Is Morphology Really at Odds with Molecules in Estimating Fern Phylogeny?

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Abstract—Using a morphological dataset of 136 vegetative and reproductive characters, we infer the tracheophyte phylogeny with an emphasis on early divergences of ferns (monilophytes). The dataset comprises morphological, anatomical, biochemical, and some DNA structural characters for a taxon sample of 35 species, including representatives of all major lineages of vascular plants, especially ferns. Phylogenetic relationships among vascular plants are reconstructed using maximum parsimony and Bayesian inference. Both approaches yield similar relationships and provide evidence for three major lineages of extant vascular plants: lycophytes, ferns, and seed plants. Lycophytes are sister to the euphyllophyte clade, which comprises the fern and seed plant lineages. The fern lineage consists of five clades: horsetails, whisk ferns, ophioglossoids, marattioids, and leptosporangiate ferns. This lineage is supported by characters of the spore wall and has a parsimony bootstrap value of 76%, although the Bayesian posterior probability is only 0.53. Each of the five fern clades is well supported, but the relationships among them lack statistical support. Our independent phylogenetic analyses of morphological evidence recover the same deep phylogenetic relationships among tracheophytes as found in previous studies utilizing DNA sequence data, but differ in some ways within seed plants and within ferns. We discuss the extensive independent evolution of the five extant fern clades and the evidence for the placement of whisk ferns and horsetails in our morphological analyses.

Keywords—Equisetaceae, horsetails, leptosporangiate ferns, lycophytes, monilophytes, Ophioglossaceae, Psilotaceae, seed plants.

Our general understanding of phylogenetic relationships across vascular plants has increased enormously in recent years, due in large part to numerous molecular systematic studies focused on the green branch of the tree of life (Mishler et al. 1994; Bowe et al. 2000; Nickrent et al. 2000; Pryer et al. 2001, 2004; Burleigh and Mathews 2004; Dombrovskaja and Qiu 2004; Wikström and Pryer 2005; Qiu et al. 2006, 2007). A parallel development has been the increasingly unpopular use of morphological data to reconstruct phylogenetic relationships (Hillis and Wiens 2000; Wiens 2004). Largely forgotten are arguments for the precedence of morphology in studies on ancient rapid radiations (Bateman 1998, 1999). Instead, DNA sequence data are seen as better suited to reconstruct the evolutionary history of deep radiations because of their universality, stochastic behavior, and abundance (Bromham 2003; Whitfield and Lockhart 2007). Some have argued that the increasing ambiguity of homology assessments in compilations of ever-larger morphological data matrices renders morphology a poor indicator of phylogeny (Scotland et al. 2003). Others are willing to accept a limited role for morphology in phylogenetic reconstruction, one where more rigorous and critical studies of fewer, but unambiguous, morphological characters are integrated with molecular data (Scotland et al. 2003; Olmstead and Scotland 2005). The ongoing discussion tends to ignore the needs of researchers working primarily with extinct taxa or those who strive to integrate fossil and extant taxa into a single phylogeny. For these kinds of studies, there is either no choice, or little choice, other than to continue to add to and attempt to improve upon morphological data matrices (Jenner 2008; Lee 2004; Wiens 2004; Smith and Turner 2005; Magallón 2007; Schneider 2007; Hermann and Hendricks 2008).

Our objective in this paper is to explore empirically to what degree incongruence and conflict between morphological and molecular data pose a problem in estimating a phylogeny for extant ferns and their relatives. For the molecular component of this study we use our published phylogeny for which we have sequenced more than 5,000 base pairs of nucleotide data from four genes (three plastid and one nuclear) for 21 taxa of ferns, plus six species of seed plants, three species of lycophytes, and five species of “bryophytes” as outgroup taxa (Pryer et al. 2001). This phylogeny is viewed as among the most robust for green plants (Palmer et al. 2004). For the same set of taxa, we critically reevaluate the available morphological data. Most of these characters were considered in previous cladistic analyses (Garbary et al. 1993; Mishler et al. 1994; Pryer et al. 1995; Schneider 1996; Stevenson and Loconte 1996; Kenrick and Crane 1997; Garbary and Renzaglia 1998; Rothwell 1999).

The hypothesis of a congruent phylogenetic signal in molecular and morphological data is fostered by the fact that lycophytes have been found to be sister to all other vascular plants using molecules alone, as well as using morphological data alone (Kenrick and Crane 1997; Nickrent et al. 2000; Renzaglia et al. 2000; Pryer et al. 2001; Qiu et al. 2006, 2007). Similarly, a clade comprising horsetails and ferns was first proposed on the basis of morphological data (Kenrick and Crane 1997), and this relationship was recovered subsequently with molecular data (Nickrent et al. 2000; Pryer et al. 2001; Dombrovskaja and Qiu 2004; Wikström and Pryer 2005; Qiu et al. 2006, 2007; Rothwell and Nixon 2006; Schuettpelz et al. 2006). Conflicting results have been reported in some, but not all, studies utilizing mitochondrial DNA markers. Peculiarities of mitochondrial genome evolution, such as the frequent horizontal transfer of mitochondria among lineages, are most likely the cause for these conflicts (Bergthorsson et al. 2003, 2004; Dombrovskaja and Qiu 2004; Knoop 2004; Davis et al. 2005; Wikström and Pryer 2005). Since Kenrick and Crane (1997), only two studies have found evidence for an alternative interpretation of relationships, one that does not support ferns as including horsetails (Rothwell 1999; Rothwell and Nixon 2006). These two studies utilized a morphological dataset for living and fossil plants. Therefore, one could argue that the more commonly found relationship of “ferns
plus horsetails” is an artifact caused by excluding extinct taxa, although the original concept of “monilophytes”, ferns plus horsetails, was based strictly on fossil evidence (Stein et al. 1984; Stein 1993; Kenrick and Crane 1997; Berry and Stein 2000; Cordi and Stein 2005).

Using maximum parsimony (Fitch 1971) and Bayesian MCMC inference (Yang and Rannala 1997) approaches, we independently reconstruct the phylogeny of ferns and horsetails from a morphological dataset, the same dataset used by Pryer et al. (2001). This data matrix was also employed in a study inferring the evolution of the vascular plant body plan (Schneider et al. 2002), as well as in a paper on the limits of approaches integrating fossil evidence in a phylogenetic framework that is based mainly on extant taxa (Schneider 2007). Our dataset comprises morphological characters, as well as various anatomical, biochemical, cytological, and DNA structural characters (e.g. absence/presence of introns and inversions). The classification used throughout this study is based on Smith et al. (2006) for ferns and Kenrick and Crane (1997) for other land plants. Our results are compared to those from independent analyses of molecular data (Pryer et al. 2001; Wikström and Pryer 2005; Schuettpelz et al. 2006; Qiu et al. 2006, 2007). We were particularly interested in ascertaining whether morphological data may be misleading in the phylogenetic placement of whisk ferns (Psilotales) and horsetails (Equisetopsida).

Materials and Methods

Taxon and Character Selection—We sampled 30 representatives from all major lineages of vascular plants (ingroup), including most early-diverging fern genera, as well as five outgroup taxa from all three “bryophyte” lineages (Appendix 1). We restricted this dataset to extant taxa to be able to take full advantage of all information provided by living organisms and to avoid potentially compromising our results by excluding extinct taxa, which can be scored for fewer than 20% of the characters used in our analysis (see Schneider 2007, for a more comprehensive study on the influence of fossil taxa on the results). Of the 258 morphological characters examined, we selected 136 that were parsimony informative for this study (Appendix 2). The remaining 122 characters were excluded (electronic supplement: Appendix 3) because information was either highly incomplete or unavailable, thereby preventing us from confidently defining unambiguous character states. The majority of characters were adopted from modern previous phylogenetic accounts of land plants (Parenti 1980; Garbary et al. 1993; Mishler et al. 1994; Kenrick and Crane 1997; Garbary and Renzaglia 1998), ferns (Hill and Camus 1993; Pryer et al. 1993; Schneider 1996; Stevenson and Loconte 1996; Pryer 1999; Rottwell 1999) and seed plants (Crane 1985, 1988; Doyle and Donoghue 1986, 1992; Loconte and Stevenson 1990; Doyle et al. 1994; Nixon et al. 1994; Rottwell and Serbet 1994; Doyle 1996; Nandi et al. 1998). Newly adopted characters were critically studied using primary literature and, whenever possible, also checked against herbarium specimens (F, UC). The dataset of 136 parsimony informative characters is deposited in TreeBASE (study number S2277).

Phylogenetic Analyses—We used equal-weighted maximum parsimony (MP), as implemented in PAUP* 4.0 b10 (Swofford 2002), and Bayesian inference (BI), as implemented in MrBayes 3.1 (Rannala and Yang 1995; Lewis 2001) implemented in MrBayes 3.1. Three independent Bayesian MCMC analyses were conducted using this model and four chains. Each chain was run for 5 million generations, and trees were sampled every 500 generations. Following completion, the sampled trees from each analysis were examined by estimating the standard deviation of all model-parameters in MrBayes and by determining convergence of parameters using Tracer v. 12.1 (Rambaut and Drummond 2005). All trees prior to convergence (< 1,500 generations in each analysis) were discarded as the “burn-in” phase. Each of the three analyses showed the same convergence diagnostics. A majority-rule consensus tree was calculated from a tree set in which all trees were pooled from the three independent analyses after discarding all trees from the “burn-in” phase.

The results of our analyses of the morphological dataset were compared for congruence to those recovered with DNA sequence data, as well as for testing competing hypotheses using the Kishino-Hasegawa (KH) test (Kishino and Hasegawa 1989) as implemented in PAUP*. A value of p < 0.05 was considered to be a significant difference between alternative hypotheses fitted to a given dataset. We designed reduced datasets to explicitly test for incongruence between the morphological dataset and various published phylogenetic hypotheses proposed for the fern clade (e.g. Pryer et al. 2001; Qiu et al. 2006, 2007). In particular, we tested for the following relationships among the major lineages of ferns (cf. Table 2): i) horsetails sister to whisk ferns (EQ:PS); ii) horsetails sister to leptosporangiate ferns (EQ:PO); iii) horsetails sister to the Ophioglossales plus Psilotales clade (EQ:(OP:PS)); iv) horsetails sister to marattiod ferns (EQ:MA); and v) horsetails sister to marattiod ferns, this clade sister to a monophyletic Psilotopsida (Ophioglossales plus Psilotales), and leptosporangiate ferns (Polyposidiopsida) sister to all (EQ-MA:(PS:OP)PO).

To localize conflicting signals within our morphological dataset, we performed Neighbor-Net (NNet) analyses (Bryant and Moulton 2004) and reconstructed consensus networks (Holland and Moulton 2003; Holland et al. 2004) to generate split graphs in which alternative relationships are visible. These analyses were performed using SplitsTree 4.0 beta (Huson 1998). Split graph approaches have been applied recently to explore counterfactual phylogenetic results based on molecular data, especially when long-branch attraction is suspected (Huson and Bryant 2005; Kennedy et al. 2005; Martin et al. 2005). Distance matrices were calculated in PAUP* using the “mean character difference” or “total character difference” measurement and used to perform Neighbor-Net analysis with the following settings: edge fitting as ordinary least squares; splits transformation as convex hull; modify weights as least squares; and filtering splits via maximum dimension set to either two or four. Alternative settings were explored to determine the influence of these settings on the recovered split graphs. Consensus networks were reconstructed with a threshold value of x = 0.1 and using all trees recovered in the plateau phase of the BI or in the bootstrap analyses using MP.

Results

Seven equally most parsimonious trees were found with a tree length of 348 steps (trees not shown; C1 = 0.5401, RI = 0.8218, RC = 0.4558). Bayesian inference found the same topology as maximum parsimony (Fig. 1), except for some relationships within leptosporangiate ferns (Fig. 2, morphological-only topology). Tracheophytes are found to be monophyletic with BS = 100% and DI = 11 from MP, and a Bayesian posterior probability (PP) = 1.00; this clade is supported by a broad range of character states such as the presence of vascular tissue and the occurrence of more than one apical meristem per sporephore (Appendix 2: characters 23, 36, 52, 111, 112,
and 121). The lycophytes (BS = 69%, DI = 1, PP = 0.66) are strongly supported as sister to the remaining vascular plants, the euphylllophytes. The euphylllophyte clade (BS = 89%, DI = 2, PP = 0.98) is characterized by the following putative apomorphic character states: a 30-kb inversion in the chloroplast genome, monoplastidic sperm cells, and the position of the basal body in sperm cells (characters 83, 115, 116, 118, and 136). Euphylllophytes include two sister clades, the ferns...
The fern clade (BS = 72%, DI = 2, PP = 0.53) is supported by several apomorphic character states, including lateral roots borne from endodermal cells, a plasmodial tapetum, the presence of a pseudoendospore, and an exclusively centrifugal sporoderm development (characters 40, 92, 94, and 95). The seed plant lineage (BS = 100%, DI = 18, PP = 1.00) has an impressive number of apomorphies (Table 1); several of these correlate with the derived reproductive biology of seed plants.

The topology of the seed plant lineage is identical to results found in previous cladistic morphological studies (Crane 1985; Nixon et al. 1994; Doyle 1996).

**DNA**

**Morphology**

The comparison of relationships among vascular plants. A) Morphology-only topology (this study) vs. B) molecular-only topology as recovered in various DNA based studies such as Fig. 1 in Pryer et al. 2001, Fig. 3 in Pryer et al. 2004, Figs. 3, 4 in Wikström and Pryer 2005). The topology shown as morphology-only corresponds to the topology of the strict consensus tree estimated in the maximum parsimony analyses of the morphological dataset. Taxon names are deleted but corresponding taxa are connected between both cladograms using thin lines. Thickened branches within the cladograms indicate good bootstrap support in MP (>75%) and posterior probability in BI (PP ≥ 0.95). Abbreviations: E = Euphyllophytes, EQ = Equisetopsida, F = Ferns, L = Lycophytes, MA = Marattiopsida, OPH = Ophioglossales, PO = Polypodiopsida, PSI = Psilotales, S = Spermatophytes, T = Tracheophytes.
Table 1. Morphological character state changes for selected clades. Numbers of unambiguous (before slash) and ambiguous (after slash) character state changes were reconstructed by plotting characters onto topologies obtained in the cladistic analysis of morphology (morphological topology, Figs. 1 and 2A) and analyses using either DNA sequence data alone or in combination with morphological evidence (molecular topology, Fig. 2B). For the latter, a topology was chosen in which Marattiopsida and Equisetopsida are sister clades and the gymnosperms are monophyletic (as found in Pryer et al. 2004). In both topologies (morphology and molecular), mosses are sister to tracheophytes. ‘Clade with Psilotales and Ophioglossales sister (topology obtained in analyses using DNA only or combined evidence); ‘clade with Psilotales sister to Equisetopsida (topology found in analyses using morphology only); ‘apomorphic character state changes found only with the molecular topology are in italics, all others are in both the morphological and molecular topologies.

<table>
<thead>
<tr>
<th>Clade</th>
<th>Morphological topology (Figs. 1 and 2A)</th>
<th>Molecular topology (Fig. 2B)</th>
<th>Unambiguous apomorphic character state changes for selected clades</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tracheophytes</td>
<td>12 / 8</td>
<td>10 / 9</td>
<td>59: 0 → 1 / 92: 0 → 1 / 94: 0 → 1 / 95: 0 → 1 / 121: 0 → T²</td>
</tr>
<tr>
<td>Lycophytes</td>
<td>3 / 7</td>
<td>3 / 9</td>
<td></td>
</tr>
<tr>
<td>Euphyllophytes</td>
<td>6 / 6</td>
<td>7 / 5</td>
<td></td>
</tr>
<tr>
<td>Seed Plants</td>
<td>17 / 14</td>
<td>18 / 17</td>
<td></td>
</tr>
<tr>
<td>Ferns (monilophytes)</td>
<td>4 / 8</td>
<td>5 / 12</td>
<td></td>
</tr>
<tr>
<td>Marattiopsida</td>
<td>10 / 11</td>
<td>15 / 14</td>
<td></td>
</tr>
<tr>
<td>Psilotales</td>
<td>5 / 3</td>
<td>7 / 3</td>
<td></td>
</tr>
<tr>
<td>Ophioglossales</td>
<td>4 / 7</td>
<td>3 / 5</td>
<td></td>
</tr>
<tr>
<td>Equisetopsis</td>
<td>6 / 4</td>
<td>10 / 11</td>
<td></td>
</tr>
<tr>
<td>Polypodiopsida</td>
<td>9 / 7</td>
<td>10 / 9</td>
<td></td>
</tr>
<tr>
<td>Psilotopsis¹</td>
<td>NA</td>
<td>1 / 10</td>
<td>102: 0 → 1</td>
</tr>
<tr>
<td>Psilotales + Equisetopsis²</td>
<td>5 / 7</td>
<td>NA</td>
<td>5: 1 → 0 / 8: 2 → 0 / 9: 1 → 0 / 19: 0 → 1 / 25: 0 → 2</td>
</tr>
</tbody>
</table>

Similarities in the leaves (reticulate venation, stomatal development) and reproductive biology (embryo development) support Gymnosperms as sisters to angiosperms (BS = 70%, DI = 1, PP = 1.00). Although several characters (presence of short shoots, pit structure of tracheids, and similar parenchymatous cells in the phloem) potentially could have supported an alternative topology with Gnetum as a clad comprising Pinus and Ginkgo, this topology is not found with either MP or BI.

Three out of four extant classes of ferns are well supported: Marattiopsida (BS = 100%, DI = 7, PP = 1.00), Equisetopsida (BS = 100%, DI = 5, PP = 1.00), and Polypodiopsida (BS = 99%, DI = 6, PP = 1.00). The Psilotopsida was not recovered as monophyletic. Although the orders Ophioglossales and Psilotales are each strongly supported lineages (Psilotales with BS = 97%, DI = 4, PP = 0.98 and Ophioglossales with BS = 89%, DI = 4, PP = 0.88) they are not sister to one another. Although the Psilotaceae and Equisetaceae differ radically in their gametophyte morphology, they appear here as sister taxa (BS = 75%, DI = 3, PP = 0.99), united by similarities in their sporoaphy, especially the simplified leaves and similarities of the shoot system organization (see Table 1). Other deep relationships among the extant lineages of ferns, however, lack statistical support (BS < 75%, PP < 0.95).

Leptosporangiate ferns are supported by several synapomorphies, such as the reduction in sporangium size, spore output per sporangium, number of sperm cells, and structure of the archegonial neck (characters 72, 75, 112, and 122). The earliest diverging order of leptosporangiates, Osmundales (Fig. 1), represented here by Osmunda, is somewhat intermediate between the eusporangiate and leptosporangiate condition in having about 1,000 spores per sporangium. All other leptosporangiate ferns produce fewer than 500 spores per sporangium. Osmunda is well supported as sister to all other extant leptosporangiate ferns (BS = 91%, DI = 4, PP = 1.00), but weak support was found for relationships among the remaining fern groups. Our results indicate that there are six other distinct lineages, of which two are represented by a single genus. In relative branching order, these clades are: (1) Gleicheniales represented by Gleichenia and Phanerorosorus; (2) Hymenophyllales represented by Hymenophyllum; (3) Schizaceae represented by Lygodium; (4) Cyatheales represented by Cyathea, Dicksonia, and Plagiogyria; (5) Salviniales represented by Marsilea and Salvinia; and (6) Polypodiales represented by Blechnum and Pteridium (Fig. 1). The results of the MP and BI analyses differed with respect to the monophyly of two orders of leptosporangiate ferns: Cyatheales were monophyletic in MP but not in BI, whereas Gleicheniales were monophyletic in BI but not in MP.

Discussion

Comparing Phylogenetic Hypotheses: Morphology vs. Molecules—Our analysis based on morphology resulted in a phylogenetic hypothesis (Fig. 1) that is quite similar to those derived from a combined dataset [as on p. 5] that included four genes, atpB, rbcl, rps4, nrSSU (cf. Fig. 2; Pryer et al. 2001), the same four genes plus one mitochondrial gene (Wikström and Pryer 2005), and the same Pryer et al. (2001) genes plus plastid atpA (Schuettpelz et al. 2006). All major lineages previously recovered with molecular data were also recovered using morphological data alone (Fig. 2), including trachyphytes (T), lycophytes (L), euphyllophytes (E), spermatophytes (S), ferns (F), marattioid (MA), horsetails (EQ), and leptosporangiate ferns (PO), except for the Psilotopsida (Ophioglossales + Psilotales). The last group was not resolved as monophyletic based on morphological characters (Figs. 1 and 2A), but rather it collapsed into two distantly related lineages, Ophioglossales (OPH) and Psilotales (PSI).

Our morphology data alone strongly support the phylogenetic classification proposed by Kenrick and Crane (1997), in which the fern clade (called Infradivision Moniliformopses by them) was preserved on the basis of a unique stelar pattern (Stein et al. 1984; Stein 1993; Kenrick and Crane 1997). However, the more common topology found in independent analyses of molecular plus morphological data differs substantially with respect to (1) relationships among seed plants, (2) relationships among lineages of leptosporangiate ferns, and (3) relationships among the four to five major lineages of ferns. Similar differences have also been observed between phylogenetic reconstructions based on single genes and combined genes (Pryer et al. 2001, 2004; Wikström and Pryer 2005; Schuettpelz et al. 2006; Qiu et al. 2006, 2007), as well as in other molecule- and morphology-based phylogenetic analyses within ferns (Pryer et al. 1995; Pryer 1999). Our KH tests
for incongruence between datasets and conflicting hypotheses indicated that the overall hypotheses based on molecular data do not fit very well with those derived from our morphological dataset \((p < 0.05)\). The same KH test for a single incongruence, the relationships among the five lineages of ferns recovered in the morphological vs. the molecular analyses, did not find significant differences \((p > 0.05)\). Comparison of differences among the hypotheses, using Fitch optimization, showed that the morphological hypothesis is more parsimonious with regard to characters describing leaf morphology (up to seven characters), whereas hypotheses generated using DNA sequence data, as published in previous studies (see Table 2), are more parsimonious with respect to several other characters, e.g. mycorrhizae of gametophytes and the presence of a foot in early embryo development. Overall, the inferred five hypotheses differ only slightly from each other with respect to the interpretation of character evolution, as indicated by their similar tree statistics (Table 2).

Two recent morphological studies are similar to ours in their selection of representatives of the same major lineages of land plants for the ingroup, but the authors reached different conclusions. Stevenson and Loconte (1996) included only living taxa, whereas Rothwell (1999) included both extant and extinct taxa. Both of these studies resulted in different overall topologies, and both placed the whisk ferns (Psilotales) between lycophytes and all other euphyllyphytes. This phylogenetic position of whisk ferns is most congruent with a general assumption of a progressive evolution from simple to more complex growth forms in a series of evolutionary steps from the apparent low morphological complexity of bryophytes, to the increasingly more complex lycophytes, whisk ferns, horsetails, ferns, different lineages of gymnosperms, and finally to the most derived clade, the angiosperms. The position of whisk ferns as part of a grade leading to derived vascular plants reflects the simplicity of their leaves and absence of roots. However, this hypothesis is inconsistent with ultrastructural characters observed in the spore wall (Tryon and Lugardon 1991; Lugardon and Piquemal 1993), haustorial placentas (Duckett and Ligrone 2003; Hilger et al. 2005), and sperm cells (Renzaglia et al. 2000, 2001), which suggest instead a close relationship of whisk ferns to other ferns. Another major difference between the results of our morphological study and others is in the phylogenetic position of the horsetails. Stevenson and Loconte (1996) placed horsetails close to whisk ferns as part of the grade leading to seed plants. Rothwell (1999) proposed an alternative hypothesis in which horsetails are sister to seed plants, which resulted from his interpretation that the equisetostele shares some similarities with the eustele, the latter being one of the major apomorphic character states of the seed plant lineage. Rothwell (1999) also stressed that the problem of comparing the elaborate stellar structure of extant and extinct horsetails in making homology assessments was difficult to address. According to other authors (e.g. Schmid 1982), the equisetostele differs from the eustele in the position of the protoxylem (centrarch in eustele, mesarch in equisetostele). The mesarch position of the protoxylem poles in mature stelae was proposed as an apomorphic character state for the ferns plus horsetail clade (Stein et al. 1984; Stein 1993; Kenrick and Crane 1997; Berry and Stein 2000; Cordi and Stein 2005). In addition, Stevenson and Loconte (1996) interpreted the leaf-like structures of horsetails and whisk ferns (only Timosperma) as microphylls and contrasted this character state to macrophylls in ferns and seed plants.

Differences between our results and those of other phylogenetic morphological studies (e.g. Mishler et al. 1994; Stevenson and Loconte 1996; Garbary and Renzaglia 1998; Rothwell 1999) are more likely caused by differences in character selection and homology assessments rather than by differences due to taxon sampling. Of the studies discussed here (Mishler et al. 1994; Stevenson and Loconte 1996; Garbary and Renzaglia 1998; Rothwell 1999; our study), only Rothwell’s (1999) included fossil taxa, and the incompleteness of these fossils resulted in a large number of unknown character states, thereby reducing phylogenetic resolution (see Schneider, 2007 and Wiens 1998, for discussion on the impact of incomplete data). Reduced resolution in phylogenetic reconstructions often diminishes the potential advantage of fossils to show character combinations that will benefit the discovery of the true phylogeny (see Schneider 2007).

Similar to other studies exploring the relationships among all major lineages of land plants (e.g. Garbary et al. 1993), we employed many ultrastructural characters, including the pattern of cell divisions, the structure of sperm cells, and spore wall ultrastructure. These characters appear to be highly conserved, and transformations of their character states have rarely occurred in the evolution of land plants. Interpretation of these characters derives from relatively few studies (Carothers and Duckett 1979; Brown and Lemmon 1990, 1991a, 1991b, 1997, 2001a, b; Renzaglia and Maden 2000; Renzaglia et al. 2000, 2001; Duckett and Ligrone 2003; Hilger et al. 2005). By comparison, Rothwell (1999) and Stevenson and Loconte (1996) focused more on gross morphology and as a result assessed several characters of controversial homology. In particular, two homology assessments in our dataset require a more detailed explanation to enable the reader to compare our results with the hypotheses obtained in those two studies.

Table 2. Comparison of tree statistics obtained for alternative relationships among ferns (monilophytes). Five hypotheses were superimposed on the morphological dataset. We used a reduced dataset including only two representatives for each of the five included lineages to avoid the influence of other nodes that conflict among the different hypotheses. EQ = Equisetopsida including Equisetum 1 and Equisetum 2, MA = Marattiopsida including Angiopteris and Danaea, OP = Ophioglossales including Botrychium and Ophioglossum, PO = Polypodiopsida including Osmunda and Phanerophlebus, and PS = Psilotales including Psilotum and Timosperma. Measurements: CI = ensemble consistency index, RC = ensemble rescaled consistency index, RI = ensemble retention index, TL = tree length.

<table>
<thead>
<tr>
<th>Hypothesis</th>
<th>TL</th>
<th>CI</th>
<th>RI</th>
<th>RC</th>
<th>Name and source</th>
</tr>
</thead>
<tbody>
<tr>
<td>MA(OP)((EQ:PS)(PO)</td>
<td>114</td>
<td>0.80</td>
<td>0.78</td>
<td>0.63</td>
<td>morphological hypothesis reported here</td>
</tr>
<tr>
<td>(OP:PS)(MA(EQ:PO)</td>
<td>116</td>
<td>0.78</td>
<td>0.76</td>
<td>0.60</td>
<td>molecular hypothesis 1 Pryer et al. 2001</td>
</tr>
<tr>
<td>((OP:PS)EQ)(MA:PO)</td>
<td>117</td>
<td>0.78</td>
<td>0.75</td>
<td>0.59</td>
<td>molecular hypothesis 2 Qiu et al. 2006</td>
</tr>
<tr>
<td>(OP:PS)(MA:EQ))</td>
<td>120</td>
<td>0.76</td>
<td>0.73</td>
<td>0.55</td>
<td>molecular hypothesis 3 Pryer et al. 2001</td>
</tr>
<tr>
<td>PO((OP:PS)(MA-EQ))</td>
<td>120</td>
<td>0.76</td>
<td>0.73</td>
<td>0.55</td>
<td>molecular hypothesis 4 Qiu et al. 2007</td>
</tr>
</tbody>
</table>
The first issue is the interpretation of the homology among different stelar types. We accept the interpretations put forth in studies that have taken morphological evidence from both fossil and extant species into account (Schmid 1982; Stein et al. 1984; Stein 1993; Kenrick and Crane 1997). We appreciate that different homology assessments for the organization of vascular tissues in plants are still to be considered as viable alternatives; however, we propose that the interpretation we have employed here is most congruent with existing anatomical, developmental, and phylogenetic evidence.

The second controversial issue involves the interpretation of leaves. We accept the distinctness of leaves in lycophytes (lycophylls) from superficially similar structures of horsetails and whisk ferns (euphylls), which differ substantially in their developmental biology (Kaplan 2001; Schneider et al. 2002; Harrison et al. 2005; Floyd and Bowman 2006, 2007; Beerling and Fleming 2007; Bowman et al. 2007; Gola et al. 2007; Harrison et al. 2007). It is now widely accepted that the term microphylls has been applied to an assemblage of non-homologous structures (e.g. Kaplan 2001; Floyd and Bowman 2006; Beerling and Fleming 2007). Lycophylls have a shared developmental origin of the venation and meristem organization, as well as having simple, usually unforked veins and a usually unstructured scale-like shape. Crane and Kenrick (1997) discussed a putative scenario for the independent origin of “microphylls” in lycophytes from sterilized sporangia.

Most recent authors have suggested multiple and independent origins of so-called megaphylls (Beerling 2005; Boyce 2005; Beerling and Fleming 2007). Here, we adopt an alternative concept, the euphyll, which differs from Zimmermann’s (1952) telome theory in that it does not invoke assumptions about the evolution of dorsiventrally organized leaves that are differentiated into lamina and petiole. We agree with most authors (Beerling 2005; Boyce 2005; Beerling and Fleming 2007) who have concluded that structures that differentiate into a petiole and lamina likely evolved independently in various groups of euryphyllophytes. However, we propose that leaves across euryphyllophytes are homologous based on shared developmental and structural characters, including apical/marginal growth, apical origin of the venation, the usual presence of leaf gaps in the stele, and determinate growth. This interpretation is similar to one suggested by Donoghue (in Judd et al. 2002), which he called pseudodichotomous branching, and is comparable to the proto-leaf concept suggested by Beerling and Fleming (2007). Our interpretation is consistent with a phylogenetic scenario in which euphylls evolved in the common trimerophyte ancestor of euryphyllophytes by transforming lateral shoots (Floyd and Bowman 2006, 2007). Understandably, this may be seen as reductionist, because it considers only some criteria usually used to define leaves and invokes only the first step in the evolutionary sequence proposed in the telome theory of Zimmermann (1952).

**Can Morphological Datasets be Positively Misleading?**—
Misleading results in studies using DNA sequence data are well known and can be attributed to several factors, including insufficient taxon sampling, long-branch attraction, saturation, and lineage-specific substitution rate changes (Felsenstein 1978; Hendy and Penny 1989; Magallón and Sanderson 2002; Burleigh and Mathews 2004; Martin et al. 2005). Less attention has been given to possibly misleading results in phylogenetic analyses utilizing morphological evidence. Phylogenetic hypotheses based on single genes are, in reality, “gene-trees” that may or may not be identical to the true phylogeny of a group of organisms (Doyle 1992). A parallel argument can be applied to many phylogenetic studies using morphological evidence, because many datasets are biased toward particular organs. This is especially true in studies based exclusively or partly on fossil data, which rely in general on characters that are preserved in the incomplete fossil record. Such studies are more appropriately considered “organ phylogenies” (Bateman et al. 1998).

With regard to our dataset, the possibility of long-branch attraction needs to be considered. Assembling morphological apomorphic characters for each of the five lineages of ferns, as well as for other land plants, results in each extant lineage having a unique combination of characters that allows for easy identification. The low number of characters that are putatively shared across all taxa, which are further fractioned into homoplastic and plesiomorphic character states, adds to our concern for a possible misleading bias that associates Psilotales with Equisetopsida (Fig. 1). Our Neighbor-Net analyses to explore alternative signals within the morphological dataset found evidence for a different relationship, one that places Psilotales more closely to Ophioglossales (Fig. 3).

In addition, we performed separate parsimony analyses that sequentially excluded each one of the five lineages of ferns. Only the exclusion of horsetails (Equisetopsida) altered the PSI + EQ topology, and in that case the Psilotales were sister to the Ophioglossales. The five lineages of ferns show strikingly different growth forms and share only a few anatomical and ultrastructural characters (Table 1). The placement of Psilotales as sister to Equisetopsida in our parsimony and Bayesian analyses may be the result of shared homoplastic character states and thus could be interpreted as a consequence of long-branch attraction. Molecular data suggest a sister relationship between Ophioglossales and Psilotales, a hypothesis that is consistent with some morphological characters, including heterotrophic gametophytes with multicellular rhizoids, the reduction of the root systems, and a peculiar placement of the sporangia on an elongate axis of uncertain homology (Pryer et al. 2001; Schneider et al. 2002). This last character was not included in the data matrix and needs further exploration.

A general issue in morphological studies is the problem of polarization of character states. In our study, about 40% of the characters scored were not applicable for the outgroup taxa (bryophytes) and thus were scored as missing data. Most of these nonapplicable characters describe gross morphological features; ultrastructural data, on the other hand, provide a much higher percentage of the characters that are applicable to the outgroups. A high percentage of nonapplicable characters are also found among the sexual reproductive characters, because all extant members of the seed plants have a highly modified reproductive system in comparison to other land plants. Several informative characters, such as the ultrastructure of sperms cells, were applicable to only two seed plant lineages, *Cycas* and *Ginkgo*, which show several plesiomorphic character states in their reproductive biology. The correct polarization of seed character states using extant land plants is likely impossible and will need to rely on the integration of fossil data (Schneider 2007). Unfortunately, correct polarization is hampered in two ways by the incompleteness of the fossil record. First, many critical taxa are not preserved or still await discovery by specialists. Second, preserved organisms are incomplete and critical structures are often not attached to each other (Kemp 1999; O’Leary 2001). In many cases, a
detailed study of the evolution of particular structures (not organisms) may be the best and only approach that will lead to the integration of fossil evidence into phylogenetic hypotheses dealing with whole organisms. Studies by Stein and coworkers on the evolution of vascular tissue in Devonian land plants are outstanding examples of this approach (Stein 1993; Berry and Stein 2000; Cordi and Stein 2005). The alternative, relying on whole plant reconstruction, may offer much less information because of the ambiguities in these reconstructions and the scarcity of taxa with an adequate fossil record.

Why are Morphological Studies Needed?—It is common for morphological data to be considered less important than DNA sequence data in phylogenetic studies (Endress 2002). Arguments against the use of morphological data include the high amount of homoplasy and the notorious problems associated with making homology assessments (Bowe et al. 2000), but objections to these arguments are raised in several contributions to this important discussion (Sanderson and Donoghue 1996; de Queiroz 2000; Donoghue and Ree 2000; Hillis and Wiens 2000). Morphological characters differ substantially from DNA sequence characters in their complexity and their frequency of evolutionary change. Many morphological characters show a much lower mutational rate than nucleotides and thus may be less-prone to problems such as saturation. Most especially, the conservation of developmental pathways and functional aspects can contribute to the persistence of certain character states in major lineages of organisms (Raff 1996; Arthur 1997; Donoghue and Ree 2000; Endress 2002). Therefore, some morphological characters are likely to be ideal phylogenetic characters because they allow us to identify single (and hence rare) evolutionary events (Bateman 1998, 1999; Endress 2002). For example, characters associated with critical steps in plant life cycles are more likely to be conserved in major lineages. Several studies demonstrate that certain anatomical and morphological characters, e.g., sperm cell ultrastructure and spore wall ultrastructure, are informative for phylogenetic studies focused at deep nodes (Kenrick and Crane 1997; Graham et al. 2000; Renzaglia et al. 2000; Schneider et al. 2002). Other morphological characters, such as the density of leaf indument, have been modified frequently during land plant evolution in response to various environmental factors, and they may be more informative in studies focused on species-level relationships. As we demonstrate with this study, morphological studies based on a careful evaluation of all potentially informative characters can generate well-supported phylogenetic results.

Four additional arguments can be put forth to support the use of morphological data in phylogenetic reconstruction. First, many theoretical and empirical studies have shown that all accessible information should be used to obtain the most robust phylogenetic hypotheses (Kluge 1989; Mishler et al. 1994; de Queiroz et al. 1995, 2001; Bateman 1998; de Queiroz 2000; Hillis and Wiens 2000; Magallón 2007; Schneider 2007; Hermsen and Hendricks 2008). Second, morphological data are the only set of characters that are observable in both fossil and living taxa (Donoghue et al. 1989; Smith 1998; Springer et al. 2001; Teeling et al. 2005; Hermsen and Hendricks 2008), although many informative morphological structures (e.g., the ultrastructure of meristems and the spindle apparatus controlling cell divisions) are rarely or never

![Diagram](https://via.placeholder.com/150)

**Fig. 3.** Neighbor-Net graph generated using SplitsTree4. The analysis was performed using Convex Hull as the chosen splits transformation, least squares as modify weights, and two maximum dimensions as the filter for selected splits. Abbreviations: F = Ferns, OPH = Ophioglossales, OSM = Osmundales, PSI = Psilotales. The arrow indicates the fern node (F) that is marked with a gray circle.
preserved in the fossil record. Similarly, fossilized DNA is certainly exceptional in phylogenetic studies (Smith 1998; Wills and Forsyth 2000). The inclusion of fossils in phylogenetic studies enables an increased taxon sampling that is especially critical in studies that address assembling the tree of life. This is particularly notable given that Jablonski (2004) estimated that more than 95% of species that ever lived are now extinct. Third, recent attempts to integrate developmental genetics and evolutionary biology in a new approach, called "evolutionary developmental genetics" (Hall 1992; Raff 1996; Arthur 1997), relies on explicit statements about character state changes in the evolution of the inferred group (Bang et al. 2000; Endress 2002; Schneider et al. 2002; Cracraft 2005). The explicit definition of discrete character states and the careful scoring of all taxa in a particular study are critical components in the reconstruction of character evolution, whether morphological data, molecular data, or both, are used in phylogenetic reconstruction. This procedure is preferred over the simple mapping of characters loosely obtained from the literature without a strict consideration of character states. Plotting morphological character states onto a phylogeny without detailed studies is adequate if explicit statements about the character states and data for all critical taxa can be obtained from the literature. However, character state definitions that are suitable for phylogenetic studies are rarely found in traditional botanical literature. In addition, without undergoing the process of collecting data for an extensive morphological matrix, important characters can be easily overlooked. Several critical but cryptic character state changes (e.g. exclusively centrifugal spore wall development) have been ignored in the past, and we have shown in this study that they can be important apomorphies of clades and thus are critical for the circumscription of taxonomic units. They may also point to major changes in developmental pathways that were involved in the formation of a particular character state. Transformation statements generated using a phylogenetic framework will provide critical information about morphological innovations and the corresponding changes in developmental programs in the evolution of land plants (e.g. Graham et al. 2000; Renzaglia et al. 2000; Schneider et al. 2002; Bowman et al. 2007; Floyd and Bowman 2007).

The last argument for the continued use of morphology when reconstructing phylogeny recalls Hennig's concept of reciprocal illumination (Hennig 1950; Daly et al. 2001). Each cycle of recompiling and reanalyzing matrices will provide us with new insights as the result of critical reflection on previous studies using morphology or other kinds of evidence, as well as the consideration of newly obtained evidence. This iterative process ultimately results in improved concepts of homology, which in turn result in the discovery of apomorphic characters that are not only critical to phylogenetics but also to a natural classification of organisms.

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Outgroup Taxa—Marchantophyta.  Haplomitrium Nees, ... in Marsileaceae. The term is used here only for pulvini as in Marattiaceae.  Hill and Camus (1986),  Kubitzki (1990).

Blechnum eufylls.  Psilotaceae are forked, and extinct relatives of Equisetaceae had forked or mentioned as a criterion of euphylls. Sporophylls of both living genera of development ( Freeberg and Wetmore 1967;  Kaplan 2001;  Imaichi, pers. comm.) and they may be derived specialized microphylls) and non-homologous to euphylls ( von Goebel 1930;  von Guttenberg 1966). Lycophylls lack apical size, the absence of blade/petiole differentiation and reduced vascular fronds and leaves may be based on differences in ptyxis and growth pat-

1. Sporophyte—A.  Leaf.  The term leaf is used here as a synonym for euphyll (= megaphyll), which is consistent with the morphological literature (Bierhorst 1971; Gifford and Foster 1988; von Goebel 1930; Troll 1935). The leaflike structures of Lycophyta are considered as lycophylls (specialized microphylls) and non-homologous to euphylls (von Goebel 1930; Kenrick and Crane 1997). Lycophylls develop differently than euphylls (von Guttenberg 1966; R. Imaichi, pers. comm.) and they may be derived from sterile sporangia (Crane and Kenrick 1997). Characters of euphylls do not apply to lycophylls, since they are not homologous. In pterido-

1. (M et al. 102) Sporophyte with lycophylls: absent (0); present (1). Lycophylls are not homologous to euphylls, resulting in “not applica-

3. (PSS 4;  R 23;  SL 26) Leaf ptyxis: erect (0); circinate, folded in same direction as the primary axis (1); circinate, but folded at a 90° angle to the primary axis (2); convolute or conduplicate folded (3). The develop-

4. (PSS 4;  R 25;  SL 38) Blade dissection: simple to dissected (1). This character applies to mature, photosynthetic blades only. Blade dissection differs remarkably among ferns, but further states do not improve the quality of the analysis at the phylogenetic level under study here. Only trends toward compounding or simple blades are informative. von Goebel (1930), Bierhorst (1971), Gifford and Foster (1988), Kubitzki (1990).

5. (D 6;  PSS 3;  SL 104;  105;  20, 21, 22) Blade dissection: simple to dissected (1). This character describes the position of secondary veins/pinnae to primary ones. Geometrically, one of two positions is usually favored, catadromous or anadromous (see Kramer 1987 for definiti- on). The patterns are most frequent among the most fern genera and only a few taxa show intermediate or mixed dromy patterns (isodromous). The charac-

6. (PSS 14;  SL 37) Dromy at base of blade (proximal pair of pinnae): catadromous (0); anadromous (1); isodromous (2). Dromy applies only to compound leaves. This character describes the position of secondary veins/pinnae to primary ones. Geometrically, one of two positions is usually favored, catadromous or anadromous (see Kramer 1987 for definiti- on). The patterns are most frequent among the most fern genera and only a few taxa show intermediate or mixed dromy patterns (isodromous). The charac-

7. (PSS 4) Primary blade vein form: solitary/unbranched (0); dichot-

8. (PSS 5) Vein orders: one (0); two or more (1). This character is used as in Doyle (1996). Vein orders higher than two reflect the width of the blade. Bower (1926), Kubitzki (1990).

9. (D 9;  PSS 5) Vein orders: one (0); two or more (1). This character is used as in Doyle (1996). Vein orders higher than two reflect the width of the blade. Bower (1926), Kubitzki (1990).

10. (D 8;  PSS 7;  R 22;  SL 110;  SL 38) Vein fusion (in sterile blades): non-

11. (PSS 11) Blade scales: absent (0); present (1). Scales are interpreted here as not homologous to scalelike structures in seed plants, which are mostly reduced leaves. Scales are modified hairs that are devel-

12. (PSS 5;  SL 32) Pulvinus: absent (0); present (1). The pulvinus in Marattiacaeae are not similar or homologous to structures called pulvini in Marsileaceae. The term is used here only for pulvini as in Marattiacaeae. Hill and Camus (1986), Kubitzki (1990).
13. (PSS 16; S 115, 117; SL 30) Pneumathodes: absent (0); present and scattered all around petiole and/or rachis (1); present and borne in discrete lines or patches on petiole and/or rachis (2). Bower (1923, 1926), Davis (1991), Kubitzki (1990), Wolf et al. (1999).

14. (PSS 17; SL 23, 24, 25) Blade/leaf/pinna articulation: absent (0); present (1). Leaf articulation is common in seed plants. In ferns, blade/leaf/pinna articulation usually occurs in Davalliaeae and Polyopodioideae (including gramminites), and it occurs occasionally in some other families (e.g., Cyatheaeeae, Dryopteridaceae, Osmundaceae (Osmunda), Lygodiaeae (Lagodium), and Thelypteridaceae). von Goebel (1930), Bierhorst (1971), Gifford and Foster (1988), Kubitzki (1990).

15. (PSS 19; R 55; S 95; SL 30) Axial outline of petiole and rachis: combined (1); separated (2). This character is sometimes scored as absent in Angiopteris and Marattia, even though they sometimes possess isolated sclereids in the hypodermis of the petiole. These cells, however, are never arranged in bundles or fibers. Ogura (1972), Hill and Camus (1986), Kubitzki (1990).

16. (PSS 21) Sclerenchymat fibers: absent (0); present (1). Sclerenchymat fibers are scored as absent in Angiopteris and Marattia, even though they sometimes possess isolated sclereids in the hypodermis of the petiole. This indicates that intercalary growth may be more widespread in Embryophyta.

I. B. Shoot. The terms shoot and thallus are used synonymously in this section.—

23. (M et al. 82; KC 3 2 3; SL 4, 5) Independent, branched sporophyte with multiple sporangia: absent (0); present (1). This character combines the independence and branching of sporophytes. Further division of this character might be useful only if some fossil taxa were included. Sporophytes of some vascular plants are generally branched, as in Ophioglossaceae and Marattiales, but mature sporophytes are independent and have multiple sporangia. In some groups of vascular plants, especially Lycopsidae, Psilotidae, and Ophioglossales, the gametophyte is long-lived and it is attached to the sporophyte for a long time. von Goebel (1930), Gifford and Foster (1988), Rothwell (1995).

24. (Q 18; GR 12; NCSF 7; PSS 92; R 35; SL 18) Position of protostomium: exarch (0); mesarch (1); endarch (2). Pryer et al. (1995) excluded this character, stating that data were insufficient or unreliable for many fern taxa. The position of protostomium poles is defined by the direction of xylem differentiation. Xylem maturation information is available for all major basal land plant groups. It is not clear if all leptosporangiate ferns have a morphological xylem, as suggested by Inoue and Eulalia (2004), or whether they may be endarch. Pryer et al. (1995) suggested that data were insufficient or unreliable for many fern taxa. The position of protostomium poles is defined by the direction of xylem differentiation. Xylem maturation information is available for all major basal land plant groups. This character combines the arrangement of leaves in whorls of six or more and a system of three kinds of lacunae/canals in the shoot (medullary canal, vascular canal, and molecular canal, protoxylem canal). Ogura (1972), Esau (1977), Khandelwal and Gorham (1972), Kato and Imaichi (1997), Takiguchi et al. (1997).

25. (PSS 26; S 5; SL 32; SL 8) Shoot symmetry: radial (0); dorsiventral (1); both (2). Semi-erect to erect shoots are treated as radial, while creeping shoots are interpreted as dorsiventral. Equisetum and Polystichum have two different kinds of shoots, a creeping subterranean shoot and an erect aerial shoot. This is treated as a combination of dorsiventral and radial shoot symmetry, rather than as a polymorphism, because both growth forms are observed consistently and uniformly in all individuals and species of both genera. Bower (1923, 1926), Kaplan (1977), Kubitzki (1990).

38. (PSS 80; SL 1) Root origin: primary allorhizic (0); homorhizic or secondarily allorhizic (1). Character reflects the embryonic orientation of the root pole to the shoot pole. Primary allorhizic patterns are the result of the root pole not in opposition to the shoot pole (unipolar embryos). Embryos of homorhizic plants have shoot poles in opposition to root poles (bipolar embryos). The first root is short-lived in Magnoliida, especially Liliopsida. Their root systems are classified as secondarily allorhizic (von Goebel 1930, Gifford and Foster (1988), Groff and Kaplan (1988).


40. (New) Lateral root origin in endodermis: absent (0); present (1). Character is applicable only to taxa with monopodial, branching roots. Lateral roots in leptosporangiate ferns, Marattiaceae, and Ophioglossaceae originate in the endodermis, while their place of origin in seed plants is the pericycle/pericambium. The endodermis is part of the inner cortex and the pericycle is part of the vascular tissue. In Equisetum, lateral roots originate in a layer internal to the endodermis, which is formed by a division of the initial cells of the endodermis. von Guttenberg (1964, 1966, 1968, 1971).

41. (PSS 34; R 17; S 4, SL 3) Root hairs: present (0); absent (1). Root hairs are absent only in Ophioglossaceae, but they are sometimes rare or uncommon in water plants such as Isoetes and Marsileaceae. The expression of root hairs in aquatic taxa is influenced by environmental conditions. Therefore, root hairs are treated as present in aquatic taxa, but scored as absent in Ophioglossaceae (terrestrial). von Guttenberg (1968a, b), Ogura (1972), Schneider (1996).

42. (S 6, SL 3) Root hair structure: non-septate (0); septate (1). Root hairs are always unicellular, though they appear multicellular in Marattiaceae. This is the result of septae formed by the cell walls. Septate root hairs also occur occasionally in some species of Actinostachys and Ochlandra. Ogura (1972), von Guttenberg (1968a, b), Ogura (1972), Schneider (1996).

43. (S 5) Rhizodermis cells: undifferentiated (0); differentiated into long and short cells (1). Trichoblasts (rhizodermis cells that develop root hairs) and atrichoblasts (rhizodermis cells without root hairs) do not differ in shape and size in most land plants, but they are differentiated in some taxa (e.g., Anemia pro parte, Azolla, Equisetum, Hyperi, Lycopodium pro parte, Schizaea). Leavitt (1904), von Guttenberg (1968a, b), Ogura (1972), Schneider (1996).

44. (S 8) Root pit: absent (0); present (1). In leptosporangiate ferns, the vascular tissue forms a compact circle with a central pith in the center of the root cross-section, whereas non-vascular tissue is central in Equisetopsida, Marattiopsida, and Ophioglossopsida. A pith is also present in most gymnosperms, such as Cupressaceae, and some angiosperms, most of which, but absent in eucommioideae (von Guttenberg 1968a, b), Ogura (1972), Esau (1977), Fahn (1990), Stevenson (1990), Norstog and Nicholls (1997). Schneider (1996).

45. (PSS 35; R 16; S 9) Number of protosteolole in roots: variable, ranging from monorchid to 18-arch (0); variable, ranging from monorchid to hexarch, rarely non-arch (1); usually diarch, rarely triarch (2). This character describes the number of protosteolole in the vascular system of a root of a mature plant. Roots of young plants tend to have fewer protosteolole than roots of mature plants. Derived leptosporangiate ferns always possess diarch vascular bundles in roots. The number of protosteolole is more variable in eusporangiate ferns and seed plants, and it is often correlated to the size of the plant. More than two protosteolole are usually present in Ophioglossaceae, Gleicheniaceae, and some other early-diverging fern families, von Guttenberg (1968a, b), Schneider (1996).

46. (S 18) Aerenchyma in root cortex: absent (0); present, septate cells not differentiated (1); present, septate cells differentiated (2), Aerenchymatous tissue is found only in Azolla, Acrocladium, Ceratopteris, Equisetum, Isoetes, Marsilea, Plagiochila, and Selaginella. Cells separating the lacunae are defined as septate cells. These characters may be composed of parenchymatous cells with a unique shape (Schneider 1996). von Guttenberg (1968a, b), Ogura (1972).

47. (PSS 94, S 12) Inner root cortex: parenchymatous (0); sclerenchymatous (1). The root cortex of ferns is divided into an inner and outer cortex, which is the product of two different cell lineages. The majority of modern leptosporangiate ferns possess a sclerenchymatous inner root cortex, von Guttenberg (1968a, b), Schneider (1996).

48. (PSS 94; R 10) Outer root cortex: parenchymatous (0); sclerenchymatous (1). A sclerenchymatous outer root cortex is common in Osmundaceae, Gleicheniaceae, and some close relatives. von Guttenberg (1968a, b), Schneider (1996).

49. (S 15) Cells of the innermost cell layer of the root cortex: not different in size, shape, and/or number from the adjacent cell layers (0); size, shape, and/or number from the adjacent cell layers in different species (1). Cells of the innermost cell layer of the root cortex obviously differ from the adjacent cell layers in size and shape in some fern families (e.g., Lindsaeaceae, Pteridaceae, Schizaeaceae, sensu Smith et al. 2006). Schneider (1996).

I. D. Anatomical and morphological characters that are applicable to more than one sporophyte organ.

50. (PSS 81; SL 1) Apical meristem of root and shoot: with single apical cell or up to 4 initial cells (0); more than 4 initial cells, complex meristem (1). A salient feature of all ferns except Marattiaceae and Osmundaceae is that a single, conspicuous apical cell is present in the zone of surface initials, and this cell can usually be identified at some point in the development of the apex (Gifford 1983, 1985). This single cell may be replaced by up to four conspicuous cells in Marattiaceae and Osmundaceae, though Imaichi (1986) has shown that the shoot apex of Angiopteris lygodiofolia possesses a single apical cell. This information supports the presence of a monolep apex (with a single initial) in all fern groups, including Equisetaceae, Ophioglossaceae, and Psilotaceae. Monolep apical meristems are also known from some species of Selaginella (von Guttenberg 1966; Philipson 1990), but Lycopodiaceae and Isoetaceae have a more complex apical meristem. Complex apical meristems are also known from Spermatophyta, but these differ in their structure from those of Lycopodium. Additionally, each order of Lycopsida (Isoetales, Lycopodiales, Selaginellales) has a unique type of apical meristem. Karrfeldt (1977) reported the exceptional occurrence of a single apical cell in Isoetes, but the genus usually possesses complex apical meristems. Bower (1923), von Guttenberg (1960, 1961), Bierhorst (1971, 1977), McAlpin and White (1974), Gifford and Corson (1977), Stevenson (1976, 1978a, 1978b), Imaichi (1977), Halperin (1978), White (1979), Gifford (1983, 1984), and Camus (1986), Imaichi and Nishida (1986), Gifford and Foster (1988), Hébant-Mauri (1993), Barlow (1994a, b).

51. (S 7) Endodermal cells in root and shoot: primary type (0); secondary type (1); tertiary type (2). The endodermal cells of all land plants show a similar development. Initially, a Casparian strip is formed (primary), followed by a suberin lamella that develops to cover all cell walls (secondary). Only in angiosperms does the cell wall thickness increase in an additional step (tertiary). Lycopsida, Equisetopsida, eusporangiate ferns (Marattiaceae, Ophioglossaceae, Psilotaceae) and some other early-diverging fern families (Osmundaceae, Salvinieae, vittarioideae, Lycopsida, Pteridaceae, and Psilotaceae) possess only the primary stage, while all other ferns and gymnosperms form endodermal cells with a suberin lamella. Most species of Hymenophyllaceae have a secondary endodermis, but the primary type is known in some species of Venushubia. Their occurrence is considered an apomorphy for this taxon. Ogura (1938), von Guttenberg (1947), Van Fleet (1961), Schneider (1996).

52. (M et al. 83; SL 12, 13) Xylem and phloem differentiation: absent (0); present (1). Specialized cells with a conductive function (hydroids and leptoids) in some Bryopsida (Pteridophyta) are often described as “precursors” to tracheids and sieve cells. Hydroids/tracheids and leptoids/sieve cells do share functional features, but they may represent convergent structures. Tracheids occurring in the gametophyte of Psilotum (Bierhorst 1971; Hébant 1976) may be present only because of the longevity of this phase, and they do not provide phylogenetic information. Esau (1977), Hébant (1979), Fahn (1990), Kenrick and Crane (1991), Cook and Friedmann (1998), Ligrone et al. (2000).
flowering plants differ in their ultrastructure from Strassburger cells found in the phloem of Coniferidra, Cycadatea, Ginkgoatea, and Gnetidra, which could be a synapomorphy of a gymnosperm clade. However, neither Strassburger cells nor companion cells are found in lycophytes and ferns. Presence of Strassburger cells is not scored as an independent character state, and detailed studies are needed to confirm the homology and coding given in Behnke and Sjolund (1990). Perry and Everit (1975), Warmbrodt (1980), Kubitzki et al. (1993).

55. (New) Sieve cells with refractive spherules: absent (0); present (1). A detailed comparative study is lacking for the ultrastructure of sieve elements in ferns; however, refractive spherules appear to be a common character in Euphyllophyta but absent in Lycophtyta, Perry and Everit (1975), Warmbrodt (1980), Behnke and Sjolund (1990), Kenrick and Crane (1997).


58. (SL 14) Muclilage-producing hairs: absent (0); present (1). Muclilage-producing hairs are present in various groups of ferns. The character may reflect ecological constraints. Tryon and Tryon (1982), Kubitzki (1990).

I. E. Sori/Sporangia/Sporores.

59. (PSS 47; R 65) Sorus: absent (0); present (1). Eames (1936), Bierhorst (1971), Tryon and Tryon (1982), Hill and Camus (1986), Kubitzki (1990).

60. (PSS 48) Sorus outline: round (0); elongate (1). As in Azolla (Salviniaeae), the Marsileaceae possesses an elongate receptacle, which is attached to the sporocarp wall only at the receptacle base. Eames (1986). Bierhorst (1971), Tryon and Tryon (1982), Hill and Camus (1986), Kubitzki (1990), Nagalingum et al. (2006).

61. (PSS 49; R 66, 61, 62; SL 47) Sporangial position on bladed fertile leaf-segments: abaxial, marginal to dorsal (0); adaxial (1). Sporangia are located either on the abaxial or the adaxial side of leaves. Some ferns show a marginal position of the sporangia, but they are located closer to the abaxial than to the adaxial side. The distinction between the dorsal and marginal position of sporangia that is used for the identification of genera is informative only for phylogenetic analyses focused on derived families or genera, but not for relationships across all ferns. The identification of dorsal position is blurred in some taxa that illustrate the changes in the development of sporangia in early-diverging leptosporangiates. The receptacle is a difficult character to code, and developmental and comparative studies are required to understand this character. Our coding reflects what we know. Campbell (1895), Eames (1936), Bierhorst (1971), Kubitzki (1990), Churchill et al. (1998).

71. (SL 61) Sporangial shape: reniform (0); globose to elongate (1). Sporangial shape of heterosporous ferns is based on the microsporangium. von Goebel (1930), Gifford and Foster (1988), Kenrick and Crane (1997), Nagalingum et al. (2006).

72. (PSS 42; R 73, 79; S 23; SL 48) Sporangial wall thickness/development: more than two cell layers, eusporangiate development (0); one cell layer, leptosporangiate development (1). The sporangial wall of leptosporangiate ferns is thin in comparison to other lower plants. The outer layers of the tapetum are persistent, which is why the sporangial wall has sometimes been reported as two-layered (Bower 1923, 1926). They also differ slightly in their development from other leptosporangiate ferns (Bierhorst 1971). Detailed comparative studies that illustrate the changes in the development of sporangia in early-diverging leptosporangiate ferns are lacking. In spite of the differences, the sporangia in Osmundaceae are clearly leptosporangiate, but with some similarities to conditions found in eusporangiate ferns, especially Marattiaeae. von Goebel (1930), Gifford and Foster (1988).

76. (PSS 56; R 74, S 24; SL 64) Annulus: absent (0); present (1). Bower (1923, 1926, 1928), Bierhorst (1980), Kubitzki (1990).

77. (PSS 57; R 74, 75, 76; S 26, 27; SL 64) Annulus aspect on sporangium: apical (0); lateral (1); oblique to transverse (2); vertical to slightly

93. (New) Paraxoplastic meiosis (0); present (1). A unique structure of Selaginellaceae and Isoëtaceae. Tryon and lugardon (1991).


95. (New) tapetum type: parietal (0); plasmodial (1). Tapetum type is highly conserved in major groups of land plants and tapetum evolution may reflect important divergence events in land plant evolution. Detailed studies are lacking for many ferns, but all reports support the presence of a plasmodial tapetum. Comparative studies may provide evidence for further subdivision of the character states. Pacini and Franchi (1991), parkinson and pacini (1995), pacini (1997).

96. (New) Harmomagnetic character of spores/pollen: maximum size in water; maximum size in sucrose concentrations (1). The term harmomagnetic (Pacini 1990) describes the behavior of pollen and spores in water. Spores of “pteridophytes” and “bryophytes” have their maximum size in water, whereas pollen of seed plants achieves a maximal size in sucrose solutions. This character reflects a change in chemical composition of the spore wall between “pteridophytes” and seed plants.

97. (PSS 61; S 46; SL 69) spores chlorophyllous: no (0); yes (1). Kubitzki (1990), Tryon and lugardon (1991).

II. Gametophyte—

98. (PSS 67; S 140; 141; SL 85) spore germination pattern: equatorial (0); polar (1); amorphous (2). Data missing for several taxa. Campbell (1985), Nayar and Kaur (1968, 1971), Bierhorst (1971), DUCKETT and PANG (1984), Whittier and PINTAUD (1999).

99. (KC 3.16; PSS 68; R 93; S 132, 133, 134, 135; SL 90) gametophyte form: tuberous (0); filamentous (1); cordate to elongate thalloid (2); reduced to relatively few cells (3). Payer et al. (1995) coded Equisetum as elongate thalloid. Similarly, Stevenson and loconte (1996) coded Equisetum as ribbon-like. Neither assessment fits well with the descriptions by DUCKETT (1973, 1979). Equisetum gametophytes are more adequately described as tuberous-cylindrical with filamentous to lamellar appendages; we treat them here as tuberous. Gametophytes of bryophytes are more complex and therefore more difficult to characterize. For purposes of our analysis, we compare the juvenile gametophyte stages of bryophytes with the adult gametophytes of tracheophytes, since they are developmentally homologous to one another. Darrell-Smith (1917), Bower (1923, 1926, 1928), Holloway (1939), Stokley (1951), Atkinson and stokley (1964), Schuster (1967, 1992), Bierhorst (1971), Nayar and Kaur (1971), Atkinson (1973), DUCKETT (1973, 1979), tryon and tryon (1982), Kubitzki (1990), Whittier and PINTAUD (1999).

100. (New) Gametophyte with gleichenia-type club-shaped hairs: absent (0); present (1). Only club-shaped hairs that develop from special wedge-shaped initial cells are scored. This kind of hair development is known only from gametophytes of Gleicheniaaceae. Club-shaped gametophytic hairs found in other fern genera differ in their development. Nayar and Kaur (1971).

101. (New) gametophyte with bristle- to scale-like hairs: absent (0); present (1). Gametophytes with bristle-like hairs are known only from Lomariaceae and Cyatheaceae. These hairs are large, pluricellular, thin-walled, chlorophyllous, 2 to several cells wide, and several cells long. Nayar and Kaur (1971).

102. (GR 4; PSS 71; SL 88) obligate mycorrhizae in gametophytes: absent (0); present (1). Obligate mycorrhizae are restricted to non-green gametophytes. Mycorrhizae are known also from different groups of non-vascular land plants with green, autotrophic gametophytes (e.g., Marattiaceae, Osmundaceae). Payer et al. (1995) included the presence of facultative mycorrhiza in this character, but the occurrence of facultative types of mycorrhiza is insufficiently studied. Spores of taxa with non-green gametophytes germinate in dark, while light induces germination of taxa with green gametophytes (whittier and PINTAUD 1999).

Gametophyte dependence: independent (0); dependent meiospore, endosporic gametophyte (1); dependent on sporophyte (2). Bell (1979), Gifford and Foster (1988).

Gametangia distribution: widely distributed (non-terminal) (0); more or less terminal (1). The position of gametangia is one of the main differences between the Lycophyta and Euphyllophyta. Gifford and Foster (1988), Kubitzi (1990).


Position of antheridia on gametophyte: embedded or slightly projecting (0); partially to fully exposed (1). This character is correlated with the development of antheridia (Keng and Crane 1997). Detailed investigations of the developmental processes associated with antheridia may provide important information about the evolution of early-diverging groups of ferns. Campbell (1895), Schuster (1967, 1992), Bierhorst (1971), Nayar and Kaur (1971), Renzaglia (1978).

Antheridial operculum: absent (0); lateral, circular (1); terminal, circular (2); triangular (3); pore (4). It is not possible to determine the character state that best applies to heterosporous taxa, which have extremely reduced antheridia. This is also the case for other antheridial characters, such as the presence of an apical cell and stalk. Hartman (1931), Atkinson and Stokey (1964), Nayar and Kaur (1971), Duckett and Bell (1977), Garbary et al. (1993), Garbary and Renzaglia (1998).


Spermatogenous cell arrangement: blocks (0); random (1); single (2). Campbell (1895), Bower (1923), Garbary et al. (1993), Garbary and Renzaglia (1998).

Number of sperm cells: more than 1,000 (0); 100 to 1,000 (1); 16 to 64 (2); 2 to 15 (3). Bower (1926), Eames (1936).

Sperm transfer with pollen tube: absent, zoodiogamum (0); suspended, zoidiogamum (1); penetrating, siphogamum (2). Gifford and Foster (1988).

Spore flagellate: biflagellate (0); multiflagellate, 3-29 (1); multiflagellate, 30 to 100 (2); multiflagellate, >100 1,000 to 10,000 (3). Detailed observations on sperm flagella are published for only a few genera. Little is known about the constancy of flagellar number per sperm cell in leptosporangiate ferns. However, it is clear that all leptosporangiate ferns examined have between 30 and 80 flagella. Preliminary studies (Renzaglia et al. 2000) have shown that *Psilotum* has about 36 flagella. Sperm cells of lycophytes differ substantially in their development from sperm cells of bryophytes and euphyllophytes (Renzaglia and Maden 2000). In addition, the development of sperm cell basal bodies is identical in taxa of ferns and seed plants (Cycadales and Ginkgoales) with free sperm cells. This character is correlated with the development of antheridia. Keng and Crane (1997). Detailed investigations of the developmental processes associated with antheridia may provide important information about the evolution of early-diverging groups of ferns. Campbell (1895), Schuster (1967, 1992), Bierhorst (1971), Nayar and Kaur (1971), Renzaglia (1978).

Spermatophyte—The embryo is recognized separately from the sporophyte because its phenotypic expression may be largely influenced by the gametophyte.


Embryo orientation: esocospotic (0); endocytotic (1); proterotic (2). Bierhorst (1971), Kubitzi (1990), Keng and Crane (1997).

Embryo suspensor: absent (0); present (1). The development of a suspensor is a fixed condition in some land plant groups. von Guttenberg (1960, 1961, 1966).

Gametophyte phase dominant: gametophyte long-lived, sporophyte short-lived (0); sporophyte long-lived, gametophyte short-lived (2). The shift from an isomorphic to a heteromorphic life cycle is a crucial innovation in the evolution of land plants (Keng and Crane 1997; Stewart and Rothwell 1993). "Bryophytes" are distinct from all other extant embryophytes by having a dominant gametophyte phase. In all tracheophytes, the sporophyte is the dominant phase, but the duration of the gametophyte compared to that of the sporophyte varies among the major lineages. Seed plants and most ferns have a short-lived gametophyte phase, whereas Equisetaceae, Lycopodiaceae, Ophioglossaceae, and Psilotaceae have long-lived gametophytes. This is also the case in some early diverging groups of ferns (e.g., Hymenophyllaceae). An extremely short-lived gametophyte is correlated with heterosporous reproduction and may be a prerequisite for the evolution of heterospory (DiMichele et al. 1988; Schuster 1992). Keng and Crane (1997).

Number of photosynthetic leaves per shoot at any given time: two or more (0); one (1). Shoots of Ophioglossaceae develop onlly one photosynthetically active leaf at any given time. Future leaves are present but do not contain chlorophyll, and they are completely enclosed by the single green leaf. The leaf growth is correlated with seasonal changes in temperature and subtropical regions. Tropical Ophioglossaceae, such as *Homalothecium*, show a similar behavior,
although a correlation with “seasons” is not obvious. All other vascular plants with euphyls possess more than one photosynthetic leaf at any given time, with the exception of the early stages of embryo development. Kubitzki (1990).

V. Biochemistry—The presence of chemical constituents reflects the activity of cellular enzymes and these could potentially provide phylogenetic information. Problems with these kinds of characters include: little is known about their biosynthesis, reports on the presence of constituents are based on doubtful identifications of taxa, not all constituents are reported, and the absence of constituents is often not noted—

133. (GR 61, 105; KC 4.36; M et al. 84) Lignin: absent (0); present (1). Lignin is an important component of the cell walls of tracheids. The chemical composition may differ among the major lineages of land plants, but detailed studies are lacking for many taxa. Angiosperms and Gnetales differ from other seed plants by the absence of the Mäule reaction; however, data are inconsistent and insufficient to add this as a character state. Cooper-Driver (1977), Logan and Thomas (1985), Soeder (1985), Markham (1988), Gottlieb et al. (1990), Wallace (1991), Cooper-Driver and Bhattacharya (1998), Rausher et al. (1999).

VI. Molecular Data—Certain macromolecular structural features of DNA (e.g., absence or presence of introns, see Qiu et al. 1998, Wikström and Pryer 2005) lend themselves well to being incorporated in a morphological study. It is unfortunate that these data (structural DNA characters) are available for a limited number of taxa—