EXPLORING ZOSTEROPHYLL RELATIONSHIPS WITHIN A MORE BROADLY SAMPLED CHARACTER SPACE: A FOCUS ON ANATOMY

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Premise of research. Zosterophylls are a major constituent of Siluro-Devonian tracheophyte floras worldwide and gave rise to the morphologically diverse lycopsid clade. Despite this pivotal position in the plant tree of life, relationships within the group have remained poorly understood. Furthermore, anatomical data have been undersampled in previous phylogenetic analyses including zosterophylls. With this in mind, we addressed the relationships among zosterophylls using a data set that maximized anatomical characters and employing phylogenetic and phenetic methods.

Methodology. A matrix of 40 total characters (12 of which are anatomical) and 19 zosterophyll species with known anatomy, representing 17 genera, was compiled and analyzed using parsimony-constrained phylogenetic methods, clustering, and ordination methods.

Pivotal results. Phenetic analyses show sensitivity to taxon sampling and support the placement of *Renalia* among the zosterophylls but do not support taxonomic inferences strongly congruent with those supported by phylogenetic analyses. Phylogenetic analyses consistently recover two major clades: one lacks internal resolution and comprises the bulk of the zosterophyll taxa included in the analyses; the other clade includes the zosterophyll *Ventarura* and the lycopsid *Sengelia*, often accompanied by *Discalis* and *Trichopherophyton*. Analyses using subsets of characters (only morphology or anatomy) recover trees that differ from those obtained using the total set of characters (morphology and anatomy).

Conclusions. The incongruence between the results of the total character analyses and those using only morphology or only anatomy highlights the importance of broadening the sampling of morphological character space. Because both anatomy and morphology are part of the identity and evolutionary history of a species, the relationships recovered by the inclusion of both morphological and anatomical characters are more likely to reflect natural evolutionary relationships. Breadth of character sampling and not the amount of phylogenetic resolution should be the primary criterion for selecting among alternative hypotheses of relationships.

Keywords: anatomy, fossil, lycopsid, paleobotany, phylogeny, Zosterophyllophytina.

Online enhancements: appendixes.

Introduction

Zosterophylls were a prominent component of Siluro-Devonian (Ludlow; Kotyk et al. 2002) to early Frasnian (Hueber and Banks 1979) landscapes and gave rise to the once extensively diverse lycophyte clade. During this relatively brief stratigraphic presence, zosterophylls were a globally dispersed group of plants that included approximately 37 accepted genera—a number that depends on the inclusion or exclusion of taxa that embody characteristics of zosterophylls as well as other groups (i.e., *Renalia* Gensel 1976) and of gametophyte fossils putatively attributed to zosterophylls (*Calyculiphyton*, *Kidstonophyton*, and *Sciadophyton* Remy et al. 1993).

While zosterophylls are well known in comparison with the other early vascular plants (Banks 1975), their taxonomy is still disputed. Banks published a proposed taxonomic classification in 1968, formally naming the Zosterophyllophytina. Generally speaking, zosterophylls are regarded to be a basal grade in the lycophyte clade, with one of the zosterophyll groups thought to have given rise to the early lycopsids (Niklas and Banks 1990; Gensel 1992; Bateman 1996). Morphologically and anatomically

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speaking, zosterophylls are delineated as plants with vegetative organography consisting of simple undifferentiated axes, reniform bivalvate lateral sporangia, and exarch protosteles (Banks 1968, 1975). Even with these demarcations, there are taxa that combine zosterophyll features with those of other early vascular plant groups (i.e., *Renalia* Gensel 1976; *Huia* Geng 1985), making it difficult to circumscribe the group with certainty.

Banks (1968) initially divided Zosterophyllophytina into two families: the Zosterophyllaceae, which included taxa with naked axes and sporangia aggregated into spikes, and the Gosslingiaceae, which contained plants with ornamented axes and sporangia scattered along the axes. However, in his 1975 publication, Banks reversed this separation into two families. Although currently there is no consensus on taxonomy within the zosterophyll plexus, most studies (Banks 1968, 1975; Hao and Xue 2013) recognize two large groups and place the origin of lycopsids among one of these. Differences between these studies primarily concern the membership of the two groups and the identity of taxa that are not included in either of the two. This situation is due, in part, to differences between the sets of taxa considered in the different studies.

In a precladistic assessment of these discrepancies, Niklas and Banks (1990), discussing the patterns of the symmetry and development of reproductive structures, distinguished two main zosterophyll groups: those with "terminate" fertile axes (strobili) and those with "nonterminate" reproductive morphology (fertile zones along indeterminate vegetative axes). They hypothesized that the immediate lycopsid ancestor had radial symmetry and nonterminate growth in its fertile axes, like the zosterophyll *Discalis* but unlike *Sawdonia* and *Crenaticaulis*, which possess bilateral fertile axes, or *Zosterophyllum*, which possesses terminate fertile axes. With the advent of cladistics, the relationships of the zosterophylls were addressed in a phylogenetic framework. Studies of the group have aimed to (1) assess the relationship of lycophytes and other basal vascular plants to zosterophylls (Gensel 1992; Kenrick and Crane 1997; Hao and Xue 2013), (2) provide classification schemes for the zosterophylls (Kenrick and Crane 1997; Hao and Xue 2013), and (3) discern the relationships between zosterophyll groups (Kenrick and Crane 1997).

Gensel published the first phylogenetic analysis focused on zosterophylls and lycopsids in 1992, recovering all the zosterophylls and lycopsids included in the analysis in a clade with a large basal polytomy that also included *Renalia*. The lycopsids and zosterophylls each grouped into separate clades, except for *Zosterophyllum*, which was also a member of the basal polytomy of the zosterophyll and lycopsid clade.

The most in-depth study of zosterophyll phylogeny to date was undertaken by Kenrick and Crane (1997). This study set out to determine the relationship of zosterophylls to the lycopsids, like Gensel's (1992) study, but it also addressed the relationships within the group. Kenrick and Crane recovered all the zosterophylls and other lycophytes (except for *Hicklingia*) as a large polytomy. Within this polytomy, a Zosterophyllopsida clade included *Zosterophyllum divaricatum* as sister to a Sawdoniales clade and featured unresolved relationships between two smaller clades (Sawdoniaceae and Barinophytaceae), *Thrinkophyton*, *Hsua*, and a few genera circumscribed as Gosslingiaceae. A few traditional zosterophylls excluded from the Zosterophyllopsida (including several species of *Zosterophyllum*) were part of the basal polytomy of Lycophytina excluding *Hicklingia*, while *Nothia* and *Zosterophyllum deciduum* were recovered as more closely related to the lycopsids than to other traditional zosterophylls. More recently, focusing specifically on the plants of the Lochkovian Posongchong flora of Yunnan (China), Hao and Xue (2013) found zosterophylls forming a monophyletic group that is sister to a clade within which relationships between early lycopsids, barinophytes, and euphyllophytes are not fully resolved. *Renalia* was recovered as the sister group of the large zosterophylls + lycopsids + barinophytes + euphyllophytes clade.

In all of these taxonomic schemes, whether phylogenetically supported or not, a few trends arise. Most of the Zosterophyllopsida fall within two major groups—one that contains *Gosslingia* and other similar plants with bilateral symmetry in their reproductive structures (Niklas and Banks 1990; Kenrick and Crane 1997) and another including plants with radially symmetrical reproductive structures similar to those of *Zosterophyllum myretonianum* (Niklas and Banks 1990). However, the membership of these groups is not identical between the studies, and there is no consensus on this potential classification scheme. For instance, whereas Hao and Xue (2013) recovered *Gosslingia* and *Sawdonia*, two zosterophylls from the bilaterally symmetrical group, as a clade, the analysis of Kenrick and Crane (1997) placed bilaterally symmetrical zosterophylls in two separate families (*Gosslingia* in the Gosslingiaceae and *Sawdonia* and *Serrulacaulis* in the Sawdoniaceae). Furthermore, the hypothesis of Niklas and Banks (1990) that lycopsids arose from zosterophylls with bilaterally symmetrical, nonterminate reproductive axes (i.e., with indeterminate growth) does not find support in these studies, some of which recover no resolution for the relationships between zosterophylls and lycopsids (Gensel 1992; Kenrick and Crane 1997). What these studies taken together demonstrate is that zosterophyll relationships are far from well understood.

Regardless of the questions posed in these studies, previous phylogenetic assessment has relied heavily on morphology, whereas anatomy was sampled very sparingly. Character matrices in these studies include 12–32 characters, of which only three to seven characters code for anatomical features, despite a substantial body of existing data on zosterophyll anatomy (e.g., Banks and Davis 1969; Edwards 1969*a*, 1969*b*; Zdebska 1982; Rayner 1983; Kenrick and Edwards 1988*a*, 1988*b*; Lyon and Edwards 1991; Powell et al. 1999). Far from being the fault of these studies' authors, the sparse sampling of anatomy rather reflected their broader taxonomic focus, wherein many of the crucial taxa were known exclusively from compressions that do not preserve anatomy. Nevertheless, anatomy is just as integral to a species' identity as any of its other features, so it is important that it be included in the characters of broader-evidence phylogenetic analyses. This is even more important for early tracheophytes with plesiomorphic organography, such as the zosterophylls, which exhibit relatively little of the complexity of external morphology that is important for character construction. Additionally, a good number of zosterophyll species are preserved as permineralizations and have relatively well-documented anatomy.

Here, we undertake the most character-rich assessment of zosterophyll phylogeny to date, using a matrix of 41 characters, of which 30 are applicable to permineralized specimens; 12 of those 30 are strictly anatomical. We use parsimony-based phylogenetic analyses to (1) explore phylogenetic relationships among zosterophylls with a more richly sampled data set, (2) look at the influence of tree rooting and taxon sampling and of morphological versus anatomical characters on the stability of relationships, and (3) examine how phylogenetic and phenetic methods compare in the taxonomic relationships they support. Our phylogenetic analyses find support for three major clades that do not align exactly with those recovered in previous studies and for the previously proposed origin of lycopsids in a zosterophyll clade with nonterminate reproductive morphology (Niklas and Banks 1990). Taxon sampling, tree rooting, and character sampling (morphology vs. anatomy) significantly impact the resolution of the relationships recovered. Taxon sampling also heavily impacts the results of the clustering approach to phenetic analysis, and taxonomic groups supported by phenetic versus phylogenetic analyses show little overlap.

Material and Methods

Taxon Selection

From 16 genera, we compiled a matrix of 18 zosterophyll species: *Crenaticaulis verruculosus* Banks and Davis (1969); *Deheubarthia splendens* Edwards et al. (1989); *Discalis longistipa* Hao (1989); *Euthursophyton hamperbachense* Mustafa (1978); *Gosslingia breconensis* Heard (1927), Edwards (1970), Kenrick and Edwards (1988*a*); *Huia gracilis* Geng (1985), Wang and Hao (2001); *Konioria andrychoviensis* Zdebska (1982); *Margophyton goldschmidtii* Zakharova (1981); *Nothia aphylla* Høeg (1967), El-Saadawy and Lacey (1979), Kerp et al. (2001), Kerp (2017); *Sawdonia* (*Ensivalia*) *deblondii* Gerrienne (1996), Gensel and Berry (2016); *Sawdonia ornata* Hueber (1971), Rayner (1983), Gensel and Berry (2016); *Serrulacaulis furcatus* Hueber and Banks (1979), Berry and Edwards (1994), Berry and Gensel (2019); *Stolbergia spiralis* Fairon (1967); *Thrinkophyton formosum* Kenrick and Edwards (1988*b*); *Trichopherophyton teuchansii* Lyon and Edwards (1991); *Ventarura lyonii* Powell et al. (1999); *Zosterophyllum fertile* Leclercq (1942), Edwards (1969*b*); and *Zosterophyllum llanoveranum* Croft and Lang (1942), Edwards (1969*a*). *Zosterophyllum* and *Sawdonia* are each represented by two species (app. 1). These taxa were chosen to maximize the sampling of zosterophyll anatomy to assess the influence of anatomy and morphology on phylogenetic resolution and patterns of relationships.

Tree searches were rooted with *Psilophyton dawsonii* (Banks et al. 1975) or *Renalia hueberi* (Gensel 1976). *Psilophyton dawsonii* is one of the best characterized of the trimerophytes, a group thought to form a grade from among which crown group euphyllophytes evolved. As such, *P. dawsonii* is part of the clade sister to the lycophytes, which makes it one of the most closely related taxa that could be employed as an outgroup. *Renalia hueberi* is a vascular plant of uncertain taxonomic affinity (Gensel 1976, 1992; Kenrick and Crane 1997). Because *Renalia* combines zosterophyll and rhyniopsid features (Gensel 1992) and because rhyniopsids (or at least some of them) form a grade basal to the lycophyte-euphyllophyte divergence (Kenrick and Crane 1997), *Renalia* is another potential candidate for an outgroup. A representative of crown group lycopsids, *Sengelia radicans* (Matsunaga and Tomescu 2017), was included in analyses for methodological completeness, as at least some zosterophylls are generally regarded as paraphyletic with respect to a lycopsid clade (Kenrick and Crane 1997).

Characters

We constructed 40 characters (app. 2) that code for vegetative morphology and anatomy (26 characters) and sporangial morphology and arrangement (14 characters). Of the vegetative characters, 12 code for anatomical features—more than in any other previous analysis of zosterophylls. In constructing the characters (app. 2), we took special care to avoid (1) implicit hypotheses of homology (other than that hypothesized among states of the same character) and (2) constructs of the "tail color problem" type (Hawkins et al. 1997).

The characters were scored for each taxon based on the published literature, and the data were recorded using Mesquite (Maddison and Maddison 2021; apps. 3–5). A significant hurdle in integrating plant fossils in phylogenetic analyses arises from the finite number of specimens representing a fossil species. Compared with extant plants, which typically have large populations of whole individuals that allow for an in-depth understanding of the range of variability for each morphological character, many fossil species are represented by few to very few (and sometimes single) specimens, which are themselves limited in size and most frequently represent only fragments of the whole plant. As a result, for fossil species we are less likely to know the full range of variation of each character, and the absence of a feature sometimes cannot be known with full certainty, especially if the feature does not occur in abundance or it can be observed only in large, complete specimens (e.g., features of rhizomatous parts of a species cannot be observed if the only specimens available for that species are aerial axes). Thus, the character scoring of fossils may sometimes represent more of an informed guess than a certainty.

Another major hurdle in integrating plant fossils in phylogenetic analyses is raised by their modes of preservation, which provide different and only partially overlapping subsets of the total characters that could be defined in the living plant. As a result, when different taxa included in a matrix are preserved in different modes, this increases the proportion of missing data in the data set. Specifically, plant fossils preserved by permineralization and those preserved as adpressions (compressions and impressions) provide sets of characters with relatively little overlap. While adpressions can reveal many aspects of plant (external) macromorphology but preserve little (if any) of the internal anatomy, permineralizations can preserve a wealth of detail about internal anatomy but make details of plant macromorphology more difficult to document. Because we aimed to emphasize the contribution of anatomical characters to phylogenetic inference for zosterophylls, the taxa included in our analyses eliminate the hurdle of preservation modes to a great extent—they all preserve anatomy and at least some morphology. For the same reasons, our taxon sampling also allows us to compare the phylogenetic signal borne by the subsets of characters preserved in permineralized versus adpression fossils. Thus, in addition to tree searches using the full list of characters, we subsampled the list of characters in separate analyses to compare the hypotheses of relationships recovered from analyses that utilize subsets of characters that can be scored only in compression fossils (hereafter, morphological characters) and of those using characters that can be scored only in permineralizations (hereafter, anatomical characters). The "morphology matrix" included 26 characters and the "anatomy matrix" 30 characters of the total of 40 (app. 2). The two subsets partially overlap, as some of the characters can be scored—at least conceivably, if not in every case—in both permineralized and compression fossils (e.g., characters encoding sporangium geometry and taxis).

Phylogenetic Analyses

Phylogenetic searches were conducted in TNT (1.5; Goloboff and Catalano 2016) using equally weighted parsimony as the optimality criterion, and 10,000 trees were held for comparison. The parsimony analyses were initiated using the command ienum to find all the most parsimonious (MP) trees. Once the tree search was complete and all MP trees were found, a consensus tree was calculated using the command nelsen*. Majority-rule trees were calculated under a second tree search algorithm using the commands xmult = hits 20 and bb to obtain the total number of MP trees. Once the tree search was complete, a majority-rule 50 tree was calculated using the command majority*.

Tree searches were conducted under different taxon sampling regimes (for both ingroup and outgroup taxa) and character sampling regimes (table 1). As part of the taxon sampling experiments, we excluded one or both of the two taxa with the highest percentage of missing data (*Stolbergia*, 54% missing data; *Euthursophyton*, 44% missing data). From among all these analyses, all of which used the full set of characters, we selected the two taxon samplings that yielded the best-resolved topologies to perform the tree searches under the different character sampling regimes: morphological characters versus anatomical characters. Trees were imaged in FigTree (ver. 1.4.4; Rambaut 2018).

Phenetic Analyses

Numerical taxonomy has had its heyday (Sneath and Sokal 1973; Sneath 1995), but its usefulness as a tool supporting taxonomic decisions has also been strongly criticized (e.g., de Queiroz and Good 1997), and with the development of computerized cladistics methods, the world of taxonomy has moved to phylogenetic systematics. Indeed, by their very nature, clustering methods impose hierarchies even where they do not exist (Hammer and Harper 2006), and both clustering and ordination base the groupings recovered on overall similarity and thus cannot rule out the contribution of individual characters, as phylogenetic methods can. Nevertheless, cluster analysis can be useful, at the very least, as a method for data exploration, and ordination methods can often be even more instructive (Hammer and Harper 2006). Indeed, they are still used, albeit rarely, to address questions of taxonomy or taxonomic identification among both fossil and extant plants (e.g., Khan et al. 2019; Zhang et al. 2021). While we do not advocate a return to numerical taxonomy, we have chosen to explore the results of phenetic analyses and compare them with those obtained using phylogenetic methods in an all-methods approach because of the general recalcitrance—perceived to be a result of previous analyses—of zosterophyll relationships (phylogenetic or otherwise) and taxonomy.

Traditionally, numerical taxonomy studies have employed both clustering and ordination (Sneath and Sokal 1973), which

Table 1

Taxon Sampling, Rooting, and Character Sampling in the Different Analyses Performed

Analysis	Taxon sampling (excluded taxa)	Rooting	Character sampling
RPS	. . .	*Renalia*	All
PRS	. . .	*Psilophyton*	All
PS	*Renalia*	*Psilophyton*	All
RS	*Psilophyton*	*Renalia*	All
RS-S	*Psilophyton*, *Stolbergia*	*Renalia*	All
RPS-S	*Stolbergia*	*Renalia*	All
PS-S	*Renalia*, *Stolbergia*	*Psilophyton*	All
PRS-S	*Stolbergia*	*Psilophyton*	All
RS-SE	*Psilophyton*, *Stolbergia*, *Euthursophyton*	*Renalia*	All
RPS-SE	*Stolbergia*, *Euthursophyton*	*Renalia*	All
PS-SE	*Renalia, Stolbergia, Euthursophyton*	*Psilophyton*	All
PRS-SE	*Stolbergia*, *Euthursophyton*	*Psilophyton*	All
RS-SE comp	*Psilophyton*, *Stolbergia*, *Euthursophyton*	*Renalia*	Morphology only
RS-SE perm	*Psilophyton*, *Stolbergia*, *Euthursophyton*	*Renalia*	Anatomy only
PRS comp	*Stolbergia*, *Euthursophyton*	*Psilophyton*	Morphology only
PRS-SE comp	*Stolbergia*, *Euthursophyton*	*Psilophyton*	Morphology only
PRS-SE perm	*Stolbergia*, *Euthursophyton*	*Psilophyton*	Anatomy only

Note. Abbreviations in column 1 are representative of taxon and character sampling outlined in columns 2–4. Letters before the hyphen indicate which nonzosterophyll or questionable taxa are included in that particular analysis (P = *Psilophyton*; R = *Renalia*; S = *Sengelia*; see col. 2). The letter in the first position indicates which taxon the tree is rooted with (P = *Psilophyton*; R = *Renalia*; see col. 3). Letters following the hyphen indicate definitive zosterophyll taxa excluded from that particular analysis (E = *Euthursophyton*; S = *Stolbergia*; see col. 2). "Comp" indicates that characters that can be gleaned only from compression fossils ("morphology"; see col. 4) were utilized, and "perm" indicates that characters that can be gleaned from permineralizations ("anatomy"; see col. 4) were utilized.

is why we have employed both of these methods of assessment. For the clustering analyses, we modified the 40 characters used in the phylogenetic analyses to render their scoring binary (apps. 6, 7). Some of the phylogenetic characters had to be split into two or more binary characters, and this resulted in 57 characters. We used the unweighted pair group method with arithmetic mean (UPGMA; Sokal and Michener 1958), computed using PAST (3.24; Hammer et al. 2001). UPGMA is a frequently used clustering method that, in this case, computes the average similarity of one included taxon to others when all are weighted equally (Sneath and Sokal 1973). In the UPGMA analyses, we used Jaccard's difference coefficient because it takes into account only positive co-occurrences that carry a taxonomic signal, whereas the absence of characters has no direct taxonomic implications (Sneath and Sokal 1973; Aldenderfer and Blashfield 1984). UPGMA clustering was performed for only two taxon samplings—ingroup taxa plus *Renalia*, either including or excluding both *Stolbergia* and *Euthursophyton*—to test for the sensitivity of the results to taxon sampling.

For the ordination analysis, we recoded the binary characters used in the clustering analyses (app. 7) to replace all "?" with "0" because "?" cannot be handled by the method used—nonmetric multidimensional scaling (NMDS). However, this did not bias the results since Jaccard's difference coefficient takes into account only positive co-occurrences. Using this matrix, we performed the NMDS analysis using PC-ORD (McCune and Grace 2002). We chose NMDS because of its effectiveness in assessing nonnormal data sets (McCune and Grace 2002). Specifically, we used the "slow and thorough" procedure to select the optimum dimensionality for the ordination. This selection process included 250 runs with original data and 250 Monte Carlo randomized runs.

We excluded *Psilophyton* and *Sengelia* from both the clustering and the ordination analyses because we did not address their similarity to zosterophylls and because phenetic analyses do not require the character polarization that is necessary for phylogenetic analyses. We included *Renalia* in the phenetic analyses to test the hypothesis of its zosterophyll affinities in a nonphylogenetic framework.

Results

Phylogeny

Taxon sampling. The strict consensus trees of analyses that include all ingroup taxa show no resolution, regardless of rooting (table 2). Nevertheless, two major clades are supported in the majority-rule trees from the same analyses (e.g., fig. 1*A*). One of these clades (hereafter, large zosterophyll clade) includes *Sengelia*, *Ventarura*, *Discalis*, and *Trichopherophyton*, while the other contains the majority of the ingroup taxa (*Deheubarthia*, both species of *Sawdonia*, *Euthursophyton*, *Gosslingia*, *Serrulacaulis*, *Thrinkophyton*, *Konioria*, and *Margophyton*).

The exclusion of taxa with the highest percentages of missing data brings resolution to the strict consensus trees. Exclusion of *Stolbergia* (highest percentage of missing data, 54%) produces a strict consensus tree (table 2; RS-S analysis; tree not shown) in which a clade consisting of *Huia* and *Nothia* is sister to a large polytomy that contains the rest of the ingroup taxa and within which the only resolved clade contains *Trichopherophyton*, *Ventarura*, and *Sengelia*. Subsequent exclusion of *Euthursophyton* (second-highest amount of missing data, 44%) leads to a marked increase in strict consensus tree resolution compared with the previous analyses. Generally, the strict consensus trees (fig. 1*B*–1*D*) include the same two major clades that were supported in the majority-rule trees of the analyses that included all the ingroup taxa. The only exception is the addition of *Discalis* to the clade that includes the lycopsid *Sengelia*.

Table 2

Results of the Different Analyses

Analysis	No. MP trees	Length of MP trees	No. nodes resolved in strict consensus tree	Figure
RPS	722	90	1	. . .
PRS	722	90	1	. . .
PS	91	88	1	. . .
RS	672	83	1	. . .
RS-S	223	83	5	. . .
RPS-S	173	90	1	. . .
PS-S	40	88	3	. . .
PRS-S	173	90	1	. . .
RS-SE	40	81	7	Figure 1*C*
RPS-SE	28	88	5	. . .
PS-SE	3	86	10	Figure 1*D*
PRS-SE	28	88	5	Figure 1*B*
RS-SE comp	9	64	8	. . .
RS-SE perm	44	58	6	. . .
PRS comp	90	67	7	. . .
PRS-SE comp	9	67	9	Figure 1*F*
PRS-SE perm	56	65	7	Figure 1*E*

Note. Analysis abbreviations are as in table 1. MP = most parsimonious.

Resolution and the position of some specific taxa vary slightly depending on rooting and taxon sampling (cf. fig. 1*B*, 1*C*, and 1*D*). Despite the fact that *Psilophyton* and *Renalia* swap positions with one other only in the PRS-SE (fig. 1*B*) and RPS-SE (tree not shown) analyses, without any other effect on consensus topology, exclusion of either *Psilophyton* (fig. 1*C*) or *Renalia* (fig. 1*D*) leads to increased resolution of the strict consensus trees. The two major clades supported in other analyses (fig. 1*A*, 1*B*) are recovered consistently when either *Psilophyton* or *Renalia* is excluded. The *Huia* and *Nothia* clade supported in the RS-S analysis is resolved in the RS-SE analysis (fig. 1*C*), but in the other analyses, these two taxa are either in a paraphyletic group basal to the rest of the ingroup or part of a large polytomy. Additionally, the position of the two species of *Zosterophyllum* is unstable: either they are part of the basal polytomy of the clade that excludes *Huia* and *Nothia* (fig. 1*B*, 1*C*), or they are placed as sister to the clade that includes *Sengelia* (*Zosterophyllum fertile*) and sister to the larger clade (*Zosterophyllum llanoveranum*; fig. 1*D*).

Character sampling. The consensus trees of the PRS-SE sampling analyses that compared alternative character sampling regimes (fig. 1*E*, 1*F*) exhibit higher resolution than those obtained using the full set of characters, but they retain similar overall clade membership, with the exception of a few taxa. In the anatomy consensus tree (fig. 1*E*), the clade that includes *Sengelia* collapses: *Trichopherophyton* and *Discalis* (along with *Renalia*) are part of the basal polytomy of the ingroup clade that excludes *Huia*

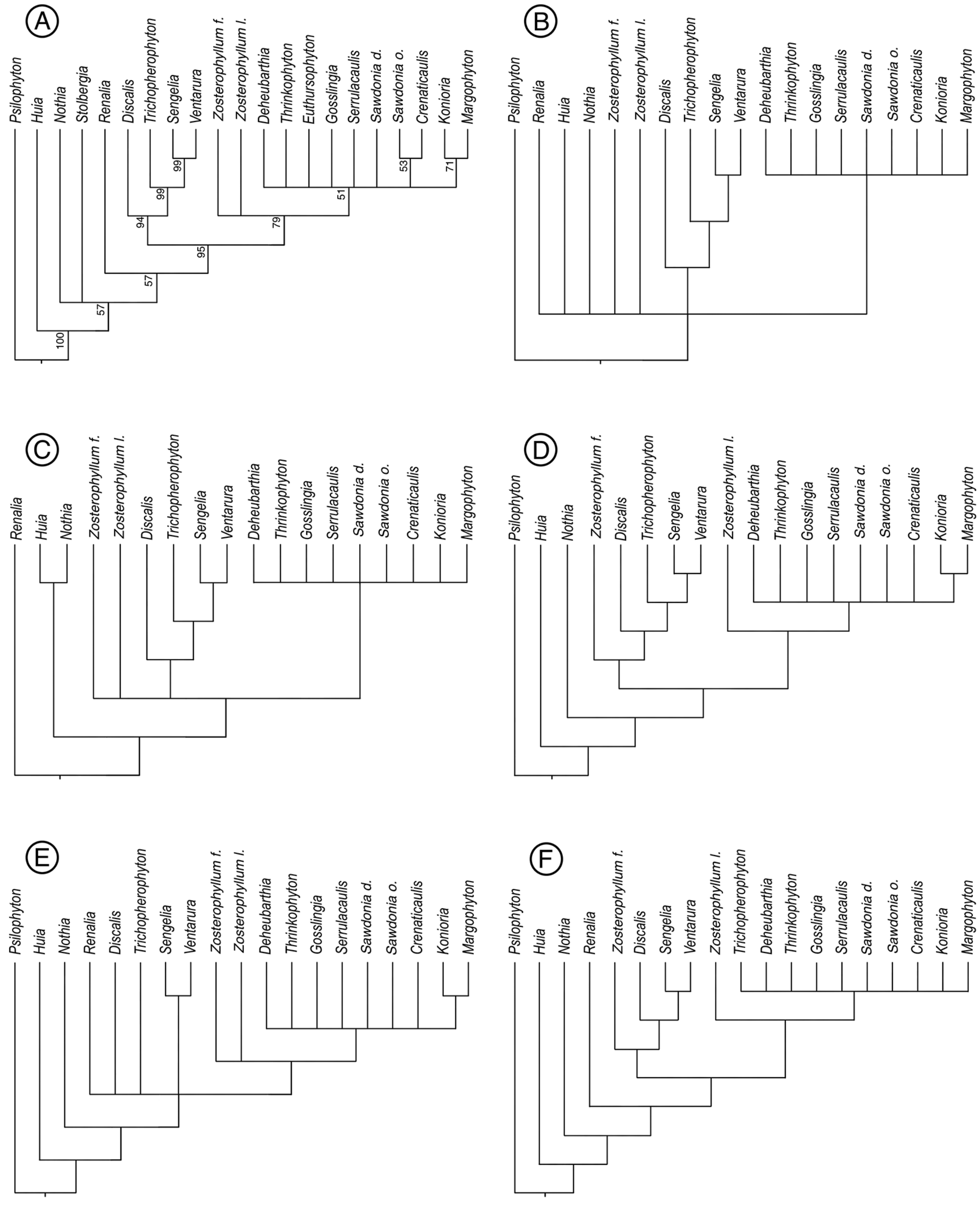

Fig. 1 Selected trees from phylogenetic analyses of zosterophylls. *A*, Fifty percent majority-rule consensus of 720 most parsimonious (MP) trees generated by the analysis, including all ingroup taxa and rooted with *Psilophyton*; numbers at the nodes indicate the percentage of MP trees in which the node is resolved. *B*, Strict consensus tree of 28 MP trees (length, 88) generated by the analysis rooted with *Psilophyton* and excluding *Stolbergia* and *Euthursophyton*. *C*, Strict consensus tree of 40 MP trees (length, 81) generated by the analysis rooted with *Renalia* and excluding *Stolbergia* and *Euthursophyton*. *D*, Strict consensus of three MP trees (length, 86) generated by the analysis rooted with *Psilophyton* and excluding *Stolbergia* and *Euthursophyton*. *E*, Strict consensus of 56 MP trees (length, 65) generated by the analysis rooted with *Psilophyton*, excluding *Stolbergia* and *Euthursophyton*, and using only characters that can be scored in permineralized fossils (“anatomy-only” analysis). *F*, Strict consensus of nine MP trees (length, 67) generated by the analysis rooted with *Psilophyton*, excluding *Stolbergia* and *Euthursophyton*, and using only characters that can be scored in compression fossils (“morphology-only” analysis). *Sawdonia d.* = *Sawdonia deblondii*; *Sawdonia o.* = *Sawdonia ornata*; *Zosterophyllum f.* = *Zosterophyllum fertile*; *Zosterophyllum l.* = *Zosterophyllum llanoveranum*.

and *Nothia*, and only *Ventarura* forms a clade with *Sengelia*. The two species of *Zosterophyllum* are recovered as part of a polytomy with the large clade that contains the remaining ingroup taxa. This clade is supported by the presence of a sclerified outer cortex (character 9). In the morphology consensus tree (fig. 1*F*), the *Discalis-Ventarura-Sengelia* clade is sister to *Z. fertile*, but *Trichopherophyton*, which is also associated with this clade in analyses that use the full set of characters, is recovered as a member of a different clade.

Synapomorphies. The clades recovered in the different analyses are supported by few strict synapomorphies (table 3), despite being consistently recovered in some cases (i.e., the *Trichopherophyton-Ventarura-Sengelia* clade and the large zosterophyll clade). This is due at least in part to the high amount of missing data but also speaks to the significant incidence of homoplasy in the data set. The consistently resolved clade that contains *Trichopherophyton*, *Ventarura*, and *Sengelia* is supported in all analyses by character 34—short sporangial stalk length. The position of *Discalis* as sister to this clade is supported by epidermal layer internal cellular differentiation (character 14) in the PRS-SE analysis (fig. 1*B*), the presence of K branching (character 24) in the PS-SE analysis (fig. 1*D*), and both character 14 and character 24 in the RS-SE analysis (fig. 1*C*). However, of the four taxa, only *Discalis* and *Sengelia* can be scored for these characters, whereas *Trichopherophyton* and *Ventarura* have missing data; thus, these are only putative synapomorphies for the clade. In the same way, in the morphology consensus tree (fig. 1*F*), *Z. fertile* is supported as sister to a clade that consists of *Discalis*, *Ventarura*, and *Sengelia* because it has a cortex with histologically distinct layers (stratified; character 8), but *Discalis* and *Sengelia* have missing data for this character, which is only a putative synapomorphy.

In different analyses, the large zosterophyll clade is supported by several traits that either present reversals within the clade or are putative synapomorphies (because of missing data). These are detailed in table 3 and include an elliptical cross-sectional stele shape (character 7), conical (spinelike) multicellular protrusions on the axes (character 16), subaxillary tubercles (character 25), circinate tips (character 26), sporangia arranged in an intercalary fertile zone (character 29), and short sporangial stalks (stalk length-to-width ratio ≤ 1; character 34). A putative synapomorphy associating the two species of *Zosterophyllum* (*Z. fertile* and *Z. llanoveranum*) with the large zosterophyll clade in the anatomy consensus tree (fig. 1*E*) is a sclerified outer cortex (character 9)—data are missing for *Sawdonia deblondii*, *Margophyton*, *Serrulacaulis*, and *Thrinkophyton*. Finally, in the RS-SE analysis (fig. 1*C*), *Huia* and *Nothia* form a clade characterized by adaxially curved sporangia (character 33).

Table 3

Synapomorphies of the Clades Recovered in Different Phylogenetic Analyses

Clade, synapomorphy	Recovered in analyses	Polymorphism	Reversal	Unknown/inapplicable
TVS:				
C34, 0	PRS-SE, RS-SE, PS-SE	. . .	. . .	. . .
Discalis and TVS:				
C14, 0	PRS-SE, RS-SE	. . .	. . .	*Trichopherophyton*, *Ventarura*
C24, 1	RS-SE, PS-SE	. . .	. . .	*Trichopherophyton*, *Ventarura*
Zosterophyllum fertile–Discalis–Ventarura–Sengelia:				
C8, 1	PRS-SE comp	. . .	. . .	*Discalis*, *Sengelia*
LZC:[a]				
C7, 1	PRS-SE, RS-SE, PS-SE	*Deheubarthia*, *Konioria*	*Sawdonia ornata*	*Serrulacaulis*
C16, 1	PRS-SE, RS-SE, PS-SE	. . .	*Gosslingia*, *Serrulacaulis*, *Thrinkophyton*	. . .
C25, 1	PRS-SE, RS-SE	. . .	*Konioria*, *S. ornata*, *Serrulacaulis*	. . .
C26, 1	PRS-SE, PRS-SE comp	. . .	*Margophyton*	. . .
C29, 0	PRS-SE, RS-SE, PS-SE	. . .	. . .	*Konioria*, *Margophyton*
C34, 1	PRS-SE, RS-SE, PS-SE	. . .	*Sawdonia deblondii*	. . .
Z. fertile–Zosterophyllum llanoveranum–LZC:				
C9, 1	PRS-SE perm	. . .	. . .	*S. deblondii*, *Margophyton*, *Serrulacaulis*, *Thrinkophyton*
Huia and *Nothia*:				
C33, 2	RS-SE	. . .	. . .	. . .

Note. Analysis abbreviations are as in table 1. C = character; TVS = *Trichopherophyton-Ventarura-Sengelia*; LZC = large zosterophyll clade.

[a] Includes *Deheubarthia*, *Thrinkophyton*, *Gosslingia*, *Serrulacaulis*, both species of *Sawdonia* (*S. ornata* and *S. deblondii*), *Crenaticaulis*, *Konioria*, and *Margophyton*.

Phenetics

UPGMA. The clustering analyses show sensitivity to taxon sampling—that is, the configurations of dendrograms differ dramatically between the analysis that included the taxa with the highest amount of missing data (*Stolbergia* and *Euthursophyton*; fig. 2*A*) and the analysis that excluded them (fig. 2*B*). Furthermore, the major clades seen in the phylogenetic analyses are not reflected in the similarity relationships conveyed by the dendrograms, apart from the grouping of *Huia* and *Nothia*, which has a high similarity score. Both analyses show high similarity scores between *Renalia* and the bulk of the zosterophyll taxa.

NMDS. NMDS ordination resulted in a three-dimensional configuration that described 76% of the variation in the data set, with a final stress of 7.18. The stress value, which is an estimate of how well the ordination summarizes the observed distances between groups, is relatively high, indicating that there is some risk of drawing false inferences. Axis 1 explains 41% of the variation in the data set, and axis 2 explains an additional 18%, for a cumulative 59% of variation explained using the two-dimensional solution. Ordination did not reveal very strong trends in the data set. However, unlike in the clustering analyses, ordination supports some of the taxonomic relationships implied by the results of the phylogenetic analyses. The bulk of the taxa present in the large clade that is recovered as a large polytomy in the phylogenetic analyses (except for *Konioria* and *Margophyton*) are closely associated in the NMDS ordination plot (fig. 2*C*). Additionally, we found a close association of *Renalia* to this group.

Discussion

Consistent Patterns of Relationships Indicate the Presence of a Phylogenetic Signal

The level of resolution and node support produced by our analyses is relatively low (tables 2, 4), even compared with those of other analyses addressing the deep phylogeny of early tracheophytes (Durieux et al. 2021; Toledo et al. 2021). Notably, the strict consensus trees of analyses including all the taxa lacked any resolution (table 2). This was driven primarily by the high amount of missing data (especially for some of the taxa included in the analyses), as demonstrated by the improved resolution of analyses that excluded the taxa with the highest amount of missing data (see discussion in Nixon 1996), but high levels of homoplasy due to the relatively simple morphology and anatomy of the plants (resulting from the incongruent distribution of the states of many characters among the taxa and reflected in the scarcity of strict synapomorphies identified; see above) probably also contributed to lowering the resolution. Nevertheless, the consensus trees of all analyses that did produce a better resolution (fig. 1) recovered the same overall patterns of relationships and broadly consistent clades (with a few notable exceptions discussed below), irrespective of tree rooting choice, taxon exclusion, and even character subsampling. Together, these demonstrate the presence of a consistent (albeit weak) phylogenetic signal among the characters. It is not surprising, therefore, that the same signal is recovered in the majority-rule consensus trees of the analyses that did not recover any resolution in the strict consensus. In turn, this confirms that in recalcitrant data sets, some phylogenetic signal

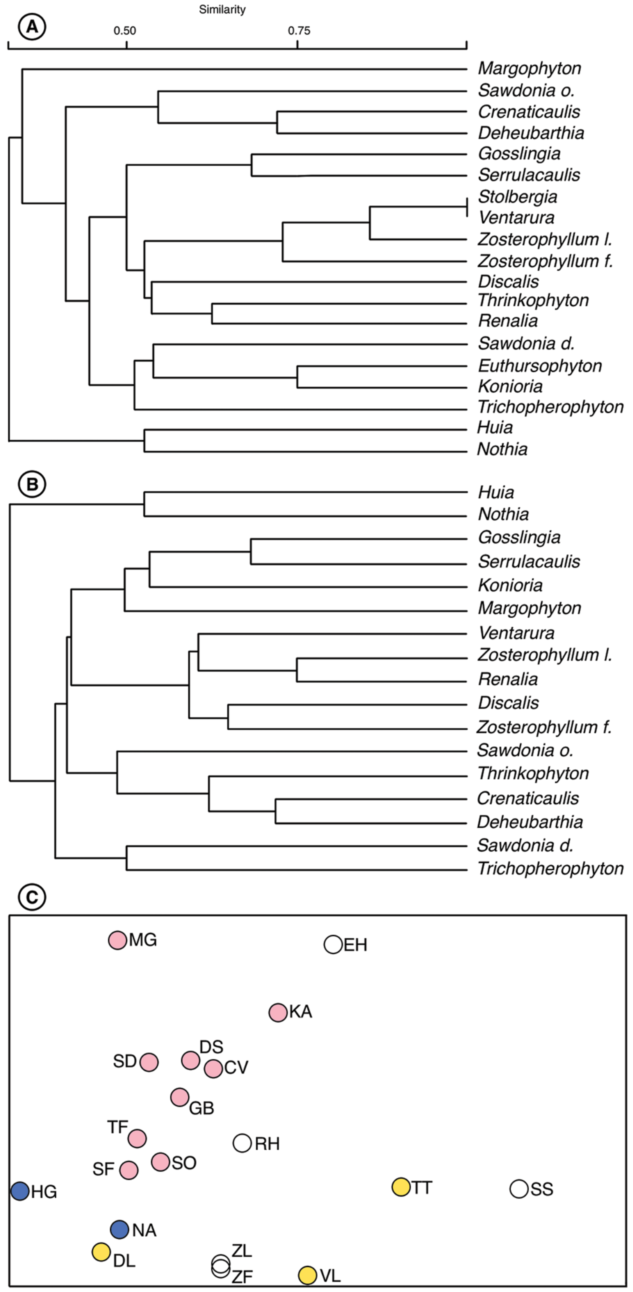

Fig. 2 Results of phenetic analyses using Jaccard's difference coefficient. *A*, Dendrogram generated by unweighted pair group method with arithmetic mean (UPGMA) analysis that excluded *Psilophyton* and *Sengelia*. *B*, Dendrogram generated by UPGMA analysis that excluded *Psilophyton* and *Sengelia*, as well as *Stolbergia* and *Euthursophyton*. *Sawdonia d.* = *Sawdonia deblondii*; *Sawdonia o.* = *Sawdonia ornata*; *Zosterophyllum f.* = *Zosterophyllum fertile*; *Zosterophyllum l.* = *Zosterophyllum llanoveranum*. *C*, Nonmetric multidimensional scaling ordination plot of the analysis that excluded *Psilophyton* and *Sengelia*. Blue circles indicate *Huia* and *Nothia*. Yellow circles indicate taxa of the clade that includes the lycopsid *Sengelia*. Pink circles indicate the large zosterophyll clade. White circles indicate taxa not recovered in consistent placements in the different phylogenetic analyses or excluded from some

can be gleaned from majority-rule consensus trees. In the same vein, Kenrick and Crane's (1997) results demonstrate that the exclusion of taxa to minimize the amount of missing data in analyses of the relationships of lycophytes and zosterophylls recovered overall patterns of relationships similar to those obtained with more missing data, but with a higher resolution.

Support for Two Major Zosterophyll Clades

In all the consensus trees, variations of two distinct clades are recovered as sister to one another (fig. 1*D*, 1*F*) or as members of the same polytomy (fig. 1*B*, 1*C*, 1*E*). The first clade, the large zosterophyll clade, is a polytomy that consists of the majority of the included taxa: *Deheubarthia*, *Thrinkophyton*, *Gosslingia*, *Serrulacaulis*, both species of *Sawdonia*, *Crenaticaulis*, *Konioria*, and *Margophyton*. Despite some differences in taxon sampling, this clade is similar to the clade designated as the Sawdoniales by Kenrick and Crane (1997) and fits the bilaterally patterned terminate fertile axis bauplan outlined by Niklas and Banks (1990). In our strict consensus trees, the large zosterophyll clade has little to no internal resolution. Some phylogenetic patterns are nevertheless apparent in majority-rule consensus trees of five analyses (corresponding to the strict consensus trees shown in fig. 1*B*–1*F*). All of these analyses recover a *Konioria-Margophyton* clade within the large zosterophyll clade in >50% of the strict consensus trees. In four of these analyses, >50% of the strict consensus trees recover a clade including *Crenaticaulis* and either both species of *Sawdonia* (forming a grade paraphyletic to *Crenaticaulis*) or just *Sawdonia ornata*. The morphology-only analysis excluding *Stolbergia* and *Euthursophyton* (strict consensus tree shown in fig. 1*F*) recovers a *Sawdonia* grade paraphyletic to *Trichopherophyton* in >50% of the strict consensus trees, with the clade formed by these three taxa sister to *Crenaticaulis*.

The other clade contains the lycopsid representative, *Sengelia*, as well as the zosterophyll *Ventarura* in all trees, but *Discalis* and *Trichopherophyton* are also joined to this group in several analyses (fig. 1*B*–1*D*, 1*F*); in some analyses *Zosterophyllum fertile* is recovered as sister to the *Sengelia-Ventarura-Trichopherophyton-Discalis* clade (fig. 1*D*, 1*F*). Previous analyses have not recovered this clade, but *Discalis* is placed in a polytomy that includes the lycopsids in Kenrick and Crane's (1997) results, and the clade also aligns with Niklas and Banks's (1990) hypothesis for the phylogenetic place of origin of lycopsids among a group that may have included *Discalis*.

We also note a consistent association of *Nothia* and *Huia*, which form either a clade sister to the clade including all other zosterophylls or a grade basal to that clade. *Nothia* and *Huia* differ notably, both anatomically and morphologically, from other zosterophyll taxa included in this study, as well as from one another. Both taxa have adaxially recurved sporangia, seen only in *S. ornata* among the other taxa. *Nothia* has axes with uneven surface relief (El-Saadawy and Lacey 1979), which is thought to be a natural feature of the plant and not a taphonomic artifact and is not seen in other zosterophylls. *Huia* was originally described as having a centrarch pattern of xylem maturation, although that description states that protoxylem tracheids could not be isolated from the center of the vascular strand (Wang and Hao 2001). However, if the pattern of xylem maturation is indeed centrarch, it suggests that *Huia* combines characters of zosterophylls and rhyniophytes. Somewhat consistent with the placement of *Huia* and *Nothia* in our analyses, both of these taxa are placed outside the Zosterophyllopsida in Kenrick and Crane's (1997) study. There, *Nothia* is recovered as a member of the polytomic clade that also contains the lycopsids, whereas *Huia* is part of a polytomy that includes that clade.

Table 4

Support Values for the Trees Obtained in the Different Analyses

Analysis/tree	Consistency index	Retention index	Bootstrap average group support
RPS	.522	.574	7.4
PRS	.522	.574	6.8
PS	.534	.573	8.1
RS	.542	.578	7.4
RS-S	.542	.568	10.0
RPS-S	.522	.566	8.7
PS-S	.534	.564	10.7
PRS-S	.522	.566	9.0
RS-SE	.556	.576	10.5
RPS-SE	.534	.573	8.8
PS-SE	.547	.571	10.9
PRS-SE	.534	.573	9.4
RS-SE comp	.516	.587	11.7
RS-SE perm	.603	.614	14.9
PRS comp	.493	.600	6.4
PRS-SE comp	.493	.590	9.3
PRS-SE perm	.569	.606	12.1

Note. Analysis abbreviations are as in table 1.

Neither clade recovered in our study is reflected in Gensel's (1992) results. It is important to note, however, that while our analyses emphasized the relationships within zosterophylls, Gensel's focus was on the relationships between zosterophylls and other groups and, as such, coded for characters relevant to that question and included a taxon sampling different from this study's.

Banks (1968) outlined a proposal to classify zosterophylls (Zosterophyllales) into two major groups: the Zosterophyllaceae, which included *Zosterophyllum* and *Bucheria* (renamed *Rebuchia* by Hueber [1970]), and the Gosslingiaceae, which included *Gosslingia*, *Serrulacaulis*, "*Psilophyton* non-Dawson" (currently, *S. ornata* Hueber [1971]), and what he termed a "new genus of Lyon" (in reference to Lyon [1964], currently known as *Nothia aphylla*; Kerp et al. 2001). Banks (1975) later rescinded the classification after considering additional zosterophylls discovered subsequent to his 1968 classification (e.g., Edwards 1969*a*, 1969*b*, 1970). Like Banks's earlier treatment, our analyses also support two major groups among the zosterophylls, but these show no overlap with Banks's groups.

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of the analyses. CV = *Crenaticaulis verruculosus*; DL = *Discalis longistipa*; DS = *Deheubarthia splendens*; EH = *Euthursophyton hamperbachense*; GB = *Gosslingia breconensis*; HG = *Huia gracilis*; KA = *Konioria andrychoviensis*; MG = *Margophyton goldschmidtii*; NA = *Nothia aphylla*; RH = *Renalia hueberi*; SD = *Sawdonia deblondii*; SO = *Sawdonia ornata*; SF = *Serrulacaulis furcatus*; SS = *Stolbergia spiralis*; TF = *Thrinkophyton formosum*; TT = *Trichopherophyton teuchansii*; VL = *Ventarura lyonii*; ZF = *Zosterophyllum fertile*; ZL = *Zosterophyllum llanoveranum*.

Relationships within Zosterophyllum *and* Sawdonia

Zosterophyllum was the first-erected zosterophyll genus (Penhallow 1892) and is presently the most speciose. Although in our analyses we included only two *Zosterophyllum* species (*Z. fertile* and *Zosterophyllum llanoveranum*), they are found in two different patterns of placement. In one, they are both part of the same polytomy, whether that is the polytomy that also includes the two major clades formed by most of the other zosterophylls (fig. 1*B*, 1*C*) or a polytomy with the large zosterophyll clade (fig. 1*E*). In the other, each of the two major clades recovered in our analysis is sister to one of the two *Zosterophyllum* species (fig. 1*D*, 1*F*). Both Kenrick and Crane (1997) and Hao and Xue (2013) included multiple species of *Zosterophyllum* that similarly were not resolved as a clade. In our character matrix, *Z. fertile* and *Z. llanoveranum* differ in only a few characters that relate to sporangial distribution and shape and also to histology, as *Z. llanoveranum* has a sclerified outer cortex that is absent in *Z. fertile*. The inconsistent and notably never monophyletic resolution of these two species of the same genus suggests that a reevaluation of *Zosterophyllum* may be needed.

The other genus represented by two species in this study is *Sawdonia*, typified by *S. ornata*. The second species, initially described as *Ensivalia deblondii* Gerrienne (1996), was reassigned by Gensel and Berry (2016) to the genus *Sawdonia* on the basis of its overall similarity to *S. ornata* and the demonstration of unequal sporangial valves, like those documented in *E. deblondii*, in the latter. Unlike that of the two *Zosterophyllum* species, the placement of both *Sawdonia* species is stable: they are consistently recovered as members of the same clade—the large zosterophyll clade. However, their finer taxonomic relationships to the other taxa of this clade are unknown, as the clade lacks internal resolution (with a few exceptions; fig. 1*D*, 1*E*). Interestingly, the majority of the consensus trees of several analyses support a paraphyletic grade formed by *S. ornata* and *S. deblondii*. The vegetative morphology of the two species is broadly similar, with the exception of the presence of trichomes (*S. deblondii* does not have them, and *S. ornata* does) and subaxillary tubercles (seen in *S. deblondii* but not in *S. ornata*); we scored trichomes as present in *S. ornata*, as suggested by the presence of rosettes of cells in the epidermis (Rayner 1983) reminiscent of those present at the trichome bases of most plants. However, *S. ornata* and *S. deblondii* differ in aspects of sporangial morphology (the sporangium shape is more flattened dorsiventrally in *S. deblondii*, and the sporangial stalk is longer relative to the size of the sporangium in *S. deblondii*) as well as in sporangial orientation (recurved in *S. ornata*) and the number of sporangium files. Thus, our results provide only circumstantial support for close relationships between the two *Sawdonia* species, possibly because our character construction did not emphasize their shared features enough.

Zosterophyll Affinities of Renalia

Renalia is an early vascular plant with an interesting combination of characters. On one hand, it has reniform, bivalvate sporangia (Gensel 1976) that are zosterophyll-like, but unlike in zosterophylls, the sporangia of *Renalia* are borne terminally on axes, like those of rhyniopsids, and have a seemingly paired arrangement. The taxonomic placement of *Renalia* has been hindered at least in part by the lack of detailed anatomical information—preserved as compressions, *Renalia* has yielded only some cellular patterns observed in cleared specimens and short strands of tracheids (Gensel 1976). In the original description of *Renalia*, Gensel (1976) compared it to cooksonioid rhyniophytes and to zosterophylls, opting for placement in the former but suggesting the possibility of an "intermediate" position between rhyniophytes and zosterophylls; Hueber (1992) later suggested that it had closer affinities to zosterophylls. Our study provides some support for the zosterophyll affinities of *Renalia*. Some of our phylogenetic analyses (fig. 1*E*, 1*F*) recovered it nested among the zosterophylls (as opposed to sister outside the clade formed by all the zosterophylls), and both the UPGMA and NMDS analyses (fig. 2) found relatively high levels of similarity between *Renalia* and other zosterophylls.

The Importance of "Total" Morphological Evidence Analyses

Under identical taxon sampling, neither the morphology-only (fig. 1*F*) nor the anatomy-only (fig. 1*E*) analyses returned topologies identical to the all-character tree, although general patterns of relationships were similar to those recovered by the all-character analyses in both cases. The large zosterophyll clade seen in the all-character trees was also recovered in both the anatomy and the morphology trees, except that this clade also includes *Trichopherophyton* in the morphology tree (fig. 1*F*). The other main clade we recovered in most of the all-character analyses is represented only by *Sengelia* and *Ventarura* in the anatomy tree (fig. 1*E*), but the morphology tree recovers a clade more consistent with the all-character trees, including *Sengelia*, *Ventarura*, *Discalis*, and *Z. fertile* (fig. 1*F*).

We additionally see a marked difference in the amount of resolution of the morphology and anatomy trees. In the anatomy-only tree (fig. 1*E*), much of the resolution between groups is lost, a situation not present in the morphology-only tree. This is somewhat to be expected—the anatomy seen in these plants is highly homoplastic and on its own will not produce results that reflect the resolution of those seen in more comprehensive data sets (fig. 1*C*, 1*D*); the resolution in the anatomy-only consensus tree is probably driven primarily by the inclusion of sporangium characters in the anatomy data set. On the other hand, while the morphology-only tree has the highest resolution of any of the trees in this study (fig. 1*F*), it recovers a topology that is not entirely consistent with the majority of the other trees we approximated. For one, the species of *Zosterophyllum* are recovered as sister to one of each of the major clades, a topology seen in only one of the other trees (fig. 1*D*). Additionally, the morphology-only analysis is the only one that recovers *Trichopherophyton*, which is most often placed as sister to *Ventarura* and *Sengelia*, as a member of the large zosterophyll clade.

A similar situation was reported by Niklas and Crepet (2020). In comparing the results obtained when using subsets of characters with those obtained under full character sampling, those authors showed that utilizing vegetative morphological characters resulted in trees with the highest resolution, consistent with our findings (table 2; fig. 1*F*). Niklas and Crepet (2020) also concluded that the reproductive and anatomical features of the early sporophytes were less useful than morphological features in resolving the phylogeny of ancient tracheophytes.

The relative rarity of permineralization fossils, which preserve anatomical features, compared with compression fossils, which preserve morphological features, has resulted in a slower accumulation of anatomical data for zosterophylls (and other plants). Additionally, it is uncommon for the same species to be found preserved both anatomically and morphologically. As a result, the small number of taxa that can be scored for characters that encompass both anatomy and morphology further discourages the use of anatomical data from those species known only from permineralizations. Zosterophylls are no exception to these gaps in the data, and many species are known either only from compressions or only from permineralizations. Alongside the relative scarcity of permineralized taxa, the homoplastic nature of the anatomical features of zosterophylls has placed the focus of phylogenetic study on more robust data sets extracted from compression fossils and thus on morphology

Independent of these considerations, anatomical characters stand to improve analyses by (1) creating more comprehensive and detailed representations of the plants in a phylogenetic matrix and (2) increasing the number of characters that can be employed to investigate relationships. Therefore, even though the morphology-only consensus tree (fig. 1*F*) has a resolution very similar to that of one of the all-character trees (fig. 1*D*), while the anatomy-only tree (fig. 1*E*) shows significantly lower resolution than either of the two, biological considerations support usage of the most inclusive character list available—that is, all characters that can be defined and scored. Conversely, the slight discrepancies between the morphology-only tree and the all-character tree indicate that even when well resolved, trees produced by analyses that undersample the character space may support inaccurate relationships.

Phenetic Results Do Not Convey the Same Taxonomic Signal as the Phylogenetic Relationships among Zosterophylls

This is not a surprising finding. Phenetic methods are not designed to recover phylogenetic relationships but to recover measures of similarity, whether as a hierarchy (clustering) or not (ordination). Nevertheless, the results of both phylogenetic and phenetic analyses can be (and have been) used to draw taxonomic inferences. Phylogenetic methods currently occupy a central place in extant plant systematics and taxonomy. By contrast, in paleobotany, phylogenetic methods have until now been significantly underutilized as means of exploring the taxonomic affinities of fossils. This situation was discussed recently by Durieux et al. (2021), who point out the prevalence of comparative taxonomy in many areas of paleobotany and provide some reasons for this situation, as well as justifications for the use of both approaches. Using measures of similarity to group taxa, phenetic methods can be regarded as quantitative approaches to comparative taxonomy and, thus, less prone to the "specialist bias" that could plague the traditional evolutionary systematics approaches (sensu Mishler 2009). However, because they distill differences or similarities between taxa into pairwise distances based on multiple characters, phenetic methods hide diagnostic characters, which are one of the main currencies that underlie the definition of taxonomic groups in systematics. For this and other reasons (e.g., they depict statistical patterns of similarity and not patterns of relationships due to inherited changes in distinct characters), phenetic methods have been largely abandoned, in their pure form, in systematics, or they have morphed into more nuanced approaches of limited and distinct utility (e.g., morphospace analyses). Nevertheless, the congruence of the results between phenetic and phylogenetic approaches has rarely been tested for a given data set, and no such test has been performed recently. For all these reasons, we decided to compare the results of these two types of approaches—phylogenetics and phenetics—for the zosterophylls, a group proved to be phylogenetically recalcitrant by previous analyses (Gensel 1992; Kenrick and Crane 1997).

UPGMA clustering is very sensitive to taxon sampling (cf. fig. 2*A* and 2*B*), and neither the clustering analysis nor the ordination reflects the results seen in the phylogenetic studies. One possible exception is the tight grouping in the ordination plot of some of the taxa (*Crenaticaulis*, *Serrulacaulis*, *S. ornata*, *S. deblondii*, *Thrinkophyton*, and *Gosslingia*; fig. 2*C*) that are part of the large zosterophyll clade. Furthermore, there was no marked consistency between the results of the clustering and ordination analyses.

These results confirm the lack of congruence in taxonomic signal between phenetic and phylogenetic methods, indicating that only the latter can be used in addressing taxonomic questions. It is interesting, nevertheless, that although they do not account for homology, both the clustering and the ordination analyses are congruent with the results of the phylogenetic analyses in providing some support for the zosterophyll affinities of *Renalia*: the latter is nested among zosterophylls at high levels of similarity in the UPGMA dendrograms, independent of taxon sampling, and it plots closest to members of the large zosterophylls clade (as opposed to distanced from all zosterophylls) in the NMDS ordination.

Conclusions

We explored the relationships of zosterophylls using a phylogenetic matrix that consists of 21 species selected primarily for preserved anatomy and scored for 40 characters that include the most extensive sampling of anatomical character space for the group to date. To characterize the strength of the phylogenetic signals in the data set, we performed alternative rooting, taxon inclusion-exclusion, and character subsampling experiments under parsimony constraints. The analyses recover a relatively low resolution but find consistent support for two clades. One of these clades consistently includes *Ventarura* and the lycopsid *Sengelia*, typically accompanied by *Trichopherophyton* and *Discalis*. The consistent association of *Sengelia* with these zosterophylls suggests that the zosterophyll ancestor of lycopsids had bilaterally symmetrical, nonterminate fertile axes, as suggested by Niklas and Banks (1990). The other main clade includes *Gosslingia*, *Crenaticaulis*, *Sawdonia*, *Deheubarthia*, *Serrulacaulis*, *Thrinkophyton*, *Konioria*, and *Margophyton*, with majority-rule consensus trees suggesting close relationships between *Margophyton* and *Konioria* and between *Sawdonia* (*ornata*) and *Crenaticaulis*. The two species of *Zosterophyllum* included in the analyses are not recovered as a clade under any rooting, taxon, or character sampling regime, which suggests that the genus (the most speciose among the zosterophylls) requires taxonomic reevaluation. Subsampling of the matrix for characters that can be scored only in compression fossils or only in permineralized fossils yields differences in the patterns of relationships that indicate that anatomical and morphological characters carry slightly divergent phylogenetic signals and confirm that the incomplete sampling of character space may recover spurious patterns of relationships. Phenetic analyses (ordination and clustering) performed for the same data set produced patterns of similarity largely incongruent with the patterns of phylogenetic relationships, indicating that numerical taxonomy approaches fail to discern taxonomic patterns generated by evolutionary history, recovered in phylogenetic hypotheses, and relevant to taxonomy as applied in phylogenetic systematics.

In addressing zosterophyll relationships, future phylogenetic studies could explore the placement of additional zosterophyll species that are less completely characterized and were not included in this study by adding them to this matrix one by one. This would minimize their wild card effects (e.g., Nixon 1996) while also showing the levels of support for several alternative placements (if applicable). Aside from clarifying the relationships of additional zosterophylls, such analyses could contribute to reevaluations of the phyletic status of speciose genera, especially *Zosterophyllum*, which this study has flagged as probably nonmonophyletic. The broadening of outgroup sampling by inclusion of rhyniopsid and euphyllophyte taxa may improve resolution by further polarizing characters and would allow for additional tree rooting experiments. These additions, along with the inclusion of other lycopsids, may bring a better resolution to the position of the zosterophylls among the tracheophytes. Finally, discoveries of additional fossils, ideally with preserved anatomy, and the definition of additional characters that may be revealed by these discoveries or by the development of new investigative methods will further illuminate the relationships and evolutionary history of the zosterophylls.

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