How green was *Cooksonia*? The importance of size in understanding the early evolution of physiology in the vascular plant lineage

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Abstract.—Because of the fragmentary preservation of the earliest *Cooksonia*-like terrestrial plant macrofossils, younger Devonian fossils with complete anatomical preservation and documented gametophytes often have received greater attention concerning the early evolution of vascular plants and the alternation of generations. Despite preservational deficits, however, possible physiologies of *Cooksonia*-like fossils can be constrained by considering the overall axis size in conjunction with the potential range of cell types and sizes, because their lack of organ differentiation requires that all plant functions be performed by the same axis. Once desiccation resistance, support, and transport functions are taken into account, smaller fossils do not have volume enough left over for an extensive aerated photosynthetic tissue, thus arguing for physiological dependence on an unpreserved gametophyte. As in many mosses, axial anatomy is more likely to have ensured continued spore dispersal despite desiccation of the sporophyte than to have provided photosynthetic independence. Suppositions concerning size constraints on physiology are supported by size comparisons with fossils of demonstrable physiological independence, by preserved anatomical detail, and by size correlations between axis, sporangia, and sporangial stalk in Silurian and Early Devonian taxa. Several *Cooksonia*-like taxa lump fossils with axial widths spanning over an order of magnitude—from necessary physiological dependence to potential photosynthetic competence—informing understanding of the evolution of an independent sporophyte and the phylogenetic relationships of early vascular plants.

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Introduction

Much is unknown about the ecology and physiology of the oldest preserved macrofossil land plants. These cooksonioid fossils first appear in the Wenlockian (Edwards and Feehan 1980; Edwards et al. 1983) and remain a common component of the paleoflora through the rest of the Silurian and the Early Devonian (Edwards 1973, 1979a). These fossils consist of unadorned axes that can branch isotomously, are terminated by sporangia, and are quite small—often of mesofossil proportions. The small size of the axes and their limited number of dichotomies has led to distinct interpretations of plant habit, ranging from the fossils representing determinate, aerial axes broken from indeterminately growing rhizomes not preserved (Niklas 1997; Kenrick and Davis 2004) to complete, determinate sporophytes rooted in a gametophyte, although without discussion of whether sporophyte physiological dependence extended beyond water and nutrient acquisition from the substrate (Mish-

ler and Churchill 1985; Rothwell 1995). That cooksonioid fossils are small but relatively intact sporophytes is supported by their uniform size in beds containing many fossils and by spore morphologies distinct from the larger taxa of which they could conceivably be distal fragments (Edwards 1996). Their most common treatment is an intermediate one in which the plants are considered small and determinate but without implication of gametophyte dependence for rooting and substrate interaction (Edwards and Fanning 1985; Edwards 1996). (Originally reflecting equivocal evidence for vasculature [Taylor 1988] and similar to the more common ''rhyniophytoid'' [Edwards 1996], ''cooksonioid'' here denotes only simple, smaller taxa usually less than 1 mm in width and lacking signs of independent growth described below, thereby including *Cooksonia*, *Salopella*, *Tarrantia*, *Tortilicaulis*, but excluding larger fossils such as *Aglaophyton*.)

Despite their obvious centrality for under-

standing the early evolution of the vascular plant lineage, the importance of these cooksonioid fossils has in many ways been overwhelmed by the abundance of information from the Lower Devonian Rhynie Chert of Scotland, almost 50 Myr younger than the oldest cooksonioids. The Rhynie Chert provides the earliest detailed anatomy (Edwards 1993) for a diverse flora (Kidston and Lang 1917, 1920a,b; Lyon and Edwards 1991; Powell et al. 2000; Kerp et al. 2001), which has in turn informed phylogenetic understanding (Gensel 1992; Kenrick and Crane 1997) and allowed well-constrained modeling of physiological function (Konrad et al. 2000; Roth-Nebelsick 2001; Roth-Nebelsick and Konrad 2003), together indicating an ancestral condition with little tissue diversity or organ differentiation but general photosynthetic and physiological continuity with later vascular plants. The Rhynie Chert also has yielded axial gametophyte fossils that are predominantly of equivalent tissue complexity and size to the corresponding sporophyte (Remy 1982; Remy et al. 1993; Kerp et al. 2004; Taylor et al. 2005), thereby implying that all land plants (Remy 1982) or at least vascular plants (Kenrick 2000) diverged from a common ancestor with equal generations, as opposed to following the trend from gametophyte to sporophyte dominance expected from the characteristics and relationships of living land plants.

Although available anatomy has been described for individual cooksonioid specimens, the preservation of that anatomy is partial and gametophyte fossils are unknown. As a result, in syntheses of early evolutionary patterns, cooksonioids are often atomized and serve as mileposts for the accumulation of individual vascular plant characteristics (e.g., Banks 1981; Knoll et al. 1984; Niklas 1997; Edwards 2003), leaving unresolved the implication that the general continuity with modern sporophyte photosynthetic function demonstrable in the Rhynie Chert persists in the still deeper past despite the greater morphological simplicity of the fossils.

Such continuity was supported by early modeling efforts upholding photosynthetic independence of early vascular plant sporophytes (Raven 1984, 1993). However, that work

was based upon axes of 1 mm diameter and then generalized to other taxa. Furthermore, these calculations were ultimately limited by a lack of anatomical detail: in modeling photosynthetic assimilation, only the endpoints of the CO_2 diffusion pathway were considered, whereas inclusion of progressive CO_2 depletion with loss to cells intermediate between the stomata and the innermost chlorenchyma is crucial (Konrad et al. 2000; Roth-Nebelsick and Konrad 2003). Similar problems can be expected with hydraulic calculations for which proximal water loss can be a far greater concern (Zwieniecki et al. 2004, 2006) than provided for by the assumption in early studies of linear water loss along the axis.

Because anatomical context has proven essential, recent modeling necessarily has been limited to permineralized fossils. In the future, techniques such as X-ray computed tomography (Tafforeau et al. 2006) might provide greater anatomical information from cooksonioid mesofossils than is provided by the current reliance on surficial exposure and intentional breakages (Edwards and Axe 2000). However, even if the detailed physiological consideration received by Rhynie fossils and other early permineralized plants (Edwards et al. 2006) currently cannot be replicated for the anatomically incomplete cooksonioid compressions and mesofossils, their preservation may yet be sufficient to constrain what physiologies would not have been possible.

Size Constraints on Tracheophyte Physiological Independence

Plant Function and Division of Labor.—The functions essential for nearly all independently growing terrestrial plants—desiccation resistance, structural support, hydraulic and solute transport, and photosynthesis—can have contradictory requirements and necessitate segregation into distinct tissues. For example, photosynthesis is severely limited if photosynthetic cells do not have direct access to airspaces because CO_2 diffusion is much more rapid through air than water (Raven 1996); however, the epidermis must have contiguous, cuticle covered cells if it is to act as a barrier to water loss. Mechanical and hydrau-

lic functions can be similarly compromised by intercellular airspaces.

In all Silurian and Early Devonian fossil plants except lycophytes, these mutually exclusive functions must be performed by (and all discrete tissues contained within) the axis as the only vegetative organ (Edwards 1993), placing geometric constraints and potentially a minimum viable size upon that axis. Minimum size constraints are easy to overlook because diverse, small structures are abundant in more complex vascular plants, but they represent specializations for limited functions. For example, the rachis of a maidenhair fern frond can be less than 500 μ thick, but is not photosynthetic and does not require airspaces.

The axes of early fossil plants could not easily circumvent these functions and resulting tissue requirements. For example, the mycorrhizal fungi seen in the Rhynie Chert (Taylor et al. 1995, 2004) would simplify the additional requirement of nutrient acquisition from the substrate, but would not affect any of the aboveground functions discussed here. Also, if a transpiring axis simply did not have vasculature, then it would lose too much water proximally and have inadequate transport rates along the axis to support distal tissues because of the high hydraulic resistance of symplastic and apoplastic transport through parenchyma (Zwieniecki et al. 2004); even the small sporophyte axes of mosses typically have dedicated conducting cells (Hébant 1977; Crum 2001).

Psilotum sporophytes, in which a leafless axis is the solitary vegetative organ type, provides the best extant analogue of early vascular plants. *Psilotum* axes consist of a series of concentric tissues fulfilling discrete functions; from periphery to center, they are epidermis for protection and desiccation resistance, aerated chlorenchyma for photosynthesis, sclerenchymatous stereome for lignin-based structural support, parenchyma for hydrostatic-based structural support, and central vasculature for transport (Fig. 1A). These tissues are housed in a cylindrical axis 2 to 3 mm in diameter that can range down to about 1 mm wide in the distal segments—di-
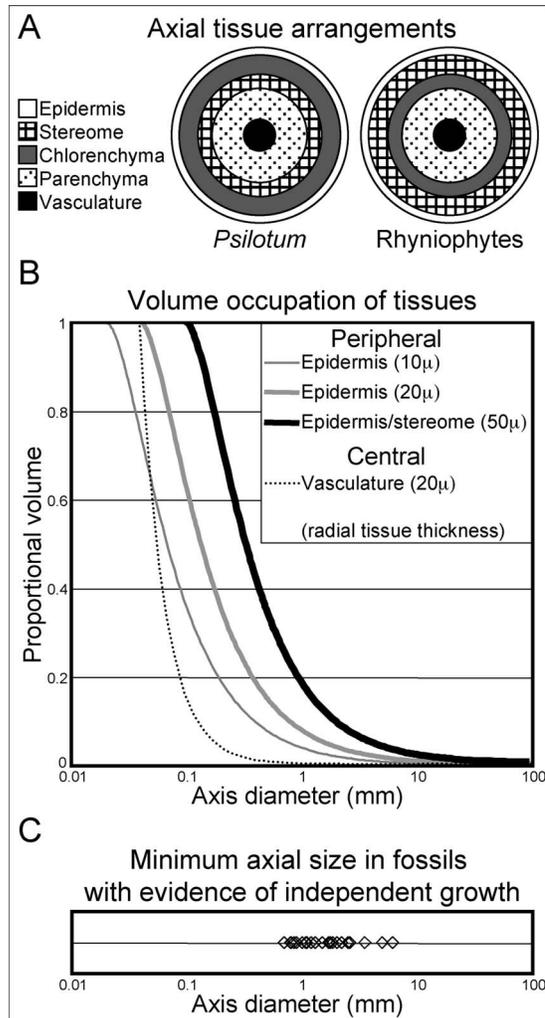

FIGURE 1. Size constraints on axial anatomy. A, Schematics of tissue distribution in axial cross-sections for *Psilotum* and Devonian rhyniophytes. B, Proportional occupation of cross-sectional area calculated in cylindrical axes of different thicknesses for concentric tissues. For calculations, the rhyniophyte arrangement was used, with stereome immediately adjacent to epidermis and chlorenchyma more deeply seated, rather than *Psilotum* arrangement with chlorenchyma between epidermis and support tissues. C, Minimum axial size in Silurian and Early Devonian taxa demonstrating substrate interaction through rhizoids, rhizomatous growth, or other rooting structures, and thereby demonstrating physiological independence from the gametophyte generation.

mensions similar to axial plants from the Rhynie Chert.

Geometric Constraints on Axial Tissues.—For plants with an axial organization similar to *Psilotum* or Rhyniophytes, the minimum functional size can be inferred from the minimum

cell sizes and volume occupation of different tissue types. Although as large as 140 μ by the end of the Devonian, smaller water-conducting tracheid cells are 8–10 μ in diameter (Niklas 1985). However, tracheids cannot be festooned across airspaces; maintenance of a continuous surrounding layer of cells is needed for radial apoplastic transport. This sheath typically occupies a much larger volume than the actual conducting cells (Armacost 1944). Phloem also requires associated nonconducting cells for maintenance of and the loading and unloading of solutes into the conducting cells. Therefore, a minimized stele with xylem and phloem each represented by a single conducting element would still likely have a 40 μ minimum diameter once the investing layer of nonconducting cells is included. The epidermis can be a single layer of cells that are often as little as 10–20 μ thick (although cell thicknesses of >100 μ also are known among Early Devonian examples [e.g., Kerp et al. 2001]). Requirements for stereome structural support will depend upon the dimensions of the axis (Niklas 1997). In the smaller axes for which a stereome has been demonstrated, that tissue is approximately three cell layers and 50 μ thick including the epidermal layer (Edwards et al. 1986).

The geometric limitations on small axial plants become clear if the cross-sectional area occupied by the minimal thicknesses discussed above for concentric vasculature, stereome, and epidermis is calculated for cylindrical axes of different diameters (Fig. 1B). Because the vasculature is central in the axis, it occupies little proportional volume except in axes <200–300 μ in diameter. However, the peripheral epidermis and stereome requirements rapidly begin to crowd out other tissues as the surface area to volume ratio increases in axes narrower than approximately 800 μ.

Photosynthesis and hydrostatic support require additional tissues. Although the leaf epidermis is photosynthetic in some shade plants (Esau 1953), particularly ferns (Ogura 1972), chloroplasts are limited to portions of the cell in contact with mesophyll airspaces because aqueous diffusion of CO_2 more than a couple of microns is prohibitively slow (Raven 1996). Therefore, the bulk of the epidermis repre-sents a peripheral skin that cannot be engaged in photosynthesis. (Similar limitations also apply to internal tissues lacking airspaces.) Furthermore, epidermal contact with internal airspaces would be precluded in axes possessing a stereome or other hypodermal tissues (Fig. 1A), so that photosynthesis would not be available in the peripheral tissues of many Silurian and Devonian taxa (Edwards et al. 1996). Depth of light penetration is not a concern in itself; however, less and less volume is available with greater depth within the axis—particularly as the surface area to volume ratio increases with decreasing size. Hydrostatic support represents another demand upon axial volume not accounted for in Figure 1B, although the non-transporting cells on the periphery of the vasculature may grade into a larger hydrostatic support tissue. As examples, volume occupation of chlorenchyma and central hydrostatic tissues are 16% and 69%, respectively, for *Aglaophyton* and 20% and 59% for *Rhynia* (estimated from Edwards 1993; Roth-Nebelsick and Konrad 2003).

Other studies have tried to set theoretical minimum plant sizes on the basis of maximally efficient packing of minimally sized cells (Raven 1999), but these were intended for rough estimations of photosynthetic output rather than full consideration of all plant functions including transport and mechanical support or of documented anatomical constraints such as peripheral tissues being unavailable for photosynthesis (Edwards et al. 1996). The calculations here (Fig. 1B) emphasize that many plant functions require a trivial proportion of axial volume in even modestly sized axes, but each function represents an increasingly significant constraint with axial diameters much less than 1 mm—a challenge compounded if all functions must be performed in a single axis—providing a baseline for comparison to axial sizes in the early fossil record.

Methods

Dimensions of Silurian and Early Devonian fossil plants were compiled from the literature. Unless unbranched, minimum and maximum axial diameter for all taxa were measured in between dichotomies on relatively straight axial portions; maximum diameters

thereby exclude the axial swelling that is common proximal to incipient dichotomies or to sporangia and minimum diameters exclude sterile terminal segments that can taper significantly. Sporangial length and width were also measured (along with the third dimension, when available), as was minimum sporangial stalk width for non-terminal sporangia. Sterile form taxa (e.g., *Hostinella*) and fragments were excluded unless they possessed characteristics such as H- or K-branching useful for analyses described in following sections.

Ranges and means tabulated in the literature were used when available; otherwise, measurements were made directly from figured specimens. Measurements were lumped as size ranges for each species (Appendix 1 online at http://dx.doi.org/10.1666/07056. s1) except where amplified with measurement records for individual figured specimens of cooksonioids (Appendix 2). In comparative analyses, midpoint values between the maximum and minimum were used when mean values were not available.

Size and Habit of Early Land Plant Fossils

Minimum Sizes of Fossils with Demonstrable Physiological Independence.—Beyond coarse geometric constraints (Fig. 1B), the distribution of morphological characteristics across the size range of fossils may provide additional evidence concerning minimum viable axis diameters. The presence of roots, rhizoids, or indeterminate rhizomatous growth (reviewed in Gensel et al. 2001; Raven and Edwards 2001; Kenrick 2002; Boyce 2005) demonstrates substrate interaction without a gametophyte intermediary and thereby independent physiological existence. For example, Rhynie Chert tracheophytes and protracheophytes—all of roughly similar axis diameter to extant *Psilotum* (Appendix 1)—possess rhizoids (*Aglaophyton*, *Horneophyton*, *Rhynia*), other rooting structures (*Asteroxylon*), or rhizomatous growth (*Aglaophyton*, *Asteroxylon*, *Nothia*, *Rhynia*) (Kidston and Lang 1920b, D. S. Edwards 1980, 1986; Gensel et al. 2001; Kerp et al. 2001).

Minimum axis sizes for species with demonstrable independence are plotted in Figure 1C. Use of minimum diameter (i.e., a value of 0.9 mm would be used for a species that bears rooting evidence on 2.0-mm-wide rhizomes if its aerial axes taper to 0.9 mm) limits the taphonomic risk of size constraints being inflated by the preferential fragmentation of smaller aerial axes. The unlikelihood that rhizoids will be preserved without permineralization presents a second taphonomic concern; however, there is still ample evidence for indeterminate growth without it and few taxa (e.g., *Horneophyton*) are recognized as independent based upon rhizoids alone. Evidence of physiological independence is abundant in taxa with axes as small as 0.8 mm, but the smallest with such evidence is 0.7 mm (Fig. 1C)—results that are consistent with estimates that the geometric constraints of tissue packing become prohibitive in axes much smaller than 1 mm (Fig. 1B).

Size Range of Silurian and Early Devonian Plant Fossils.—Most taxa conform to the size limits implied by both estimation of geometric constraints and morphological proxies for independence (Fig. 1). However, cooksonioid taxa can range down well below 100 μ in axial diameter, and individual genera (Fig. 2) or even morphospecies (Fig. 3) can span almost two orders of magnitude, straddling the inferred physiological size threshold. This extreme variance reflects that size has not been a direct criterion for taxonomy. The likelihood has been widely discussed that *Cooksonia* and other morphotaxa that are based only upon few and simple characteristics may artificially group fossils that are not closely related (Edwards 1979b; Taylor 1988; Fanning et al. 1992; Edwards et al. 2001; Habgood et al. 2002); size may prove a valuable additional characteristic for parsing fossils with such simple morphologies.

Size extremes are unlikely to reflect taphonomy alone. Many cooksonioids are preserved as charcoal (Edwards 1996; Glasspool et al. 2006), which can entail shrinkage (e.g., Glasspool et al. 2006: Plate 1.7). However, this shrinkage is typically in the range of 14–47% (Lupia 1995) and hence not relevant here; at issue are not axial widths of 100 versus 150 μ, but rather 100 μ versus 1 mm. Instead, the sizes of cooksonioids must be interpreted as a genuine physiological dilemma. Regardless of
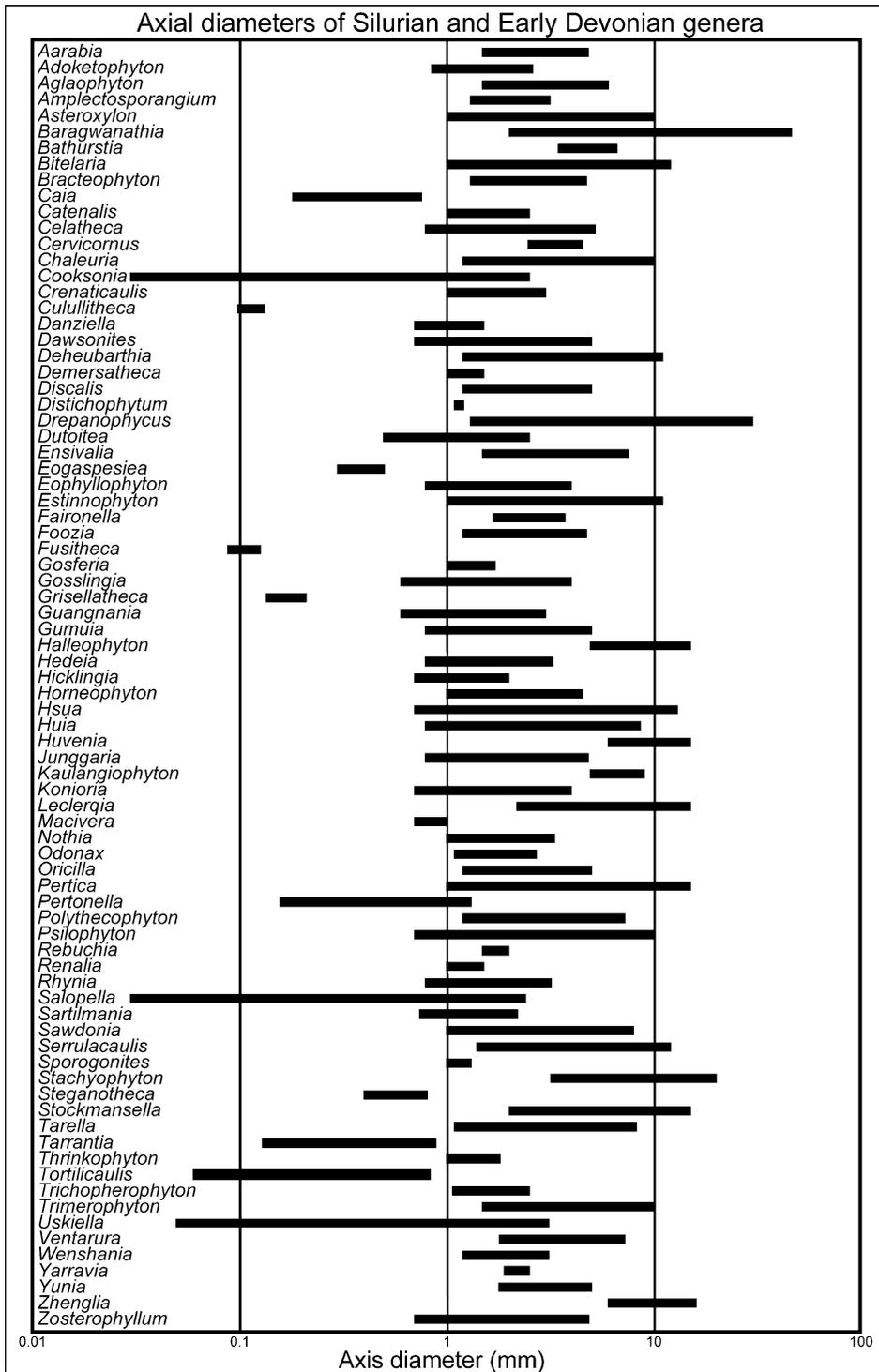
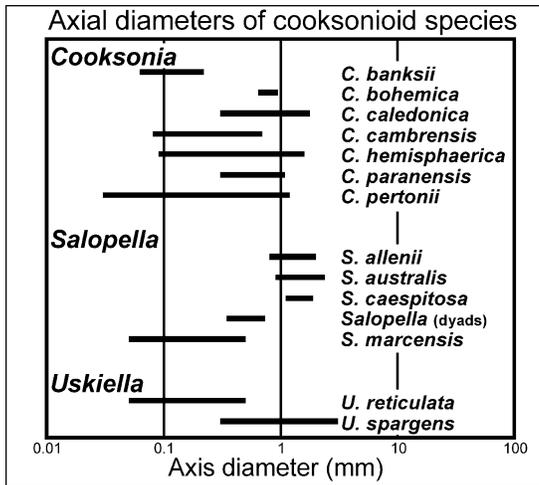FIGURE 2.   Range in axial diameters for Silurian and Early Devonian genera.

FIGURE 3. Range in axial diameters for morphospecies of particularly variable cooksonioid genera.

how their biology is ultimately interpreted, continuity with later vascular plant processes—just on a smaller scale—is problematic.

Physiological Independence Despite Small Size

Various mechanisms, all involving extreme departures from known tracheophyte biology, can be envisioned to allow photosynthetic independence in very small plants; however, none are likely to apply to cooksonioids. If cell size were greatly reduced, that could allow rhyniophyte-like tissue complexity in a smaller axis. However, those smaller cells would probably have to average 1μ or less in diameter in cooksonioid axes that can be much less than 100μ wide. The absence of vacuoles implied by such small cell size would represent the highly unusual loss of a fundamental aspect of land plant biology that would be counterproductive as far as the cost of dry-matter investment (Raven 1999). Regardless, the cells of cooksonioid plants are of normal size, typically 10–20μ, when preserved (Edwards et al. 1986, 1992, 1994; Fanning et al. 1992; Edwards 2000; Habgood et al. 2002). Cooksonioids obviously cannot express the larger end of the plant cell size range, because some entire cooksonioid axes are narrower than the 60 to 120μ width common for individual parenchyma and epidermis cells of Rhynie Chert fossils (e.g., Kerp et al. 2001); nonetheless, they

do squarely occupy the smaller end of the normal range.

Some examples of smaller cells are known from cooksonioids–significantly all represent central, thick-walled cells for which programmed cell death may have come before the vacuolate expansion that characterizes other cell types. For such anatomical curiosities as ''vasculature'' almost occluded by thickenings (Edwards 2000) or only 2μ wide (Edwards et al. 1992), identifying function is difficult (Edwards 2003). Water-conducting cells are often spindle shaped, and some of these examples likely reflect the tapering ends of larger elements (Edwards and Axe 2000).

Using bryophytes as a guide, limited opportunities do exist for the exclusion of entire tissue types. External transport through capillary action along enation bases obviates the need for internal vasculature in many moss gametophytes (and can actually proceed faster than internal transport in mosses that have both methods available [reviewed in Hébant 1977]). However, external transport would not be available to the unadorned axes of cooksonioids, as is also the case for moss sporophytes typically containing hydrome and/or leptome analogues of tracheophyte xylem and phloem. Additionally, bryophytes possess highly divergent modes of sporophyte growth—the basal growth of hornworts, the delayed tissue expansion of liverworts only after differentiation is complete, the tiered distal growth seen in bryopsid mosses in which apical tissue is set aside early for sporangial differentiation and seta elongation progresses from more proximal tissue, and the lack of sporophyte growth beyond the sporangia itself in other mosses (Crandall-Stotler 1980; Renzaglia et al. 2000; Crum 2001)—all of which should minimize the need for nutrient transport to growing sporophyte tissues. However, the branching seen in cooksonioid axes would not be possible without apical growth (Kenrick discussion in Edwards 2000), and so none of these bryophyte growth modes would be possible (except perhaps for unbranching *Tortilicaulis* specimens for which a liverwort affinity has been considered [Edwards 1979b]). Furthermore, both external transport and divergent growth strategies

would each only limit the need for vasculature, which already takes up little volume because of its central axial location (Fig. 1) and which is indeed already known from many mesofossils—cooksonioid (Edwards et al. 1992; Edwards and Axe 2000; Edwards 2003; Glasspool et al. 2006) and otherwise (Edwards 2000).

In the absence of a cuticle, axial surface area could be available for photosynthesis (Raven 1993) and internal airspaces be avoided. However, the cylindrical morphology of cooksonioid axes minimizes external surface area and they are seen to have tightly packed epidermal cells when cell outlines are preserved (e.g., Edwards and Wellman 2001), so that photosynthesis would thereby be restricted to only that limited axial surface. Cooksonioid taxa show none of the elaboration of external photosynthetic surface area common to liverwort and moss gametophytes (Renzaglia et al. 2000) and instead resemble other members of the tracheophyte lineage for which the norm is internalization of what photosynthetic spaces are present.

The one entirely expendable tissue is a hypodermis or stereome; plant axes in the size range of smaller cooksonioid axes can rely exclusively on turgor from the hydrostatic core (Niklas 1997). Nonetheless, a stereome is preserved in some of the narrowest cooksonioids (Edwards et al. 1992; Fanning et al. 1992; Edwards 2000). In order to account for their small size, the only function available to exclude may be photosynthesis.

An Alternative Interpretation of Cooksonioid Biology

Significance of Hypodermal Tissues.—In addition to mechanical support (Niklas 1976; Edwards and Fanning 1985; Edwards 1996), the stereome preserved in many cooksonioid axes (but not all [Edwards and Axe 2000]) has been interpreted as an adaptation to resisting drought (Edwards and Fanning 1985; Edwards et al. 1986) and potentially could have shielded the plant from ultraviolet light or other environmental threats (Raven 1984). Regardless of the potential efficacy of a stereome for withstanding drought in larger plants such as *Psilophyton* (e.g., Edwards et al. 1997),

the need to survive such transient threats is not consistent with the overall cooksonioid morphology of determinate growth in a herbaceous plant at most a few centimeters tall with a uniquely exposed apical meristem. Such a plant might be better adapted for stress avoidance with life-cycle completion only during favorable conditions. UV and other environmental threats are more constant, but the cuticle, anthocyanins, and other aspects of plant biochemistry (Edwards et al. 1996; Raven 2000) are involved in UV absorption beyond the presumed lignin (Niklas 1976; Edwards et al. 1997; Boyce et al. 2003) of stereome cell walls. Furthermore, protection afforded by differentiated stereome would only be available to more proximal, mature tissues, whereas it is the growing apex that likely would be the most vulnerable. Finally, monocarpic plants typically die after reproduction; there is no value to continued axial activity once the terminal sporangia are mature. As a result, the sterome of the small, determinate cooksonioids represents such a prohibitive investment of axis volume and thickened cell wall biosynthesis that it must have played a role distinct from that in larger, indeterminately growing plants. Even if the stereome is deemed homologous as an anatomical structure between *Cooksonia* and *Psilophyton*, its function was likely distinct.

The stereome documented in cooksonioids has been treated as a link to more complex tracheophytes and a pteridophytic lifestyle (Edwards et al. 1986; Kenrick and Crane 1997) (although other cooksonioid characteristics have been treated with increasing circumspection, owing to their prevalence in some bryophytes [Edwards 2000]); however, 60% of the volume of the fossil in question is taken up by a stereome that is not needed for structural support in a hydrostatic plant with a 150 μ diameter and that is of questionable alternative utility. Furthermore, chlorenchyma at least has the volume advantage of being more peripheral than the sclerenchyma in *Psilotum*, but the opposite is true in early land plants (Fig. 1A), making a stereome in such volume-constrained plants all the more problematic. Given the vascular and peripheral anatomy preserved, smaller cooksonioid axes likely would

not have had the capacity for an extensive, aerated photosynthetic tissue—suggesting instead photosynthetic dependence on an unpreserved gametophyte.

An explanation for cooksonioid function may best be afforded by the wiry setae of mosses where stereome allows the sporophyte to remain erect after axis desiccation because spore dispersal is more efficacious in dry air (Edwards 1996; Crum 2001). For taxa in which dehiscence may have been dependent on sporangial drying (Edwards et al. 1996) or which show no specialized dehiscence mechanism and appear dependent on general sporangial wall disintegration (Edwards 1996; Habgood et al. 2002), maintaining axes erect postmortem may have been particularly important. Rather than hardy survivors pioneering both the terrestrial landscape and the overall physiological strategy so successfully exploited by later vascular plants, smaller cooksonioid sporophytes more likely represent ephemeral, gametophyte-dependent reproductive structures with an anatomical construction allowing continued spore dispersal after sporophyte death.

Size Correlations Involving Sporangia.—The scaling of size of sporangium with that of sporangial stalk and supporting axis can provide an independent assessment of photosynthetic potential. In trimerophytes and zosterophylls, the axes upon which sporangia are borne can be expected to have fulfilled all plant functions including photosynthesis, whereas the short stalks connecting sporangium to axis were much less likely to have been significantly photosynthetic and instead probably were involved only in the structural and vascular support of the sporangia. As such, for a particular sporangium size, sporangial stalks typically should be substantially thinner than the photosynthetic main axes because of the fewer functions being performed (and fewer tissues types needed). Also, sporangial stalk size should show close correlation with sporangium size because the limited number of straightforward functions of the stalk are all directly related to the sporangia; the scaling of sporangial size with the main axis, on the other hand, should show more variance because of the more complex array of suboptimal mor-
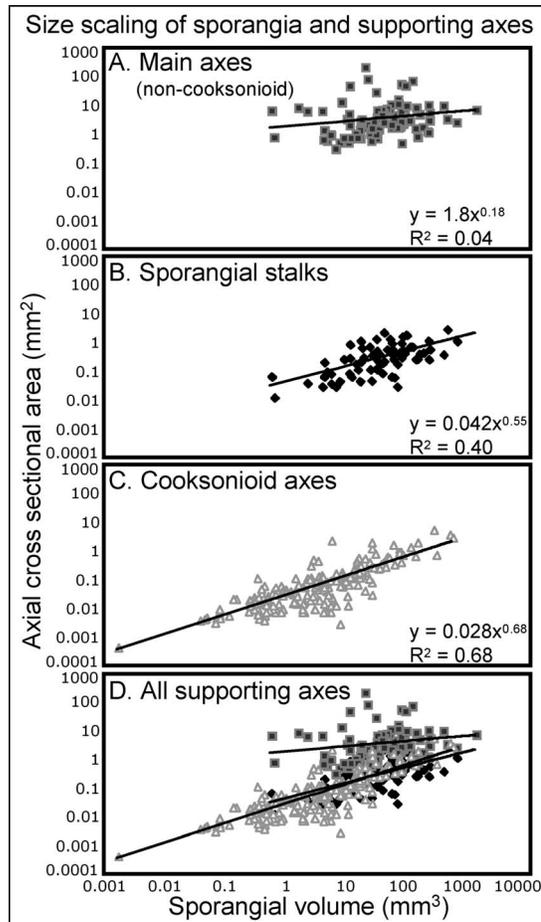

FIGURE 4. Correlation of sporangial volume with cross-sectional area of non-cooksonioid axes presumed to be photosynthetic (A), of sporangial stalks presumed to be poorly photosynthetic (B), of cooksonioid axes (C), and of all subtending axes in composite (D). Calculations based upon mean or midpoint values for species except where amplified in cooksonioids with measurements of individual specimens.

phological solutions for the diverse functions of these axes (Niklas 1997).

Among non-cooksonioid Silurian and Early Devonian taxa, the above expectations are borne out: relative to photosynthetic axes, the cross-sectional area of sporangial stalks is smaller and more tightly correlated with sporangial volume (Fig. 4). Sporangia were treated as ellipsoids to estimate sporangial volume. Where unknown, the third sporangial dimension was estimated as the lesser of the two known dimensions; i.e., depth was assumed to equal width in fusiform sporangia and length in most reniform sporangia. (The

volume of some of the more discoidal zoster-ophyll sporangia will be overestimated by this method.) Axis width is measured at the point of sporangial attachment rather than repre-senting the overall range in axis size for the whole plant. For estimates of cross-sectional area from preserved widths, axes and sporan-gial stalks were assumed to be cylindrical. Axes grossly departing from this shape (e.g., *Huvenia* or *Serrulacaulis*) were excluded from the analysis, as were individual measure-ments involving sessile, sunken, or otherwise obscured sporangial attachment or for which physiology is ambiguous (e.g., branching *Le-clerqia* sporophylls).

If photosynthetic, then the axis/sporangi-um size scaling of cooksonioids should be similar to that of larger photosynthetic axes although extending into a much smaller size range. If cooksonioid axes were not extensive-ly photosynthetic, but instead were involved primarily in sporangial mechanical and vas-cular support, then their size scaling should more closely resemble that of the sporangial stalks of larger plants rather than photosyn-thetic axes. For cooksonioid specimens, spo-rangial volume and axis cross-sectional area were estimated as described above with the exception that the axis width used was the me-dian value between proximal and distal ex-tremes (except for any swelling immediately proximal to a sporangium).

The correlation of cooksonioid axis and spo-rangium size closely resembles that of the sporangial stalks of large photosynthetic plants rather than that of the photosynthetic axes (Fig. 4). Although they include smaller cross-sectional areas, their axis/sporangial size ratios overlap with those of sporangial stalks—with which they also share similar slopes and variance—while being shifted sub-stantially away from the range of photosyn-thetic axes toward smaller axial size for a par-ticular sporangial volume. [The relative small-ness of cooksonioid axes for their sporangial sizes may be even more pronounced than shown here because of the overestimation of zosterophyll sporangial volume discussed above.] A few of the largest cooksonioid axes do overlap with the size ratios of photosyn-thetic axes (as commented upon below in the

Discussion), but axial and sporangial dimen-sions support interpretation of all smaller cooksonioid sporophytes as organs of sporan-gial support rather than of photosynthesis.

Significance of Stomata.—Several cookson-ioid axes have preserved stomata (Edwards et al. 1986, 1994; Edwards 1996; Habgood et al. 2002), a clear indication of regulated internal gas exchange. Although often essentially syn-onymous with photosynthesis in living vas-cular plants, the presence of stomata is not, however, inconsistent with limited or no pho-tosynthetic capability. Because the transport of solutes through the xylem is reliant upon transpiration-driven flow, stomata may have been necessary before playing a role for pho-tosynthetic gas exchange, in increasing tran-spiration and thereby increasing xylem nutri-ent flux (Edwards et al. 1996). Stomata can be considered a clear indicator of a cuticular bar-rier to evaporation, but not necessarily pho-tosynthesis.

When preserved in cooksonioid fossils, sto-mata are often concentrated distally at the base of the sporangium where they are some-times associated with a swollen neck of tissue (Edwards 1996; Edwards et al. 1996, 1998), a pattern that is shared with many moss spo-rophytes (Edwards 1996, 2000). The differen-tiation of these distal stomata may have pro-vided an increase of transpiration-mediated solute delivery to the apex just when a spo-rangium was initiated and required an in-creased nutrient flux (Edwards et al. 1996). This stomatal distribution may also have been important for photosynthesis; when present, a sporangial neck tends to be the widest part of the axis and hence possesses the greatest ca-pacity for possible airspaces and chlorenchy-ma. This is borne out in some moss sporo-phytes that have highly elaborate air chambers in the dilated tissues adjacent to and enclosing the sporangial cavity (e.g., *Splachnum* or *Fu-naria* of the Bryidae or various Polytrichidae [Crum 2001]). In addition to any role these air-spaces may play in reproduction and spore dispersal, these mosses can possess substan-tial—but still insufficient—photosynthetic ac-tivity (Bold 1940; Paolillo and Bazzaz 1968; Proctor 1980; Renzaglia et al. 2000).

Although the sporangial neck may well

have been a localized site of photosynthetic production, all but the distalmost axial epidermis in these cooksonioids has a very low stomatal density. The generally low stomatal densities of Early and Middle Devonian fossils is attributed to high atmospheric CO_2 (McElwain and Chaloner 1995; Edwards et al. 1998); however, cooksonioid stomatal densities are low even by Devonian standards: when stomata are not absent, cooksonioids typically possess about 1 stoma/mm^2 (Edwards 1996), whereas most other Early Devonian sporophytes range from 3 to 31.5 stomata/mm^2 (McElwain and Chaloner 1995; Edwards et al. 1996, 1998). *Aglaophyton* and *Rhynia* do have the extremely low stomatal densities of 1.0 and 1.8 mm^{-2} respectively (Edwards et al. 1998); however, that still amounts to 11 to 14 stomata per 1 mm of axial length, whereas a density of 1 mm^{-2} in a cooksonioid axis of 150 µ thickness equates to 1 stoma per 2 mm of axis. These isolated stomata are of questionable utility (Edwards et al. 1998) and indicate little potential for gas exchange over most of the life of the sporophyte. Hence, even if stomatal densities are similar, cooksonioids represent a special case difficult to equate with larger Silurian and Devonian taxa.

In bryopsid moss sporophytes, the tiered apical growth may allow for differentiation of distal tissues and airspaces associated with the capsule before the intercalary elongation of the seta is complete (Crum 2001). However, no such possibilities are available to branching polysporangiophytes, which must have strictly apical growth. Hence, a cooksonioid with stomata localized primarily to a sporangial neck possessed no or almost no stomata for most of its growing life span: stomatal evidence may well indicate that many cooksonioid sporophytes were probably green, but not necessarily green enough to be photosynthetically independent.

Discussion

The Fossil Record of the Early Evolutionary History of Polysporangiophytes.—That the evolution of a multicellular land plant sporophyte was ancestrally an intercalary structure physiologically dependent upon the gametophyte throughout its ontogeny is overwhelmingly indicated by the following three observations: (1) the lack of a multicellular sporophyte among the Charales sister group to the land plants; (2) the extremely divergent mechanisms of sporophyte growth between liverworts, hornworts, mosses, and vascular plants; and (3) the paraphyletic nature of the bryophytes relative to the tracheophytes (Mishler and Churchill 1984, 1985; Hemsley 1994; Kenrick 1994, 2000; Renzaglia et al. 2000). As essential as that work has been in establishing sporophyte origins, such inference from the phylogeny of living plants cannot inform the timing or order of events—such as the appearance of photosynthetic independence, branching, and tracheids—in the evolution of sporophyte independence within stem-group polysporangiophytes that predate the common ancestor of the living vascular plant crown group; that is exclusively the domain of the fossil record. However, the general view has been that photosynthetic independence and physiological continuity with later pteridophyte vascular plants had already been achieved prior to our oldest preserved macrofossils, as has been directly stated or implicitly required in paleobotanical studies of character evolution, physiology, and systematics (Niklas et al. 1980; Raven 1984, 1993; Edwards and Fanning 1985; Kenrick 1994, 2000; Edwards et al. 1996; Kenrick and Crane 1997; Bateman et al. 1998). The results of the current study, in contrast, suggest that the establishment of a physiologically independent sporophyte generation has in fact been recorded directly in the existing macrofossil record.

No discrete and inviolate boundaries can be drawn between cooksonioids large enough to possess tissue complexity and photosynthetic capacity sufficient for functional independence and those so small as to require photosynthetic dependence on a gametophyte; however, several important transitions are likely to be recorded among described fossils. Several examples (e.g., *Cooksonia* cf. *hemisphaerica* [Edwards et al. 2004: Fig. 2]) possess axes more than 1 mm in diameter for which there would be no reason to doubt photosynthetic independence and for which rhizomatous growth proves it. Other taxa (e.g., *Uskiella spargens* [Shute & Edwards 1989] or *Cooksonia*

caledonica [Edwards 1970]) are large enough for photosynthetic independence, but nonetheless possess a determinate growth pattern suggesting they may still have been dependent on a gametophyte for substrate interaction. A variety of specimens in the 300–700 μ diameter range likely contributed significantly to their photosynthetic needs—as in many moss and hornwort sporophytes—but the possibility of actual photosynthetic independence is increasingly remote with decreasing size, and particularly given the frequent presence of anatomical constraints such as hypodermal tissues. And finally, near-complete nutritional dependence upon a gametophyte would be the most parsimonious interpretation for the smallest cooksonioids with axial diameters as small as 30–70 μ (e.g., *Cooksonia pertoni*, *Salopella marcensis*, and *Uskiella reticulata* [Fanning et al. 1992] or *Tortilicaulis offaeus* and *Salopella* cf. *marcensis* [Edwards et al. 1994]).

Alternation of Generations and Ancestral Gametophytes.—The recognition of permineralizations (Remy 1982; Remy et al. 1993; Kerp et al. 2004; Taylor et al. 2005) and compressions (Remy et al. 1980; Kenrick and Crane 1991) of relatively large axial gametophytes with tissues as complex as in the corresponding sporophyte recast a century old debate over the origin of the land plant alternation of generations. According to the homologous hypothesis (Church 1919), the ancestral sporophyte and gametophyte generations were morphologically and physiologically similar and have since diverged. Alternatively, with the antithetic or intercalary hypothesis (Bower 1908), the sporophyte represents a distinct innovation of multicellular complexity based upon the delay of meiosis in an embryo retained by and dependent upon a larger gametophyte. With discovery of the Rhynie gametophytes, Remy (1982) argued that gametophyte-dominant bryophytes and sporophyte-dominant tracheophytes were both descended from the isomorphic rhyniophytes. That view is no longer tenable given subsequent advances in understanding embryophyte phylogeny (Mishler and Churchill 1984, 1985; Kenrick 1994, 2000; Renzaglia et al. 2000); however, isomorphic generations continue to be considered as

a possibility for the common ancestor of polysporangiophytes or perhaps polysporangiophytes plus mosses (Kenrick 1994, 2000; Kenrick and Crane 1997).

Regardless of whether any particular cooksonioid possessed genuine tracheids, their sporophyte branching places them in the larger polysporangiophyte clade (Kenrick and Crane 1997) of which vascular plants are the only extant members. The smallest cooksonioid sporophytes, highly unlikely to have been photosynthetically competent, suggest that gametophyte dominance is the primitive condition for the clade that includes the vascular plants and that isomorphic generations were derived within the group. This does not necessarily indicate the isomorphic rhyniophytes were a ''dead end'' (e.g., Gerrienne et al. 2006), because a switch from gametophyte to sporophyte dominance presumably would have involved an intermediate in which both generations were independent, albeit not necessarily isomorphic.

Validation of the ideas advanced here ultimately must involve the search for a broader diversity of gametophyte fossils among basal polysporangiophytes, for which small cooksonioids suggest the need for a revised search image. The isomorphic generations seen in later Rhynie Chert plants has led to the expectation that, if the oldest sporophytes looked like *Cooksonia*, then the oldest gametophytes should also look like *Cooksonia* (Edwards 2000; Kenrick 2000)—a solution unlikely if those sporophytes are too small to be independent. If axial, the ancestral gametophytes likely would have been either considerably larger than the sporophytes or possessed external elaborations of photosynthetic tissue as in the leafy bryophytes. Alternatively, a prostrate and possibly thalloid gametophyte would eliminate some of the structural support and transport requirements of an erect axis. Thalloid candidate fossils are known (Tomescu and Rothwell 2006), although most of those with sporophyte attachment involve more-derived taxa (Gensel et al. 2001) or at least sporophytes of larger size (Gerrienne et al. 2006) that likely are capable of photosynthetic independence (but see Fanning et al. 1992: Fig. 54).
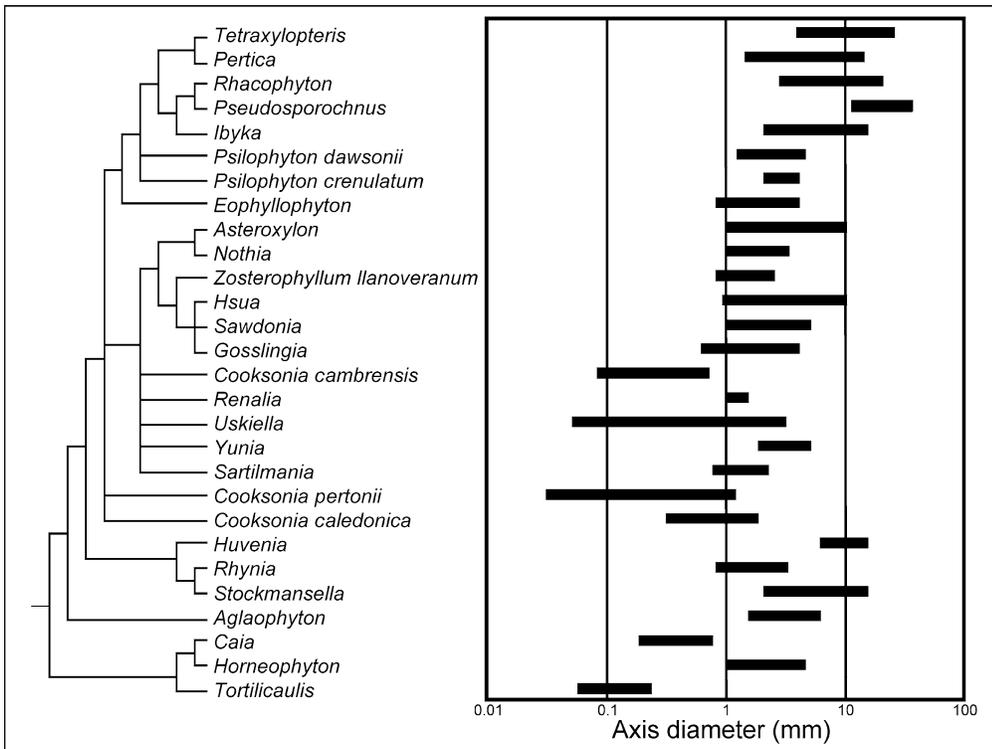
FIGURE 5. Phylogenetic distribution of axial diameters in basal polysporangiophyte fossils. Phylogeny from Kenrick and Crane (1997).

Systematics and Taxonomy of Mid-Paleozoic Land Plants.—Extremely small axial diameters suggestive of sporophyte dependence upon a gametophyte are rather dispersed across the basal vascular plant phylogeny, so far as currently understood (Fig. 5). This result is not simply a taxonomic artifact whereby the mistaken assignment of very small specimens has inflated the size ranges of taxa with larger axial diameters; it is specifically the smallest fossils that have been included in the phylogenetic analyses in some cases (*Tortilicaulis offaeus*, *Cooksonia pertoni* [Kenrick and Crane 1997]). This polyphyletic distribution may highlight a lack of phylogenetic resolving power and the usefulness of size as a character to help resolve the tree topology. If small size, as a proxy for photosynthetic dependence, were considered ancestral and granted weight as a character heavy enough to ensure a monophyletic origin of sporophyte independence within the polysporangiophytes, such a rearrangement would question the current perceived significance of other characteristics,

such as the columella of *Horneophyton*, which likely would have to be a derived state rather than an ancestral one shared with a bryophyte sister group. Alternatively, if the currently favored topology is accepted, it must be understood that this implies that an independent sporophyte evolved multiple times—perhaps even among extant vascular plants, with sporophyte independence in the lycophytes and euphyllophytes being separate events. Although rejected in phylogenetic analyses based solely upon living plants (Mishler and Churchill 1985), multiple evolutions of sporophyte independence within the polysporangiophyte lineage are a necessary, albeit implicit, consequence of current phylogenetic hypotheses involving sufficient fossil representation to address the issue (Kenrick and Crane 1997).

That cooksonioid sporophytes possessed photosynthetic independence is rarely explicitly stated, but is pervasively implicit in treatments of early land plant taxonomy, systematics, morphology, anatomy, ecology, and the

alternation of generations. However, photosynthetic dependence should be considered an equal possibility for many cooksonioids and it may simply be a requirement for the smallest. That it is perilous to treat bryophytes as little vascular plants is understood (e.g., Mishler 2001), but it may be equally problematic to treat the early fossil relatives of the vascular plants as such.

Acknowledgments

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