

INVITED SPECIAL ARTICLE

For the Special Issue: *The Tree of Death: The Role of Fossils in Resolving the Overall Pattern of Plant Phylogeny*

Origin of *Equisetum*: Evolution of horsetails (Equisetales) within the major euphyllophyte clade Sphenopsida

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PREMISE OF THE STUDY: *Equisetum* is the sole living representative of Sphenopsida, a clade with impressive species richness, a long fossil history dating back to the Devonian, and obscure relationships with other living pteridophytes. Based on molecular data, the crown group age of *Equisetum* is mid-Paleogene, although fossils with possible crown synapomorphies appear in the Triassic. The most widely circulated hypothesis states that the lineage of *Equisetum* derives from calamitaceans, but no comprehensive phylogenetic studies support the claim. Using a combined approach, we provide a comprehensive phylogenetic analysis of Equisetales, with special emphasis on the origin of genus *Equisetum*.

METHODS: We performed parsimony phylogenetic analyses to address relationships of 43 equisetalean species (15 extant, 28 extinct) using a combination of morphological and molecular characters.

KEY RESULTS: We recovered Equisetaceae + *Neocalamites* as sister to Calamitaceae + a clade of Angaran and Gondwanan horsetails, with the four groups forming a clade that is sister to Archaeocalamitaceae. The estimated age for the *Equisetum* crown group is mid-Mesozoic.

CONCLUSIONS: Modern horsetails are not nested within calamitaceans; instead, both groups have explored independent evolutionary trajectories since the Carboniferous. Diverse fossil taxon sampling helps to shed light on the position and relationships of equisetalean lineages, of which only a tiny remnant is present within the extant flora. Understanding these relationships and early character configurations of ancient plant clades as Equisetales provide useful tests of hypotheses about overall phylogenetic relationships of euphyllophytes and foundations for future tests of molecular dates with paleontological data.

KEY WORDS cladistics; combined-analysis; Equisetales; Equisetaceae; Euphyllophytina; fossil; phylogeny; sphenophytes.

Equisetum L. is a genus of vascular plants comprising approximately 15 extant species with nearly worldwide distribution (Hauke, 1963, 1978; Husby, 2013; PPG I, 2016). Even though the morphology and anatomy of *Equisetum* have been documented in detail (e.g., Bierhorst, 1971; Gifford and Foster, 1989; Evert and Eichhorn, 2012), the distinctiveness of this genus has vexed researchers since the

early 20th century in terms of its phylogenetic placement in the plant tree of life (Scott, 1900; Browne, 1908; Eames, 1936; Boureau, 1964; Bierhorst, 1971; Rothwell, 1999; Pryer et al., 1995, 2001; Karol et al., 2010; Rothfels et al., 2015). Based on overall morphological differences, this genus has been divided into two subgenera, *Equisetum* L. and *Hippochaete* Milde (Hauke, 1963, 1978). More recently,

DNA-based phylogenetic analyses have placed *E. bogotense*, a species morphologically allied with subgenus *Equisetum*, in equivocal positions (Des Marais et al., 2003; Guillon, 2004, 2007).

Despite the modest modern diversity of horsetails, paleobotanical studies have long demonstrated that the genus *Equisetum* is the sole surviving representative of an extremely ancient and diverse group, the Sphenopsida (Neuberg, 1964), which includes at least 60 genera with a plethora of species (Boureau, 1964), and which has a long evolutionary history that can be traced in time as far back as the Late Devonian (Gu and Shi, 1974; Wang et al., 2005). Sphenopsids comprise two orders, the Sphenophyllales (Boureau, 1964) and the Equisetales (Boureau, 1964) (Stein et al., 1984; Stewart and Rothwell, 1993; Taylor et al., 2009), which reached a peak in species richness during the Carboniferous period, when sphenopsids were some of the most common constituents of paleotropical swamp forest ecosystems (Behrensmeyer et al., 1992; Cleal et al., 2012).

The oldest representatives of Equisetales, the archaeocalamites, probably originated in the Early Carboniferous (Kidston, 1883; Chaphekar, 1965; Bateman, 1991), a time they are known from humid environments of Europe and North America. Since that time, the group has exhibited a variety of growth architectures. These include arborescent Calamitaceae with compact bracteate strobili, from the northern hemisphere (Williamson and Scott, 1894; Good, 1975; Feng et al., 2012), herbaceous *Cruciaetheca* Cúneo et Escapa and *Tschernovia* Zalesky from the Permian of South America and Angara that had lax reproductive structures (Meyen and Menshikova, 1983; Cúneo and Escapa, 2006), and large *Equisetites* Sternberg with multiple compact strobili on lateral branches, from the European Triassic (Kelber and van Konijnenburg-van Cittert, 1998). The unique morphology of modern horsetails, usually including bractless compact strobili on main aerial stems, as well as numerous vegetative synapomorphies, dates at least from the Jurassic, according to fossils from Patagonia (Channing et al., 2011; Elgorriaga et al., 2015), and probably from the Triassic (Stanich et al., 2009).

A study based on molecular data (Sanderson, 2002) estimates the divergence of extant *Equisetum* species to have occurred in mid-Paleogene (late Eocene, ca. 40 Ma), with the main radiation occurring during the Neogene (Des Marais et al., 2003). While that estimate may seem reasonable from analysis of living species, the absence of a phylogenetic framework including well-preserved fossils renders that hypothesis difficult to test. Fortunately, our understanding of *Equisetum* evolution has been enriched in the past years by the discovery of several fossils of successively more recent ages that display progressively larger numbers of putative crown group features (Stanich et al., 2009; Channing et al., 2011). The initial studies of those fossils suggested that the origin of the group may have been considerably older than previously calculated, perhaps as old as the mid-Mesozoic (Stanich et al., 2009; Channing et al., 2011; Elgorriaga et al., 2015; Rothwell and Ash, 2015), interpretations that still need to be tested in a phylogenetic framework.

The origin and systematic relationships of modern *Equisetum* have been debated extensively, with the most widely accepted paleontological hypothesis proposing that the line leading to living species had calamitacean ancestors (Good, 1975). According to that scenario, evolution of *Equisetum* involved the loss of several important features, such as arborescent stature, secondary growth, heterospory, and bracts within strobili (Good, 1975; Bateman, 1991; Stewart and Rothwell, 1993). An alternative hypothesis

proposes that *Equisetum* belongs to a lineage from the Angaran and Gondwanan paleofloristic provinces, including species of *Koretrophyllites* Radczenko, *Tschernovia* Zalesky, *Paracalamitina* Zalesky, *Equisetinostachys* Rasskasova, *Cruciaetheca* Cúneo et Escapa, and *Peltotheca* Escapa et Cúneo (Naugolnykh, 2004; Cúneo and Escapa, 2006), a hypothesis partly supported by the similarity of reproductive structures of those fossils to teratological forms of modern *Equisetum* (Tschudy, 1939; Boureau, 1964; Naugolnykh, 2004). A third hypothesis proposes that both Equisetaceae and Calamitaceae originated during the Early Carboniferous, having evolved independently since that time, perhaps sharing an ancestor of archaeocalamitacean affiliation (Bierhorst, 1971; Page, 1972a; Gifford and Foster, 1989).

These previously developed hypotheses were mired in problems arising from the traditional view of the equisetalean fertile shoots (i.e., strobili) as node–internode alternations and the homology assumptions derived from that (e.g., Page, 1972b; Good, 1975). However, a recent study combining developmental, genetic, morphoanatomical, and paleontological information, provides a new perspective on the structure and morphological evolution of equisetalean reproductive structures, focusing on phytomers as modular units of the shoots. Accordingly, the different reproductive morphologies seen in the clade can be explained by the hierarchical expression of at least three different regulatory modules (Tomescu et al., 2017), with the effects of each module being readily identifiable by distinct “structural fingerprints” (Rothwell et al., 2014; Rothwell and Tomescu, 2018). Accordingly, in the development of the *Equisetum*-type strobilus, the three regulatory modules are all activated, resulting in a stack of phytomers that lack node–internode differentiation. In *Peltotheca*-type plants, the activation of two modules results in a determinate reproductive structure composed of a series of fertile phytomers, while a single functioning module produces *Cruciaetheca*-type plants having series of fertile phytomers alternating with vegetative ones as part of an indeterminate reproductive shoot (Tomescu et al., 2017).

Molecular-based phylogenetics has provided a broad array of hypotheses concerning the position of *Equisetum* among extant vascular plants. In some of the earliest and most controversial positions for *Equisetum* recovered in such analyses, this genus is nested among modern ferns (i.e., “monilophytes” sensu Pryer et al., 2004), either as sister to the Marattiales (Pryer et al., 2001, 2004; Wikström and Pryer, 2005), or as sister to leptosporangiate ferns (Nickrent et al., 2000). Subsequent analyses recovered *Equisetum* as sister to either an Ophioglossales + Psilotales clade (Grewe et al., 2013; Lu et al., 2015), or to the Psilotales only (Karol et al., 2010), with the clade including *Equisetum* sister to the remaining seed-free euphyllophytes. The results of these analyses were at odds with the position of *Equisetum* according to early morphology-based phylogenetic studies that included both extinct and extant species, which set *Equisetum* apart from modern fern clades (e.g., Rothwell, 1999). In contrast, another morphology-based analysis, based on only living species, recovered *Equisetum* nested within the monilophyte clade (Schneider et al., 2009), similar to the results of DNA-based studies. The most recent analyses, based on ever-growing mitochondrial, plastid, and nuclear DNA data sets, recover *Equisetum* as sister to all remaining seed-free euphyllophytes with which it forms a clade that is sister to the seed plants (Rai and Graham, 2010; Wickett et al., 2014; Knie et al., 2015; Rothfels et al., 2015; Testo and Sundue, 2016). The main results of the molecular-based analyses seemed to be in line with and supported by those derived from the

morphology-based studies of fossil polysporangiophytes (Kenrick and Crane, 1997), which recovered a clade including putative sphenopsids as sister to a fern clade, with the clade formed by the two, termed the “moniliformopses”, as sister to lignophytes. However, the affiliations of the different fossil species used as placeholders for modern clades (e.g., *Ibyka* Skog et Banks for the sphenopsids) were not tested, and in turn, the apparent support that the recovery of the “moniliformopses” sensu Kenrick and Crane gave to the clade of the “monilophytes” sensu Pryer et al. (2004) is not as strong (e.g., Rothwell and Nixon, 2006) as usually considered (Pryer et al., 2001, 2004; Schneider et al., 2009; Knie et al., 2015).

In the Equisetales, successive divergence and extinction events left *Equisetum* at the tip of one of the longest branches in the plant kingdom to be phylogenetically analyzed in an evolutionary context (Stewart and Rothwell, 1993; Taylor et al., 2009). The divergent results obtained by molecular-based analyses, from which the rich and well-documented fossil diversity of the group has been consistently excluded, may be a reflection of this situation. In light of these considerations, the reconstruction of progressively deeper nodes in the order Equisetales and in class Sphenopsida may represent a more successful approach for understanding the phylogenetic position of this enigmatic lineage and its role in the evolution of vascular plants.

In this contribution, we employ parsimony phylogenetic analyses with a combination of discrete and continuous morphological characters, as well as nucleotide sequence characters, to shed light on the evolutionary history of the sphenopsid clade and to test the previously calculated mid-Paleogene divergence age of the genus *Equisetum*. We also employ the fossil as well as the extant diversity of *Equisetum* to test alternative hypotheses of internal phylogenetic relationships within the Equisetales and to provide evidence for future analyses of relationships among major clades within the Euphyllophytina.

MATERIALS AND METHODS

Character sampling

Two different matrices were constructed for this study: one including morphological data only (discrete + continuous characters), and the other a combined matrix of morphological + nucleotide sequence characters. Supplemental Data with this article provide: (i) GenBank accession numbers (Appendix 1); (ii) the two matrices in TNT format (Appendix S1); (iii) the discrete character matrix in Nexus format (Appendix S2, also presented in Table 1); (iv) complete morphological character list and herbarium voucher numbers (Appendix S3).

The morphological matrix was built using Mesquite v3.3 (Maddison and Maddison, 2017), based on literature and personal observations of fossil and herbarium specimens (voucher numbers in Appendix S3). Two different types of morphological characters were coded: 77 discrete characters (DC) and 11 continuous characters (CC), both of which are based on morphoanatomical features of vegetative and reproductive organs (Table 2).

We opted to use continuous characters in this study, even though they are not usually taken into account when dealing with plant phylogenies containing fossils; the continuous characters have proven to be useful in providing phylogenetic resolution at lower taxonomic levels dealing with closely related species which do not display abundant and clear-cut discrete morphological differences (de Bivort et al., 2010; Escapa and Catalano, 2013; Mongiardino

Koch et al., 2015; Flores et al., 2017; Parins-Fukuchi, 2018). Additionally, continuous characters now can be analyzed as such by phylogenetic software, with no need to divide character states into discrete units, thus avoiding many of the problems that such a practice produced (Goloboff et al., 2006, 2008). Whenever possible, we scored the states of the continuous characters as ranges, reflecting the natural variation between individuals; otherwise, we coded them as means. We also standardized these characters to avoid the excessive influence of their magnitudes in determining optimal topologies (Goloboff et al., 2006; Mongiardino Koch et al., 2015). The standardization process implemented with TNT transforms the full range of each continuous character so it becomes equal to a single step of a discretely coded one (Goloboff et al., 2008). All discrete characters were treated as unordered.

The morphological matrix contains 4312 cells; data are missing for 1258 of these cells and inapplicable for 608 (Table 1), representing 29.2% and 14.1% of the data, respectively. The missing entries for the fossil species are due to their incomplete preservation or to the fact that some fossils represent organs and not whole plants. The missing entries for extant species represent characters that are not available in the literature and could not be scored otherwise.

The molecular (i.e., nucleotide sequence) matrix was built and aligned with GenBank-to-TNT and MAFFT (Goloboff and Catalano, 2012; Katoh and Standley, 2013); ambiguous areas were removed, and the alignments were manually refined. The DNA sequences were obtained from GenBank (Appendix 1) and represent six plastid regions (*atpB*, *matK*, *nadhJ*, *rbcL*, *rps4*, and *trnL-F*), totaling 5086 aligned sites that include 709 informative characters. Gaps were treated as missing characters.

Taxon sampling

We included 49 species in this study, 17 extant and 32 extinct (Table 3; Appendix S4), with six species representing outgroups and the other 43 representing the ingroup. We selected our samples to cover most of the known diversity of equisetalean reproductive morphology, while including the most complete and confidently reconstructed fossil species.

The four extinct outgroup species are *Hamatophyton verticillatum* (Gu et Shi) Li et al., *Rotafolia songziensis* (Feng) Wang et al., *Bowmanites moorei* Mamay, and *B. dawsonii* (Williamson) Taylor. *Hamatophyton verticillatum* and *R. songziensis* are some of the oldest anatomically preserved plant fossils that exhibit sphenopsid-type vegetative architecture (i.e., whorled leaves and articulated shoots with longitudinal ridges and grooves) and are known from the Late Devonian of China (Gu and Shi, 1974; Li et al., 1995; Wang et al., 2005, 2006). *Bowmanites* Binney includes some of the best-understood anatomically preserved sphenophyllalean cone species described to date (Mamay, 1959; Taylor, 1969, 1970; Good, 1978). Up to the present, there have been no reliable whole-plant reconstructions for most sphenophyllaleans, including species selected for this study, but see the article by Good (1973) for progress toward that goal. Thus, the exact type of leaves, stems, and rhizomes associated with *Bowmanites* cone species are still unknown. Consequently, we assumed that *Bowmanites* cones were borne on plants characterized by the stem and leaf types most common in this group, i.e., *Sphenophyllum* Brongniart-type stems and leaves (Taylor et al., 2009). The two extant outgroups, *Psilotum nudum* (L.) Beauv. and *Ophioglossum reticulatum* L., are species of very distant families that were used as outgroups in previous studies concerning

TABLE 1. Discrete morphological character matrix (characters 1 to 26).

Taxon/Character	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26		
<i>Psilotum nudum</i>	0	1	0	0	-	2	0	0	0	-	-	1	2	0	-	-	-	0	0	0	0	0	1	0	?	0		
<i>Ophioglossum reticulatum</i>	0	1	0	0	-	2	0	0	0	-	-	2	2	0	-	-	-	0	0	0	0	-	0	0	1	0		
<i>Hamatophyton verticillatum</i>	0	?	0	?	?	?	0	0	0	?	?	1	0	1	0	?	-	?	0	?	?	?	0	1	1	0		
<i>Rotafolia songziensis</i>	0	?	0	?	?	?	0	0	0	?	?	1	0	1	0	?	-	1	0	0	0	0	0	1	1	0		
<i>Bowmanites dawsonii</i>	?	1	0	0	?	?	0	0	0	?	?	1	0	1	1	1	0	1	1	0	0	0	0	1	1	0		
<i>Bowmanites moorei</i>	?	1	0	0	?	?	0	0	0	?	?	1	0	1	1	1	0	1	1	0	0	0	0	1	1	0		
<i>Protocalamostachys farringtonii</i>	0	?	0	?	?	?	1	0	1	?	?	A	1	1	1	?	0	1	0	0	1	0	0	1	0	0		
<i>Protocalamostachys arranensis</i>	0	?	0	?	?	?	1	0	1	?	?	A	1	1	1	?	0	1	0	0	1	0	0	1	0	0		
<i>Peltotheca furcata</i>	0	?	?	?	?	?	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	0	0	1	0	0		
<i>Calamocarpon insignis</i>	1	1	0	?	?	?	1	0	1	?	?	2	1	1	1	0	1	1	0	0	0	1	1	1	0	1		
<i>Calamostachys americana</i>	1	1	0	1	?	?	1	0	1	?	?	2	1	1	1	0	0	1	0	0	0	1	0	1	0	1		
<i>Calamostachys binneyana</i>	1	1	0	1	?	?	1	0	1	?	?	2	1	1	1	0	0	1	0	0	0	1	1	1	0	1		
<i>Calamostachys casheana</i>	1	1	0	?	?	?	?	?	1	?	?	2	1	1	1	?	?	1	0	0	0	1	1	1	0	?		
<i>Palaeostachya andrewsi</i>	1	1	0	1	?	?	1	0	1	?	?	2	1	1	1	0	0	1	0	0	0	?	?	1	0	1		
<i>Pendulostachys cingulariformis</i>	1	1	0	?	?	?	1	0	1	?	?	2	1	1	1	0	0	1	0	0	0	1	0	1	0	1		
<i>Calamostachys inversibractis</i>	1	1	0	?	?	?	1	0	1	?	?	2	1	1	1	0	0	1	0	0	0	1	?	1	0	1		
<i>Palaeostachya decacnema</i>	1	1	0	?	?	?	1	0	1	?	?	2	1	1	1	0	0	1	0	0	0	1	0	1	0	1		
<i>Mazostachys pendulata</i>	1	1	0	?	?	?	?	?	1	?	?	2	1	1	1	?	?	1	0	0	0	?	?	1	0	1		
<i>Weissistachys kentuckiensis</i>	1	1	0	?	?	?	1	0	1	?	?	2	1	1	1	1	1	1	0	0	0	?	?	1	?	?		
<i>Cruciaetheca feruglioi</i>	0	?	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	0	?	0	1	1	0	1	
<i>Cruciaetheca patagonica</i>	0	?	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	0	?	0	1	1	0	1	
<i>Paracalamitina striata</i>	0	?	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	0	?	0	1	1	0	1	
<i>Neocalamostachys arrondoi</i>	?	?	0	?	?	?	1	1	1	?	?	?	1	?	?	?	?	?	?	?	0	0	0	0	1	0	?	
<i>Neocalamites</i> sp.	?	?	0	?	?	?	?	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	0	1	1	0	0
<i>Spaciinodum collinsonii</i>	0	?	1	?	1	1	1	1	1	0	0	?	1	0	-	-	-	0	0	0	0	1	1	1	1	1	1	
<i>Equisetites arenaceus</i>	?	1	?	?	?	?	1	1	1	?	?	?	?	?	?	?	?	?	?	?	0	0	1	1	1	1	1	
<i>Equisetum thermale</i>	0	1	0	?	1	2	1	1	1	0	0	?	1	0	-	-	-	0	0	0	0	1	0	1	1	1		
<i>Equisetum haukeanum</i>	0	?	?	?	?	?	1	1	1	0	0	?	1	0	-	-	-	0	0	0	0	1	0	1	?	1		
<i>Equisetum clarnoi</i>	?	?	?	?	1	2	1	1	1	0	0	?	1	0	-	-	-	0	0	0	0	?	?	1	?	1		
<i>Equisetites lyelli</i>	0	?	?	?	?	?	1	1	1	?	?	?	1	0	-	-	-	0	0	0	0	1	0	1	1	1		
<i>Equisetum dimorphum</i>	0	?	?	?	?	?	1	1	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	0	1	1	1	
<i>Equisetum laterale</i>	0	?	?	?	?	?	1	1	1	?	?	?	?	?	?	?	?	?	?	?	0	0	?	0	1	1	1	
<i>Equisetum fluviatoides</i>	0	?	?	?	?	?	1	1	?	?	?	?	1	?	?	?	?	?	?	?	1	0	1	1	1	1	1	
<i>Equisetum vancouverense</i>	0	?	?	?	0	?	1	1	1	0	0	?	1	0	-	-	-	0	0	0	0	1	0	1	?	1		
<i>Equisetum bogotense</i>	0	A	0	0	2	2	0	1	1	0	0	0	1	0	-	-	-	0	0	0	0	1	A	1	1	1		
<i>Equisetum hyemale</i>	0	1	0	0	1	0	1	1	1	0	1	0	1	0	-	-	-	0	0	0	0	1	0	1	0	1		
<i>Equisetum variegatum</i>	0	1	0	0	1	1	1	1	1	1	0	0	1	0	-	-	-	0	0	0	0	1	0	1	0	1		
<i>Equisetum scirpoides</i>	0	1	0	0	2	2	0	1	1	0	0	0	1	0	-	-	-	0	0	0	0	1	0	1	1	1		
<i>Equisetum ramosissimum</i>	0	1	0	0	1	0	1	1	1	A	1	0	1	0	-	-	-	0	0	0	0	1	1	1	A	1		
<i>Equisetum giganteum</i>	0	1	0	0	0	0	1	1	1	A	1	0	1	0	-	-	-	0	0	0	0	1	1	1	1	1		
<i>Equisetum myriochaetum</i>	0	1	0	0	1	0	1	1	1	1	1	0	1	0	-	-	-	0	0	0	0	1	1	1	0	1		
<i>Equisetum laevigatum</i>	0	0	0	0	1	0	1	1	1	1	A	0	1	0	-	-	-	0	0	0	0	1	0	1	0	1		
<i>Equisetum fluviatile</i>	0	0	0	0	0	0	1	A	1	1	0	0	1	0	-	-	-	0	0	0	0	1	A	1	1	1		
<i>Equisetum palustre</i>	0	0	0	0	2	2	1	1	1	0	0	0	1	0	-	-	-	0	0	1	0	1	A	1	1	1		
<i>Equisetum diffusum</i>	0	A	0	0	2	2	0	1	1	0	0	0	1	0	-	-	-	0	0	1	0	1	A	1	1	1		
<i>Equisetum telmateia</i>	0	0	1	0	2	2	1	1	1	0	0	0	1	0	-	-	-	0	0	1	0	1	1	1	1	1		
<i>Equisetum arvense</i>	0	0	1	0	2	2	1	1	1	0	0	0	1	0	-	-	-	0	0	1	0	1	1	1	1	1		
<i>Equisetum sylvaticum</i>	0	0	2	0	2	1	1	1	1	0	0	0	1	0	-	-	-	0	0	1	0	1	1	1	1	1		
<i>Equisetum pratense</i>	0	0	2	0	2	2	1	1	1	1	0	0	1	0	-	-	-	0	0	0	0	1	1	1	1	1		

TABLE 1. (continued). Discrete morphological character matrix continued (characters 27 to 52).

Taxon/Character	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	
<i>Psilotum nudum</i>	-	-	-	-	-	-	-	1	?	0	A	0	0	-	-	-	-	-	-	0	-	-	-	-	-	-	
<i>Ophioglossum reticulatum</i>	-	-	-	1	0	1	-	1	?	2	1	0	0	-	-	-	-	-	-	0	-	-	-	-	-	-	
<i>Hamatophyton verticillatum</i>	-	?	?	1	A	2	-	?	?	?	?	?	?	?	?	?	1	1	0	A	-	0	0	-	-	-	
<i>Rotafolia songziensis</i>	-	?	?	1	1	C	-	?	?	?	?	?	?	?	?	?	1	1	0	1	-	0	0	-	-	-	
<i>Bowmanites dawsonii</i>	-	?	?	1	0	3	-	1	?	0	?	?	?	?	?	?	1	1	0	1	-	0	1	0	-	-	
<i>Bowmanites moorei</i>	-	?	?	1	0	3	-	1	?	0	?	?	?	?	?	?	1	1	0	1	-	0	1	1	-	-	
<i>Protocalamostachys farringtonii</i>	-	?	?	1	1	2	-	?	?	?	?	?	?	?	?	?	1	1	?	1	-	1	?	?	1	1	
<i>Protocalamostachys arranensis</i>	-	?	?	1	1	2	-	?	?	?	?	?	?	?	?	?	1	1	?	1	-	1	1	2	1	1	
<i>Peltotheca furcata</i>	-	?	?	1	1	2	-	?	?	?	?	?	?	?	?	?	1	1	?	0	-	1	1	2	1	1	
<i>Calamocarpon insignis</i>	1	?	?	0	0	1	?	?	?	?	?	?	?	?	?	?	?	1	1	1	-	0	1	1	-	1	
<i>Calamostachys americana</i>	1	?	?	0	0	0	?	1	?	2	0	?	?	?	?	?	?	1	1	1	-	0	1	1	-	1	
<i>Calamostachys binneyana</i>	1	?	?	0	0	0	?	1	?	2	?	?	?	?	?	?	1	1	1	1	-	0	1	1	-	1	
<i>Calamostachys casheana</i>	?	?	?	0	0	0	?	?	?	?	?	?	?	?	?	?	?	1	1	1	-	0	1	?	-	?	
<i>Palaeostachya andrewsi</i>	1	?	?	0	0	1	?	1	?	2	0	?	?	?	?	?	?	1	1	1	-	0	1	1	-	2	
<i>Pendulostachys cingulariformis</i>	1	?	?	0	0	0	?	?	?	?	?	?	?	?	?	?	?	1	1	1	-	0	1	0	-	1	
<i>Calamostachys inversibractis</i>	1	?	?	0	0	1	?	1	?	2	?	?	?	?	?	?	?	1	1	1	1	-	0	1	1	-	1
<i>Palaeostachya decacnema</i>	1	?	?	0	0	1	?	1	?	?	?	?	?	?	?	?	?	1	1	1	-	0	1	1	-	2	
<i>Mazostachys pendulata</i>	1	?	?	0	0	3	?	?	?	?	?	?	?	?	?	?	?	1	1	1	-	0	1	0	-	1	
<i>Weissistachys kentuckiensis</i>	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	1	1	1	-	0	1	0	-	2	
<i>Cruciaetheca feruglioi</i>	1	?	?	0	0	A	?	?	?	?	?	?	?	?	?	?	?	0	1	1	1	-	1	1	1	0	1
<i>Cruciaetheca patagonica</i>	1	?	?	0	0	A	?	?	?	?	?	?	?	?	?	?	?	0	1	1	1	-	1	1	1	0	1
<i>Paracalamitina striata</i>	?	?	?	0	0	0	?	?	?	?	?	?	?	?	?	?	?	0	1	1	1	-	1	1	1	1	1
<i>Neocalamostachys arrondoi</i>	-	?	?	0	0	2	-	1	?	1	?	?	?	?	?	?	?	1	0	0	1	?	-	1	2	-	1
<i>Neocalamites</i> sp.	-	?	?	0	0	2	-	?	?	?	?	?	?	?	?	?	?	1	0	0	1	0	-	1	2	-	1
<i>Spaciinodum collinsonii</i>	0	0	0	0	0	0	0	1	?	1	?	?	?	?	?	?	?	1	0	0	?	?	-	1	2	-	1
<i>Equisetites arenaceus</i>	0	0	0	0	0	0	1	?	?	?	?	?	?	?	?	?	?	1	0	0	1	0	-	1	2	-	1
<i>Equisetum thermale</i>	0	1	0	0	0	0	1	1	1	1	0	1	0	-	-	-	1	0	0	0	?	-	1	2	-	1	
<i>Equisetum haukeanum</i>	?	?	0	?	?	0	0	1	1	1	0	?	?	?	?	?	?	?	?	?	?	-	?	?	-	?	
<i>Equisetum clarnoi</i>	?	?	?	?	?	0	0	0	0	1	?	?	?	?	?	?	?	?	?	?	?	-	?	?	-	?	
<i>Equisetites lyelli</i>	0	?	0	0	0	0	1	1	1	1	0	1	1	?	?	?	?	?	?	?	?	?	1	?	?	?	
<i>Equisetum dimorphum</i>	0	1	0	0	0	0	1	1	1	?	0	?	?	?	?	?	?	1	0	0	0	0	-	1	2	-	1
<i>Equisetum laterale</i>	1	1	0	0	0	0	1	1	2	?	0	?	?	?	?	?	?	1	0	0	0	0	-	1	2	-	1
<i>Equisetum fluviatoides</i>	0	0	0	0	0	0	?	?	?	?	?	?	?	?	?	?	?	1	0	0	0	0	-	1	2	-	1
<i>Equisetum vancouverense</i>	?	?	0	?	?	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	-	?	?	-	?	
<i>Equisetum bogotense</i>	0	1	0	0	0	0	1	1	2	1	0	1	1	0	0	0	1	0	0	0	A	-	1	2	-	1	
<i>Equisetum hyemale</i>	0	1	1	0	0	0	1	0	0	1	0	0	0	-	-	-	1	0	0	0	1	-	1	2	-	1	
<i>Equisetum variegatum</i>	0	1	1	0	0	0	0	0	0	1	0	0	0	-	-	-	1	0	0	0	1	-	1	2	-	1	
<i>Equisetum scirpoides</i>	0	1	1	0	0	0	0	0	0	1	0	0	0	-	-	-	1	0	0	0	1	-	1	2	-	1	
<i>Equisetum ramosissimum</i>	0	1	0	0	0	0	0	0	1	1	0	0	0	-	-	-	1	0	0	0	A	-	1	2	-	1	
<i>Equisetum giganteum</i>	0	1	1	0	0	0	1	0	1	1	0	0	0	-	-	-	1	0	0	A	1	-	1	2	-	1	
<i>Equisetum myriochaetum</i>	0	1	1	0	0	0	0	0	1	1	0	0	1	-	-	-	1	0	0	A	A	-	1	2	-	1	
<i>Equisetum laevigatum</i>	0	A	1	0	0	0	1	0	0	1	0	0	0	-	-	-	1	0	0	0	A	-	1	2	-	1	
<i>Equisetum fluviatile</i>	0	0	-	0	0	0	0	1	2	1	1	1	1	0	0	1	1	0	0	0	0	-	1	2	-	1	
<i>Equisetum palustre</i>	0	A	0	0	0	0	1	1	2	1	0	1	1	1	1	2	1	0	0	0	0	-	1	2	-	1	
<i>Equisetum diffusum</i>	0	0	-	0	0	0	1	1	2	1	0	1	1	1	1	1	1	0	0	0	0	-	1	2	-	1	
<i>Equisetum telmateia</i>	A	1	1	0	0	0	1	1	B	1	0	1	1	1	1	2	1	0	0	0	0	-	1	2	-	1	
<i>Equisetum arvense</i>	0	1	0	0	0	0	0	1	1	1	1	1	1	0	0	1	1	0	0	0	0	-	1	2	-	1	
<i>Equisetum sylvaticum</i>	1	1	1	0	0	0	0	1	A	1	0	1	1	0	0	0	1	0	0	0	0	-	1	2	-	1	
<i>Equisetum pratense</i>	0	1	1	0	0	0	0	1	A	1	1	1	1	0	0	0	1	0	0	0	0	-	1	2	-	1	

TABLE 1. (continued). Discrete morphological character matrix continued (characters 53 to 77)

Taxon/Character	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77
<i>Psilotum nudum</i>	-	-	0	-	?	-	0	-	-	-	-	-	-	-	-	2	0	0	-	-	-	-	2	1	0
<i>Ophioglossum reticulatum</i>	-	-	0	-	?	-	0	-	-	-	-	-	-	-	-	2	0	0	-	-	-	-	0	0	0
<i>Hamatophyton verticillatum</i>	?	?	-	-	0	-	0	1	1	0	0	-	-	-	-	?	?	0	-	-	-	-	0	?	?
<i>Rotafolia songziensis</i>	?	-	-	-	-	-	?	1	1	0	1	-	-	-	-	?	?	?	?	?	?	?	?	?	?
<i>Bowmanites dawsonii</i>	1	0	0	?	0	-	0	1	1	2	0	-	-	-	-	?	1	0	-	-	-	-	0	?	?
<i>Bowmanites moorei</i>	1	0	0	?	0	-	0	1	1	2	1	-	-	-	-	?	?	0	-	-	-	-	0	?	?
<i>Protocalamostachys farringtonii</i>	0	0	1	1	2	-	1	0	?	-	-	-	-	-	-	?	?	0	-	-	-	-	0	?	?
<i>Protocalamostachys arranensis</i>	0	0	1	1	2	-	0	0	?	-	-	-	-	-	-	?	?	0	-	-	-	-	0	?	?
<i>Peltotheca furcata</i>	?	?	?	1	2	-	?	0	?	-	-	-	-	-	-	?	?	?	?	?	?	?	?	?	?
<i>Calamocarpon insignis</i>	0	2	1	1	2	2	1	0	1	-	-	0	0	1	0	0	1	1	1	0	0	0	0	?	?
<i>Calamostachys americana</i>	0	1	1	?	2	1	1	0	1	-	-	1	1	0	1	0	1	1	1	0	0	0	0	?	?
<i>Calamostachys binneyana</i>	0	0	1	1	2	2	0	0	1	-	-	0	1	0	0	0	1	1	1	0	0	0	0	?	?
<i>Calamostachys casheana</i>	0	0	1	1	2	2	1	0	1	-	-	0	1	0	?	?	?	1	?	0	?	0	0	?	?
<i>Palaeostachya andrewsi</i>	0	1	1	1	2	0	1	0	1	-	-	0	1	0	0	?	1	1	?	?	0	0	0	?	?
<i>Pendulostachys cingulariformis</i>	0	0	1	1	2	3	0	1	1	0	0	1	1	1	1	0	1	1	1	0	0	0	0	?	?
<i>Calamostachys inversibractis</i>	0	2	1	?	2	2	0	0	1	-	-	1	0	1	?	0	1	1	1	0	0	0	0	?	?
<i>Palaeostachya decacnema</i>	0	0	1	1	2	1	0	0	1	-	-	1	1	?	1	0	1	1	1	0	0	0	0	?	?
<i>Mazostachys pendulata</i>	0	0	1	1	1	3	0	0	1	-	-	0	1	0	?	0	1	1	1	0	0	0	0	?	?
<i>Weissistachys kentuckiensis</i>	0	0	1	1	1	0	0	0	1	-	-	0	1	1	?	0	1	1	1	0	0	0	0	?	?
<i>Cruciaetheca feruglioi</i>	?	?	?	1	2	-	?	0	0	-	-	-	-	-	-	?	?	?	?	?	?	?	?	?	?
<i>Cruciaetheca patagonica</i>	?	?	?	1	2	-	?	0	0	-	-	-	-	-	-	?	?	?	?	?	?	?	?	?	?
<i>Paracalamitina striata</i>	?	?	?	1	2	-	?	0	0	-	-	-	-	-	-	?	?	?	?	?	?	?	?	?	?
<i>Neocalamostachys arrondoii</i>	-	?	?	?	3	-	0	0	-	-	-	-	-	-	-	?	?	?	?	?	?	-	?	?	?
<i>Neocalamites</i> sp.	-	?	?	0	3	-	?	0	-	-	-	-	-	-	-	?	?	?	?	?	?	?	?	?	?
<i>Spaciinodum collinsonii</i>	-	0	0	0	3	-	0	0	-	-	-	-	-	-	-	?	?	1	?	1	1	?	0	?	?
<i>Equisetites arenaceus</i>	?	?	0	0	3	-	?	0	-	-	-	-	-	-	-	?	?	?	?	?	?	?	A	?	?
<i>Equisetum thermale</i>	-	0	0	0	3	-	0	0	-	-	-	-	-	-	-	?	?	?	?	?	?	?	1	?	?
<i>Equisetum haukeanum</i>	?	?	?	?	?	-	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
<i>Equisetum clarnoi</i>	?	?	?	?	?	-	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
<i>Equisetites lyelli</i>	?	?	?	?	3	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	1	?	?
<i>Equisetum dimorphum</i>	?	?	?	0	3	-	?	0	-	-	-	-	-	-	-	?	?	?	?	?	?	?	?	?	?
<i>Equisetum laterale</i>	?	?	?	0	3	-	0	0	-	-	-	-	-	-	-	?	?	?	?	?	?	?	1	?	?
<i>Equisetum fluviatoides</i>	-	?	?	0	?	-	?	0	-	-	-	-	-	-	-	?	?	?	?	?	?	?	?	?	?
<i>Equisetum vancouverense</i>	?	?	?	?	?	-	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
<i>Equisetum bogotense</i>	-	0	0	0	3	-	0	0	-	-	-	-	-	-	-	1	0	1	0	1	1	1	1	1	3
<i>Equisetum hyemale</i>	-	0	0	0	3	-	0	0	-	-	-	-	-	-	-	?	?	1	?	1	1	1	1	0	1
<i>Equisetum variegatum</i>	-	0	0	0	3	-	0	0	-	-	-	-	-	-	-	?	?	1	?	1	1	1	1	0	1
<i>Equisetum scirpoides</i>	-	0	0	0	3	-	0	0	-	-	-	-	-	-	-	?	?	1	?	1	1	1	1	0	1
<i>Equisetum ramosissimum</i>	-	0	0	0	3	-	0	0	-	-	-	-	-	-	-	1	0	1	?	1	1	1	1	0	1
<i>Equisetum giganteum</i>	-	0	0	0	3	-	0	0	-	-	-	-	-	-	-	?	?	1	?	1	1	1	1	0	1
<i>Equisetum myriochaetum</i>	-	0	0	0	3	-	0	0	-	-	-	-	-	-	-	?	?	1	?	1	1	1	1	0	1
<i>Equisetum laevigatum</i>	-	0	0	0	3	-	0	0	-	-	-	-	-	-	-	?	?	1	?	1	1	1	1	0	1
<i>Equisetum fluviatile</i>	-	0	0	0	3	-	0	0	-	-	-	-	-	-	-	?	?	1	?	1	1	1	1	1	3
<i>Equisetum palustre</i>	-	0	0	0	3	-	0	0	-	-	-	-	-	-	-	1	0	1	?	1	1	1	1	1	3
<i>Equisetum diffusum</i>	-	0	0	0	3	-	0	0	-	-	-	-	-	-	-	?	?	1	?	1	1	1	1	1	2
<i>Equisetum telmateia</i>	-	0	0	0	3	-	0	0	-	-	-	-	-	-	-	1	0	1	?	1	1	1	1	A	B
<i>Equisetum arvense</i>	-	0	0	0	3	-	0	0	-	-	-	-	-	-	-	1	0	1	0	1	1	1	1	1	2
<i>Equisetum sylvaticum</i>	-	0	0	0	3	-	0	0	-	-	-	-	-	-	-	?	?	1	?	1	1	1	1	1	2
<i>Equisetum pratense</i>	-	0	0	0	3	-	0	0	-	-	-	-	-	-	-	?	?	1	?	1	1	1	1	1	D

Notes: A = 0&1, B = 1&2, C = 1&3, D = 2&3.

TABLE 2. Summary of the informative characters of the morphological matrix.

Character type	Plant architecture	Anatomy and roots	Vegetative morphology	Stomata and micromorphology	Reproductive	Spores and gametophytes	Total
Discrete	3	18	12	9	25	10	77
Continuous	0	0	3	2	5	1	11

TABLE 3. List of fossil species included in the phylogenetic analyses.

Fossil species	Distribution; Age*	Preserved Organs	Preservation	Main Reference(s)*
<i>Hamatophyton verticillatum</i>	China; Late Devonian	Fertile, stems, branches	I/C, M/C, permineralization	Li et al., 1995; Wang et al., 2006
<i>Rotafolia songziensis</i>	China; Late Devonian	Fertile, stems, branches	I/C, M/C, permineralization	Wang et al., 2005; Wang et al., 2006
<i>Bowmanites dawsonii</i>	UK, Belgium, USA; Early Pennsylvanian	Fertile w/spores	Permineralization	Taylor, 1969, 1970
<i>Bowmanites moorei</i>	USA; Middle Pennsylvanian	Fertile w/spores	Permineralization	Mamay, 1959
<i>Protocalamostachys farringtonii</i>	UK; Early Mississippian	Fertile w/spores, stems, branches, rhizomes	Permineralization	Bateman, 1991
<i>Protocalamostachys arranensis</i>	UK; Early–Middle Mississippian	Fertile w/spores, stems, branches, rhizomes	Permineralization	Bateman, 1991
<i>Peltotheca furcata</i>	Argentina; Early Permian	Fertile, stems, branches	I/C, M/C	Escapa and Cúneo, 2005
<i>Calamocarpon insignis</i>	USA; Early–Late Pennsylvanian	Fertile w/spores, stems, branches	Permineralization	Baxter, 1963, 1964; Good, 1975
<i>Calamostachys binneyana</i>	UK, USA; Early–Mid. Pennsylvanian	Fertile w/spores, stems, branches	Permineralization, I/C, M/C	Good, 1975
<i>Calamostachys casheana</i>	UK; Middle Pennsylvanian	Fertile w/spores	Permineralization	Williamson and Scott, 1894; Lacey, 1941
<i>Palaeostachya andrewsi</i>	USA; Middle Pennsylvanian	Fertile w/spores, stems, branches	Permineralization	Good, 1975
<i>Calamostachys inversibractis</i>	USA; Middle Pennsylvanian	Fertile w/spores, stems, branches	Permineralization	Good, 1975
<i>Mazostachys pendulata</i>	USA; Middle Pennsylvanian	Fertile w/spores, stems, branches	Permineralization	Kosanke, 1955; Good, 1975
<i>Weissistachys kentuckyensis</i>	USA; Middle Pennsylvanian	Fertile w/spores	Permineralization	Rothwell and Taylor, 1971a, 1971b
<i>Palaeostachya decacnema</i>	USA; Middle–Late Pennsylvanian	Fertile w/spores, stems, branches	Permineralization	Good, 1975
<i>Pendulostachys cingulariformis</i>	USA; Late Pennsylvanian	Fertile w/spores, stems, branches	Permineralization	Good, 1975
<i>Calamostachys americana</i>	USA; Late Pennsylvanian	Fertile w/spores, stems, branches, rhizomes	Permineralization	Good, 1975
<i>Cruciaetheca feruglioi</i>	Argentina; Early Permian	Fertile, stems, branches	I/C, M/C	Cúneo and Escapa, 2006
<i>Cruciaetheca patagonica</i>	Argentina; Early Permian	Fertile, stems, branches	I/C, M/C	Cúneo and Escapa, 2006
<i>Paracalamitina striata</i>	Russia; Early–Middle Permian	Fertile, stems, branches	I/C, M/C	Naugolnykh, 2002
<i>Neocalamites</i> sp.	Argentina; Middle Permian	Fertile, stems, branches	I/C, M/C	Escapa and Cúneo, 2006
<i>Neocalamostachys arrondoii</i>	Argentina; Middle–Late Triassic	Fertile w/spores?, stems, branches	I/C, M/C	Brea and Artabe, 1999; Bomfleur et al., 2013
<i>Spaciinodum collinsonii</i>	Antarctica; Early–Middle Triassic	Fertile, stems, branches, rhizomes	Permineralization	Schwendemann et al., 2010
<i>Equisetites arenaceus</i>	Germany; Middle–Late Triassic	Fertile w/spores, stems, branches, rhizomes	I/C, M/C	Kelber and van Konijnenburg-van Cittert, 1998
<i>Equisetum laterale</i>	Australia; Early–Middle Jurassic	Fertile w/spores, stems, branches	I/C, M/C	Gould, 1968
<i>Equisetum dimorphum</i>	Argentina; Early Jurassic	Fertile, stems, rhizomes?	I/C, M/C	Elgorriaga et al., 2015
<i>Equisetum thermale</i>	Argentina; Middle–Late Jurassic	Fertile w/spores, stems, rhizomes	Petrification	Channing et al., 2011
<i>Equisetum haukeanum</i>	Canada; Early Cretaceous	Aerial stems	Permineralization	Stanich et al., 2009
<i>Equisetum vancouverense</i>	Canada; Early Cretaceous	Aerial stems	Permineralization	Stanich et al., 2009
<i>Equisetites lyelli</i>	England; Early Cretaceous	Fertile w/spores, stems, rhizomes	Petrification	Watson, 1983; Watson and Batten, 1990
<i>Equisetum fluviatoides</i>	Canada; Paleocene	Fertile w/spores, stems, rhizomes	I/C, M/C	Mclver and Basinger, 1989
<i>Equisetum clarnoi</i>	USA; Late Eocene	Aerial stems, rhizomes	Petrification	Brown, 1975

Notes: I/C = impression/compression, M/C = mold/cast, *see supplemental material for additional information.

the phylogenetic relations of *Equisetum* and for which DNA sequences were readily available in GenBank to incorporate into the molecular matrix.

For the Equisetales ingroup, we selected 43 species, including archaeocalamitaceans, calamitaceans, taxa of uncertain affinities from Gondwana and Angara, species with *Neocalamites* Halle-type

stems, one Antarctic species morphologically similar to *Equisetum*, several *Equisetites*/*Equisetum* fossil species, and all living species of *Equisetum* (Appendix S2).

Archaeocalamitacean plants are represented by two species of *Protocalamostachys* Walton emend. Bateman from the Lower Carboniferous of Scotland, both based on anatomically preserved

strobili associated with stems, rhizomes, and leaves (Chaphekar, 1963, 1965; Bateman, 1991). Ten Carboniferous species from six genera traditionally assigned to Calamitaceae were coded, i.e., *Calamostachys* Schimper emend. Good, *Palaeostachya* Weiss emend. Good, *Weissistachys* (Rothwell et Taylor) Rothwell et Taylor emend. Good, *Mazostachys* Kossanke emend. Good, *Pendulostachys* Good, and *Calamocarpon* Baxter emend. Good (Kossanke, 1955; Arnold, 1958; Baxter, 1963, 1964; Good, 1971a, b, 1975; Leisman and Boucher, 1971a, b; Rothwell and Taylor, 1971a, b; Good and Taylor, 1974, 1975). Most of the species are based exclusively on anatomically preserved strobili, but some have been associated with stems, rhizomes, and leaves in whole-plant concepts that are based on their co-occurrence in the same fossiliferous beds and on morphoanatomical similarities suggesting close affinities [e.g., *Calamostachys binneyana* (Carruthers) Schimper emend. Good, *Calamocarpon insignis* Baxter emend. Good; Good, 1975].

Four equisetalean species with unresolved affinities, known from Angara and Gondwanan floras, i.e., *Cruciaetheca patagonica* Cúneo et Escapa, *Cruciaetheca feruglioi* Cúneo et Escapa, *Paracalamitina striata* (Schmalhausen) Zalesky emend. Naugolnykh, and *Peltotheca furcata* Escapa et Cúneo, are based on impressions and casts of Permian age (Naugolnykh, 2002; Escapa and Cúneo, 2005; Cúneo and Escapa, 2006). Other equisetalean genera from Angara and Gondwana, such as *Koretrophyllites*, *Sendersonia* Meyen et Menshikova, *Tschernovia*, and *Equisetinostachys*, are poorly understood in terms of the overall sporophyte architecture (Meyen, 1971, 1987; Meyen and Menshikova, 1983) with most species being based on isolated strobili or sporangiophores very similar to the ones of *Cruciaetheca* and *Paracalamitina* and were, thus, not included.

Two equisetalean species associated with *Neocalamites*-type stems, preserved as impressions and casts, were scored. One of them is known from the Permian of Argentina (Escapa and Cúneo, 2006), whereas the other comes from the Late Triassic of Argentina (Brea and Artabe, 1999; but see Bomfleur et al., 2013); both include aerial stems, leaf whorls, and reproductive structures.

We included nine fossil species of *Equisetites*/*Equisetum*. *Equisetites lyellii* (Mantell) Seward emend. Watson et Batten, *Equisetum clarnoi* Brown, *Equisetum thermale* Channing et al., *Equisetum haukeanum* Stanich et al., and *Equisetum vancouverense* Stanich et al., are anatomically preserved (Brown, 1975; Watson and Batten, 1990; Stanich et al., 2009; Channing et al., 2011), whereas *Equisetites arenaceus* (Jaeger) Schenk, *Equisetum dimorphum* Elgorriaga et al., *Equisetum fluviatoides* McIver et Basinger, and *Equisetum laterale* Phillips emend. Gould, are preserved as impressions/compressions (Gould, 1968; McIver and Basinger, 1989; Watson and Batten, 1990; Elgorriaga et al., 2015). We also scored the Antarctic species, *Spaciinodum collinsonii* Osborn et Taylor emend. Schwendemann et al., which is based on anatomically preserved strobili, stems, leaves, and spores from the Middle Triassic (Osborn and Taylor, 1989; Osborn et al., 2000; Ryberg et al., 2008; Schwendemann et al., 2010).

Lastly, we scored all 15 extant species of *Equisetum*, i.e., the seven traditionally placed in subgenus *Hippochaete* and the eight from subgenus *Equisetum* (Hauke, 1963, 1978; but see Des Marais et al., 2003; Guillon, 2004, 2007).

Analyses

The analyses were performed in TNT v. 1.5 (Goloboff et al., 2008; Goloboff and Catalano, 2016), following the same basic procedure

for each matrix: a heuristic search was conducted based on 100 random addition sequences of taxa (RAS), followed by tree-bisection-reconnection (TBR) branch swapping, holding 10 trees per replication. The most parsimonious trees found were used for a final round of TBR branch swapping. Branch support of the most parsimonious trees (MPTs) was evaluated with standard jackknife resampling analysis (1000 replicates, using absolute frequencies and $P = 36$), and Bremer support (= decay index). Trees were rooted with *Psilotum nudum*.

The tree obtained in the combined analysis was time-plotted (Fig. 3) with R software utilizing the functions *timePaleoPhy* and *geoscalePhylo* in the *paleotree* and *strap* packages, respectively (Bapst, 2012; Bell and Lloyd, 2015; R Core Team, 2017).

To our knowledge, the theoretical basis and the available software to perform simultaneous phylogenetic searches combining DNA, discrete, and continuous characters, under a likelihood or Bayesian approach it is in early stages of development and testing compared to parsimony-based approaches (Goloboff et al., 2008, 2017; Parins-Fukuchi, 2018). In the current literature, there is a constant back-and-forth between studies advocating between parsimony or model-based approaches (Goloboff et al., 2017; Puttick et al., 2017; O'Reilly et al., 2018), with some of them relying heavily on simulation experiments. In that line, one of the latest studies advocating for a Bayesian approach using the Mk model stated that "our experiments do not attempt to simulate non-contemporaneous taxa or address the problem of missing data, qualities of paleontological data that are of a level of complexity that is beyond the current debate" (O'Reilly et al., 2018, p. 106), and therefore, those approaches are apparently not yet readily suitable for analyzing data sets such as the ones from this study.

RESULTS

Each of the searches produced a single MPT; the matrix based on morphology produced one MPT of 197.237 steps, whereas the combined matrix produced one MPT of 2658.782 steps (Fig. 1; the fractional steps are due to the use of continuous characters).

As a general result, Equisetales, Archaeocalamitaceae, Calamitaceae, and Equisetaceae were each recovered as monophyletic, with moderate to low support values (Fig. 1). The two archaeocalamitacean species of *Protocalamostachys* are recovered, together with the Patagonian species *Peltotheca furcata*, as a clade sister to all other Equisetales. However, *Protocalamostachys* is not recovered as monophyletic, and the monophyly of Archaeocalamitaceae depends on the inclusion of *Peltotheca* in the family, a relationship that was not recognized in previous studies (see Discussion).

The other equisetalean species form two major clades. One of these (termed the A.G. clade) includes the Permian species from Angara and Gondwana (i.e., *Cruciaetheca* and *Paracalamitina*), as sister to all the species classically included in family Calamitaceae. Within the Calamitaceae, *Weissistachys* + *Mazostachys* are recovered as sister to the rest of the clade. Interestingly, neither *Calamostachys* nor *Palaeostachya*, two textbook genera of calamite strobili, were recovered as monophyletic. Also, *Calamocarpon* and *Pendulostachys* are placed as sister to *Calamostachys inversibractis* and *Calamostachys americana*, respectively.

The other major clade, defined by four synapomorphies (i.e., presence of vallecular canals, lack of node-internode differentiation

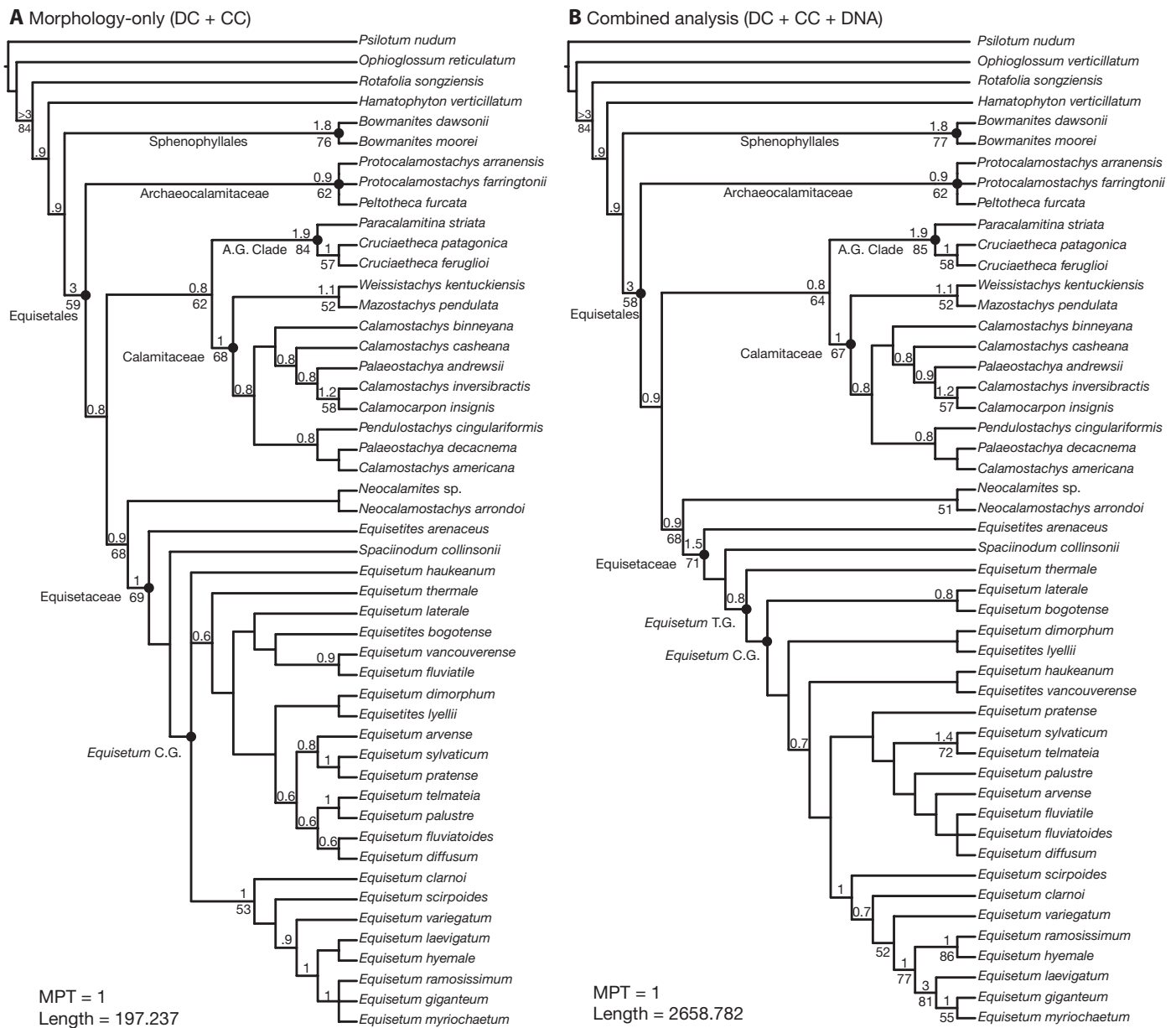


FIGURE 1. Trees resulting from parsimony-based analyses. (A) Single most-parsimonious tree (MPT) from the morphology-only analysis based on 77 discrete (DC) and 11 continuous characters (CC). (B) Single MPT of the analysis combining morphological characters and DNA sequences from six plastid regions (*atpB*, *matK*, *nadhJ*, *rbcL*, *rps4*, and *trnL-F*). Bremer support values >0.5 are above branches, jackknife values $>50\%$ are below them. Black circles indicate major clades.

within fertile phytomers, sporangiophore shields alternating in successive whorls, and six or more sporangia per sporangiophore; Fig. 2), includes the two species with *Neocalamites*-type stems as sister to Equisetaceae; the latter clade includes *Spaciinodum* + all the species of *Equisetites* and *Equisetum*.

The fossil species *Equisetites arenaceus* and *Spaciinodum collinsonii* are recovered as sister to the rest of Equisetaceae in both analyses, with *E. thermale* forming part of the *Equisetum* stem group in the combined analysis (Figs. 1, 2); the remaining species of *Equisetum* and *Equisetites* form the *Equisetum* crown group. The fossil species *E. clarnoi* is recovered as part of subgenus *Hippochaete*, in both analyses, either nested in or sister to the

rest of the subgenus (Fig. 1). The morphology-only analysis recovers the two traditional subgenera as forming a polytomy with *E. haukeanum*, with numerous fossil species being members of subgenus *Equisetum*. The combined analysis recovers a monophyletic subgenus *Hippochaete*, whereas subgenus *Equisetum* remains monophyletic only if *E. bogotense* is not considered to be part of it, being most closely related to fossil species, whereas *E. laterale*, *E. dimorphum*, *E. haukeanum*, *E. vancouverense*, and *E. lyellii* fall outside both subgenera.

The position of the extant species of *Equisetum* varies between the two analyses (Fig. 1), being markedly different within subgenus *Equisetum*. Nodes within this subgenus present markedly low

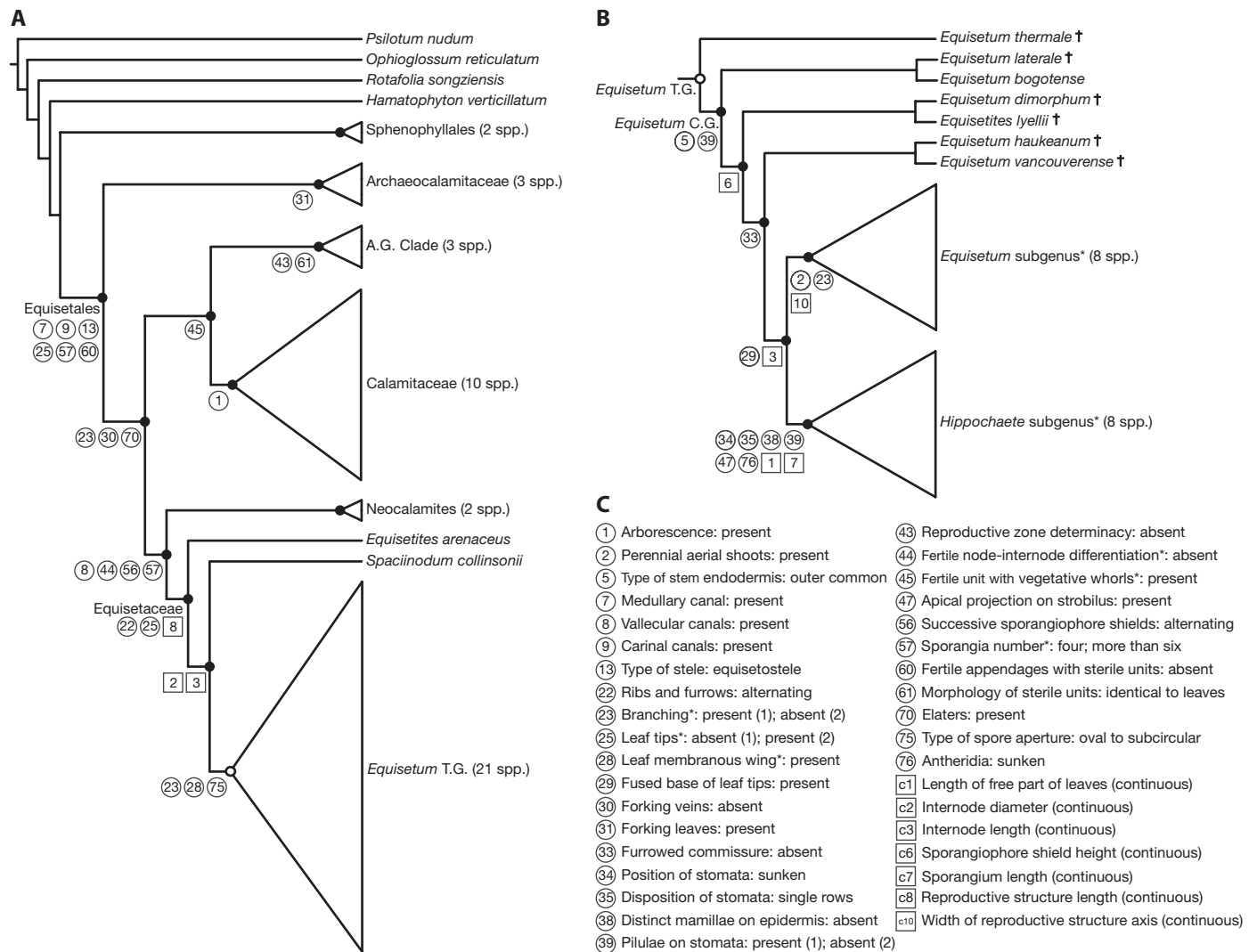


FIGURE 2. Synapomorphies mapped on abridged trees resulting from parsimony-based analyses. (A) Tree depicting overall relationships obtained from both matrices. (B) *Equisetum* total group tree obtained from combined matrix. (C) Characters and states. *Equisetum* subgenus* = *Equisetum* subgenus minus *E. bogotense*. *Hippochaete* subgenus* = *Hippochaete* subgenus + *E. clarnoi*. Characters with an asterisk (*) are fully described in Appendix S3.

Bremer support values (i.e., <0.5; Fig. 2). However, both analyses recover similar relationships for most species of subgenus *Hippochaete*, and they also show that *Equisetum bogotense* has the longest phylogenetic branch of all living species of *Equisetum* (Figs. 1–3). Low support values are expected to occur for trees produced by these matrices due to the presence of several unstable and largely incomplete fossil species (Escapa and Pol, 2011).

DISCUSSION

On the origin of *Equisetum*

By recovering a Calamitaceae + the A.G. clade as sister to Equisetaceae + Neocalamites and resolving the clade formed by these four groups as sister to Archaeocalamitaceae (Figs. 1, 2), the overall topology is not in agreement with previous hypotheses, and it is particularly relevant for hypotheses concerning the evolution of

Equisetales in general and for the origin of *Equisetum* in particular (Page, 1972b; Good, 1975; Rothwell and Stewart, 1993; Naugolnykh, 2004; Escapa and Cúneo, 2005). Within this context, the species *Neocalamites* sp. from the Upper Permian of Argentina (Escapa and Cúneo, 2006) appears to partially bridge the stratigraphic gap between Archaeocalamitaceae and the Equisetaceae + *Neocalamites* lineage (Fig. 3). Despite this, there is still a stratigraphic gap in evolution of the order Equisetales extending from the mid-Permian to the Early Carboniferous (i.e., between Archaeocalamitaceae and the first records of *Neocalamites* fossils; Fig. 3). It should be noted that there are a few Carboniferous records of apparently bractless strobili with polygonal sporangiophores attached to equisetalean-like stems with apparently free leaves (e.g., *Equisetites hemingwayi*; Kidston, 1892; Seward, 1898; Crookall, 1969). If those fossils represent the oldest records of either *Neocalamites* or Equisetaceae-type of fossils, they would narrow the stratigraphic hiatus with archaeocalamitaceans. However, suboptimal preservational quality uncertainties about the exact localities and horizons where those

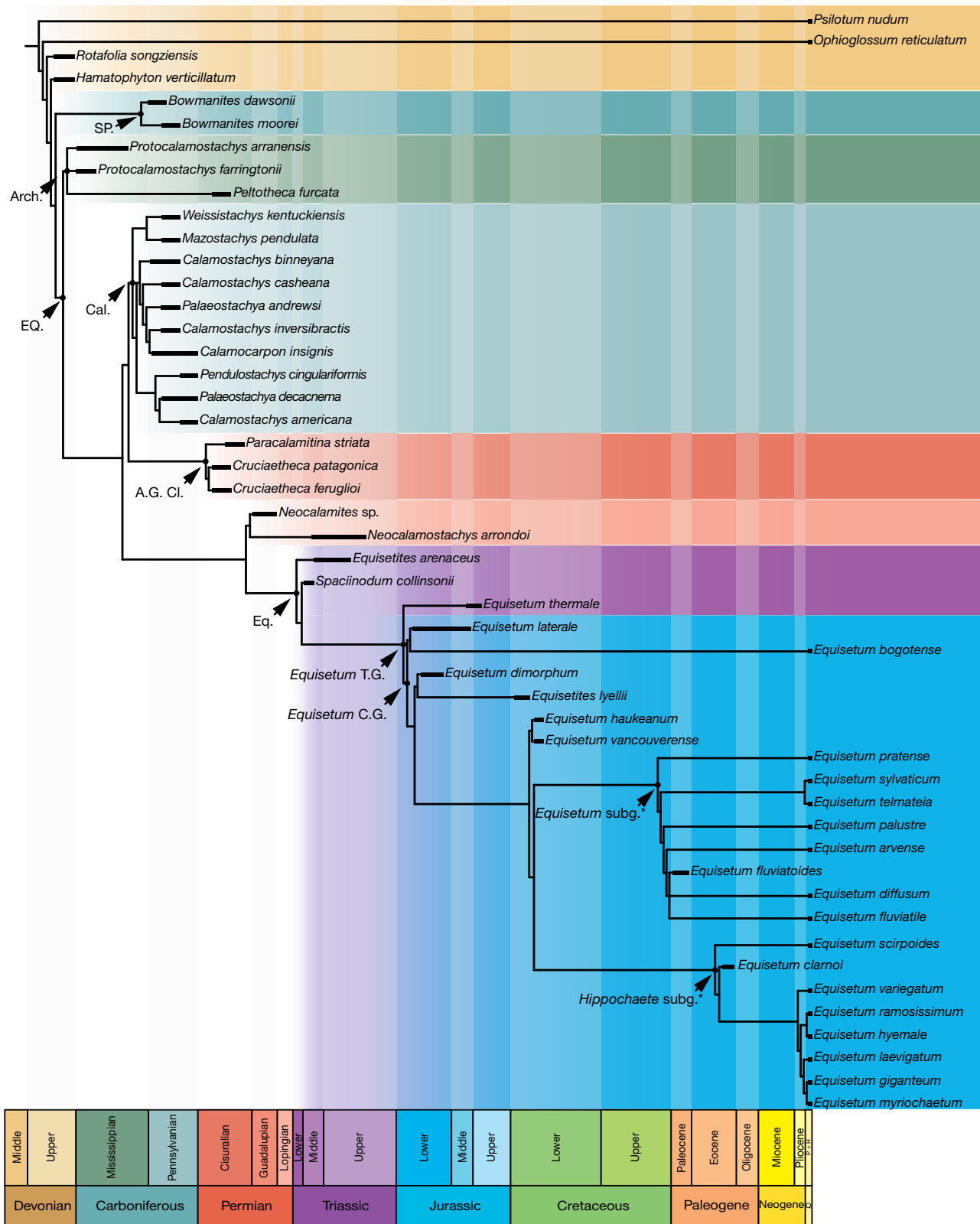


FIGURE 3. Single most-parsimonious tree (MPT) of the combined analysis plotted against geologic time. Thick bars represent the stratigraphic range of taxa whereas thin lines represent the ghost lineages implied by the phylogenetic tree. Black circles indicating major sphenopsid clades: SP. = Sphenophyllales. EQ. = Equisetales. Arch. = Archaeocalamitaceae (including *Peltotheca*). Cal. = Calamitaceae. A.G. Cl. = Angaran-Gondwanan Clade. Eq. = Equisetaceae. *Equisetum* T.G. = *Equisetum* total group. *Equisetum* C.G. = *Equisetum* crown group. *Equisetum* subg.* = *Equisetum* subgenus minus *E. bogotense*. *Hippochaete* subg.* = *Hippochaete* subgenus + *E. clarnoi*.

fossils were collected preclude their inclusion in our phylogenetic analyses.

There are several interesting correlates implied by the obtained topology: (1) The presence of whorls of vegetative units alternating with whorls of sporangiophores on fertile organs of Calamitaceae + the A.G. clade is recovered as a synapomorphy of the group. (2) Secondary growth was a shared feature of all equisetaleans until the Triassic, whence the first records of *Equisetum*-like fossils that lack secondary growth have been described (Osborn and Taylor, 1989; Schwendemann et al., 2010). (3) Elater-bearing spores could have been homologous and shared by most non-archaeocalamitacean equisetaleans, with the scarcity of elaters in the fossil record resulting from a combination of taphonomic factors and inappropriate methods employed in isolating the spores from rocks (Fig. 2; Good and Taylor, 1975; Kelber and van Konijnenburg-van Cittert, 1998).

This topology (Fig. 1) implies that the lineages of Calamitaceae and Equisetaceae had their most recent common ancestor at some point during the middle of the Carboniferous (Fig. 3), and since that time, they have followed independent evolutionary pathways. While Calamitaceae has developed the arborescent growth architecture and explored various configurations of reproductive structures (i.e., isosporic vs heterosporic strobili, sporangiophore fused vs free from fertile appendages), the Equisetaceae + *Neocalamites* lineage appears to have become progressively reduced in size and developed compact strobili. As a result of these changes, equisetacean plants have acquired character configurations that are practically like those of modern *Equisetum* since Jurassic times (Fig. 3).

Age of the *Equisetum* crown group

Our results suggest that the *Equisetum* crown group originated no later than the Early Jurassic, based on the position, nested within the crown group in both of our analyses, of *Equisetum dimorphum*, a fossil species represented by vegetative and reproductive specimens preserved as casts and impressions (Elgorriaga et al., 2015). However, due to the lack of information from species based on that mode of preservation (i.e., impressions), the possibility that such fossils could represent a stem group representative of *Equisetum* cannot be ruled out.

There also are two more conservative minimum age possibilities that derive from these results. The *Equisetum* crown group could have originated as recently as the Late Jurassic, based on the stratigraphic position of the anatomically preserved *Equisetum thermale* (Channing et al., 2011), which was recovered as part of the crown group in the morphology-only analysis (Fig. 1A). Alternatively, this crown group could have originated as recently as the Early Cretaceous, based on the position of *Equisetum vancouverense* (Stanich et al., 2009), which was recovered nested within the *Equisetum* crown group in the results of both analyses (Fig. 1). Regardless of either an Early/Late Jurassic or Early Cretaceous origin (ranging ca. 190–140 Ma), these minimum ages for the *Equisetum* crown group are far older than the previously estimated mid-Paleogene (i.e., Cenozoic) divergence calculation (~40 Ma; Des Marais et al., 2003). They are also far more consistent with age estimates suggested by studies that also consider the character configurations of extinct and extant members of the group (Stanich et al., 2009; Channing et al., 2011; Rothwell and Ash, 2015).

However, it should be noted that the estimated divergence age for members of the *Equisetum* crown group minus *E. bogotense* is late Cretaceous–early Paleogene (ca. 66–59 Ma). This last estimate

is more in line with molecular-based estimations and based on the position of the Paleocene fossil *E. fluviatoides*, since all Mesozoic species occupy an intermediate position between *E. bogotense* and the rest of extant species (Figs. 1, 3).

Equisetum subgenera and fossil species

The 15 living species of *Equisetum* are traditionally divided into two subgenera (i.e., *Equisetum* and *Hippochaete*; Page, 1972a). However, many of the features used to characterize these subgenera are inconsistent within each, according to molecular-based phylogenies (Des Marais et al., 2003; Guillon, 2004, 2007). To further complicate matters, the position of *Equisetum bogotense* remains uncertain. Studies based on molecular data variously recover *E. bogotense* as sister to the rest of the genus, sister to subgenus *Hippochaete*, or as part of a polytomy with both subgenera (Des Marais et al., 2003; Guillon, 2004, 2007). By contrast, older morphology-based treatments place *E. bogotense* within the subgenus *Equisetum* (Hauke, 1963, 1978; Page, 1972a).

We have recovered *E. bogotense* either as nested within subgenus *Equisetum*, in the morphology-only analysis, or together with *Equisetum laterale*, as sister to the rest of the crown group species, in the combined analysis (Fig. 1), and part of a lineage at least 190 Ma old (Fig. 3). These phylogenetic placements add further support to the hypothesis that *E. bogotense* is the most phylogenetically isolated living species of the genus, having a distinct evolutionary history, as compared to the rest of extant *Equisetum* species, since the Mesozoic (Des Marais et al., 2003; Guillon, 2004, 2007). Additionally, these placements may help to explain why *E. bogotense* is the only species that does not appear to hybridize with other species of the genus (Hauke, 1978; Lubienski and Dörken, 2016) and why it has several features that set it apart from the other living species in the genus (e.g., gametophyte morphology; Hauke, 1969, 1978).

When all available evidence is taken into account, some of the features present in *E. bogotense* and traditionally used to characterize subgenus *Equisetum* are plesiomorphic (Fig. 2; e.g., superficial stomata with a scattered arrangement, non-apiculate strobili, persistence of leaf teeth). *Equisetum bogotense* is clearly the most distantly related living species to the rest of the species in the genus according to the combined analysis, and it appears to be more closely related to some fossil species rather than other extant ones. On the other hand, several features shared by most of the *Hippochaete* species, and traditionally used for the characterization of the subgenus, were recovered as synapomorphies of *Hippochaete* with the fossil *E. clarnoi* nested within it (Fig. 2; e.g., sunken stomata arranged in single rows, massive ornamentation on stomatal and epidermal cells, apiculate strobili, sunken antheridia).

The *Neocalamites* lineage

Neocalamites sp. from the mid-Permian and *Neocalamites arrondoi* Brea et Artabe from the Middle Triassic of Argentina share the same basic organization of both vegetative and reproductive structures. Shared features are (1) aerial stems with continuous ribs across nodes, (2) whorls of unfused leaves as in archaeocalamitacean plants, (3) compact strobili consisting of successive whorls of peltate sporangiophores, and (4) bearing six or more sporangia per sporangiophore as in equisetacean strobili. Additionally, there is evidence that *Neocalamites*-type stems had secondary growth

(Brunner and Kelber, 1988; Kelber and Hansch, 1995) and that they produced trilete spores (Brea and Artabe, 1999), like some calamitacean and archaeocalamitacean plants. Thus, species of *Neocalamites* appear to have a mosaic of equisetalean features not present in any other clade, supporting the idea that they were part of a distinct natural group.

Polyphyly of calamitaceans

All the genera traditionally assigned to Calamitaceae were recovered as part of a monophyletic group with moderate support values (Fig. 1). We discuss some of the most interesting aspects of the recovered topology within the clade, but emphasize that these aspects remain highly speculative due to the low support values found for most of these relationships (<50% jackknife, <1 Bremer support; Fig. 1).

The calamitacean genera, *Calamostachys* and *Palaeostachya*, were recovered as polyphyletic (Fig. 1). *Calamostachys* includes strobili of both homosporous and heterosporous plants in which whorls of sporangiophores alternate with bracts, being spaced at equal distances, and having four sporangia per sporangiophore. However, it should be noted that those features were recovered as homoplastic within the family. Other calamitacean species, such as *Calamocarpon insignis* Baxter emend. Good, apart from having quite distinctive autapomorphies such as their megaspore size and number per megasporangium, also share the homoplastic features that characterize *Calamostachys*. The hypothesis of a polyphyletic *Calamostachys* was previously advanced by Good (1975, p. 46): “*Calamostachys* appears to be a loosely defined and probably artificial genus.”

A similar situation occurs with *Palaeostachya*, in which features traditionally used to characterize the genus, such as small size of strobili, spore size, position, and insertion angle of sporangiophores, are recovered as homoplastic within Calamitaceae. *Palaeostachya decacnema* Delevoryas is recovered in a clade with *Calamostachys americana* Arnold and *Pendulostachys cingulariformis* Good, held together by features such as the presence of bract heels, overlapping bracts at successive internodes, sporangiophore position, and length of strobili.

The two other calamitacean strobili coded for this study, i.e., *Weissistachys kentuckiensis* Rothwell et Taylor and *Mazostachys pendulata* Kosanke, from the middle Pennsylvanian of USA, are recovered as sister taxa. A close relationship between these two genera was suggested earlier (Rothwell and Taylor, 1971a), based on the diminutive development of their sporangiophore shields and the number of sporangia per sporangiophore, features that we recovered as synapomorphies of the group.

Given our results, it becomes evident that further systematic work is required to better circumscribe whole-plant concepts for these calamitacean plants. Within this context, it is important to emphasize that calamitaceans currently are represented by dozens of species and appear to form the most species-rich branch of the equisetalean clade.

Peltotheca and archaeocalamitaceans

Archaeocalamitaceans have been reported exclusively from northern hemisphere localities (i.e., equatorial paleotropics), and some are as old as the earliest Carboniferous (Paterson, 1844; Bateman, 1991; Mamay and Bateman, 1991). Their youngest fossils, which do

not include reproductive structures, date from the Early Permian (Mamay and Bateman, 1991). Members of this group are recognized by the combination of whorls of dichotomizing leaves on stems with longitudinally continuous ribbing, and their reproductive units consist of successive internodes completely covered by sporangiophores (Bateman, 1991). Within this context, the recovery of *Peltotheca furcata*, an Early Permian species from the southern hemisphere (i.e., high latitude floras), nested within Archaeocalamitaceae as sister to *Protocalamostachys farringtonii* Bateman, is notable.

Peltotheca furcata was originally described from impressions and casts in the Rio Genoa Formation, Chubut, Argentina (Escapa and Cúneo, 2005), but family-level relationships for the species were not resolved (Escapa and Cúneo, 2005). Linear leaves of *P. furcata* that exhibit one to several dichotomies are most similar to those of archaeocalamitaceans (Escapa and Cúneo, 2005). This species also has aerial stems with longitudinally continuous ribbing, like archaeocalamitacean plants, although it should be noted that a minority of specimens also appear to have alternate ribbing. Most importantly, the reproductive organs of *Peltotheca* consist of series of internodes covered in whorls of sporangiophores with well-developed sporangiophore shields. *Peltotheca* can be easily distinguished from other archaeocalamitacean plants by its slender stems, wider leaves, and the grade of development of its sporangiophore shields.

If *Peltotheca* is indeed an archaeocalamitacean, as suggested by our results, then the hypothesis advanced by Mamay and Bateman (1991) that the Archaeocalamitaceae survived at least until the Early Permian is further supported. It is interesting to note that there are no Pennsylvanian records of the group, with a hiatus of ca. 55 Myr separating the youngest record from the others (Mamay and Bateman, 1991). However, species such as *Barakaria neuburgiana* Meyen, from the late Carboniferous of Russia (Meyen, 1969), with linear dichotomous leaves extremely similar to those of *P. furcata*, may also be part of this lineage, thereby reducing the gap some 10–15 Myr. *Barakaria neuburgiana* is recognized as having leaves that are very similar to archaeocalamitacean leaves, but its affinity with that group has been dismissed based on its geographic and stratigraphic position and on the absence of reproductive structures supporting that affinity (Meyen, 1969).

In addition to its stratigraphic importance, *Peltotheca* also demonstrates that archaeocalamitaceans had a broader geographic distribution than previously thought (i.e., including the southern hemisphere). However, the phylogenetic position of *Peltotheca* should be regarded with caution for the time being, since numerous features of this intriguing plant have yet to be documented (e.g., type of spores, morphology of underground organs, internal anatomy). We anticipate that future studies focused on those yet unknown aspects of this plant will provide additional data for testing the hypothesis of archaeocalamitacean affinities.

Monilophytes, Moniliformopses, and *Equisetum*

While some authors consider the “monilophyte” clade sensu Pryer et al. (2004) to be supported by results of the phylogenetic analysis of Kenrick and Crane (1997), it is important to realize that “Moniliformopses” sensu Kenrick and Crane (1997) includes an entirely disjunct set of fossil euphyllophyte taxa (i.e., *Ibyka*, *Rhacophyton*, *Pseudosporochnus*; see Fig. 4.31 of Kenrick and Crane, 1997) as compared to the living species that form the monilophyte clade of Pryer et al. (2004). Whereas “moniliformopses” sensu

Kenrick and Crane (1997) consists of exclusively Devonian age fossil species fern-like and/or sphenopsid-like plants with no confirmed living descendants (Rothwell and Nixon, 2006; Rothwell and Stockey, 2008), “monilophytes” sensu Pryer et al., 2001) includes only living crown group species of psilotophytes, equisetophytes, ophioglossids, marattioid ferns, and leptosporangiate ferns. Moreover, phylogenetic analyses that include all of the groups of “monilophytes” (i.e., a combination of the taxa included in Kenrick and Crane, 1997 and Pryer et al., 2001, 2004) do not find support for the clade, suggesting a polyphyletic arrangement of its constituents instead (e.g., Rothwell and Nixon, 2006). As a result, moniliformopses sensu Kenrick and Crane (1997) and monilophytes sensu Pryer et al. (2004) appear to be completely different groups of plants with no confirmed phylogenetic relationships beyond their common inclusion within the Euphyllphytina sensu Kenrick and Crane (1997). Therefore, the monilophyte hypothesis, i.e., that all seed-free euphyllphytes form a clade that is sister to the spermatophyte clade, still lacks support from phylogenetic analyses that include taxa from both the fossil record and the extant diversity of euphyllphytes.

According to the topology obtained by early systematic studies of living plants, in which *Equisetum* was recovered nested within a clade containing traditional fern groups (Pryer et al., 2001, 2004; Wikström and Pryer, 2005; Schneider et al., 2009), it seemed logical to interpret equisetaleans as highly modified ferns. However, data on plant structure and development, as well as the fossil record, have suggested alternative scenarios (Rothwell, 1999; Rothwell and Nixon, 2006; Tomescu, 2011). Studies that include both living and extinct plants fail to recover *Equisetum* and its extinct sister groups nested within a clade with living marattiaceous and leptosporangiate ferns (Rothwell, 1999; Rothwell and Nixon, 2006). It should be noted that as compared to analyses of only living species, mainly based on DNA and which usually rely on a number of characters higher by orders of magnitude, systematic analyses of euphyllphytes including fossil species have a reduced level of resolution and confidence for individual nodes of the resulting phylogenetic trees (e.g., Coiro et al., 2018). However, results of the most recent analyses of relationships among living euphyllphytes, based on molecular data, also fail to recover *Equisetum* nested within modern fern clades. Rather, these analyses recover equisetaleans (i.e., *Equisetum* spp.) as sister to a clade composed by Ophioglossidae and living ferns (Rai and Graham, 2010; Wickett et al., 2014; Knie et al., 2015; Rothfels et al., 2015; Testo and Sundue, 2016; PPG I, 2016). Such results suggest that equisetaleans have been evolving independently from modern ferns clades since at least the Devonian, according to a relaxed clock model (Rothfels et al., 2015), and possibly the Silurian according to a penalized approach (Testo and Sundue, 2016), dates that still lack thorough geologic and paleontologic testing.

It seems that the study of the relationships of Euphyllphytes would derive great benefit from an integrative approach that includes both the ever-increasing amount of molecular data and anatomical/morphological information available from extant species and data provided by the rich fossil record of the group. A much denser taxon sampling of extant and extinct species also would be beneficial. That sampling necessarily would require the inclusion of a much larger number of ancient representatives of the groups under study to accurately elucidate the early character configuration of modern and extinct clades and their subsequent evolutionary pathways. Additionally, a better understanding of the positions of fossil Euphyllphytes will be required to further test molecular datings,

which seldom are rigorously tested by geology-based data (Wilf and Escapa, 2015a, b).

CONCLUSIONS AND OUTLOOK

Our results shed some light on the long and complicated evolutionary history of *Equisetum*, highlighting the fact that *Equisetum* is part of a diverse, species-rich sphenopsid clade with two major subclades and a rich fossil record that dates back to the Devonian. In this study, we addressed phylogenetic relationships of only one of those major subclades (i.e., the Equisetales). Relationships within the other major sphenopsid clade (i.e., Sphenophyllales) have yet to be addressed using modern phylogenetic techniques. Nevertheless, our current understanding of Sphenophyllales is sufficient to hypothesize that analysis of that clade will further illuminate the phylogenetic isolation of modern *Equisetum* within the extant flora adding further evidence to the independent phylogenetic history of the sphenopsid clade with respect to all other groups of pteridophytic euphyllphytes (i.e., ferns and fern-like plants; Tomescu, 2011).

Our analyses resolve Equisetaceae + *Neocalamites* as sister to Calamitaceae + the A.G. clade, with the four groups being sister to Archaeocalamitaceae (Figs. 1–3), which, for the most part, are phylogenetic relationships not previously recovered. The estimated age for the origin of the *Equisetum* crown group is at least as old as the Early Cretaceous and probably as old as the Jurassic (Fig. 3). These results emphasize the usefulness of combining all available evidence for phylogenetic inferences, with different types of characters proving useful for addressing different questions.

Critical assessment of our morphological matrix points to several key areas in which future studies would be most helpful for understanding homologies of equisetalean characters. One area concerns the Permian equisetaleans, for which key characters are still unknown. Another area where additional information could be useful is that of spore characters, which are incompletely documented in both extinct and extant species. From a molecular standpoint, either sequencing the chloroplast genome of *Equisetum bogotense* or sequencing nuclear genes of additional key species of the genus could help to test hypotheses on the phylogenetic placement of this enigmatic species and to determine the usefulness of morphological characters in this endeavor.

Our study is a stark reminder that *Equisetum* is deeply nested within a clade that diverged from the rest of the euphyllphytes at least as early as the Upper Devonian, some 370 Ma, and possibly even earlier than that (Taylor et al., 2009; Rothfels et al., 2015; Testo and Sundue, 2016). Therefore, sphenopsids pre-date all other clades of living pteridophytic euphyllphytes. Members of this ancient species-rich clade underwent numerous evolutionary radiations and extinction events and possess unique organography with a very distinctive suite of morphological, anatomical, and developmental characters (Tomescu et al., 2017), which evolved along evolutionary pathways with no parallel compared to the rest of the euphyllphytes. The fossil record of sphenopsids provides a rich body of evidence for changes during the evolutionary history of the clade. Only in light of these data can the unique, highly canalized morphology of modern *Equisetum* be understood as a reflection of a complex process of incremental morphological exploration and evolutionary change. Given the long independent evolutionary history and the largely extinct diversity of the sphenopsid clade,

studies using *Equisetum* as its sole representative cannot avoid a significant taxon-sampling bias when attempting to estimate some of its large-scale evolutionary patterns and phylogenetic relationships. Our understanding of the deep phylogeny and evolution of euphyllophytes in general, and of the position of *Equisetum* within the euphyllophyte tree in particular, will continue to be meaningfully benefited by the routine integration of data from the paleontological and extant plant record.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

LITERATURE CITED

- Arnold, C. A. 1958. Petrified cones of the genus *Calamostachys* from the Carboniferous of Illinois. *Contributions from the Museum of Paleontology, University of Michigan* 14: 149–165.
- Bapst, D. W. 2012. Paleotree: an R package for paleontological and phylogenetic analyses of evolution. *Methods in Ecology and Evolution* 3: 803–807.
- Bateman, R. M. 1991. Palaeobiological and phylogenetic implications of anatomically-preserved *Archaeocalamites* from the Dinantian of Oxroad Bay and Loch Humphrey Burn, southern Scotland. *Palaeontographica Abteilung B* 223: 1–59.
- Baxter, R. W. 1963. Calamocarpon insignis, a new genus of heterosporous, petrified calamitean cones from the American Carboniferous. *American Journal of Botany* 50: 469–473.
- Baxter, R. W. 1964. The megagametophyte and microsporangia of *Calamocarpon insignis*. *Phytomorphology* 14: 481–487.
- Behrensmeyer, A. K., J. D. Damuth, W. A. Dimichele, R. Potts, H.-D. Sues, and S. L. Wing [eds.] 1992. Terrestrial ecosystems through time: evolutionary paleoecology of terrestrial plants and animals. University of Chicago Press, Chicago, IL, USA.
- Bell, M. A., and G. T. Lloyd. 2015. strap: an R package for plotting phylogenies against stratigraphy and assessing their stratigraphic congruence. *Palaeontology* 58: 379–389.
- Bierhorst, D. W. 1971. Morphology of vascular plants. Macmillan, NY, NY, USA.
- Bomfleur, B., I. H. Escapa, R. Serbet, E. L. Taylor, and T. N. Taylor. 2013. A reappraisal of *Neocalamites* and *Schizoneura* (fossil Equisetales) based on material from the Triassic of East Antarctica. *Alcheringa* 37: 1–17.
- Boureau, E. 1964. Traité de Paléobotanique. Tome II. Sphenophyta, Noeggerathiophyta. Masson et C^{ie}, Paris, France.
- Brea, M., and A. E. Artabe. 1999. Apocalamitaceae (Sphenophyta) triasicas de la Formación Paramillo, Agua de la Zorra, provincia de Mendoza, Argentina. *Ameghiniana* 36: 389–400.
- Brown, J. T. 1975. *Equisetum clarnoi*, a new species based on petrifications from the Eocene of Oregon. *American Journal of Botany* 62: 410–415.
- Browne, I. 1908. The phylogeny and inter-relationships of the Pteridophyta. II. Equisetales. *New Phytologist* 7: 103–113.
- Brunner, H., and K.-P. Kelber. 1988. Eisenerzkonglomerate im württembergisch-fränkischen Unterkeuper – Bemerkungen zum fossilen Environment. In H. Hagdorn [ed.], *Neue Forschungen zur Erdgeschichte von Crailsheim, 185–205*. Gesellschaft für Naturkunde, Württemberg, Germany.
- Channing, A., A. Zamuner, D. Edwards, and D. Guido. 2011. *Equisetum thermale* sp. nov. (Equisetales) from the Jurassic San Agustín hot spring deposit, Patagonia: anatomy, paleoecology, and inferred paleoecophysiology. *American Journal of Botany* 98: 680–697.
- Chaphekar, M. 1963. Some calamitean plants from the Lower Carboniferous of Scotland. *Palaeontology* 6: 408–429.
- Chaphekar, M. 1965. On the genus *Pothocites* Paterson. *Palaeontology* 8: 107–112.
- Cleal, C. J., D. Uhl, B. Cascales-Miñana, B. A. Thomas, A. R. Bashforth, S. C. King, and E. L. Zodrow. 2012. Plant biodiversity changes in Carboniferous tropical wetlands. *Earth-Science Reviews* 114: 124–155.
- Coiro, M., G. Chomicki, and J.A. Doyle. 2018. Experimental signal dissection and method sensitivity analyses reaffirm the potential of fossils and morphology in the resolution of the relationship of angiosperms and Gnetales. bioRxiv preprint first posted online 31 March 2018, <https://www.biorxiv.org/content/early/2018/03/31/134262>.
- Crookall, R. 1969. Fossil plants of the Carboniferous rocks of Great Britain [second section]. *Memoirs of the Geological Survey of Great Britain, Palaeontology* 4: 573–792.
- Cúneo, N. R., and I. H. Escapa. 2006. The equisetalean genus *Cruciaetheca* nov. from the Lower Permian of Patagonia, Argentina. *International Journal of Plant Sciences* 167: 167–177.
- De Bivort, B. L., R. M. Clouse, and G. Giribet. 2010. A morphometrics-based phylogeny of the temperate Gondwanan mite harvestmen (Opiliones, Cyphophthalmi, Pettalidae). *Journal of Zoological, Systematics and Evolutionary Research* 48: 294–309.
- Des Marais, D. L., A. R. Smith, D. M. Britton, and K. M. Pryer. 2003. Phylogenetic relationships and evolution of extant horsetails, *Equisetum*, based on chloroplast DNA sequence data (*rbcl* and *trnL-F*). *International Journal of Plant Sciences* 164: 737–751.
- Eames, A. J. 1936. Morphology of vascular plants: lower groups (Psilophytales to Filicales). McGraw Hill, NY, NY, USA.
- Elgorriaga, A., I. H. Escapa, B. Bomfleur, R. Cúneo, and E. G. Ottone. 2015. Reconstruction and phylogenetic significance of a new *Equisetum* Linnaeus species from the Lower Jurassic of Cerro Bayo (Chubut Province, Argentina). *Ameghiniana* 52: 135–152.
- Escapa, I. H., and S. A. Catalano. 2013. Phylogenetic analysis of Araucariaceae: integrating molecules, morphology, and fossils. *International Journal of Plant Sciences* 174: 1153–1170.
- Escapa, I. H., and R. Cúneo. 2005. A new equisetalean plant from the early Permian of Patagonia, Argentina. *Review of Palaeobotany and Palynology* 137: 1–14.
- Escapa, I. H., and R. Cúneo. 2006. Primer registro de *Neocalamites* (Halle) Vladimirovicz en el Pérmico de Gondwana. *Ameghiniana* 43: 85–92.
- Escapa, I. H., and D. Pol. 2011. Dealing with incompleteness: new advances for the use of fossils in phylogenetic analysis. *Palaaios* 26: 121–124.
- Evert, R. F., and S. E. Eichhorn. 2012. Raven biology of plants. W. H. Freeman, NY, NY, USA.
- Feng, Z., T. Zierold, and R. Rößler. 2012. When horsetails became giants. *Chinese Science Bulletin* 57: 2285–2288.
- Flores, J. R., S. A. Catalano, and G. M. Suarez. 2017. Cladistic analysis of the family Cryphaeaceae (Briophyta) with emphasis on *Cryphaea*: a study based on a comprehensive morphological dataset. *Darwiniana* 5: 51–64.
- Gifford, E. M., and A. S. Foster. 1989. Morphology and evolution of vascular plants. W. H. Freeman, San Francisco, CA, USA.
- Goloboff, P. A., and S. A. Catalano. 2012. GB-to-TNT: facilitating creation of matrices from GenBank and diagnosis of results in TNT. *Cladistics* 28: 503–513.

- Goloboff, P. A., and S. A. Catalano. 2016. TNT version 1.5, including a full implementation of phylogenetic morphometrics. *Cladistics* 32: 221–238.
- Goloboff, P. A., S. Farris, and K. Nixon. 2008. TNT, a free program for phylogenetic analysis. *Cladistics* 24: 774–786.
- Goloboff, P. A., C. I. Mattoni, and A. S. Quinteros. 2006. Continuous characters analyzed as such. *Cladistics* 22: 589–601.
- Goloboff, P. A., A. Torres, and J. S. Arias. 2017. Weighted parsimony outperforms other methods of phylogenetic inference under models appropriate for morphology. *Cladistics*. <https://doi.org/10.1111/cla.12205>.
- Good, C. W. 1971a. The ontogeny of Carboniferous articulates: Calamite leaves and twigs. *Palaeontographica Abteilung B* 133: 137–158.
- Good, C. W. 1971b. The ontogeny of Carboniferous articulates: *Calamostachys binneyana*. *Botanical Gazette* 132: 337–346.
- Good, C. W. 1973. Studies of *Sphenophyllum* shoots: species delimitation within the taxon *Sphenophyllum*. *American Journal of Botany* 60: 929–939.
- Good, C. W. 1975. Pennsylvanian-age calamitean cones elater-bearing spores, and associated vegetative organs. *Palaeontographica Abteilung B* 153: 28–99.
- Good, C. W. 1978. Taxonomic characteristics of sphenophyllean cones. *American Journal of Botany* 65: 86–97.
- Good, C. W., and T. N. Taylor. 1974. The establishment of *Elaterites triferens* spores in *Calamocarpon insignis* microsporangia. *Transactions of the American Microscopical Society* 93: 148–151.
- Good, C. W., and T. N. Taylor. 1975. The morphology and systematic position of calamitean elater-bearing spores. *Geoscience and Man* 11: 133–139.
- Gould, R. E. 1968. Morphology of *Equisetum laterale* Phillips, 1829, and *E. bryanii* sp. nov. from the Mesozoic of south-eastern Queensland. *Australian Journal of Botany* 16: 153–176.
- Grewe, F., W. Guo, E. A. Gubbels, A. K. Hansen, and J. P. Mower. 2013. Complete plastid genomes from *Ophioglossum californicum*, *Psilotum nudum*, and *Equisetum hyemale* reveal an ancestral land plant genome structure and resolve the position of Equisetales among monilophytes. *BMC Evolutionary Biology* 13: 8.
- Gu, Z., and L. Shi. 1974. Fossil plants of China, vol. 1. Palaeozoic plants from China. Science Press, Beijing, China.
- Guillon, J. M. 2004. Phylogeny of horsetails (*Equisetum*) based on the chloroplast *rps4* gene and adjacent noncoding sequences. *Systematic Botany* 29: 251–259.
- Guillon, J. M. 2007. Molecular phylogeny of horsetails (*Equisetum*) including chloroplast *atpB* sequences. *Journal of Plant Research* 120: 569–574.
- Hauke, R. L. 1963. A taxonomic monograph of the genus *Equisetum* subgenus *Hippochaete*. *Beihfte zur Nova Hedwigia* 8: 1–123.
- Hauke, R. L. 1969. Gametophyte development in Latin American horsetails. *Bulletin of the Torrey Botanical Club* 96: 568–577.
- Hauke, R. L. 1978. A taxonomic monograph of *Equisetum* subgenus *Equisetum*. *Nova Hedwigia* 30: 385–455.
- Husby, C. 2013. Biology and functional ecology of *Equisetum* with emphasis on the giant horsetails. *Botanical Review* 79: 147–177.
- Karol, K. G., K. Arumuganathan, J. L. Boore, A. M. Duffy, K. D. E. Everett, J. D. Hall, S. K. Hansen, et al. 2010. Complete plastome sequences of *Equisetum arvense* and *Isoetes flaccida*: implications for phylogeny and plastid genome evolution of early land plant lineages. *BMC Evolutionary Biology* 10: 321.
- Katoh, K., and D. M. Standley. 2013. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution* 30: 772–780.
- Kelber, K.-P., and W. Hansch. 1995. Keuperpflanzen. Die Enträtselung einer über 200 Millionen Jahre alten Flora. *Museo* 11: 1–157.
- Kelber, K. P., and J. H. A. van Konijnenburg-van Cittert. 1998. *Equisetites arenaceus* from the Upper Triassic of Germany with evidence for reproductive strategies. *Review of Palaeobotany and Palynology* 100: 1–26.
- Kenrick, P., and P. R. Crane. 1997. The origin and early diversification of land plants. A cladistic study. Smithsonian Institution Press, Washington D.C., USA.
- Kidston, R. 1883. On the affinities of the genus *Pothocites*, Paterson; with the description of a specimen from Glencartholm, Eskdale. *Annals and Magazine of Natural History* 65: 297–314.
- Kidston, R. 1892. On the occurrence of the genus *Equisetum* (*E. hemingwayi*, Kidston) in the Yorkshire Coal-measures. *Annals and Magazine of Natural History, Series 6* 9: 138–141.
- Knie, N., S. Fischer, F. Grewe, M. Polskiewicz, and V. Knoop. 2015. Horsetails are the sister group to all other monilophytes and Marattiales are sister to leptosporangiate ferns. *Molecular Phylogenetics and Evolution* 90: 140–149.
- Kosanke, R. M. 1955. *Mazostachys*—a new calamite fructification. *Illinois State Geological Survey, Report of Investigations* 180: 7–24.
- Lacey, W. S. 1941. On *Calamostachys oldhamia* Hick & Lomax, and its inclusion in *C. casheana* Williamson. *Annals and Magazine of Natural History: Series 11* 7: 536–540.
- Leisman, G. A., and J. L. Bucher. 1971a. On *Palaeostachya decacnema* from the Middle Pennsylvanian of Kansas. *Bulletin of the Torrey Botanical Club* 98: 140–144.
- Leisman, G. A., and J. L. Bucher. 1971b. Variability in *Calamocarpon insignis* from the American Carboniferous. *Journal of Paleontology* 45: 494–501.
- Li, X., C. Cai, and Y. Wang. 1995. *Hamatophyton verticillatum* (Gu & Zhi) emend. A primitive plant of Sphenopsida from the Upper Devonian-Lower Carboniferous in China. *Palaeontographica Abteilung B* 235: 1–22.
- Lu, J.-M., N. Zhang, X.-Y. Du, J. Wen, and D.-Z. Li. 2015. Chloroplast phylogenomics resolves key relationships in ferns. *Journal of Systematics and Evolution* 53: 448–457.
- Lubiński, M., and V. M. Dörken. 2016. The hybrid between *Equisetum scirpoides* and *E. variegatum* in northern Europe. *American Fern Journal* 106: 116–130.
- Maddison, W.P., and D.R. Maddison. 2017. Mesquite: a modular system for evolutionary analysis, version 3.3. Available at <http://mesquiteproject.org>.
- Mamay, S. H. 1959. A new Bowmanitean fructification from the Pennsylvanian of Kansas. *American Journal of Botany* 46: 530–536.
- Mamay, S. H., and R. M. Bateman. 1991. *Archaeocalamites lazarii*, sp. nov.: the range of Archaeocalamitaceae extended from the lowermost Pennsylvanian to the mid-Lower Permian. *American Journal of Botany* 78: 489–496.
- McIver, E. E., and J. F. Basinger. 1989. The morphology and relationships of *Equisetum fluviatoides* sp. nov. from the Paleocene Ravenscrag Formation of Saskatchewan, Canada. *Canadian Journal of Botany* 67: 2937–2943.
- Meyen, S. V. 1969. The Angara members of Gondwana genus *Barakaria* and its systematical position. *Argumenta Palaeobotanica* 3: 1–14.
- Meyen, S. V. 1971. *Phyllothea*-like plants from the Upper Palaeozoic flora of Angaraland. *Palaeontographica Abteilung B* 133: 1–33.
- Meyen, S. V. 1987. Fundamentals of palaeobotany. Chapman and Hall, NY, NY, USA.
- Meyen, S. V., and L. V. Menshikova. 1983. Systematics of the Upper Palaeozoic articulates of the family Tchernoviaceae. *Botanicheskiy Zhurnal* 68: 721–729.
- Mongiardino Koch, N., I. M. Soto, and M. J. Ramirez. 2015. First phylogenetic analysis of the family Neriidae (Diptera), with a study on the issue of scaling continuous characters. *Cladistics* 31: 142–165.
- Naugolnykh, S. V. 2002. *Paracalamitina striata*—a newly reconstructed equisetophyte from the Permian of Angaraland. *Journal of Paleontology* 76: 377–385.
- Naugolnykh, S. V. 2004. On some aberrations of extant horsetails and the origin of the family Equisetaceae. *Paleontological Journal* 38: 335–342.
- Neuberg, M. F. 1964. Permian flora of the Petchora Basin, part II. Sphenopsida [in Russian]. *Trudy Geologicheskogo Instituta Akademii Nauk SSSR* 30: 1–90.
- Nickrent, D. L., C. L. Parkinson, J. D. Palmer, and R. J. Duff. 2000. Multigene phylogeny of land plants with special references to bryophytes and the earliest land plants. *Molecular Biology and Evolution* 17: 1885–1895.
- O'Reilly, J. E., M. N. Puttick, D. Pisani, and P. C. J. Donoghue. 2018. Probabilistic methods surpass parsimony when assessing clade support in phylogenetic analyses of discrete morphological data. *Paleontology* 61: 105–118.
- Osborn, J. M., C. J. Phipps, T. N. Taylor, and E. L. Taylor. 2000. Structurally preserved sphenophytes from the Triassic of Antarctica: reproductive remains of *Spaciodum*. *Review of Palaeobotany and Palynology* 111: 225–235.
- Osborn, J. M., and T. N. Taylor. 1989. Structurally preserved sphenophytes from the Triassic of Antarctica: vegetative remains of *Spaciodum* gen. nov. *American Journal of Botany* 76: 1954–1601.
- Page, C. N. 1972a. An assessment of inter-specific relationships in *Equisetum* subgenus *Equisetum*. *New Phytologist* 71: 355–369.

- Page, C. N. 1972b. An interpretation of the morphology and evolution of the cone and shoot of *Equisetum*. *Botanical Journal of the Linnean Society* 65: 359–397.
- Parins-Fukuchi, C. 2018. Use of continuous traits can improve morphological phylogenetics. *Systematic Biology* 67: 328–339.
- Paterson, R. 1844. Description of *Pothocites grantonii*, a new fossil vegetable, from the Coal Formation. *Transactions of the Botanical Society of Edinburgh* 1: 45–52.
- PPG, I. 2016. A community-derived classification for extant lycophytes and ferns. *Journal of Systematic and Evolution* 54: 563–603.
- Pryer, K. M., H. Schneider, A. R. Smith, R. Cranfill, P. G. Wolf, J. S. Hunt, and S. D. Sipes. 2001. Horsetails and ferns are a monophyletic group and the closest living relatives to seed plants. *Nature* 409: 618–622.
- Pryer, K. M., E. Schuettpelz, P. G. Wolf, H. Schneider, A. R. Smith, and R. Cranfill. 2004. Phylogeny and evolution of ferns (Monilophytes) with a focus on the early leptosporangiate divergences. *American Journal of Botany* 91: 1582–1598.
- Pryer, K. M., A. R. Smith, and J. E. Skog. 1995. Phylogenetic relationships of extant ferns based on evidence from morphology and *rbcl*. *American Fern Journal* 85: 205–282.
- Puttick, M. N., J. E. O'Reilly, A. R. Tanner, J. F. Fleming, J. Clark, L. Holloway, J. Lozano-Fernandez, L. A. Parry, J. E. Tarver, D. Pisani, and C. J. Donoghue. 2017. Uncertain-tree: discriminating among competing approaches to the phylogenetic analysis of phenotype data. *Proceedings of the Royal Society, B, Biological Sciences* 284: 1846.
- R Core Team. 2017. R: a language and environment for statistical computing. v. 3.4.1. R Foundation for Statistical Computing, Vienna, Austria. Website <https://r-project.org>.
- Rai, H. S., and S. W. Graham. 2010. Utility of a large, multigene plastid data set in inferring higher-order relationships in ferns and relatives (monilophytes). *American Journal of Botany* 97: 1444–1456.
- Rothfels, C. J., F. W. Li, E. M. Sigel, L. Huiet, A. Larsson, D. O. Burge, M. Ruhsam, et al. 2015. The evolutionary history of ferns inferred from 25 low-copy nuclear genes. *American Journal of Botany* 102: 1089–1107.
- Rothwell, G. W. 1999. Fossils and ferns in the resolution of land plant phylogeny. *Botanical Review* 65: 188–218.
- Rothwell, G. W., and S. R. Ash. 2015. Internal anatomy of the Late Triassic *Equisetocaulis* gen. nov., and the evolution of modern horsetails. *Journal of the Torrey Botanical Society* 142: 27–37.
- Rothwell, G. W., and K. C. Nixon. 2006. How does the inclusion of fossil data change our conclusions about the phylogenetic history of euphyllophytes? *International Journal of Plant Sciences* 167: 737–749.
- Rothwell, G. W., and R. A. Stockey. 2008. Phylogeny and evolution of ferns: a paleontological perspective. In T. A. Ranker and C. H. Haufler [eds.], *Biology and evolution of ferns and lycophytes*, 332–366. Cambridge University Press, Cambridge, UK.
- Rothwell, G. W., and T. N. Taylor. 1971a. Studies of Paleozoic calamitean cones: *Weissia kentuckiense* gen. et sp. nov. *Botanical Gazette* 132: 215–224.
- Rothwell, G. W., and T. N. Taylor. 1971b. *Weissistachys kentuckiensis*: a new name for *Weissia kentuckiense* Rothwell and Taylor. *Botanical Gazette* 132: 371–372.
- Rothwell, G. W., and A. M. F. Tomescu. 2018. Structural fingerprints of development at the intersection of evolutionary developmental biology and the fossil record. In L. Nuño de la Rosa and G. B. Müller [eds.], *Evolutionary developmental biology—a reference guide*. Springer International Publishing AG, Cham, Switzerland. https://doi.org/10.1007/978-3-319-33038-9_169-1.
- Rothwell, G. W., S. E. Wyatt, and A. M. F. Tomescu. 2014. Plant evolution at the interface of paleontology and developmental biology: an organism-centered paradigm. *American Journal of Botany* 101: 899–913.
- Ryberg, P. E., E. J. Hermsen, E. L. Taylor, T. N. Taylor, and J. M. Osborn. 2008. Development and ecological implications of dormant buds in the high-Paleolatitude Triassic sphenophyte *Spaciinodum* (Equisetaceae). *American Journal of Botany* 95: 1443–1453.
- Sanderson, M. J. 2002. Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. *Molecular Biology and Evolution* 19: 101–109.
- Schneider, H., A. R. Smith, and K. M. Pryer. 2009. Is morphology really at odds with molecules in estimating fern phylogeny? *Systematic Botany* 34: 455–475.
- Schwendemann, A. B., T. N. Taylor, E. L. Taylor, M. Krings, and J. M. Osborn. 2010. Modern traits in Early Mesozoic sphenophytes: the *Equisetum*-like cones of *Spaciinodum collinsonii* with in situ spores and elaters from the Middle Triassic of Antarctica. In C. T. Gee [ed.], *Plants in Mesozoic time: innovations, phylogeny, ecosystems*, 15–33. Indiana University Press, Bloomington, IN, USA.
- Scott, D. H. 1900. *Studies in fossil botany*. Adam and Charles Black, London, UK.
- Seward, A. C. 1898. *Fossil plants*, vol. 1. C. J. Clay and Sons, London, UK.
- Stanich, N. A., G. W. Rothwell, and R. A. Stockey. 2009. Phylogenetic diversification of *Equisetum* (Equisetales) as inferred from Lower Cretaceous species of British Columbia, Canada. *American Journal of Botany* 96: 1289–1299.
- Stein, W. E., D. C. Wight, and C. B. Beck. 1984. Possible alternatives for the origin of Sphenopsida. *Systematic Botany* 9: 102–118.
- Stewart, W. N., and G. W. Rothwell. 1993. *Paleobotany and the evolution of plants*. Cambridge University Press, Cambridge, UK.
- Taylor, T. N. 1969. On the structure of *Bowmanites dawsoni* from the Lower Pennsylvanian of North America. *Palaeontographica Abteilung B* 125: 65–72.
- Taylor, T. N. 1970. The morphology of *Bowmanites dawsoni* spores. *Micropaleontology* 16: 243–248.
- Taylor, T. N., E. L. Taylor, and M. Krings. 2009. *Paleobotany. The biology and evolution of fossil plants*, 2nd ed. Academic Press, Amsterdam, Netherlands.
- Testo, W., and M. Sundue. 2016. A 4000-species dataset provides new insight into the evolution of ferns. *Molecular Phylogenetics and Evolution* 105: 200–211.
- Tomescu, A. M. F. 2011. The sporophytes of seed-free vascular plants – major vegetative developmental features and molecular genetic pathways. In H. Fernández, A. Kumar, and A. Revilla [eds.], *Working with ferns: issues and applications*, 67–94. Springer International Publishing AG, Cham, Switzerland.
- Tomescu, A. M. F., I. H. Escapa, G. W. Rothwell, A. Elgorriaga, and N. R. Cúneo. 2017. Developmental programmes in the evolution of *Equisetum* reproductive morphology: a hierarchical modularity hypothesis. *Annals of Botany* 119: 489–505.
- Tschudy, R. 1939. The significance of certain abnormalities in *Equisetum*. *American Journal of Botany* 26: 744–749.
- Wang, D.-M., S. G. Hao, and Q. Wang. 2005. *Rotafolia songziensis* gen. et comb. nov., a sphenopsid from the Late Devonian of Hubei, China. *Botanical Journal of the Linnean Society* 148: 21–37.
- Wang, D.-M., S.-G. Hao, Q. Wang, and J.-Z. Xue. 2006. Anatomy of the Late Devonian sphenopsid *Rotafolia songziensis*, with a discussion of stelar architecture of the Sphenophyllales. *International Journal of Plant Sciences* 167: 373–383.
- Watson, J. 1983. Two Wealden species of *Equisetum* found in situ. *Acta Palaeontologica Polonica* 28: 265–269.
- Watson, J., and D. J. Batten. 1990. A revision of the English Wealden flora, II. Equisetales. *Bulletin of the British Museum (Natural History). Geology* 46: 37–60.
- Wickett, N. J., S. Mirarab, N. Nguyen, T. Warnow, E. Carpenter, N. Matasci, S. Ayyampalayam, et al. 2014. Phylotranscriptomic analysis of the origin and early diversification of land plants. *Proceedings of the National Academy of Sciences, USA* 111: E4859–E4868.
- Wikström, N., and K. M. Pryer. 2005. Incongruence between primary sequence data and the distribution of a mitochondrial *atp1* group II intron among ferns and horsetails. *Molecular Phylogenetics and Evolution* 36: 484–493.
- Wilf, P., and I. H. Escapa. 2015a. Green Web or megabiased clock? Plant fossils from Gondwanan Patagonia speak on evolutionary radiations. *New Phytologist* 207: 283–290.
- Wilf, P., and I. H. Escapa. 2015b. Molecular dates requires geologic testing. *New Phytologist* 209: 1359–1362.
- Williamson, W. C., and D. H. Scott. 1894. Further observations on the organization of the fossil plants of the Coal-Measures. Part I. *Calamites, Calamostachys and Sphenophyllum*. *Philosophical Transactions of the Royal Society, B, Biological Sciences* 185: 863–959.

APPENDIX 1. GenBank accession numbers from the *Equisetum*, *Psilotum*, and *Ophioglossum* DNA sequences used for this study.

Species	<i>rbcl</i>	<i>trnL-F</i>	<i>rps4</i>	<i>atpB</i>	<i>matK</i>	<i>ndhJ</i>
<i>E. arvense</i>	AY226140 ¹	AY226125 ¹	AJ583677 ⁵	AM422389 ⁷	AM883547 ⁹	AM883603 ⁹
<i>E. bogotense</i>	AY226139 ¹	AY226124 ¹	AJ583678 ⁵	AM422390 ⁷	KP757846 ¹⁰	-
<i>E. diffusum</i>	AY226141 ¹	AY226126 ¹	AJ583679 ⁵	AM422391 ⁷	-	-
<i>E. fluviatile</i>	AY226142 ¹	AY226121 ¹	AJ583680 ⁵	AM422392 ⁷	-	-
<i>E. giganteum</i>	AY226127 ¹	AY226118 ¹	AJ583681 ⁵	AM422393 ⁷	AM883549 ⁹	AM883605 ⁹
<i>E. hyemale</i>	AY226128 ¹	AY226110 ¹	AJ583682 ⁵	AM422394 ⁷	AM883542 ⁹	AM883598 ⁹
<i>E. laevigatum</i>	AY226130 ¹	AY226112 ¹	AJ583683 ⁵	AM422395 ⁷	-	-
<i>E. myriochaetum</i>	AY226131 ¹	AY226114 ¹	AJ583684 ⁵	AM422396 ⁷	-	-
<i>E. palustre</i>	AY226138 ¹	AY226123 ¹	AJ583685 ⁵	AM422397 ⁷	AM883541 ⁹	AM883597 ⁹
<i>E. pratense</i>	AY226137 ¹	AY226122 ¹	AJ583686 ⁵	AM422398 ⁷	-	-
<i>E. ramosissimum</i>	AY226132 ¹	AY226115 ¹	AJ583687 ⁵	AM422399 ⁷	AM883551 ⁹	AM883607 ⁹
<i>E. scirpoides</i>	AY226133 ¹	AY226116 ¹	AJ583688 ⁵	AM422400 ⁷	AM883552 ⁹	AM883608 ⁹
<i>E. sylvaticum</i>	AY226136 ¹	AY226120 ¹	AJ583689 ⁵	AM422401 ⁷	AM883553 ⁹	AM883609 ⁹
<i>E. telmateia</i>	AY226135 ¹	AY226119 ¹	AJ583690 ⁵	AM422402 ⁷	AM883540 ⁹	AM883596 ⁹
<i>E. variegatum</i>	AY226134 ¹	AY226117 ¹	AJ583691 ⁵	AM422403 ⁷	AM883554 ⁹	-
<i>P. nudum</i>	AB626657 ²	FJ384430 ⁴	EU439174 ⁶	EU439075 ⁶	AB716747 ²	-
<i>O. reticulatum</i>	AY138410 ³	AY138446 ³	EU439177 ⁶	U93825 ⁸	AB716737 ²	-

¹Des Marais, D. L., A. R. Smith, D. M. Britton, and K. M. Pryer. 2003. Phylogenetic relationships and evolution of extant horsetails, *Equisetum*, based on chloroplast DNA sequence data (*rbcl* and *trnL-F*). *International Journal of Plant Sciences* 164: 737–751.

²Shinohara, W., N. Nakato, Y. Yatabe-Kakugawa, T. Oka, J.K. Kim, N. Murakami, H. Noda, and N. Sahashi. 2013. The use of *matK* in Ophioglossaceae phylogeny and the determination of *Mankyua* chromosome number shed light on chromosome number evolution in Ophioglossaceae. *Systematic Botany* 38: 564–570.

³Hauk, W. D., C. R. Parks, and M. W. Chase. 2003. Phylogenetic studies of Ophioglossaceae: Evidence from *rbcl* and *trnL-F* plastid DNA sequences and morphology. *Molecular Phylogenetics and Evolution* 28: 131–151.

⁴Perrie, L. R., P. J. Brownsey, and J. D. Lovis. 2010. *Tmesipteris horomaka*, a new octoploid species from Banks Peninsula. *New Zealand Journal of Botany* 48: 15–29.

⁵Guillon, J.M. 2004. Phylogeny of horsetails (*Equisetum*) based on the chloroplast *rps4* gene and adjacent noncoding sequences. *Systematic Botany* 29: 251–259.

⁶Murdock, A. G. 2008. Phylogeny of marattioid ferns (Marattiaceae): inferring a root in the absence of a closely related outgroup. *American Journal of Botany* 95: 626–641.

⁷Guillon, J. M. 2007. Molecular phylogeny of horsetails (*Equisetum*) including chloroplast *atpB* sequences. *Journal of Plant Research* 120: 569–574.

⁸Wolf, P. G. 1997. Evaluation of *atpB* nucleotide sequences for phylogenetic studies of ferns and other pteridophytes. *American Journal of Botany* 84: 1429–1440.

⁹Cowan, R., and L. J. Kelly¹². DNA barcoding of land plants. Unpublished data. 1: Royal Botanic Gardens, Kew, Richmond TW9 3DS, UK. 2: Royal Botanic Garden Edinburgh, Edinburgh EH3 5LR, UK.

¹⁰Knie, N., S. Fischer, F. Grewe, M. Polsakiewicz, and V. Knoop. 2015. Horsetails are the sister group to all other monilophytes and Marattiales are sister to leptosporangiate ferns. *Molecular Phylogenetics and Evolution* 90: 140–149.