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Phylogenetic Studies of Extant Pteridophytes

Paul G. Wolf, Kathleen M. Pryer, Alan R. Smith, and Mitsuyasu Hasebe

Pteridophytes represent the most poorly understood group of vascular plants from a phylogenetic perspective (Stewart and Rothwell, 1993). The group is probably polyphyletic and includes four extant divisions (following Cronquist et al., 1966): Polypodiophyta (ferns), Psilotophyta (Psilotaceae, or whisk ferns), Lycopodiophyta (lycopods), and Equisetophyta (horsetails). Estimating phylogenetic relationships among these groups, and their relationship to seed plants and to many extinct groups of land plants, remains one of the greatest challenges in plant systematics. In this chapter we review some of the literature bearing on relationships among pteridophytes, focusing on studies of ferns. We also present an exploratory analysis, using nucleotide sequences from three genes and data from 77 morphological characters, to examine the feasibility of a combined approach to inferring pteridophyte phylogeny. We then discuss the problems associated with resolving ancient divergence events and with analyzing large and diverse data sets. We conclude with what we believe to be the most fertile directions for future research on pteridophyte phylogeny.

An improved phylogenetic framework of pteridophytes is required for developing classifications of land plants that reflect evolutionary history. Phylogenetic hypotheses are also necessary for understanding the sequence of events associated with major changes in vegetative morphology (Kenrick and Crane, 1991), reproductive characters (Crane, 1990; Lugardon, 1990; Kenrick, 1994), ecology (Brooks and McLennan, 1991), life histories (Stearns, 1992), and habitat (Mishler and Churchill, 1985). Robust phylogenies also allow us to learn more about evolution at the molecular level (Avise et al., 1994; Sharp et al., 1995), which in turn can provide more realistic models for inferring phylogeny using molecular data (Swofford et al., 1996). Moreover, some authors have argued that phylogenetic hypotheses permit inferences on aspects of evolutionary processes themselves such as patterns of speciation, biogeography, and adaptation (Harvey and Pagel, 1991; Philippe and Adoutte, 1996). Thus, improving the resolution of pteridophyte phylogeny will enhance our understanding of vascular plant diversification and the evolution of terrestrial ecosystems.

Much of our understanding of vascular plant relationships comes from studies of the fossil record. For example, characteristics of pteridosperm fossils strongly suggest that ferns and seed plants share a common ancestor (Stewart,

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1981). Incorporating data from both extant and fossil taxa should provide the most informative approach to resolving relationships (Rothwell, 1994; Rothwell and Stockey, 1994; Smith and Littlewood, 1994). However, our goal here is to focus initially on developing better phylogenetic hypotheses for extant taxa. Because this synthesis will be based partly on morphological characters, subsequent studies that include fossil taxa should then become more feasible (Donoghue et al., 1989; Doyle et al., 1994; Huelsenbeck, 1994).

NONMOLECULAR PHYLOGENETIC HYPOTHESES FOR PTERIDOPHYTES

Detailed overviews of pteridophyte phylogenetic studies and taxonomic treatments can be found elsewhere (Takhtajan, 1953; Foster and Gifford, 1974; Crane, 1990; Kramer, 1991; Stewart and Rothwell, 1993; Kenrick, 1994; Kenrick and Friis, 1995; Kenrick and Crane, 1997). The view that pteridophytes are not monophyletic developed long before the application of formal cladistic approaches. For example, Jeffrey (1902) recognized two phyla of vascular plants: the Lycopsida (including Psilotaceae, lycopods, and horsetails) and the Pteropsida (ferns and seed plants). Although Jeffrey (1902) acknowledged that his Lycopsida was unnatural (not monophyletic), it was not until the work of Eames (1936), Smith (1938), and Zimmerman (1959) that the current four-taxon system for pteridophytes (sensu Cronquist et al., 1966; Kramer and Green, 1990) was adopted. Many variations on this basic four-group theme have emerged, the most evident (and perhaps phylogenetically accurate) being the inclusion of ferns and seed plants as a taxon (e.g., Jeffrey, 1902). More recently, Bierhorst (1968, 1977) suggested that Psilotaceae (*Psilotum* and *Tmesipteris*) are closely related to some leptosporangiate ferns, rather than representing a separate and old lineage of vascular plants. Bierhorst's evidence came from the structure and development of gametophytes, embryos, "fronds," and stems, and the similarities of these characters in Psilotaceae and the fern genera *Stromatopteris* and *Actinostachys*. However, other pteridologists have disagreed with these interpretations (Kaplan, 1977; Wagner, 1977); as a result, Bierhorst's phylogenetic hypotheses were never incorporated into taxonomic treatments other than his own (Bierhorst, 1971). Another phylogenetic hypothesis for pteridophytes was proposed by Kato (1983) in which the lycopods and Psilotaceae together form a subdivision, as do ferns plus horsetails. This "biphyletic" classification was also based on a reevaluation of comparative morphology, but again without a formal cladistic analysis of characters. Given the wide range of treatments for pteridophytes it is no wonder that the most recent classification does not propose relationships among the four classes (Kramer and Green, 1990). Alternatively, if the four groups all diverged more or less simultaneously in geologic time, then lack of resolution may be an accurate reflection of relationships.

Somewhat independent of taxonomic treatments, several phylogenetic analyses of vascular plants have been conducted. We discuss studies using nonmolecular data, followed by a review of molecular studies. Parenti (1980) used 24 cellular, morphological, and anatomical characters to generate a cladogram of the major groups of land plants, including fossil taxa. The branching order among extant vascular plants was (Psilotaceae (lycopods (horsetails (ferns (seed plants))))). Thus, ferns are sister to extant seed plants and Psilotaceae are sister to all other extant vascular plants. Bremer et al. (1987) used 88 morphological characters and obtained the same branching order of extant vascular plant groups. Crane (1990) built on the data of Bremer et al. (1987), but presented an unresolved tree for the taxa of concern here. In Crane's analysis, Psilotaceae were included with true ferns (as noted above, a classification proposed by Bierhorst, 1971), and ferns, horsetails, and seed plants formed an unresolved trichotomy, leaving lycopods as sister to other extant vascular plants. Similar trees were developed by Kenrick (1994) and Kenrick and Friis (1995), where the focus was the inclusion of extinct and extant taxa, as it was by Crane (1990). The most notable departure from hypotheses discussed so far was presented by Garbary et al. (1993) in a cladistic study of land plants using strictly characters of male gametogenesis. Their results are contro-

versial because they infer polyphyly of lycopods (*Selaginella* is sister to a bryophyte clade), with the remaining vascular plants branching as follows: (*Lycopodium* (*Marsilea* ((horsetails + ferns) seed plants))). *Psilotum* was not included in their analysis.

Ongoing studies by Rothwell (1998) involve cladistic analysis of 101 morphological characters of ferns and fernlike plants (including many fossil groups). The results suggest that ferns sensu lato are polyphyletic, but that extant ferns represent a distinct clade. Also, Psilotaceae appear near the base of the vascular plant tree. Perhaps Rothwell's most significant finding is that there are some changes in the extant fern tree (e.g., positions of *Osmunda* and *Schizaea*) when the extinct taxa are excluded.

In summary, nonmolecular phylogenetic hypotheses for vascular plants depict rather varied phylogenetic relationships. Yet, there appears to be a general consensus on two points: that extant lycopods are the sister to other extant vascular plants (Banks, 1975; DiMichele and Skog, 1992) and that ferns and seed plants form a monophyletic group.

NONMOLECULAR PHYLOGENETIC HYPOTHESES FOR FERNS

Many evolutionary trees (phyletic schemes), as well as evolutionarily-based classifications, have been proposed for the approximately 30 families of ferns (e.g., Ching, 1940; Wagner, 1969; Holttum, 1973; Mickel, 1974; Crabbe et al., 1975; Lovis, 1977; Pichi Sermolli, 1977; Kramer and Green, 1990). These schemes were based mostly on morphological (but also cytological) characters, and despite the many differences among schemes, there is consensus on some points. Details of the characters used and areas of concordance among schemes were most recently provided by Smith (1995). Seventeen categories of characters were discussed; the most widely used have been sorus position; indusial presence, shape, and orientation; spore structure; rhizome structure; root anatomy; and stipe vasculature. Many of the differences among phyletic schemes arise from emphases on different characters. Nevertheless, four areas of agreement were noted among these systems: (1) Ophioglossaceae and Marattiaceae are distant relatives of leptosporangiate ferns. (2) About ten families are regarded as "primitive," i.e., branching early with respect to other families. These include: Osmundaceae, Schizaeaceae, Gleicheniaceae, Matoniaceae, Dipteridaceae, Plagiogyriaceae, Loxomataceae, Hymenophyllaceae, Dicksoniaceae, and Cyatheaceae. (3) Families considered to have arisen more recently include Dennstaedtiaceae, Pteridaceae, Vittariaceae, Polypodiaceae, Grammitidaceae, Thelypteridaceae, Dryopteridaceae, Aspleniaceae, and Blechnaceae. (4) The "higher" leptosporangiate ferns (see foregoing discussion) have had more than one origin, with Pteridaceae "derived" from schizaeoid stock, Polypodiaceae and Grammitidaceae from gleicheniioid progenitors, and most of the remaining families from dennstaedtioid ancestors.

In addition to the partly intuitive "phyletic schemes" mentioned above, a few studies have attempted to estimate fern phylogeny more objectively using morphological data. The first was by Wagner (1969), who used his ground plan/divergence method. This approach examined character distributions within groups to identify "primitive" versus "specialized" conditions, and then summed across characters to develop a relative score for each group, from which a tree was generated. The first study to use parsimony methods to analyze morphological data in ferns was by Pryer et al. (1995). That study was based on 77 parsimony-informative characters, and the results of one of their analyses (analysis 1A of Pryer et al., 1995) is reproduced in Figure 19.1. Unlike Wagner's (1969) analysis, the more recent study made no a priori assumptions about ontogeny or whether characters are ancestral or derived. Character polarity was only inferred later from the most parsimonious trees. Several aspects of Pryer et al.'s (1995) tree are consistent with that of Wagner's and with the general consensus of previous works discussed by Smith (1995). Ophioglossaceae and Marattiaceae diverged near the base of the fern tree. The general positions of the ten "primitive" families were basal, and the "advanced families" were sister to a clade that included the tree ferns. Thus, although the "higher" fern families were more recently derived than the "primitive" families, the former

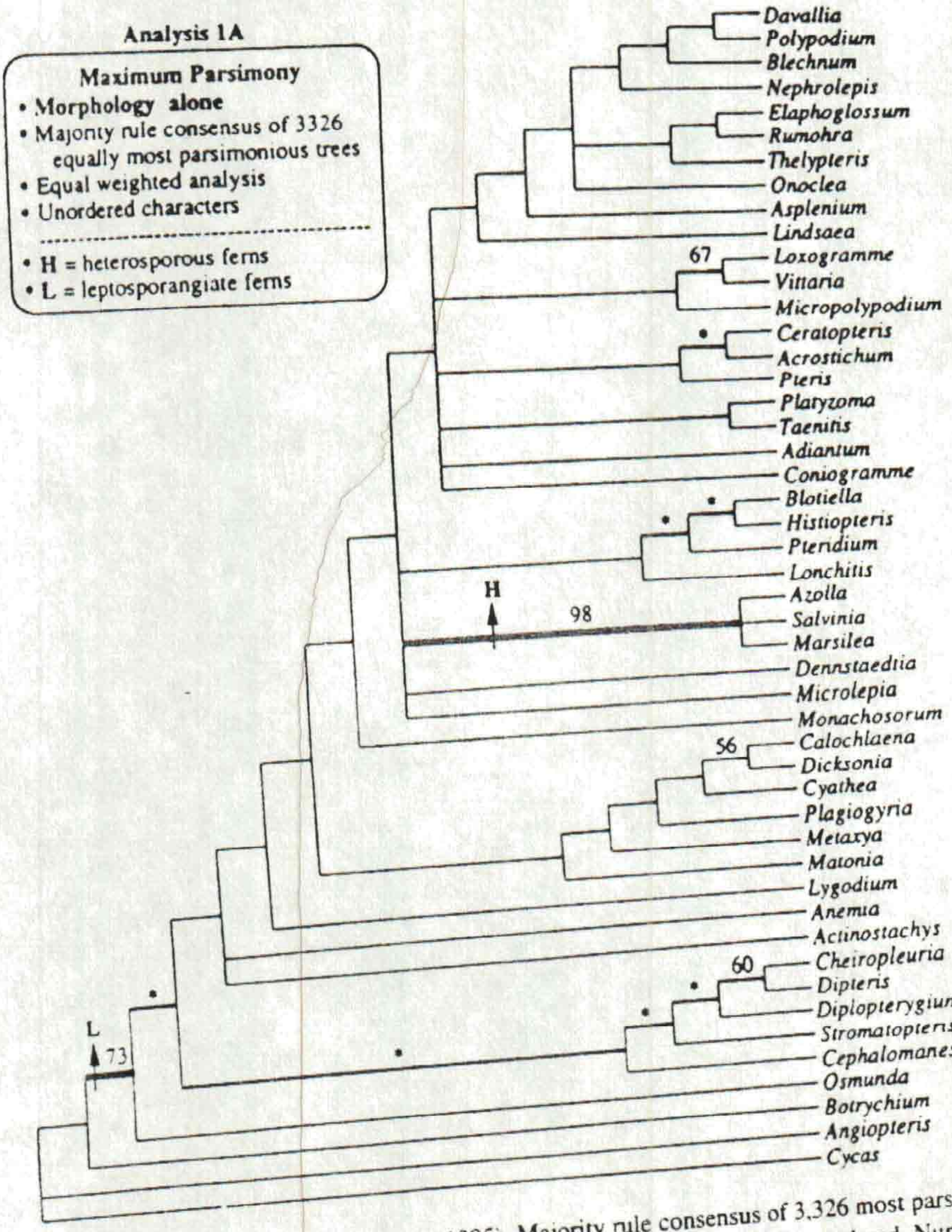

Figure 19.1. Analysis 1A (fig. 2, p. 222) of Pryer et al. (1995). Majority rule consensus of 3,326 most parsimonious trees at 449 steps based on 77 parsimony-informative characters that were equally weighted and unordered. Numbers above branches indicate bootstrap percent values. Asterisks denote branches also found in the strict consensus, but not the bootstrap majority rule tree. Tree was rooted with *Cycas*: CI = 0.325; RI = 0.541. Figure reproduced with permission from the American Fern Society.

were monophyletic rather than polyphyletic, as suggested by previous workers. Also, the heterosporous ferns appeared to be monophyletic, and Dryopteridaceae were paraphyletic (Pryer et al., 1995). As will be seen later, this phylogenetic analysis based on morphology agreed more than it conflicted with analyses based on molecular data.

Another recent cladistic analysis of morphological data used 146 characters, including 22 root characters, to infer relationships among all (Schneider, 1996). Several aspects of Schneider's results are different from those of Pryer et al. (1995). For example, in Schneider's (1996) analysis, *Lindsaea* is in a pteridoid clade. *Asplenium* is sister to *Polypodium*, *Platyzoma* is sister to *Azolla* + *Marsilea*, and *Plagiogyria* is at the base of the tree, above *Osmunda*. Clades that are similar in the two studies include a tree fern clade and some aspects of the dryopteroid group. Schneider (1996) used some characters not studied by Pryer et al. (1995), most notably

might benefit from evaluating and integrating characters from the two data matrices.

MOLECULAR SYSTEMATICS OF PTERIDOPHYTES

Stein (1985) reviewed the early work that used nucleic acid data for fern systematics, and in the same paper used reassociation kinetic data to test the hybrid origin of *Osmunda* species. In addition to these DNA hybridization studies, Stein's lab pioneered the use of restriction site data for phylogenetic studies of ferns (Yatskievych et al., 1988; Stein et al., 1989) using heterologous chloroplast DNA probes from *Lactuca* and *Petunia*. Later, probes from *Adiantum* chloroplast DNA (Hasebe and Iwatsuki, 1990a, 1990b) permitted more restriction site studies in ferns (e.g., Gastony et al., 1992; Murakami and Schaal, 1994; Conant et al., 1995; Haufler et al., 1995). The restriction site approach has provided robust data for inferring relationships among species within genera and among closely related genera, but it has not been used extensively for studies at the family level or higher. At these higher levels two approaches have been used: characterization of chloroplast DNA structure and variation of nucleotide sequences in coding genes (both chloroplast and nuclear). Structural rearrangements are relatively rare events and therefore can be powerful phylogenetic markers (Downie and Palmer, 1992; see Chapter 1). Several rearrangements have been detected in ferns and used to make phylogenetic inferences (Stein et al., 1992; Raubeson and Stein, 1995). The colinearity of gene order in *Marchantia* and lycopods supports the hypothesis (e.g., Crane, 1990) that lycopods are the sister group to all other vascular plants (Raubeson and Jansen, 1992). Although there is clear phylogenetic potential for such cpDNA structural characters, to date the number detected and the number of pteridophyte taxa surveyed are too low to make robust conclusions.

With the incorporation of nucleotide sequence data into plant systematics (e.g., Ritland and Clegg, 1987; Hamby and Zimmer, 1988), it was not long before such approaches were used by fern systematists. The first gene to be sequenced from a sufficient number of pteridophyte taxa for phylogenetic studies was *rbcL* (Hasebe et al., 1993). Attempts have been made to use *rbcL* sequence data to infer relationships among groups of vascular plants (Hasebe et al., 1993) and to examine relationships among all major groups of green plants (Manhart, 1994). However, at these higher levels, sites are generally saturated, producing only weak phylogenetic signal. Using amino acid sequences (Hasebe et al., 1993) or focusing on first and second codon positions (Manhart, 1994) did not solve this problem. Better phylogenetic resolution was achieved with sequences from nuclear 18S ribosomal RNA genes (Kranz et al., 1995; Kranz and Huss, 1996). In the most recent analysis, Kranz and Huss (1996) examined 22 complete sequences using maximum parsimony and maximum likelihood, comparing support for alternative topologies. The best supported trees had Lycopsida as the sister group to the remaining vascular plants and *Psilotum* as sister to the seed plants: (lycopods (ferns (horsetails (Psilotaceae (seed plants))))). However, short branches suggest that the ferns, horsetails, Psilotaceae, and seed plants radiated more or less simultaneously, perhaps from a trimerophyte ancestor (Kranz and Huss, 1996).

Hiesel et al. (1994) used mitochondrial *coxIII* (cytochrome oxidase subunit III) sequences to examine the phylogeny of land plants. Parsimony analysis resulted in *Lycopodium* as sister to a clade that included bryophytes and vascular plants. However, maximum likelihood trees were more congruent with inferences from other data: (lycopods (horsetails (Psilotaceae (ferns + seed plants)))). An alternative topology was inferred by Kolukisaoglu et al. (1995) using nuclear encoded phytochrome genes: ((lycopods + horsetails) (ferns (Psilotaceae + seed plants))). Two trees were presented by Kolukisaoglu et al. (1995), one of which includes a basal clade with bryophytes, *Selaginella*, and *Equisetum*. Many aspects of the phytochrome gene tree are consistent with other studies. However, phytochrome genes are part of a gene family (Schneider-Poetsch et al., 1994; Mathews et al., 1995), and sequences from different taxa may not be orthologous, potentially resulting in conflict between the gene tree and organismal

Boivin et al. (1996) used sequences of the chloroplast *chlB* gene (which encodes a subunit of the light-independent protochlorophyllide reductase) to examine relationships among diverse land plants. The gene could not be PCR-amplified from *Ephedra* or *Psilotum*. Neighbor-joining and parsimony analyses of an internal fragment of approximately 350 bp resulted in optimal trees that were consistent with many previous analyses; bryophytes were paraphyletic, lycopods (except *Isoetes*) were the most basal vascular plant clade, and ferns appeared as a monophyletic group: (lycopods (*Equisetum* (ferns + seed plants))). However, unlike in most previous studies, *Isoetes* appeared as a sister to all land plants. The *chlB* gene appears to contain good phylogenetic signal, but failure to detect the gene in plastids of various angiosperms and critical taxa such as *Ephedra* and *Psilotum* may limit its use as a phylogenetic tool at the level of vascular plants.

Manhart (1995) examined chloroplast 16S rRNA gene sequences from 23 land plants using maximum parsimony. Phylogenetic resolution appeared to be better for the deeper nodes than with *rbcL* sequences (Manhart, 1994), but the base of the vascular plant clade was still poorly resolved; the topology was dependent on the inclusion of *Selaginella*, which was connected by a long branch. With *Selaginella* included, the vascular plant tree was (horsetails (ferns ((Psilotaceae + Ophioglossaceae) (lycopods and seed plants)))). Lycopods did not form a clade: ((*Lycopodium* + *Selaginella*) (*Isoetes* + seed plants)). With *Selaginella* removed, *Lycopodium* became the sister to the remaining vascular plants, and *Isoetes* remained sister to seed plants. The clade that included Psilotaceae and Ophioglossaceae (the eusporangiate ferns *Ophioglossum* and *Botrychium*) was reasonably well supported with a bootstrap of at least 80%. This clade had also been detected (with less support) in Manhart's (1994) *rbcL* study. More recently, Wolf (1996) detected the same clade using parsimony analysis of nucleotide data from the chloroplast gene *atpB*. The *atpB* tree with the greatest support was (*Selaginella* (horsetails (*Angiopteris* (Psilotaceae + Ophioglossaceae) (other lycopods + ferns))))). Seed plants were not included in the *atpB* analysis, and lycopods were polyphyletic as in the 16S rDNA analysis. The Psilotaceae + Ophioglossaceae clade has not been proposed on the basis of data other than nucleotide sequences. Congruence among the three data sets (*rbcL*, 16S rDNA, and *atpB*) could be interpreted as strong support for this clade. An alternative explanation for this congruence could be long-branch attraction (Felsenstein, 1978; Hendy and Penny, 1989). Long branches on a tree can result from multiple extinctions along a lineage, increased substitution rate, or ancient divergence. Successive long branches can cause parsimony methods to converge on an incorrect tree that unites taxa with long branches together in a false clade. Under these conditions, adding more data actually increases support for the false clade. Maximum likelihood approaches should be less susceptible to these effects (Swofford et al., 1996). Preliminary maximum likelihood analyses using the *atpB* data set (P. Wolf, unpubl.) still support the Psilotaceae + Ophioglossaceae clade, suggesting that long-branch attraction may not be the explanation. Further analyses are needed for *rbcL*, 16S rDNA, and 18S rDNA to test the support for this possibly spurious clade.

MOLECULAR SYSTEMATICS OF FERNS

Although *rbcL* nucleotide data alone were not highly informative for inferring relationships among groups of vascular plants, these data have provided evidence for many well-supported clades within the ferns (Hasebe et al., 1994, 1995; Wolf et al., 1994). Pryer et al. (1995) used parsimony analysis of *rbcL* data for the same set of taxa for which they analyzed 77 morphological characters. The *rbcL* tree is reproduced in Figure 19.2, which can be compared more directly (than the other *rbcL* studies above) with the morphological analysis (Fig. 19.1). Most genera from the more derived families formed well-resolved clades, whereas basal clades were poorly supported by bootstrap analysis. The consensus *rbcL* tree is not significantly different from the consensus tree based on morphological data, including some results that differed from most (but not all) previous phylogenetic hypotheses. For example, *Polypodium* appeared closely related to *Davallia* (Davalliaceae) in

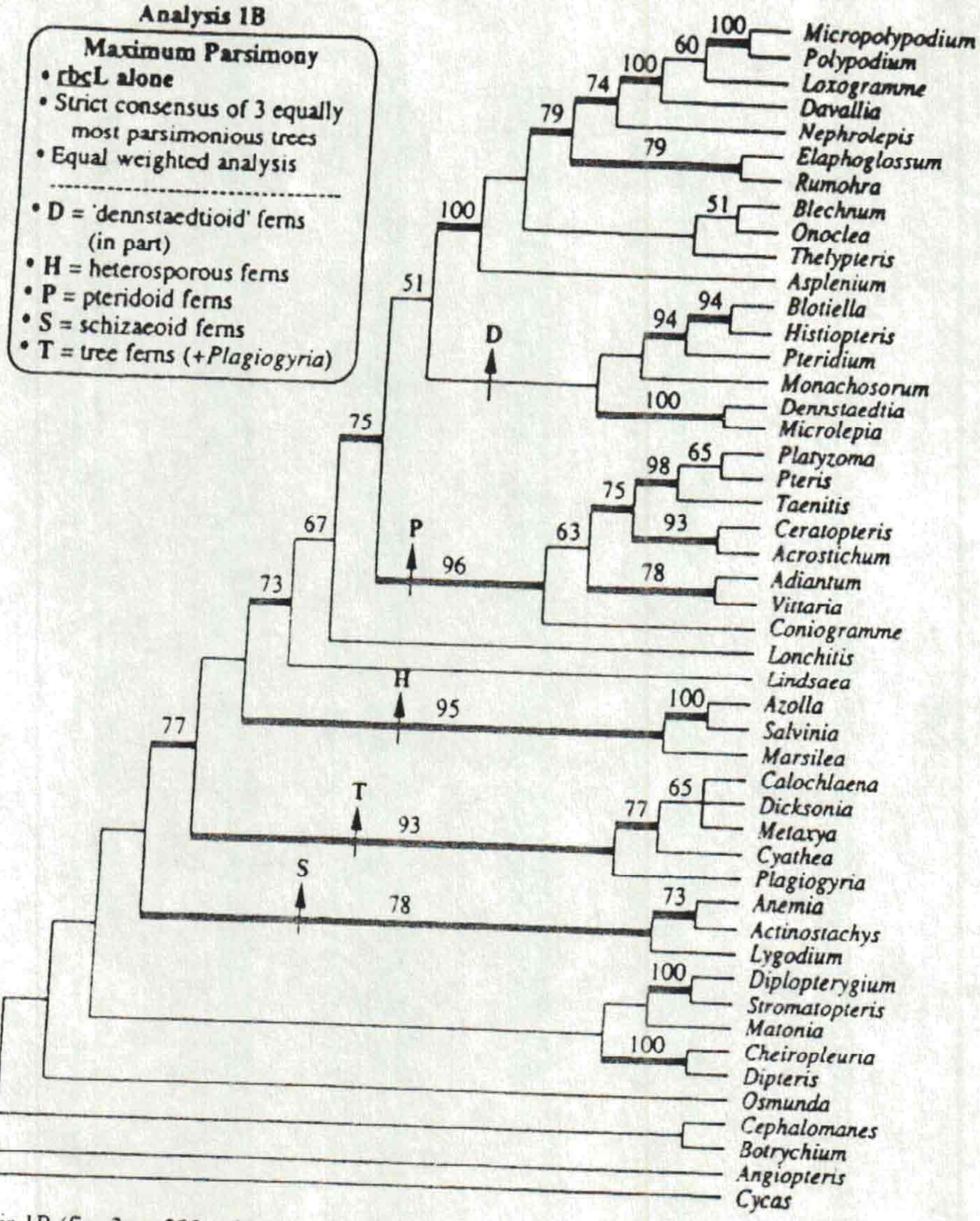

Figure 19.2. Analysis 1B (fig. 3, p. 223) of Pryer et al. (1995). Strict consensus of three most parsimonious trees at 3,639 steps based on 489 parsimony-informative characters. See Fig. 19.1 for additional notation. CI = 0.235; RI = 0.464. Figure reproduced with permission from the American Fern Society.

both morphological and *rbcL* analyses. This result does not support the traditional view of the Polypodiaceae being more closely related to gleichenioid ferns (Wagner, 1969; Lovis, 1977), but is consistent with evidence from sporangial and gametophyte structures, as suggested by Jarrett (1980). Most traditionally recognized fern families (sensu Kramer and Green, 1990) appear consistently as monophyletic groups based on analysis of *rbcL*, for example, Polypodiaceae (with *Micropolypodium* included), Oleandraceae, Blechnaceae, Aspleniaceae, Vittariaceae, Marsileaceae, Schizaeaceae, Gleicheniaceae, Hymenophyllaceae, and Ophioglossaceae (Hasebe et al., 1995). Consistently nonmonophyletic families include Dennstaedtiaceae, Dryopteridaceae (by inclusion of at least eight other families), and Pteridaceae (by inclusion of Vittariaceae).

Although *rbcL* appears to lack signal for inferring basal clades of ferns and pteridophytes, the gene seems to be extremely informative for inferring relationships among genera within families. In a parsimony analysis of *rbcL* sequences in Vittariaceae (Crane et al., 1995), most clades were highly resolved with 100% (or

nearly 100%) bootstrap support, and seven clades had decay indices greater than 10. Crane et al.'s (1995) results indicate that *Vittaria* is probably polyphyletic. Gastony and Rollo (1995) analyzed *rbcL* from 25 species of cheilanthoid ferns, also finding strongly supported clades. The resulting trees suggest that both *Pellaea* and *Cheilanthes* are polyphyletic, and the segregation of *Argyrochosma* from *Notholaena* is supported. Wolf (1995) used sequence data from *rbcL* and nuclear 18S rRNA genes to infer relationships among genera of Dennstaedtiaceae. The results suggest that the family is polyphyletic with the lindsaeoid genera representing one main lineage and Dennstaedtiaceae sensu stricto another. The genera *Orthiopteris* and *Tapeinidium* do not appear to be supported within either clade. *rbcL* sequence data have also been used to infer relationships among closely related genera (e.g., onocleoid ferns, Gastony and Ungerer, 1997) and among species within genera (e.g., *Polypodium*, Haufler and Ranker, 1995; *Botrychium* subg. *Botrychium*, Hauk, 1995).

ANALYSES OF COMBINED DATA SETS

It has become increasingly clear that progress in systematics rarely comes from focusing on only one type of data. Rather, taking into account information from fossils, extant taxa, morphology, and molecules has resulted in many of the more robust insights into phylogeny (Patterson, 1987). Thus, several studies have used approaches that combine extinct and extant taxa (Rothwell, 1987; Donoghue et al., 1989; Doyle and Donoghue, 1992; Nixon et al., 1994; Rothwell and Serbet, 1994; Nixon, 1996; Rothwell, 1998), other studies have combined morphological and molecular data (e.g., Baldwin, 1993; Doyle et al., 1994; Mishler et al., 1994; Lutzoni and Vilgalys, 1995; Pryer et al., 1995), and some studies have combined different molecular data sets (e.g., Olmstead and Sweere, 1994; Hoot, 1995; Hoot and Crane, 1995; Mason-Gamer and Kellogg, 1996; Soltis et al., 1996). Pryer et al. (1995) combined data from 77 parsimony-informative, morphological characters with 1,206 bp for *rbcL* (of which 490 were parsimony-informative) for 49 pteridophyte taxa. The strict consensus of 34 most parsimonious trees for a smaller "fern" data set (47 taxa plus one outgroup) is reproduced in Figure 19.3. The most significant finding of this study was that the combined analysis provided better resolution than the separate analyses (compare Figs. 19.1, 19.2, and 19.3). For almost all clades detected, bootstrap support was greatest in the combined analysis (table 4 of Pryer et al., 1995). For example, a leptosporangiate fern clade (including *Osmunda* at the base) did not appear on the strict consensus *rbcL* tree, the clade had 73% bootstrap support on the morphology tree, and 89% support on the combined tree. Apparently, *rbcL* sequence data and morphology provided optimal information at different levels in the phylogeny: *rbcL* for more recent divergences and morphology for older events, and together, the information is complementary. Increased phylogenetic resolution and increased internal support in analyses of combined (versus separate) data sets have also been observed in other studies (e.g., Kim et al., 1992; Doyle et al., 1994; Mishler et al., 1994; Olmstead and Sweere, 1994; Soltis et al., 1996, 1997; see also Chapters 11 and 17). The approach used by Pryer et al. (1995) suggests that increasing the number of gene sequences and the number of morphological characters may help to resolve further the base of both the fern and pteridophyte phylogeny, where most of the phylogenetic uncertainty remains.

A tree based on nucleotide sequence data estimates a gene phylogeny, which may not necessarily agree with the organismal phylogeny, because, for example, of introgression, lineage sorting, or gene duplication (e.g., Hillis, 1987; Pamilo and Nei, 1988; Doyle, 1992; Lutzoni and Vilgalys, 1995). In addition, different data sets can provide evidence for different trees because of sampling error (Rodrigo et al., 1993) or use of an inappropriate evolutionary model for the data (Bull et al., 1993). The issue of whether to combine molecular and morphological data (or multiple molecular data sets) is not trivial. Arguments have been presented for combining data sets to analyze the "total evidence" (Kluge, 1989; Barrett et al., 1991), for analyzing data sets separately and then comparing trees and testing for congruence (Miyamoto and Fitch, 1995), and for first testing for homogeneity among data sets

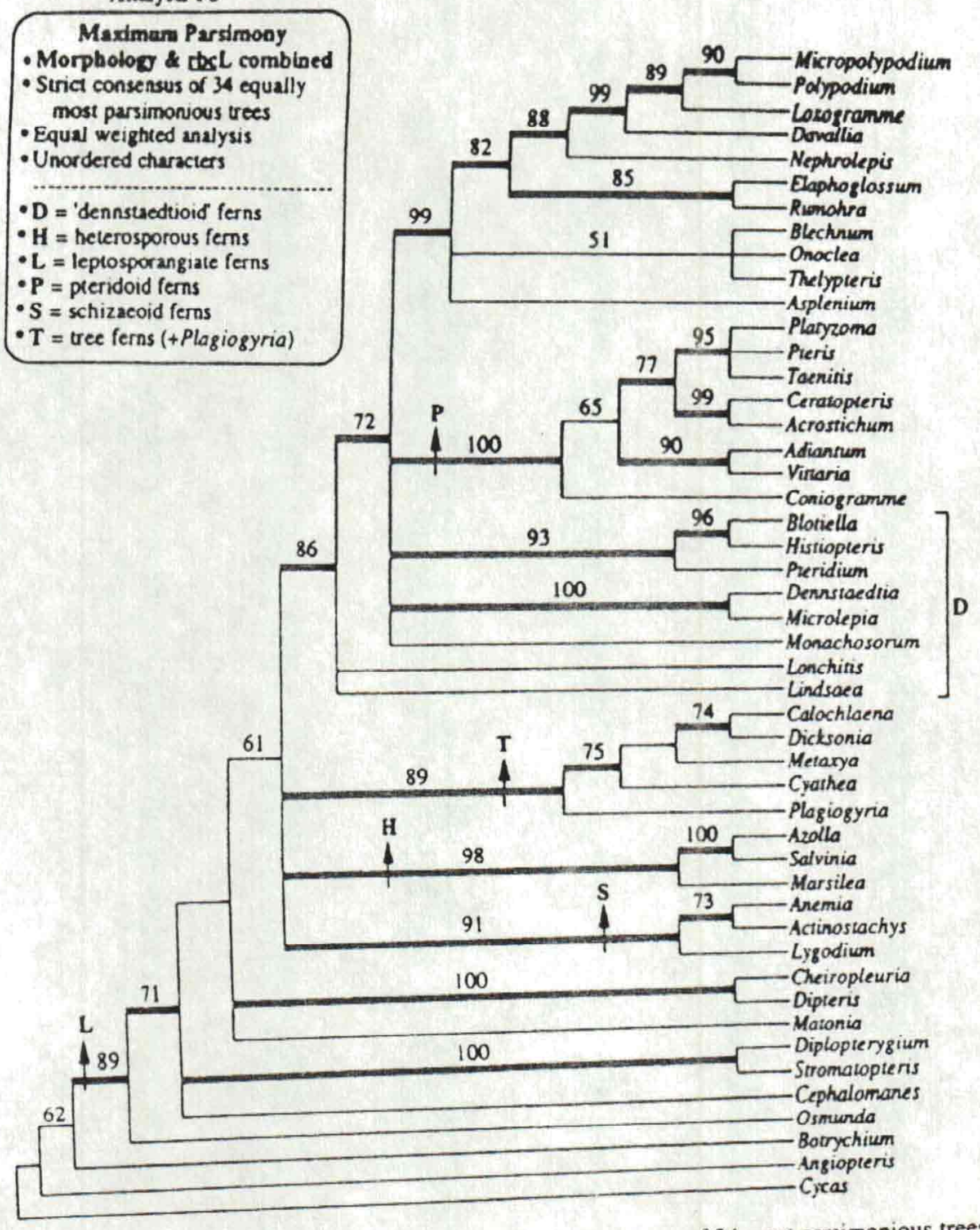

Figure 19.3. Analysis 1C (fig. 4, p. 225) of Pryer et al. (1995). Strict consensus of 34 most parsimonious trees at 4,128 steps based on 564 parsimony-informative characters. See Fig. 19.1 for additional notation. CI = 0.242; RI = 0.466.
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to see if combining is appropriate (Bull et al., 1993; de Queiroz, 1993; Rodrigo et al., 1993; Lutzoni and Vilgalys, 1995; for reviews, see de Queiroz et al., 1995, Huelsenbeck et al., 1996, and Chapter 11). Regardless of how multiple data sets should be analyzed, the use of several independent sources of phylogenetic data should greatly increase the chance of correctly inferring evolutionary relationships (Penny et al., 1991). This is especially important for ancient divergence events (such as the base of the vascular plant phylogeny) where any single data set is likely to have a weak phylogenetic signal.

EXPLORATORY ANALYSIS OF PTERIDOPHYTE PHYLOGENY USING MULTIPLE DATA SETS

To explore the feasibility of combining several diverse data sets for phylogenetic analysis of pteridophytes, we chose 12 representative taxa for which we could gather data on 77 morphological characters and nucleotide sequence data from *rbcL* (1,206 bp), *atpB* (588 bp), and the 18S nuclear rRNA gene (1,617 bp). Previous studies have examined the effect of combining two data sets in ferns. Our goal was to see if

combining four data sets could provide even better phylogenetic resolution.

Our taxon sample was limited because few pteridophytes have been examined for many genes. However, restricting the number of taxa to 12 had the advantage of allowing for thorough searches of tree space. We chose one representative of each class of fern allies (*Huperzia, Equisetum,* and *Psilotum*), eight ferns (*Ophioglossum, Vandenboschia, Osmunda, Dicksonia, Adiantum, Lonchitis, Pteridium,* and *Blechnum*), and one seed plant (*Ginkgo*). We used *Huperzia* to root the trees. Obviously, as data sets grow, more outgroups need to be included, especially bryophytes. We used an exemplar approach, with each taxon represented by the same genus for all data sets. For nine of the 12 taxa we used a single exemplar species (Table 19.1). The exceptions (where no specific epithet is given in Table 19.1) were *Ophioglossum—O. reticulatum* for *atpB*, *O. petiolatum* for 18S rDNA, *O. engelmannii* for *rbcL*, all three for morphology; *Equisetum—E. hyemale* for 18S rDNA, *E. arvense* for the other three data sets; *Huperzia—H. campiana* for *rbcL*, *H. lucidula* for the other three data sets.

Complete matrices for all data sets are available upon request from any of the authors. The Pryer et al. (1995) morphology data matrix (with the four new taxa included here) is also available at http://ucjeps.berkeley.edu/bryolab/greenplantpage.html. Nuclear 18S rDNA, *atpB*, and *rbcL* sequences were found in GenBank for all 12 taxa, except for the *atpB* sequence of *Ginkgo*, which was kindly provided by M. Chase and V. Savolainen. Morphological data for 77 characters are available for eight of the 12 taxa (Pryer et al., 1995), and this data matrix was extended with information for *Huperzia, Ginkgo, Vandenboschia,* and *Ophioglossum*. Sources of information, additional to those cited in Pryer et al. (1995), used for the extended morphological data matrix were: Arnott (1959), Fryns-Claussens and Van Cotthem (1973), and Rohr (1977) for *Ginkgo;* and Gewirtz and Fahn (1960) and Pant and Khare (1969) for *Ophioglossum*.

We used maximum parsimony to infer phylogeny from each data set separately and from a combined data set of 3,488 characters. We used PAUP version 3.1.1 (Swofford, 1993), searching for shortest trees using the branch-and-bound algorithm. Multistate morphological characters were treated as polymorphic; ambiguous nucleotides were treated as uncertain in the separate analyses. Bootstrapping (100 branch-and-bound replicates) was used to assess the support of each branch. Because current versions of PAUP do not allow for mixing different treatments of multistate characters, we used the "uncertain" coding for the combined analysis (see Swofford and Begle, 1993; Doyle et al., 1994, for further discussion).

Numbers of shortest trees, tree lengths, and other tree statistics are included in Figures 19.4, 19.5, and 19.6. There were several areas of congruence among the resultant trees. A "higher indusiate" clade (*Dicksonia, Lonchitis, Pteridium, Blechnum,* and *Adiantum*) was seen in all three molecular-based trees, and the morphology-based tree differed slightly with the inclusion of *Vandenboschia*. The positions of basal clades

Table 19.1. Taxa used and GenBank accession numbers for nucleotide sequence data.

Taxon	18S rDNA	*rbcL*	*atpB*
Osmunda cinnamomea	U18516	D14882	U93827
Vandenboschia davallioides	U18629	U05948	U93828
Dicksonia antarctica	U18624	U05919	U93829
Pteridium aquilinum	U18628	U05939	U93835
Adiantum raddianum	X78889	U05906	U93840
Blechnum occidentale	U18622	U05909	U93838
Lonchitis hirsuta	U18632	U05929	U93830
Psilotum nudum	X81963	U30835	U93822
Ophioglossum	U18515	L11058	U93825
Huperzia	U18505	X98282	U93819
Equisetum	X78890	L11053	U93824
Ginkgo biloba	D16448	D10733	Chase unpubl.

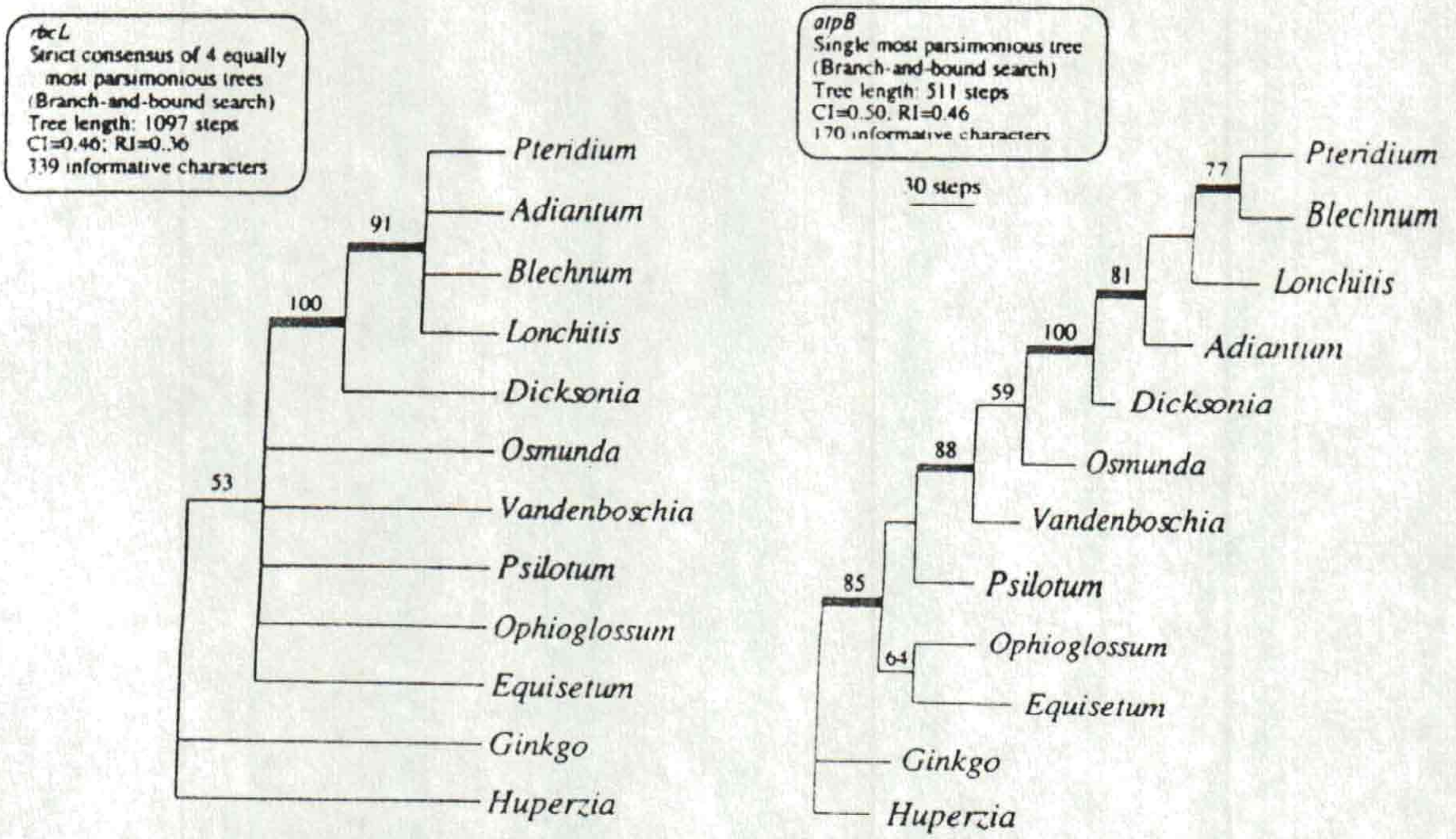

Figure 19.4. Strict consensus and single most parsimonious trees resulting, respectively, from phylogenetic analysis of *rbcL* (left) and *atpB* (right). Numbers above branches denote bootstrap percent values. Trees were rooted with *Huperzia*. For *rbcL* the strict consensus of four most parsimonious trees is shown; tree length of 1,097 steps; CI = 0.46; RI = 0.36. For *atpB* the single most parsimonious tree is depicted; tree length of 511 steps; CI = 0.50; RI = 0.46.

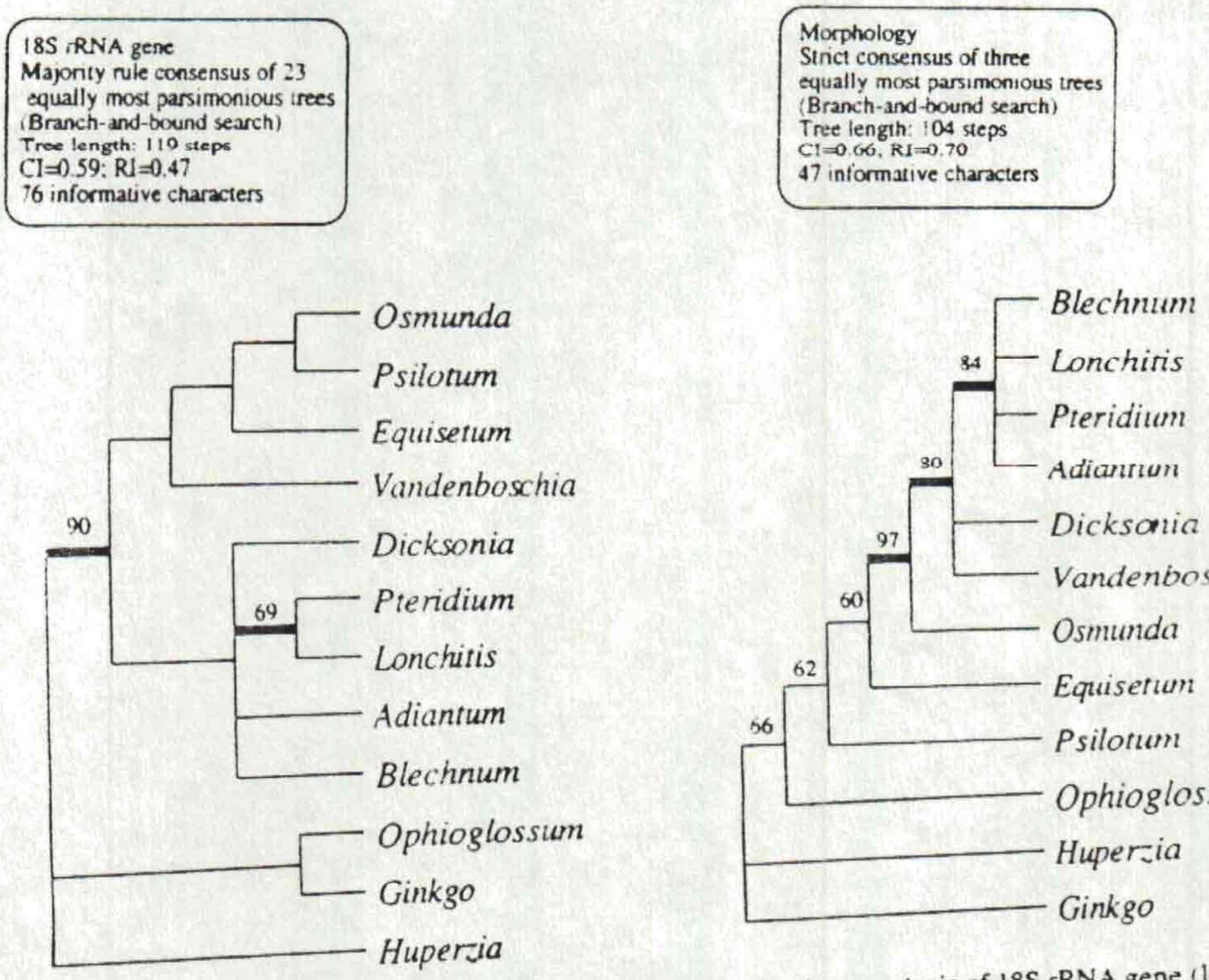

Figure 19.5. Majority rule and strict consensus trees resulting, respectively, from analysis of 18S rRNA gene (left) and morphology (right). Numbers above branches denote bootstrap percent values. Trees were rooted with *Huperzia*. For the 18S rRNA gene the majority rule consensus of 23 shortest trees is shown; tree length of 119 steps; CI = 0.59; RI = 0.47. For morphology the strict consensus of three most parsimonious trees is shown; tree length of 104 steps; CI = 0.66; RI = 0.70.

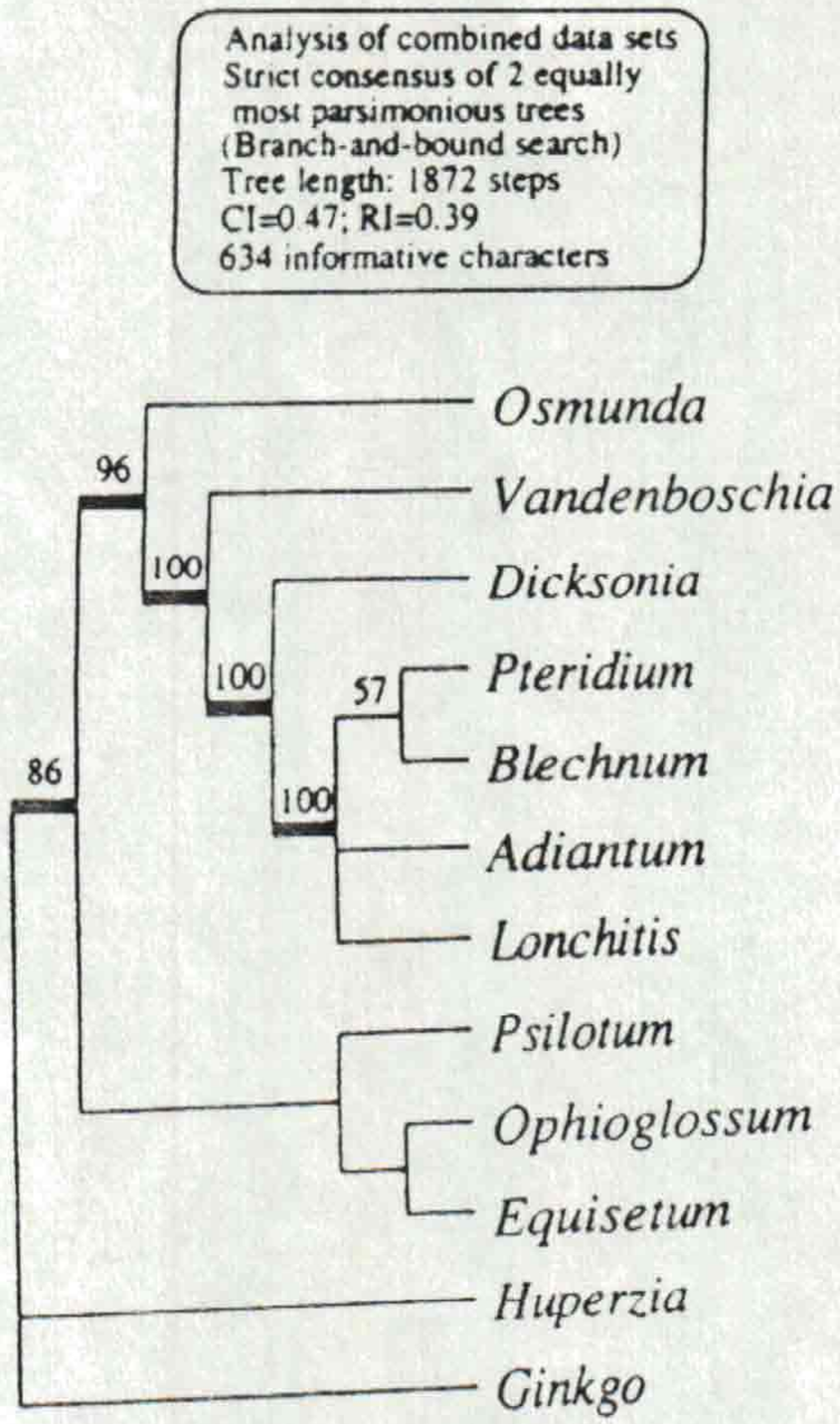

Figure 19.6. Strict consensus tree resulting from analysis of four combined data sets (*rbcL*, *atpB*, 18S rRNA gene, and morphology) at 1,872 steps. Numbers above branches denote bootstrap percent values. Tree was rooted with *Huperzia*; CI = 0.47; RI = 0.39.

varied more among trees. For example, the *atpB* tree had an (*Equisetum* + *Ophioglossum*) clade, whereas the 18S rDNA tree had a (*Psilotum* + *Osmunda*) clade. The *atpB* and morphology trees had resolution spread relatively evenly across the tree, as indicated by high bootstrap values. The *rbcL* and 18S rDNA trees were not as well resolved. However, the combined analysis using all four data sets had branches with the highest bootstrap support at deep nodes of the tree (Fig. 19.6).

Because our taxon sampling was limited, we avoid making strong statements about vascular plant phylogeny. Rather, this exploratory exercise illustrates that combining several data sets can result in trees with better support than those from single data sets. This finding is not new; it has been noted previously from combining two or more data sets in other studies (e.g., Marshall, 1992; Doyle et al., 1994; Olmstead and Sweere, 1994; Hoot and Crane, 1995; Pryer et al., 1995; Soltis et al., 1996, 1997). Currently, seven genes appear to have some phylogenetic utility for vascular plant studies: *rbcL*, *atpB*, 16S rDNA, 18S rDNA, 26S rDNA, phytochrome, and the mitochondrial *coxIII;* this list is growing continually (see Chapter 1). If combining several molecular data sets plus morphology increases the phylogenetic signal, then the potential exists to improve significantly the resolution at the base of the extant vascular plant tree using such a combined approach. Such a task will require coordination among workers to ensure accuracy of data, use of the same vouchered taxa (preferably same DNA sources), and careful consideration of taxon sampling to achieve good representation of all major extant groups. Hillis (1996) showed that, up to a point, increasing the length of variable molecular data sets should improve phylogenetic accuracy, and that large data sets (in terms also of taxa) can be used effectively to infer phylogeny. Hillis's (1996) simulations (based on 18S rDNA data) suggest that phylogenies can be accurately reconstructed for over 200 taxa using 5,000 informative characters. The total length of the seven genes listed above is approximately 9,000 bp.

Several studies have found that genomic rearrangements, which by their nature tend to be rare, can be more useful for unraveling ancient phylogenetic patterns (Raubeson and Jansen, 1992; Stein et al., 1992; Raubeson and Stein, 1995; Manhart and McCourt, 1996; Qiu and Palmer, 1996). A limitation with the current genomic mapping strategies is that they involve one-by-one screening of taxa for intron positions and large-scale rearrangements, using probing techniques. An alternative strategy would be to use complete chloroplast genome sequences. Through automated DNA sequencing, this approach is now feasible and not completely out of reach in terms of cost. Complete sequences of the chloroplast genomes of several pteridophytes (150–180 kb each) could be added to the six cpDNA sequences already published. Genomic mapping algorithms, developed for the human genome sequencing project, can be applied to large sequence data sets to search for rearrangements and large-scale losses and gains of fragments. In the long run, this should be a faster and more systematic approach to looking for needles in the haystack. An added

advantage over traditional probing methods is that evolutionarily homologous losses and gains of regions could be identified by sequence identity in the splicing regions. Also, by generating complete chloroplast genome sequences, we can search for new regions where the sequences themselves contain phylogenetic signal.

The accuracy of our knowledge of relationships of pteridophytes, and vascular plants in general, will depend on continued study in all areas: single gene sequences, genomic sequences, developmental patterns, and morphological characters. These large-scale studies are feasible only for extant taxa. Many of the phylogenetically key taxa are from extinct lineages; hence, the incorporation of fossil data is also essential for continued progress in estimating vascular plant phylogenies.

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