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Revisiting the phylogeny of the Royal Ferns (Osmundales) through the lens of dependence: incorporating character dependence questions family and subfamily concepts within Osmundales

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Abstract — The royal ferns (Osmundales) are a morphologically diverse group of leptosporangiate ferns whose fossil record dates back to the Permian. Despite there being numerous described permineralized species with exquisitely preserved characters, the phylogenetic relationships between extinct species remain contentious. Although several analytical approaches have been employed to infer well-resolved topologies – even methods that are arguably conceived to be better at dealing with data conflict and uncertainty, many taxa have not been assigned to specific taxonomic categories within the current classification scheme. Here, we revisit the phylogeny of Osmundales by reanalysing a dataset comprising an extensive taxon sampling of fossil Osmundalean rhizomes. The impact of both character dependence – the logical relationship between character statements – and character downweighting on the inferred topologies is also evaluated. Our analyses cast doubts on the monophyly of the families traditionally accepted: Osmundaceae is not recovered throughout the analytical spectrum while Guaireaceae is recovered only if character dependence is considered along with weighting against homoplasy. Only one subfamily, Itopsidemoideae, is consistently rendered monophyletic across our analyses. To recover the monophyly of the remaining three subfamilies as originally proposed, incorporating character dependence and/or weighting against homoplasy was necessary. Consistent with previous studies, multiple taxa were unstable, leaving their phylogenetic affinities unclear. Our analyses underscore the significance of accounting for both character dependence and weighting against homoplasy, especially when considering the contribution of missing data to observed homoplasy. Ultimately, these considerations yield markedly different topologies which imply contrasting classification schemes, highlighting the complexity inherent in resolving the evolutionary history of royal ferns.

Keywords: Character dependence, Ferns, Fossils, Osmundaceae

Introduction

The order Osmundales is a group of leptosporangiate ferns that are characterized by a rhizome with distinctive anatomy and sporangia with lateral annulus. This character differs from all other sporangial morphologies of leptosporangiate ferns (Gifford and Foster, 1989; Smith et al., 2006). Osmundales date back to the Late Carboniferous and have one of the most diverse fossil records known to date. This record comprises approximately 150 species that include foliar compressions, isolated spores, sporangia and mainly mineralized stems (Miller, 1971). However, the extant diversity of this order is much more limited, with around 20 species worldwide, classified into four genera: *Osmunda* L.*, Osmundastrum* Presl*, Leptopteris* Presl, and *Todea* Willd. Ex Bernth.

Despite its abundant fossil record, Osmundales systematics has been particularly challenging to address both from a taxonomic and a phylogenetic viewpoint. After Miller's (1971) extensive study, Osmundales accommodated 21 genera in two families (Guaireaceae and Osmundaceae; Tidwell and Ash, 1994). However, based on new material and interpretations of mineralized stem samples, different species have been added (e.g., Vera, 2010, 2012). Some recently described species have combinations of characters (mosaicism) that prevent them from being unambiguously associated with other taxa. For instance, *Osmunda pulchella* Bomfleur, G. Grimm & McLoughlin has diagnostic features of both *Osmunda* and *Osmundastrum* – which led Bomfleur et al. (2015) to reincorporate *Osmundastrum* within *Osmunda*.

The currently accepted classification scheme of Osmundales is mostly based on the studies by Wang et al. (2014) and Bomfleur et al. (2017). Wang et al. (2014) struggled to recover well-resolved consensus trees under parsimony; although they recovered Guaireaceae and Osmundaceae as monophyletic, relationships between most genera within Osmundales were equivocal. In their network analyses, Bomfleur et al. (2017) included most of the fossil rhizomes known to date and 14 extant taxa. Likewise, they conducted a thorough synthesis of the diagnostic characters; upon which the largest morphological matrix for the group was compiled. Bomfleur et al. (2017) proposed nomenclatural changes at the family and genus level based on new affinities between fossil taxa. Even though the studies by Wang et al. (2014) and Bomfleur et al. (2017) improved the classification scheme for the group, some factors not considered by the authors can considerably affect the inferred phylogenies.

Character dependence has been one of the main drawbacks related to morphology-based phylogenies, and different authors have attempted to tackle this issue in different manners (e.g., O'Keefe and Wagner, 2001; Covain et al., 2008). A classic example of dependence is (in)applicability, where a transformational character can be observed only if its presence is given by a neomorphic character (Maddison, 1993). Even though complete dependencies between characters (e.g., applicability) may be the common case in most matrices, more intricate dependencies can also be found. Serial homology, wherein characters likely share a common developmental mechanism but their co-variation is not perfect (see Goloboff and De Laet, 2023), is of particular concern for osmundalean rhizomes. Leaf trace shape at the cortex, for instance, depends on its previous stage closer to the stele (Bomfleur et al., 2017). While recent methodological advances allow considering such types of dependencies in phylogenetic analyses (De Laet, 2005, 2015; Goloboff et al., 2021; Goloboff and De Laet, 2023), character dependence remains unexplored in plant phylogenies.

In this work, we revisit the phylogeny of Osmundales by taking into account character dependence in Bomfleur et al.'s (2017) dataset of fossil rhizomes and exploring different weighting strategies. Our analyses question the monophyly of Osmundaceae – the family traditionally accepted for Osmundales, and reveal that Guaireaceae is highly sensitive to the analytical condition. Instead, the

monophyly of the subfamilies depended on the incorporation of dependencies, downweighting characters, or both. Furthermore, proposing diagnostic characters for the groups of reference is also affected by character dependencies. These results unequivocally demonstrate that character interactions carry significant systematic implications and highlight the importance of considering the logical consistency of characters when compiling morphological data matrices.

Materials and Methods

Dataset and character complexes

To assess the phylogenetic relationships of Osmundales in the context of character dependence, the most extensive morphological matrix compiled for the group was analysed (Bomfleur et al., 2017). Taxon sampling consisted of 132 terminals, 14 extant and 118 fossil rhizomes. The ingroup included 127 Osmundales that represented a complete sampling at the genus level – arranged in two families (Guaireaceae and Osmundaceae) and four subfamilies (Itopsidemoideae, Guaireoideae, Thamnospteroideae, and Osmundoideae). Four genera (Osmundacaulis sp., Shuichengella sp., Bathypteris sp., and Anomorrhoea sp.) were not assigned to a specific subfamily within Osmundaceae (Bomfleur et al., 2017). The outgroup was represented by five filicalean fossil species: *Grammatocaulis donponii*, *Grammatopteris freitasii*, *G. rigollotii*, *G. baldaufii*, and *Rastropteris pinquanensis*. Character sampling included 45 characters drawn from the internal anatomy of rhizomes that scored for traits related to the vascular system. A total of 1232 cells were missing entries (20.73% relative to the complete matrix). Characters were treated as non-additive.

Seven character complexes were defined to express different logical relationships between character statements. Three complexes (complexes 0, 2 and 4) represented 'static dependencies of inapplicability'; complexes 0 and 2 yielded three combined character states with up to three non-homoplastic steps whereas complex 4 consisted of 19 combined character states with up to six non-homoplastic steps. Two complexes (complexes 1 and 3) represented ´dynamic dependencies´; complex 1 comprised 108 combined character states with up to eight non-homoplastic steps and complex 3 involved 162 combined character states with eight non-homoplastic steps. Finally, two other complexes (5 and 6) represented more intricate relationships between characters. Complex 5, with 46 combined character states and six non-homoplastic steps, consisted of an inapplicability link between two characters and penalised transformations in four others. Complex 6 (23 combined states and six non-homoplastic steps) comprised two links of inapplicability and one penalised transformation link. A verbose description and justification of the complexes is provided in Appendix I (Supplementary Information).

Tree searches and pruning wildcards

Topologies were inferred under the parsimony criterion as implemented in TNT 1.6 (Goloboff and Morales, 2023). Tree searches were performed in parallel (with eight slaves) by subjecting 100 RAS trees to 15 cycles of Tree Drifting, Random Sectorial Searches, and 10 cycles of Tree Fusion (Goloboff, 1999). The most optimal topologies recovered were subsequently subjected to an additional round of TBR. Clade support values were estimated through Bremer Support after iteratively inferring topologies under equal weighting that were one extra step suboptimal. Tree searches were conducted by assuming that all characters had the same weight regardless of their homoplasy (i.e., equal weighting) and by downweighting characters according to their homoplasy under extended implied weighting (Goloboff, 1993, 2014). While the main systematic conclusions were derived from topologies recovered under equal weighting, different concavities under extended implied weighting were explored to assess sensitivity (see below). Characters were individually downweighted with different concavities and missing entries were assumed to contribute to half (0.5)

of the observed homoplasy (Goloboff, 2014). Under extended implied weighting, three concavities of reference were explored (k10, k15, and k20). Note that, under extended implied weighting, while independent characters are each given a different concavity, dependent characters are downweighted using the same concavity of reference. In summary, six groups of topologies were inferred: equal weighting and no dependencies ("ew-nodep"), equal weighting and dependencies included ("ew-dep"), extended implied weighting with concavity k10 and dependencies included ("k10-dep"), extended implied weighting with concavity k5 and dependencies included ("k15-dep"), and extended implied weighting with concavity k20 and dependencies included ("k20-dep"). A common -albeit not exclusive- phenomenon of palaeontological datasets is the instability of terminals (i.e., alternative optimal positions in different trees) that prevent well-resolved consensus trees from being estimated. To find and prune such unstable terminals, the most optimal topologies inferred in each analytical approach (i.e., equal weighting, implied weighting with k5, k10, k15 and k20) were subjected to IterPCR (Pol and Escapa, 2009).

Topological and taxonomic congruence

To assess the sensitivity of the data to the inclusion of character dependence and character downweighting, the topological congruence between the topologies in the group "ew-nodep" and the remaining trees ("ew-dep", "k5-20-dep"). In the context of this paper, "topological congruence" refers exclusively to the overall similarity between trees – thus is not considered a synonym for "taxonomic congruence" as in Wheeler (1995). Topological congruence was here estimated as the proportion of common nodes between the topologies under comparison, relative to the maximum possible resolution in the smaller topology (since trees under comparison can have different sizes due to the pruning of wildcards). To complement this metric, congruence was also assessed as the standardised Robinson-Foulds distance. Taxonomic congruence was estimated as the recovery frequency of the taxonomic groups proposed by Bomfleur et al. (2017). Because "ew-nodep" trees did not necessarily match the taxonomic classification of the group, recovery frequency was assessed for all the tree groups ("ew-nodep", "ew-dep", "k10-dep", "k15-dep" and "k20-dep").

Results

Phylogeny

Our analyses recovered Osmundales as monophyletic regardless of the incorporation of character dependencies (Fig. 1). However, the inferred relationships at the family level, and below, differed between analytical approaches. In omitting character dependencies, tree searches resulted in optimal trees of 327 steps. After pruning 45 wildcards, the strict consensus had a resolution of 0.9 (77 nodes relative to 85 possible non-redundant nodes); that is an improvement of 143.23% relative to the non-pruned strict consensus (resolution: 0.37, 48 nodes out of 130). In the pruned consensus, Guaireaceae and Osmundaceae (sensu Bomfleur et al., 2017) were not recovered as monophyletic (Fig. 1A). The former included four taxa assigned to Osmundaceae: *Osmundacaulis richmondii*, *O. tasmanensis*, *O. andrewii*, and *Shuichengella primitiva* (Fig. 1A). In this analysis, Guaireaceae (plus *Shuichengella*, *O. richmondii*, *O. tasmanensis*, and *O. andrewii*) are nested within Osmundaceae (Fig. 1A). The subfamily Thamnopteroideae was not monophyletic due to the exclusion of *Thamnopteris splendida* (which was sister to all members of Osmundales) and *Chasmatopteris principalis* (sister to Guaireaceae + Shuichengella + Osmundoideae), and the inclusion of *Bathypteris rhomboidea* (Fig. 1A). The subfamily Osmundoideae was monophyletic and included *Osmundacaulis janae*, which had unknown affinities within Osmundaceae (Bomfleur et al., 2017; Fig. 1A). At the genus level, only three taxa (*Palaeosmunda sp.*, *Plenasium sp.* and *Todea sp.*) – out of 11 – were recovered monophyletic (Fig. 1A).

The analysis that considered character dependencies yielded optimal trees of 332 steps that, upon excluding 52 wildcards, led to a strict consensus tree of 68 nodes (out of 78 non-redundant nodes, 0.86; Fig. 1B) – this is an improvement of 177.42% relative to the non-pruned strict consensus (42 out of 130 non-redundant nodes; 0.31). In this analysis, neither Osmundaceae nor Guaireaceae are found monophyletic (Fig. 1B). The "Clade I" is assignable to a monophyletic subfamily Itopsidemoideae (Guaireaceae), which is sister to all remaining members of Osmundales (Fig. 1B). The former includes a clade with representatives of the subfamily Guaireoideae (Guaireaceae) and the genus *Osmundacaulis* (hereafter "Clade III"), which is sister to *Lunea jonesii* + Osmundoideae (Fig. 1B). The clade including the subfamily Thamnopteroide (Osmundaceae) and *Bathypteris rhomboidea* ("Clade II") is recovered as sister to a clade comprised by representatives of the subfamily Guaireoideae (Guaireaceae) and the genus *Osmundacaulis* ("Clade III"; Fig. 1B). "Clade IV" includes *Lunea* and members of Osmundoideae (Fig. 1B); within the latter, none of subcategories previously proposed were recovered as monophyletic (Bomfleur et al., 2017). However, all members of the subtribe Todeinae were recovered in a clade except for *Millerocaulis tuhajkulensis* (Fig. 1B). Aside form four genera (*Guairea*, *Todea*, *Leptoperis*, and *Osmunda*), the monophyletic status of genera within Osmundales was not corroborated – either because they were not monophyletic or were pruned due to instability.

Sensitivity to character weighting: topological and taxonomic congruence

Evaluations of topological congruence across weighting schemes indicated that character dependence affects dramatically the inferred relationships (Table 2). In assessing topological congruence, relative to the trees in the group "ew-nondep", trees inferred with character dependencies had values RF values above 0.6 and shared less than 0.5 of the possible nodes (Table 2). The topologies inferred under the strongest concavity (k5) and using character dependencies maximised similarity with the reference topologies by sharing 0.45 nodes and having an RF distance of 0.66 (Table 2). In contrast, the most dissimilar topologies were those inferred under k10 shared 0.17 nodes and had an RF average distance of 0.86 to the target trees (Table 2).

Taxonomic congruence, relative to the groups proposed by Bomfleur et al. (2017), revealed that Osmundaceae were not recovered as monophyletic throughout the analyses (Fig. 2). The family Guaireaceae, however, was rendered monophyletic in three weighting schemes – a similar pattern was observed for the subfamily Guaireoideae (k5, k10 and k15; Fig. 2). Itopsidemoideae, the second family within Guaireaceae, was the most stable category by being retrieved monophyletic across the entire range of analytical conditions (Fig. 2). The remaining subfamilies, Osmundoideae and Thamnospteroideae (Osmundaceae), had contrasting patterns. On the one hand, Thamnospteroideae was rendered only under equal weighting and when dependencies were incorporated (Fig. 1B, Fig. 2). On the other hand, Osmundoideae was recovered monophyletic in two weighting conditions (k10 and k15). In three conditions, different species of Osmundacaulis – which have uncertain taxonomic affinities – are included within an otherwise monophyletic Osmundoideae: two species are included under ew-nondep and k20-dep while one species under ew-dep (Figs. 1, 2).

Synapomorphies

In general, fewer characters are reconstructed as synapomorphies when dependencies are considered – 72 nodes were supported by at least one synapomorphy when dependencies were ignored whilst 68 nodes were supported by synapomorphic characters upon including dependencies. Despite more nodes having synapomorphies when dependencies are ignored, more characters were reconstructed as synapomorphies for the clades of reference upon considering dependencies (Table 3). For instance, Clade I – assignable to the subfamily Itopsidemoideae (*sensu* Bomfleur et al., 2017) – was supported by five characters under "ew-dep" while only two characters ("*Stele leaf gaps*" and "*Stele metaxylem with interspersed parenchyma*") were synapomorphic under "ew-nondep" (Table 3). A single reference clade (Clade III) had the same number of synapomorphic characters, although

only one synapomorphy was common to both approaches ("*Cortex sclerenchymatous outer cortical layer*"; Table 3).

Discussion

In this study, we revisited the phylogeny of Osmundales – an iconic, fossil-rich group of ferns – and carried out the first phylogenetic analyses for plants that dealt with character dependencies. Our analyses are in disagreement with previous authors (Wang et al., 2014; Bomfleur et al., 2017) and cast doubts on the classification scheme proposed for the group. More specifically, our analyses indicated that, except for Osmundaceae and Itopsidemoideae, the monophyly of other groups depends on the inclusion of character dependence and the weighting regime. Overall, these results highlight the importance of considering character dependence and character weighting (while extrapolating the contribution of missing data to homoplasy) for conducting phylogenetic analyses of extinct land plants.

Phylogenetic affinities

Our phylogenetic analyses contradicted previous studies (Wang et al., 2014; Bomfleur et al., 2017) by rejecting the monophyly of most of the proposed taxonomic categories within Osmundales (Fig. 1). While Guaireaceae was recovered as monophyletic only upon considering character dependence and weighting characters against homoplasy (k5-15, Fig. 2), Osmundaceae was consistently rejected as a monophyletic group (Figs. 1, 2).

Except for two subfamilies (Itopsidemoideae and Thamnopteroideae; Fig. 1B), the topologies resulting from the analysis with character dependence and using equal weighting did not reflect the relationships amongst Osmundales as inferred by Bomfleur et al. (2017; Fig. 1B). However, because of both the exclusion of wildcards and inclusion of species with uncertain taxonomic affinities (i.e., *incertae sedis*), our analysis did recover clades that reflected some degree of similarity with previously proposed groups (Wang et al., 2014; Bomfleur et al., 2017). Even though our inferred phylogenies differed – and occasionally agreed – with previous analyses at different levels, we will limit our systematic discussion to four clades of reference (Table 1) and the genus *Osmundacaulis*.

Clade I. This clade comprised members of the subfamily Itopsidemoideae, a group originally included under the family Guaireaceae to accommodate the genera *Donwellaicaulis*, *Tiana* and *Itopsidema* (Bomfleur et al., 2017). Amongst the taxonomic groups proposed for Osmundales, this is the only category above the tribe level that is consistently recovered in our analyses (Figs. 1). Throughout our analyses, however, its placement varied from being sister to all the members of Osmundales ("ew-dep", Fig. 1B) to being closely related members of Guaireoideae in a less inclusive node within Osmundales (e.g., "ew-nondep", Fig. 1A; Fig. S1). Even though this clade is weakly supported – both in our analyses and previous ones (BS < 50; Bomfleur et al., 2017), Itopsidemoideae is recovered regardless of the analytical condition (Fig. 2). In raising this group, Bomfleur et al. (2017) proposed "absence of stele leaf gaps" and "stele with patches of parenchyma" as diagnostic characters, among others. In our topologies inferred without considering dependencies, these two characters are reconstructed as the sole synapomorphies for the subfamily (Table 3). Upon considering dependencies under equal weighting ("ew-dep"), five characters are synapomorphic – one of which is also synapomorphic in "ew-nondep" (Table 3). Amongst these, four have not been considered diagnostic for the subfamily according to Bomfleur et al. (2017).

Clade II. This clade includes species of the subfamily Thamnopteroideae, which is monophyletic under "ew-dep", and the genus *Bathypteris* (Fig. 1B). Thamnopterioideae was originally proposed to accommodate *Bathypteris*, *Chasmatopteris*, *Thamnopteris*, *Ieosipteris* and *Zalesskya* (Miller, 1971). In its original formulation, however, Thamnopteroideae has been recovered only under

specific analytical conditions (Wang et al., 2014; Bomfleur et al., 2017). Wang et al. (2014), for instance, did not recover Thamnopteroideae monophyletic; being either unresolved in their strict consensus or excluding *Bathypteris* in their semi-strict consensus. In contrast, Bomfleur et al. (2017) rendered the subfamily as either monophyletic ('holophyletic' in their network analysis) or paraphyletic. Throughout these previous studies, the genus *Bathypteris* has had uncertain affinities. While Wang et al. (2014) retrieved *Bathypteris* in a polytomy along with other members of the subfamily, Bomfleur et al. (2017) placed it within the Family Osmundaceae but without assignation at the subfamily level. Our "ew-dep" analysis, while not contradicting the concept of Thamnopteroideae as defined by Bomfleur et al. (2017), disagrees with the placement of *Bathypteris* within Osmundoideae (Bomfleur et al., 2017). Instead, our analysis recovers *Bathypteris* as a sister to Thamnopteroideae (Fig. 1B) – thus suggesting that the genus could well be incorporated into the subfamily. Such incorporation could be supported by three synapomorphies: "absence of stem core parenchyma", "continuous sclerenchyma abaxial lining in the leaf trace", and "absence of sclerenchyma scattered in the inner cortex of the petiole" (Table 3).

Clade III. This clade included four (out of five) species of the subfamily Guaireoideae – albeit not constituting a monophyletic group – as well as the genera *Osmundacaulis* and *Shuichengella* (Fig. 1B). Following Wang et al. (2014), the family Guaireaceae included seven genera: *Guairea*, *Lunea*, *Donwellicaulis*, *Itopsidema*, *Shuichengella*, *Zhongmingella*, and *Osmundacaulis*. Bomfleur et al. (2017) excluded *Shuichengella* from Guaireaceae and proposed to include *Zhongmingella, Guairea, and Lunea* within the subfamily Guaireoideae based on their perforated steles. In this regard, our analyses disagreed with both Wang et al. (2014) and Bomfleur et al. (2017) in three points. First, Guaireaceae and Guaireoideae – as defined by Bomfleur et al. (2017) – are not robustly supported as it is monophyletic only under three weighting schemes that include dependencies (Fig. 2). Second, under "ew-dep", *Lunea* is not related to Guaireaceae but to a clade composed by *Palaeosmunda* and other Osmundoideae taxa (Clade IV; Fig. 1B). Only upon ignoring dependencies is *Lunea* included within a clade that comprises Guaireaceae (sensu Bomfleur et al., 2017), *Osmundacaulis* and *Shuichengella* (i.e., the traditional genera of Guaireaceae; e.g., Wang et al., 2014; Fig. 1A). Third, throughout our analyses, *Shuichengella* is recovered in different positions that suggest a close affinity with Guaireaceae: it is related to Itopsidemoideae and other Guaireaceae in a polytomy if dependencies are ignored (Fig. 1A), is sister to *Zhongmingella* under "ew-dep" (Fig. 1B), sister to Itosidemoideae in a monophyletic Guaireaceae (k5, k20; Fig. 2; Fig. S1), or sister to Osmundacaulis in a clade related to Guaireaceae *sensu* Bomfleur et al. (2017) under k10 and k15 (Fig. 2; Fig. S1). As defined by Bomfleur et al. (2017), the subfamily Guaireoideae is diagnosed by having perforated stele and stipe bundles with recurved tips.

Clade IV. The clade comprises *Lunea* – a member of Guaireaceae (Wang et al., 2014; Bomfleur et al., 2017) – and the remaining members of Osmundales that constitute the subfamily Osmundoideae (Fig. 1B). The concept of Osmundoideae has varied throughout studies (Miller, 1967; Tidwell and Ash, 1994; Wang et al., 2014; Bomfleur et al., 2017), spanning between four and nine genera (Table 1). Bomfleur et al. (2017) avoided providing further groups below the subfamily rank for purely extinct clades. However, regarding lineages with both extant and fossil taxa, Bomfleur et al. (2017) raised the subtribes Todeinae (including *Todea* and *Leptopteris*) and Osmundinae (including *Claytosmunda*, *Osmundastrum*, *Osmunda*, and *Plenasium*). In our analyses, only the subtribe Todeinae was robustly recovered across analytical conditions (Fig. S1). Subtribe Osmundinae, however, was rendered polyphyletic with multiple unrelated clades interspersed between extinct clades of uncertain placement below the subfamily rank (Fig. 1B, Fig. S1). Under ew-dep, Clade IV was defined by three synapomorphies, which had not been mentioned as possible diagnostic features: two protoxylem strands at the central part of the cortex, free adaxial masses of sclerenchyma in leaf traces, and having interrupted or scattered sclerenchyma masses in trace concavities. As in the study by Bomfleur et al.

(2017), informal categories aimed at reflecting relationships within the genus level were not recovered as monophyletic groups (e.g., *Millerocaulis* "Aschicaulis" group). This outcome implies that categories within the subfamily Osmundoideae might not be reliable indicators of affinities within Osmundales and, instead, alternative groups may be needed.

Osmundacaulis. Another result of our analyses is that several genera are not monophyletic regardless of the inclusion of dependencies or character downweighting (e.g., *Millericaulis*, *Claytosmunda*; Fig. 1; Fig. S1). An interesting case is that of *Osmundacaulis*, which has been placed in different families (Miller, 1971; Tidwell and Ash, 1994; Wang et al., 2014; Bomfleur et al., 2017) depending on the study approach. Whereas parsimony-based phylogenetic analyses have recovered a sister relationship to *Zhongmingella* within Guaireaceae (Wang et al., 2014), classic studies and network analyses have placed *Osmundacaulis* within Osmundaceae (Miller, 1971; Tidwell and Ash, 1994; Bomfleur et al., 2017). Furthermore, after combining *O. estipularis* with *Leptoperis*, Bomfleur et al. (2017) retrieved a 'putatively holophyletic' *Osmundacaulis* (p. 63). Our analyses recovered *Osmundacaulis* (sensu Bomfleur et al., 2017) as non-monophyletic across analytical conditions (Fig. 1; Fig. S1). Under "ew-dep", and throughout weighting schemes, three species (*O. tehuelchensis, O. tasmanensis,* and *O. andrewii*) were nested within Clade III, which indicates a clear relationship with Guaireaoid taxa (Fig. 1B; Fig. S1). This relationship between Guaireaoid taxa and the three species of *Osmundacaulis* is defined by three characters: a thin sclerenchymatous cortical layer, two protoxylem strands in leaf traces upon departing from the stele, and concave leaf traces after departing from stele (Table 3). Also, note that *Osmunda* (= *Osmundacaulis*) *kidstonii* – which has been excluded in previous analyses due to their incompleteness (Bomfleur et al., 2017) – is retrieved within Clade IV as a sister to Todeinae, *Claytosmunda embreei* and *Osmundastrum indentata* (Fig. 1B). This outcome suggests that many putative monophyletic genera – which are mostly morphogenera – should be carefully reviewed to achieve a robust classification for the group.

Even though our results question taxonomic groups that have been traditionally accepted, it should be noted these have not been robustly recovered in former studies (Miller, 1971; Tidwell and Ash, 1994; Wang et al., 2014; Bomfleur et al., 2017). Phylogenetic studies focusing on extinct Osmundales have not recovered strongly supported clades (Wang et al., 2014; Bomfleur et al., 2017). As Bomfleur et al. (2017) pointed out, the present dataset has a high proportion of missing data (20.73%) and a low taxon:character ratio. Nevertheless, previous datasets with better ratio sampling and fewer missing entries also failed in recovering well-supported clades (e.g., Wang et al., 2014). This implies that, besides improving sampling, novel sources of evidence should be explored. Continuous characters, for instance, have been observed to improve analyses in other taxonomic groups (e.g., Flores et al., 2021). Such a data type, as well as different character systems, may contribute to improving support and reducing sensitivity.

Topological and taxonomic congruence, and (extended) implied weighting

In agreement with previous studies that applied dependencies to empirical data (e.g., Brazeau et al., 2019), our results indicate that ignoring dependencies or not downweighting characters leads to incongruent topologies and poor recovery of taxonomic groups (Table 2, Fig. 2). Even though subtle, inferring topologies under extended implied weighting improved the recovery of taxonomic groups – especially Osmundoideae, Guaireaceae and Guaireoideae (Fig. 2). Implied weighting has been observed to increase jackknife frequencies, enhance stability (i.e., the recovery of correct groups after adding data), and lower error (i.e., recovering incorrect groups; Goloboff et al., 2008). In that sense, the improvement observed in our analyses is not surprising. More interesting, however, is the fact that when dependencies are ignored and inferences are conducted under equal weighting, only one taxonomic group is found (Itposidemoideae; Fig. 2). To recover additional groups, searches needed to be run by either considering dependencies or weighting against homoplasy – or even both (Fig. 2; Fig. S1).

Finally, it is worth noting that the monophyly of the subfamily Osmundoideae – as defined by Bomfleur et al. (2017) – is recovered only when dependencies are considered and/or using extended implied weighting (k10-15; Fig. 2). In our analyses under "ew-nondep" and "k20-dep", *Osmundcaulis janae* is nested within the subfamily (Fig. 1A; Fig. S1). The genus *Osmundacaulis*, which is not monophyletic in our analyses, has uncertain affinities within the family Osmundaceae (Bomfleur et al., 2017). Our analyses support previous authors who had placed this genus within Osmundoideae (Miller, 1971; Tidwell and Ash, 1994); however, this only holds for certain species of Osmundacaulis (*O. janae*). Along with recovering a non-monophyletic *Osmundacaulis*, this result suggests possible nomenclatural emendations for this taxon.

Character dependence and group diagnoses

A methodological novelty of the present study is the use of character dependencies through step-matrix complexes (De Laet, 2005, 2015; Goloboff et al., 2021; Goloboff and De Laet, 2023), which is the first application of such a method to empirical analyses of land plants. As noted above, some of the characters subject to dependencies in our analyses are indicated by Bomfleur et al. (2017) as diagnoses of different groups. Due to forbidden, penalised or rewarded character-state combinations in dependence complexes, diagnoses for different taxonomic groups may differ from those inferred when dependencies are ignored.

Clade I in Fig. 3A, which corresponds to Itopsidemoideae, is reconstructed as having no cortical sclerenchyma layer (left tree) – being a synapomorphy of the group. Omitting dependence leads to the type of sclerenchyma to be inferred as "homogeneous" at Clade I (right tree) while it is inapplicable when dependencies are considered (middle tree). In Fig. 3B, the node that includes Clades II, III, and IV, is defined by having wings as stipular expansions (left tree). "*Sclerenchyma clusters*" are biologically *possible* when stipular expansions develop into wings. If dependencies are ignored, such sclerenchyma clusters are inferred as "absent" in all of the node's ancestors — as if stipular expansions were either wings or spines already in the root (right tree). In considering dependencies, "sclerenchyma clusters" changes from inapplicable to applicable (and "absent") only at the node where stipular expansions developed into wings (middle tree). This latter is thus a synapomorphy for the node in question.

These examples underline the impact of considering dependence in inferring synapomorphies. Even though there is no explicit mention of diagnoses as synapomorphies in the current classification of Osmundales (Bomfleur et al., 2017), synapomorphies could certainly serve as diagnostic characters. In this sense, taking into account dependencies between characters allows reconstructing biologically meaningful synapomorphies; this prevents impossible or unlikely character state combinations from being retrieved.

Discrepancies with Bomfleur et al's (2017) study

Regardless of the incorporation of dependencies and character weighting, our phylogenetic analyses are in stark contrast with the analyses conducted by Bomfleur et al. (2017) in terms of systematic outcomes. While some low-rank taxonomic groups were recovered in our analyses (e.g., Todeinae; Fig. 1), the concepts of families and subfamilies as proposed by Bomfleur et al. (2017) are not robustly supported. It is worth noting that our "ew-nondep" analysis was effectively performed upon analysing Bomfleur et al.'s (2017) dataset with no modifications. Yet, aside from Osmundales, only one group above the tribe level is recovered: subfamily Itopsidemoideae (Fig. 1A; Fig. 2). Because Bomfleur et al.'s (2017) study is the most comprehensive to date, and provides much of the basis for the current classification, we briefly comment on the possible causes creating conflict between our results and Bomfleur et al.'s.

Bomfleur et al. (2017) performed Hamming-distance-based network analyses instead of ("standard") phylogenetic analyses. As opposed to ("standard") phylogenetic analyses, networks are

assumed to be useful in the presence of 'conflicting signals' (i.e., homoplasy) and uncertainty. Morrison (2005) and Schliep *et al.* (2017) argued that, since reticulations are allowed, networks are appropriate for summarising conflicting hypotheses and enabling the discovery of sources of incongruence within datasets. However, it should be noted that Hamming-distance matrices are indeed affected by homoplasy: the higher the homoplasy, the higher the average Hamming distance between terminals. Also, while networks can visually summarise conflicting trees, they do not solve the problem of identifying sources of data conflict *per se* — both network and non-network analyses rely on inspecting data subsets to assess support for a given group (Baker and DeSalle, 1997; Gatesy et al., 1999; Schliep et al., 2017). The differences with Bomfleur et al. (2017), nonetheless, can be better attributed to the transformation of raw data into distance values.

Also, because distance matrices are affected by the number of missing entries, Bomfleur et al. (2017) filtered taxa that had over 60% missing data. In our analyses, instead of filtering *a priori*, unstable taxa that reduced the resolution of the strict consensus were pruned. It is accepted that increasing taxon sampling – even if slightly – can yield drastically different topologies (Gauthier et al., 1988; Rowe et al., 1989; Pol and Escapa, 2009). In contrast with filtering terminals *a priori*, pruning allows considering the phylogenetic information content of the terminals. Therefore, the different treatments of problematic taxa in both studies ultimately imply contrasting taxon samplings, which can cause much of the discrepancy observed relative to Bomfleur et al.´s (2017) results.

Final remarks

In this study, we have revisited the phylogeny of Osmundales while taking into account dependence between characters. Incorporating character dependence, which has been a major issue in phylogenetics, proved to have a strong impact on the inferred relationships and led to drastic differences with previous studies (Miller, 1967, 1971; Tidwell and Ash, 1994; Wang et al., 2014; Grimm et al., 2015; Bomfleur et al., 2017). The traditionally accepted Osmundaceae was not recovered in our analyses and Guaireaceae was monophyletic only under extended implied weighting (Figs. 1 and 2). Subfamilies, as defined by Bomfleur et al. (2017), were recovered only if dependencies were considered or characters were weighted against homoplasy (Fig. 2) – except for Itopsidemoideae which was robust throughout the analyses.

Our study applies two methodologies that have not been employed in previous evaluations of the phylogeny of Osmundales: weighting against homoplasy and character dependencies. The former allows missing data, which tend to be numerous in palaeontological matrices, to contribute to observed homoplasy (Goloboff, 2014). This approach has enhanced fossil stability in previous studies that combined different data types (e.g., Flores et al., 2021). Character dependence, aside from leading to novel topologies, has also impacted the inference of synapomorphic (or diagnostic) characters (Fig. 3). When considering dependencies, synapomorphic characters supported fewer nodes than inferences without dependencies (Table 3). While it could be interpreted that ignoring dependencies yields more robustly diagnosed classifications, this result suggests the opposite: more conservative estimations – which are biologically meaningful (as shown in Fig. 3) – are achieved through the incorporation of dependencies.

Osmundales is one of the groups whose fossil record has been most thoroughly studied (e.g., Arnold, 1952; Archangelsky and de la Sota, 1962; Miller, 1967, 1971; Ash, 1994; Cantrill, 1997; Tian et al., 2008). Yet, despite the numerous described fossils, previous authors have struggled to clarify the phylogenetic relationships within the group. Our study emphasises the need for a better sampling of characters – including new sources of characters – and exploration of novel methodological approaches. The royal ferns are a morphologically diverse group, and even subfamilies and genera include species that considerably differ from each other (Bomfleur et al., 2017). Therefore, it is likely

that many taxonomic categories under the order level are not monophyletic. In this regard, Bomfleur et al. (2017) raised taxonomic groups by also considering paraphyletic taxa. While the clades of reference discussed above could serve as taxonomic ranks, we have refrained from formally proposing them (or emending previous ones) given the low support for most clades. Improving the sampling of characters may result in clades with higher support, thereby refining the classification of the group.

Conflict of interest

None declared.

Data availability statement

The dataset and TNT scripts are freely available from https://github.com/JR-5888/Osmundaceae. Appendix S1 includes a description of character step-matrix complexes and Fig. S1.

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Supplementary Information

Appendix I. Relationships between characters in each step-matrix complex employed during the study.

Fig. S1. Topologies inferred under extended implied weighting and using character dependencies. From left to right: concavity 5, 10, 15, and 20. Subfamilies highlighted in each tree in colour: (light red) Thamnopteroideae, (dark red) Itopsidemoideae, (blue) Guairoideae, (green) Osmundoideae.

Table 1. Classification schemes as proposed by previous characters and as implied by the current study. * denotes that a taxon has been assigned to Osmundaceae but no subfamily according to Bomfleur et al. (2017). ** denotes that a taxon was found in a polytomy in the strict consensus of Wang et al. (2014).

Table 2. Topological congruence between phylogenies inferred under different analytical approaches. The Robinson-Foulds distance is normalised so that "0.0" minimises distance (i.e., maximises congruence) and "1.0" maximises distance (i.e., minimises congruence). The proportion of common nodes is estimated relative to the maximum number of possible nodes for the smallest tree in each pairwise comparison. Pairwise comparisons against the reference topologies (under equal weighting and no dependencies; "ew-nondep") entail topologies inferred with dependencies included under: equal weighting ("ew-dep"), extended implied weighting with concavity 5 ("k5-dep"), concavity 10 ("k10-dep"), concavity 15 ("k15-dep") and concavity 20 ("k20-dep").

Table 3. Clades of reference in the topologies under equal weighting and including or excluding character dependencies. The subfamily assignable to such clades and their synapomorphies are given, as well as the genera included. The symbol "-" denotes the exclusion of a terminal.

Fig. 1. Strict consensus of topologies inferred equal weighting after pruning the unstable terminals that reduce the resolution. (a) Strict consensus of topologies inferred without considering character dependencies. (b) Strict consensus of topologies inferred after including character dependencies as step-matrix complexes. The numbers below branches correspond to Bremer Support values. Roman numerals are clades of reference discussed throughout the paper. Taxonomic categories based on Bomfleur et al. (2017) are indicated in colour.

Fig. 2. Sensitivity plots for the families (Osmundaceae and Guaireaceae) and four subfamilies (Osmundoideae, Thamnopteroideae, Guaireoideae, and Itopsidemoideae). White boxes indicate non-monophyly, dark blue represents monophyly as defined by Bomfleur et al. (2017), and light blue indicates the addition of an *incertae sedis* taxon.

Fig. 3. Effect of ignoring character dependence in the reconstruction of characters in Osmundales. (a) Reconstruction of the sclerenchyma type, which is absent in Clade I (red clade) but inferred as 'homogeneous' if dependencies are ignored (right tree). (b) Reconstruction of stipular expansions as 'wings' at the node including Clades II-IV (red clade) and "sclerenchyma clusters", which are possible only when stipular expansions are 'wings'. If dependencies are ignored (right tree), "sclerenchyma clusters" are inferred to be 'absent' in nodes where there are no stipular expansions. Alternatively, "sclerenchyma clusters" is inapplicable when there are no stipular wings (middle tree).

Table 1. Classification schemes as proposed by previous characters and as implied by the current study. * denotes that a taxon has been assigned to Osmundaceae but no subfamily according to Bomfleur et al. (2017). ** denotes that a taxon was found in a polytomy in the strict consensus of Wang et al. (2014).

Family	Subfamily	Miller (1961, 1971), Tidwell and Ash (1994)	Wang et al. (2014)	Bomfleur et al. (2017)	This study (Dependencies)	This study (Non-dependencies)
Guaireaceae	Itopsidemoideae	*Itopsidema*	*Guairea*	*Donwelliacaulis*	*Donwelliacaulis*	*Donwelliacaulis*
		Donwelliacaulis	*Itopsidema*	*Itopsidema*	*Itopsidema*	*Itopsidema*
		Shuichengella	*Shuichengella*	*Tiania*	*Tiania*	*Tiania*
	Guairoideae	*Guairea*	*Lunea*	*Guairea*	*Guairea*	*Guairea*
		Lunea	*Zhongmingella*	*Lunea*	*Lunea*	*Lunea* **
					Osmundacaulis tehuelchensis, O. tasmanensis, andrewii *	*Osmundacaulis richmondii, O. tasmanensis, O. andrewii* *
			Osmundacaulis	*Zhongmingella*	*Shuichengella**	*Shuichengella* *, **
					Zhongmingella	*Zhongmingella* **
Osmundaceae	Thamnopteroideae	*Anomorrhoea*		*Chasmatopterys*	*Chasmatopterys*	*Thamnopteris (- T. splendida)*
		Bathypteris		*Thamnopteris*	*Bathypteris* *	*Bathypteris* *
		Chasmatopteris			*Thamnopteris*	
		legosigopteris				
		Petcheropteris				
		Thamnopteris				
		Zalesskya				
	Osmundoideae	*Osmunda*	*Osmunda* (+ *Claytosmunda, Plenasium*)	*Palaeosmunda* (+*Millerocaulis stipabonettiorum*)	*Lunea jonesii*	*Ashicaulis*

	Todea	*Todea*	*Millerocaulis*	*Millerocaulis*	*Claytosmunda*
	Leptopteris	*Leptopteris*	*Todea*	*Todea*	*Leptopteris*
				Leptopteris (L. estipularis)	
	Palaeosmunda	*Osmundastrum*	*Leptopteris*		*Millerocaulis*
		Millerocaulis (Osmundaceae?) **			
			Osmundacaulis estipularis		
	Ashicaulis			*Claytosmunda*	*Osmunda*
		Ashicaulis (Osmundaceae?) **			
	Millerocaulis		*Claytosmunda*	*Osmunda*	*Osmundacaulis janae**
		Palaeosmunda (Osmundaceae?) **			
	Australosmunda		*Osmunda*	*Osmundastrum*	*Osmundastrum*
		Aurealcaulis (Osmundaceae?) **			
					Palaeosmunda williamsii, P. playfordii
	Osmundacaulis		*Osmundastrum*	*Palaeosmunda*	
			Plenasium (+Aurealcaulis)		
	Aurealcaulis				*Plenasium*
			Osmundacaulis*		*Todea*
			Shuichengella*		
			Bathypteris*		
Incertae sedis		*Chasmatopterys (Osmundales?) ***			
		Thamnopteris (Osmundales?) **			
		Bathypteris (Osmundales?) **			

Table 2. Topological congruence between phylogenies inferred under different analytical approaches. The Robinson-Foulds distance is normalised so that "0.0" minimises distance (i.e., maximises congruence) and "1.0" maximises distance (i.e., minimises congruence). The proportion of common nodes is estimated relative to the maximum number of possible nodes for the smallest tree in each pairwise comparison. Pairwise comparisons against the reference topologies (under equal weighting and no dependencies; "ew-nondep") entail topologies inferred with dependencies included under: equal weighting ("ew-dep"), extended implied weighting with concavity 5 ("k5-dep"), concavity 10 ("k10-dep"), concavity 15 ("k15-dep") and concavity 20 ("k20-dep").

Comparison	RF distance	Proportion of common nodes
ew-nondep vs ew-dep	0.66	0.45
ew-nondep vs k5-dep	0.87	0.17
ew-nondep vs k10-dep	0.76	0.28
ew-nondep vs k15-dep	0.79	0.23
ew-nondep vs k20-dep	0.71	0.31

1
2
3
4
5

Table 3. Clades of reference in the topologies under equal weighting, and including or excluding character dependencies. The subfamily assignable to such clades and their synapomorphies are given, as well as the genera included. The symbol "-" denotes the exclusion of a terminal.

Clade of reference	Subfamily assignable	Included Taxa (Dependencies)	Synapomorphy (Dependencies)	Included Taxa (No Dependencies)	Synapomorphy (No dependencies)
Clade I	Itopsidemoideae	*Tiania*	Char. 0: Stem core tracheids: main tissue (2) -> accessory (1)	*Tiania*	Char. 6: Stele leaf gaps (peripheral invaginations/dissections): prominent (deeper than leaf-trace thickness) (2) -> shallow (shallower than leaf-trace thickness) (1)
		Itopsidema	Char. 9: Stele metaxylem cylinder spongy, with interspersed parenchyma: absent (0) -> present (1)	*Itopsidema*	Char. 9: Stele metaxylem cylinder spongy, with interspersed parenchyma: absent (0) -> present (1)
		Donwelliacaulis	Char. 15: Cortex sclerenchymatous outer cortical layer: thicker than parenchymatous layer (2) -> absent (0)	*Donwelliacaulis*	
			Char. 26: Tips of petiolar vascular bundle after departure from stem: straight or incurved (0) -> recurved (1)		
			Char. 32: Sclerenchyma ring: present (1) -> absent (0)		
Clade II	Thamnopteroideae	*Thamnopteris*	Char. 2: Stem core parenchyma: main tissue (2) -> absent (0)	*Thamnopteris (- T. splendida)*	Char. 27: Sclerenchyma lining leaf-trace abaxially: absent (0) -> continuous (2)
		Bathypteris	Char. 27: Sclerenchyma lining leaf-trace abaxially: absent (0) -> continuous (2)	*Bathypteris*	
		Chasmatopterys	Char. 31: Scattered sclerenchyma in the inner cortex of the petiole: present (1) -> absent (0)		
Clade III	Guairoideae	*Guairea*	Char. 15: Cortex sclerenchymatous outer cortical layer: thicker than parenchymatous layer (2) -> thinner than parenchymatous layer (1)	*Guairea*	Char. 8: Stele internal invaginations into metaxylem cylinder: fewer than peripheral invaginations (1) -> as many as or more than peripheral invaginations (2)

		Osmundacaulis tehuelchensis, O. tasmanensis, andrewii *Shuichengella* *Zhongmingella*	Char. 19: Number of leaf-trace protoxylem strands upon departure from stele: one (0) -> two (1) Char. 21: Leaf-trace shape immediately after departure from stele: oblong or slightly concave (0) -> strongly concave (1)	*Lunea* *Osmundacaulis richmondii, O. tasmanensis, O. andrewii* *Shuichengella* *Zhongmingella* *Tiania* *Itopsidema* *Donwelliacaulis*	Char. 15: Cortex sclerenchymatous outer cortical layer: thicker than parenchymatous layer (2) -> absent (0) Char. 31: Scattered sclerenchyma in the inner cortex of the petiole: absent (0) -> present (1)
Clade IV	Osmundeoideae	*Lunea jonesii*	Char. 22: Number of leaf-trace protoxylem strands in central part of cortex: more than two (2) -> two (1)	*Osmundacaulis (- O. richmondii, O. tasmanensis, O. andrewii)*	Char. 22: Number of leaf-trace protoxylem strands in central part of cortex: more than two (2) -> two (1)
		Osmundacaulis kidstonii *Millerocaulis*	Char. 28: Sclerenchyma in leaf-trace concavity adaxially: lining band (2) -> free mass (1) Char. 29: Sclerenchyma in trace concavity special states: solid (0) -> interrupted/scattered (1)	*Millerocaulis*	
		Todea		*Todea*	
		Leptopteris (L. estipularis) *Claytosmunda* *Osmunda* *Osmundastrum* *Palaeosmunda*		*Leptopteris (L. estipularis)* *Claytosmunda* *Osmunda* *Osmundastrum* *Palaeosmunda*	

Fig. 1. Strict consensus of topologies inferred equal weighting after pruning the unstable terminals that reduce the resolution. (a) Strict consensus of topologies inferred without considering character dependencies. (b) Strict consensus of topologies inferred after including character dependencies as step-matrix complexes. The numbers below branches correspond to Bremer Support values. Roman numerals are clades of reference discussed throughout the paper. Taxonomic categories based on Bomfleur et al. (2017) are indicated in colour.
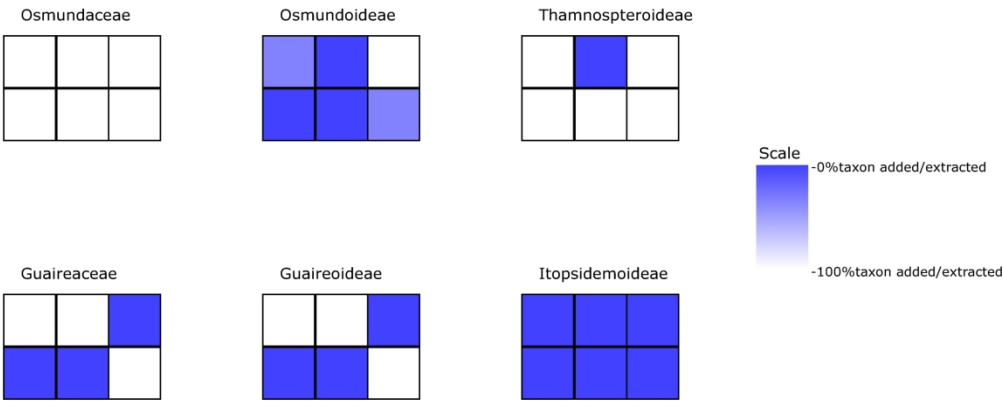
1153x830mm (600 x 600 DPI)

Fig. 2. Sensitivity plots for the families (Osmundaceae and Guaireaceae) and four subfamilies (Osmundoideae, Thamnopteroideae, Guaireoideae, and Itopsidemoideae). White boxes indicate non-monophyly, dark blue represents monophyly as defined by Bomfleur et al. (2017), and light blue indicates the addition of an incertae sedis taxon.
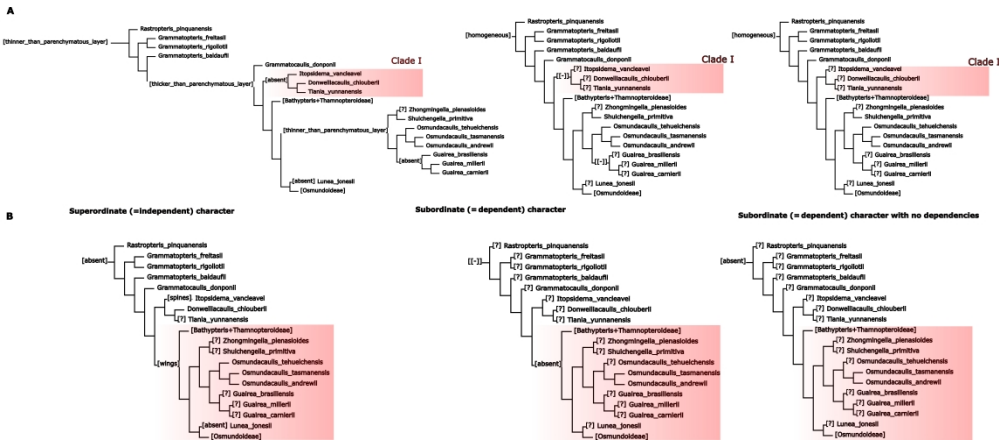
204x80mm (300 x 300 DPI)

Fig. 3. Effect of ignoring character dependence in the reconstruction of characters in Osmundales. (a) Reconstruction of the sclerenchyma type, which is absent in Clade I (red clade) but inferred as 'homogeneous' if dependencies are ignored (right tree). (b) Reconstruction of stipular expansions as 'wings' at the node including Clades II-IV (red clade) and "sclerenchyma clusters", which are possible only when stipular expansions are 'wings'. If dependencies are ignored (right tree), "sclerenchyma clusters" are inferred to be 'absent' in nodes where there are no stipular expansions. Alternatively, "sclerenchyma clusters" is inapplicable when there are no stipular wings (middle tree).

1323x572mm (600 x 600 DPI)