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Research

Vascularization of the *Selaginella* rhizophore: anatomical fingerprints of polar auxin transport with implications for the deep fossil record

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Summary

- The *Selaginella* rhizophore is a unique and enigmatic organ whose homology with roots, shoots, or neither of the two remains unresolved. Nevertheless, rhizophore-like organs have been documented in several fossil lycophytes. Here we test the homology of these organs through comparisons with the architecture of rhizophore vascularization in *Selaginella*.
- We document rhizophore vascularization in nine *Selaginella* species using cleared whole-mounts and histological sectioning combined with three-dimensional reconstruction.
- Three patterns of rhizophore vascularization are present in *Selaginella* and each is comparable to those observed in rhizophore-like organs of fossil lycophytes. More compellingly, we found that all *Selaginella* species sampled exhibit tracheids that arc backward from the stem and side branch into the rhizophore base. This tracheid curvature is consistent with acropetal auxin transport previously documented in the rhizophore and is indicative of the redirection of basipetal auxin from the shoot into the rhizophore during development.
- The tracheid curvature observed in *Selaginella* rhizophores provides an anatomical fingerprint for the patterns of auxin flow that underpin rhizophore development. Similar tracheid geometry may be present and should be searched for in fossils to address rhizophore homology and the conservation of auxin-related developmental mechanisms from early stages of lycophyte evolution.

Introduction

Rhizophores, seen only in the lycophyte *Selaginella*, are downward growing axial organs located at branching points of the shoot and which produce roots near their apex upon reaching the soil (Jernstedt *et al.*, 1994). Developmentally, rhizophores originate exogenously from meristems positioned in the branching angle of the shoot, termed angle meristems. Two angle meristems form at each branching point, a dorsal and a ventral one, either or both of which can develop into a rhizophore. The homology of the *Selaginella* rhizophore has puzzled plant morphologists for well over a century, owing to a set of morphological and developmental characters that combines root and stem features. Stem-like features of the rhizophore include exogenous developmental origination (Jernstedt *et al.*, 1992), the expression of class 1 KNOX genes at the apex (like in the shoot apical meristem and unlike in the root apex of *Selaginella*; Kawai *et al.*, 2010), and the capacity of rhizophores and angle meristems to develop as shoots under specific conditions (Harvey-Gibson, 1902; Williams, 1937; Webster, 1969; Wochok & Sussex, 1976; Jernstedt *et al.*, 1994; Sanders & Langdale, 2013). However, like roots, the rhizophore does not typically produce leaves, it exhibits positive gravitropism, and has been demonstrated to have acropetal polar auxin transport (PAT) (Wochok & Sussex, 1974), an auxin transport pattern which in euphyllophytes is seen only in roots. Various authors have argued for or against interpreting the rhizophore as a stem homolog, a root, or as a novel *sui generis* organ, but currently there is no scientific consensus on the nature of the rhizophore (Jernstedt *et al.*, 1994, and references therein).

Although no structures comparable to the selaginellalean rhizophore are known in extant plants, the deep fossil record of the lycophyte clade includes several plants exhibiting two types of structures that share some features with the rhizophore. These include subaxillary tubercles and the branches derived from them, which have been reported in several zosterophyll-grade lycophytes of the Devonian: *Crenaticaulis*, *Gosslingia*, *Deheubarthia*, *Thrinkophyton* and *Anisophyton* (Høeg, 1942; Banks & Davis, 1969; Edwards, 1970, 1994; Edwards & Kenrick, 1986; Hass & Remy, 1986; Kenrick & Edwards, 1988; Edwards *et al.*, 1989). Such structures are regularly positioned in the immediate vicinity of, and basal to, the angle formed at branching points of axes, and for this reason they are also referred to as angular organs (Hass & Remy, 1986) (Fig. 1a). While the function and developmental fate of subaxillary tubercles is unknown in many of these taxa, branches derived from them may have functioned as rooting organs in some cases.

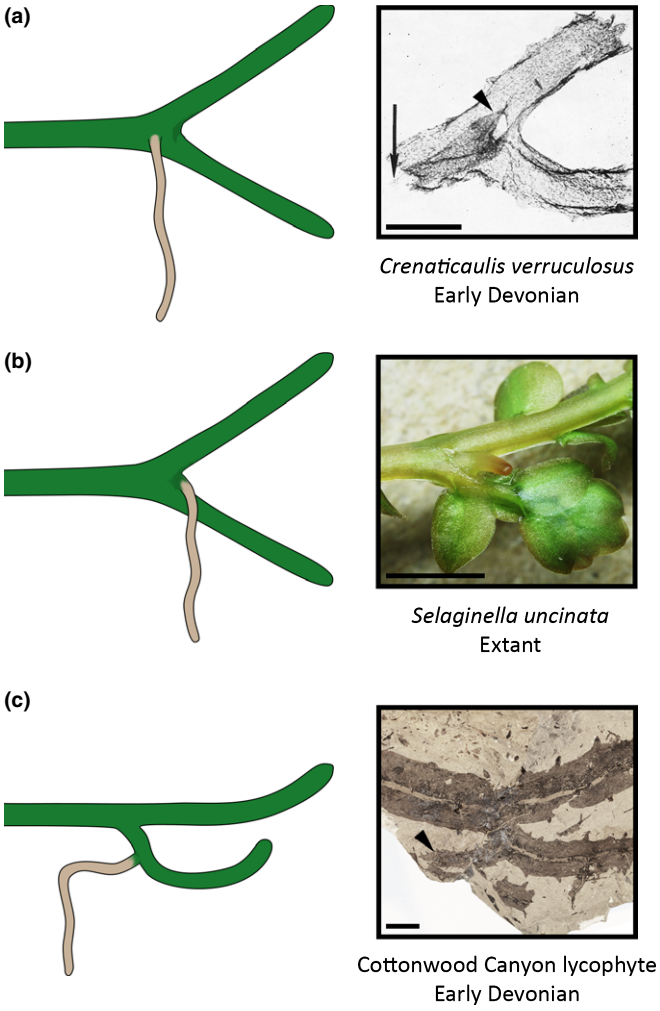

Fig. 1 Branching patterns associated with rhizophore-like structures among lycophytes. (a) Angular organs of basal lycophytes. Diagrammatic example (at left) shows the divergence of angular organs proximal to the branching angle of axes. In fossils of *Crenaticaulis verruculosus* (at right) the divergence point of the angular organ, which overlaps the main axis, is indicated by the arrowhead (Banks & Davis, 1969, fig. 18; published with permission from the Botanical Society of America). Bar, 5 mm. (b) Divergence of the *Selaginella uncinata* rhizophore occurs laterally in the immediate vicinity of the branching angle of the shoot. Bar, 2 mm. (c) Rooting axes derived from K-branches arise from lateral branches of the shoot (arrowhead), shown here in a drepanophycalean lycophyte from Cottonwood Canyon, Wyoming, USA. Bar, 10 mm.

Additionally, there are the root-bearing axes of some Devonian drepanophycalean lycophytes (Schweitzer & Giesen, 1980; Xu *et al.*, 2013; Matsunaga & Tomescu, 2016, 2017). These are produced by two closely spaced dichotomies of the shoot, in a manner that is referred to as H- or K-branching (Fig. 1c), and resemble morphologically equivalent nonroot-bearing axes with inferred downward growth seen in some Devonian zosterophylls: *Zosterophyllum*, *Sawdonia* and *Bathurstia* (Lang, 1927; Walton, 1964; Rayner, 1983; Gensel *et al.*, 2001; Hao *et al.*, 2010).

Like the *Selaginella* rhizophore (Fig. 1b), both the subaxillary axes and those produced by K-branching, documented in fossil plants, (1) arise consistently in association with branching points of the subtending axis; and (2) develop, in many cases, in a direction different from that of the regular axes or shoots of the parent plant. Subaxillary branches have been found to extend in a direction perpendicular to the plane defined by the forking of the subtending axis (e.g. Banks & Davis, 1969; Kenrick, 1988) and the axes arising from K-branching point away from the regular axes or shoots of the parent plant, which sometimes means downwards (Walton, 1964; Rayner, 1983; Gensel *et al.*, 2001; Hao *et al.*, 2010; Matsunaga & Tomescu, 2016). In such cases, the downward direction of growth is probably cued by a positive gravitropic response (Walton, 1964; Matsunaga & Tomescu, 2016), like that of the *Selaginella* rhizophore. Furthermore, like the rhizophore, some drepanophycalean axes arising from K-branching bear roots (Schweitzer & Giesen, 1980; Xu *et al.*, 2013; Matsunaga & Tomescu, 2016).

The notable similarities between rhizophores and the organs described above have prompted direct comparisons with *Selaginella* (Banks & Davis, 1969; Rayner, 1983; Hass & Remy, 1986; Kenrick, 1988; Edwards *et al.*, 1989). However, these similarities have not been seriously explored due to the great phylogenetic distance between *Selaginella* and the fossil lycophytes characterized by subaxillary branching and K-branching (Kenrick & Crane, 1997). Nevertheless, while *Selaginella* is highly derived among lycophytes, the existence of shared morphological patterns that span broad ranges of systematic diversity within the clade indicates that fundamental body plan features can be conserved over hundreds of million years of evolutionary history. For instance, morphological identity and homology of the rooting system of extant *Isoetes* with that of Paleozoic rhizomorphic lycophytes (e.g. *Paralycopodites* and *Sigillaria*; Stewart, 1947; Karrfalt, 1980; Jennings *et al.*, 1983; Rothwell & Erwin, 1985; Hetherington *et al.*, 2016; Hetherington & Dolan, 2017) demonstrates conservation of developmental characters and body plan organization over > 300 Myr. Or, to consider an even broader context, every major lineage of lycophytes exhibits rooting systems derived from or incorporating downward growing stems or undifferentiated axes (Matsunaga & Tomescu, 2017). Together, these observations imply that hypotheses of homology between the *Selaginella* rhizophore and the rhizophore-like organs of early lycophytes should not be regarded as far-fetched, and that addressing them within a comparative morphology framework is a worthy pursuit.

Branching patterns and vascular architecture have been documented in basal lycophytes with rhizophore-like organs (e.g. Lang, 1927; Banks & Davis, 1969; Edwards, 1970; Kenrick, 1988; Matsunaga & Tomescu, 2016), but comparable studies that can address the question of homology have not been conducted on *Selaginella*. Here we test the hypothesis of homology between the selaginellalean rhizophore and rhizophore-like organs of early lycophytes based on comparative anatomy. We further explore whether anatomical signatures unique to rhizophore development, related to the hormone auxin and applicable to studying the plant fossil record, are present in *Selaginella*.

Materials and Methods

We surveyed the architecture of rhizophore vascularization in nine *Selaginella* species kept in the Dennis K. Walker Glasshouse

(Humboldt State University): *S. involvens*, *S. wallacei*, *S. kraussiana*, *S. pallescens*, *S. delicatula*, *S. doederleinii*, *S. wildenovii*, and two additional species. While the latter two could not be identified and are hereafter referred to as *Selaginella* sp. 1 and *Selaginella* sp. 2, they are distinctly different from the other seven. Information on the origin and identity of these two species was lost from the glasshouse records, and *Selaginella* species (totaling *c.* 700–800 world-wide; Zhou *et al.*, 2016) are notoriously difficult to identify in the absence of information on their geographic origin. The *Selaginella* species sampled here span phylogenetic diversity within the family, covering six different clades as resolved in the phylogeny of Weststrand & Korall (2016), and include species with both dorsal and ventral rhizophores and a range of stele types (Korall & Kenrick, 2002, 2004; Weststrand & Korall, 2016).

Shoot segments centered around branching points were sampled from each species and prepared by clearing and by paraffin embedding and serial sectioning on a rotary microtome. For clearing we followed Ruzin's (1999) sodium hydroxide–chloral hydrate protocol, including full-strength commercial bleach (sodium hypochlorite), followed by safranin staining, and dehydration in an ethanol series; slides were mounted in epoxy resin. Paraffin embedding followed the standard protocol for plant tissue. Serial sections were cut at 10 μm thickness and stained using Walker's Sam quadruple stain protocol, which uses Weigert's iron hematoxylin (1% in 10% ethanol), followed by Bismark Brown (1% in 50% ethanol), then phloxine (1% in 95% ethanol) and 1 : 1 Fast Green – Orange G (saturated in clove oil). Slides were mounted using Eukitt mounting medium (O. Kindler GmbH, Freiburg, Germany).

Micrographs were taken using a Nikon Coolpix E8800 digital camera mounted on a Nikon Eclipse E400 compound microscope (Nikon Inc., Melville, NY, USA) and an Olympus DP73 digital camera on an Olympus SZX16 microscope (Olympus, Center Valley, PA, USA). High-magnification images of tracheids in *Selaginella* were digitally stacked along the *z*-axis to provide a consistent focal plane. Focal stacking and figure construction were done in Photoshop CC (Adobe, San Jose, CA, USA). Volume renderings based on serial sections were produced using Amira 3D software (FEI, Hillsboro, OR, USA).

Results

Three patterns of rhizophore vascularization

In the nine *Selaginella* species we surveyed, the architecture of the vascular supply of the rhizophore falls into three main types: (1) the vascular bundle supplying the rhizophore (rhizophore trace) diverges centrally, from the angle between stem stele and branch stele (referred to as central divergence); (2) the rhizophore trace diverges from the stele of the main stem, close to but above the divergence of the branch stele (stem divergence); and (3) the rhizophore trace diverges from the stele of the side branch above the divergence of the latter from the main stem stele (branch divergence). These patterns are independent of the external position of rhizophores or angle meristems, which are always positioned in the branching angle. Of the nine species, seven exhibit central divergence, one species is characterized by stem divergence (*S.* sp. 2), and one by branch divergence (*S. wallacei*) (Table 1). While the number of meristeles (one to three) forming the stele of the main stem (Table 1) adds complexity to the architecture of vascularization in some cases, this does not interfere with the three main types of rhizophore vascularization. We describe below the architecture of rhizophore vascularization in four species that illustrate the range of variation observed: *Selaginella kraussiana* (two meristeles) and *S. wildenovii* (three meristeles) for central divergence, *S.* sp. 2 (variable, one or two meristeles) for stem divergence, and *S. wallacei* (one meristele) for branch divergence.

Selected species descriptions

S. kraussiana stems have two meristeles (Fig. 2a). At shoot branching points, one of the meristeles of the main stem splits to produce two vascular segments. One of the segments continues as one of the main stem meristeles while the other segment fuses with the second meristele producing a plexus of vascular tissue. From this plexus three vascular segments diverge and continue distal to the branching point: the second main stem meristele, the stele of the side branch and the stele of the rhizophore. The three diverge at the same level, with the rhizophore stele diverging from the angle between the other two and extending laterally and perpendicular to the plane defined by the main stem and lateral branch (Fig. 3a,b; Supporting Information Video S1).

S. wildenovii stems have three meristeles represented by plates of vascular tissue flattened in the plane of shoot branching (defined by the main stem and lateral branch) (Fig. 2b). At shoot branching points, each stem meristele diverges to give rise to one vascular segment that becomes a meristele of the lateral branch. At the same level, the three meristeles anastomose. The plexus of vascular tissues connecting them forms a bridge. This bridge extends vertically in the angle between the stem and lateral branch, forming a ridge of vascular tissue developed in a direction perpendicular to the plane of branching. The vascular supply of the two rhizophores diverges from the two ends of this bridge, laterally and perpendicular to the plane of branching (Fig. 3c,d; Video S2). Overall, the rhizophore vascular strands thus diverge from a central position in the angle between the steles of the main stem and lateral branch.

Table 1 The position of rhizophore divergence in nine species of *Selaginella* surveyed

Selaginella species	No. of meristeles	Position of rhizophore divergence
S. involvens	1	Central
S. wallacei	1	Lateral branch
S. kraussiana	2	Central
S. pallescens	2	Central
S. sp. 1	2	Central
S. sp. 2	1 or 2	Main stem
S. delicatula	3	Central
S. doederleinii	3	Central
S. wildenovii	3	Central

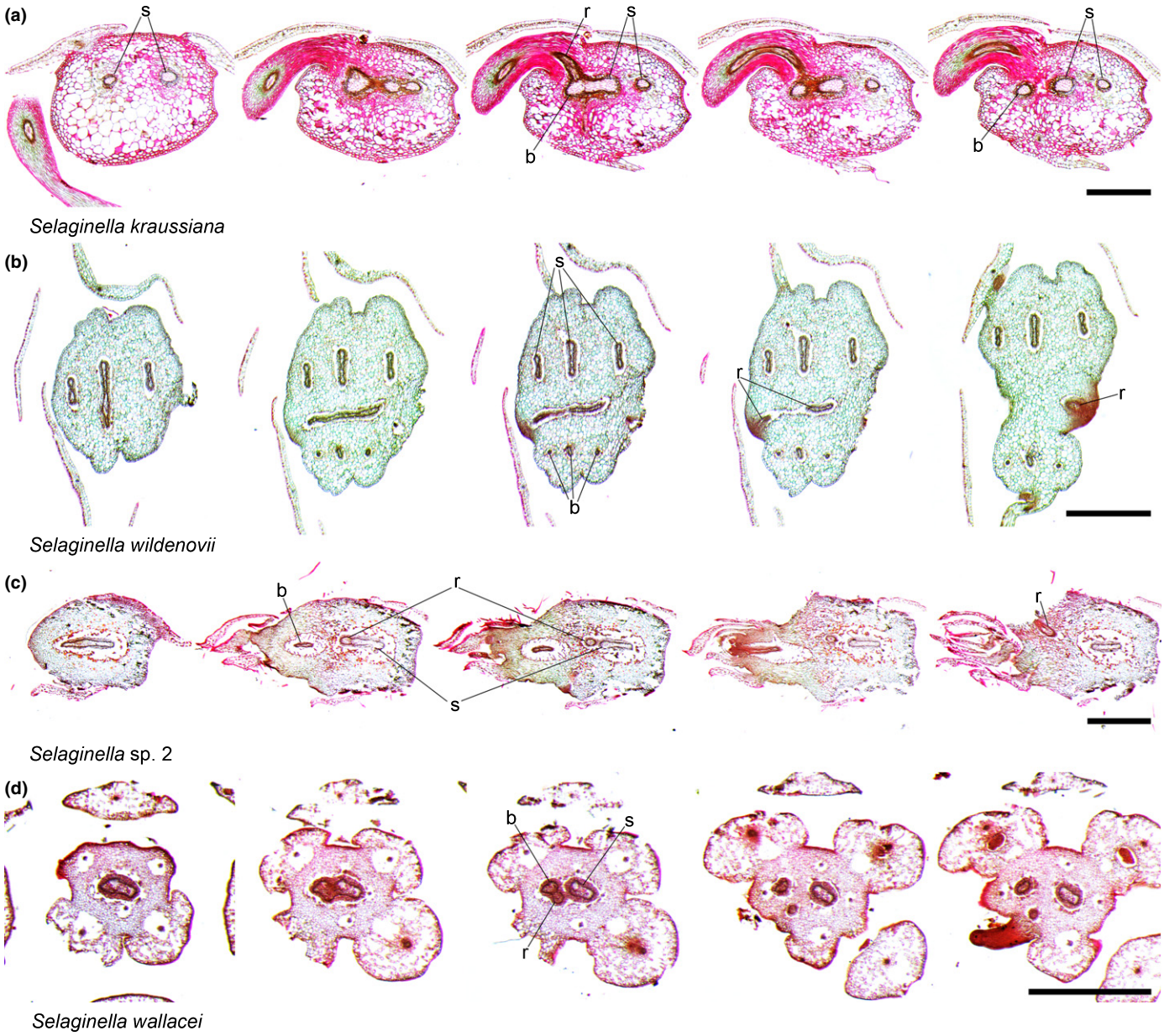

Fig. 2 Serial cross sections through branching points of four *Selaginella* species showing the vascular architecture of rhizophore vascularization. (a) *Selaginella kraussiana*. The main stem has two meristeles (s) and the rhizophore stele (r) arises from the divergence point of the main stem and lateral branch (b) steles. Bar, 500 μm. (b) *Selaginella wildenovii* has a complex stelar architecture consisting of three meristeles in both the main stem and the lateral branch. Two rhizophores are produced, and diverge centrally from the angle between the steles of the main stem and lateral branch (see also Fig. 3g,h). Bar, 500 μm. (c) *Selaginella* sp. 2 showing divergence of the rhizophore stele from the stele of the main stem, distal to the divergence of the lateral branch. Bar, 500 μm. (d) *Selaginella wallacei*. The rhizophore stele diverges from the stele of the lateral branch. Bar, 500 μm.

In *S.* sp. 2 rhizophores are produced at branching points of shoots that have either one meristele (Fig. 2c) or two meristeles. In both cases, the vascular supply of the rhizophore arises from the stele of the main stem, distal to the divergence of the lateral branch. The rhizophore stele initially diverges from the stem stele in the plane of shoot branching and in the same direction as the stele of the branch (Fig. 2c); distally, the rhizophore stele curves in a direction perpendicular to the plane of branching (Fig. 3e,f; Video S3).

S. wallacei stems are characterized by a simple stele consisting of a single vascular segment (Fig. 2d). In this species, the rhizophore stele diverges from the stele of the lateral branch, and therefore distal to the divergence of the branch stele from the stele of the main stem. Furthermore, the direction of rhizophore divergence is oblique to the plane of shoot branching (Fig. 2d, 3g,h; Video S4).

Rhizophore divergence and tracheid geometry

In all the species surveyed, the shape, arrangement and orientation of tracheids at the point of rhizophore divergence exhibit a consistent geometry. The xylem bundle of the rhizophore is

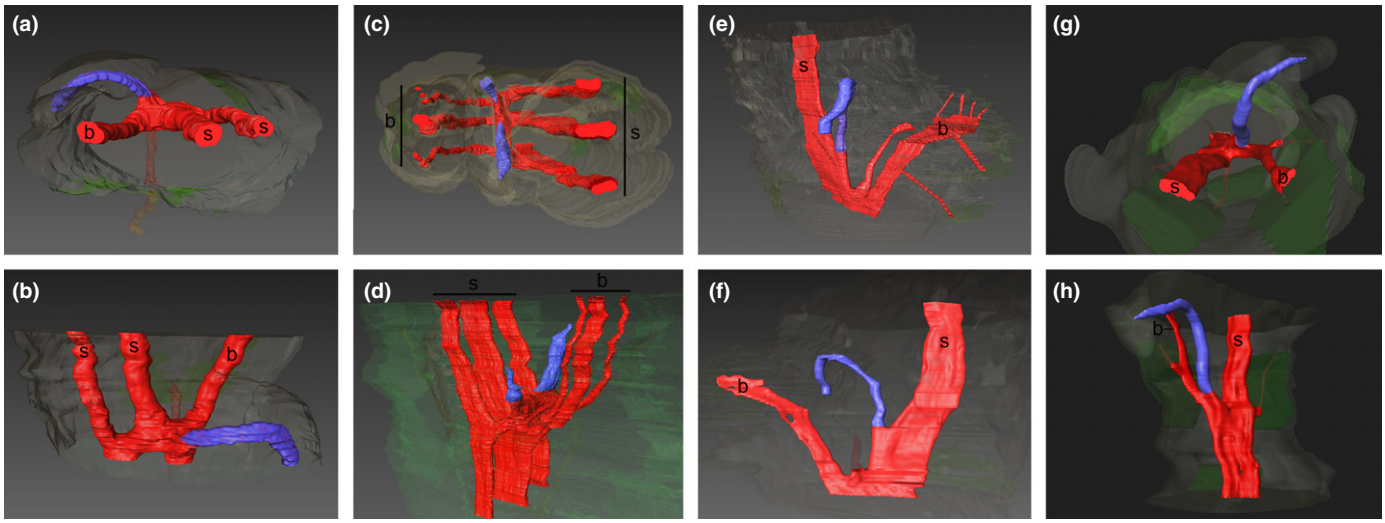

Fig. 3 Three-dimensional reconstructions of the vascular architecture at rhizophore divergence in *Selaginella kraussiana* (a, b), *Selaginella wildenovii* (c, d), *Selaginella* sp. 2 (e, f) and *Selaginella wallacei* (g, h). (a, c, g) Views looking down at branching points from positions distal to them. (b, f, h) Views in the horizontal plane from positions lateral to branching points. (d), (e) are oblique views from positions slightly above branching points. The stele of the main stem (s) and lateral branch (b) are shown in red, while the rhizophore stele is shown in blue. Note the presence of more than one rhizophore trace in *S. wildenovii*. Leaf traces are shown translucent (red) in (a, b, f–h) for clarity. Green structures on the periphery of the stems represent leaves, or parts of leaves, present on the stem segments.

connected to the subtending shoot by tracheids that arc from the stele of the main shoot and branch shoot back into the rhizophore vascular supply, toward the rhizophore apex (Fig. 4a–c, g–k). This consistent pattern is very conspicuous starting with early stages of rhizophore development and, when two rhizophores are associated with one branching point, is seen in the vascular supply of both rhizophores. It is worth noting that in some cases the high tracheid density at branching points, especially when associated with thick sclerified cortical tissues that hindered clearing, obscured fine details of tracheid arrangement and shape, making it difficult to obtain good photographs that illustrate the curved tracheids clearly (Fig. 4l). Importantly, the geometry of tracheids associated with rhizophore divergence is clearly different from the geometry of tracheids documented around leaf traces and shoot branching points that lack rhizophores. In these instances, tracheids consistently form a basipetally convergent pattern with no tracheids that arc back apically (Fig. 4d–f).

Discussion

The architecture of the rhizophore vascular supply is variable

The rhizophore-like axes of Devonian lycophytes exhibit several patterns of vascular architecture. These patterns provided the starting point for our comparative approach to the homology of the *Selaginella* rhizophore. In some plants that produce subaxillary branches, such as *Gosslingia breconensis*, the vascular supply of the subaxillary branch diverges from the stele of the main axis distal to the divergence of the branch stele (Edwards, 1970) (Fig. 5a). In lycophytes with K-branching, the vascular strand of the rhizophore-like organ diverges from the stele of the side branch (Matsunaga & Tomescu, 2016) (Fig. 5b). Finally, in other lycophytes with subaxillary branches, such as *Deheubarthia splendens* (Edwards *et al.*, 1989), the vascular supply of the subaxillary branch seems to diverge centrally, from in between and at the same level as the stele of the main axis and the stele of the side branch (Fig. 5c).

The different patterns of vascular architecture at rhizophore divergence, documented in the nine *Selaginella* species, cover all three types of architecture seen in the vascular supply of Devonian rhizophore-like axes (Table 1). The pattern documented in *S.* sp. 2 (stem divergence) corresponds to the architecture of *Gosslingia* subaxillary branches; the pattern seen in *S. wallacei* (branch divergence) fits the vascular architecture of K-branching; the central divergence observed in the other seven *Selaginella* species is comparable to the architecture of the subaxillary branch vascular supply in *Deheubarthia*. This broad range of variation documented within *Selaginella*, along with the taxonomic diversity of Devonian lycophytes that exhibit matching patterns, indicates that vascular architecture cannot directly provide useful evidence relevant to discussions of homology between Devonian rhizophore-like axes and the *Selaginella* rhizophore. However, the variation seen in *Selaginella* indicates that a broad spectrum of vascular patterns can be produced by the same set of developmental processes – in this case those associated with rhizophore development.

Tracheid geometry and patterns of auxin transport in the development of the *Selaginella* rhizophore

PAT is the unidirectional flow of the hormone auxin and is mediated by PIN auxin efflux carriers positioned in the plasma membrane. The localization of PIN proteins at one end of the cell polarizes and canalizes the flow of auxin away from a source of

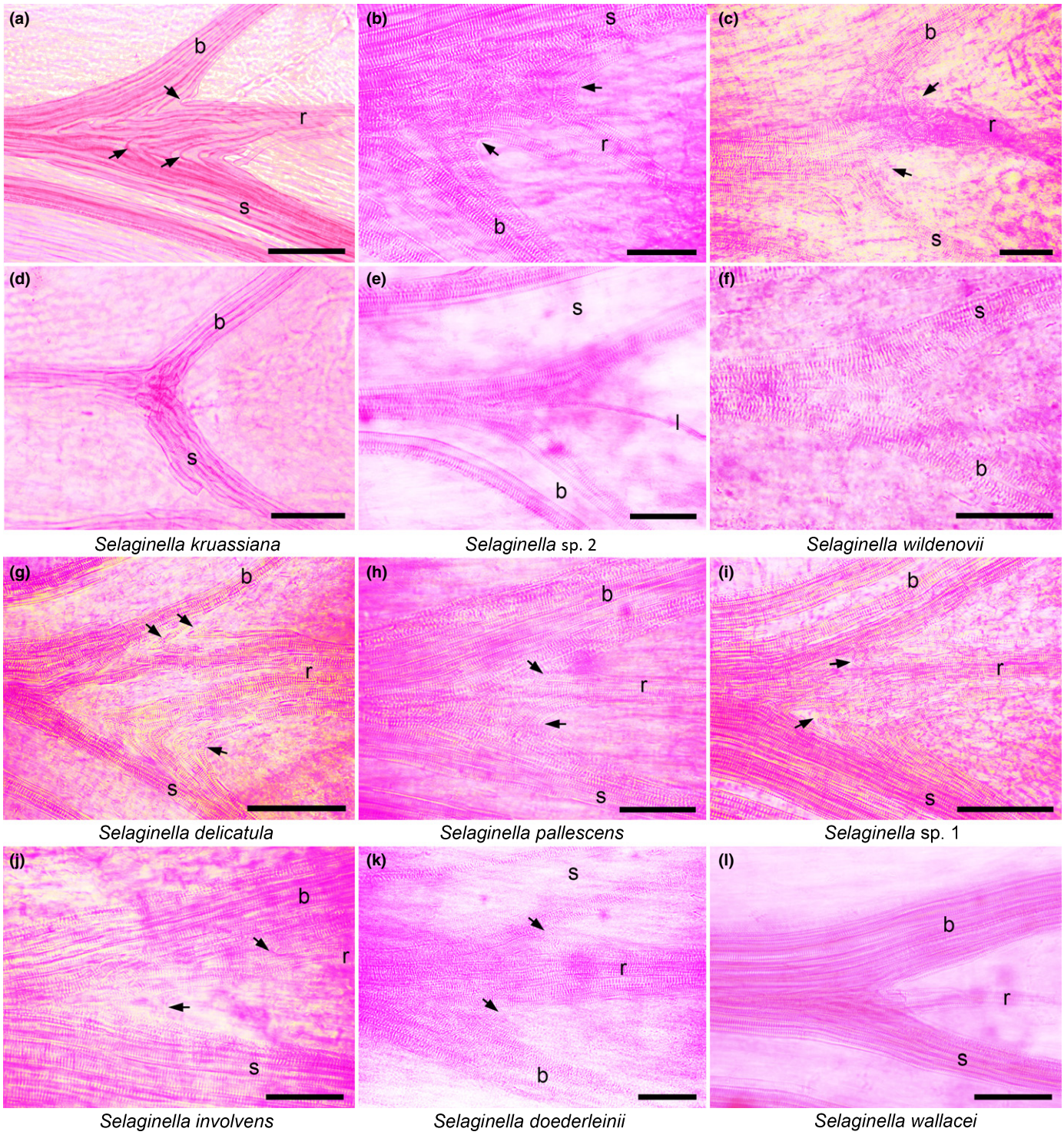

Fig. 4 Tracheid curvature at shoot–rhizophore junctions in nine *Selaginella* species. Comparisons between branching points with rhizophores (a–c, g–l) and those without rhizophores (d–f). Note the arcing of individual tracheids (arrows) from the stele of the main stem (s) and lateral branches (b) into the rhizophore stele (r), and the absence of such tracheids in branching points without rhizophores. The thin vascular strand (l) in (e) represents a leaf trace and not a rhizophore trace. Also note that curvature is more apparent in some species than in others, and cannot always be clearly shown (e.g. in l) owing to tracheid density, the orientation of mounted specimens or the limitations on photographing a thick three-dimensional structure through multiple focal planes. (a) *Selaginella kraussiana*; bar, 250 μm. (b) *Selaginella* sp. 2; bar, 50 μm. (c) *Selaginella wildenovii*; bar, 50 μm. (d) *S. kraussiana* branching point without a rhizophore; bar, 100 μm. (e) *S.* sp. 2, no rhizophore; bar, 50 μm. (f) *S. wildenovii*, no rhizophore; bar, 50 μm. (g) *Selaginella delicatula*; bar, 100 μm. (h) *Selaginella pallescens*; bar, 50 μm. (i) *Selaginella* sp. 1; bar, 100 μm. (j) *Selaginella involvens*; bar, 50 μm. (k) *Selaginella doederleinii*; bar, 50 μm. (l) *Selaginella wallacei*; bar, 50 μm.

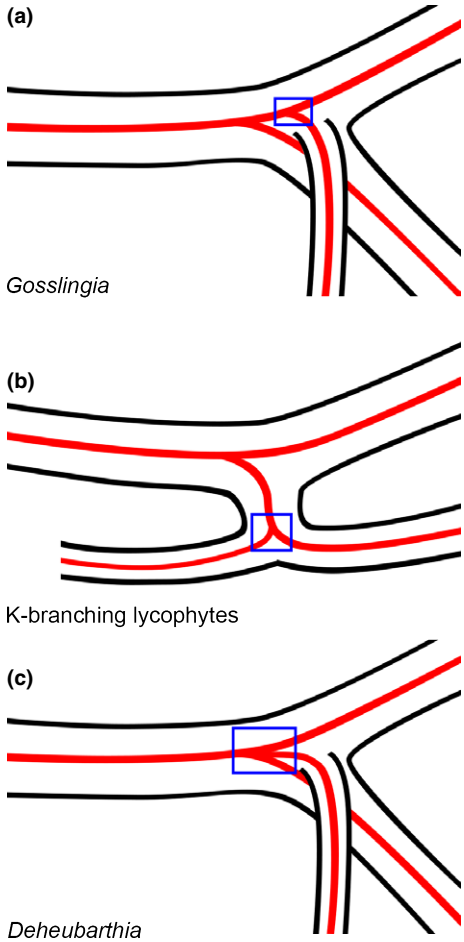

Fig. 5 Diagrammatic representations of vascular architecture in basal lycophytes with rhizophore-like rooting structures: zosterophyll-grade lycophytes – *Gosslingia* (a) and *Deheubarthia* (c) – with subaxillary branches (angular organs) and basal lycophytes with K-branching (b). *Gosslingia* exhibits stem divergence of the vascular supply to the rhizophore-like axis, K-branching involves branch divergence and *Deheubarthia* exhibits central divergence. If these rooting structures exhibit acropetal polar auxin transport resulting from redirection of auxin from the shoot system, then curved tracheids should be found in the regions indicated by the blue boxes.

higher concentration, leading to elongation of those cells that transport auxin and their differentiation as vascular tissues (Leyser & Day, 2003; Bennett *et al.*, 2014). Polar gradients of auxin within the plant body have been demonstrated, through numerous auxin application and inhibition experiments, as sufficient for inducing vascular tissue differentiation in plants (e.g. Wangermann, 1967; Sachs, 1969; Mattsson *et al.*, 1999; Berleth *et al.*, 2000). Moreover, the auxin flux through tissues is responsible for the shape and orientation of xylem tracheary elements, as documented in both natural and experimental conditions (Sachs, 1981; Sachs & Cohen, 1982; Lev-Yadun & Aloni, 1990). Specifically, developing tracheids elongate along a polar auxin gradient. The direct relationship between PAT and vascular architecture indicates that vascular tissues provide an anatomical record of physiological processes, i.e. polar auxin transport during development.

Patterns of PAT have been documented for both shoots and rhizophores of *Selaginella*. In shoots, PAT is basipetal, consistent with patterns of PAT documented in shoots of seed plants (Wochok & Sussex, 1973; Sanders & Langdale, 2013). By contrast, PAT is acropetal in the rhizophore, as in the roots of seed plants (Wochok & Sussex, 1974). These patterns of PAT are consistent with the orientation of tracheids at rhizophore junctions, in which tracheids arc from the steles of the main stem and side branch into the rhizophore vascular supply (Fig. 4). Together, these suggest the following developmental scenario: at the rhizophore–shoot junction, auxin is redirected from its basipetal flow in the main stem and lateral branch to flow acropetally into the rhizophore, and these changes in auxin flow are recorded in the geometry of xylem tracheids (Fig. 6). This pattern is seen in all species sampled, regardless of whether the rhizophore develops from a dorsal or ventral angle meristem. Although the specific polarity of auxin flow between rhizophores and shoots (e.g. from shoot to rhizophore vs rhizophore to shoot) cannot be directly inferred based on tracheid curvature alone, our interpretation is the only one that is consistent with the patterns of auxin transport documented in *Selaginella*.

There is a significant amount of experimental evidence suggesting that angle meristem specification and rhizophore development are at least partially under the control of, and can be manipulated by, auxins (Williams, 1937; Webster, 1969; Wochok & Sussex, 1973, 1974, 1976; Jernstedt *et al.*, 1994; Sanders & Langdale, 2013) – higher concentrations of auxin are associated with development of the angle meristems into rhizophores and maintenance of rhizophore identity, while lower auxin concentrations or auxin inhibition are correlated with development of shoots. We suspect that the developmental identity of angle meristems (shoot vs rhizophore) is specified, at least in part, by the local distribution of auxin concentrations around branching points. At least one study has shown differences in auxin concentrations on dorsal vs ventral sides of *Selaginella* shoots at branching points (Wochok & Sussex, 1973), where the pattern of converging meristeles may generate an asymmetric distribution of auxin. It will be interesting to test, in the future, (1) whether dorsi-ventral variation in auxin concentrations at shoot branching points is correlated with dorsal and ventral rhizophore position, and (2) if auxin maxima are formed at rhizophore apices, like they are in roots (e.g. Petrasek & Friml, 2009; Robert & Friml, 2009; Hayashi *et al.*, 2014). Answers to these questions would provide important insights into rhizophore development and, more broadly, into developmental pathways common to all rooting structures, regardless of homology.

An anatomical fingerprint for testing hypotheses on the evolution of the lycophyte body plan

The arced tracheid geometry we documented in *Selaginella* at the shoot–rhizophore junction is consistent with vascular patterns associated with changes in the polarity of auxin transport in flowering plants (Sachs, 1981). More broadly, this provides another example of tracheid geometry serving as an anatomical fingerprint (e.g. Rothwell *et al.*, 2014) for patterns of auxin flow during organ

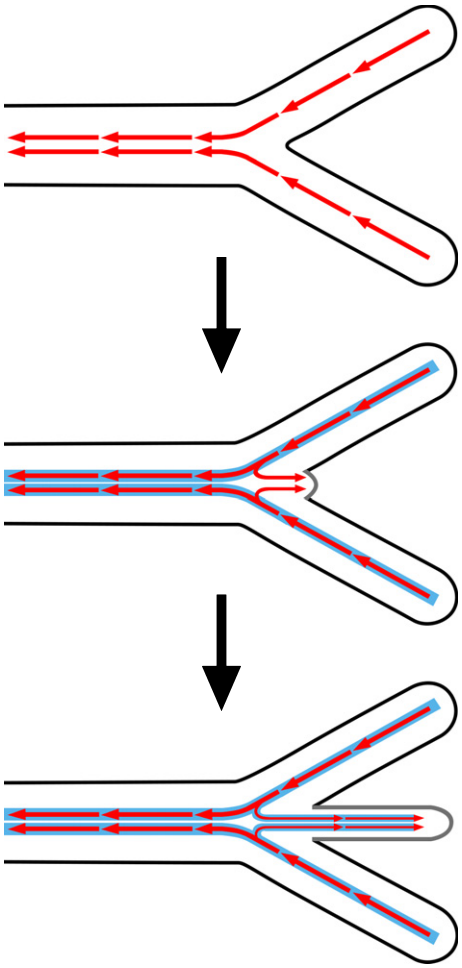

Fig. 6 Patterns of polar auxin transport at the rhizophore–shoot junction. Auxin is redirected from its basipetal flow in the main stem and lateral branch to flow acropetally into the rhizophore. This redirection is reflected in the geometry of tracheids at the base of the rhizophore: these tracheids arch from the steles of the main stem and lateral branch into the rhizophore stele. Red arrows indicate the flow of auxin and solid blue lines represent differentiated vascular tissues.

development. A well-documented example of this uses the 'auxin swirls' seen in the wood of several plant lineages that evolved secondary growth independently (lignophytes (progymnosperms and seed plants), lycophytes and sphenophytes; Lev-Yadun & Aloni, 1990; Rothwell & Lev-Yadun, 2005; Rothwell *et al.*, 2008; Decombeix *et al.*, 2010) to infer a shared underlying mechanism of polar auxin transport through the cambial layer (Rothwell & Lev-Yadun, 2005; Sanders *et al.*, 2011; Rothwell *et al.*, 2014).

Sanders *et al.* (2011) used the same anatomical fingerprint, auxin swirls, to demonstrate that rhizomorphs, the rooting organs of extinct lepidodendralean lycophytes, had acropetal PAT. Aside from lepidodendralean rhizomorphs, acropetal PAT has only been demonstrated in the *Selaginella* rhizophore (Wochok & Sussex, 1974) and is well known in the roots of seed plants. Interestingly, whereas these three types of organs are not homologous (the rhizomorph is best explained as a shoot-homolog modified for rooting – Rothwell & Erwin (1985) – and the homology of the rhizophore is unresolved), they all serve rooting functions and exhibit positive gravitropism. Based on these we hypothesize that acropetal PAT is independent of organ identity and is correlated with positive gravitropic responses in organs with roles in absorption and anchoring. An interesting test of this hypothesis would be to investigate the polarity of auxin flow in the positively gravitropic rhizomes seen in the monocot *Cordyline*, for which developmental plasticity of axillary buds borne on horizontal rhizomes is at least in part controlled by auxin concentrations (Fisher, 1972), as well as in tubers of the lycophyte *Phylloglossum* – downward growing shoots modified for starch storage and perennation (Bower, 1885; Eames, 1936).

Because auxin is an important regulator of plant development, anatomical signatures of PAT in vascular tissues provide important means of studying developmental mechanisms in the fossil record. If acropetal PAT is associated with positively gravitropic organs independent of organ identity, then we can hypothesize that the rhizophore-like organs of early lycophytes – subaxillary branches with rooting function and rooting axes derived from K-branches – must have had acropetal PAT regardless of their developmental homology as stems or above-ground axes. The tracheid geometry of the shoot–rhizophore junction in *Selaginella* provides an excellent anatomical fingerprint that can be used to test this hypothesis (Fig. 5). Xylem tracheary elements have among the highest fossil preservation potential of all plant tissues and are preserved even in the oldest known vascular plant fossils (Edwards *et al.*, 1992). Thus, arcing tracheids such as those seen at the base of the *Selaginella* rhizophore could, and should, be searched for in the vascular tissues of anatomically preserved fossils of basal lycophytes with rhizophore-like organs (Fig. 5). The anatomy of subaxillary branch vascularization in *Gosslingia breconensis*, as documented by Edwards (1970), is encouraging in this respect and re-examination of those specimens could be a first step in the hypothesis-testing process. Should such tracheids be discovered, they would provide evidence for shared developmental mechanisms and patterns of PAT.

Conclusions

The rhizophore of *Selaginella* is a unique organ that has putative counterparts in fossil basal lycophytes of the Devonian period. A survey of the anatomy of rhizophore vascularization in nine *Selaginella* species revealed that the rhizophore stele can diverge in three positions in the branching angle of a shoot, depending on the species: centrally, from the angle formed by the steles of the main stem and lateral branch; from the stele of the main stem, distal to the divergence of the branch stele; or from the stele of the lateral branch. This broad range of anatomical variation within the genus precludes the use of the architecture of rhizophore vascularization as a feature relevant to discussions of the homology of rhizophore-like axes identified in Devonian basal lycophytes, but nevertheless indicates that the same set of developmental processes can produce a wide range of vascular architectures.

Concurrently, this investigation revealed evidence for an anatomical fingerprint of physiological processes involved in the development of the rhizophore. This fingerprint consists of arced tracheids that connect the stele of the rhizophore to the steles of

the main stem and lateral branch. The geometry of these tracheids reflects redirection of the basipetal PAT of the stem and branch into the acropetal auxin stream of the rhizophore. This anatomical fingerprint has potential for applications in the study of Devonian lycophytes with rhizophore-like organs, in which it could be used to infer patterns of PAT. Documenting patterns of PAT is relevant to characterizing developmental patterns and mechanisms in these fossil plants. In turn such information is crucial for addressing hypotheses of homology over broad taxonomic scales and the evolution of development in deep time.

Applying knowledge of plant development derived from studies of extant plants to the study of fossils is a necessary step in advancing our understanding of the evolution of plant development. In studies of animals, integration of morphological and anatomical data from the fossil record with developmental and genetic data from extant organisms has played an important role in the assembly of comprehensive evo-devo perspectives that span phylogeny and geologic time, and wherein features of the fossils are used to understand the evolution of developmental regulators and vice versa (e.g. Shubin, 2008; Erwin & Valentine, 2013). However, similar progress in plant studies lags behind. As we advance our understanding of how plant physiology and developmental processes are reflected in anatomy, investigating these aspects of plant biology in the deep fossil record will become more feasible. This will provide an important means for harnessing the rich store of data inherent in the fossil record, and enable us to both generate and test hypotheses on development and homology in the evolution of plant body plans.

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Author contributions

K.K.S.M., N.P.C. and A.M.F.T. planned and designed the research. N.P.C. and A.M.F.T. collected and analyzed the data. K.K.S.M. and A.M.F.T. analyzed the data and wrote the manuscript. K.K.S.M. rendered figures and illustrations.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information tab for this article:

Video S1 Three-dimensional reconstruction of *Selaginella kraussiana*.

Video S2 Three-dimensional reconstruction of *Selaginella wildenovii*.

Video S3 Three-dimensional reconstruction of *Selaginella* sp. 2.

Video S4 Three-dimensional reconstruction of *Selaginella wallacei*.

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