsevier Academic Press.

Anonymous (Schuh OH, née Freiin von Reichenbach). 1845. Untersuchungen über die zellenartigen Ausfüllungen der Gefässe [Studies on the cell-like fillings of the vessels].

The version of record of this manuscript has been published and is available in *Botany Letters*, 2022, https://www.tandfonline.com, https://doi.org/10.1080/23818107.2022.2099461

Araujo L, Bispo WM, Cacique IS, Moreira WR, Rodrigues FÁ. 2014. Resistance in mango against infection by *Ceratocystis fimbriata*. *Phytopathol* 104: 820–833.

Beckman CH. 1964. Host responses to vascular infection. *Annu Rev Phytopathol* 2: 231–252.

Bechtel A, Widera M, Sachsenhofer RF, Gratzer R, Lücke A, Woszczyk M. 2007. Biomarker and stable carbon isotope systematics of fossil wood from the second Lusatian lignite seam of the Lubstów deposit (Poland). *Org Geochem* 38: 1850–1864.

Bierhorst DW. 1960. Observations on tracheary elements. *Phytomorphology* 10: 249–305.

Blanchette RA. 1992. Anatomical responses of xylem to injury and invasion by fungi. In: Blanchette RA, Biggs AR, editors. Defense mechanisms of woody plants against fungi. Springer Series in Wood Science. Berlin, Heidelberg: Springer; p. 76–95.

Boddy L. 2016. Interactions between fungi and other microbes in *The Fungi*, eds SC Watkinson, L Boddy, NP Money. Oxford, UK: Academic Press; Elsevier: 337–360

Bonsen KJM. 1991. Gefässverschluss-Mechanismen in Laubbäumen. [Mechanisms of vessel occlusion in deciduous trees]. *Vierteljahresschr Naturforsch Ges Zürich* 136: 13–50. In German.

Bonsen KJ M, Kučera LJ. 1990. Vessel occlusions in plants: morphological, functional and evolutionary aspects. *IAWA J* 11: 393–399.

Boucot AJ, Xu C, Scotese CR, Morley RJ. 2013. Phanerozoic paleoclimate: An atlas of lithologic indicators of climate. SEPM Society for Sedimentary Geology.

Boura A, Pons D, Vozenin-Serra C, Phú M, B. 2013. Mesozoic fossil wood of Kiên Giang Province, southwestern Vietnam. *Palaeontogr Abt B* 290: 11–40.

Carlquist S. 1996. Wood, bark and stem anatomy of New World species of *Gnetum. Bot J Linn Soc* 120: 1–19.

Cascales-Miñana B, Gerrienne P, Sirjacq B, Steemans P. 2019. On the hydraulic conductance of three woody Devonian plants. *IAWA J* 40: 446–465.

Césari SN, Álvarez-Vázquez C, Méndez-Bedia I, Álvarez-Laó D, Turrero P, Arbizu M. 2015. First report of permineralised plants in the Stephanian of Arnao (Asturias, northwestern Spain). *Palaeogeogr Palaeoclimatol Palaeoecol* 440: 475–486.

Chattaway MM. 1949. The Development of tyloses and secretion of gum in heartwood formation. *Austral J Biol Sci* 2: 227–240.

Chin K, Estrada-Ruiz E, Wheeler EA, Upchurch GR, Wolfe DG. 2019. Early angiosperm woods from the mid-Cretaceous (Turonian) of New Mexico, USA: *Paraphyllanthoxylon*, two new taxa, and unusual preservation. *Cretaceous Res* 98: 292–304.

Chrysler MA. 1908. Tyloses in tracheids of conifers. 1. *New Phytol* 7: 198–204.

Chrysler MA. 1932. A new cycadeoid from New Jersey. *Am J Bot* 19: 679–692.

Cichan MA. 1985. Vascular cambium and wood development in Carboniferous plants. II. *Sphenophyllum plurifoliatum* Williamson and Scott (Sphenophyllales). *Bot Gaz* 146: 395–403.

Cichan MA. 1986a. Vascular cambium and wood development in Carboniferous plants. III. *Arthropitys* (Equisetales, Calamitaceae). *Can J Bot* 64: 688–695.

Cichan MA. 1986b. Conductance in the wood of selected Carboniferous plants. *Paleobiology* 12: 302–310.

Cohen KM, Harper DAT, Gibbard PL. 2022. ICS International Chronostratigraphic Chart 2022/02. *International Commission on Stratigraphy, IUGS*. www.stratigraphy.org (visited: 02/03/2022).

De Micco V, Balzano A, Wheeler EA, Baas P. 2016. Tyloses and gums: a review of structure, function and occurrence of vessel occlusions. *IAWA J* 37: 186–205.

Decombeix A-L, Boura A, Tomescu AMF. 2019. Plant hydraulic architecture through time: lessons and questions on the evolution of vascular systems. *IAWA J* 40: 387–420.

The version of record of this manuscript has been published and is available in *Botany Letters*, 2022, https://www.tandfonline.com, https://doi.org/10.1080/23818107.2022.2099461

Decombeix A-L, Galtier J, Prestianni C. 2015. The Early Carboniferous progymnosperm *Protopitys*: new data on vegetative and fertile structures, and on its geographic distribution. *Hist Biol* 27: 345–354.

Decombeix A-L, Meyer-Berthaud B, Galtier J, Talent J, Mawson R. 2011. Diversity of arborescent lignophytes in the Tournaisian vegetation of Queensland (Australia): paleoecological and paleogeographical significance. *Palaeogeogr Palaeoclimatol Palaeoecol* 301: 39–55.

Dufraisse A, Coubray S, Girardclos O, Dupin A, Lemoine M. 2018. Contribution of tyloses quantification in earlywood oak vessels to archaeological charcoal analyses: Estimation of a minimum age and influences of physiological and environmental factors. *Quatern Int* 463: 250–257.

Edwards D. 2003. Xylem in early tracheophytes. *Plant Cell Environ* 26: 57–72.

Eggert DA, Taylor TN. 1966. Studies of Paleozoic ferns: on the genus *Tedelea* gen. nov. *Palaeontogr Abt B* 118: 52–73

Esau K. 1965. Plant anatomy. Ed. 2. New York, London, Sydney: Wiley & Sons.

Feng Z, Wang J, Rößler R, Kerp H, Wei H-B. 2013. Complete tylosis formation in a latest Permian conifer stem. *Ann Bot* 111: 1075–1081.

Fisher JB, Ewers FW. 1991. Structural responses to stem injury in vines. In: Putz FE, Mooney HA, editors. The biology of vines. New York: Cambridge University Press; p. 99–124.

Fourie G, Steenkamp ET, Ploetz RC, Gordon TR, Viljoen A. 2011. Current status of the taxonomic position of *Fusarium oxysporum formae specialis cubense* within the *Fusarium oxysporum* complex. *Infect Genet Evol* 11: 533–542

Franco MJ, Moya E, Brea M, Martínez CM. 2020. *Astroniumxylon, Schinopsixylon*, and *Parametopioxylon* n. gen. fossil woods from the upper Cenozoic of Argentina: taxonomic revision, new taxon and new records. *J Paleontol* 94: 185–201.

Galtier J, Harper CJ, Rößler R, Kustatscher E, Krings M. 2018. Enigmatic, structurally preserved stems from the Triassic of central Europe: A fern or not a fern. In: Krings M, Harper CJ, Cuneo NR, Rothwell GW, editors. Transformative paleobotany: Papers to commemorate the life and legacy of Thomas N. Taylor. Cambridge (MA): Academic Press; p.187–209.

Galtier J, Hueber FM. 2001. How early ferns became trees. *Proc R Soc Lond B* 268: 1955–1957

Galtier J, Phillips TL. 1999. The acetate peel technique. In: Jones T, Rowe NP, editors. Fossil plants and spores: modern techniques. London: Geol Soc London; p. 67–70.

Gerrienne P, Gensel PG, Strullu-Derrien C, Lardeux H, Steemans P, Prestianni C. 2011. A simple type of wood in two Early Devonian plants. *Nature* 333: 837.

Gerry E. 1914. Tyloses; their occurrence and practical significance in some American woods. . *Agric Res* 1: 445– 469.

Greppi CD, Garcia Massini, JL, Pujana RR, Marenssi SA. 2018. Fungal wood-decay strategies in Nothofagaceae woods from Miocene deposits in southern Patagonia, Argentina. *Alcheringa* 42: 427–440.

Gryc V, Vavrčík H, Sakala J. 2009. Cenomanian angiosperm wood from the Bohemian Cretaceous Basin, Czech Republic. *IAWA J 30:* 319–329.

Hass H, Rowe NP. 1999: Thin sections and wafering. In: Jones T, Rowe NP, editors. Fossil plants and spores: modern techniques. London: Geol Soc London; p 76–81.

Harper, C.J.,Krings, M., 2021. Fungi as parasites: a conspectus of the fossil record. In *The Evolution and Fossil Record of Parasitism* (pp. 69-108). Springer, Cham.

Harper CJ, Bomfleur B, Decombeix A-L, Taylor EL, Taylor TN, Krings M. 2012. Tylosis formation and fungal interactions in an Early Jurassic conifer from northern Victoria Land, Antarctica. *Rev Palaeobot Palynol* 175: 25–31.

The version of record of this manuscript has been published and is available in *Botany Letters*, 2022, https://www.tandfonline.com, https://doi.org/10.1080/23818107.2022.2099461

Harper CJ, Decombeix A-L, Taylor TN, Taylor EL, Krings M. 2017. Fungal decay in Permian glossopteridalean stem and root wood from Antarctica. *IAWA J* 38: 29–48.

He XY, Wang SJ, Hilton J, Galtier J, Jiang HG. 2020. An advanced species of the fern *Botryopteris* Renault from the Permian of southwestern China. *Rev Palaeobot Palynol* 273: 104136.

Hillbrand-Grill F. 1998. Schuh, (Ottone) Hermine; geb. Freiin von Reichenbach (1819-1902), Botanikerin. In: Österreichisches Biographisches Lexikon (ÖBL) 1815–1950, Vol. 11 (part 53), p. 310. In German.

Hoffman LA, Tomescu AMF. 2013. An early origin of secondary growth: *Franhueberia gerriennei* gen. et sp. nov. from the Lower Devonian of Gaspé (Quebec, Canada). *Am J Bot* 100: 754–763.

Holden HS. 1925. On the occurrence of cavity parenchyma and tyloses in ferns. *J Linn Soc, Bot* 47: 141–153.

Holden HS. 1930. On the structure and affinities of *Ankyropteris corrugata*. *Phil Trans R Soc London* 218B: 79–113.

IAWA [Commitee on Nomenclature, International Association of Wood Anatomists]. 1964. Multilingual glossary of terms used in wood anatomy. Winterthur: Verlagsanstalt Buchdruckerei Konkordia; 186 p.

IAWA Committee [International Association of Wood Anatomists]. 1989. IAWA list of microscopic features for hardwood identification. *IAWA Bull* 10: 219–332.

Jud NA, D’Emic MD, Williams SA, Mathews JC, Tremaine KM, Bhattacharya J. 2018. A new fossil assemblage shows that large angiosperm trees grew in North America by the Turonian (Late Cretaceous). *Sci Adv* 4: eaar8568.

Kashyap A, Planas-Marquès M, Capellades M, Valls M, Coll NS. 2021. Blocking intruders: inducible physico-chemical barriers against plant vascular wilt pathogens. *J Exp Bot* 72: 184–198.

Kenrick P, Crane PR. 1997. The origin and early diversification of land plants: a cladistic study. Washington, DC: Smithsonian Institution Scholarly Press.

Khan MA, Bera M, Spicer RA, Spicer TEV, Bera S. 2018. Evidence of simultaneous occurrence of tylosis formation and fungal interaction in a late Cenozoic angiosperm from the eastern Himalaya. *Rev Palaeobot Palynol* 259: 171–184.

Klein G. 1923. Zur Aetiologie der Thyllen [On the etiology of the thylles]. *Z Bot* 15: 418–439. In German.

Laloux B, Decombeix A-L. Accepted 11/2021. *Pitus* and *Eristophyton*-type woods in the Tournaisian of northeastern Queensland, Australia: taxonomical, biogeographical and palaeoclimatic implications. *C R Palevol*.

Leroux O, Knox JP, Masschaele B, Bagniewska-Zadworna A, Marcus SE, Claeys M, van Hoorebeke L, Viane RL. 2011. An extensin-rich matrix lines the carinal canals in *Equisetum ramosissimum*, which may function as water-conducting channels. *Ann Bot* 108: 307–319

Leśniewska J, Öhman D, Krzesłowska M, Kushwah S, Barciszewska-Pacak M, Kleczkowski LA, Sundberg B, Moritz T, Mellerowicz EJ. 2017. Defense responses in Aspen with altered pectin methylesterase activity reveal the hormonal inducers of tyloses. *Plant Physiol* 173: 1409–1419.

Mace ME, Bell AA, Beckman CH. 1981. Fungal wilt diseases of plants. New York/ London, Academic Press.

Malpighi M. 1686–87 Opera omnia [The works]. London: Apud R. Littlebury. In Latin.

Marguerier J. 1968. Révision du genre *Calamodendron* Brongniard 1849 d'après l'étude de la structure de *Calamodendron punctatum* (Renault) 1896 [Revision of the genus *Calamodendron* Brongniard 1849 based on the study of the structure of *Calamodendron punctatum* (Renault) 1896]. *C R 93^{e} Congr Nat Soc Sav Tours*. Tome 3: 39–66. In French.

Mawson R, Talent JA. 1997. Famennian–Tournaisian conodonts and Devonian-Early Carboniferous transgressions and regressions in north-eastern Queensland. *Geol Soc Am Spec Pap* 321: 189–233.

Mawson R, Talent JA. 1999. Early Carboniferous (mid-Tournaisian) conodonts from north-eastern Queensland (Ruxton and Teddy Mountain Formations): age implications and stratigraphic alignments. *Boll Soc Paleontol Ital* 37: 407–425.

McNicol M. 1908. On cavity parenchyma and tyloses in ferns. *Ann Bot* 22: 401–413.

Medlyn DA, Tidwell WA. 1975. Conifer wood from the Upper Jurassic of Utah. Part I: *Xenoxylon morrisonense* sp. nov. *Am J Bot* 62: 203–208.

Mickle JE. 1980. *Ankyropteris* from the Pennsylvanian of Eastern Kentucky. *Bot Gaz* 141: 230–243.

Miller CJ, Duckett JG. 1979. A study of stelar ultrastructure in the heterosporous water fern *Marsilea quadrifolia* L. *Ann Bot* 44: 231–238.

Mindell RA, Stockey RA, Rothwell GW, Beard G. 2006. *Gleichenia appianensis* sp. nov. (Gleicheniaceae): A permineralized rhizome and associated vegetative remains from the Eocene of Vancouver Island, British Columbia. *Int J Plant Sci* 167: 639–647.

Molisch H. 1889. Zur Kenntniss der Thyllen, nebst Beobachtungen über Wundheilung in der Pflanze. [Concerning the knowledge of tyloses, with observations on wound healing in plants]. *Sitzungsber K Akad Wiss Wien, Math-Naturw Cl, Abt I,* 97: 264–299. In German.

Morris H, Brodersen C, Schwarze FW, Jansen S. 2016. The parenchyma of secondary xylem and its critical role in tree defense against fungal decay in relation to the CODIT model. *Front Plant Sci* 7: 1665.

Nunes CI, Pujana RR, Escapa IH, Gandolfo MA, Cúneo NR. 2018. A new species of *Carlquistoxylon* from the Early Cretaceous of Patagonia (Chubut province, Argentina): the oldest record of angiosperm wood from South America, *IAWA J* 39*:* 406–426.

Oh C, Philippe M, McLoughlin S, Woo J, Leppe M, Torres T, Park T-YS, Choi H-G. 2020. New fossil woods from lower Cenozoic volcano-sedimentary rocks of the Fildes Peninsula, King George Island, and the implications for the trans-Antarctic Peninsula Eocene climatic gradient. *Pap Palaeontol* 6: 1–29.

Orlova OA, Jurina A. 2011. Genus *Callixylon* Zalessky (Archaeopteridophyta): main criteria for distinguishing its species and revision of its species composition. *Paleontol J* 45: 580–89.

Pearce RB. 1996. Antimicrobial defences in the wood of living trees. *New Phytol* 132: 203–233.

Phillips TL, Galtier J. 2005. Evolutionary and ecological perspectives of late Paleozoic ferns. Part I. Zygopteridales. *Rev Palaeobot Palynol* 135: 165–203.

Phillips TL, Galtier J. 2011. Evolutionary and ecological perspectives of late Paleozoic ferns: Part II. The genus *Ankyropteri*s and the Tedeleaceae. *Rev Palaeobot Palynol* 164: 1–29.

Phipps PM, Stipes RJ. 1976. Histopathology of mimosa infected with *Fusarium oxysporum* f. sp. *perniciosum*. *Phytopathology* 66: 839–843.

Pigg KB, Rothwell GW. 1983. *Chaloneria* gen. nov.; Heterosporous lycophytes in the Pennsylvanian of North America. *Bot Gaz* 144: 132–144.

The version of record of this manuscript has been published and is available in *Botany Letters*, 2022, https://www.tandfonline.com, https://doi.org/10.1080/23818107.2022.2099461

Raatz W. 1892. Ueber Thyllenbildung in den Tracheïden der Coniferenhölzer [On the formation of tyloses in the tracheids of conifer wood]. *Ber Deutsch Bot Ges* 10: 183–191. In German.

Renault B. 1893–1896. Bassin Houiller et Permien d'Autun et d'Epinac. Flore Fossile, 2e partie. [Coal and Permian Basin of Autun and Epinac. Fossil Flora, part 2]. Paris: Imprimerie Nationale. 578 pp. In French.

Rioux D, Chamberland H, Simard M, Ouellette GB. 1995. Suberized tyloses in trees: An ultrastructural and cytochemical study. *Planta* 196: 125–140.

Rioux D, Nicole M, Simard M, Ouellette GB. 1998. Immunocytochemical evidence that secretion of pectin occurs during gel (gum) and tylosis formation in trees. *Phytopathology* 6: 494–505.

Roberts D, Barghoorn E. 1952. *Medullosa olseniae*: a Permian *Medullosa* from north central Texas. *Bot Mus Leaf Harvard Univ* 15: 191–200.

Rößler R, Feng Z, Noll R. 2012. The largest calamite and its growth architecture — *Arthropitys bistriata* from the Early Permian Petrified Forest of Chemnitz. *Rev Palaeobot Palynol* 185: 64–78.

Rößler R, Noll R. 2007. *Calamitea* Cotta, the correct name for calamitean sphenopsids currently classified as *Calamodendron* Brongniart. *Rev Palaeobot Palynol* 144: 157–180.

Rößler R, Noll R. 2010. Die Calamiten der Gattung *Arthropitys* Goeppert 1864 im Versteinerten Wald von Chemnitz. [Calamites of the genus *Arthropitys* Goeppert in the petrified forest of Chemnitz]. *Veröff Mus Naturkunde Chemnitz* 33: 89–112. In German.

Rößler R, Noll R. 2012. Anatomy and branching of *Arthropitys bistriata* (Cotta) Goeppert – New observations from the petrified forest of Chemnitz, Germany. *Int J Coal Geol* 83: 103–124.

Scheckler SE, Galtier J. 2003. Tyloses and ecophysiology of the early Carboniferous progymnosperm tree *Protopitys buchiana*. *Ann Bot* 91: 739–747.

Schmitt U, Liese W. 1994. Wound tyloses in *Robinia pseudoacacia* L. *IAWA J* 15: 157–160.

Schneider CA, Rasband WS, Eliceiri KW. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nat Methods* 9: 671–675.

Schwarze FWMR, Baum S. 2000. Mechanisms of reaction zone penetration by decay fungi in wood of beech (*Fagus sylvatica*). *New Phytol* 146: 129–140.

Schopf JM. 1975. Modes of fossil preservation. *Rev Palaeobot Palynol* 20: 27–53.

Schweingruber FH, Börner A. 2018. The plant stem. A microscopic aspect. Springer, Cham, published online at https://doi.org/10.1007/978-3-319-73524-5

Scott AC. 1990. Preservation, evolution, and extinction of plants in Lower Carboniferous volcanic sequences in Scotland. In: Lockley MG, Rice A, editors. Volcanism and fossil biotas. Boulder CO: Geological Society of America; special paper 244.

Shi X, Yu J, Sun Y. 2020. Tyloses in the Lopingian cordaitalean root from Xinjiang, Northwest China. *Rev Palaeobot Palynol* 273: 104134.

Shigo AL 1984. Compartmentalization: a conceptual framework for understanding how trees grow and defend themselves. *Annu Rev Phytopathol* 22: 189–214.

Shivanna M, Singh A, Singh BD, Singh VP, Matthews RP, Souza PA. 2017. Peat biomass degradation: Evidence from fungal and faunal activity in carbonized wood from the Eocene sediments of western India. *Palaeoworld* 26: 531–542.

Smoot EL, Taylor TN. 1981. Phloem histology of a Lower Pennsylvanian *Psaronius*. *Palaeobotanist* 28/29: 81–85.

Speranza M, Ascaso C, Delclòs X, Peñalver E. 2015. Cretaceous mycelia preserving fungal polysaccharides: taphonomic and paleoecological potential of microorganisms preserved in fossil resins. *Geol Acta* 13: 363–385.

The version of record of this manuscript has been published and is available in *Botany Letters*, 2022, https://www.tandfonline.com, https://doi.org/10.1080/23818107.2022.2099461

Steidtmann WE. 1944. The anatomy and affinities of *Medullosa noei* Steidtmann, and associated foliage, roots, and seeds. *Contrib Mus Paleontol Univ Mich* 6: 131–166.

Strullu-Derrien C, Kenrick P, Tafforeau P, Cochard H, Bonnemain J-L, Le Hérissé A, Lardeux H, Badel E. 2014. The earliest wood and its hydraulic properties documented in *c*. 407-million-year-old fossils using synchrotron microtomography. *Bot J Linn Soc* 175: 423–437

Sun Q, Rost TL, Reid MS, Matthews MA. 2007. Ethylene and not embolism is required for wound-induced tylose development in stems of grapevines. *Plant Physiol* 145:1629–1636.

Talboys PW. 1972. Resistance to vascular wilt fungi. *Proc R Soc London* B 181: 319–332.

Tanrattana M, Barczi J-F, Decombeix A-L, Meyer-Berthaud B, Wilson JP. 2019. A new approach for modelling water transport in fossil plants. *IAWA J* 40: 466–487.

Taylor TN, Krings M, Dotzler N, Galtier J. 2011. The advantage of thin section preparations over acetate peels in the study of late Paleozoic fungi and other microorganisms. *Palaios* 26: 239–244.

Taylor TN, Taylor EL, Krings M. 2009. Paleobotany. The biology and evolution of fossil plants. Cambridge (MA): Academic Press.

Terreaux de Félice H, Decombeix A-L, Galtier J. 2019. Anatomy, affinities, and evolutionary implications of new silicified stems of *Sphenophyllum* from the early Carboniferous (Mississippian) of France and Germany. *Geodiversitas* 41: 587–599.

Thayne GF, Tidwell WD, Stokes WL. 1983. Flora of the lower Cretaceous Cedar Mountain Formation of Utah and Colorado, Part I. *Paraphyllanthoxylon utahense*. *Great Basin Naturalist* 43: Article 3.

Tosal A, Decombeix A-L, Meyer-Berthaud B, Galtier J, Martín-Closas C. 2021. First record of silicified woods in the Late Carboniferous basins of the Pyrenees. *7th International*

The version of record of this manuscript has been published and is available in *Botany Letters*, 2022, https://www.tandfonline.com, https://doi.org/10.1080/23818107.2022.2099461

Meeting of Agora Paleobotanica, Oct 2021, Liège, Belgium. https://hal.inrae.fr/hal-03412572/document.

VanderMolen GE, Beckman CH, Rodehorst E. 1987. The ultrastructure of tylose formation in resistant banana following inoculation with *Fusarium oxysporum* f. sp. *cubense*. *Physiol Mol Plant Pathol* 31: 185–200.

Walton J. 1924. IV. On *Rhexoxylon* Bancroft—a Triassic genus of plants exhibiting a liane-type of vascular organization. *Phil Trans R Soc London* B212: 79–109.

Walton J. 1949. On some Lower Carboniferous Equisetineae from the Clyde area. I *Protocalamostachys arranensis* gen. et sp. nov. – a hitherto undescribed type of strobilus. II. The nodal structure of *Asterocalamites Göpperti* Solms sp. *Trans R Soc Edinburgh* 61: 729–73.

Wang SJ, Hilton J, Galtier J, He XY, Shao LY. 2014. *Tiania yunnanense* gen. et sp. nov., an osmundalean stem from the Upper Permian of southwestern China previously placed within *Palaeosmunda*. *Rev Palaeobot Palynol* 210: 37–49.

Weaver LS, McLoughlin S, Drinnan AN. 1997. Fossil woods from the Permian Bainmedart Coal Measures, northern Prince Charles Mountains, East Antarctica. *AGSO J Aust Geol Geophys* 16: 655–676.

Wei HB, Gou XD, Yang JY, Feng Z. 2019. Fungi-plant-arthropods interactions in a new conifer wood from the uppermost Permian of China reveal complex ecological relationships and trophic networks. *Rev Palaeobot Palynol* 271: 104100.

Weiner G, Liese W. 1997. Wound reactions in bamboo culms and rhizomes. *J Trop For Sci* 9: 379–397.

Weiss FE. 1906. On the tyloses of *Rachiopteris corrugata*. *New Phytol* 5: 1–5.

Wheeler EA, Manchester AR. 2002. Woods of the Middle Eocene Nut Beds Flora, Clarno Formation, Oregon, USA. *IAWA J,* Supplement 3:1–188.

Williamson WC. 1877. On the organisation of the fossil plants of the Coal Measures. Part VIII. Ferns (continued) and gymnospermous stems and seeds. *Proc R Soc London* 25 68–73.

Williamson WC. 1888. On some anomalous cells developed within the interior of the vascular and cellular tissues of the fossil plants of the Coal-Measure. *Ann Bot* 1: 315–323.

Wilson JP. 2013. Modeling 400 Million Years of Plant Hydraulics. *The Paleontological Society Papers* 19: 175–194.

Wilson JP. 2016. Hydraulics of *Psilophyton* and evolutionary trends in plant water transport after terrestrialization. *Rev Palaeobot Palynol* 227: 65–76.

Yadeta KA, Thomma BPHJ. 2013. The xylem as battleground for plant hosts and vascular wilt pathogens. *Front Plant Sci* 4: 97.

Zimmermann MH. 1979. The discovery of tylose formation by a Viennese Lady in 1845. *IAWA Bull* 3: 51–56.

Zürcher E, Kučera LJ, Bosshard HH. 1985. Bildung und Morphologie der Thyllen: Eine Literaturübersicht. [Development and morphology of tyloses: a survey of the literature]. *Vierteljahresschr Naturforsch Ges Zürich* 130: 311–333. In German.

The version of record of this manuscript has been published and is available in *Botany Letters*, 2022, https://www.tandfonline.com, https://doi.org/10.1080/23818107.2022.2099461

FIGURE CAPTIONS

Figure 1. Schematic representation of tylosis formation seen in transverse and longitudinal sections. C: conducting cell (tracheid or vessel), P: parenchyma cell.

Figure 2. Tyloses in the secondary xylem of the (pro)gymnosperm *Dameria hueberi* from the Tournaisian (early Mississippian) of Australia. Features in transverse section.

A. General view of specimen MOD5 in transverse section. A very high resolution, zoomable version of this image is available at https://doi.org/10.6084/m9.figshare.c.5869925

B. General view of a portion of wood with no tyloses and no evidence of growth ring boundaries

C. General view of a zone with tyloses

D. Detail of tyloses in radial files of tracheids

E. Detail of tyloses in radial files of tracheids

F. Close-up showing tyloses completely filling the lumen of the tracheids. Arrows indicate cell walls.

G. Close-up of a ray (R) and tracheids showing different stages of tylosis formation. From a small bubble-like structure coming from the ray (a, black arrow), to a cell with several balloon-like structures (b, white arrows), to a completely filled lumen with visible angular cell walls (c) All views from slide MOD5d CT1. Scale bars: A: 1cm; B, C: 500 µm; D, E: 100 µm; F, G: 25 µm.

Figure 3. Tyloses in secondary xylem of the (pro)gymnosperm *Dameria hueberi* from the Tournaisian (early Mississippian) of Australia. Features in longitudinal section

A. General aspect in tangential section showing numerous small uniseriate rays and cells with a brown content that are filled with tyloses (t). Slide MOD5a CLT1

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B. Detail of a tangential section showing a tracheid filled with tyloses with angular cell walls. Slide MOD5a CLT1

C. Detail of a tangential section showing a tracheid filled with tyloses with some bubble-like forms. Slide MOD5a CLT1

D. Detail of a tangential section showing a tracheid filled with tyloses with angular cell walls. Slide MOD2 CLT1

E. Detail of a tangential section showing a tracheid filled with tylosis. Slide MOD2 CLT1

F. Radial section showing the uniseriate circular radial pits and some tracheids filled with tyloses. Slide MOD5c CLR1

G. Radial section showing details of radial pits (white arrowhead) and cross-field pits (orange arrowhead) in a ray (R). Slide MOD5c CLR1

Scale bars: A: 100 µm; B, C, D, F, G: 50 µm; E: 10 µm.

Figure 4. Tyloses in extant and extinct vascular plants. Dots indicate the presence of tyloses in extant representatives, either in the vascular tissues (black dots) or the carinal canals (white dot). Black stars indicate the oldest evidence to date of tylosis formation in vascular tissue. Psi: *Psilophyton*; Cladox: Cladoxylopsida s.l.; Progymnosp.: progymnopserms; Pteridosp.: pteridosperms. Black arrow indicates the age of the new evidence presented in this paper in the (pro)gymnosperm *Dameria hueberii*.

Figure 5. Tyloses in early ferns: (A-C) *Ankyropteris corrugata* (Teledaceae) from the Lower Pennsylvanian of Bouxharmont (Belgium) and Burnley (England), and (D-F) *Zygopteris/Etapteris* (Zygopteridales) from the Middle-Upper Pennsylvanian of Illinois (USA, UI-)

A. Stem protostele of *A. corrugata* with tyloses in the metaxylem. Slide BX 336D2B02

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B. Departing clepsydroid vascular trace of the phyllophore /petiole with tyloses in the metaxylem. Slide B 309C1I03.

C. Tyloses inside the xylem strand of the « free » proximal phyllophore, filling most of the large metaxylem tracheids of the median rectangular « apolar » zone. Slide BX 336D1B02

D. Basal transverse section of an *Etapteris* petiole, with tyloses filling all large metaxylem tracheids of the rectangular « apolar » zone. Specimen UI 3457, slide 3457BTOP-6.

E. Longitudinal section of another *Etapteris* petiole (borne on a *Zygopteris illinoiensis* stem) showing a metaxylem tracheid filled with tyloses (t) and one with small balloon-like tylosis at an earlier stage of formation (white arrows). Specimen UI 22655, slide 22655HBOT-37.

F Tyloses in secondary xylem tracheids of a *Zygopteris* root with a thick periderm. Specimen UI 22655, slide 22655HBOT-97

Scale bars: A: 500 µm; B-F: 250 µm.

Fig. 6 Tyloses in secondary xylem of the progymnosperm *Protopitys buchiana* from the Visean of the Vosges, France.

A. General view of the secondary xylem of *Protopitys buchiana* showing radial files of tracheids with a brown content. Slide BOU 1500 AT.

B. Detail of secondary xylem in transverse section showing tracheids filled with tyloses forming angular cell walls (black arrows). Slide BOU 1500 AT.

C. Detail of secondary xylem tracheids filled with tyloses (t) in radial section. Slide BOU 1500 BLR.

Scale bars: A: 250 µm; B, C: 50 µm.

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FIG 1

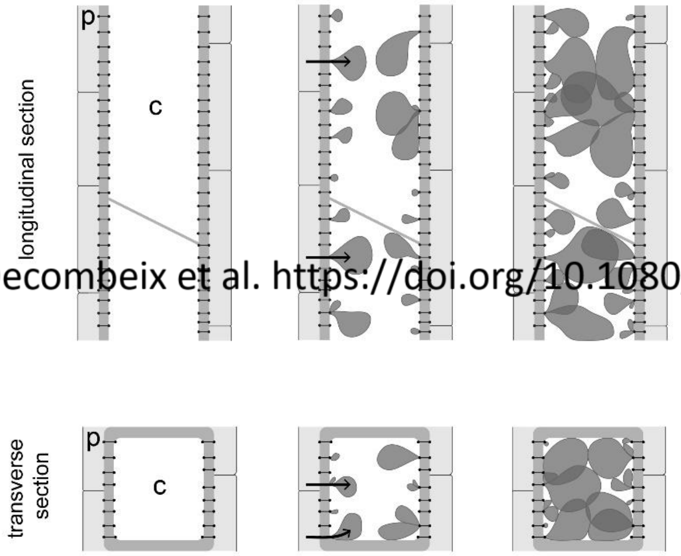

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FIG 2

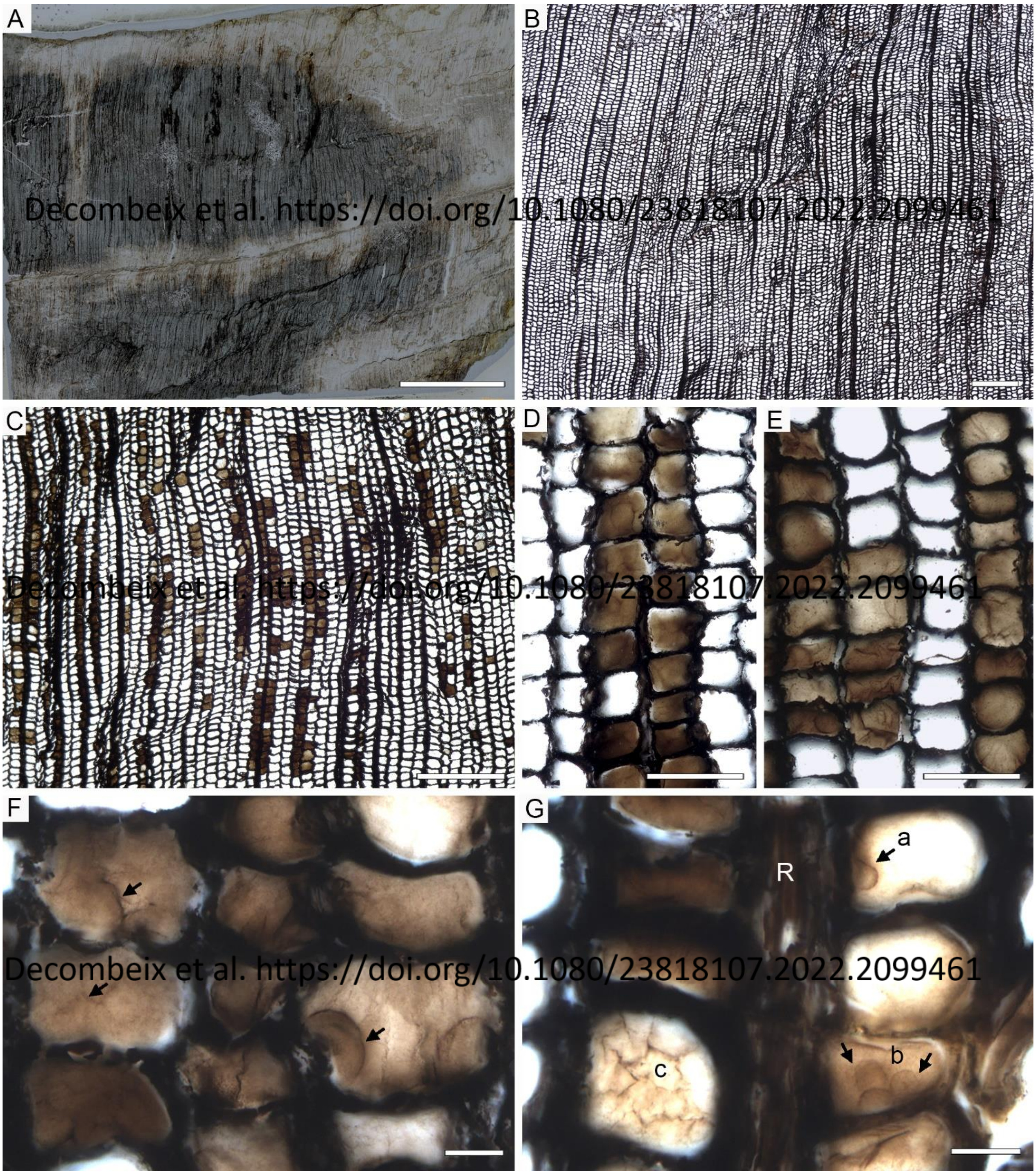

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FIG 3

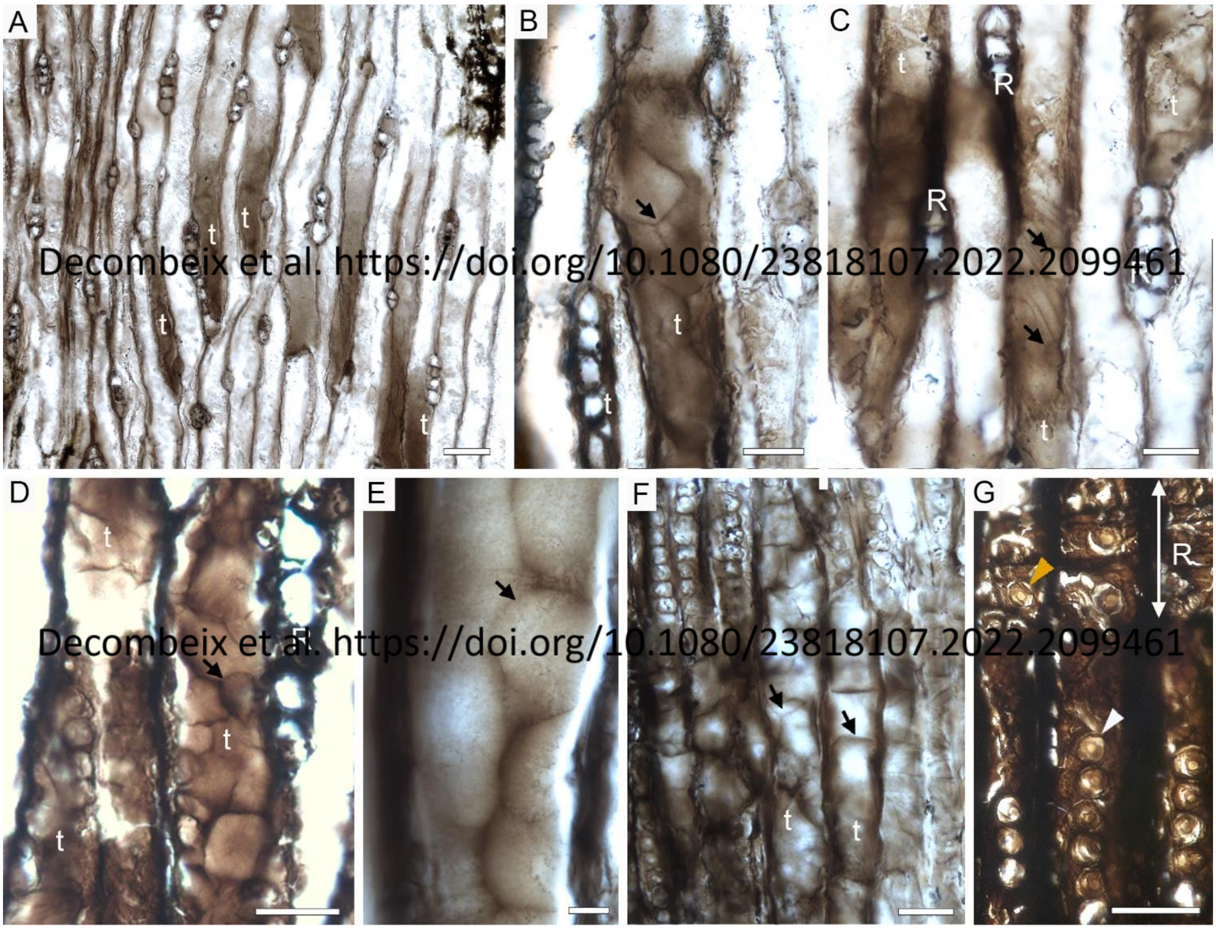

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FIG 4

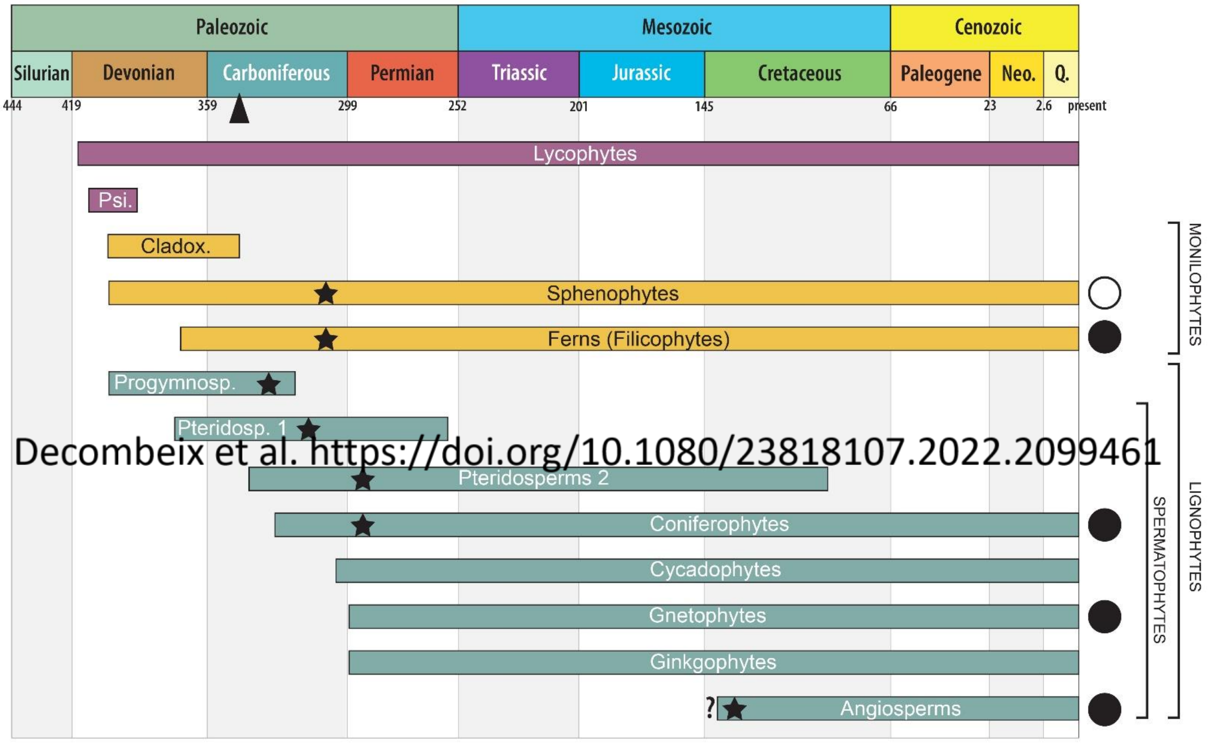

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FIG 5

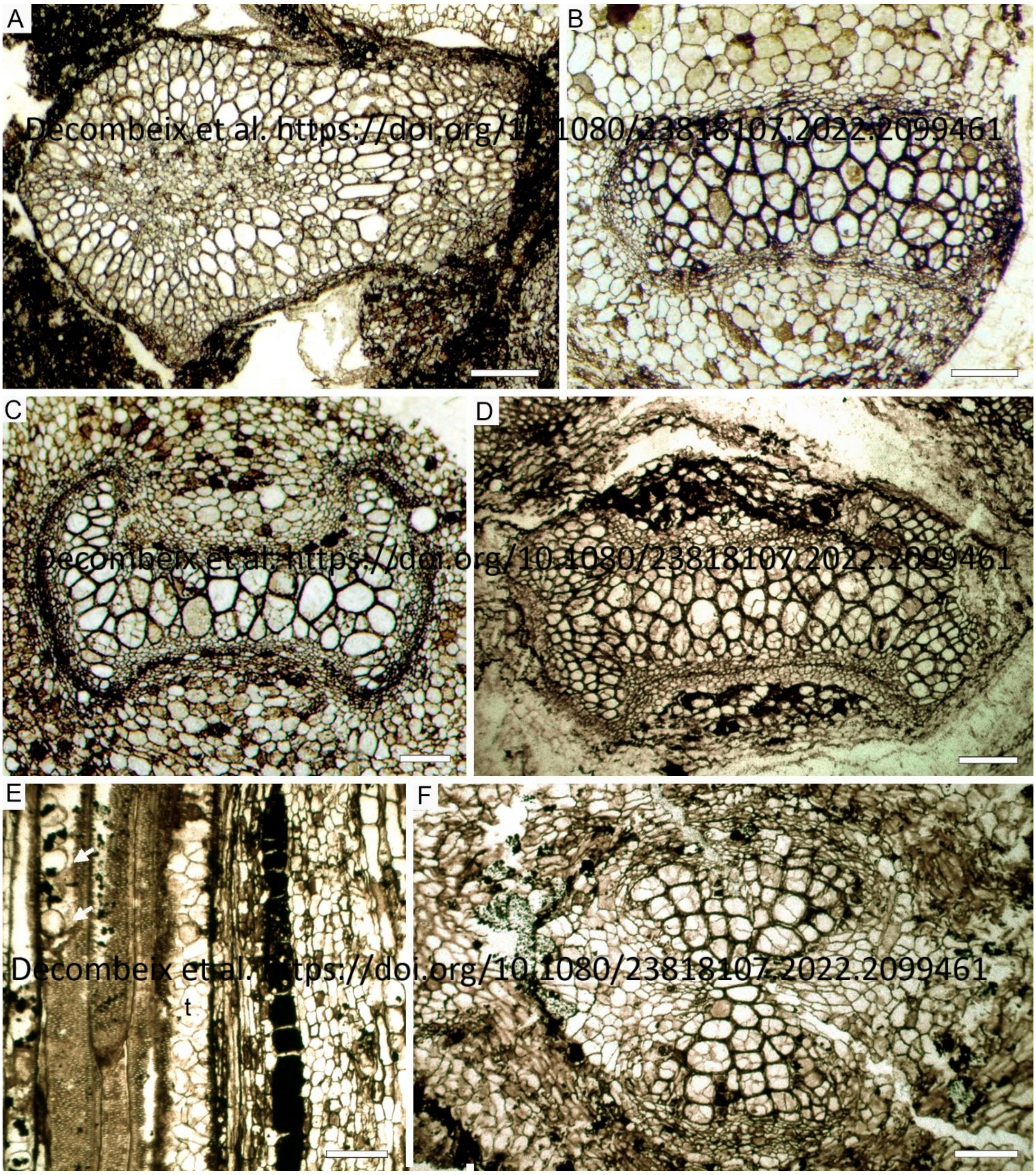

FIG 6