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Viewpoint

Carboniferous plant physiology breaks the mold

A response to Boyce & Zwieniecki (2019) 'The prospects for constraining productivity through time with the whole-plant physiology of fossils. *New Phytologist* 233: 40–49; doi: https://doi.org/10.1111/nph.15446'

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Summary

How plants have shaped Earth surface feedbacks over geologic time is a key question in botanical and geological inquiry. Recent work has suggested that biomes during the Carboniferous Period contained plants with extraordinary physiological capacity to shape their environment, contradicting the previously-dominant view that plants only began to actively moderate the Earth's surface with the rise of angiosperms during the Mesozoic Era. A recently published *Viewpoint* disputes this recent work, thus here, we document in detail, the mechanistic underpinnings of our modeling and illustrate the extraordinary ecophysiological nature of Carboniferous plants.

Keywords: carboniferous, paleoclimate, paleophysiology, vegetation-climate feedbacks, plant hydraulics

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Recent physiological modelling of early-diverging Paleozoic plants, including ferns, arborescent lycophytes, and seed plants (Montañez et al. 2016; McElwain et al. 2016b), suggests that these plants were capable of a high degree of environmental forcing during the Carboniferous Period of the Paleozoic Era. At this time in Earth history, low-latitude biomes contained abundant plants with extraordinary anatomy and morphology, including arborescent lycophytes ("lycopsids"); climbing sphenopsids, ferns, and seed plants; and seed plants and seedless vascular plants whose xylem contained tracheids that functioned as quasi-vessels. These extraordinary plants are notable for their lack of close living relatives and close morphological/anatomical analogues making functional interpretations complex. In Wilson et al. (2017) we argued that the use of a mechanistic modelling approach based on fossil-plant anatomy was an important way forward to better understand these extinct plants. Results demonstrated that Carboniferous taxa were capable of significantly modulating the local and regional climate and/or environment. A contrary view suggested that the rise of angiosperms during the Cretaceous Period and Cenozoic Era was likely the first and only time during which plants evolved the capacity to significantly influence the environment, largely through the evolution of increased vein length per area ("vein density") in angiosperm leaves (Boyce et al., 2009).

In this debate, Wilson et al. (2015; 2017) have argued that, based on fossil plant anatomy, Carboniferous forests contained plants with the capacity to exhibit environmental forcing. The opposing view (Boyce & Zwieniecki, 2019 and their works cited within) has argued that one feature of these Carboniferous plants, their comparatively low vein length per area (when compared with certain groups of extant angiosperms), would preclude high hydraulic conductance and, consequently, greatly reduce the environmental impact of Carboniferous terrestrial ecosystems.

A recent *Viewpoint* (Boyce & Zwieniecki, 2019) raised three objections to a "high-functioning Carboniferous ecosystem" perspective. First, that a recent Tansley Review (Wilson et al. 2017) lacked clear methods for determining stomatal conductance from fossil plants and that snapshots of our database—which was developed and refined over three years—are inconsistent with one another. In response, we describe our methods, publish our full database here (as it stands in May 2019), and show that this critique of our work is not supported. Boyce and Zwieniecki (2019) focus on statistical outliers in our database, and generally misunderstand our paper, as well as other recently published studies, when they argue for a "fundamental limit" to stomatal conductance of 6.0 mol m^{-2} s^{-1}.

For the second objection, Boyce & Zwieniecki (2019) argue for the primacy of leaf hydraulic resistance as a constraint on whole-plant physiology and that low vein length per area in Carboniferous plants prevents the evolution of high plant conductance. In response, we show that this is an incomplete perspective on plant hydraulics: leaves vary tremendously in the amount of whole-plant resistance they offer, but even leaves with high hydraulic resistance of the mesophyll path do not render the rest of the plant unimportant. We explicate this view by returning to two fundamental questions: what is the best way to represent the pathway of water through a plant, and which components of this system dominate hydraulic resistance?

For the third objection, Boyce & Zwieniecki (2019) argue that Wilson et al. (2017) states that all Paleozoic plants are more productive than the modern members of the same lineages. In response, we argue that this misrepresents our paper —it would be difficult to argue that Carboniferous mosses, of which there are few, are more productive than extant mosses. Rather, Wilson et al. (2017) point out that there are dramatic anatomical differences between Carboniferous plants and some of their distant living relatives. We show that in the case of sphenopsids, for example, tracheid diameters from Carboniferous plants are four to thirty times wider than their living representatives. In turn, this would translate into a higher per-conduit hydraulic conductance of Carboniferous sphenopsids by more than four orders of magnitude (up to 31,600X) over living representatives, based on differences between average-diameter tracheids. We thus argue that it is necessary to rely on the fossils themselves, rather than a distant living relative in order to reconstruct their paleo-physiological functioning. The unique anatomies found within Carboniferous plants and the physiological capabilities inferred from their fossils challenge our assumptions about the evolution of past ecosystems and highlight the critical role of paleobotanical and non-angiosperm physiological inquiry to understanding the role of terrestrial ecosystems in Earth history.

Section 1: Determining stomatal conductance from fossil plants: methods and raw data

Over the last several years, there has been great interest in applying mechanistic models of stomatal function in two directions: reconstructing paleo-$p\mathrm{CO}_2$ when combined with estimates of photosynthetic rate and deriving stomatal conductance from morphological features preserved in the fossil record (Franks et al. 2014; McElwain et al., 2016b). The ability to robustly measure stomatal conductance and recent global compilations and surveys of stomatal function (Lin et al., 2015; McElwain et al., 2016a; Murray et al., 2019), has led to a substantial increase in stomatal dimension data over the past several years (e.g., de Boer et al. 2016). Although this advance has been thought-provoking, two gaps have resulted: (1) most compilations of stomatal conductance (g_s) data have focused on modern plants, and few have focused on extinct taxa, and (2) most extinct compilations have focused on periods in Earth history when atmospheric pCO$_2$ was high (e.g., the Mesozoic Era) and maximum theoretical stomatal conductance to water (g_{wmax}) would have been low. Therefore, most data from modern plants do not include values that can be used to parameterize extinct plants, the fossilized leaves of which may have different stomatal morphologies and frequencies than modern plants, and few extant plants to compare with as proxies. Thus, when capturing stomatal data from fossils, it is possible to exclude, inadvertently, parts of the g_{wmax} parameter space that could match or exceed modern values, leading to an oversimplified view that the stomatal conductance of all modern plants exceeds that of all extinct plants. This oversimplification may become amplified because few papers examining fossil material publish full derivations of equations, observational data, or diagrams demonstrating the process for making measurements of maximum theoretical stomatal conductance to water (g_{wmax}) on fossil material. Consequently, the parameter space from some of these published papers has been overinterpreted (Boyce and Zweiniecki, 2019), leading to suggestions that there are "fundamental limits" to g_{wmax} that result from overlooking assumptions in the initial models themselves. Here, we outline in detail the equations used in our study and illustrate how these parameters are measured on fossil material. The latter is critical because parameters such as vein length per area ("vein density") contain aspects in their measurement that are can be compromised by "a level of subjectivity and uncertainty" (Scoffoni et al. 2013). This issue, among others, is rarely addressed in methods sections of other papers.

Calculating water vapor conductance and measuring stomatal parameters on fossil cuticle

The maximum water vapor conductance (g_{wmax}; mol m^{-2} s^{-1}) values we report in this paper and previous work were calculated from the following equation:

(Eq. 1):

$$\mathrm{g_{wmax}} = \frac{D_{wv} \cdot d \cdot \mathrm{a_{max}}}{\mathrm{M_V}\left(\mathrm{l_p} + \left(\pi/4\right) \cdot \sqrt{\mathrm{a_{max}}/\pi}\right)}$$

where D_{wv} is the diffusivity of water within air ($\mathrm{m^2\ s^{-1}}$), d is stomatal density ($\mathrm{m^{-2}}$), $\mathrm{a_{max}}$ is the maximum stomatal aperture size ($\mathrm{m^2}$), $\mathrm{l_p}$ is pore depth (m), and $\mathrm{M_V}$ is the molar volume of air ($\mathrm{m^{-3}}$ mol) at 22°C.

The value of amax was calculated for lycopsids, sphenopsids, cordaitaleans, and tree ferns by:

(Eq. 2):

$$a_{max} = \frac{\pi}{2} \cdot (\beta\ l_{gc}) \cdot (\gamma\ w_{gc})$$

Our approach is illustrated in Figure 1. First, guard cell length (l_{gc}) and widths (w_{gc}) were measured from the fossil cuticles. Next, the observed stomatal aperture guard cell lengths (l_a) and widths ($\mathrm{w_a}$), those that could be visually identified in the fossilized samples, were also measured to derive length (β) and width (γ) scalars (e.g. $\beta = l_a / l_{gc}$) for estimating aperture linear dimensions from guard cell measurements. This is similar to the method described by Franks and Beerling (2009) where $\mathrm{a_{max}} = \alpha \mathrm{S}$, where S is the total area of guard cells, and α is an allometric scaling factor between stomatal and aperture area. In this case, $\alpha = \beta \cdot \gamma$, whereby, to the best of our knowledge, we measured more dimensions of the guard cells and stomatal apertures than in any previous studies of fossil plants. For $\mathrm{l_p}$ in equation 1, this was estimated from pore dimensions of $(l_a - \mathrm{w_a}) / 2.0$.

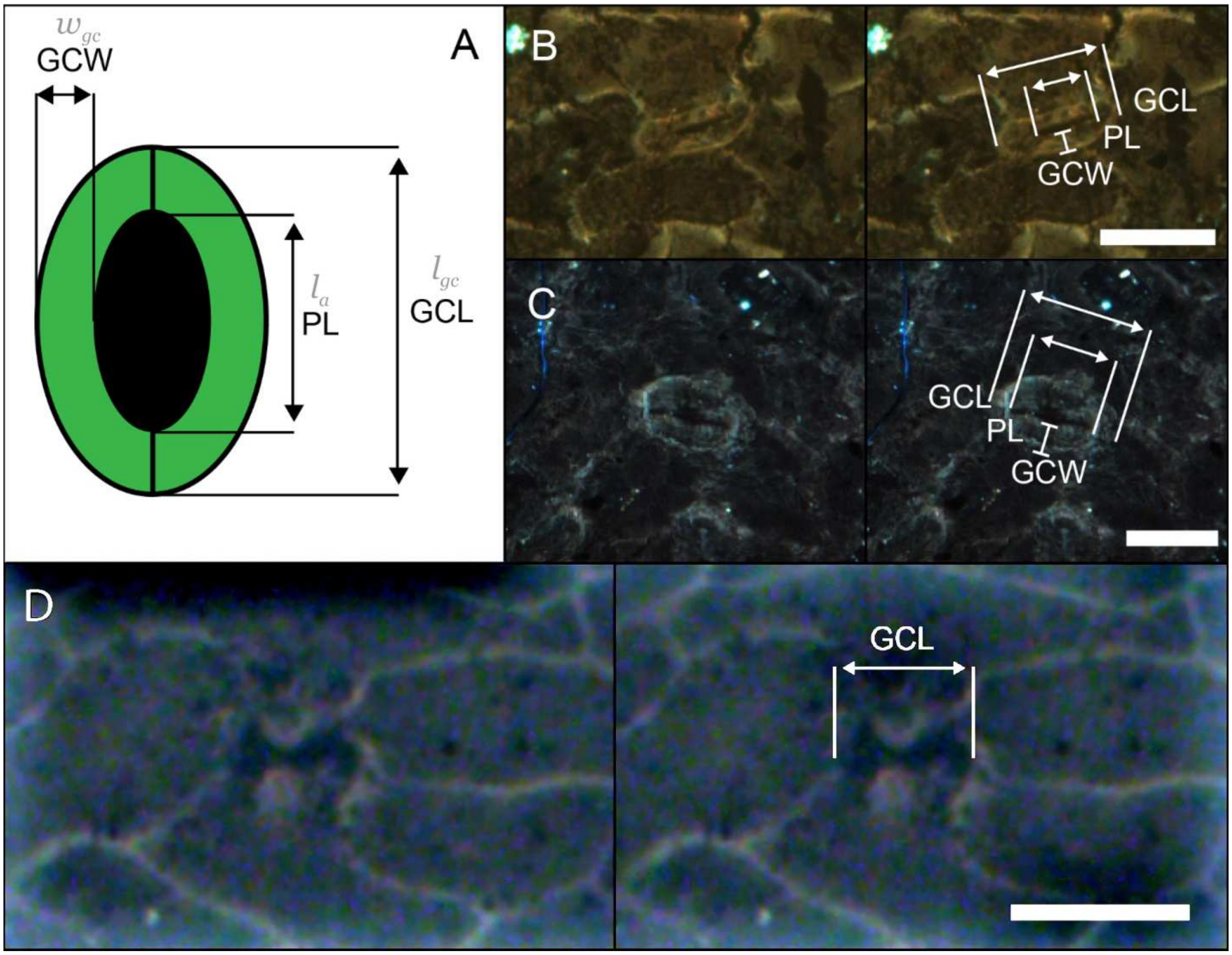

Figure 1: Diagrammatic representation of stomatal parameters and measurements made directly on fossilized cuticle. A) Diagram of two open stomatal guard cells highlighting guard cell width (w_{gc} in Equation 2; GCW), pore length (l_a in text; PL), and guard cell length (l_{gc} in Equation 2; GCL). B, C, D) Measurements made on fossilized cuticle of species Alethopteris serlii, Macroneuropteris scheuchzeri, and Neuropteris ovata, respectively, showing epifluorescent images at left and annotated images at right. Note the sunken stomata for Neuropteris ovata (D) *where only GCL (l_{gc}) can be measured. Scale bars are 25 microns in each image pair.*

Under ideal modes of preservation, guard cell and aperture dimensions can be measured directly from cuticle (Figure 1B, 1C), as they have been, where available, in our previous studies (Montañez et al. 2016; McElwain et al. 2016b; Wilson et al. 2017). In contrast, most studies of gas exchange in fossil plants have interpolated stomatal pore area based on guard cell length and width, characteristics that are more easily observed in fossil material (Figure 1D). This is a fundamental limitation of paleobotanical work: well-preserved material is neither abundant nor uniformly available for all taxa, forcing databases, such as ours, to add new data and revise methods and measurements over time. Over the course of several years, then, new measurements and improved methods are expected to shift the database. The question should therefore be, do the data display the same patterns and order over time?

Boyce & Zwieniecki (2019) describe our data as “individually and mutually inconsistent,” which is based on their survey of our Tansley review paper (Wilson et al. 2017) in addition to two other papers not published in the *New Phytologist*. With regard to variation in values presented in our publications, we agree that these have changed because of 1) reevaluation of methods used to estimate conductance (e.g., directly measuring the proportion of stomatal size forming the aperture pore in fossil material, rather than treating it as a scalar, as others have), and 2) new data added to our database as part of our on-going study on Carboniferous plant physiological functioning. For conductance values of six species of medullosans, *Alethopteris lesquereuxii, Alethopteris sullivanti, Blanzyopteris praedentata, Laveineopteris loshii, Laveinopteris tenuifolia, and Lescuropteris genuina*, we originally reported higher g_{wmax} values ranging from 1.79 to 13.37 mol m^{-2} s^{-1} in Montañez et al. (2016), which have been reduced to 0.64 to 5.15 mol m^{-2} s^{-1} in Wilson et al. (2017) as more information regarding stomatal and aperture area ratios became available for these taxa in the time intervening between these publications. Similarly, two species, *Cordaabaxicutis brzyskii* and *Cordaabaxicutis czeczottensis*, have been eliminated from reporting here from the original Montañez et al. (2016) paper, because of difficulty in confirming the aperture area ratios of these specimens. A summary of values, including those reported from the Boyce & Zwieniecki (2019) *Viewpoint* and those presented here are found in Table 1, derived from our current May 2019 database of calculated maximum water vapor conductance (g_{wmax}) of Carboniferous plants (the complete dataset is shown in Supporting Information Table S1). Some values are slightly different from those originally presented in 2016, however the patterns among the ranges of g_{wmax} have not changed (Figure 2). Lycopsids have the highest maximum stomatal conductance, tree ferns the lowest median value, and the other taxa are distributed in between.

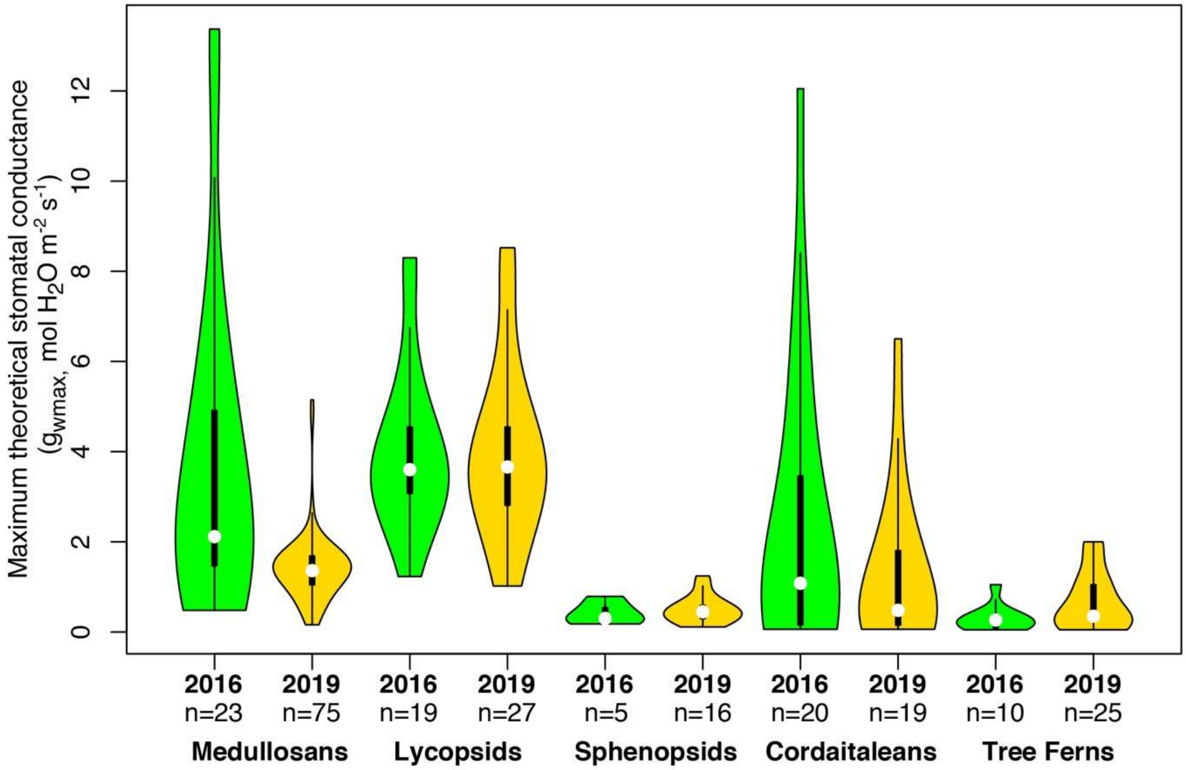

Figure 2: Violin plots (combined boxplot and kernel density function) of maximum theoretical stomatal conductance to water (g_{wmax}) of selected Carboniferous plant taxa from Montañez et al. 2016 Supplementary Information and this study. Median values are indicated by white dots, interquartile ranges (25th–75th) by thick bars, and 5th to 95th by thin bars. Number of measured values for each taxon in each study is indicated below the year of publication. Width of the "violin" indicates abundance of values.

The maximum theoretical stomatal conductance values presented here compare favorably with experimental values derived from modern woody angiosperms. Among the Carboniferous plants in Supporting Information Table S1, the central tendency of all taxonomic groups assessed ranges from 0.50 to 3.97 mol H_2O m^{-2} s^{-1} (statistics presented in Table 1). A recent survey of 218 woody angiosperm species, based on 4273 measurements of operational stomatal conductance, shows a range of ~0.02 to ~0.76 mol H_2O m^{-2} s^{-1} with a central tendency of ~0.25 mol H_2O m^{-2} s^{-1} (Murray et al. 2019). These two datasets can be compared directly by multiplying the maximum theoretical stomatal conductance values from the Carboniferous plants by 0.2 to reach an estimated operational stomatal conductance (Franks et al. 2014, McElwain et al. 2016). Doing so results in a range of operational stomatal conductance for Carboniferous plants of 0.1 to 0.78 mol H_2O m^{-2} s^{-1}, similar to the range found in modern angiosperms. As we discuss below, the maximum g_{wmax} value of 8.52 mol H_2O m^{-2} s^{-1} for *Lepidodendron dichotomum*, an extinct lycopsid, is certainly both theoretically possible and presents an opportunity to assess the spectrum of physiological potential and constraints of plants living in in non-analog environments of deep time.

Variation in our reporting should be viewed positively as we actively reanalyze information. As fossil material is found as a result of field work, additional studies are made of existing collections, and new ways of looking at these fossils are developed, these add to what is, in some instances, scant information on taxa from deep time. This is particularly true in an area such as the physiology of extinct plants, where few quantitative studies have been undertaken. This is the central principle behind large-scale database efforts, such as the Paleobiology Database: as new data are added, the central trend will become clearer over time. The values we present here (Table 1) are the most recent versions of our dataset, with more to come among different taxonomic groups as new fossils are found, measured, and modeled.

Table 1 Minimum, maximum, median, and mean values of theoretical g_{wmax} (mol m^{-2} s^{-1}) based on anatomical measurements applied to Equation 1.

	Montañez et al (2016) as reported in Boyce & Zwieniecki (2019) Table 1					Table 1 this study				
	Min	Max	Median	Mean	n	Min	Max	Median	Mean	n
Medullosans	0.48	13.37	2.1	3.8	23	0.16	5.15	1.36	1.42	75
Lycopsids	1.23	8.3	3.6	4.03	19	1.02	8.52	3.66	3.97	27
Sphenopsids	0.18	0.79	0.3	0.41	5	0.11	1.24	0.45	0.50	16
Cordaitaleans	0.06	12.01	0.57	2.43	20	0.06	6.50	0.48	1.35	19
Tree Ferns	0.05	1.05	0.26	0.28	10	0.05	2.00	0.35	0.68	25

The left side of the table contains values presented in Boyce & Zwieniecki (2019) (derived from Montañez et al. (2016)); on the right are current values from our database of Carboniferous taxa (see Supporting Information Table S1 for complete, raw data). Sources for this data are: Stidd and Stidd (1976), Reihman and Schabilion (1976), Krings and Kerp (1999), Cleal and Shute (1992), Shute and Cleal (2002), Krings and Kerp (1997), Montañez et al. (2016), Cleal and Zodrow (1989), Zodrow and Cleal (1993), Yao et al. (2000), Batenberg (1981, 1982), Batenburg and Hetterscheid (1984), Šimůnek (2015), Pant and Mehra (1963), Thomas (1966, 1967, 1968, 1970, 1977), Šimůnek and Florian (2013), Šimůnek (2007), Šimůnek et al. (2009), Pšenička (2005), Pšenička et al. (2005), Pšenička and Bek (2003), Pšenička et al. (2003).

What are the possible limits to maximum theoretical stomatal conductance in leaves?

Boyce & Zwieniecki (2019) argued that the range of our fossil-based data is beyond a geometric limit—that the maximum possible theoretical stomatal conductance to water cannot exceed 6.0 mol H_2O m^{-2} s^{-1}. We reject this assertion for several reasons. First, our data of stomatal sizes (S) and density (D) fit within the morphospace of these parameters described by Franks and Beerling

(2009) defined by Dmax (Figure 3, upper graph; see Data and Methods in Franks and Beerling, 2009 for additional details). Furthermore, our data regarding stomatal sizes and density are also consistent with the stomatal size and density values of those from the same geologic periods as analyzed by Franks and Beerling (2009; their Fig. 5 B&C) and fall within the expected range for Carboniferous plants.

Regarding the assertion that our data must be in error because $g_{wmax} > 6.0$ mol H_2O m^{-2} s^{-1} is "impossible," is not supported by analysis of the theoretical model presented in Franks and Beerling (2009; Figure 3). In a complementary paper, Franks et al. (2009) present a broader explanation of this model (Eq. 4 in Franks et al. 2009) by which the value of g_{wmax} is assessed as a function of the proportion of leaf area occupied by stomata:

(Eq. 3):

$$g_{wmax} = \frac{D_{wv}\alpha\, d\,\sqrt{2S}}{M_V(0.5 + 0.627 \cdot \sqrt{\alpha})}$$

where α is the proportion of the stomatal size (S) (i.e., guard cell length) forming the aperture pore (a_{max}) or $a_{max} = \alpha S$. In Franks and Beerling (2009), α is assumed as a constant value of 0.12, however, in the Franks et al. (2009) companion paper, this parameter is demonstrated as varying by more than an order of magnitude across the four taxa they studied (α: 0.03 – 0.35, Franks and Farquhar (2007)). Therefore, what Boyce & Zwieniecki (2019) have treated as a scalar does, in fact, vary by taxon, and cannot therefore impose a "geometric limit" as they have stated. It should be noted, that in our previous work, we have developed taxon-appropriate scalars for the proportion of stomatal size forming the aperture pore (α), based on fossils where the full guard cell length and pore length is preserved and that we report below.

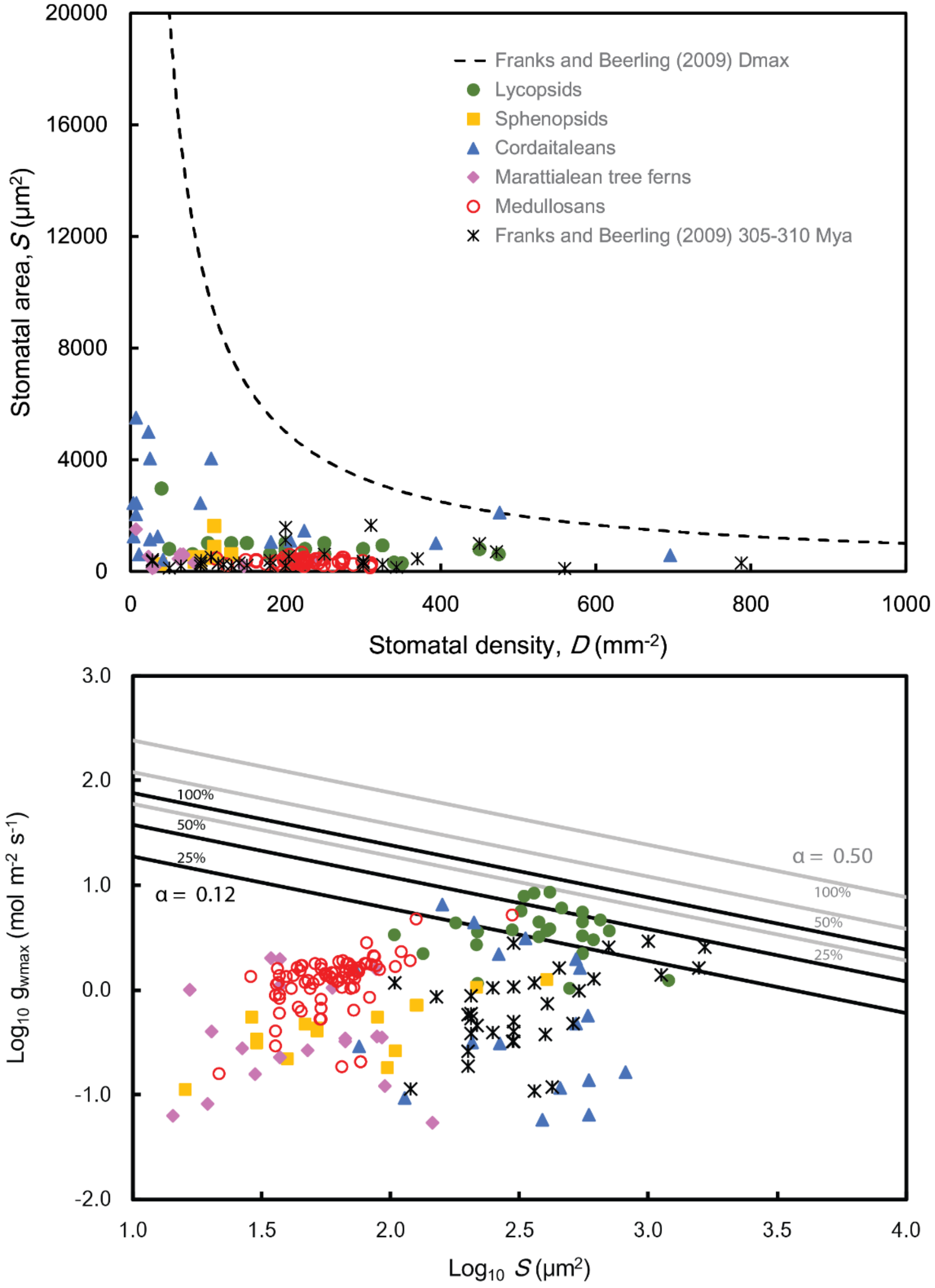
Franks and Beerling (2009) Dmax
Lycopsids
Sphenopsids
Cordaitaleans
Marattialean tree ferns
Medullosans
Franks and Beerling (2009) 305-310 Mya
Stomatal area, S (μm2)
20000
16000
12000
8000
4000
0
0
200
400
600
800
1000
Stomatal density, D (mm-2)
Log10 gwmax (mol m-2 s-1)
3.0
2.0
1.0
0.0
-1.0
-2.0
1.0
1.5
2.0
2.5
3.0
3.5
4.0
Log10 S (μm2)
100%
50%
25%
α = 0.12
α = 0.50
100%
50%
25%

Figure 3: Upper plot: stomatal area (S; µm^2*) versus stomatal density (D; mm*$^{-1}$*) in our taxa (color symbols), compared with raw data from Carboniferous plants in Franks and Beerling (2009) and Franks and Beerling's Dmax (black symbols). Note that stomatal area has also been described as "stomatal size" in previous publications (e.g., Fig. 6 in Franks and Beerling, 2009). Lower plot: log plot of maximum water vapor conductance vs. stomatal area in our Carboniferous taxa with two "maximum theoretical trendlines" for α = 0.12 (black) and α = 0.50 (grey). Lower, middle, and upper lines represent percentage of epidermal cells occupied by stomata: 25%, 50%, 100%. Lycopsid stomatal conductance (green circles) is high, but well within the morphospace if stomatal pores are large relative to guard cell width.*

It is worth exploring this parameter space further, graphically (Figure 3, lower graph). First, when we plot our data presented here and used in previous papers, they fit within the theoretical boundaries prescribed by the Franks et al. (2009) based on an α = 0.12 (Figure 3, lower graph). Several lycopsid specimens fall into an area in which stomata occupy > 50% of leaf area when assuming an α = 0.12, suggesting that α = 0.12 is too low. One, in particular, is *Lepidodendron dichotomum*, which was described by Thomas (1966) and is included as part of the Franks and Beerling (2009) dataset. As a sensitivity analysis, we recalculated the upper limits of g_{wmax} for an α = 0.50, representing an aperture size that is 50% of the total guard cell area (grey lines Figure 3, lower graph) and found that this species would have stomata covering 25% of the leaf area using Franks et al.'s (2009) determination of S and D. Although high, this proportion of leaf area occupied by stomata is not out of the range of stomatal parameters, even in living plants. Notably, this value of 25% is low for other described Carboniferous plants, such as the lycopsid *Swillingtonia denticulata* (Scott and Chaloner 1983; Hubers et al. 2011) where well over 25% of the abaxial leaf surface is occupied by stomata (Figure 4).

Figure 4: Cuticle of Swillingtonia denticulata, a Carboniferous lycopsid, for which over 25% of the leaf's abaxial surface is occupied by stomata. Image modified from Scott and Chaloner (1983).

At this time, it does not appear that α varies according to phylogenetic groups. If it were so, this would simplify treatment of values measured from fossil material (e.g., using the same α for extinct and living conifers). A recent study by deBoer et al (2016) addressed stomatal pore allometry but did not find a consistent relationship among gymnosperms and/or pteridophytes. Thus, because α varies widely among living plants, we measured this directly from fossil material. For our data, mean α values ($\alpha = \beta \cdot \gamma$) were 0.38, 0.53, 0.15, 0.17, and 0.14 for medullosans, lycopsids, sphenopsids, cordaitaleans, and tree ferns, respectively. In their derivations of the original model, Franks and Farquhar (2007) illustrate that the relationships between guard cell sizes, density, and g_{wmax} are quite sensitive to the formation of the stomatal pore and its maximum extension associated with high guard cell turgor pressure. The variability we observe among fossil material suggests that they were correct to observe that there are developmental factors responsible for this variation.

Finally, our analysis shows that theoretical g_{wmax} calculated using fossil stomatal anatomy can exceed 6.0 mol H_2O m^{-2} s^{-1}. For example, using the Franks et al. (2009) model, a plant with a stomatal size (S) of 10 μm^2, an $\alpha = 0.5$, and a stomatal density of 25% of leaf area (e.g., Swillingtonia denticulata in Figure 4) would have an estimated g_{wmax} of 19.0 mol H_2O m^{-2} s^{-1} (See Figure 2, lower graph, intercept of 25% line with log-scale y-axis). Therefore, the conductance limit stipulated by the previous *Viewpoint* of Boyce & Zwieniecki (2019) is not supported by the underlying theory clearly provided by Franks and Farquhar (2007) and appears to be an incomplete assessment of information from Franks and Beerling (2009).

To summarize, Boyce & Zwieniecki's (2019) purported "limit" of stomatal conductance to 6.0 mol H_2O m^{-2} s^{-1} does not exist. This purported limit is not a conclusion from the Franks and Beerling (2009) dataset, but rather is an extrapolation that treats variable parameters in their model as constants. Note that we are not challenging Franks and Beerling in their work—we trust that their calculations are correct. They clearly state their assumptions regarding how stomatal aperture size and depth were estimated from their data pool that influenced calculation of the conductance range. Instead, Boyce & Zwieniecki (2019) appear to have taken the Franks and Beerling variables as constants rather than review the methods that Franks and Beerling used to assemble their dataset. For example, in Franks and Beerling (2009), where no stomatal pore could be measured in their samples, a_{max} was approximated by $\pi(L/4)^2$ based on guard cell length (L). Likewise, pore depth (l) was approximated using guard cell width (W) divided by 2.0. These assumptions allowed Franks and Beerling to compare plant data with minimal information across the Phanerozoic and kick-start a number of interesting studies that have transformed our view of vegetation-climate feedbacks over the past 400 million years. However, our data, which includes direct measurements of stomatal and aperture length and width values, allowed us to produce more refined estimates of maximum g_{wmax} that yielded a broader range of g_{wmax} values, and that expands previous approximations—just as simple, linear proxies for plant function have given way to multivariate, multiparameter analyses. Overall, our mean g_{wmax} values range between 0.5 to 4.0 mol H_2O m^{-2} s^{-1}, which compare favorably with the estimated range of maximum theoretical stomatal conductance found in a survey of 218 woody angiosperm species of ~0.1 to ~3.8 mol H_2O m^{-2} s^{-1}, if operational stomatal conductances are multiplied by 5 to estimate the theoretical maximum (Murray et al. 2019). The correspondence between these ranges highlights the variety of plant form, function, and productivity that existed during the Carboniferous Period.

Section 2: What is the best way to represent the flow of water through a plant?

Methods for quantifying whole-plant hydraulics have been refined for more than 70 years (van den Honert, 1948). From the perspective of the liquid phase flow within a plant, the major organ systems within a plant—the roots, stem, and leaves— are in series with one another but, within each organ system, individual organs or hydraulic pathways are in parallel with one another. Specifically, leaves contain numerous parallel hydraulic pathways to stomata and are not in a series with one another within the hydraulic architecture of the tree, even in plants with a single vein. Therefore, from a *whole plant perspective*, veins function in parallel and must be modeled as resistors in parallel, in which more veins reduce the overall resistance of the leaf (Eq. 4). This is particularly true for many Paleozoic plants, whose leaves and fronds contain multiple parallel pathways and frequently contain several independent connections to branch and stem xylem (e.g., Stewart and Delevoryas, 1952; Figure 5). To account for the major organ systems acting in series and the leaves acting in parallel, a mathematical representation of the hydraulic resistance on a whole-plant-scale for a leaf with multiple veins would therefore be:

$$R_{plant} = \boxed{\frac{R_{leaf}}{N_{leaf}}} + \boxed{R_{stem} + R_{root}}$$

Percentage of total hydraulic resistance:
25% (Sack et al. 2003) 75%
30% (Sack and Holbrook, 2006) 70%

(Eq. 4)

This equation represents the entire liquid-phase hydraulic pathway through a plant focused on the hydraulic resistances of a leaf, a stem, and the root system (R_{leaf}, R_{stem}, R_{root}). Total plant hydraulic resistance (R_{plant}) is the sum of three terms: the average whole-leaf hydraulic resistance (including both mesophyll and xylem; R_{leaf}) divided by the number of leaves (N_{leaf}), the stem hydraulic resistance (R_{stem}), and root hydraulic resistance (R_{root}). One consequence of this mathematical framework is that there are three components to plant hydraulic resistance and each one may vary considerably over long evolutionary timescales.

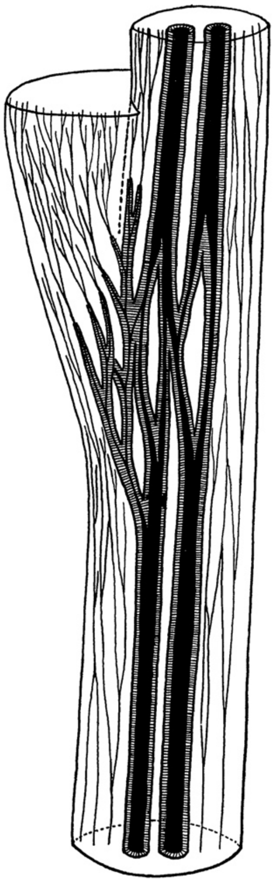

Figure 5: Reconstruction of the nodal and internodal xylem of the Pennsylvanian stem group seed plant Medullosa heterostelica, from Stewart and Delevoryas (1952). Thin lines represent leaf traces, heavy black shading represents primary xylem, horizontal lines represent secondary xylem. This reconstruction was developed by serial sectioning through a single fossilized stem nearly 16cm long and less than 4cm wide. Even a stem this small contains secondary xylem tracheids greater than 100μm in diameter (Stewart and Delevoryas, 1952).

To be sure, stems, roots, and mesophyll pathways also contain their own topological complexities and should be deconvolved into their own mathematical expressions, accounting for structures within them that are in parallel and those that are in series. The representation here (in Eq. 4) is merely to highlight that the argument in the Boyce & Zwieniecki's (2019) *Viewpoint*, that fluid flow in a plant is strictly modeled as resistors in series, rather than accounting for structures in parallel with one another, is contrary not only to our view, but to those of many other researchers over the past seventy years (e.g., Van den Honert, 1948; Philip, 1966; Weatherley, 1976; Gibson et al., 1984; Tardieu, 1993; Tyree and Ewers, 1991; Whitehead, 1998; Sperry, 2000; Sperry et al., 2002; Wright et al., 2006; Pitterman, 2010; Manzoni et al., 2013; Landsberg et al., 2017; Lehnebach et al., 2018).

How is an equation like this evaluated?

The next logical question is, which component of this equation is most important to the overall hydraulic resistance of the plant? The Boyce & Zwieniecki (2019) *Viewpoint* suggests that leaves are the dominant hydraulic resistance of plants and that our previous studies, which highlighted the contribution of root, stem, and branch anatomy to the total hydraulic path of Carboniferous plants, constitute a "fundamental error" by considering the parallel aspects of these structures. However, there is a great deal of plant physiological research that indicates that leaves vary tremendously in their contribution to the whole-plant liquid-phase hydraulic resistance. Compilations of analyses of angiosperms and conifers, for example, have suggested that leaves contribute only 25% (Sack et al. 2003; Sack and Tyree, 2005) to 30% (Sack and Holbrook, 2006) of total plant hydraulic resistance. Recent organ-specific analysis of whole-plant hydraulic resistance has supported this view, including the surprising discovery of coordinated, high cavitation resistance in roots, stems, and leaves of olive plants (Rodriguez-Dominguez et al. 2018).

Therefore, *stems and roots contribute 70% to 75% of total plant hydraulic resistance*. This distribution of percentage of hydraulic resistance from leaves and stems is annotated in Equation 4. The stem and root systems contribute the majority of total plant hydraulic resistance, contrary to the Boyce & Zwieniecki (2019) *Viewpoint*. Further details on this quantitative derivation can be found in Sack et al. (2003), Sack and Holbrook (2006), and Sack and Tyree (2005).

Other, recently published work has demonstrated that there is a great deal of variability in the amount of hydraulic resistance among leaves. For example, leaf hydraulic resistance varies between species, between sun and shade leaves, and based on internal leaf anatomy (Sack et al. 2005, Scoffoni et al 2016). This variability argues for the importance of leaves in understanding the whole-plant liquid phase pathway, but it cannot and does not render the rest of the plant unimportant.

The relatively low vein length per area (VLA) found among Carboniferous plants, when taken together with the highly conductive xylem of some lineages, presents an apparent paradox: how could highly conductive stems be attached to leaves that are presumed by others to have low transpirational capacity and, therefore, also low photosynthetic capacity? Boyce & Zwieniecki's (2019) *Viewpoint* argues, incorrectly (see below), that Carboniferous plants must have had low-conductivity xylem to match their presumably low-transpiration leaves. However, a recent analysis comparing the leaves of the "living fossil" *Ginkgo biloba* with those of the northern red oak (*Quercus rubra*) demonstrated that there are quantitative tradeoffs between leaf mesophyll resistance and vascular resistance that allow plants with lower VLA to reduce the overall leaf-scale hydraulic resistance (Rockwell and Holbrook, 2017). In angiosperms, high VLA reduces one component of total leaf resistance, the mesophyll resistance, by decreasing the distance between minor veins and the sites of evaporation. However, *Ginkgo* leaves lack minor veins, which is compensated for by simply increasing the number and width of tracheary elements in each leaf vein. These *Ginkgo* traits reduce the leaf vascular resistance, which results in a water supply per unit vein length greater than that of red oak, despite *Ginkgo*'s higher mesophyll resistance. Because of the Hagen-Poiseuille relationship between flow rate and conduit diameter ($\sim r^4$), small increases in non-angiosperm leaf tracheid width (as in *Ginkgo*), when taken to the fourth power, could compensate for longer mesophyll pathways that are a result of the lack of minor veins (Rockwell and Holbrook, 2017). Therefore, a leaf with relatively low vein length per area, such as many Carboniferous plants, but with low-resistance vein xylem, can supply more water per unit vein length area by simply increasing the diameter of its leaf tracheids, obviating the need for increased vein density. *Contrary to the prevailing opinion, then, high vein length per area is not required to yield high hydraulic supply in leaves*. High vein length per unit area is merely one evolutionary solution to the hydraulic supply problem, and it appears to be a "simpler" path to high hydraulic supply in leaves when considering photosynthetic productivity from an angiosperm-centric point of view. However, the limitations of low VLAs have been mitigated in other plant lineages by changes in leaf tracheid structure or in mesophyll resistance. Rather than a paradox, then, when considering the comparatively large diameter of leaf-vein tracheids in certain Carboniferous plants in relation to their thin leaves (Oestry-Stidd, 1979;

Raymond et al. 2014), it is likely that these extinct plants contained an ecologically successful suite of coordinated stem and leaf adaptations.

Boyce & Zwieniecki's (2019) recently published *Viewpoint* also presents a related argument, suggesting that large and wide conduits in plants do not always translate to high plant productivity. This view is partially correct because very little in biology correlates perfectly. That said, many studies have shown that large conduits are strongly correlated with high leaf conductance and photosynthetic rates across the phylogenetic tree of vascular plants (Wright et al. 2006) and within lineages, from ferns (Pittermann et al. 2011, 2013; Watkins et al. 2010) to conifers (Hubbard et al. 2001) to angiosperms (Brodribb and Feild, 2000; Sperry 2000). This relationship has been observed for more than 25 years (Sperry et al. 1993) and holds across a wide variety of environments, from moist tropical forests (Santiago et al. 2004) to dry forests (Brodribb et al. 2002) and even to nearshore, nutrient-stressed environments (Lovelock et al. 2006). Furthermore, unusually large vasculature can permit competitive ecological behavior that results in surprising winners. *Pteridium aquilinum*, the bracken fern, can act as an invasive species and routinely outcompetes angiosperms in a wide variety of environments (Watt, 1979; Page, 1986; Taylor, 1990; Goddard, 2000, Levy-Tacher et al. 2014), despite low VLA and likely because its xylem combines resistance to cavitation and low hydraulic resistance (Brodersen et al. 2014; Pittermann et al. 2011, 2013). It is expected that, as it is today, plants in the geologic past with large conduits (e.g., in the case of *Medullosa* and other extinct plants*;* Wilson et al. 2008; Wilson and Knoll 2010; Wilson and Fischer 2011a, Wilson 2013) could supply large leaf areas—and that even if individual-leaf productivity was lower than modern angiosperms, these plants could achieve large per-plant productivity (Pittermann et al. 2016). Numerous plants with large conduits supplying large leaf areas, as observed in the tropical and temperate forests of the Carboniferous Period, would translate into biomes capable of acting as a large carbon sink and producing major effects on local- to global-scale hydrology.

Section 3: How do Carboniferous plants compare with extant representatives of their lineages?

Finally, the substantial evolutionary and morphological differences between Carboniferous plants and their extant relatives raises a question: are Carboniferous plants more productive than extant members of their lineages? In order to answer this question, it is normally best to take a within-lineage perspective, comparing apples to apples. However, there either are (1) no close living relatives of key Carboniferous plants such as the medullosans, (2) the relatives are vastly different in form from the fossil taxa, such as the arborescent lycopsids, (3) or there are limited morphological analogues, such as the cordaitaleans (no living gymnosperm has broad, meter-long, strap-shaped leaves attached to pycnoxylic wood), or (4) the architecture and internal anatomy of the Carboniferous plants, such as the tree fern taxon *Psaronius*, differ in such an extreme case from their extant relatives (for *Psaronius*: the marattialean tree ferns).

One of the strongest lines of evidence that Carboniferous plants are distinct from their living relatives comes from study of their xylem anatomy, which demonstrates that these plants evolved highly efficient hydraulic tissues long before the evolution of angiosperms. These tracheids are notable for their size, and the consequent effect of large tracheids on plant function. It is a biophysical fact that a 200-µm diameter and more than 20-mm long xylem cell will support high hydraulic conductivity. Several previous studies, including the Boyce & Zwieniecki (2019) *Viewpoint*, have argued that Carboniferous plants' anatomical features are less than consequential for whole-plant physiology or ecosystem evolution by directly comparing them with other non-angiosperm taxa, specifically by Boyce & Zwieniecki (2019) to ferns or cycads as direct analogues of Carboniferous plants. For example, Boyce & Zwieniecki (2019) state "The [climbing fern] vine Lygodium has tracheids that are frequently > 100 µm in diameter (Pitterman et al., 2011), yet this fern does not have high photosynthetic rates – indeed, it was one of the original taxa used to demonstrate that low vein densities are associated with low transpiration and assimilation capacities." This sentence argues that relationship between the large xylem cells, transpiration, and assimilation in a modern seed-free vascular plant, *Lygodium*, is the same as the relationship between the xylem cells, transpiration, and assimilation in Carboniferous plants, based on their approximate relationship on the phylogenetic tree of land plants and comparatively low VLA. It is misleading to analogize *Lygodium* with the Carboniferous medullosans, whose stems were constructed of large secondary xylem cells, which were attached to large leaf areas (Sterzel, 1918, Laveine, 1986; Laveine and Dufour, 2013, Pfefferkorn et al., 1984, Wnuk and Pfefferkorn, 1984), in tandem with modeling results that suggest that medullosan leaves supported high stomatal conductance. Extant ferns with limited vascular tissue but a small number of large tracheids (Gibson and Nobel, 1984), such as *Lygodium*, or cycads, in which stem xylem is made up of a small number of narrow tracheids (much closer to the morphology of coniferophyte xylem than any Carboniferous plant; Greguss, 1968), are both inappropriate ecophysiological analogues to many of the Carboniferous tropical plants, particularly seed-bearing medullosans. Using these plants as analogues for tropical Carboniferous plants is further perplexing as the anatomical distinction and complexity of medullosans, for example, is well known. Furthermore, this anatomical information is well preserved in the fossil record—failure to consider the totality of the information these fossils provide closes off entire avenues of investigation and hypothesis formulation. Readers need not rely on our quantitative work to point this out. This has been noted by Cichan in 1986 (Cichan, 1986) and Niklas in 1985 (Niklas, 1985), and even qualitatively by Andrews more than 75 years ago (Andrews, 1940).

It is instructive to consider the case of the sphenopsids as a concrete example of how divergent extinct and extant plants can be, even within this lineage notable for its stereotypical morphology. The horsetails, genus *Equisetum*, form a monophyletic group of seed-free vascular plants with 15 species distributed around the globe (Des Marais et al. 2003). Extant horsetails are closely related to two extinct clades of sphenopsids that formed prominent components of wetland vegetation during the Paleozoic Era: the Sphenophyllales (e.g., *Sphenophyllum*; Figure 6) which contained secondary xylem, and the Calamitaceae (e.g., *Arthropitys*), both of which had secondary xylem. The former had the largest tracheids of any Carboniferous plant, and the latter lineage evolved the arborescent habit through an amplified form of the internodal expansion found within extant horsetails (Taylor et al. 2009). There are no extant horsetails with secondary xylem or true arborescence. Therefore, an investigation of these extinct plants, with a strict focus on nearest living relatives analogous to the approach of Boyce & Zwieniecki (2019) to employ ferns and cycads to understand Carboniferous "seed ferns", would be forced to use *Equisetum* to stand in for *Sphenophyllum* and *Arthropitys*. These groups demonstrate how far astray we can be led by a narrow focus on nearest living relatives.

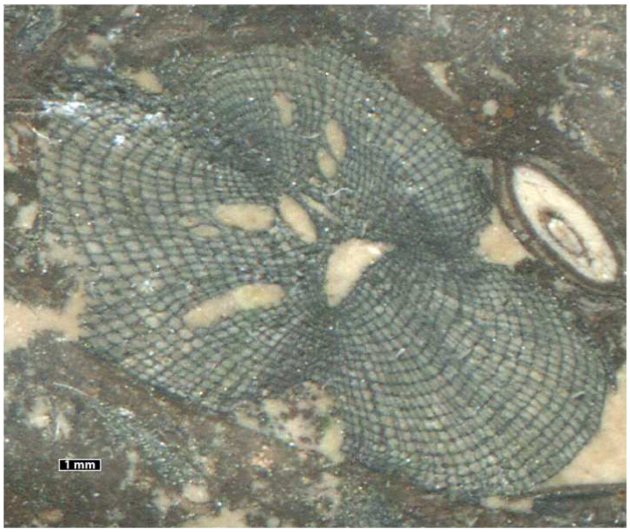

Figure 6: Cross-section of a permineralized stem of Sphenophyllum plurifoliatum, an extinct sphenophyte from the Carboniferous Period with secondary xylem. Scale bar is 1mm, median secondary xylem tracheid diameters in this specimen are 236µm, maximum measured diameter exceeds 350µm.

Such a strict reliance on living relatives can lead to completely incorrect conclusions. Figure 7 presents the tracheid diameter of four specimens of *Sphenophyllum plurifoliatum*, three taxa from the arborescent sphenopsid *Arthropitys*, and four species of extant horsetails, including two of the "giant" species of *Equisetum* alive today (*E. giganteum* and *E. myriochaetum*). As this plot clearly demonstrates, tracheids from Carboniferous *Sphenophyllum* routinely exceed 200µm in diameter, and rare, large, tracheids exceed 400µm in diameter—the same scale as some of the widest angiosperm vessels today. In contrast, extant *Equisetum*'s tracheids, even those found in the largest extant species, are between 9 and 19µm in diameter, with mean values between 12 and 18µm. Assuming a typical *Sphenophyllum* tracheid with a radius of 100µm and a typical *Equisetum* tracheid with a radius of 7.5µm, and using the Hagen-Poiseuille equation to estimate flow rate ($\sim r^4$) between these two taxa, the *Sphenophyllum* tracheid would have a flow rate 31,600 times higher than *Equisetum*. Even an average Carboniferous *Arthropitys* tracheid that is 35µm wide would support a per-conduit flow rate 29 times higher than a living *Equisetum* tracheid. Although these three clades of plants are sufficiently similar to one another in external morphology that an undergraduate student can recognize that they are closely related, their anatomy and physiology are so different that it would be a serious error to look to *Equisetum* as an analogue for *Arthropitys* or *Sphenophyllum*. The pathway advocated by Boyce & Zwieniecki's (2019) *Viewpoint*, to look toward ferns or cycads as direct analogues of Carboniferous plants because they are not angiosperms and only share one leaf anatomical feature, would lead scientists considerably astray in their interpretations.

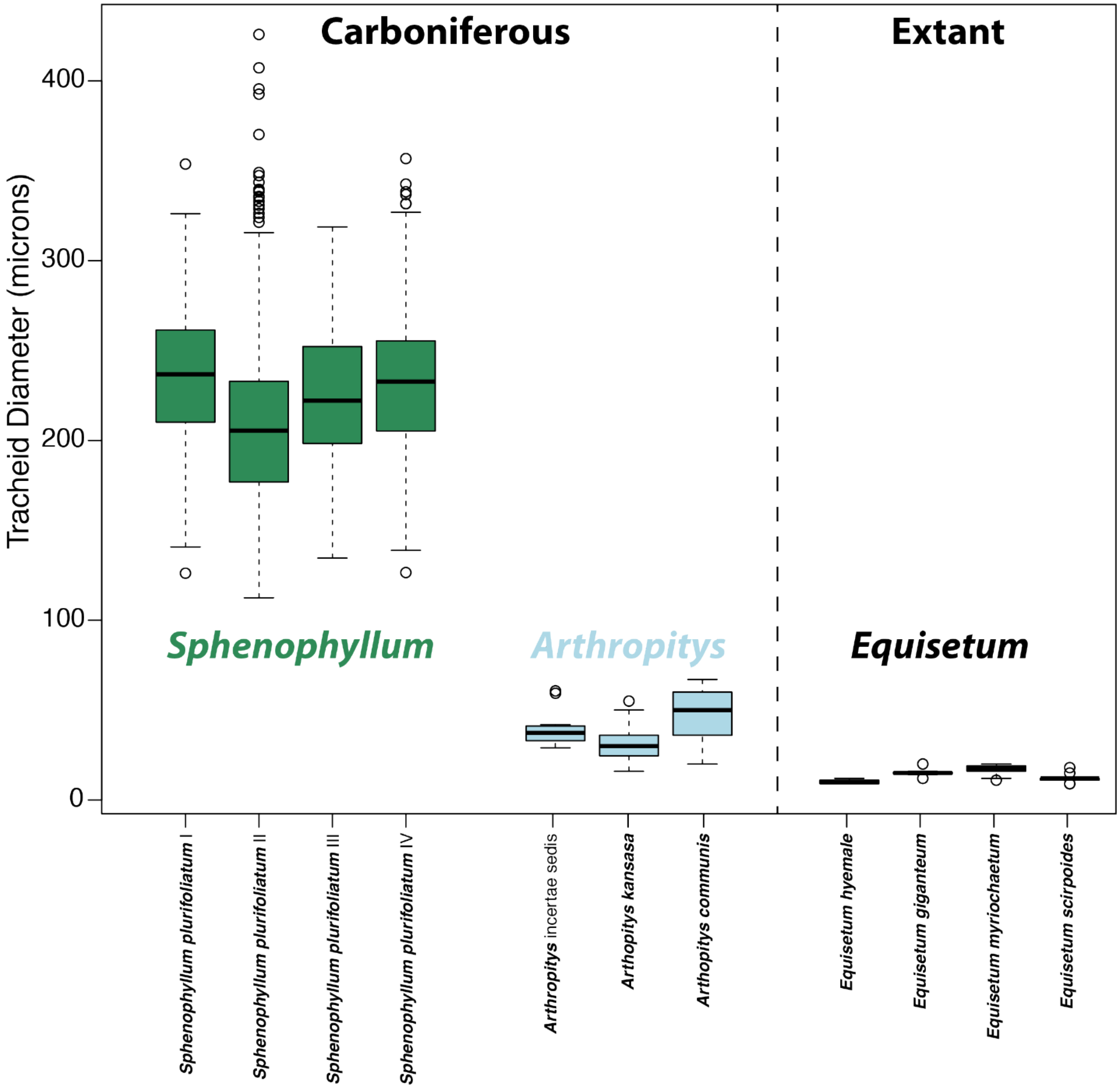

Figure 7: Comparison of tracheid diameter between extinct and extant sphenophytes. At left, Sphenophyllum and Arthropitys, representatives of Carboniferous Period

sphenophytes, both of which evolved secondary xylem and, in the latter, arborescence. At right, four species of extant horsetails. All measurements were made directly from fossil or extant material, and tracheid diameters were measured directly in the case of circular tracheids or transformed into the diameter of a circle with equivalent cross-sectional area in the case of oblong tracheid shapes (see Supporting Information Methods S1 for details). Sphenophyllum measurements are from four anatomically preserved specimens, with 494 tracheids measured from sample I, 505 measured from sample II, 296 measured from sample III, and 498 measured from sample IV. Arthropitys incertae sedis measurements are from 11 tracheids in one anatomically preserved stem. Arthropitys kansasa measurements are from 83 tracheids found in four anatomically preserved stems. Arthropitys communis measurements are from 27 tracheids found in one exceptionally preserved stem. Equisetum tracheid measurements are from 6, 7, 12, and 12 tracheids, respectively (left to right), macerated from living specimens. Central line in boxplot is the median; top and bottom of box is 75th percentile and 25th percentile, respectively; whiskers define the boundaries of the 1st and 99th percentile; outliers are shown as points.

Section 4. Climate modeling vs. geochemical mass balance modeling.

We would like to clear up a large-scale misunderstanding presented in the Boyce & Zwieniecki (2019) *Viewpoint* regarding the modeling utilized in both Montañez et al. (2016) and Wilson et al. (2017a). Boyce & Zwieniecki (2019) assert that Wilson et al. 2017 combined a 'Berner-style carbon cycle model' with a regional biome model. This is an erroneous assumption, and it led, in turn, to Boyce & Zwieniecki (2019) linking the modeling approaches of Montañez et al. (2016) and the Wilson et al. (2017) papers. Boyce and Zwieniecki (2019) state "In addition to climate, the other original application of the physiological interpretations expanded upon in Wilson *et al.* (2017a) was to the modeling of the Carboniferous *carbon cycle* (Montañez et al., 2016)." To clarify, neither Wilson *et al.* (2017) or Montañez *et al.* (2016) utilized or referred to a carbon cycle model, in which the pools and fluxes between elements of the global carbon cycle are simulated (a key tool of geochemical and geobiological analysis; ; see Supporting Information Notes S1). The results and discussion of terrestrial carbon sequestration in the Montañez *et al.* (2016) — and not discussed in the Wilson *et al.* (2017) paper — are based on vegetation simulations made using GENESIS v.3.0, a General Circulation Model linked to an ice-sheet model (Horton et al., 2010), and not based on using a carbon cycle model. Of note, the discussion in Montañez *et al.* (2016) of the potential of late Paleozoic plants — lycopsids in particular — to sequester sufficient carbon during the glacial stages of eccentricity cycles relative to the interglacial stages, and thus to impart large-scale (100 to 200 ppm) changes in atmospheric $p\mathrm{CO}_2$ between glacials and interglacials, is based on the productivity and lifespan estimates of Boyce and DiMichele (2016). Therefore, Boyce & Zwieniecki's (2019) objection to the capability of lycopsids to sequester carbon during the Carboniferous period, as demonstrated in Montañez *et al.* (2016), is to their own published work.

Conclusions

We restate an implicit message of our original Tansley Review (Wilson et al. 2017): extinct plants of the past are not necessarily functional analogs of extant plants found within modern ecosystems. Although phylogenetic clades have been used as an organizing principle for assessing physiological functional diversity in modern plants, several hundred million years separate Carboniferous plants from their nearest living relatives, allowing for considerable differences, both genetically and functionally. The purpose of the original Tansley Review was to convey to a diverse audience of modern plant biologists that there is potential diversity in plants of the past that should expand our appreciation of how plants, in general, may relate to topics such as paleoclimate, the evolution of plant hydraulics, and global water and carbon cycling. We showed in our review that in past ecosystems important differences from modern plants existed in plant organ morphology, plainly evident and measurable in the fossil record, and that these morphological differences must imply some differences in physiological function, particularly in clades of plants often described as "primitive" or "lower" in functional capacity. Although angiosperms represent the current most-derived clade of plants within the Cenozoic adaptive landscape, the extant remnants of non-angiosperm clades should not necessarily be interpreted as the functional equivalents of extinct members of their phylogenetic lines. *Isoetes* is physiologically divergent from *Lepidodendron*, as *Angiopteris* is from *Psaronius*, and *Dioon* is from *Medullosa*. Distinctions such as these are important to avoid progressionist or typological biases when evaluating the evolutionary history of land plants.

The debate over the potential capacity of ecosystems to influence the Earth system before the rise of angiosperms argues for the centrality of paleobotanical studies—along with deeper physiological analyses of non-angiosperms alive today—in order to understand the trajectory of Earth surface processes after plant terrestrialization. The Carboniferous Period is a time of exceptional plants forming exceptional ecosystems: these extinct plants pushed the physiological envelope in many ways. Application of modern methods to paleobotanical materials will help derive the real driving factors that undergird plant and ecosystem evolution over long evolutionary timescales.

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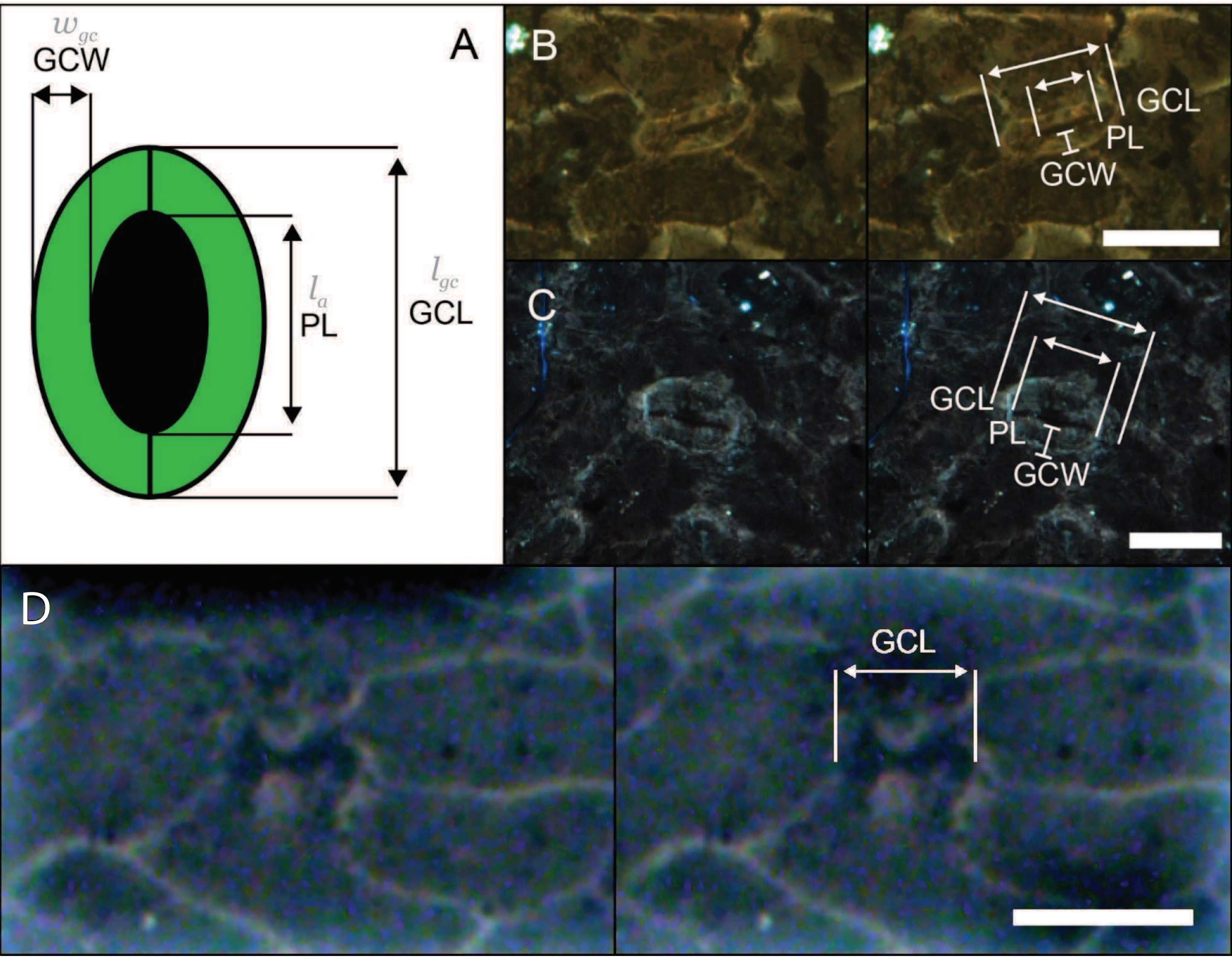
Supporting Information

Table S1: Values and sources of maximum theoretical water vapor conductance (g_{wmax}; mol m^{-2} s^{-1}) for representative species of Carboniferous flora based on anatomical measurements.

Methods S1: Methods for: converting tracheid cross-sectional area to area of an equivalent circle, and differentiating between maximum theoretical stomatal conductance and average operational stomatal conductance.

Notes S1: Additional notes.



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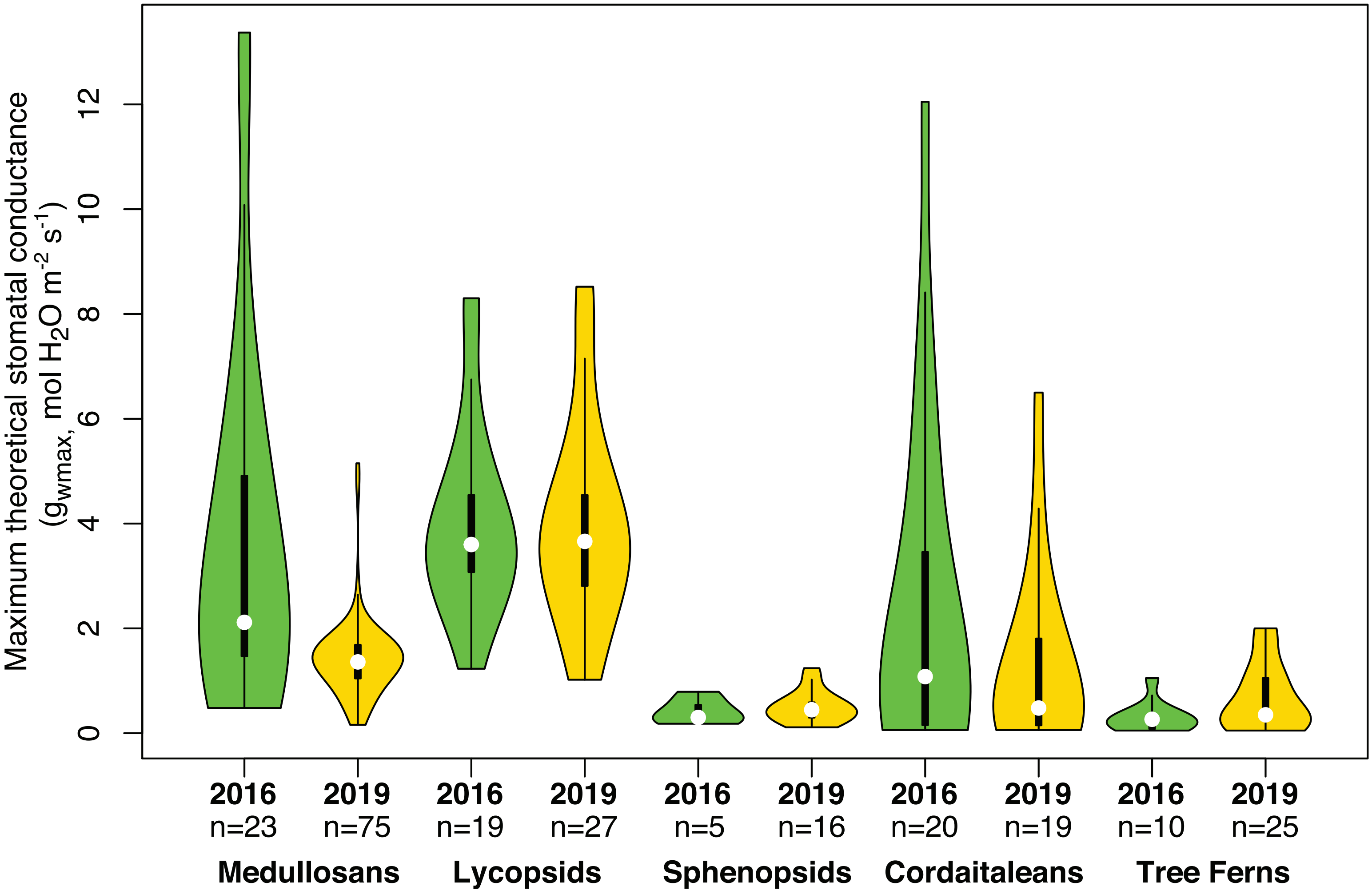

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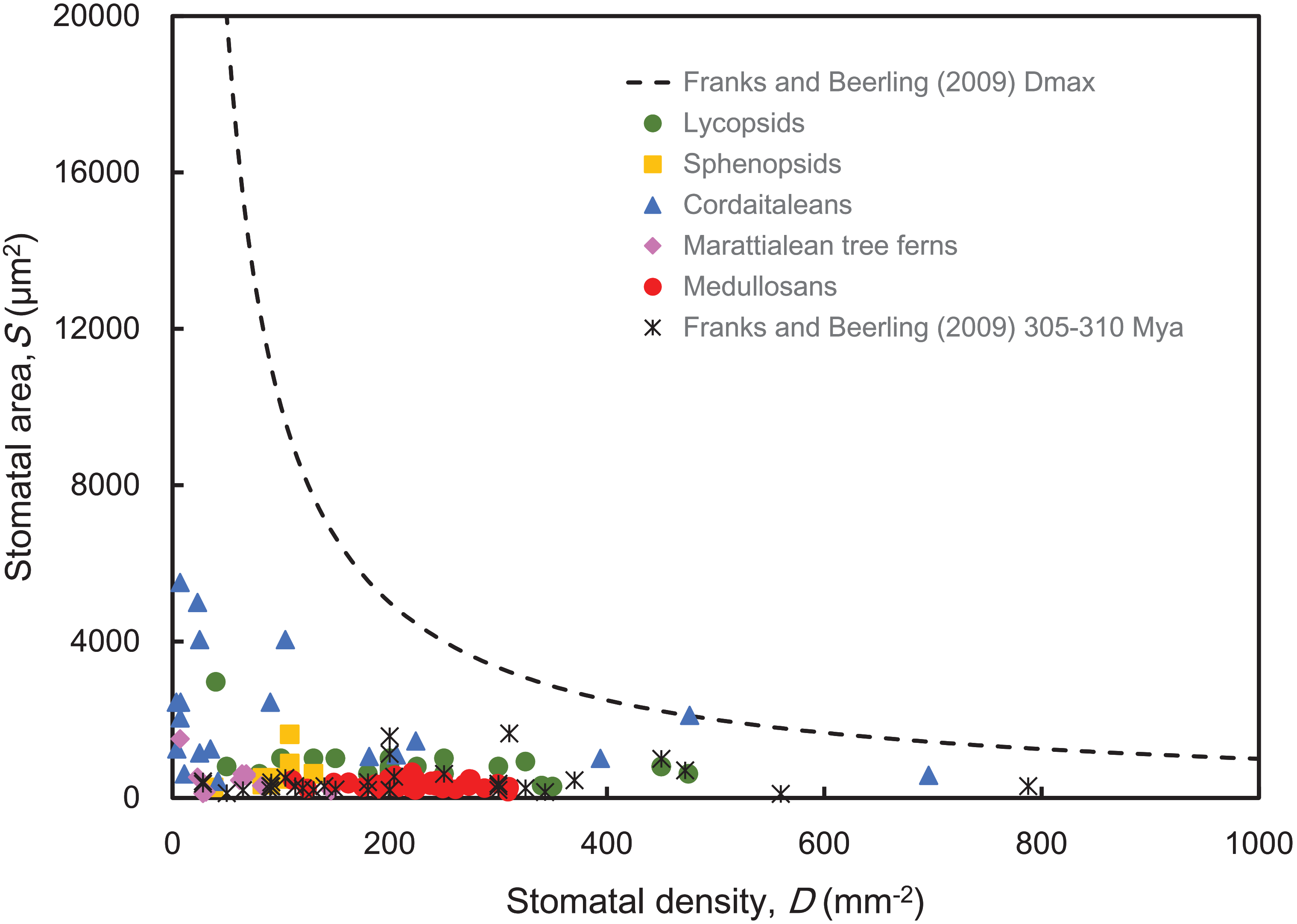
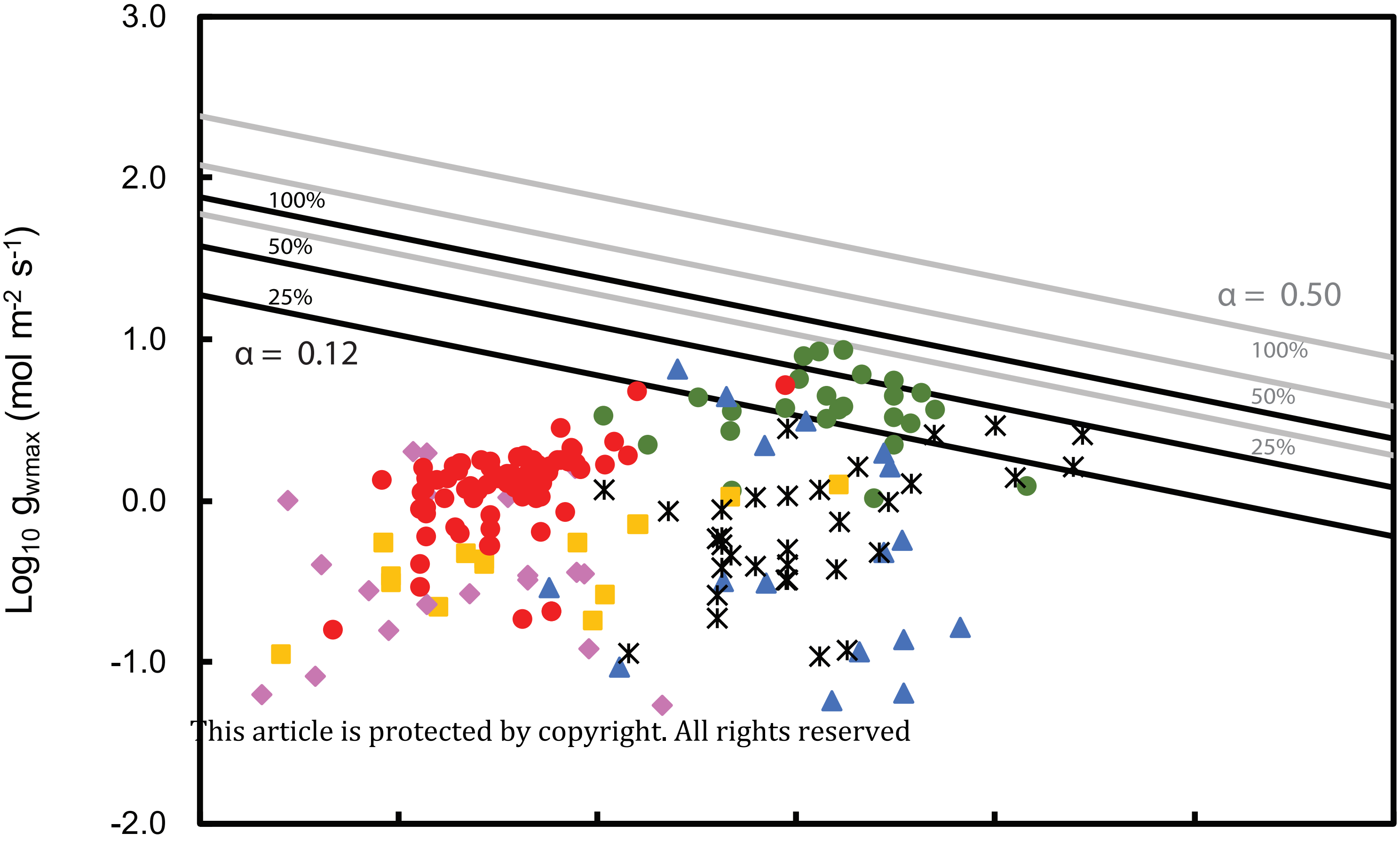
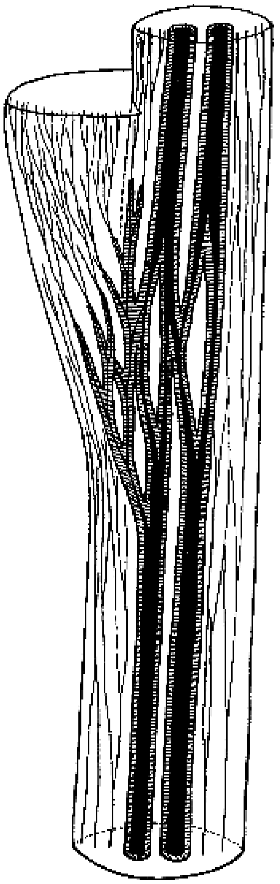

20000
16000
12000
8000
4000
0
Stomatal area, S (μm^2)
0
200
400
600
800
1000
Stomatal density, D (mm^{-2})
Franks and Beerling (2009) Dmax
Lycopsids
Sphenopsids
Cordaitaleans
Marattialean tree ferns
Medullosans
Franks and Beerling (2009) 305-310 Mya


3.0
2.0
1.0
0.0
-1.0
-2.0
Log$_{10}$ g$_{wmax}$ (mol m^{-2} s^{-1})
100%
50%
25%
α = 0.12
α = 0.50
100%
50%
25%

nph_16460_f4.png



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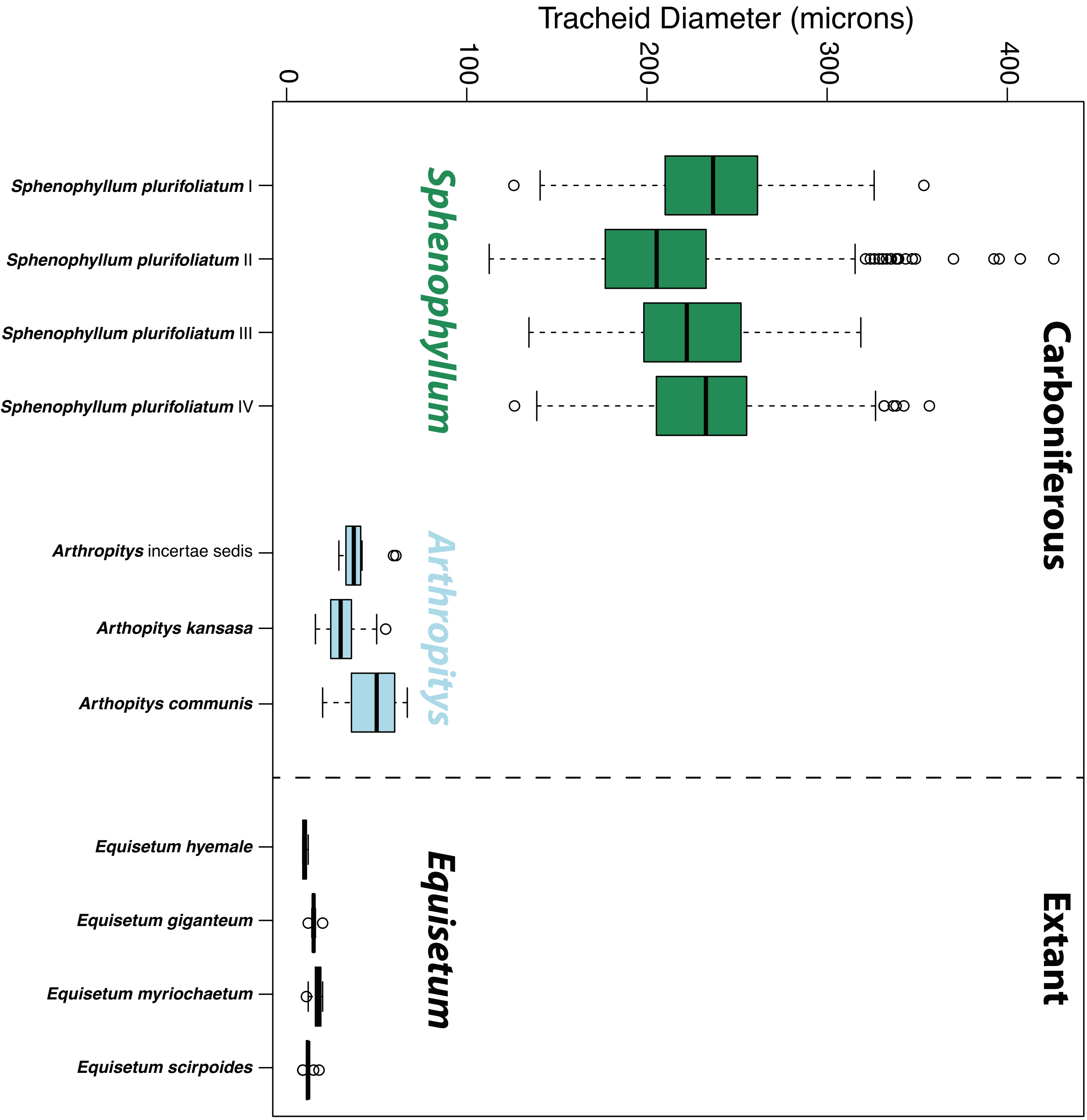
nph_16460_f6.eps



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