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1 Oldest fossil evidence of tylosis formation in plants

2
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5
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15
16 **Tyloses are swellings of parenchyma cells into adjacent water conducting cells that**
17 **develop in vascular plants as part of the heartwood formation or specifically in response**
18 **to embolism and pathogen infection. Here, we document tyloses in Late Devonian (ca.**
19 **360 Ma) *Callixylon* wood. This discovery suggests that already some of the earliest**
20 **woody trees were capable of protecting their vascular system by occluding individual**
21 **conducting cells.**

22 Tyloses are protoplasmic inflations formed by parenchyma cells into the lumen of
23 neighboring conducting cells [1, 2]. They extend through the pit pair and can completely fill
24 the lumen of the conducting cell. The mechanisms underlying tylosis formation remain
25 largely unresolved [e.g., 3, 4], but it is generally accepted that they represent a response to
26 embolism and have a major role in blocking air-filled conducting cells. Moreover, tylosis
27 formation is among the earliest processes in the Compartmentalization Of Decay In Trees
28 (CODIT; [5]), which can delay the spreading of pathogens. Tyloses in fossil plants, therefore,
29 provide direct insights into the evolution of one of the most important mechanisms with
30 which plants protect their vascular system from biotic and abiotic stresses.

31 Structurally preserved fossils provide evidence that tylosis formation occurred in most
32 lineages of vascular plants as early as the Mississippian (ca. 350 Ma) [6]. However, the oldest
33 fossils believed to represent vascular plant conductive elements (tracheids) are dispersed
34 microscopic tubes from the uppermost Silurian (ca. 430 Ma) [7], and the first record of
35 secondary xylem (wood) comes from the Lower Devonian (410 Ma) [8-10]. By Middle
36 Devonian times, a great many plants possessed wood [11] in which the arrangement of
37 conducting cells and parenchyma could, in theory, have allowed the formation of tyloses. The
38 development of larger and more complex plant bodies during the later Devonian, along with
39 the evolution of leaves and more extensive root systems [12, 13], likely increased the risk of
40 embolism in the vascular system. There is also evidence from this period of geologic time of
41 the existence of various types of vascular plant-fungal interactions, possibly including wood

42 decay [14, 15]. It is therefore surprising that no tyloses have been hitherto reported in plant
43 fossils older than the Mississippian.

44 The lack of documented evidence of tyloses in early vascular plants has spurred us to
45 specifically look for these structures in Devonian structurally preserved woods. We found that
46 many early woods are fossilized in a manner not conducive to the preservation (in a
47 recognizable form) of tenuous parenchymatous structures, including tyloses. Nevertheless, we
48 discovered evidence of early stages of tylosis formation in one piece of pyritized wood from
49 the Upper Devonian (Famennian, ca. 360 Ma) of Sandeel Bay, County Wexford, Ireland.

50 This wood consists of tracheids and small parenchymatous rays (Fig. 1); distinct
51 growth ring-like zones of tracheids with reduced radial diameters are indicative of intermittent
52 periods of less-favorable growth conditions (Fig 1a). The radial tracheid walls show groups of
53 pits separated by unpitted areas, a feature typical of *Callixylon*, the wood of the
54 archaeopteridalean progymnosperms ((Fig 1c) [16]; see methods section). Rays are 1–2 cells
55 wide and 1–15 (usually less than 10) cells high in tangential section (Fig 1d), abundant, and
56 each xylem tracheid is in contact with a significant number of ray parenchyma cells.
57 Conspicuous bubble-like outgrowths of ray cells into the lumen of adjacent tracheids occur in
58 one area of a longitudinal section (Fig 1e-k). Although they appear relatively dark compared
59 to the surrounding parenchyma cells, we interpret these outgrowths as tyloses, rather than
60 gum deposits, based on their regular outline, physical connection to the rays, and the presence
61 also of several translucent examples (Fig 1k). The majority of the tyloses are spheroidal in
62 shape (Fig 1e, f), sometimes with a constriction at the base that likely corresponds to their
63 passage through the pits connecting rays and tracheids; others are more flattened (Fig 1h, i).
64 Still others completely occlude the tracheid (Fig 1g). There are often several tyloses emerging
65 from a single ray, sometimes visible along both sides of the ray in tangential sections (Fig 1j).
66 No evidence of tylosis formation has been observed in any other structurally preserved plant
67 fossil from the Sandeel Bay locality.

68 Archaeopteridales are a Middle–Late Devonian group of progymnosperms, extinct
69 plants that had a gymnosperm-like wood but reproduced via spores [16]. Archaeopteridalean
70 trees produced a significant amount of wood, formed a deep and complex root system, and
71 had true leaves, making them the first trees with a “modern” vegetative body [12, 18]. Recent
72 studies of their hydraulic properties suggest that archaeopteridalean wood had a conductivity
73 comparable to that of extant conifers, with some plasticity provided by variations in the size
74 of the conducting cells and rays [19, 20]. Like extant trees, archaeopteridaleans were
75 susceptible to wood decaying fungi that invaded their vascular system [14]. By the Late
76 Devonian, these trees were a major component of ecosystems worldwide, having been
77 reported from a diversity of latitudes, from the paleotropics to the high latitudes of Gondwana
78 [21]. This wide altitudinal and geographic range implies that the group was able to adaptate to
79 very diverse growth conditions. The ability to protect the vascular system from biotic and
80 abiotic stresses by occluding (some of) the conducting cells may, therefore, have been an
81 important advantage. The depositional environment of the Sandeel Bay anatomically
82 preserved plants has been interpreted as a cut-off channel chute of a meandering river near the
83 coastline [22], within a dry tropical area [23]. Growth ring-like zones in the
84 archaeopteridalean woods from this deposit suggest that the trees were exposed to periodic
85 shifts from favorable to less favorable growth conditions. It is likely that there were episodes

86 of drought governed by variations of the water table that could have caused embolism in
87 certain regions of the wood and led to tylosis formation. The fact that most tracheids are not
88 completely blocked indicates that the specimen depicts an early stage in this process.

89 This discovery illustrates how structurally preserved fossils can provide snapshots of
90 plant physiology in deep time, and allow us to piece together episodes in the evolutionary
91 history of key biological processes. Moreover, it provides further evidence of the antiquity of
92 tylosis formation in plants, and suggests that the emergence of this capacity could have been
93 linked to the evolution of larger plant bodies with a more complex organization. As soon as
94 plants had evolved a water conducting system [[11](#), [12](#)], they had also created a gateway
95 through which physical (i.e., embolism) and biological (i.e., pathogens) threats could spread.
96 Early vascular plants were perhaps able to mitigate this drawback simply through their small
97 size and the short life span of their structures. However, as soon as plants grew larger, an
98 effective system of protection of the vascular system became essential [[24](#)], with tylose
99 formation being an integral part of it.

100 **Online Methods**

101 *Age of the fossil wood, sedimentological and floristic context*

102 The specimen was collected in 2021 at Sandeel Bay on Hook Head peninsula, Wexford
 103 County, Ireland. Outcrops containing Late Devonian plant macrofossils in this area belong to
 104 the Harrylock Formation [25] (Supplementary Fig. 1a), which corresponds to fluvial
 105 sediments deposited in a proximal alluvial plain setting. Anatomically preserved specimens
 106 occur as pyritic permineralizations in grey-green mudstones, which also contain
 107 compressions, dispersed spores, and cuticles (Supplementary Figs. 1b, 2). The miospore
 108 assemblage at Sandeel Bay corresponds to the LL Miospore Biozone of latest Famennian age
 109 [25]. New findings in 2021 of *Retispora lepidophyta* (Kedo) Playford 1976 and
 110 *Vallatisporites pusillites* (Kedo) Dolby and Neves 1970 in the layers containing the
 111 macrofossils (Supplementary Fig. 2h, j) confirm their age as latest Famennian. Other
 112 permineralized plants recovered from the source deposit of the specimen with tyloses include
 113 axes of the lycopsid *Wexfordia hookense* (Matten) Klavins 2004 at different developmental
 114 stages [26, 27] (Supplementary Fig. 2a), as well as axes and wood with the typical *Callixylon*
 115 Zalessky 1911 anatomy of archaeopteridalean progymnosperms [16]. *Callixylon* was first
 116 reported from this locality by Klavins [22], who also described an anatomically preserved
 117 branch assigned to *Archaeopteris* cf. *hibernica*. Newly collected specimens from this locality
 118 include the first anatomically preserved *Archaeopteris* roots (Supplementary Fig. 2d-g).
 119 Finally, Klavins [22] reported 3 different types of pyritized seeds, which indicate the presence
 120 also of seed plants in the paleoenvironment. Paleosol layers at Sandeel Bay show traces of
 121 relatively deep and branched root systems that could belong to either *Archaeopteris* and/or
 122 seed plants.

123

124 *Fossil preparation and observation*

125 The fossil is approximately 17 cm long and 2–3 cm wide, and consists of 7 consecutive
 126 portions of a single axis. Selected axis portions were prepared at UMR AMAP in 2022
 127 according to the following protocol. They were embedded in Epoxy (DBF, Escil), and
 128 transverse and longitudinal sections of 1–2 mm thickness were subsequently cut with an
 129 Isomet 1000 diamond saw and polished on both sides. To improve contrast, the section were
 130 then etched following a modified version of the protocol for pyrite permineralizations
 131 published by Stein and collaborators [28]. They were initially etched in 70% nitric acid
 132 (HNO₃) heated to 70°C for 60–90 seconds. After neutralization in sodium hydroxide (NaOH)
 133 they were additionally etched in 37% hydrochloric acid (HCl) for a few seconds,
 134 neutralized, and then etched again in 5–10 % HCl before being rinsed in water and let to dry.
 135 Selected sections were mounted on glass slides and observed in reflected light. Due to the
 136 distortion of the wood, there was no good quality view of the radial pitting on the mounted
 137 sections. Photographs of the radial pitting (Fig. 1 c) were thus taken directly on the surface of
 138 fragment HH5-E after a fracture in the radial plane. All photographs were taken with a
 139 Keyence VX 7000 electronic microscope and the associated software. Composite figures were
 140 assembled in Adobe Photoshop 21.2.2. In slide numbers such as HH5-D-G1, the first part
 141 refers to the specimen number (5), the second to the part of the specimen (D), and the last to
 142 the section (G1). Letters r or v in photographs indicates the side of the wafer that was
 143 photographed (recto, bearing the number, or verso).

144 ***Systematic affinity of the wood***

145 The systematic affinity of the specimen was determined based on wood anatomical traits in
 146 longitudinal and transverse sections. While no primary tissues are preserved, the presence of
 147 wood tracheids with groups of radial pits separated by unpitted areas on their radial walls is a
 148 distinctive feature of the fossil genus *Callixylon*, which represents the wood of
 149 archaeopteridalean progymnosperms [16]. Other woody plants discovered from the source
 150 layers of the specimen described here are lycopsids, which clearly differ from *Callixylon* in
 151 the radial pitting (Supplementary Fig. 2c). Seed plants, currently only evidenced from this
 152 locality based on the seeds described by Klavins [22], are not known to produce wood with
 153 unpitted areas on the radial walls. Species of *Callixylon* are distinguished based on differences
 154 in certain wood anatomical features, such as ray width, and the presence, abundance, and
 155 distribution of ray tracheids [29]. Rare ray cells with thicker walls and a squarish outline in
 156 the new specimen (e.g., Fig 1d) could correspond to ray tracheids. However, the preservation
 157 of the specimen prevents a confident assignment at the species level.

158

159 ***Interpretation of the outgrowths as tyloses***

160 An alternative interpretation of the bubble-like outgrowths views them as partly coagulated
 161 (due to water loss) cell contents of the ray cells that have oozed out into adjacent tracheids as
 162 a result of the pyritization process. Strongly arguing against this interpretation are the facts
 163 that (1) similar structures have not been observed in any other pyritized wood from the
 164 Sandeel Bay site; and (2) the occurrence of outgrowths is limited to one particular region of
 165 the specimen that does not differ from the rest in regard to preservation. Small circular
 166 structures lacking a clear connection to parenchyma cells could also correspond to tyloses, but
 167 could also be gum or other organic residues.

168

169 **Data availability.**

170 All data that support the findings of this study are included in this published article and its
 171 supplementary information files. The fossil and associated slides are currently on loan at
 172 UMR AMP Montpellier and accessible under specimen number HH5 (for HookHead #5).
 173 This material and other fossils illustrated in the supplementary figures will ultimately be
 174 deposited in the Earth Science collections of the National Museum of Ireland, Dublin, once
 175 the study of the assemblage is completed.

176

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 182 comments on a previous draft of the manuscript.

183

184 **Author contributions**

185 ALD and CJH initiated the research project. ALD, CP, and TD participated in the fieldwork
 186 during which the material was collected. MR prepared the sections. ALD and MR

187 photographed the sections. ALD, CJH and MK analysed the data with inputs from TD and
 188 CP. CP analysed the sedimentological context and prepared the spores and cuticles illustrated
 189 in the supplementary figure. ALD and MK prepared the manuscript with contributions from
 190 all co-authors.

191

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 248

249 Figure legends

250 Fig 1. Late Devonian *Callixylon* wood containing tyloses. a, transverse section, showing
 251 growth ring boundaries (white arrows) b, detail of transverse section with two rays. c,
 252 radial section, showing groups of pits separated by unpitted areas (white arrows) typical of
 253 *Callixylon*. d, longitudinal section of a ray containing what appear to be ray tracheids
 254 (black arrow). e, f, general views of area with abundant tyloses. g, tyloses entirely
 255 blocking conducting cell. h, ray with several small outgrowths (developing tyloses) into
 256 adjacent tracheid. i, small outgrowth from ray cell into tracheid. j, ray with tyloses on both
 257 sides. k, tylosis lacking dark content (translucent).
 258 All images were selected from the direct observation under the microscope of preparations
 259 of a single fossil (#HH5): a, b: slide HH5-B-CT1r; c: HH5-E (surface of specimen); d-g, j,
 260 k: slide HH5-D-G1r; h: slide HH5-D-G1v .
 261 Scale bars: a: 200 µm; b, e, f: 100 µm; c, g-k: 50 µm.
 262 Legend: R: ray. Arrowheads: tyloses.