**Revisiting the phylogeny of Royal Ferns (Osmundales) through the lens of character dependence and restudied fossil taxa questions existing family and subfamily concepts**

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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, the phylogenetic relationships between extinct species remain contentious. Although several analytical approaches have been applied to infer well-resolved phylogenetic hypotheses – 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. Here, we evaluate the phylogenetic affinities in Osmundales by reanalysing a dataset comprising an extensive taxon sampling of fossil Osmundalean rhizomes. The impact of both character dependence and weighting characters against homoplasy on the inferred topologies is also evaluated. Our analyses cast doubts on the monophyly of Osmundaceae and Guaireaceae. Subfamily Itopsidemoideae is rendered monophyletic when inferences are conducted by considering character dependence and downweighting characters. Subfamily Osmundoideae is retrieved monophyletic only under one concavity value and using character dependence while the remaining subfamilies included fossils with uncertain affinities within Osmundales. The position of *Osmundacaulis*, for instance, is recovered in as sister to member of guaireoid fossils. To recover the monophyly of the categories below the subfamily level, 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 underline the impact 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 Permian and have one of the most diverse fossil records amongst ferns 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 six genera: *Osmunda* L.*, Osmundastrum* Presl*, Leptopteris* Presl, *Todea* Willd. Ex Bernth*., Plenasium* Presl, and *Claytosmunda* (Yatabe, Murak. Iwats.) Metzgar et Rouhan (PPG I, 2016).

Despite its abundant fossil record, Osmundales systematics has been particularly challenging to address both from a taxonomic and 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 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 systematics of extant Osmundaceae is mostly based on molecular studies that included few molecular markers (Yatabe et al., 1995, Metzgar et al., 2008). In the most extensive molecular study, which included seven plastid markers and the four extant genera, Metzgar et al. (2008) found evidence for a paraphyletic *Osmunda* and, consequently, proposed the monospecific genus *Osmundastrum*. The systematics of extinct Osmundales is largely derived from the morphological 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. Leaf trace shape at the cortex in Osmundales, 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 empirical phylogenetics of land plant. Therefore, the impact of incorporating the logical link between characters on the phylogenetic affinities is still unclear.

In this study, we assess the phylogenetic relationships within Osmundales by taking into account character dependence in Bomfleur et al.’s (2017) dataset of fossil rhizomes and exploring different character weighting strategies. By employing a fixed molecular backbone for extant Osmundaceae, based on Metzgar et al. (2008), our analyses question the monophyly of Guaireaceae and Osmundaceae – families traditionally accepted for Osmundales. Similarly, the monophyly of the subfamilies depended on the incorporation of dependencies, downweighting characters, or both. Even though multiple fossils were unstable in our analyses, the systematic affinities of some fossils with uncertain placement were clarified. These results unequivocally demonstrate that character interactions carry significant systematic implications and highlight the importance of considering dependencies when compiling morphological data matrices.

**Materials and Methods**

*Backbone of extant Osmundaceae*

Phylogenetic analyses were performed by running constrained searches. That is, a backbone for extant Osmundaceae was enforced during tree searches while the position of the fossils was allowed to be decided based on morphology alone. Backbone topologies were inferred from the molecular dataset of Metzgar et al. (2008), which includes all the extant genera of Osmundaceae, four Gleicheniales, and seven plastid markers: atpA, rbcL, rbcL–accD, rbcL–atpB, rps4–trnS, trnG–trnR, and trnL–trnF. The original dataset by Metzgar et al. (2008) included multiple individuals per species, which creates redundant entries at the species level if scored in the morphological dataset. Therefore, in order to infer the backbone, we retained a single individual per species based on their sequence length. The final molecular dataset, thus, consisted of the six extant Osmundaceae and four Glecheniales species as outgroups. The backbone trees were rooted in *Gleichenella pectinata* (Willd.) Ching.

*Morphological 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 reanalysed (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). In the morphological dataset, the outgroup included five filicalean fossil species: *Grammatocaulis donponii*, *Grammatopteris freitasii*, *G. rigollotii*, *G. baldaufii*, and *Rastropteris pinquanensis*. Rooting, as in the molecular dataset of Metzgar et al. (2008), employed *Gleichenella pectinata*. 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. All of them represented ‘static dependencies’ with a single superordinate character (Fig. 1):

*Complex 0 (2 characters):* The possibility of having any of the types of stem core tracheids listed in Character 1 is subject to the presence of stem core tracheids, which is scored in Character 0. In this sense, the rationale of this complex is that “the type of stem core tracheid” can be scored if and only if “stem core tracheids” are present (state 1 or 2 of Character 0).

*Complex 1 (2 characters):* Following Bomfleur et al. (2017), internal embayments in xylem siphon (scored in Character 8; red arrow in Fig. 1A) are indicative of incipient leaf gaps formation and, therefore, they are ontologically subject to the presence of that leaf gaps (scored in Character 6; blue arrow in Fig. 1A). For this reason, Characters 6 and 8 express a logical relationship such that Character 8 is scorable for states 0 or 1 when Character 6 is different from state 0 (“absent”).

*Complex 2 (2 characters):*  The Osmundales families can be differentiated by having different cortex types depending on the tissues that compose them. Particularly in Osmundaceae, for example, cortex is differentiated into an inner, primarily parenchymatous layer (painted in yellow in Fig. 1B) and an outer sclerenchymatous layer (painted in brown in Fig. 1B). Bomfleur et al. (2017) use Character 15 to score the presence of sclerenchymatous outer cortical layer, whereas they define whether it is homogeneous or heterogeneous in Character 16. Therefore, Character 16 is contingent on sclerenchymatous cortex not being scored as "absent" in 15 (Fig. 1B).

*Complex 3 (4 characters):* This complex involves characters related to the development of leaf traces and their associated xylem strands, which are intricately related. In this complex, the development of traces and strands is determined by the type and number of leaf traces and strands at the inner parts of the stem (Fig. 1C). Specifically, the number of initial protoxylem poles per leaf trace (Character 17) determines the number of leaf trace protoxylem strands upon departure from stele (Character 19; red arrow in Fig. 1C), in the central part of cortex (Character 22; purple arrow in Fig. 1C) and upon departure from the stem (Character 24; blue arrow in Fig. 1C). As noted by Bomfleur et al. (2017), the development of xylem strands in leaf traces is such that the number of strands in leaf traces can never be lower than the number of strands at an inner section of the cortex. Hence, for instance, when the number of initial protoxylem poles per leaf trace is “two” (Ch. 17, state 1), the number of leaf trace protoxylem strands upon departure from stele can be “two” (Ch. 19, state 1) or “more than two” (Ch. 19, state 2). For the number of leaf trace protoxylem strands upon departure from stele to be inferred as “one” (Ch. 19, state 0), the number of initial protoxylem poles per leaf trace should be “one” (Ch. 17, state 0) – but never “two” (Ch. 17, state 1) or “more than two” (Ch. 17, state 2).

*Complex 4 (3 characters):* According to Bomfleur et al. (2017), a key factor in the classification of Osmundaceae is the presence, distribution, and degree of distal differentiation of sclerenchyma at specific levels and tissue regions of the stipe (e.g., in the concavity of the vascular bundle). In this complex, the type (Character 29) and appearance (30) of sclerenchyma in the stipe bundle concavity depend on their presence (Character 28; sclerenchyma indicated in brown in Fig. 1D).

*Complex 5 (6 characters):* In some groups, sclerenchyma may also be present in the petiole cortex, forming an external cylinder (Character 32; indicated in brown in Fig. 1E). This cylinder may be homogeneous or heterogeneous depending on whether it has a distinct abaxial arch of thick-walled fibres observable in cross-sections (Character 33; indicated as black patches or clusters in Fig. 1E). The applicability of Character 33 depends on the Characters 32: sclerenchyma in the petiole cortex should be present to be able to score the Character 33. Additionally, the abaxial arch of thick-walled fibres can adopt different configurations which are reflected in Characters 34, 35, 36 and 37. Therefore, Character 33 determines the applicability of last mentioned characters (Fig. 1E).

*Complex 6 (4 characters):* The stipe of Osmundales can be expanded in spines or wings (Character 38). When stipular wings are present, fibres (Character 39) or masses (Character 40) of sclerenchyma are sometimes developed (indicated in brown in Fig. 1F). As a result, Character 38 determines the applicability of Characters 39 and 40. Additionally, the shape or arrangement of distinct sclerenchyma masses within stipular wings (scored in Character 41) depends on the presence of masses (Character 40; Fig. 1F).

*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 — and considering dependencies, 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 k5 and dependencies included (“k5-dep”), extended implied weighting with concavity k10 and dependencies included (“k10-dep”), extended implied weighting with concavity k15 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*

Evaluating the topological and taxonomic congruence between different data sources is common practice when the taxonomic diversity of a group is relatively well covered by the data partitions under comparison. However, the taxonomic diversity of Osmundales is mainly represented by fossils – making such a comparison pointless. Consequently, to assess the impact of character dependence on the systematics of Osmundales, changes in topology and taxonomic congruence were evaluated for the constrained phylogenies inferred based on morphology.

Topological congruence was evaluated between the phylogenies inferred with dependencies included (“k5-20-dep”) relative to the topologies inferred under equal weighting and with no dependencies (“ew-nodep”). 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 (RF) 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”, “k5-dep”, “k10-dep”, “k15-dep” and “k20-dep”).

**Results**

*Phylogeny*

Even though filicalean fossils (*Grammatopteris*, *Rastropteris* and *Grammatocaulis*) had ambiguous positions amongst outgroup terminals, our analyses consistently recovered Osmundales as monophyletic regardless of the incorporation of dependencies (Fig. 2). However, the inferred relationships at the family level, and below, differed between analytical approaches.

In omitting character dependencies, tree searches yielded optimal trees of 329 steps. After pruning 54 wildcards, the reduced strict consensus had a resolution of 0.76 (60 nodes relative to 79 nodes in a fully resolved, reduced tree) — that is an improvement of 39% relative to the non-pruned strict consensus (resolution: 0.37, 49 nodes out of 133). In the pruned consensus, Guaireaceae and Osmundaceae (sensu Bomfleur et al., 2017) were not recovered as monophyletic (Fig. 2A). The subfamily Itopsidemoideae, included in Guaireaceae, had ambiguous placements and was pruned from the consensus tree. The remaining Guaireaceae (plus *Shuichengella* and *Osmundacaulis*) were nested within Osmundaceae (Fig. 2A). The subfamily Guaireoideae included the genera *Osmundacaulis* and *Shuichengella*,previously assigned to Osmundaceae (Fig. 2A). The subfamily Thamnopteroideae (Osmundaceae) was not monophyletic due to the exclusion of *Thamnopteris splendida* (which was sister to all members of Osmundales) and *Chasmatopteris principalis*, and the inclusion of *Bathypteris rhomboidea* (Fig. 2A). In ignoring dependencies, the genera *Osmunda* and *Plenasium* had multiple positions and improved the consensus upon pruning. The remaining genera assigned to the subfamily Osmundoideae constituted a clade that included *Osmundacaulis janae* (Fig. 2A). At the genus level, two genera within Osmundeae were not monophyletic (*Osmundastrum* and *Claytosmunda*; Fig. 2A).

The analysis that considered character dependencies inferred optimal trees of 346 steps that, upon excluding 29 wildcards, led to a reduced strict consensus tree of 90 nodes (out of 104 possible nodes, 0.86; Fig. 2B) — an improvement of 51% relative to the non-pruned strict consensus (47 out of 133 nodes; 0.35). In this analysis, neither Osmundaceae nor Guaireaceae are found monophyletic (Fig. 2B). The “Clade I”, which is sister to all remaining Osmundales, includes members of the subfamily Thamnopteroideae (Guaireaceae) and the genus Bathypteris (Fig. 2B). Members of the subfamily Itposidemoideae (Guaireaceae) and the genus *Lunea*, included within “Clade II”, are closely related to “Clade III”, which encompasses *Zhongmingella*, *Shuichengella*, *Guairea*, and *Osmundacaulis* (Fig. 2B). Except for *Palaeosmunda williamsii*, the remaining members of the subfamily Osmundoideae (Osmundaceae) are included in “Clade IV” along with the genus *Anomorrhoea*. Except for the subtribe Todeinae, the tribes and subtribes within Osmundoideae were not monophyletic (Fig. 2B). Five genera of Osmundales (*Guairea*, *Todea*, *Leptopteris*, *Plenasium*, and *Osmundacaulis*) were rendered monophyletic.

*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 had the lowest similarity with the “ew-nondep” topologies in terms of RF distance (Table 2). If measured as the proportion of shared nodes, however, the topology inferred under k20 was the most dissimilar to the “ew-nondep” trees (Table 2). Even though the most similar topologies to the “ew-nondep” trees were those inferred under equal weighting, the proportion of shared nodes was still below 0.5 and the RF distance higher than 0.6 (Table 2).

Taxonomic congruence, relative to the groups proposed by Bomfleur et al. (2017), revealed that neither Guaireaceae nor Osmundaceae were recovered as monophyletic throughout the analyses (Fig. 3). Likewise, the subfamily Guaireoideae was not recovered in the analyses (Fig. 3). Itopsidemoideae, the other subfamily recognised within Guaireaceae (Bomfleur et al., 2017), was retrieved monophyletic across the entire range of analytical conditions that incorporated dependencies (Fig. 3). The remaining subfamilies, Osmundoideae and Thamnospteroideae (Osmundaceae), were unstable throughout the analyses. Osmundoideae was strictly contradicted only under the strongest concavity and including dependencies while it was rendered monophyletic under k10 (Fig. 3). In the remaining conditions, Osmundoideae either included *incertae sedis* taxa (e.g., *Osmundacaulis janae*, or *Anomorrhoea*) or excluded different species of *Palaeosmunda*. Thamnopteroideae was strictly contradicted under equal weighting and not considering dependencies while it included different *incertae sedis* taxa across the other conditions (Fig. 3).

*Synapomorphies*

In general, few nodes were supported by synapomorphies under equal weighting, although a slight improvement was observed when dependencies were considered. If dependencies were ignored, 50 nodes (out of 79; 0.62) are supported by at least one character when dependencies are ignored. In contrast, upon incorporating dependencies, 70 nodes (out of 104, 0.66) are supported by at least one character. Few characters were reconstructed as synapomorphies for the clades of reference (Clades I-IV, Fig. 2; Table 3). For instance, Clade IV — assignable to subfamily Osmundoideae, is supported by one synapomorphic character when dependencies are ignored while four characters are synapomorphic upon including dependencies (Table 3).

**Discussion**

In this study, we evaluated the phylogenetic affitnities between 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 Osmundaceae, Guaireaceae, and Guaireoideae were not monophyletic across analytical conditions whereas the monophyly of other groups depends on the inclusion of dependencies 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. 2). None of the families, as proposed by previous authors (Wang et al., 2014; Bomfleur et al., 2017), were recovered as monophyletic across the analyses (Fig. 3). Except for the subfamily Itopsidemoideae, the topologies resulting from the analysis with character dependencies did not reflect the relationships amongst Osmundales as inferred by Bomfleur et al. (2017; Fig. 2B). The monophyly of the subfamily Guaireoideae was contradicted throughout the analyses while the remaining subfamilies were recovered only under certain analytical conditions (Fig. 3). 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 (Tables 1, 3) and the genus *Osmundacaulis*.

***Clade I***. This clade includes taxa currently assigned to the subfamily Thamnopteroideae (Osmundaceae): *Thamnopteris* and *Chasmatopteris* (Bomfleur et al., 2017). 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 and has had unclear affinities with other Osmundales (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 their analyses, Thamnopteroideae was also excluded from Osmundaceae (Wang et al., 2014). In contrast, Bomfleur et al. (2017) rendered Thamnopteroideae as either monophyletic (‘holophyletic’ in their network analysis) or paraphyletic, although nested within Osmundaceae. In this regard, our analyses – whether including dependencies or not – are in agreement with Wang et al. (2014) by rendering thamnopteroid taxa as sister to all other Osmundales (Clade I; Fig. 2). In previous studies, the genus *Bathypteris* also 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 Osmundaceae but without assignation at the subfamily level. In our analyses, the concept of Thamnopteroideae as defined by either Wang et al. (2014) or Bomfleur et al. (2017) is not supported. Instead, our analyses recovered *Bathypteris* in a clade along with *Thamnopteris* (Fig. 2). Consequently, resembling traditional concepts of the subfamily (e.g., Miller, 1971) and disputing recent inferences (Wang et al., 2014; Bomfleur et al., 2017). In contrast with previous inferences, our analyses also rendered the genus *Thamnopteris* non-monophyletic due to the inclusion of *Chasmatopteris* and *Bathypteris*, and – when ignoring dependencies – the exclusion of *T. splendida* (Fig. 2; Fig. S1). This outcome suggests that the concept of both the genera included in Thamnopteroideae and the subfamily itself should be reconsidered.

***Clade II***. This clade comprised members of the subfamily Itopsidemoideae, a group originally included under the family Guaireaceae to accommodate the genera *Donwelliacaulis*, *Tiana* and *Itopsidema* (Bomfleur et al., 2017). Amongst the taxonomic groups proposed for Osmundales, Itopsidemoideae is the only category above the tribe level consistently recovered in our analyses when dependencies are incorporated (Fig. 2, 3). Conversely, upon ignoring dependencies, taxa within Itopsidemoideae are unstable across the optimal trees and the subfamily is not recovered (Fig. 3). In the analyses with dependencies, however, the placement of Itopsidemoideae varied from being nested in a clade that involves the remaining Gauireaceae and the genera *Osmundacaulis* and *Shuichengella* (“ew-dep”, Fig. 2B) to sister to all members of Osmundales (“xpi5\_dep”; Fig. S1). Across our analyses, *Lunea jonesii* (Guaireoideae) is nonetheless rendered as a sister taxon to Itopsidemoideae (Fig. S1). Further, in four (out of five) topologies inferred with dependencies, the clade Itopsidemoideae + *Lunea* (Clade II) is retrieved as sister to the remaining Guaireoideae and *Osmundacaulis* (Fig. S1). Unless non-monophyletic groups are accepted as valid categories (as in Bomfleur et al., 2017), retaining the current concept of Itopsidemoideae necessarily entails rejecting Guaireoideae. An alternative is to expand Itopsidemoideae to include the genus *Lunea*, a clade supported by four synapomorphies: “absence of sclerenchyma in the outer cortex”, “recurved vascular bundle tips”, and absence of “sclerenchyma ring in the inner cortex” and “stipular expansions” (Table 3). In raising Itopsidemoideae, Bomfleur et al. (2017) proposed “absence of stele leaf gaps” and “stele with patches of parenchyma” as diagnostic characters, among others. Our analyses under equal weighting and dependencies recover the same characters as synapomorphies for the clade including *Tiania, Donwelliacaulis and Itopsidema*. However, stele leaf gaps are reconstructed as “shallow” instead of “absent”.

***Clade III***. This clade includes representatives of the subfamily Guaireoideae and fossils with uncertain affinities within Osmundaceae (Fig. 2; Table 1). As is the case of other categories within Osmundales, guaireoid fossils have had equivocal affinities. Even though Wang et al. (2014) did not establish a subfamily category within Osmundales, they included seven genera within the family Guaireaceae: *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 either Wang et al. (2014) or Bomfleur et al. (2017) – are not monophyletic due to both the exclusion of guaireoid fossils and the inclusion of osmundacean fossils (Figs. 2, 3). Second, as mentioned earlier, *Lunea* is either sister to *Zhongmingella* + *Shuichengella* (Osmundaceae) when dependencies are ignored (Fig. 2A) or sister to Itopsidemoideae in Clade II when dependencies are considered (Fig. 2B). Third, throughout our analyses, *Shuichengella* – an osmundacean fossil according to Bomfleur et al. (2017) – constitutes a clade with guaireoid fossils and the genus *Osmundacaulis* regardless of the analytical approach (Fig. 1; Fig. S1). Consequently, as compared to Bomfleur et al. (2017), our analyses imply a wider concept of Guaireoideae where Osmundacaulis and Shuichengella are included (Table 1). This new concept, closer to Guaireaceae sensu Wang et al. (2014), is supported by having “two leaf-trace protoxylem strands” and “strongly concave leaf traces” (Table 3).

***Clade IV***. This clade comprises the remaining members of the subfamily Osmundoideae along with fossils of uncertain position within Osmundaceae (Fig. 2; Table 1). 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). In our analyses, Osmundoideae *sensu* Bomfleur et al. is recovered only when dependencies are included under a strong weighting concavity (“k10\_dep”) and rendered non-monophyletic under the strongest concavity (“k5\_dep”; Fig. 3). In the remaining analytical conditions, fossils with uncertain affinities within Osmundaceae were nested in a clade otherwise composed by Osmundoideae (Figs. 2, 3). Within Osmundaceae, 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*) within tribe Osmundeae. In our analyses, only the subtribe Todeinae was robustly recovered across analytical conditions (Fig. S1). Subtribe Osmundinae, however, was not monophyletic throughout as it included either *Palaeosmunda* or *Millerocaulis* – both *incertae sedis* osmundacean fossils – and Todeinae (Fig. 2, Fig. S1). As in 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). These results indicate that categories within the subfamily Osmundoideae might not be reliable indicators of affinities within Osmundales and, instead, alternative groups may be needed. If Clade IV is taken as an alternative concept of Osmundoideae, the subfamily is then supported by having its vascular tissue with “one protoxylem strand in the leaf traces” in the central part of the cortex and upon departing the stem, “leaf trace sclerenchyma in petioles”, and “radially oriented root in mantle cross-sections” (Table 3).

***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 monophyletic only upon considering dependencies (Fig. 2; Fig. S1). If dependencies are ignored (“ew\_nondep”), *O. janae* is nested in Clade IV and excluded from the remaining Osmundacaulis species in Clade III (Fig. 2). In the remaining analyses with dependencies, Osmundacaulis is consistently recovered in Clade III along with guaireoid fossils (Fig. 2; Fig. S1). The affinity with guaireoid fossils resembles the previous concept of Guaireaceae (e.g., Wang et al., 2014), which also included *Shuichengella* (Table 1), is given by two synapomorphies: “two protoxylem strands in leaf traces upon departure of the stem”, and “strongly concave leaf traces” (Table 3). Also, note that upon including dependencies, *Osmunda* (= *Osmundacaulis*) *kidstonii* – which has been excluded in previous analyses due to their incompleteness (Bomfleur et al., 2017) – is retrieved within Clade IV without constituting a clade with other species of Osmunda (Fig. 2; Fig. S1). These topologies imply 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. These factors, which along with character informativeness amount to the information content of a dataset, affect the recovery of well supported, stable clades (see Flores et al., 2020). Nevertheless, previous datasets of Osmundales with better sampling ratio and fewer missing entries also failed in recovering well-supported clades (e.g., Wang et al., 2014). Phylogenetic analyses using molecular data necessarily sample a small fraction of the total diversity of the group (as most genera and species are extinct taxa; e.g., 13 extant species in Metzgar et al., 2008). This implies that, besides improving sampling, novel sources of morphological 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. 3). Even though subtle, inferring topologies under extended implied weighting improved the recovery of taxonomic groups: three (out of four) subfamilies are recovered when weighting against homoplasy (Fig. 3). 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 – albeit including *incertae sedis* fossils (Osmundoideae; Fig. 3). Moreover, the monophyly of the subfamily Osmundoideae – as defined by Bomfleur et al. (2017) – is recovered only when dependencies are considered and using a strong concavity under extended implied weighting (k10; Fig. 3). To recover additional groups, searches needed to be run by either considering dependencies or weighting against homoplasy – or even both (Fig. 3; Fig. S1).

Finally, it is worth noting that, for this dataset, higher concavities led to taxonomic categories being recovered with more precision (i.e., being recovered as defined by Bomfleur et al., 2017) while also minimised congruence with the topologies inferred under equal weighting and no dependencies (Table 2). If topologies are inferred under extended implied weighting without considering dependencies, congruence given by Robinson-Foulds distance is slightly improved relative to the topologies under equal weighting and no dependencies (Table S1). However, such improvement is still lower than the congruence with the topologies inferred under equal weighting and considering dependencies (Table 2). Thus, while character weighting influences incongruence, much of the dissimilarity between topologies is likely driven by the inclusion of dependencies.

*Character dependence, optimal topologies 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 compared to a typical approach run under equal weighting, the use of dependence complexes in our analyses led to alternative topologies (Fig. 2B). While recovering different topologies after incorporating dependencies is somehow expected, it is not necessarily obvious. This is so since the dataset at hand might not violate (in)dependence assumptions. In Bomfleur et al.’ (2017) dataset, and considering our dependence hypotheses, all but one complex is violated under equal weighting (Complex 3). This, of course, affects how trees are evaluated and the resulting topologies. Here, violating complexes leads to a difference of nine extra steps – which translates to less than half of the nodes shared (Table 2).

By extension, incorporating dependencies also leads to alternative reconstructions of character states along the trees – which may affect macroevolutionary assessments or group diagnoses if ancestral states are employed as diagnostic characters. Among guaireoid fossils, included in Clade II and III (Fig. 2), relationships and character reconstructions differ upon considering dependencies (Fig. 4). If dependencies are ignored, for instance, *Guairea* is rendered paraphyletic and Itopsidemoideae – despite not having sclerenchyma outer layer – are reconstructed as scorable for the type of “cortex sclerenchymatic outer layer” (Fig. 4A). In contrast, upon considering dependencies, *Guairea* is monophyletic and taxa with no sclerenchyma outer layer are inferred as inapplicable (Fig. 4B) – an ancestral character reconstruction more reasonable given the lack of sclerenchyma in the outer cortex.

These examples underline the impact of considering dependence in inferring phylogenies. While the different classifications of Osmundales –and most systematic classification proposals in general– do not explicitly derive diagnoses from synapomorphies, these could serve as quantitatively assessed diagnostic characters. As opposed to diagnosing taxonomic groups based on a mere general description of the included taxa, synapomorphies can yield testable diagnoses. In this sense, taking into account dependencies between characters allows reconstructing biologically meaningful synapomorphies; this prevents impossible character state combinations from being retrieved.

*Discrepancies with Bomfleur et al´s (2017) study*

Regardless of the use 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 only one group above the tribe level is recovered: subfamily Itopsidemoideae (Figs. 2, 3). However, such subfamily is recovered only when including dependencies and downweighting characters (Fig. 3). Altogether calling into question of classification scheme of Osmundales as proposed by Bomfleur et al. (2017). 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 better be 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.

Finally, it should be noted that, in addition to monophyletic groups, Bomfleur et al. (2017) proposed taxonomic categories based on paraphyletic groups. Interestingly, Bomfleur et al. (2017, p. 19) stated that “[...]The study of evolutionary processes, however, requires phylogenetically meaningful (as opposed to merely phenetic) ‘natural’ taxa, and the definition of such ‘natural’ groups requires an implicit phylogenetic framework via the recognition of evolutionary lineages of common ancestry[...]”. The appealing for using paraphyletic groups, according to Bomfleur et al. (2017), resides in its alleged pragmatism. For instance, classifications based on monophyletic groups might be unstable since “[...]Just a single newly scored trait in a morphological matrix may change a clade in a phylogenetic tree into a grade or vice versa[...]” (Bomfleur et al., 2017, p. 23). As is evident in our analyses, groups that were paraphyletic in Bomfleur et al. (2017) were not recovered as such in our analyses under equal weighting and ignoring dependencies (e.g., *Millerocaulis*).

**Final remarks**

In this study, we conducted phylogenetic analyses on a dataset of Osmundales while taking into account the 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 and Guaireaceae were not recovered in our analyses (Figs. 2, 3). Subfamilies, as defined by Bomfleur et al. (2017), were unstable and often included taxa that had been previously excluded as *incertae sedis*. Upon considering dependencies, Itposidemoideae was the only subfamily that was recovered as proposed by Bomfleur et al. (2017). Our study thus questions the current classification scheme for Osmundales and suggests a careful review of the systematic affinities among these fossils.

Our study applies two methodologies that have not been employed in previous evaluations 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., 2023) and has herein improved the recovery of taxonomic categories within Osmundales (Fig. 3, Fig. S1). Because dependencies affect how trees are evaluated, in addition to yielding novel topologies, using dependence complexes has also impacted the inference of synapomorphic characters (Table 3). While it could be interpreted that ignoring dependencies yields more robustly supported classifications (in terms of the number of synapomorphies), our results suggest the opposite: more conservative estimations – which are biologically consistent – 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.

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**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, Table S1 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.** Reduced consensus trees from topologies inferred under equal and extended implied weighting and using or excluding character dependencies. Subfamilies highlighted in each tree in colour: (blue) Thamnopteroideae, (light blue) Itopsidemoideae, (green) Guairoideae, and (red) Osmundoideae. Groups in black are either incertae sedis or non-osmundalean fossils.

**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). Genera not included in the column indicate that the taxon was either unsampled in the respective study or pruned from the consensus tree.

**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 (character number within brackets), as well as the genera included. The symbol “-” indicates that the species was not found forming a clade with the remaining species of the genus

**Fig. 1.** Structures related to the character complexes defined in this article. Diagrams modified from Bomfleur et al. (2017). (a) Diagram showing examples of arrangements of stelar xylem siphon in osmundalean stems as seen in cross-section through the stele. Internal embayments in xylem siphon indicated by red arrow, leaf-gaps indicated by blue arrow; (b) Diagram illustrating different layering and composition of cortical tissues in osmundalean stems. Parenchyma indicated in yellow, sclerenchyma indicated in brown and thicker walled fibre patches as stipples; (c) Diagram illustrating examples of modes of leaf-trace formation and development in osmundalean stems. Cross-sections at the level of the stele indicated by red arrow, upon departure from the stele indicated by pink arrow, mid-way through the cortex indicated by purple arrow, and upon departure from the stem indicated by blue arrow, metaxylem in brown, protoxylem strands in light green; (d) Diagram illustrating sclerenchyma arrangements in stipe bundle concavity. Parenchyma in yellow, sclerenchyma in brown, vascular bundle in white; (e) Diagram showing examples of tissue composition and heterogeneity of the external cylinder of petiole cortex. Parenchyma in yellow, sclerenchyma in brown, patches or clusters of particularly thick-walled fibres in black, vascular bundle in white; (f) Diagram illustrating examples of extensions in Osmundales stipes and tissue distribution on it. Parenchyma in yellow, sclerenchyma in brown.

**Fig. 2.** Strict consensus trees from the topologies inferred under equal weighting and after pruning the unstable terminals. (a) Reduced strict consensus of topologies inferred without considering character dependencies. (b) Reduced strict consensus of topologies inferred after including character dependencies. 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. 3.** Sensitivity plot for the families (Osmundaceae and Guaireaceae) and four subfamilies (Osmundoideae, Thamnopteroideae, Guaireoideae, and Itopsidemoideae) to changes in the analytical approach. 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. 4.** Effect of including character dependence in the systematic affinities and reconstruction of characters in Osmundales. The reconstruction of the type of “Cortex sclerenchymatic outer layer” (ch. 16) is contingent on the development of a “sclerenchymatous outer layer in the cortex” (ch. 15). The dependence rule states that character 16 can only be inferred when character 15 is different from "absent" (state 0), otherwise character 16 is inapplicable. Ancestral states for character 15 are presented alongside those inferred for character 16, separated by a dot ("."). Combinations that violate the assumption of dependence are highlighted in red, combinations that follow the assumption are highlighted in blue. (a) Topology that violates character dependence as inferred under equal weighting. (b) Topology inferred under equal weighting after including dependencies. Species of *Guairea* and subfamily Itopsidemoideae are highlighted in coloured boxes. “[-]” indicate inapplicable character states. “L” indicates tree lengths: values estimated when dependencies are included are given in bold, and grey values within brackets are calculated when dependencies are ignored. Trees for comparison were randomly chosen between the optimal topologies inferred under “ew-nodep” and “ew-dep”.