PHYLOGENY OF MARSILEACEOUS FERNS AND RELATIONSHIPS OF THE FOSSIL *HYDROPTERIS PINNATA* RECONSIDERED

Kathleen M. Pryer[1]

Department of Botany, Duke University, Durham, North Carolina 27708, U.S.A.

Recent phylogenetic studies have provided compelling evidence that confirms the once disputed hypothesis of monophyly for heterosporous leptosporangiate ferns (Marsileaceae and Salviniaceae). Hypotheses for relationships among the three genera of Marsileaceae (*Marsilea*, *Regnellidium*, and *Pilularia*), however, have continued to be in conflict. The phylogeny of Marsileaceae is investigated here using information from morphology and *rbcL* sequence data. In addition, relationships among all heterosporous ferns, including the whole-plant fossil *Hydropteris pinnata* are reconsidered. Data sets of 71 morphological and 1239 *rbcL* characters for 23 leptosporangiate ferns, including eight heterosporous ingroup taxa and 15 homosporous outgroup taxa, were subjected to maximum parsimony analysis. Morphological analyses were carried out both with and without the fossil *Hydropteris*, and it was excluded from all analyses with *rbcL* data. An annotated list of the 71 morphological characters is provided in the appendix. For comparative purposes, the Rothwell and Stockey (1994) data set was also reanalyzed here. The best estimate of phylogenetic relationships for Marsileaceae in all analyses is that *Pilularia* and *Regnellidium* are sister taxa and *Marsilea* is sister to that clade. Morphological synapomorphies for various nodes are discussed. Analyses that included *Hydropteris* resulted in two most-parsimonious trees that differ only in the placement of the fossil. One topology is identical to the relationship found by Rothwell and Stockey (1994), placing the fossil sister to the *Azolla* plus *Salvinia* clade. The alternative topology places *Hydropteris* as the most basal member of the heterosporous fern clade. Equivocal interpretations for character evolution in heterosporous ferns are discussed in the context of these two most-parsimonious trees. Because of the observed degree of character ambiguity, the phylogenetic placement of *Hydropteris* is best viewed as unresolved, and recognition of the suborder Hydropteridineae, as circumscribed by Rothwell and Stockey (1994), is regarded as premature. The two competing hypotheses of relationships for heterosporous ferns are also compared with the known temporal distribution of relevant taxa. Stratigraphic fit of the phylogenetic estimates is measured by using the Stratigraphic Consistency Index and by comparison with minimum divergence times.

Keywords: fossil, heterosporous, *Hydropteris*, leptosporangiate, Marsileaceae, marsileaceous, morphology, parsimony, phylogeny, *rbcL*, Salviniaceae, stratigraphy, systematics.

Introduction

Marsileaceae is a small family (ca. 80 species) of rooted amphibious leptosporangiate ferns distinguished by their unusual leaves and reproductive structures and particularly by heterospory. Salviniaceae (*sensu* Tryon and Tryon 1982; ca. 15 species), the only other family of heterosporous leptosporangiate ferns, comprise exclusively free-floating aquatic ferns. Phylogenetic studies based on morphological, paleobotanical, and *rbcL* evidence all strongly support Salviniaceae (*Azolla* and *Salvinia*) as sister group to Marsileaceae, confirming the long-disputed hypothesis of a single origin of heterospory in leptosporangiate ferns (Rothwell and Stockey 1994; Hasebe et al. 1995; Pryer et al. 1995; Stevenson and Loconte 1996). Although the three genera of Marsileaceae (*Marsilea*, *Regnellidium*, and *Pilularia*) are highly distinctive and well charac-

terized (fig. 1), relationships within the family have perplexed developmental and evolutionary botanists for much of this century (Bower 1926; Eames 1936; Johnson and Chrysler 1938; Chrysler and Johnson 1939; Higinbotham 1941; Copeland 1947; Puri and Garg 1953; Bonnet 1956; Mahabalé 1974; Schmidt 1978; Bhardwaja 1980; Wallace et al. 1984; Johnson 1986). Much of the discussion has explored morphological, cytological, and paleobotanical evidence to answer such questions as: Which of the three genera is "most primitive"? How could the genera have been "derived" from one another? Which genus is "intermediate" between the other two? To a large extent, these questions reflect outmoded approaches to phylogenetic analysis where extant taxa (rather than character states) are thought of as being "primitive," "advanced," or "intermediate," and some taxa are thought to be directly ancestral to other taxa. The critical question really is: What is the relative recency of common ancestry of these extant, monophyletic terminal taxa?

An explicit hypothesis of phylogenetic relationships within Marsileaceae has not been available (Johnson 1986) until very recently; however, those now published are in conflict. New

[1] Present address: Department of Botany, Field Museum of Natural History, 1400 S. Lake Shore Drive, Chicago, Illinois 60605; e-mail kpryer@fmnh.org.

Marsilea   *Regnellidium*   *Pilularia*
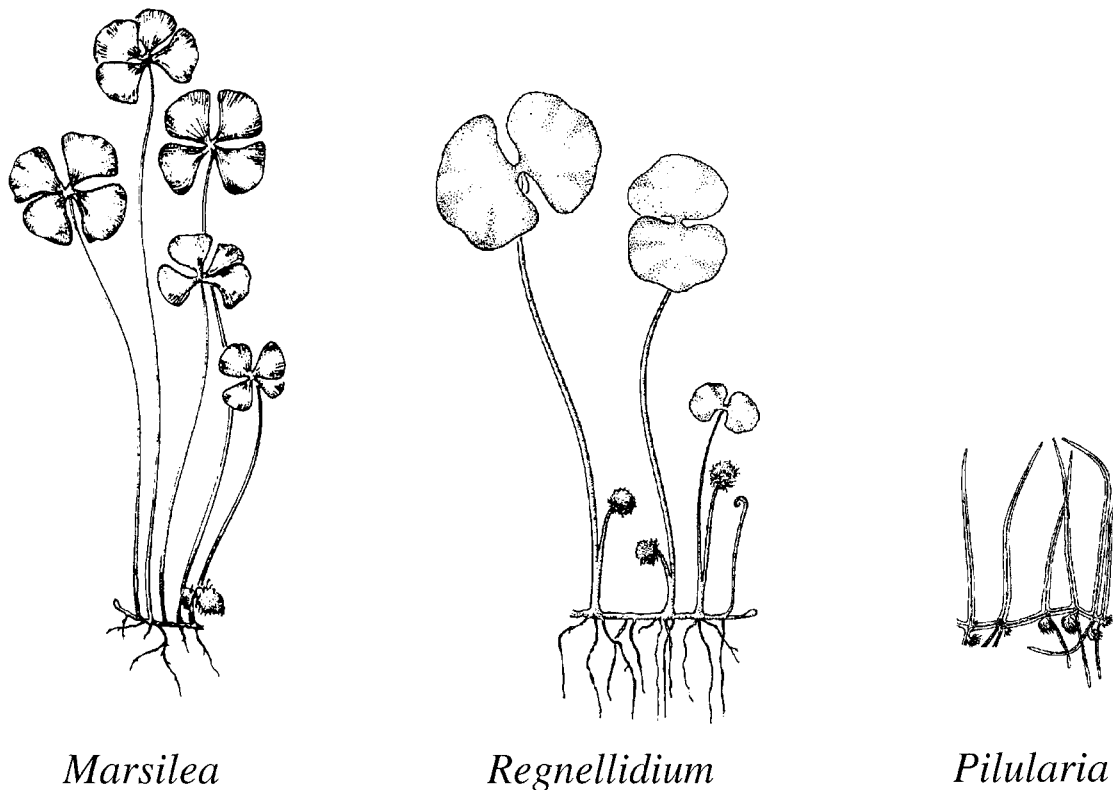
Fig. 1 Marsileaceae genera. Leaves of *Marsilea* have four leaflets and one to many sporocarps per node, whereas *Regnellidium* has two leaflets and a single sporocarp per node; these illustrations reproduced and modified from figures 118.3 and 119.1 in Tryon and Tryon (1982) with permission from the publisher. *Pilularia* has filiform leaves and a single sporocarp per node; illustration reproduced and modified from figure 1 in Johnson (1933*b*) with permission from the publisher.

insights from *rbcL* data analyzed for 107 fern taxa (Hasebe et al. 1995) not only convincingly demonstrated the monophyly of all heterosporous leptosporangiate ferns but yielded the same intergeneric relationships, (*Marsilea*, (*Regnellidium*, *Pilularia*)), as those inferred by Rothwell and Stockey (1994) in a study of 10 fern taxa (including a newly described heterosporous fossil fern, *Hydropteris*) based on 17 morphological characters (fig. 2*A*). In an analysis of 111 taxa (including 106 pteridophytes) scored for 116 morphological characters, however, Stevenson and Loconte (1996, p. 448, fig. 4) reported a contradictory result, (*Pilularia*, (*Regnellidium*, *Marsilea*)), for intergeneric relationships within Marsileaceae (fig. 2*B*). The remaining possible combination of relationships, (*Regnellidium*, (*Pilularia*, *Marsilea*)), was hypothesized by Schmidt as recently as 1978 and was based on morphological and anatomical studies of both sporophytes and gametophytes (fig. 2*C*).

All members of Marsileaceae produce spores within specialized, long-persistent structures called sporocarps and display remarkably rapid establishment of new sporophytes, making them well suited to growth in intermittent and ephemeral aquatic habitats. *Marsilea* is a cosmopolitan genus of perhaps 70 species. A phylogeny is not available for the genus, and the last comprehensive treatment of *Marsilea* was that of Braun

(1871, 1873), who recognized 13 species groups. Contributions by Baker (1886) and Sadebeck (1902) were a worldwide synopsis of taxa and an updated compilation of Braun's papers, respectively. All subsequent taxonomic studies of *Marsilea* were undertaken on a regional basis (Gupta 1962; Launert 1968, 1983–1984; Johnson 1986). *Marsilea* is found primarily in seasonally wet habitats, where it grows in shallow water and at the edges of ponds, lakes, or rivers (Johnson 1986). Plants may be submerged, except for the floating leaflets, or emergent (Kornas 1988). *Regnellidium* is a monotypic genus known only from three localities in southern Brazil and adjacent Argentina, where it grows among aquatic vegetation along lakeshores and in stagnant ponds (Lindman 1904; de la Sota and Mitchell 1970). *Pilularia* is a genus of six species (likely fewer) of wide but disjunct distribution (Tryon and Tryon 1982), and the distinguishing features among its species need to be critically compared (Large and Braggins 1989). It grows submerged in low depressions that are seasonally dry, such as shallow rock pools, lake margins where water levels fluctuate, and vernal pools, and can colonize man-made lakes and impoundments with seasonal drawdown zones (Dennis and Webb 1981). Given the complexity of most fern leaves, the marsileaceous leaf is remarkable for its simplicity. It consists of a petiole and four leaflets in *Marsilea*, a petiole and

two leaflets in *Regnellidium*, and a simple, filiform leaf in *Pilularia* (fig. 1).

The focus of this study is to estimate a phylogenetic hypothesis for marsileaceous ferns based on all currently available information from morphology and *rbcL* sequence data. The goal is to determine which of the hypotheses presented in figure 2 is best supported. In doing this, relationships among all heterosporous ferns, including the whole-plant fossil *Hydropteris*, will be reconsidered.

Materials and Methods

Terminal Taxa

The 23 leptosporangiate fern taxa included in this study (table 1) are, for the most part, a subset of those in Pryer et al. (1995). The only heterosporous fern genera included in that study, however, were *Marsilea quadrifolia* L., *Azolla caroliniana* Willd., and *Salvinia cucullata* Roxb. ex Bory. In order to include all heterosporous fern genera in this study, the following additional taxa were incorporated: *Pilularia americana* L., *Regnellidium diphyllum* Lindm., and the recently described whole-plant fossil *Hydropteris pinnata* Rothwell & Stockey. To better represent the diversity within the largest genus, *Marsilea*, *Marsilea ancylopoda* A. Braun, and *Marsilea polycarpa* Hook. & Grev. were also studied.

The most critical heterosporous fern fossils for understanding phylogenetic relationships are whole plants with associated vegetative and fertile parts from which details of megaspores and microspores can be obtained (Collinson 1992; Rothwell and Stockey 1994). All other known whole-plant species of extinct heterosporous ferns are assignable to extant genera already represented here by a living member (*Marsilea*: Skog and Dilcher 1992, 1994; *Salvinia* and *Azolla*: Collinson 1992). Extinct microfossil genera of heterosporous ferns are known (e.g., Marsileaceae: *Arcellites*, *Molaspora*; Salviniaceae: *Parazolla*, *Ariadnaesporites*; Collinson 1992; Johnson 1986), but these are based on megaspore (and sometimes adherent microspores) material only and thus were not included here because of recognized problems associated with excessive missing data in phylogenetic analyses (Nixon and Davis 1991; Maddison 1993; Wilkinson 1995; Wiens 1998).

Ingroup and Outgroup Circumscriptions

In the analyses by Rothwell and Stockey (1994), *H. pinnata* was shown to be most closely related to *Azolla* and *Salvinia* and was included with them in the newly described suborder Hydropteridineae. These three taxa were included here, along with *Regnellidium*, *Pilularia*, and three species of *Marsilea* (a total of eight taxa making up the ingroup) to test Rothwell and Stockey's (1994) hypothesis regarding this fossil's relationships to other heterosporous ferns.

Among the paraphyletic homosporous ferns, the sister group to heterosporous ferns has not been identified yet with confidence (Rothwell and Stockey 1994; Hasebe et al. 1995; Pryer et al. 1995; Stevenson and Loconte 1996; Doyle 1998). The choice was narrowed to a polytomy of three monophyletic groups by Pryer et al. (1995, p. 225, fig. 4): the schizaeoid ferns, the tree ferns, and a large clade including dennstaedtioid,
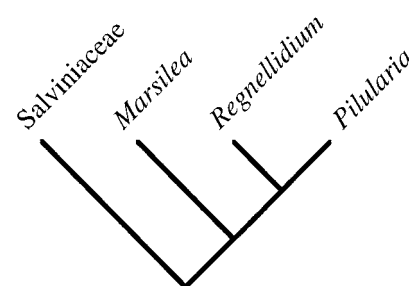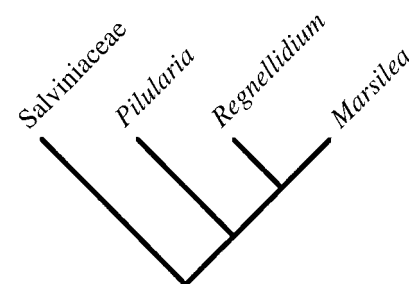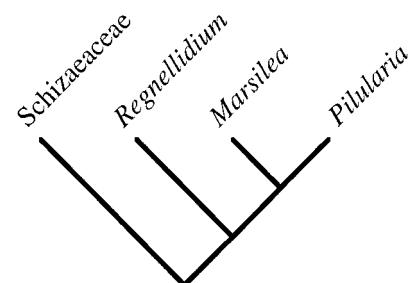
Fig. 2 Alternative phylogenetic hypotheses for relationships within Marsileaceae.

pteridoid, dryopteridoid, and polypodioid ferns, henceforth referred to as the "polypodiaceous" clade (fig. 3). Representatives from these three groups were used here as outgroups. The schizaeoid and tree fern taxa included here (table 1) were the same as in Pryer et al. (1995). The large number of taxa (27) in the robustly supported polypodiaceous clade in Pryer et al. (1995, p. 225, fig. 4) was reduced here to a total of seven taxa. This was achieved by including only a single member from each of their most inclusive subclades with greater than 90% bootstrap support (*Asplenium*, *Coniogramme*, *Pteridium*, *Dennstaedtia*); all taxa not assigned to any of the subclades were also included here (*Monachosorum*, *Lonchitis*, *Lindsaea*). Although the topology of the ingroup is the prin-

Table 1

Taxa Included in Phylogenetic Study

Anemiaceae:[b]
  Anemia mexicana Klotzsch
Aspleniaceae:[d]
  Asplenium filipes Copel.
Azollaceae:[a]
  Azolla caroliniana Willd.
Cyatheaceae:[c]
  Sphaeropteris lepifera (J. Sm. ex Hook.) R.M. Tryon (formerly *Cyathea*)
Dennstaedtiaceae s.l.:[d]
  Dennstaedtia punctilobula (Michx.) T. Moore
  Lindsaea odorata Roxb.
  Lonchitis hirsuta L.
  Monachosorum henryi H. Christ
  Pteridium aquilinum (L.) Kuhn
Dicksoniaceae:[c]
  Calochlaena dubia (R. Br.) M.D. Turner & R.A. White
  Dicksonia antarctica Labill.
Hydropteridaceae:[a]
  Hydropteris pinnata Rothwell & Stockey[e]
Lygodiaceae:[b]
  Lygodium japonicum (Thunb.) Sw.
Marsileaceae:[a]
  Marsilea quadrifolia L.
  M. ancylopoda A. Braun—Guatemala (Huehuetenango), *Williams et al. 22416* (F)
  M. polycarpa Hook. & Grev.—USA (Puerto Rico), *Britton and Cowell 1486* (F)
  Pilularia americana L.—USA (Texas), *Hill 8654* (US)
  Regnellidium diphyllum Lindm.—Brazil (Rio Grande do Sul), *Rau s.n.* (US 1593512)
Metaxyaceae:[c]
  Metaxya rostrata (Kunth) C. Presl
Plagiogyriaceae:[c]
  Plagiogyria japonica Nakai
Pteridaceae:[d]
  Coniogramme japonica (Thunb.) Diels
Salviniaceae:[a]
  Salvinia cucullata Roxb. ex Bory
Schizaeaceae:[b]
  Actinostachys digitata (L.) Wall. (formerly *Schizaea*)

Note.   Voucher exsiccatae examined to verify morphological character data are listed only for *Marsilea ancylopoda*, *Marsilea polycarpa*, *Pilularia americana*, and *Regnellidium diphyllum*; all others are listed in Pryer et al. (1995). Morphological data scored for the whole-plant fossil *Hydropteris pinnata* were obtained from Rothwell and Stockey (1994).

[a] Heterosporous fern.
[b] Schizaeoid fern.
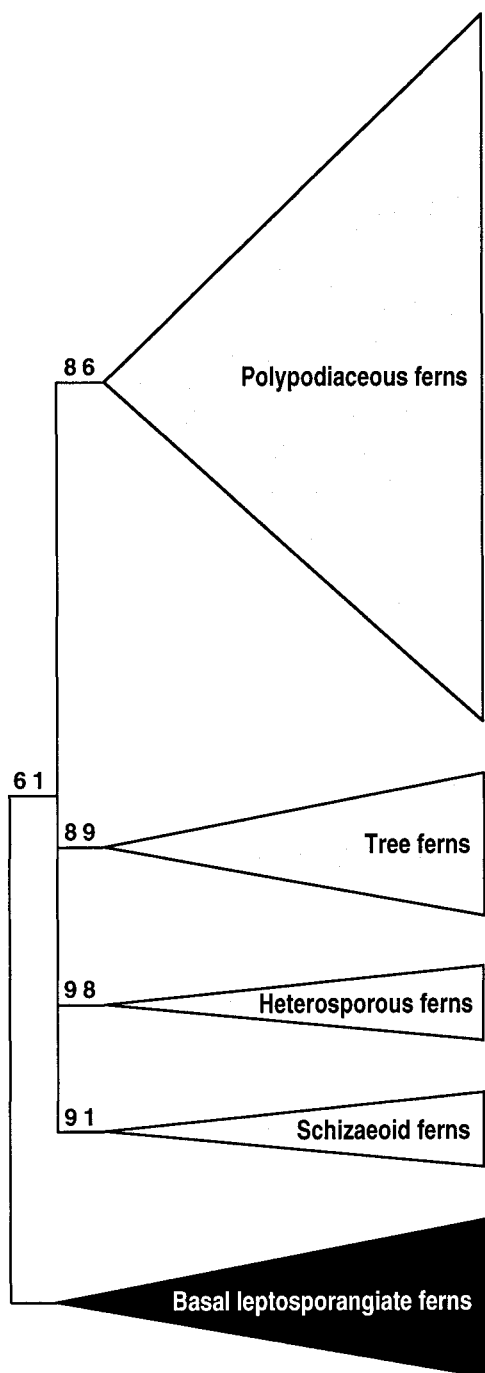[c] Tree fern.
[d] "Polypodicaceous" ferns.
[e] Fossil.

cipal focus here, the relationships within the ingroup and the outgroup were resolved simultaneously. The outgroup was not constrained to have the same topology as in Pryer et al. (1995, p. 225, fig. 4) since the morphological characters differ substantially between these two studies.

Platyzoma was not included in this study even though it has been suggested that, because of its incipient heterospory, it may be more closely related to other heterosporous ferns (Tryon 1964; Tryon and Vida 1967). Stevenson and Loconte (1996) tentatively included it in the Pilulariales as a result of their analyses. In all other recent studies, however, *Platyzoma* has been shown convincingly to be nested within the polypodia-

ceous ferns and, in particular, in the Pteridaceae (Hasebe et al. 1995; Pryer et al. 1995).

Morphological Characters

Morphological information was coded for the same species for which *rbcL* data were available. Morphological characters are defined and documented in the appendix. A total of 71 characters sampling vegetative and reproductive morphology and anatomy were included in this study, and many of these were adopted or modified from Pryer et al. (1995), Rothwell and Stockey (1994), and Stevenson and Loconte (1996). Some

were modified only by adjusting characters state divisions in light of the narrower set of taxa, whereas others were more radically altered from previous publications. Twelve new characters, pertinent especially to heterosporous ferns, were added here (characters 25, 27, 28, 33, 34, 36, 59, 60, 65, 68, 70, and 71). The morphological data, complete with footnotes, were compiled using MacClade, version 3.05 (Maddison and Maddison 1992), and the data matrix is shown in figure 4. Of the 71 morphological characters, 53 were binary, 15 were three-state, and three were four-state. All multistate characters were treated as unordered (Hauser and Presch 1991; Hauser 1992; Mabee 1989). No differential character weighting was used, and trees were rooted via the outgroup method. Character-state optimization was investigated using both Acctran and Deltran. An annotated Nexus file for the morphological matrix showing the source of character coding is posted at http://www.fmnh.org./candr/academic_affairs/ collection_report/cv_pryer.htm.

The morphological and anatomical data were derived from personal observations, a voucher study set of herbarium specimens (table 1), and from the literature. Literature sources consulted here for compiling the data matrix are cited in the appendix.

Autapomorphic characters were excluded. For example, *Regnellidium* is reported to have latex and lactiferous ducts in the sporophyte (Labouriau 1952a, 1952b; de la Sota 1965) and accessory or B-chromosomes (Jain and Raghuvanshi 1973; Lesho 1994), features unknown in any other pteridophyte and more commonly observed in unrelated groups of angiosperms. Several morphological characters that were included in Pryer et al. (1995; abbreviated as PSS) were excluded in this study either because they were not applicable to any of the heterosporous taxa (e.g., annulus characters PSS 57 and PSS 58), they were not informative for the particular taxon combination included in this study, or they were reevaluated. Examples of two reevaluated characters are PSS 48 (Sorus outline), which is apparently not independent from sporocarp shape in heterosporous taxa (and therefore sporocarp shape only was scored here), and PSS 70 (Gametophytes photosynthetic). *Marsilea* and *Azolla* were scored as having nonphotosynthetic gametophytes in Pryer et al. (1995). There are reports in the literature, however, that the sole of the archegonium has chloroplasts (e.g., Higinbotham 1941), and so it is incorrect to score them as not green (see also Campbell 1893b, p. 174; Eames 1936; Loyal and Chopra 1976). The rescoring of taxa resulted in this character being uninformative for the subset of taxa in this study.

The fossil *H. pinnata* could not be scored for 33 characters, nearly half of the 71 characters in this study. These are characters presumed to be knowable but that are inaccessible based on material and available studies (e.g., gametophyte characters). Of the 17 characters in Rothwell and Stockey's (1994) data matrix, which was reanalyzed here, only one was scored as unknown for the fossil.

Molecular Characters

All *rbcL* sequences included 1239 bp for each taxon and, except for *M. ancylopoda* and *M. polycarpa*, were obtained from Hasebe et al. (1995). The following taxa in the present

Fig. 3 Simplified and partial outline of strict consensus tree from Pryer et al. (1995, p. 225, fig. 4), based on 489 *rbcL* and 75 morphological parsimony-informative characters. Major clades are represented by isosceles triangles with a base roughly proportional to the number of taxa included in their analysis. Numbers indicate bootstrap percent values. Sister group to heterosporous ferns unknown as reflected by a polytomy among major groups of derived ferns.

Character number	1 1234567890	2 1234567890	3 1234567890	4 1234567890	5 1234567890	6 1234567890	7 1234567890	1
Marsilea quadrifolia	1111121201	0010000101	1010101010	1201011111	1011A13011	0011000000	131?111111	1
Marsilea ancylopoda	1111121201	0010000101	1010111010	1201011111	1011A13011	0011000000	131?111111	1
Marsilea polycarpa	1111121201	0010000101	1010101010	1210001111	1011A13011	0011000000	131?111111	1
Regnellidium diphyllum	11101-01-0	0010000101	1010101110	0200101111	1?11??3011	1011110000	131?111111	0
Pilularia americana	110---00--	0000001101	1010111110	0200101111	1011A13011	1011110000	131?111111	0
Hydropteris pinnata FOSSIL	1112021201	0?000???01	???0?0??10	?200?1????	1???????10	2011120111	??1???????	?
Azolla caroliniana	01101-01-0	0000001001	1000011021	0100201111	1211212010	2111120111	1310012112	0
Salvinia cucullata	0B0---1211	0000001001	101012--20	0110201111	1211212010	1111120100	1310012112	0
Anemia mexicana	1112011210	0000100100	1010000-00	00----0000	0-00---10-	--10011000	1101010010	0
Lygodium japonicum	1112001210	0000001101	0010000-00	00----0000	0-00---10-	--11111000	1101010010	0
Actinostachys digitata	110---00--	0000000100	1010000-00	00----0000	0-00---10-	--00001000	2001010010	0
Cyathea lepifera	1012001210	1001113100	2101000-01	00----1001	1110---10-	--11110000	0101000000	0
Calochlaena dubia	1012011210	0101100100	1010000-01	00----1001	1011A1210-	--10011000	0101000000	0
Dicksonia antarctica	1012011210	0001013100	2010000-00	00----1101	1011A1210-	--10011000	0101000000	0
Metaxya rostrata	1012011210	0101100111	1010000-00	00----0001	1100---10-	--11110000	?101000000	0
Plagiogyria japonica	1212011210	0001100100	2000000-00	00----0101	1120---10-	--11110000	1101000000	0
Asplenium filipes	1012011210	1000102101	2001000-00	00----0111	112110110-	--01110000	1101010010	0
Dennstaedtia punctilobula	1012001210	1001100111	1110000-00	00----0011	1011A1210-	--11?10000	1101010010	0
Lindsaea odorata	1012011200	1000100101	0001000-00	00----0111	102110110-	--01010000	1101010010	0
Lonchitis hirsuta	1012001210	1000110101	1010000-00	00----0011	102100010-	--11110000	1101010010	0
Monachosorum henryi	1012001210	1000100101	B010000-00	00----0111	1100---10-	--11101000	1201010010	0
Pteridium aquilinum	1012001210	0001110111	1110000-00	10----0111	102100010-	--10110000	1101010010	0
Coniogramme japonica	10120C1211	1000100101	B001000-00	00----0011	1120---10-	--1000?000	1101010010	0

Fig. 4 Morphological data matrix. First row indicates character number. Voucher specimens for each taxon are listed in Pryer et al. (1995) or table 1. Characters and character states are described in the appendix. Question mark = character state unknown; hyphen = character state not applicable (interpreted as missing data in analyses). For certain characters, some taxa were scored and analyzed as polymorphic: A = 0&1; B=1&2; C = 0&1&2. Ingroup and three outgroup clades (schizaeoid ferns, tree ferns, polypodiaceous ferns) are listed separately.

study were listed more than once in Hasebe et al. (1995) since they were sequenced from different vouchers, and the one I selected is indicated by its GenBank number in parentheses: *Lygodium japonicum* (L13479), *Dicksonia antarctica* (U05919), *Pteridium aquilinum* (U05939), and *M. quadrifolia* (L13480).

New *rbcL* sequence data were obtained here for *M. ancylopoda* (U.S.A., Puerto Rico, Pryer et al. 963, DUKE; GenBank Acc. No. AF104212) and *M. polycarpa* (U.S.A., Puerto Rico, Pryer and Tremblay 960, DUKE; GenBank Acc. No. AF104213). These two *Marsilea* species were growing in wet, muddy areas and were therefore carefully washed in a dilute solution of Tween 20 to reduce the risk of algal contamination. The material was then rinsed several times in running water

and dried overnight in a lyophilizer. DNA was extracted using the DTAB-CTAB protocol described by Armaleo and Clerc (1995). Approximately 1.4 kb of the *rbcL* gene was amplified from genomic DNA by polymerase chain reaction (PCR), using primers M34 and M1390R (Lewis et al. 1997). The amplified products were purified using 30,000 NMWL (nominal molecular weight limit) low-binding, regenerated cellulose membrane filter units (Millipore). Both strands of purified PCR products were directly sequenced using the sequencing primers listed in table 2 of Lewis et al. (1997). Sequencing was conducted using an ABI 373A automated DNA sequencer (Perkin Elmer, Applied Biosystems). Sequence fragments were edited and assembled into contiguous alignments, using Sequencher 3.0 (Gene Codes).

The *rbcL* sequence data were straightforward to align because of the presence of codon structure. All *rbcL* characters were unordered and weighted equally. The *rbcL* data matrix is available as a Nexus file at http://www.fmnh.org./candr/academic_affairs/collection_report/cv_pryer.htm. The fossil *H. pinnata* was excluded from *rbcL* analyses because of absence of data.

Phylogenetic Analyses

Phylogenies were reconstructed using equally weighted maximum parsimony as implemented in PAUP* 4.0 (Swofford 1998). The following four analyses were carried out on the morphological and *rbcL* data sets (number of parsimony-informative characters for each analysis is indicated in parentheses): morphology only, without fossil (69); *rbcL* only, without fossil (404); morphological and *rbcL* data combined, without fossil (473); and morphology only, with fossil (71). The morphological and *rbcL* data sets were combined only after a partition-homogeneity test (implemented in PAUP* 4.0 with 100 replicates, each with 10 random-addition-sequence starting trees) indicated that the null hypothesis of homogeneity was supported and that results from *rbcL* and morphology were not significantly different (Bull et al. 1993). The most-parsimonious trees were calculated for each analysis, using a full heuristic search with 10,000 random-addition-sequence starting trees and TBR swapping and MULTREES selected. Equally most-parsimonious trees from all analyses were summarized using strict consensus.

For comparative purposes, the Rothwell and Stockey (1994) data set was reanalyzed here using the branch-and-bound option of PAUP* 4.0 (Swofford 1998). The purpose of this reanalysis was to explore more fully the results in their paper, particularly those morphological characters that supported the resolved topology within Marsileaceae, as well as the relationship of the fossil, *H. pinnata*, to Salviniaceae. Their data set included the same six heterosporous genera, plus four composite taxa of homosporous leptosporangiate ferns, and comprised 16 parsimony-informative morphological characters (their character 6 was autapomorphic). As in their study, the Osmundaceae were used to root the topology.

Branch support was estimated by both bootstrap (Felsenstein 1985) and decay (Bremer 1988; Mishler et al. 1991) analyses. Bootstrap percentages were calculated using 1000 bootstrap replicates and 10 random-addition-sequence starting trees per replicate. The decay indices were determined by examining the strict consensus of trees one to three steps longer than the shortest trees found for the "morphology only" data sets, and the strict consensus of trees one to 10 steps longer than the shortest trees for data sets with *rbcL* data. The searches for longer trees were carried out with the same rigor as for the shortest trees. Accelerated transformation (Acctran) and delayed transformation (Deltran) were used to investigate character-state optimization. All the branch lengths in the topologies presented in this article reflect Acctran optimization.

Results

Phylogeny of Marsileaceae

A single and completely congruent topology for extant Marsileaceae, (*Marsilea*, (*Regnellidium*, *Pilularia*)) was revealed in all the phylogenetic analyses: morphology (with and without fossil), *rbcL*, combined data, and reanalysis of the Rothwell and Stockey (1994) data set (figs. 5–9). Only the degree of support for some internodes, as indicated by bootstrap values, decay indices, and character-state changes, fluctuated among analyses.
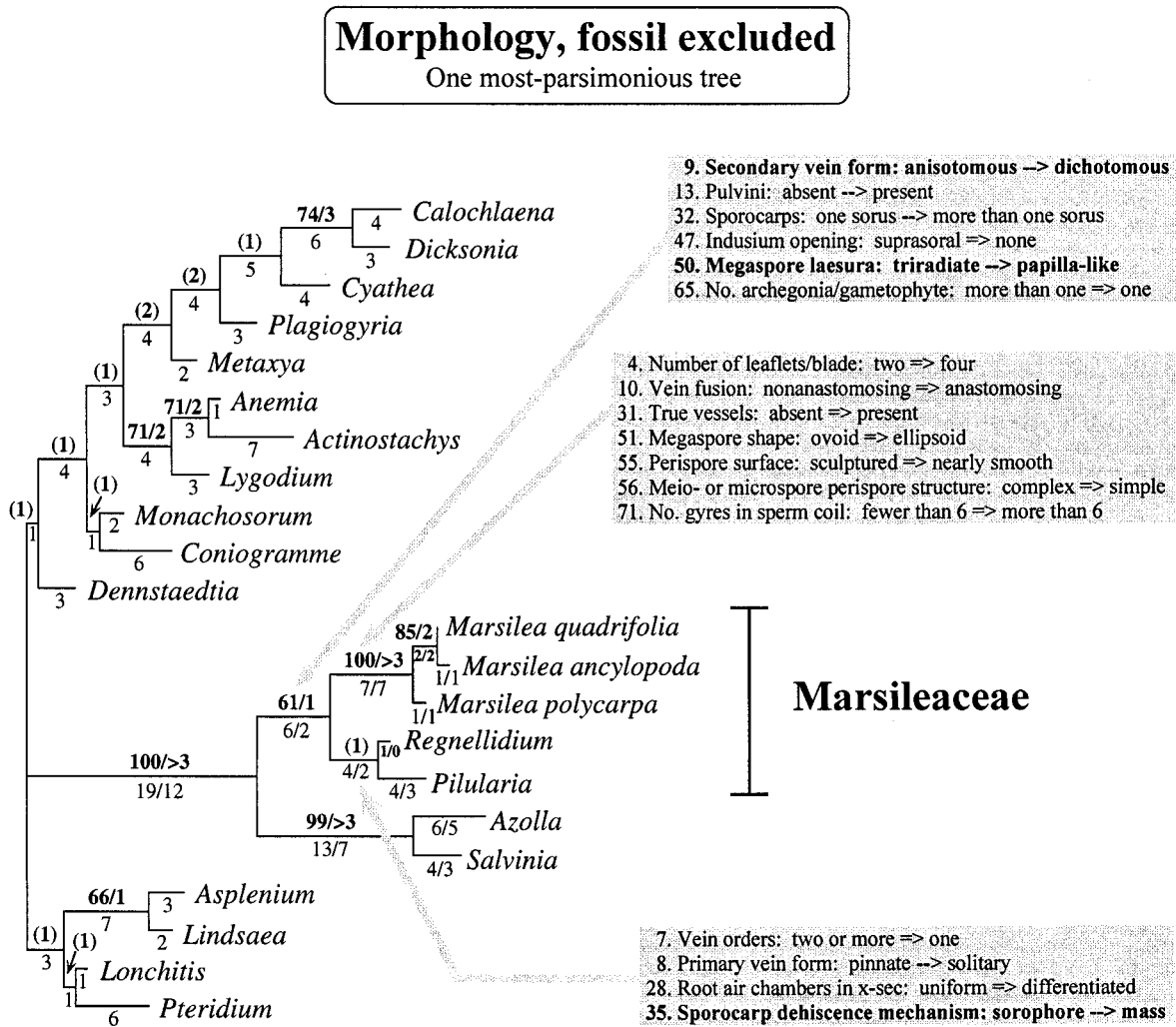
Analyses with Fossil Excluded

Morphology only. A single most-parsimonious tree was found and is shown in figure 5. Bootstrap support for *Marsilea* is 100%, the decay index is >3, and there are seven unambiguous character-state changes located along this branch. Within the genus *Marsilea*, there is robust bootstrap support (85%) for *Marsilea quadrifolia* and *Marsilea ancylopoda* as sister taxa. Four character-state changes support *Regnellidium* and *Pilularia* as sister taxa, though only two are unambiguous; bootstrap support is <50%, and the decay index is 1. The branch supporting Marsileaceae as a monophyletic group has bootstrap support of 61% (decay index = 1), and only two of the six character-state changes that occur along this branch are unambiguous. *Azolla* and *Salvinia* are strongly supported as sister taxa (bootstrap = 99%, decay index > 3). Among the outgroup taxa, the schizaeoid ferns and tree ferns are both monophyletic and weakly supported as sister taxa (<50% bootstrap support, decay index = 1). There is insufficient character support to keep the polypodiaceous ferns together as a clade.

rbcL only. Four most-parsimonious trees were found, and the strict consensus is shown in figure 6. The four trees differ only in their placement of the tree fern taxa. The topology of the ingroup is identical in all four trees, and all of the ingroup branches are robustly supported. Fourteen unambiguous character transformations support *Regnellidium* and *Pilularia* as sister taxa, and bootstrap support for this relationship is 88% (decay index = 5). The schizaeoid ferns, tree ferns, and polypodiaceous ferns are each monophyletic, with low bootstrap (56%) and decay (2) support uniting tree ferns with polypodiaceous ferns.

Combined data (morphology and rbcL*).* The partition-homogeneity test, as implemented in PAUP* 4.0, indicated with a *P* value of 0.41 that the results from morphology and *rbcL* were essentially homogeneous and that the data sets could be combined. A single most-parsimonious tree was found and is shown in figure 7. All of the ingroup branches have bootstrap support >90%. Character-state changes along these branches are additive from the two separate analyses. Support for *Regnellidium* and *Pilularia* as sister taxa is greater in the combined analysis (bootstrap = 94%, decay index = 8) than in either separate analysis (morphology: bootstrap < 50%, decay = 1; *rbcL*: bootstrap = 88%, decay = 5). The schizaeoid ferns, tree ferns, and polypodiaceous ferns are each monophyletic, with increased support uniting the tree ferns with the polypodiaceous ferns (bootstrap = 72%, decay = 4).

Analyses with Fossil Included

Morphology only. Two most-parsimonious trees were found, and both are shown in figure 8. These two trees differ only in their placement of the fossil *Hydropteris pinnata*. The topology for Marsileaceae is identical in both trees and to that

Fig. 5   Single most-parsimonious phylogram resulting from analysis of morphology data only, fossil excluded. Tree length = 176 steps, CI (excluding uninformative characters) = 0.591, RI = 0.737. Bootstrap percent values greater than 50% are shown above branches; number following slash is decay value. If bootstrap support <50%, decay value is given above branch in parentheses. Number of character-state changes is shown below branches (number of unambiguous changes is shown after slash for ingroup taxa only). Character-state changes along branches supporting Marsileaceae, *Marsilea*, and (*Regnellidium*, *Pilularia*) clades are described in detail in shaded boxes: a double-lined arrow indicates that the change is unambiguous, whereas a single-lined arrow indicates an ambiguous change. Character-state changes in bold are notable since their ambiguity may be influenced by having coded critical sister taxa or outgroups as nonapplicable for these characters (see fig. 4), which is interpreted as missing data.

from the analysis of morphology without the fossil (fig. 5). Similarly, the relationships of the outgroup taxa are identical to those in the morphology analysis with the fossil excluded (fig. 5). Bootstrap support for *Marsilea* is 92% (decay index = 2), and four of the five character-state changes located along this branch in both trees are unambiguous (fig. 8A). Six character-state changes support *Regnellidium* and *Pilularia* as sister taxa, though only two are unambiguous (same two as shown in fig. 5), and bootstrap and decay support is weak (51% and 1, respectively). The branch supporting Marsileaceae as a monophyletic group has bootstrap support of 53%, and one-half the character-state changes on this branch are unambiguous (node A in fig. 8A, 8B).

Addition of the fossil in this analysis reduces support for

Azolla and *Salvinia* as sister taxa (bootstrap = 74%, decay index = 1) when compared with the analysis in figure 5, where the fossil was excluded. In figure 8A, *Hydropteris* is weakly supported (<50%) as sister to the *Azolla* plus *Salvinia* clade, and together these three taxa form a sister group to the Marsileaceae. This relationship of the fossil to the other heterosporous ferns is the same as that found by Rothwell and Stockey (1994). In figure 8B, the *Azolla* plus *Salvinia* clade is weakly supported (<50%) as sister group to Marsileaceae, and the fossil *Hydropteris* is the most basal member of the heterosporous fern clade. All the character-state changes occurring on those branches that differ in length or position between the two trees are described in detail in figure 8 in shaded boxes A–F. The two trees provide equivocal interpretations of char-
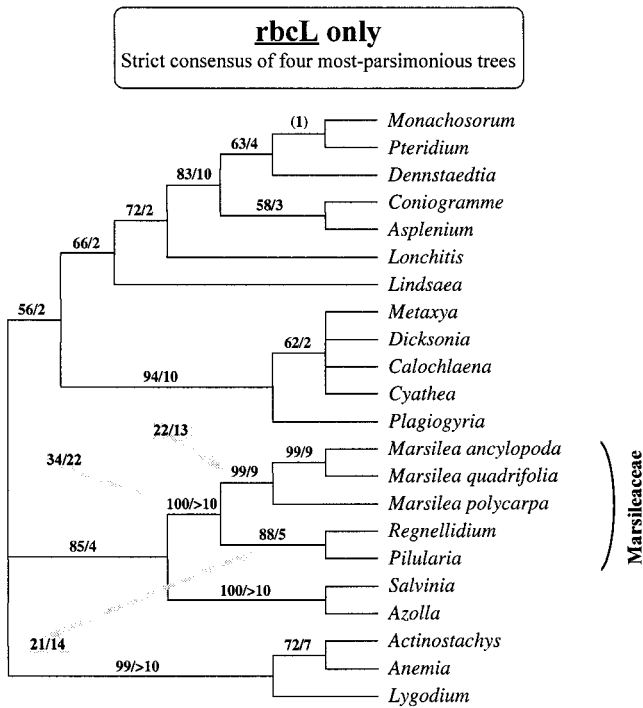
Fig. 6 Strict consensus of four most-parsimonious trees resulting from analysis of *rbcL* data only. Tree length = 1584 steps, CI (excluding uninformative characters) = 0.384, RI = 0.496. Bootstrap percent values greater than 50% are shown above branches; number following slash is decay value. If bootstrap support <50%, decay value is given above branch in parentheses. Number of character-state changes supporting Marsileaceae, *Marsilea*, and (*Regnellidium, Pilularia*) clades is highlighted in shaded boxes (number of unambiguous changes is shown after slash).

or discuss in detail their character-state definitions for their character 14 (simple, filamentous, and lamellar or vacuolate). Therefore, it was not possible for me to determine to what degree the character-state definitions and assignments that I used here (see my character 56) are consistent with their study. At any rate, in my extended analysis, this character was not one of those providing support to the *Regnellidium-Pilularia* clade in the "fossil excluded" morphology analysis (fig. 5). However, it was one of the four ambiguous character-state changes supporting the clade in one of the two most-parsimonious trees in the "fossil included" morphology analysis (fig. 8*B*; detailed character list not shown for this node).

Rothwell and Stockey's (1994) conclusion that *Hydropteris* is more closely related to Salviniaceae is shown here to have a 69% bootstrap value, a decay index of 2, and is supported by two unambiguous character-state changes (fig. 9, box *B*, character 11, microspores: free⇒embedded; character 14, microspore perispore structure: simple⇒vacuolate). These are essentially the same two unambiguous characters that support
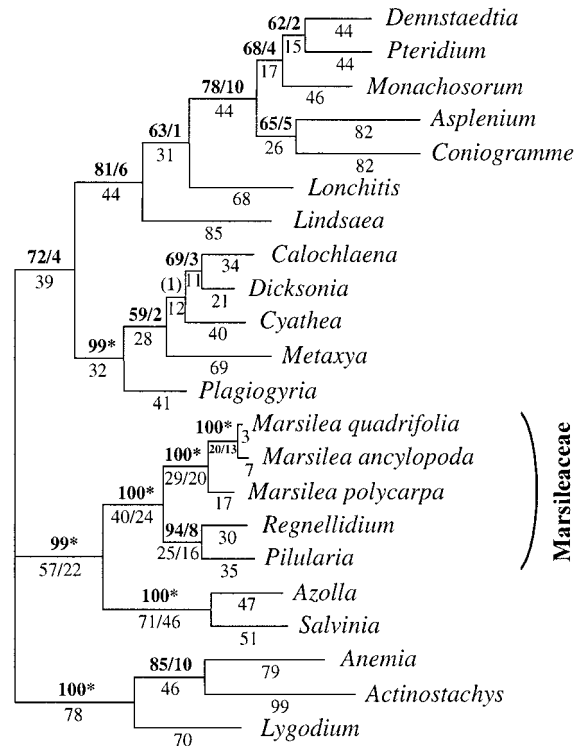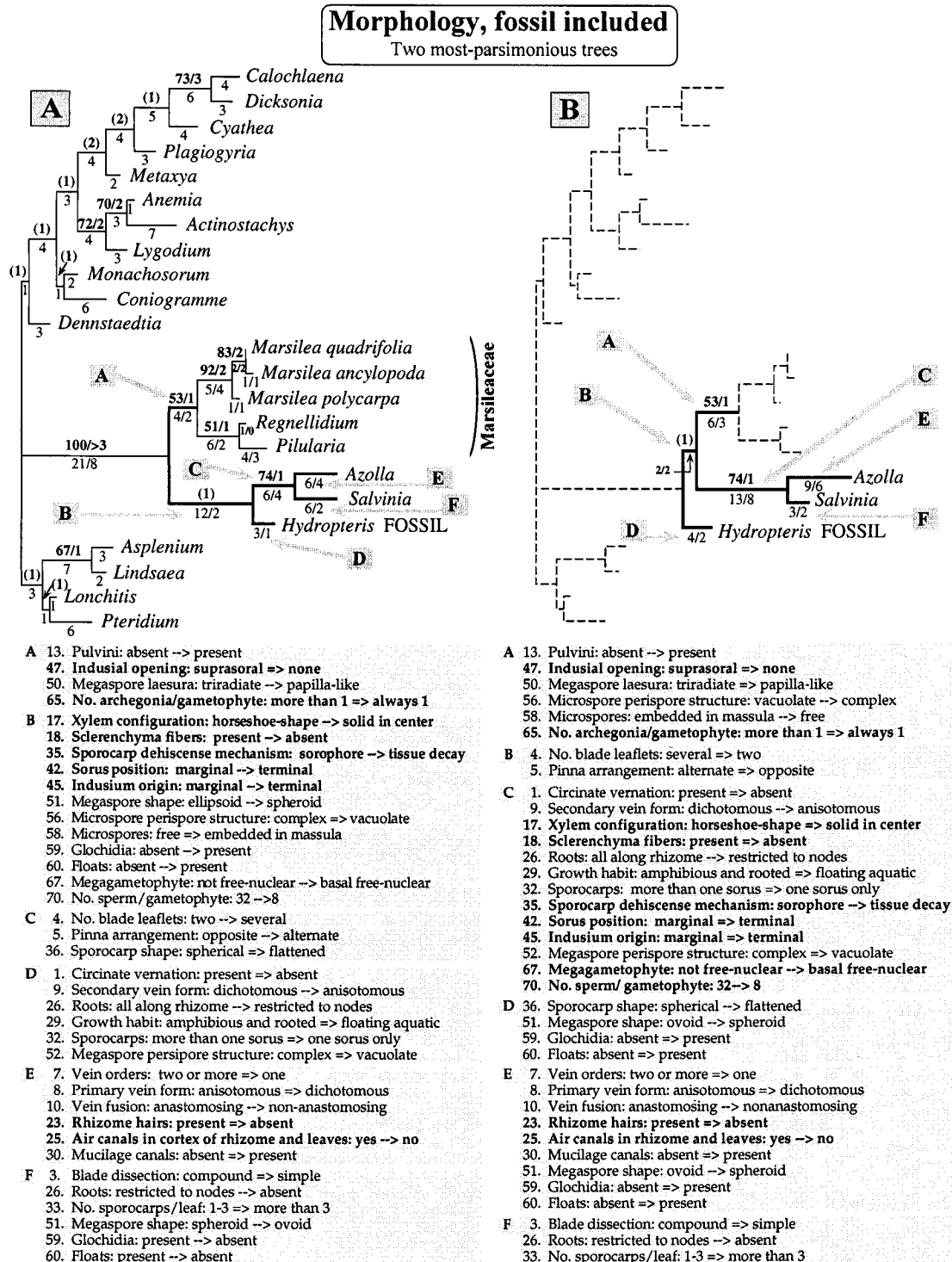
acter evolution in heterosporous ferns. Characters on these branches for which the fossil was scored as missing data are indicated in bold (figs. 4, 8).

Reanalysis of Rothwell and Stockey (1994) data set. In a study of heterosporous ferns by Rothwell and Stockey (1994) based on 17 morphological characters (one character parsimony-uninformative) for 10 fern taxa, their strict consensus of three most-parsimonious trees resolved relationships within Marsileaceae that are completely congruent with the present study (figs. 5–8). Their data set was reanalyzed here to critically assess support for the internodes using bootstrap and decay analyses and to examine character-state changes along the various branches. As in their study, three most-parsimonious trees were found, and the strict consensus is shown in figure 9. The three trees differ only in their placement of homosporous fern taxa. The topology of heterosporous ferns is identical in all three trees. Bootstrap (64%) and decay (1) support for the *Pilularia-Regnellidium* node is weak and is provided by a single unambiguous character-state change (fig. 9, box *A*, character 14, microspore perispore structure: simple⇒filamentous). When this single character was removed from the analysis, several more equally most-parsimonious trees were obtained, and relationships within Marsileaceae were unresolved (trichotomy). Rothwell and Stockey (1994) did not elaborate on

Fig. 7 Single most-parsimonious phylogram resulting from combined analysis of morphology and *rbcL* data. Tree length = 1771 steps, CI (excluding uninformative characters) = 0.402, RI = 0.521. Bootstrap percent values greater than 50% are shown above branches; number following slash is decay value. An asterisk indicates decay value >10. If bootstrap support <50%, decay value is given above branch in parentheses. Number of character-state changes is shown below branches (number of unambiguous changes is shown after slash for ingroup taxa only).

Morphology, fossil included
Two most-parsimonious trees

A 13. Pulvini: absent --> present
47. Indusial opening: suprasoral => none
50. Megaspore laesura: triradiate --> papilla-like
65. No. archegonia/gametophyte: more than 1 => always 1
B 17. Xylem configuration: horseshoe-shape --> solid in center
18. Sclerenchyma fibers: present --> absent
35. Sporocarp dehiscense mechanism: sorophore --> tissue decay
42. Sorus position: marginal --> terminal
45. Indusium origin: marginal --> terminal
51. Megaspore shape: ellipsoid --> spheroid
56. Microspore perispore structure: complex => vacuolate
58. Microspores: free => embedded in massula
59. Glochidia: absent --> present
60. Floats: absent --> present
67. Megagametophyte: not free-nuclear --> basal free-nuclear
70. No. sperm/gametophyte: 32 -->8
C 4. No. blade leaflets: two --> several
5. Pinna arrangement: opposite --> alternate
36. Sporocarp shape: spherical => flattened
D 1. Circinate vernation: present => absent
9. Secondary vein form: dichotomous --> anisotomous
26. Roots: all along rhizome --> restricted to nodes
29. Growth habit: amphibious and rooted => floating aquatic
32. Sporocarps: more than one sorus => one sorus only
52. Megaspore perispore structure: complex => vacuolate
E 7. Vein orders: two or more => one
8. Primary vein form: anisotomous => dichotomous
10. Vein fusion: anastomosing --> non-anastomosing
23. Rhizome hairs: present => absent
25. Air canals in cortex of rhizome and leaves: yes --> no
30. Mucilage canals: absent => present
F 3. Blade dissection: compound => simple
26. Roots: restricted to nodes --> absent
33. No. sporocarps/leaf: 1-3 => more than 3
51. Megaspore shape: spheroid --> ovoid
59. Glochidia: present --> absent
60. Floats: present --> absent

A 13. Pulvini: absent --> present
47. Indusial opening: suprasoral => none
50. Megaspore laesura: triradiate => papilla-like
56. Microspore perispore structure: vacuolate --> complex
58. Microspores: embedded in massula --> free
65. No. archegonia/gametophyte: more than 1 => always 1
B 4. No. blade leaflets: several => two
5. Pinna arrangement: alternate => opposite
C 1. Circinate vernation: present => absent
9. Secondary vein form: dichotomous --> anisotomous
17. Xylem configuration: horseshoe-shape => solid in center
18. Sclerenchyma fibers: present => absent
26. Roots: all along rhizome --> restricted to nodes
29. Growth habit: amphibious and rooted => floating aquatic
32. Sporocarps: more than one sorus => one sorus only
35. Sporocarp dehiscense mechanism: sorophore --> tissue decay
42. Sorus position: marginal => terminal
45. Indusium origin: marginal => terminal
52. Megaspore perispore structure: complex => vacuolate
67. Megagametophyte: not free-nuclear --> basal free-nuclear
70. No. sperm/ gametophyte: 32--> 8
D 36. Sporocarp shape: spherical --> flattened
51. Megaspore shape: ovoid --> spheroid
59. Glochidia: absent => present
60. Floats: absent => present
E 7. Vein orders: two or more => one
8. Primary vein form: anisotomous => dichotomous
10. Vein fusion: anastomosing --> nonanastomosing
23. Rhizome hairs: present => absent
25. Air canals in rhizome and leaves: yes --> no
30. Mucilage canals: absent => present
51. Megaspore shape: ovoid --> spheroid
59. Glochidia: absent => present
60. Floats: absent => present
F 3. Blade dissection: compound => simple
26. Roots: restricted to nodes --> absent
33. No. sporocarps/leaf: 1-3 => more than 3

Fig. 8 Two most-parsimonious phylograms resulting from analysis of morphology data only, fossil included. Tree length = 186 steps, CI (excluding uninformative characters) = 0.570, RI = 0.724. Bootstrap percent values greater than 50% are shown above branches; number following slash is decay value. If bootstrap support <50%, decay value is given above branch in parentheses. Number of character-state changes is shown below branches (number of unambiguous changes is shown after slash for ingroup taxa only). Portions of the topology in the second tree that are identical to those in the first tree are shown with dashed lines. Branches that differ (in length or position) between the two trees have thicker lines, and all character-state changes on these branches are described in detail in shaded boxes *A–F*. A double-lined arrow indicates an unambiguous change, whereas a single-lined arrow indicates one that is ambiguous. Characters on these branches for which the fossil was scored as missing data are shown in bold (see also fig. 4).

Rothwell and Stockey 1994, reanalyzed
Strict consensus of three most-parsimonious trees

A
3. No. pinnae: 4 --> 2
5. Venation: closed --> open
14. Microspore perispore structure: simple => filamentous
15. Megaspore shape: ellipsoidal --> ovate

B
11. Microspores: free => embedded in common massula
14. Microspore perispore structure: simple => vacuolate
15. Megaspore shape: ellipsoidal --> spheroidal

Fig. 9 Strict consensus of three most-parsimonious trees from reanalysis of Rothwell and Stockey (1994) data set. Tree length = 32 steps (reported as 34 steps in Rothwell and Stockey [1994]), CI (excluding uninformative characters) = 0.781, RI = 0.788. Bootstrap percent values >50% are shown above branches; number following slash is decay value. Character-state changes along branches supporting (*Regnellidium*, *Pilularia*) and (*Hydropteris*, (*Salvinia*, *Azolla*)) clades are described in detail in shaded boxes *A* and *B*. A double-lined arrow indicates that the change is unambiguous, whereas a single-lined arrow indicates an ambiguous change.

the same hypothesis shown on one of the two parsimonious trees in the extended analysis (fig. 8*A*, box *B*, characters 56 and 58). The distribution of these particular character-state changes is completely modified in the alternative equally most-parsimonious tree (fig. 8*B*, see box *A*).

Discussion

Phylogeny of Marsileaceae

Speculation on putative relationships among genera of Marsileaceae has been controversial and long standing (Bower 1926; Schmidt 1978; Wallace et al. 1984; Johnson 1986). From the results presented in this article, it now can be stated with confidence that *Pilularia* and *Regnellidium* are more closely related to one another than either is to *Marsilea* and that the

hypothesis of phylogenetic relationships of Marsileaceae by Hasebe et al. (1995) and Rothwell and Stockey (1994), shown in figure 2, is favored over the alternative hypotheses of Stevenson and Loconte (1996) and Schmidt (1978).

The apparent phenotypic simplicity of the three genera of Marsileaceae, and heterosporous ferns in general, made homology assessments and the assembly of an informative morphological data set for this group of plants particularly challenging. It is not surprising that past interpretations of their relationships were contradictory (fig. 2). In the cladistic analyses in this study, the morphology data alone do indicate that *Pilularia* and *Regnellidium* are more closely related to one another, although the bootstrap and decay support for this relationship is weak (figs. 5, 8), unlike the analysis of *rbcL* characters (fig. 6). The extreme reduction in phenotypic morphology in *Pilularia*, and to some extent in *Regnellidium*, is most likely the reason for this weak support, since some characters were necessarily inapplicable and had to be scored as missing data (fig. 4). For example, *Pilularia* has simple leaves and therefore could not be scored for characters 4, 5, and 6, which pertain to the pinnae or leaflets of compound leaves. Likewise, because *Pilularia* has a single vein, characters 9 and 10 were inapplicable because they relate to secondary vein form and vein fusion. The reduced morphology found in *Regnellidium* resulted in it also being scored as inapplicable for two of these five characters. Leaflet pulvini presence is a character state shared by both *Regnellidium* and *Marsilea*, but because *Pilularia* has no leaflets, the character is inapplicable. The reduced size of *Pilularia* likely also affects such characters as xylem configuration (character 17), which is horseshoe shaped with a parenchymatous center in *Marsilea* and *Regnellidium* but consists simply of a small central bundle in *Pilularia*.

A glance at figure 4 indicates that within Marsileaceae, *Regnellidium* and *Pilularia* are scored the same state for a number of characters, but only two of these (character 7, single vein order, and character 28, differentiated root air chambers) are shown to be umambiguous synapomorphies for the clade (figs. 5, 8). The character states shared by *Regnellidium* and *Pilularia* for characters 31 (lack of vessels), 55 (sculptured perispore surface), 56 (complex perispore structure), and 71 (fewer than six gyres in sperm coil) are shown to be symplesiomorphic and not informative for this particular relationship (fig. 10). Conversely, these same characters have states that are synapomorphic for the genus *Marsilea* (figs. 5, 10).

In the Rothwell and Stockey (1994) data set, the single unambiguous character state supporting the *Regnellidium* plus *Pilularia* clade was their character 14, a "filamentous" microspore perispore structure (fig. 9), which was defined as "complex" and identified as a plesiomorphic character (character 56; fig. 10) in the current extended analysis. This could be caused by taxon-sampling differences between the two data sets and therefore an apt demonstration of how choice of taxa, including more proximate outgroups, can have an effect on the character evidence. This difference might also be explained by conflicting interpretations of character states, which were not explicitly defined in Rothwell and Stockey (1994).

It is generally acknowledged that the phylogenetic loss of characters can obscure genealogical hierarchy in attempts to reconstruct it from morphological characters; these phyloge-

Fig. 10   Distribution of selected characters plotted onto same topology shown in figure 7 (the best estimate of the phylogeny). The names of outgroup taxa have been removed. The character states shared by *Regnellidium* and *Pilularia* for these four characters are shown here to be symplesiomorphic and not informative for that clade. Conversely, these characters have states that are synapomorphic for all *Marsilea* taxa.

nies are often beset with extensive phenotypic homoplasy (Nelson 1978; Stevens 1980; Nelson and Platnick 1981; Kluge 1985; Bateman 1996). There are simply too few informative characters to permit a hypothesis of relationships within Marsileaceae based on morphology alone that is robustly supported by bootstrap and decay values (fig. 5). However, morphological synapomorphies that support the relationships now have been identified. Additional detailed information from the internal structure of the sporocarps and relating to the gametophytic phase may prove useful in future morphological analyses. Results from the *rbcL* data set, however, very strongly support a sister relationship between *Pilularia* and *Regnellidium* (fig. 6). This support is not contraindicated when the morphological data are combined with molecular data (fig. 7). In fact, it is interesting to note how bootstrap and decay sup-

port values are improved in the combined analysis (fig. 7) when compared with the separate analyses (figs. 5, 6).

Missing Data

The problem of missing data has been discussed most often in the context of paleontological data sets that include poorly preserved fossils as terminal taxa (Donoghue et al. 1989; Huelsenbeck 1991; Novacek 1992). It has also been discussed in relation to interpreting gaps as missing data in molecular DNA sequence alignments (e.g., Barriel 1994; Hibbett et al. 1995; Lutzoni et al., in press). The fossil *Hydropteris* could not be scored for nearly half of the morphological characters (33 out of 71) in this study and for none of the *rbcL* characters. For example, there is a total absence of gametophytic data for

Hydropteris (fig. 4), which is not surprising because vascular plant gametophytes and gametangia are exceedingly rare in the fossil record (Kenrick 1994; Kenrick and Crane 1997).

A side effect of including taxa with much missing data is usually a dramatic increase in the number of equally most-parsimonious trees, resulting in a loss of resolution in consensus trees (Huelsenbeck 1991; Novacek 1992) that obfuscates phylogenetic relationships. However, it is not simply the proportion of missing entries, but the kinds of characters that are preserved that can determine the potential influence of an added taxon (Wilkinson 1995). Adding *Hydropteris* to the data set resulted in only two equally most-parsimonious trees.

Nonapplicable Data

The interpretation of "not applicable" character states as missing data in phylogenetic analyses is complex. Data are not applicable when taxa are known to lack a structure under study altogether. Scoring "not applicable" as a separate character state could result in two taxa that lack a complex structure being falsely grouped by sharing a large number of "not applicable" states. As shown by Waggoner (1996), this is not a satisfactory solution to the problem. Another solution, which is more commonly practiced and that was also done in this study, is to code those taxa lacking the structure as having missing data, even though the implications are not the same in that this information is never expected to be gained—it is truly lacking. The risks involved in doing this were explicitly defined by Maddison (1993) and are sometimes referred to as the Maddison effect (Doyle 1996). A particularly troublesome result of scoring nonapplicable characters as missing data using parsimony is that ancestors and taxa without the character will sometimes be equivocally (or worse still, unequivocally) assigned impossible states (Platnick et al. 1991). However, if the character is inapplicable only among the outgroups, then coding them as having missing data will not cause problems (Maddison 1993). This is the most common scenario for several characters scored here as not applicable (see fig. 4 between characters 28 and 52). Alternative approaches to the problem of scoring inapplicable characters as missing data and/or algorithms that can effectively consider interactions between independent characters are still needed in morphological cladistic studies.

Relationships of the Fossil Hydropteris pinnata

The Late Cretaceous fossil *H. pinnata* uniquely combines certain vegetative and reproductive features of Marsileaceae (large, multisoral, bisporangiate sporocarps), Salviniaceae (megaspore complexes and microsporangiate massulae), and most other leptosporangiate ferns (pinnate leaves), and this combination is unlike that of any other known fossil or modern heterosporous fern. *Hydropteris* was shown by Rothwell and Stockey (1994) to be the sister group to Salviniaceae and, together with Salvinaceae, a sister group to Marsileaceae. Rothwell and Stockey (1994, p. 490) indicated some surprise with their result, stating "Contrary to our intuitive supposition, *Hydropteris* nests with the floating aquatic taxa rather than with the marsileaceous taxa, with which it has much more morphological similarity." An examination of their character-state distributions led them to determine that character states shared by *Hydropteris* and the marsileaceous genera were symplesiomorphic, whereas it shared two synapomorphies with the Salviniaceae clade. It is on this basis that they proposed a new suborder, Hydropteridineae, to circumscribe the *Hydropteris* plus Salviniaceae clade.

This data set was reanalyzed by Bateman (1996), who showed that merely ordering a single foliar character was sufficient to place *Hydropteris* as sister taxon to Salviniaceae plus Marsileaceae. That this minor revision to the character data could have so dramatic an impact underscores the frailty of the data and phylogenetic conclusions. A reanalysis of the Rothwell and Stockey (1994) data set was also done here (fig. 9). The bootstrap values are comparable to those calculated by Bateman (1996), though slightly lower. The branch supporting the (*Hydropteris*, (*Salvinia*, *Azolla*)) clade has a bootstrap value of 69% (75% in Bateman 1996) and a decay index of 2. It was confirmed that ordering character 3 in Rothwell and Stockey's (1994) data matrix places *Hydropteris* at the base of the heterosporous fern clade.

In my extended data set, *Hydropteris* could be scored for only 33 of the 71 characters. According to Huelsenbeck (1991), adding taxa with missing character data could increase ambiguity in the assignment of character states to nodes, thereby likely increasing the number of most-parsimonious trees. The result of this analysis was only two most-parsimonious trees. One showed the same relationship as in Rothwell and Stockey (1994), and the other an alternative arrangement, pointed out by Bateman (1996), placing *Hydropteris* as the most basal member of the heterosporous fern clade (fig. 8). The support for both placements of the fossil was weak, with less than 50% bootstrap support and a decay index of 1. The two unambiguous character-state transitions that support (*Hydropteris*, (*Salvinia*, *Azolla*)) in figure 8*A* (node *B*) are the same as in the Rothwell and Stockey (1994) analysis and differ completely from the two unambiguous character states supporting the Salviniaceae plus Marsileaceae clade in figure 8*B* (node *B*). Removing all characters scored for missing data in the fossil is not a solution because it results in hundreds of equally most-parsimonious trees with no resolution for the fossil's relationship to other heterosporous ferns (trees not shown). Interestingly, ordering characters 4, 8, 32, and 70 in the extended data set (singly or in combination) also did not help to resolve ambiguity over the fossil's placement (trees also not shown).

The two equally most-parsimonious placements of *Hydropteris* in this study indicate that it is premature to adopt the suborder classification Hydropteridineae for the *Hydropteris* plus Salviniaceae clade as proposed by Rothwell and Stockey (1994). These two hypotheses undoubtedly result from the degree of character ambiguity that *Hydropteris* presents, and the computed phylogeny is, to that extent, in agreement with the initial subjective impressions by the discoverers of the fossil (Rothwell and Stockey 1994). The phylogenetic position of *Hydropteris* has broad implications for our understanding of character evolution in the heterosporous ferns (cf. fig. 8*A*, 8*B*), and until we have robust resolution for its placement, alternative possible trees still need to be considered.

Adding fossils to a phylogenetic study often can play a pivotal role by effectively strengthening some groupings of fossil and living taxa at the expense of other groupings (Huelsenbeck

1991). However, there are several recent examples of fossils from key periods, possessing a mélange of defining character states from different groups, that have reduced phylogenetic resolution rather than increased it. For example, Clack (1998) described a 334-million-yr-old fossil amphibian with character states, in the same animal, usually associated with three different types of early tetrapods. This mosaic of character states resulted in three conflicting evolutionary interpretations in Clack's (1998) phylogenetic analysis. This situation has also been reported in other contexts in vertebrate evolutionary biology (Ji et al. 1998; Padian 1998; Shubin 1998).

A recent botanical example of this phenomenon is the report by Sun et al. (1998) of the first fossil evidence of a Jurassic angiosperm, *Archaefructus*. *Archaefructus* uniquely combines magnolialean characters as well as the nonmagnolialean feature of a missing perianth, a condition found in some species of Chloranthaceae and Piperaceae, families competing with Magnoliales for a basal position within angiosperms (Crane et al. 1995; Crepet 1998; Sun et al. 1998). The chimeric nature of *Archaefructus* suggests that when it is eventually incorporated into a phylogenetic analysis of basal angiosperms, its particular combination of character states could in fact decrease phylogenetic resolution.

The mix of characters seen in *Hydropteris* suggests that ecological experimentation during the evolution of heterosporous ferns may have involved parallel evolution of similar characters (e.g., large, multisoral, bisporangiate sporocarps) in closely related groups. The mosaicism seen in the fossil record may point to the possibility that similar genes and developmental processes can result in very different organs and organisms. There is strong evidence that morphological variation and diversity may be the product of regulatory gene evolution and changes in the relative timing of developmental gene expression (heterochrony; Carroll 1995). In this way, new characters may seemingly be "cut and pasted" on different groups at different times (Shubin 1998).

### Stratigraphic Record for Heterosporous Ferns

The stratigraphic distribution of fossils is usually thought to preserve, at least at some level, a phylogenetic signal (Norell and Novacek 1992; Benton and Storrs 1994; Huelsenbeck 1994; Brochu 1997), although the extent to which stratigraphy can be applied to cladistic studies has been recently debated (Thewissen 1992; Wagner 1995; Norell 1996; Siddall 1998). First appearances of extant taxa of heterosporous ferns were estimated here primarily from stratigraphic information summarized in Collinson (1992), Skog and Dilcher (1992, 1994), and references cited therein (fig. 11B). The fossil *Hydropteris* is known from a single site and formation (Rothwell and Stockey 1994). The two competing hypotheses of relationships for *Hydropteris* (fig. 8) were compared here with the known temporal distribution of relevant taxa to determine whether stratigraphy could provide an argument to favor one topology over the other (fig. 12).

The Stratigraphic Consistency Index (SCI) of Huelsenbeck (1994) was calculated to measure the degree to which either cladogram conflicted with superpositional information (fig. 11). The SCI is the ratio between the number of nodes on a cladogram that are consistent with stratigraphy and the total number of internal nodes (the root is excluded). This measure of stratigraphic fit is sensitive to errors in phylogenetic estimation as well as to missing parts of lineages, which is an important consideration given the incomplete nature of the fossil record for these ferns (Johnson 1986; Stewart and Rothwell 1993). Two of the four internal nodes on both trees (fig. 11; see also arrows in fig. 12) were found to be inconsistent, resulting in a SCI of 0.5 and suggesting that either hypothesis is equally valid. Based on SCI, the node supporting *Regnellidium* plus *Pilularia* is consistent (*N1*; fig. 11A, 11C); however, the node indicating a sister group relationship between *Marsilea* and the *Regnellidium-Pilularia* clade is inconsistent with stratigraphy (*N2*; fig. 11A, 11C). This is because *Marsilea* is the oldest lineage of heterosporous ferns, and stratigraphy rejects the notion that the youngest occurrences (*Regnellidium* and *Pilularia*) would pair with the oldest. Marsileaceae, however, are poorly represented in the fossil record (Collinson 1992). In figure 12A (see also *N3*; fig. 11A), the *Salvinia* plus *Azolla* node is inconsistent, whereas in figure 12B (see also *N4*; fig. 11B), the alternative placement of *Hydropteris* at the base of heterosporous ferns causes the node supporting the *Salvinia* plus *Azolla* clade to become consistent with stratigraphy but creates a new inconsistency at the most basal node.

A different approach to examine the fit of the stratigraphic record to a phylogenetic tree was also attempted here. It measures absolute temporal disparity by relying on assessments of ghost lineages. The number of ghost lineages required for a given topology can be estimated by calculating the sum of minimum implied gaps (SMIG; Benton and Storrs 1994; Brochu 1997). Each minimum implied gap is the difference between the age of the first representative of a lineage and that of its phylogenetic sister. Rough estimates of first occurrences for heterosporous ferns are shown in figure 11B. SMIG is simply the summed lengths of minimum ghost lineages over the entire tree and corresponds to the "stratigraphic debt" of the tree. It is best cited as a measure of relative completeness of the tree (Benton and Storrs 1994). The tree in figure 12A (SMIG = 125) matches the absolute temporal pattern better than the tree in figure 12B (SMIG = 134), although the difference between them is small (6.7%). In other words, the tree in figure 12A requires 6.7% less missing fossil record (stratigraphic debt) than the alternative topology. This is because longer ghost lineages extending at least to the Early Cretaceous are required for *Hydropteris* and for the lineage supporting the *Salvinia* plus *Azolla* clade in figure 12B, whereas the hypothesized relationships in figure 12A clip these same ghost lineages to before the Cenomanian (Late Cretaceous).

Both competing hypotheses for the relationship of *Hydropteris* to other heterosporous ferns predict undiscovered fossils in the lowermost Cretaceous. There are Cretaceous microfossil genera for both Marsileaceae and Salviniaceae (e.g., Hall 1963; Kovach and Batten 1989), extending the temporal ranges for these lineages, that were not included here because of excessive missing data with this particular character matrix. For example, one might constrain the megaspores of the extinct form genus *Molaspora* Schemel to be synonymous with *Regnellidium* on the basis of the cautious observation by Batten (1988) and Collinson (1992) that they are almost identical to modern *Regnellidium*. This alone would extend the *Regnellidium* line-
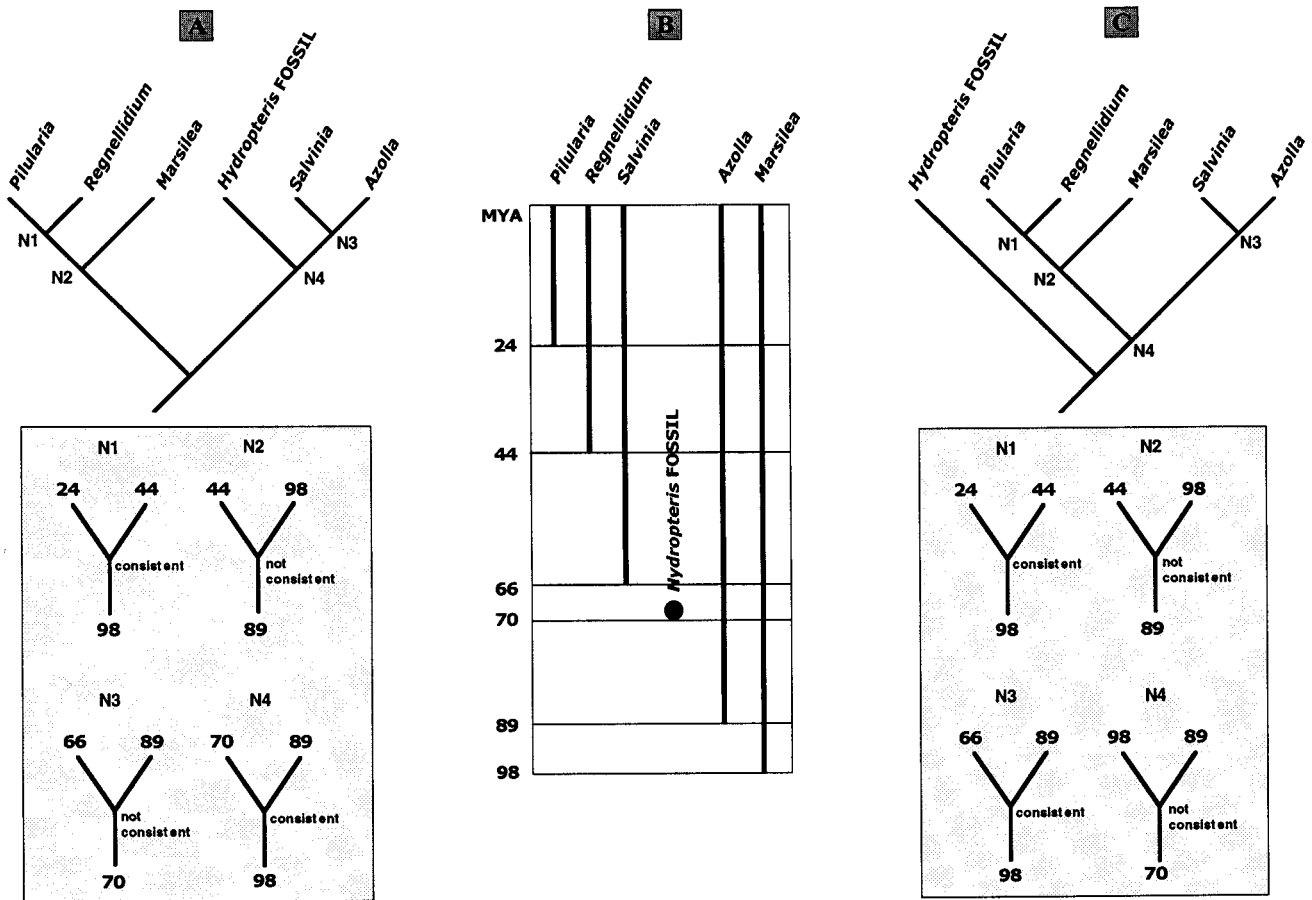
Fig. 11 Calculation of Stratigraphic Consistency Index (SCI; *sensu* Huelsenbeck 1994). *A, C,* Two competing hypotheses for the placement of the fossil *Hydropteris.* The nodes of interest include *N1* to *N4* and exclude the bottom (root) node. *B,* Stratigraphic ranges of the lineages with first occurrences (minimum age estimates) reported according to Collinson (1992), Skog and Dilcher (1992, 1994), Rothwell and Stockey (1994). A node is stratigraphically consistent when the oldest first occurrence above the node is the same age or younger than the oldest first occurrence of the sister taxon of that node. The SCI is 0.50 for both phylogenies, because two of the four nodes for each topology are inconsistent with the stratigraphic record.

age from the Paleocene to the Early Cretaceous as a "Lazarus taxon"—one that apparently goes extinct and then reappears higher up in the rock record (Benton and Storrs 1996). Likewise, if one accepts *Arcellites* Miner as an extinct genus of Marsileaceae, and not part of an extinct family (Collinson 1992), this would extend the family lineage even further into the Early Cretaceous. Five form genera (also not included here) based on megaspores with attached microspore massulae have been assigned to the Salviniaceae. These fossils indicate that this clade can also be drawn back to the Early Cretaceous (Collinson 1992). If these microfossils were subjected to careful and detailed reexamination, it would be worthwhile to attempt a cladistic analysis of heterosporous ferns that also includes all microfossil genera. This might necessarily require restricting the analysis to ultrastructural and other fine details of the megaspores and microspore complexes, but it could potentially yield a better understanding of the relationships of these taxa than is currently known.

Donoghue et al. (1989) investigated the relationship between completeness and informativeness and concluded that some

fossils are phylogenetically informative despite their incompleteness. Recent simulation studies by Wiens (1998) imply that it is probably better to sample a small set of characters for all the taxa under study than to seek out a large number of characters for a smaller sample of taxa. These kinds of restricted analyses are not uncommon. For example, nearly all taxa of the Early Tertiary mammal *Ectocion* are known only from teeth, and their phylogenetic relationships have been analyzed using dental characters only (Thewissen 1992). Likewise, in a study of phylogenetic relationships among fossil and extant basal eudicots, Magallon (1999) examined a data set restricted to floral and some general inflorescence characters.

Although the fit of phylogeny with the stratigraphic record is not exact for heterosporous ferns, this exercise serves to illustrate the degree of cladogram-stratigraphy mismatch. Benton and Storrs (1994, 1996) tested the absolute quality of the fossil record by comparing changes in paleontological knowledge over research time. These and other tests have shown that knowledge of the fossil record is changing substantially and that the relative completeness of the fossil record is increasing.
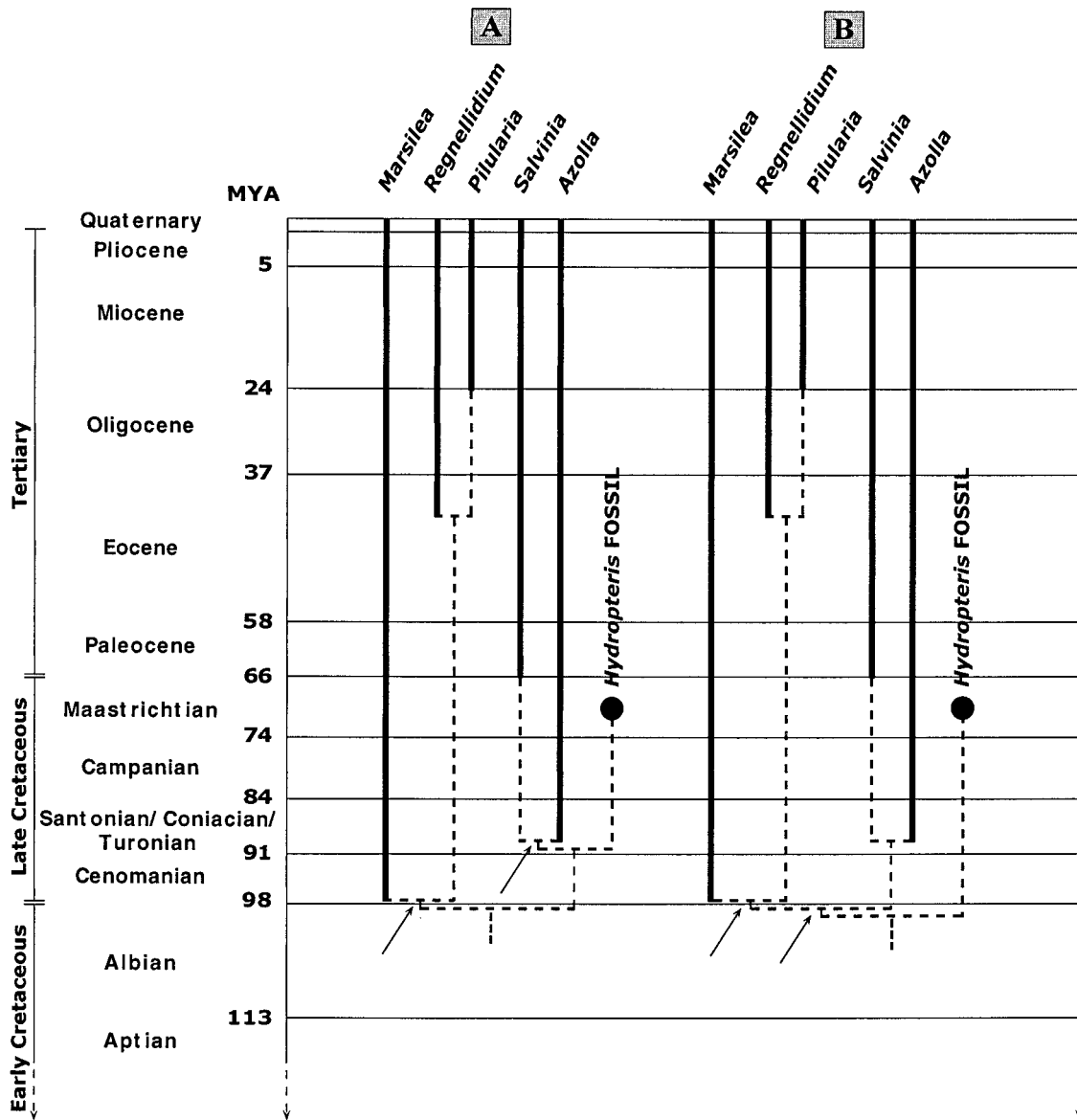
Fig. 12 Stratigraphic distribution of living heterosporous fern genera and *Hydropteris* fossil consistent with the two best estimates of phylogenetic relationships shown in figure 8. Filled circles indicate occurrence within a single formation or limited time range; solid lines indicate continuous time ranges; vertical dashed lines are minimum estimates for gaps in the fossil record and represent "ghost lineages"; *MYA* = million years ago. Stratigraphic data obtained primarily from Collinson (1992), Rothwell and Stockey (1994), Skog and Dilcher (1992, 1994). Arrows indicate nodes stratigraphically inconsistent with the proposed phylogenetic hypothesis.

Just this past decade, the rate of discovery of new megafossils and whole-plant fossils for heterosporous taxa has risen remarkably (Skog and Dilcher 1992, 1994; Rothwell and Stockey 1994), with the first discovery drawing back in time the lineage for *Marsilea* from the Oligocene of the Tertiary (Collinson 1992) to the Cenomanian of the mid-Cretaceous. These are encouraging developments that permit us to anticipate the discovery of additional heterosporous fern fossils over time that will improve the cladogram-stratigraphy mismatch. However, at the present time, stratigraphy can not be the arbiter for resolving equivocal hypotheses about the relationships of these ferns.

Acknowledgments

Appendix

Morphological Character List

The abbreviation PSS designates character numbers in Pryer et al. (1995); RS, characters in Rothwell and Stockey (1994); and SL, characters in Stevenson and Loconte (1996). Readers are referred to these papers for additional references and argumentation pertaining to these characters when definitions and scoring of taxa and characters are the same. Only supplementary references are indicated when applicable.

All scoring for the fossil, *Hydropteris pinnata*, was based on information in Rothwell and Stockey (1994). For all other taxa, vouchers at UC or F were consulted, whenever possible, to corroborate data taken from the literature: Bell and Duckett (1976), Bhardwaja (1980), Bierhorst (1971), Bonnet (1955, 1956), Bordonneau and Tourte (1988), Bower (1923, 1926, 1928), Campbell (1892, 1893*a*, 1893*b*, 1895, 1904), Chopra (1960), Chrysler and Johnson (1939), Collinson (1992), Croxdale (1978, 1979, 1981), Eames (1936), Flora of North America Editorial Committee (1993), Garbary et al. (1993), Gaudet 1964*a*, 1964*b*), Gifford and Foster (1988), Gupta (1962), Hanstein (1865), Higinbotham (1941), D. M. Johnson (1986), D. S. Johnson (1898*a*, 1898*b*, 1933*a*, 1933*b*), Johnson and Chrysler (1938), Konar and Kapoor (1972), Kondo (1962), Kornas (1988), Kotenko (1990), Kubitzki (1990), Lindman (1904), Loyal and Chopra (1976), Lugardon and Husson (1982), Meunier (1888), Mickel and Votava (1971), Mizukami and Gall (1966), Nayar and Kaur (1971), Ogura (1972), Perkins et al. (1985), Pieterse et al. (1977), Pray (1962), Puri and Garg (1953), Rice and Laetsch (1967), Rothwell and Stockey (1994), Saunders and Fowler (1992, 1993), Schmid (1982), Schmidt (1973, 1978), Schneider (1996*a*, 1996*b*), Sen and De (1992), Sharp (1914), Shattuck (1910), Sheffield and Bell (1987), Svenson (1944), Tewari (1975), Tryon and Lugardon (1991), Tryon and Tryon (1982), Van Cotthem (1973), Wagner and Wagner (1977), Warmbrodt and Evert (1978), White (1961), Yasui (1911), Yuasa (1933*a*, 1933*b*, 1935, 1954), Zimmerman et al. (1989).

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1. (PSS 1; SL 26, modified.) Circinate vernation (0) absent, (1) present. Croxdale (1978, 1979, 1981); Konar and Kapoor (1972).

2. (PSS 2, modified; SL 42, modified.) Fertile-sterile leaf differentiation (0) (nearly) monomorphic, (1) hemidimorphic, (2) dimorphic. Pryer et al. (1995) treated this as a four-state character by further subdividing the hemidimorphic state (Wagner and Wagner 1977) into fertile at tip versus fertile at base. The finer division of character states does not capture the hemidimorphic state as a synapomorphy for Schizaeaceae and segregates *Anemia* with the autapomorphic state of fertile basal pinnae.

3. (PSS 3; SL 20, modified.) Blade dissection (0) simple to deeply pinnatifid, (1) compound (once-pinnate or more divided). As in Pryer et al. (1995), this character applies to mature, sterile, photosynthetic blades only (i.e., it does not include the submerged, divided leaves of *Salvinia*; Croxdale 1981). Stevenson and Loconte (1996) scored *Azolla* as having simple leaves. It is scored here as having a compound leaf with an upper aerial leaflet and a lower submersed leaflet (Bierhorst 1971).

4. (RS 3, modified.) Number of pinnae (leaflets) per blade (0) two, (1) four, (2) several. Rothwell and Stockey (1994) defined this character with two additional states: one and none. This character is defined here as applying only to taxa scored as compound for character 3; by definition, taxa scored for simple blades do not have pinnae, and therefore this character is not applicable. To be defined as a pinna, a leaf subdivision must be stalked or at least obviously narrowed at the base and not connected to adjacent pinnae by blade tissue; simple leaf subdivisions, which are not stalked or narrowed, are segments (Wagner and Smith 1993, p. 251). *Azolla* and *Salvinia* were scored by Rothwell and Stockey (1994) as having one pinna. Pinnae are usually paired, and therefore their interpretation is unclear. *Azolla* is scored here as having a compound leaf with an upper aerial leaflet and a lower submersed leaflet (Bierhorst 1971); *Salvinia* has simple, photosynthetic, floating leaves and also highly divided, submersed leaves that bear sporocarps in fertile plants. As with character 3, this character applies to mature, sterile, photosynthetic blades only (i.e., it does not include the submerged, divided leaves of *Salvinia*). Croxdale (1978, 1979, 1981), Eames (1936), Warmbrodt and Evert (1978).

5. (RS 4.) Pinna arrangement (0) alternate or subopposite, (1) opposite or paired. Warmbrodt and Evert (1978).

6. (PSS 14; SL 37, modified.) Dromy at base of blade (lowermost pinnae) (0) catadromous, (1) anadromous, (2) isodromous. *Marsilea* is regarded here as having pinnate leaves with isodromous dichotomous venation.

7. (PSS 5, modified.) Laminar vein orders (0) one, (1) two or more. The argumentation for character 9 in Doyle (1996) for coding this as a binary rather than a multistate character is followed here. A multistate character fails to capture the similarity among some taxa having the blade covered by veins of more than one order. Splitting this character into several

finer states may obscure patterns at the present broad level of analysis.

8. (PSS 4.) Primary vein form of blade (0) solitary/unbranched, (1) dichotomous, (2) anisotomous (pinnate). Evidence of "overtopping" was required in order for a blade to be coded as anisotomous (pinnate). *Marsilea* was scored as dichotomous in Pryer et al. (1995) but is scored here as anisotomous, based on evidence for a short rachis in Gaudet (1964*a*, figs. 21.8 and 22.7; 1964*b*, figs. 7, 8, 22). The vascular anatomy of the *Marsilea* leaf was described by Eames (1936, fig. 128*B*) as being the result of three successive dichotomies of the leaf, forming a scorpioid sympodium. Konar and Kapoor (1972).

9. (PSS 6.) Secondary vein form (0) dichotomous, (1) anisotomous/nondichotomous.

10. (PSS 7; RS 5; SL 38, modified.) Vein fusion in sterile blades (0) nonanastomosing (open), (1) anastomosing (closed). The main laminar veins in *Regnellidium* are nonanastomosing. There is a marginal tie-up of veinlets, as in *Marsilea*, but the laminar veins do not fuse until this point in *Regnellidium*, whereas they anastomose well before the margin in *Marsilea*. Gaudet (1964*a*, 1964*b*), Johnson (1986), Pray (1962).

11. (PSS 9; SL 55.) Hydathodes (0) absent, (1) present.

12. (PSS 12.) Guard mother cell (GMC) division (0) diameristic, (1) parameristic. The character state anomomeristic in Pryer et al. (1995) does not occur in any of the taxa studied here.

13. (PSS 15; SL 32.) Pulvini (0) absent, (1) present. A pulvinus is defined here as an enlargement at the base of a leaflet that has a role in its nocturnal movements. Lindman (1904).

14. (PSS 16, modified; SL 30.) Pneumathodes (0) absent, (1) present. This character was not further subdivided here into finer character states as in Pryer et al. (1995).

15. (PSS 19.) Adaxial outline of petiole and/or rachis (0) convex to flattened, (1) sulcate. The solitary, unbranched vein in *Pilularia* is regarded here as homologous to the central axis "petiole + rachis" of the leaf.

16. (PSS 23, modified; SL 40.) Petiole and/or rachis anatomy (0) monostele (1) polystele. The multistate character definitions in Pryer et al. (1995) were modified here to conform to those in Stevenson and Loconte (1996) since splitting this character into several finer states may obscure patterns at the present level of analysis. Those taxa with a single vascular bundle at some point along the petiole were scored as monostele (e.g., although *Asplenium* and *Monachosorum* are distelar at the base, these steles merge to form a single bundle toward the apex; therefore, they are scored here as monostele).

17. (PSS 24.) Xylem configuration in petiole and/or rachis (from base to apex) (0) horseshoe-shape variation (C, U, V, O, Ω, or low arc) with petiole center parenchymatous, (1) solid T, O, or Δ-shape in petiole center, (2) X-shape (sometimes becoming V toward petiole apex), (3) 3 arcs: two 7-shaped adaxial strands and one arc-shaped abaxial strand.

18. (PSS 21; SL 11.) Sclerenchyma fibers (0) absent, (1) present. The score for *Marsilea* differs from Pryer et al. (1995) and is in agreement here with Stevenson and Loconte (1996), based on evidence in Johnson (1986, p. 6).

19. (PSS 22.) Epipetiolar branches (0) absent, (1) present.

20. (PSS 26; SL 8.) Rhizome symmetry (0) radial, (1) dorsiventral. Scores given to various taxa for this character differ from those of Stevenson and Loconte (1996), e.g., they score all Marsileaceae genera as having radial rhizome symmetry and *Azolla* and *Salvinia* as dorsiventral. All these taxa are scored here as dorsiventral.

21. (PSS 27; SL 19, modified.) Mature rhizome stele type (0) protostele, (1) solenostele, (2) dictyostele.

22. (PSS 28.) Rhizome stele cycles (0) monocyclic, (1) polycyclic.

23. (PSS 30.) Rhizome hairs (0) absent, (1) present.

24. (PSS 31; SL 43.) Rhizome scales (0) absent, (1) present.

25. (New character.) Air canals present in the outer cortex of rhizome and leaves (0) no, (1) yes. The large cavity in *Azolla* leaves is filled with a cyanobacterial symbiont and is not an air chamber per se (Ogura 1972). Croxdale (1978, 1979), D. M. Johnson (1986), D. S. Johnson (1898*a*, 1898*b*), Johnson and Chrysler (1938), Kubitzki (1990), Ogura (1972), Saunders and Fowler (1993).

26. (RS 2; PSS 33, modified; SL 1, modified.) Roots (0) arise all along the rhizome (both at nodes and along internodes), (1) restricted to nodes at leaf bases (normally not internodal), (2) absent. Root absence is an autapomorphy for *Salvinia*. Stevenson and Loconte (1996) incorrectly scored *Azolla* as lacking roots. Johnson (1986) and Kornas (1988) have shown that where roots arise can differ between species and populations of *Marsilea*, depending on the environment. Bierhorst (1971), Johnson (1986), Schmidt (1978), Rothwell and Stockey (1994).

27. (New character.) Root outer cortex aerenchymatous (0) no, (1) yes. Ogura (1972), H. Schneider (personal communication), Schneider (1996*a*, 1996*b*).

28. (New character.) Root outer cortex air chambers in cross section (0) uniform in size, (1) differentiated into large and small cavities. Meunier (1888), Ogura (1972), H. Schneider (personal communication), Schneider (1996*a*, 1996*b*).

29. (PSS 36, modified; RS 1.) Growth habit (0) terrestrial, (1) amphibious and rooted, (2) floating aquatic.

30. (PSS 37; SL 14, modified.) Mucilage/latex canals (0) absent, (1) present. Kubitzki (1990), Tryon and Tryon (1982).

31. (PSS 38.) True vessels (0) absent, (1) present. Tewari (1975) reported vessels from *Regnellidium*, but I concur with Johnson (1986, p. 9) that this report is not conclusive. A recent series of papers (e.g., Carlquist and Schneider 1997*a*, 1997*b*; Schneider and Carlquist 1998) documents vessels to be more extensive in ferns than previously realized. Bhardwaja (1980).

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32. (RS 9.) Sporocarps (0) absent, (1) comprising one sorus only, (2) comprising more than one sorus. Sporocarps are (not always) indurate, protective structures that enclose one or more sori.

33. (New character.) Number of sporocarps/leaf (0) one to three, (1) usually more than three. Eames (1936), Johnson (1986), Lindman (1904), Tryon and Tryon (1982).

34. (New character.) Sporocarp teeth near juncture with stalk (0) absent, (1) present. Johnson (1986).

35. (SL 62, modified.) Sporocarp dehiscence mechanism (0) distinct gelatinous sorophore, (1) gelatinous mass, (2) tissue decay. I differ here with Stevenson and Loconte (1996), who scored only *Marsilea* and *Regnellidium* as having a gelatinous dehiscence mechanism. *Pilularia* and *Regnellidium* both exude

the contents of their sporocarps in a gelatinous mass (Eames 1936; Higinbotham 1941; K. Pryer, personal observation). *Marsilea* is the only taxon with a distinct gelatinous sorophore that, on hydration, breaks through the sporocarp opening, dragging with it the attached sori. The sporocarps of salviniaceous ferns release their contents following tissue fragmentation and decay (Campbell 1893*b*; Eames 1936).

36. (New character.) Sporocarp (0) more or less spherical, (1) usually somewhat flattened with one axis longer. This character refers only to those sporocarps bearing more than a single sporangium. The megasporocarp of *Azolla* is therefore excluded here because it encloses a single megasporangium and the sporocarp essentially takes on the form of the mature megaspore. Bierhorst (1971), Eames (1936), Johnson (1986).

37. (PSS 43, modified; SL 60, modified.) Sporangium receptacle (0) (nearly) flat, (1) convex to short-columnar. Lindman (1904).

38. (PSS 44; SL 63, modified.) Isosporangium or microsporangium stalk length (0) sessile to short, (1) long. In extant heterosporous ferns, microsporangia are long stalked, whereas megasporangia appear short stalked (Bierhorst 1971; Campbell 1893*b*; Eames 1936; Gupta 1962). Only isosporangia and microsporangia were scored here since they undergo similar meiotic processes, resulting in 64 spores. Megasporangia occur only in heterosporous ferns, and they undergo meiotic events that result in far fewer mature spores (usually one).

39. (PSS 45.) Isosporangium or microsporangium stalk width (0) 4–6 cell rows wide, (1) 1–3 cell rows wide.

40. (PSS 46; SL 67, modified.) Spore output per isosporangium or microsporangium (0) between 100 and 1000, (1) <100 (usually 64).

41. (PSS 47; RS 7.) Sori (0) absent, (1) present. *Azolla* was scored as polymorphic for this character in Pryer et al. (1995) but is scored only as present here. The microsporocarps house microsporangia that are arranged in a sorus and radiate from a common columella-like receptacle. Although the mature megasporocarp encloses a solitary megasporangium, in its initial development, several sporangia develop on a common receptacle, but the partly developed microsporangia eventually abort (Bierhorst 1971; Eames 1936). The presence of sori in both developing micro- and megasporocarps is recognized here, and this character is not scored as a polymorphism for *Azolla*. The two kinds of sporocarps thus diverge rather late in ontogeny.

42. (PSS 49, modified; SL 47, modified.) Sorus position on leaves/segments (0) marginal, (1) dorsal (abaxial), (2) terminal. Within the sporocarps of *Regnellidium*, the sori appear to be borne quite differently than in *Marsilea* and *Piluaria*. They are attached to the sporocarp partition walls rather than to the sporocarp wall, and so their position relative to the blade (sporocarp wall is regarded here as homologous to a blade) is undetermined. Eames (1936), Gupta (1962), Johnson and Chrysler (1938), Lindman (1904), Puri and Garg (1953).

43. (PSS 50; SL 49.) Sporangial maturation (0) ± simultaneous (simple), (1) gradate, (2) mixed. This character refers to the maturation of sporangia within a sorus or to sporangia adjacent to one another if not arranged in sori.

44. (PSS 52, modified; RS 6, modified; SL 56 and 57, modified.) True indusia (0) absent, (1) present. A true indusium is a protective soral covering that is attached to the receptacle and/or abaxial leaf surface. It is distinguished here from a false indusium, which is formed by a strongly reflexed or otherwise modified leaf margin. The indusia of heterosporous ferns are the most complex to understand. Campbell (1893*a*, 1893*b*), Johnson (1898*a*, 1898*b*, 1933*a*, 1933*b*), Johnson and Chrysler (1938) are useful for interpreting these, but most of the details of indusia in *Regnellidium* are unclear. Rothwell and Stockey (1994) also scored all members of Marsileaceae as indusiate, whereas Stevenson and Loconte (1996) scored them as having false indusia.

45. (PSS 53, modified.) Origin of indusia or indusial components (0) leaf margin, (1) abaxial leaf surface, (2) terminal. Campbell (1893*a*), Johnson (1898*a*, 1898*b*, 1933*a*, 1933*b*), Lindman (1904).

46. (PSS 54.) Indusium attachment relative to sorus (0) lateral, (1) basal. Campbell (1893*a*), Johnson (1898*a*, 1898*b*, 1933*a*, 1933*b*), Lindman (1904).

47. (PSS 55.) Indusial opening (0) introrse, (1) extrorse, (2) suprasoral, (3) none. The indusia of marsileaceous ferns are saclike and do not have an opening. The delicate indusial tissues break down when they come in contact with water (Eames 1936). Campbell (1893*a*), Gifford and Foster (1988), Johnson (1898*a*, 1898*b*, 1933*a*, 1933*b*), Lindman (1904).

48. (PSS 56; SL 64, modified.) Annulus (0) absent, (1) present. An annulus is defined here as a structure with at least some indurated cells involved in the spore releasing mechanism of the sporangium (the contraction of the annulus by drying is the principal factor in releasing spores). *Pilularia* is scored here as not having an annulus, although there are several references that cite the form of the microsporangium and the arrangement of the cells at its apex as so closely resembling those in *Schizaea* or *Anemia* that they may be regarded as the vestigial equivalent of the annulus in these genera (see Bower 1923, p. 256; Bierhorst 1971; Campbell 1893*a*, 1895, 1904; Johnson 1898*b*). Although the cell outline at the apex of the microsporangium does very vaguely resemble the apical cells of sporangia in schizaeaceous ferns, there is no convincing evidence that *Pilularia* does in fact possess an annulus. Like other marsileaceous ferns, the sporangia open to release spores only when their delicate walls are broken down by decay in water. In contrast, Stevenson and Loconte (1996) scored *Pilularia* as having an apical annulus.

49. (PSS 59; RS 8; SL 68, modified.) Sporogenesis (0) homosporous, (1) heterosporous.

50. (PSS 60, modified; RS 16, modified; SL 71, modified.) Megaspore laesura (0) triradiate, (1) papilla-like. Tryon and Lugardon (1991).

51. (RS 15.) Megaspore shape (0) ellipsoid, (1) ovoid, (2) spheroid. Not applicable to homosporous ferns. Tryon and Lugardon (1991).

52. (RS 17, modified.) Megaspore perispore (epispore) structure (0) complex (2–3 layers), (1) vacuolate. Rothwell and Stockey's (1994) two character states were filamentous and lamellar (vacuolate), however, these were not discussed, making it impossible to determine whether the character-state definitions and designations here are consistent with their study. The character states used here are defined as follows: "complex" is my interpretation of Rothwell and Stockey's "filamentous" character state, whereby the perispore is made up of more than one layer, as in *Marsilea*, with an inner, dense

zone of filamentous strands and a larger, diffuse, cavate outer zone (Tryon and Lugardon 1991, p. 566, figs. 221.10–221.13); "vacuolate" or "lamellar" indicates that the epispore is not distinctly layered but, rather, is made up of a highly intricate meshwork of interconnected lamellae forming large vacuoles under a more compact surface, as in *Salvinia* (Tryon and Lugardon 1991, p. 575, figs. 224.8–224.11). Not applicable to homosporous ferns.

53. (PSS 60, modified; RS 16, modified; SL 71, modified.) Isospore or microspore laesura (0) linear, (1) triradiate. Rothwell and Stockey (1994) united these two character states into a single state and provided "papillate" as the alternate state. Since papillate refers only to the marsileaceous megaspore laesura condition, the megaspore aperture is considered here as a separate character.

54. (PSS 63; RS 13; SL 73, modified.) Perispore (epispore) relative to exospore (0) not prominent (conforming to exospore contours), (1) prominent (producing observable surface contours). Stevenson and Loconte (1996) regarded epispore and perispore as separate characters, with epispore applicable solely to heterosporous ferns. I concur with Rothwell and Stockey (1994, p. 487) in regarding epispore and perispore as essentially synonymous here, referring simply to the sporopollenin wall external to the exospore.

55. (PSS 64.) Perispore (epispore) surface (0) (nearly) smooth or plain, (1) obviously patterned or sculptured.

56. (RS 14, modified.) Isospore or microspore perispore (epispore) structure (0) simple (one layer) (1) complex (two to three layers), (2) vacuolate (not distinctly layered, forming a massula). Rothwell and Stockey's (1994) three character states were simple, filamentous, and lamellar (vacuolate), however, these were not discussed, making it impossible to determine whether the character-state definitions and designations here are consistent with their study. The character states used here are defined as follows: "simple" indicates that the perispore consists of a single layer as in *Actinostachys* (*Schizaea*) *digitata*, where the perispore is thin and conforms to exospore contours (Tryon and Lugardon 1991, p. 116, figs. 31.5, 31.21), or as in *Marsilea*, where the perispore is thick, but comprises an undulated, densely folded layer (Tryon and Lugardon 1991, pp. 565–566, figs. 221.5–221.6); "complex" is my interpretation of Rothwell and Stockey's "filamentous" character state, whereby the perispore is made up of more than one layer, as in *Metaxya rostrata*, with a thick perispore of three layers (Tryon and Lugardon 1991, p. 238, fig. 82.4), or, as in *Pilularia* and *Regnellidium*, with epispores consisting of a zone of filamentous strands below an irregularly sinuous layer (Tryon and Lugardon 1991, pp. 568–573, figs. 222.4–222.5, 223.5); and finally, "vacuolate" or "lamellar" indicates that the epispore is not distinctly layered but, rather, is made up of a highly intricate meshwork of interconnected lamellae forming large vacuoles that together envelop microspores into a common massula (massulae in *Azolla*) within the sporangium wall, as in *Hydropteris*, *Salvinia*, and *Azolla* (Bonnet 1955; Rothwell and Stockey 1994, p. 484, figs. 22, 27, 28; Tryon and Lugardon 1991, pp. 575–580, figs. 224.2–224.7, 225.4–225.8).

57. (PSS 66; SL 81, modified.) Exospore (exine) surface (0) (nearly) smooth or plain, (1) obviously patterned or sculptured. Exospore sculpture (character 81) of Stevenson and Loconte (1996) corresponds most closely to this character; how-

ever, they finely subdivided it into nine states. Although they indicated in their appendix 1 that *Pilularia* and *Marsilea* (and many other taxa) had a subset polymorphism for this character, Stevenson and Loconte (1996) actually scored these marsileaceous taxa for a single state (psilate), which was not one of the two states (rugulate, papillate) specified by them in the appendix.

58. (RS 11, modified.) Isospores or microspores in each sporangium (0) free, (1) embedded in massula. Rothwell and Stockey (1994) indicated three states for this character in their appendix 1, yet in their figure 43*c*, it is displayed as a two-state character when mapped onto a topology. The two-state character was chosen here since the finer division of character states does not capture embedded spores as a synapomorphy for Hydropteridineae and segregates *Azolla* with the autapomorphy of microspores from one sporangium embedded in multiple massulae, rather than a common massula.

59. (New character.) Glochidia (0) absent, (1) present. Glochidia are barbed or circinate trichomes associated with spores (Collinson 1992). The massulae that enclose microspores in some extant *Azolla* bear barbed glochidia. Several extinct azollaceous spores have associated glochidia. The megaspore complexes of *Hydropteris* bear circinate glochidia.

60. (New character.) Floats (0) absent, (1) present. The spores of living and extinct azollaceous ferns are the most elaborate among pteridophytes, with complex accessory structures above the megaspore, including floats (Tryon and Lugardon 1991). The megaspore complexes of *Hydropteris* also bear floats (Rothwell and Stockey 1994).

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61. (PSS 67; SL 85, modified.) Spore germination pattern (0) equatorial, (1) polar, (2) amorphous. Stevenson and Loconte (1996) finely subdivided the equatorial and polar states into substates conforming to the types described by Nayar and Kaur (1971) for a total of seven character states. More general character states were chosen here to capture shared cell division patterns.

62. (PSS 68; SL 90, modified.) Gametophyte form (0) tuberous, (1) cordate-thalloid, (2) elongate-thalloid, (3) reduced to relatively few cells. It is unclear why Stevenson and Loconte (1996) scored this character as "inapplicable" for heterosporous ferns.

63. (PSS 72; SL 88, modified.) Dependent gametophyte (0) no, (1) yes. Stevenson and Loconte (1996) listed autotrophic, mycorrhizal, and endosporic as character states for the nature of the gametophyte. Their scores are essentially the same as here, except that gametophytes of *Actinostachys* are scored in their study as autapomorphic for a fungal association.

64. (PSS 74.) Position of archegonia on gametophyte (0) embedded or slightly projecting, (1) partially to fully exposed.

65. (New character.) Number of archegonia per gametophyte (0) always more than one (usually many more), (1) always one. All marsileaceous ferns have a single archegonium/gametophyte (Higinbotham 1941). Salviniaceous ferns usually have more than one, but fewer than about five archegonia/gametophytes; however, if none of the first archegonia is fertilized, others may form in large numbers (Eames 1936). All

homosporous ferns have abundant archegonia (Nayar and Kaur 1971). Bierhorst (1971), Eames (1936), Gifford and Foster (1988), Higinbotham (1941), Nayar and Kaur (1971).

66. (PSS 76; SL 108, modified.) Number of archegonial neck cell tiers (0) more than six cells high, (1) one to five (rarely) six cells high.

67. (SL 104, modified.) Megagametophyte (0) absent, (1) not free-nuclear, (2) basal free-nuclear. Bierhorst (1971), Eames (1936).

68. (New character.) Number of antheridia per gametophyte (0) always more than two (usually many more), (1) usually two (sometimes one). All heterosporous ferns are described as having two antheridia (Higinbotham 1941; Eames 1936; Yasui 1911), with the exception of *Azolla*, which has one (Bierhorst 1971; Campbell 1893*b*; Kubitzki 1990). All homosporous ferns have abundant antheridia (Nayar and Kaur 1971).

69. (PSS 75; SL 95, modified.) Number of antheridial wall cells (0) ≥five, (1) three to (rarely) five. The number of sperm discharged by an antheridium (Stevenson and Loconte's char-

acter 102) is likely correlated with antheridium size, hence I chose to score only antheridium size (measured here according to number of wall cells) because more precise information is available for this character.

70. (New character.) Number of spermatids released per gametophyte (0) >100, (1) 32, (2) eight. This character is more readily scored than number of spermatids per antheridium, which is likely to be closely correlated with antheridium size. Bierhorst (1971), Eames (1936), Gifford and Foster (1988), Higinbotham (1941), Mizukami and Gall (1966), Yasui (1911).

71. (New character.) Number of gyres in nucleus- and flagella-bearing coil of spermatozoids (0) fewer than six, (1) more than six. Bell and Duckett (1976), Bonnet (1955), Bordonneau and Tourte (1988), Campbell (1892, 1893*b*), Garbary et al. (1993), Gupta (1962), Hanstein (1865 [cf. Tafel X]), Higinbotham (1941), Kotenko (1990), Myles and Hepler (1977), Rice and Laetsch (1967), Sharp (1914), Sheffield and Bell (1987), Yuasa (1933*a*, 1933*b*, 1935, 1954).

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