

Fern Phylogeny Based on *rbcL* Nucleotide Sequences

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ABSTRACT.—We analyzed nucleotide variation in *rbcL* (the gene encoding the large subunit of ribulose 1,5-bisphosphate carboxylase/oxygenase) from 99 genera of leptosporangiate ferns representing 31 of the 33 extant families. Phylogenetic relationships were inferred using three methods: neighbor joining, maximum parsimony, and maximum likelihood. All three methods resulted in optimal trees that were similar. Within the context of those taxa examined, these trees suggest that: 1) Polypodiaceae, Grammitidaceae and *Pleurosoriopsis* form a monophyletic group that is most derived among indusiate ferns; 2) *Davallia* is closely related to the Polypodiaceae; 3) *Tectaria* is related to Oleandraceae rather than to other members of Dryopteridaceae; 4) *Rumohra* and

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Elaphoglossum are closely related; 5) Dryopteridaceae are polyphyletic; 6) a monophyletic group consists of Polypodiaceae, Grammitidaceae, Davalliaceae, Oleandraceae, Nephrolepidaceae, Lomariopsidaceae, Dryopteridaceae, Thelypteridaceae, Blechnaceae, Aspleniaceae, and *Pleurosoriopsis*; 7) Dennstaedtiaceae, Monachosoraceae, Pteridaceae, Vittariaceae, and the previous clade form a monophyletic group; 8) Dennstaedtiaceae are polyphyletic; 9) tree ferns in the Cyatheaceae, Metaxyaceae, and Dicksoniaceae form a monophyletic group that emerged early in the diversification of leptosporangiate ferns; 10) Plagiogyriaceae and Loxomataceae emerge with the tree ferns; 11) heterosporous water ferns form a monophyletic group that diverged prior to the tree ferns; 12) Schizaeaceae, Cheiroleuriaceae, Dipteridaceae, Gleicheniaceae, Matoniaceae, and Hymenophyllaceae are basal to the heterosporous aquatic ferns; and 13) Osmundaceae are the most basal lineage of the leptosporangiate ferns.

Nucleotide variation in the gene encoding the large subunit of ribulose 1,5-bisphosphate carboxylase/oxygenase (*rbcL*) has provided the most extensive molecular data set for plant systematists (e.g., Chase et al., 1993). In the last five years almost every major lineage of land plants has been surveyed for *rbcL* sequence data (e.g., Chase et al., 1993; Manhart, 1994; Mishler et al., 1994), including three studies of ferns. Hasebe et al. (1993) analyzed *rbcL* from two leptosporangiate ferns (*Adiantum* and *Osmunda*) and two eusporangiate ferns (*Angiopteris* and *Botrypus*), and inferred their relationships with other land plants. Translated amino acid sequences, rather than DNA sequences, were used in that study, because variation in GC content across widely divergent taxa can bias phylogenetic analyses (Hasegawa et al., 1993). Maximum likelihood analyses supported monophyly of the ferns but relationships among basal taxa within the ferns were not fully resolved. Polymerase Chain Reaction (PCR)-mediated direct sequencing has recently become feasible and accumulation of fern *rbcL* data has accelerated. Hasebe et al. (1994) analyzed *rbcL* from 64 species (62 genera, 29 families sensu Kramer and Green, 1990) using neighbor joining and maximum parsimony methods. Wolf et al. (1994) analyzed *rbcL* from 45 species of dennstaedtioid ferns using maximum parsimony. The two studies produced almost concordant results, although both pointed out the need for more sequence data to increase taxon sampling, especially in large and diverse families such as Dryopteridaceae and Pteridaceae.

In this study, we compiled information from 180 fern *rbcL* sequences available to us (Appendix). We selected 107 taxa representing all major extant lineages of ferns and analyzed the data using three different tree construction methods: neighbor joining (NJ), maximum parsimony (MP), and maximum likelihood (ML). We also estimated the number of synonymous (Ks) and non-synonymous (Ka) nucleotide substitutions in fern *rbcL* sequences. The goals of our study were to 1) infer relationships among the major lineages of leptosporangiate ferns, 2) compare the results of three different tree making methods, 3) compare average interfamilial and intergeneric (intrafamilial) Ks and Ka in ferns with those in angiosperms, and 4) infer the phylogenetic positions of the historically problematic taxa *Loxoma*, *Orthiopteris*, and *Pleurosoriopsis*.

MATERIALS AND METHODS

We randomly selected one species from each genus for which *rbcL* data were available at the time of this analysis (Appendix), incorporating representatives from all extant families and subfamilies (see Fig. 1) except the Lophosoriaceae and Hymenophyllopsidaceae. We followed Kramer and Green's (1990) system for the delimitation of families. However, generic delimitation was not always concordant with Kramer and Green's (1990) system, and several genera were treated as in the original references from which sequence data were cited (see Appendix). The 107 selected sequences were used in the NJ and MP analyses. Due to limitations in computer capabilities and time, ML analyses were performed on a reduced data set of 72 species.

The *rbcL* sequences themselves were generated in ten different laboratories, and therefore specific protocols for DNA extraction, PCR amplification, cloning, and sequencing vary. Details are provided in the original citations given in the Appendix (see also Ranker, 1995).

The sequences could be aligned without any insertions or deletions. The 1206 bp region between base pair positions 73 and 1278 (from the initial methionine codon of *Marchantia polymorpha*; Ohyama et al., 1986) was used for phylogenetic analyses.

For the NJ analyses, we used PHYLIP version 3.5c (Felsenstein, 1993). In calculating the distance matrix, Kimura's 2-parameter model of nucleotide substitution (Kimura, 1980) was implemented, in which the transition (ts)/transversion (tv) ratio was fixed to 3 (Hasebe et al., 1994). Support for each internode was estimated using bootstrap resampling of nucleotide positions (Felsenstein, 1985; Felsenstein and Kishino, 1993). We also generated a NJ tree using the MOLPHY ver. 2.2 program (Adachi and Hasegawa, 1994). We calculated a distance matrix based on a maximum likelihood model of nucleotide substitution (HKY85 model; Hasegawa et al., 1985) using the "distance option" of NucML (Adachi and Hasegawa, 1994) with the assumption of ts/tv=3.

For the MP analyses, we used PAUP version 3.1 (Swofford, 1993). We searched for multiple islands of equally most parsimonious trees (Maddison, 1991) using the heuristic search method. We assigned equal weight to each codon position and conducted 500 searches using random-order-entry starting trees and nearest neighbor interchanges (NNI) branch swapping with MULPARS and STEEPEST DESCENT selected. The equally most parsimonious trees from these 500 searches were used as starting trees for tree bisection-reconnection (TBR) branch swapping with MULPARS and STEEPEST DESCENT selected (see Olmstead and Palmer [1994] for other search strategies for multiple islands with large data sets). We also implemented the differential character-state weighting model of Albert et al. (1993) and conducted 1000 searches using random-order-entry starting trees and NNI branch swapping with MULPARS and STEEPEST DESCENT selected. The equally most parsimonious trees from these 1000 searches were then used as starting trees for subtree pruning-regrafting (SPR) and TBR branch swapping with MULPARS and STEEPEST DESCENT selected. Bootstrap (Felsenstein, 1985; Sanderson, 1989; Hillis and

Bull, 1993; Felsenstein and Kishino, 1993) and decay analyses (Bremer, 1988) were used to obtain a measure of confidence for each branch. Five hundred bootstrap replications were carried out with equal weighting, simple sequence addition, and NNI branch swapping with MULPARS and STEEPEST DESCENT selected. Bootstrap values were recorded for nodes supported in more than half the replicates. For the decay analysis, the equally most parsimonious trees from the heuristic searches above were used as starting trees for a further search using TBR branch swapping with MULPARS and STEEPEST DESCENT selected. All trees up to two steps longer than the equally most parsimonious trees were saved. The strict consensus trees were calculated to determine decay values for each branch, i.e., the number of steps that could be added to the tree before causing the branch to collapse.

For the ML data set, we eliminated 35 species that always clustered in monophyletic groups with other taxa with more than 85% bootstrap probability during preliminary analyses with NJ and MP methods. The data set containing 72 genera was analyzed on a Silicon Graphics Indigo II with IRIX 5.2 and the MIPS R4400 coprocessor using fastDNAmI version 1.0.6 (Felsenstein, 1981; Olsen et al., 1992). Thirty replications of the ML analysis were executed, each with different randomly determined orders of sequence addition and local branch swapping in effect. The "Categories" option was invoked to specify the different rates of substitution by codon position. Codons were categorized (1.0:0.39:8.0 for first:second:third codon positions, respectively) based on empirical estimates of base substitution rates at each codon position for fern *rbcL* sequences (Hasebe et al., 1994). As with the NJ and MP analyses, a ts/tv ratio of 3.0 was specified. One tree resulted from each of the 30 random sequence addition searches. Using fastDNAmI version 1.1 (Felsenstein, 1981; Olsen et al., 1994), we performed a statistical test (Kishino and Hasegawa, 1989) of each of these trees against the one with the best log-likelihood. This test uses the standard error of the difference of log-likelihood from the best ML tree to determine whether the log-likelihoods of any of the trees are significantly worse (lower) than that of the best ML tree. In addition, the "Global and User Tree" option was invoked to identify the best of these 30 trees and to carry out global branch swapping to attempt to find a more likely tree.

In the MP and ML analyses, the eusporangiate ferns (Marattiaceae and Ophioglossaceae) and Psilotaceae were designated as outgroup taxa based on previous molecular phylogenetic results (Hasebe et al., 1993; Hiesel et al., 1994; Manhart, 1994). Only a single taxon could be designated as the outgroup in the NJ analyses; we chose *Angiopteris* (Marattiaceae).

The number of synonymous and nonsynonymous nucleotide substitutions per site were counted by the NG method (Nei and Gojobori, 1986) for the taxa indicated in the Appendix, using the program kindly provided by N. Saitou (National Institute of Genetics, Mishima, Japan). The expected numbers of synonymous and nonsynonymous nucleotide substitutions per site (K_s and K_a , respectively) estimated by the Jukes and Cantor equation (Jukes and Cantor, 1969) were averaged among species-pairs in different families (interfamilial distances) and among pairs of species in different genera that formed a mono-

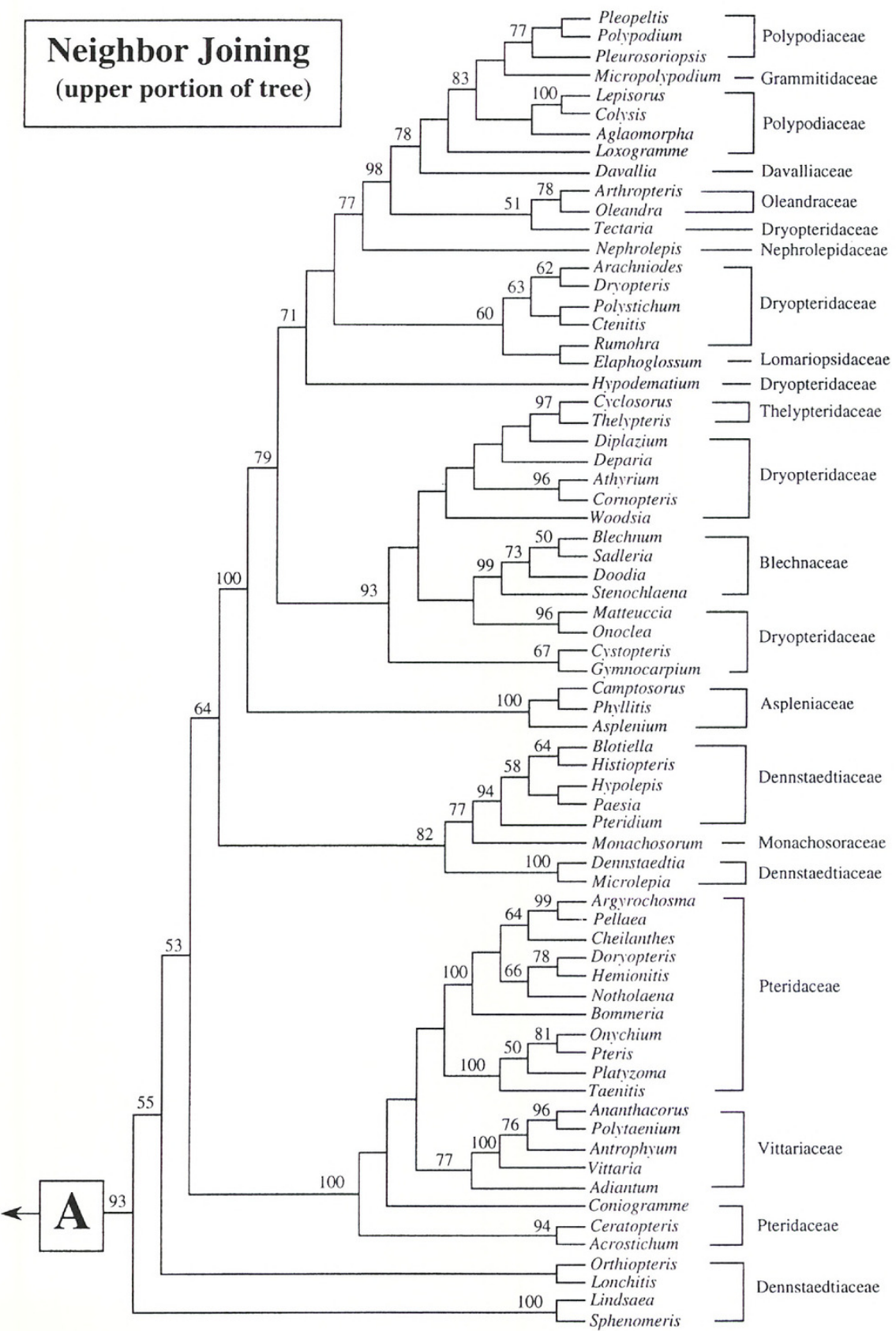


FIG. 1. Continued.

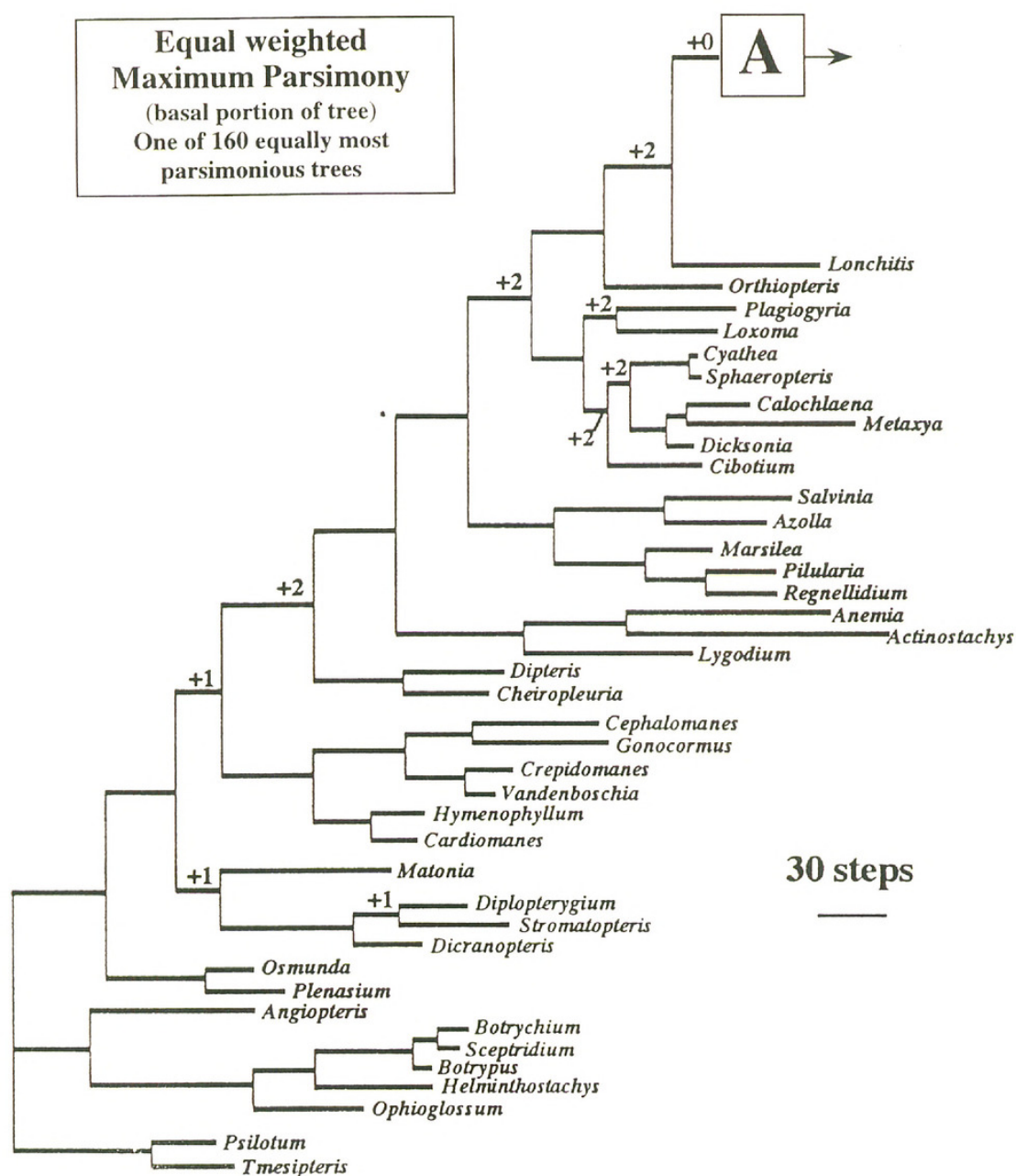


FIG. 2. An arbitrarily selected tree from the 160 equally most parsimonious trees in the maximum parsimony (MP) analyses with equal weighting. Branch lengths correspond to the number of nucleotide substitutions (ACCTRAN optimization). Decay indices are indicated on those nodes where the number of additional steps needed for a branch to collapse is ≤ 2 . A decay index of "+0" means that the node collapses in the strict consensus of the 160 equally most parsimonious trees. CI=0.196; RI=0.596; RCI=0.117. Taxa shown in the tree are indicated in the Appendix. The tree was rooted by the eusporangiate ferns and Psilotaceae. The basal and upper portions of the tree are connected along the branch labeled "A".

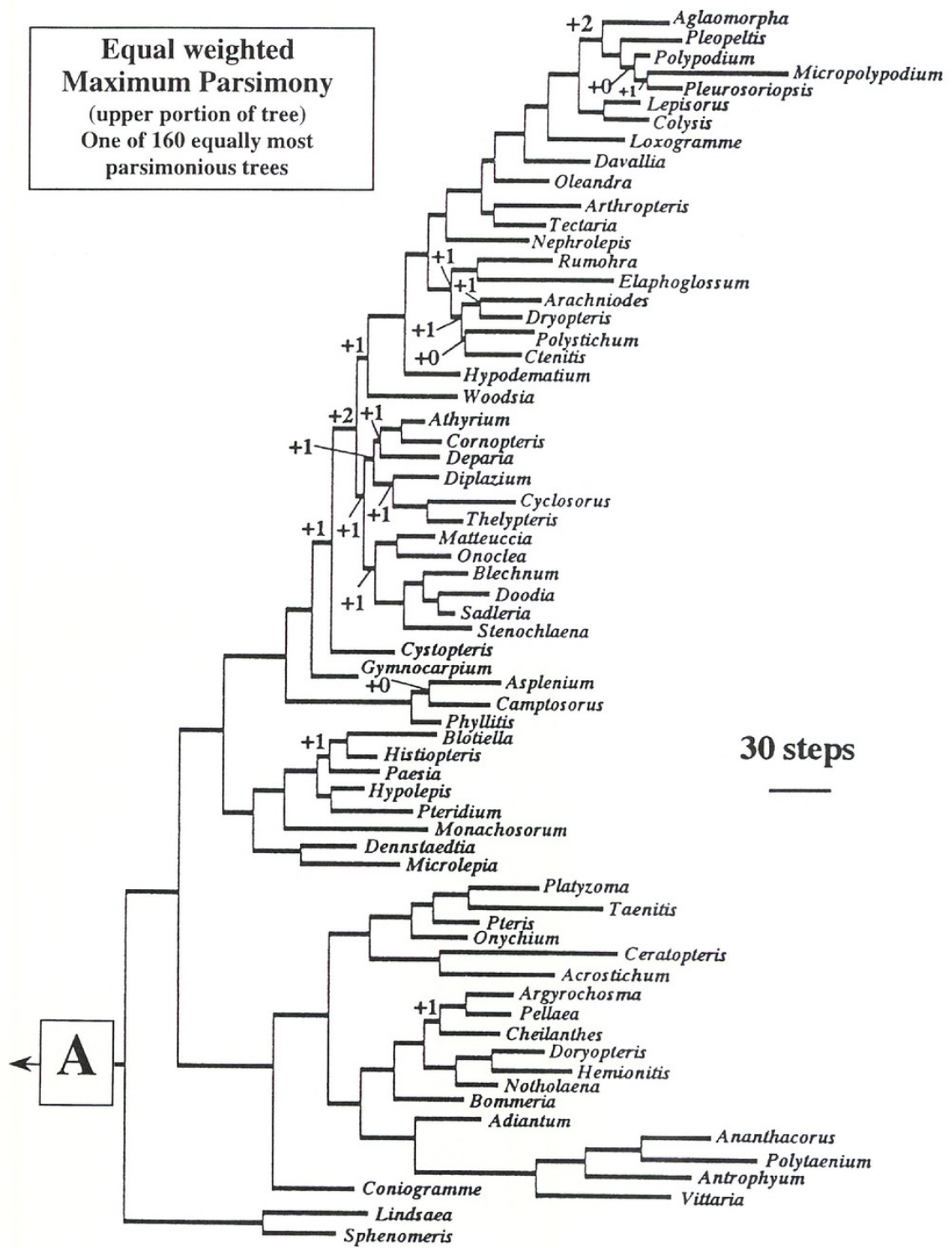


FIG. 2. Continued.

phyletic group within families (intergeneric distances). We also calculated the Ks and Ka values for angiosperm *rbcl* sequences available in the DNA databases DDBJ, EMBL, and NCBI. We selected 20 families for which more than 5

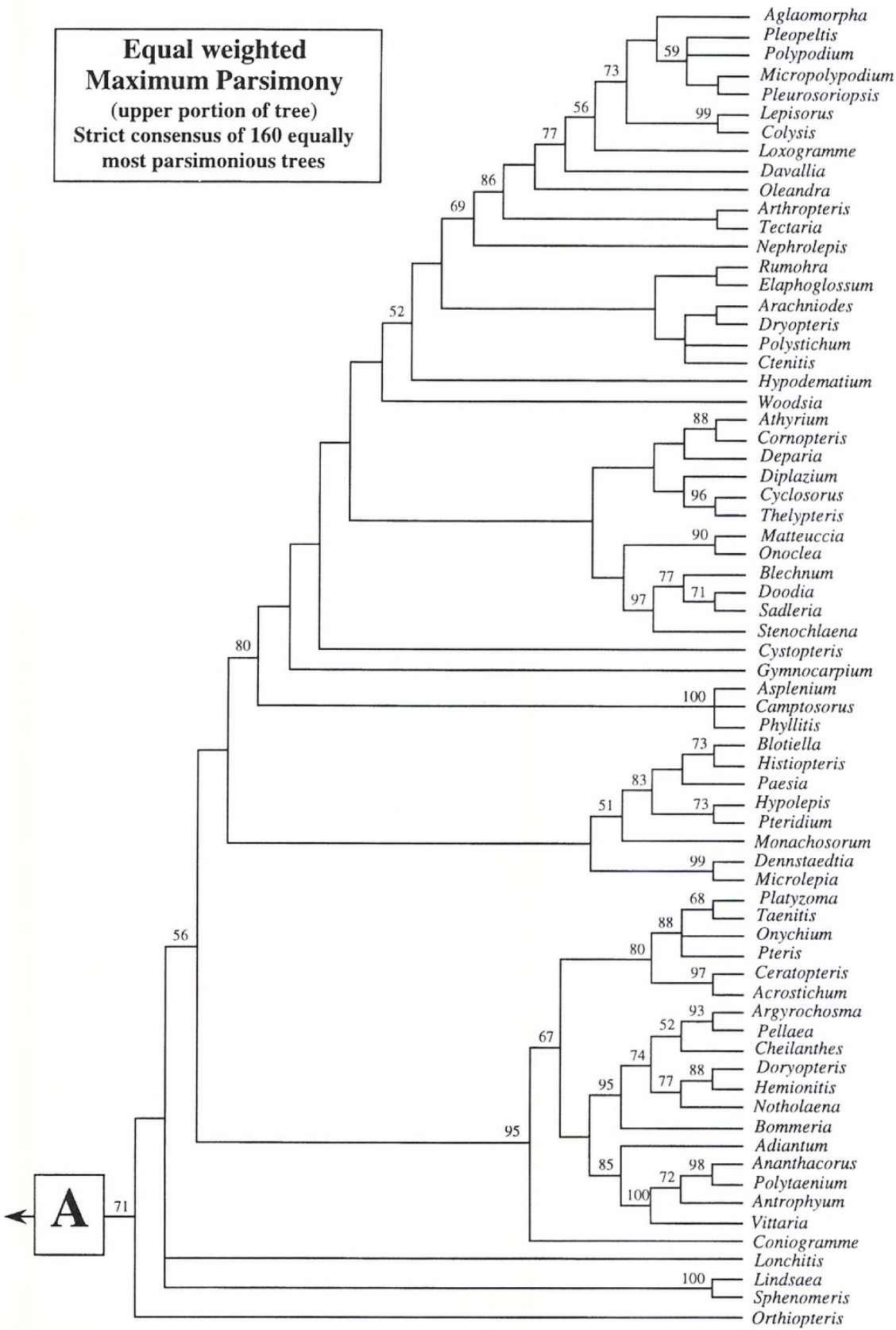


FIG. 3. Continued.

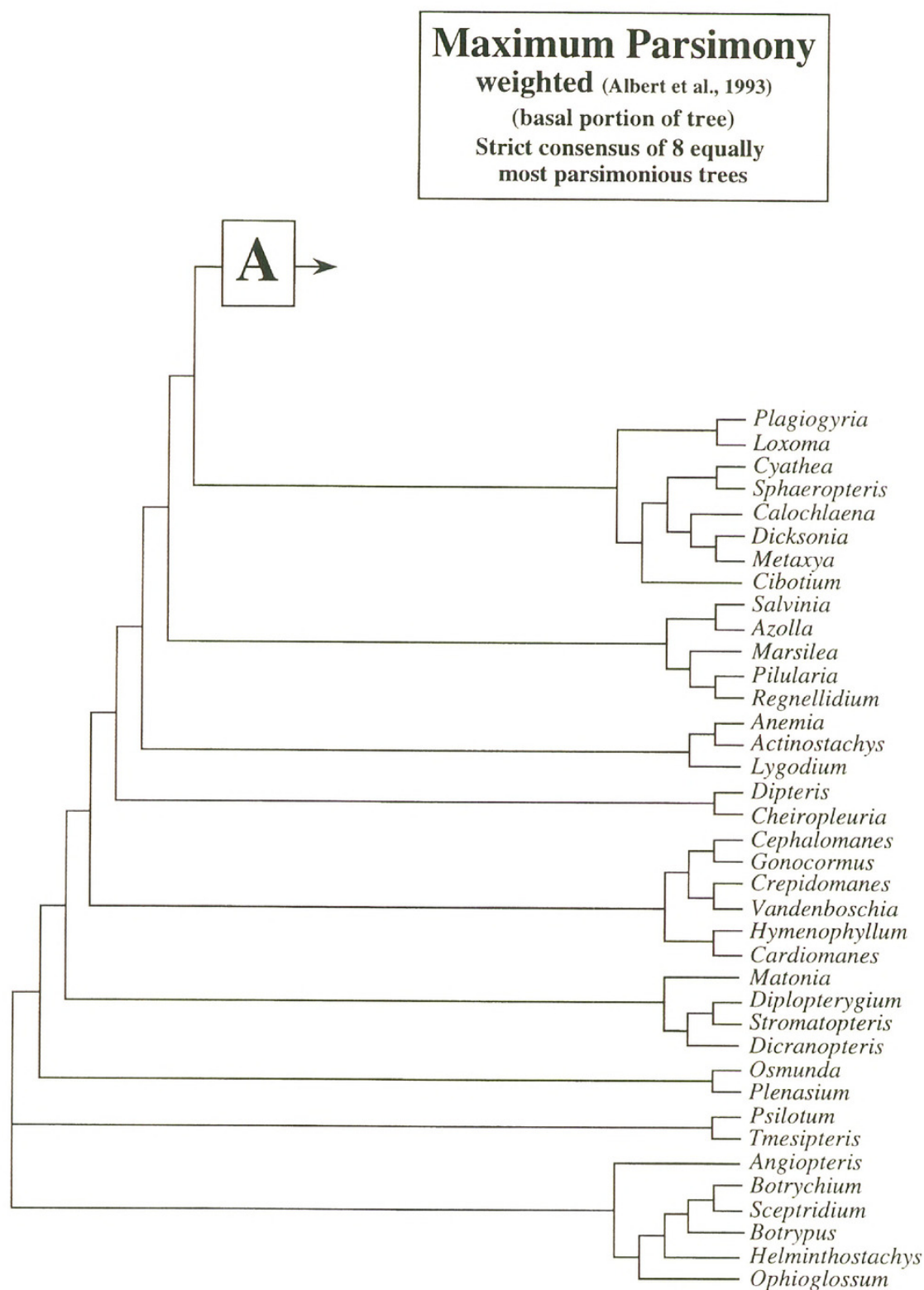


FIG. 4. Strict consensus tree of the eight equally most parsimonious trees obtained using maximum parsimony (MP) with the weighting criterion of Albert et al. (1993). Branch lengths are arbitrary. Taxa shown in the tree are indicated in the Appendix. The tree was rooted by the eusporangiate ferns and Psilotaceae. The basal and upper portions of the tree are connected along the branch labeled "A".

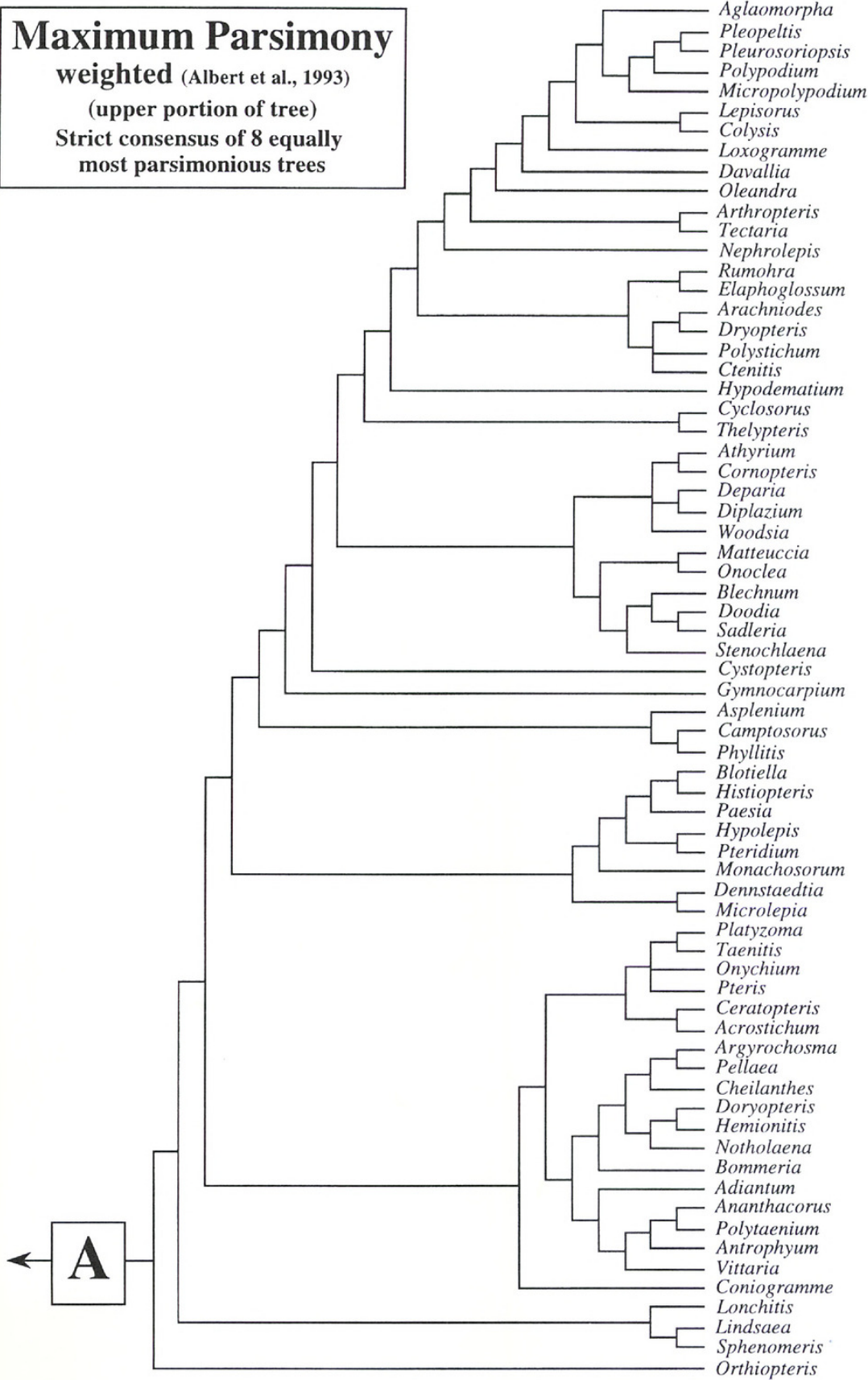


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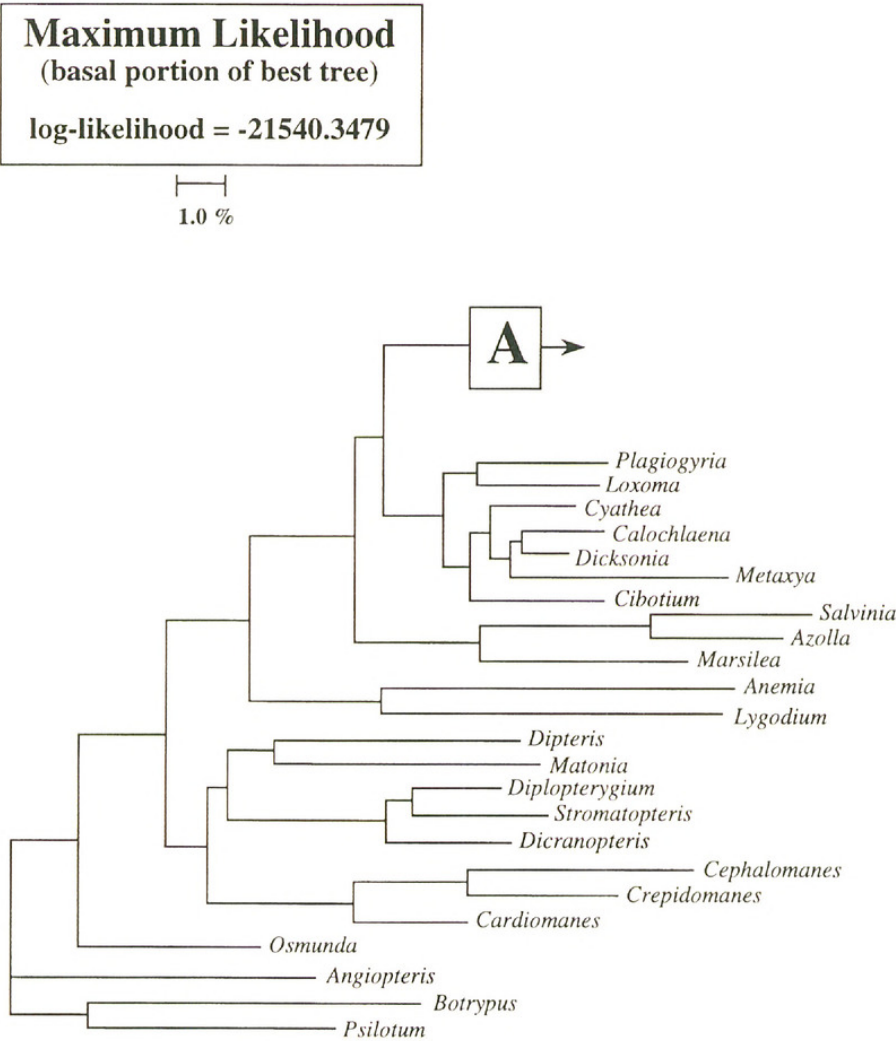


FIG. 5. The best maximum likelihood tree with a log-likelihood of -21540.3479 obtained after 30 random sequence addition searches with local branch swapping. Codons were categorized (1.0: 0.39:8.0 for first:second:third codon positions, respectively), and ts/tv was assumed as 3.0. Taxa shown in the tree are indicated in the Appendix. The tree was rooted by the eusporangiate ferns and Psilotaceae. The basal and upper portions of the tree are connected along the branch labeled "A".

genera were reported in the databases. The dataset and the complete table of Ks and Ka values are available from K. Ueda upon request.

RESULTS

The NJ tree obtained using Kimura's 2-parameter model is shown in Fig. 1. The NJ analysis with a maximum likelihood model of nucleotide substitution resulted in the same tree.

Using the MP method with equal weighting, 160 equally most parsimonious trees of 5978 steps were found in two islands (40 and 120 trees, respectively). Using random-order-entry starting trees, the bigger island was found at the first

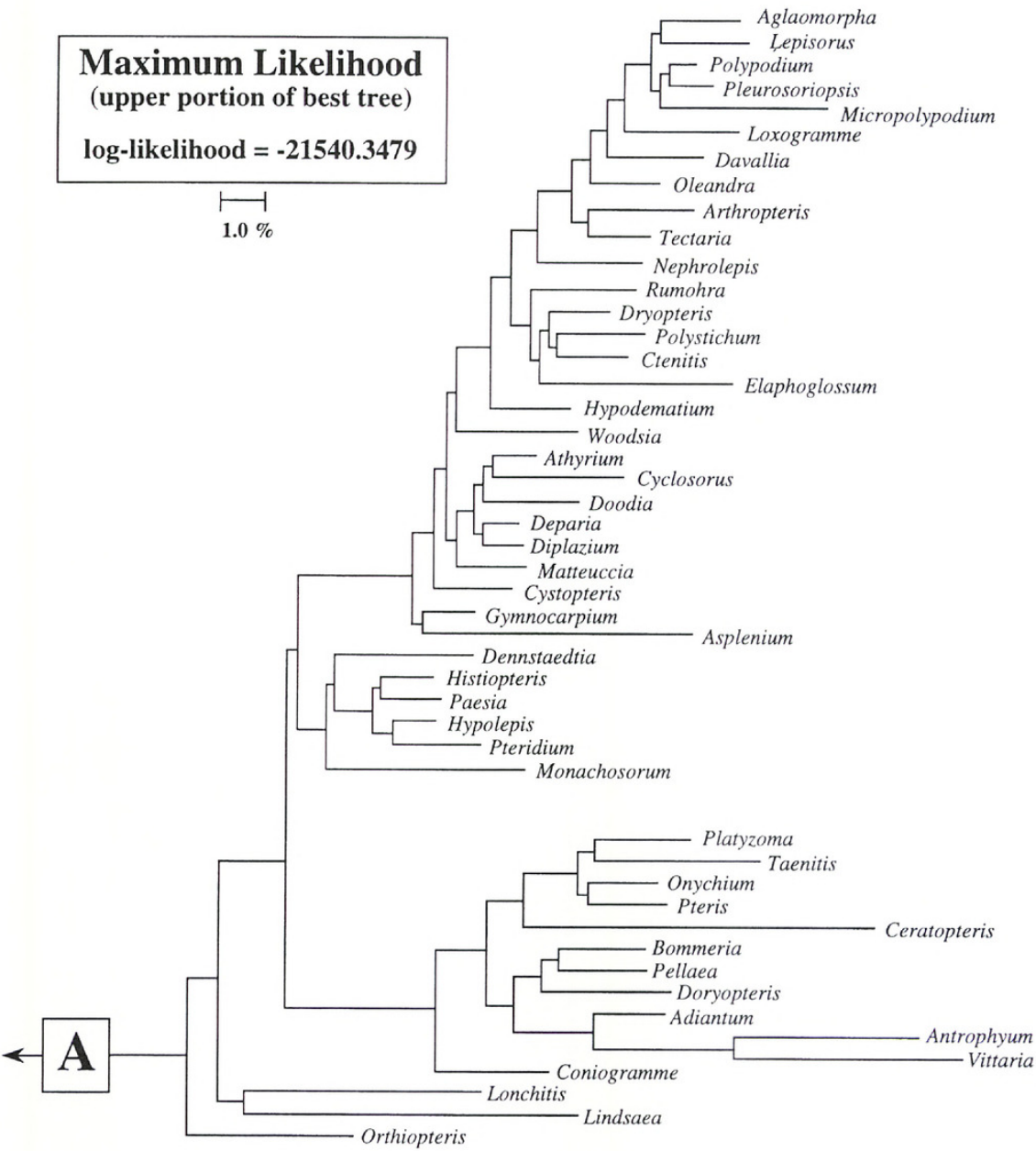


FIG. 5. Continued.

search and the smaller island at the 48th search. The same strict consensus tree was calculated for both islands. These trees had a consistency index of 0.196, a retention index of 0.596, and a rescaled consistency index of 0.117. One arbitrarily selected tree from the 160 equally most parsimonious trees is shown (with decay values) in Fig. 2. The strict consensus of the 160 equally most parsimonious trees is shown in Fig. 3. The weighted MP analysis resulted in eight equally most parsimonious trees from one island found at the first search using random-order-entry starting trees. These eight trees were then used as starting trees for further searches with both SPR and TBR swapping. Additional trees of shorter or equal length were not found. The strict consen-

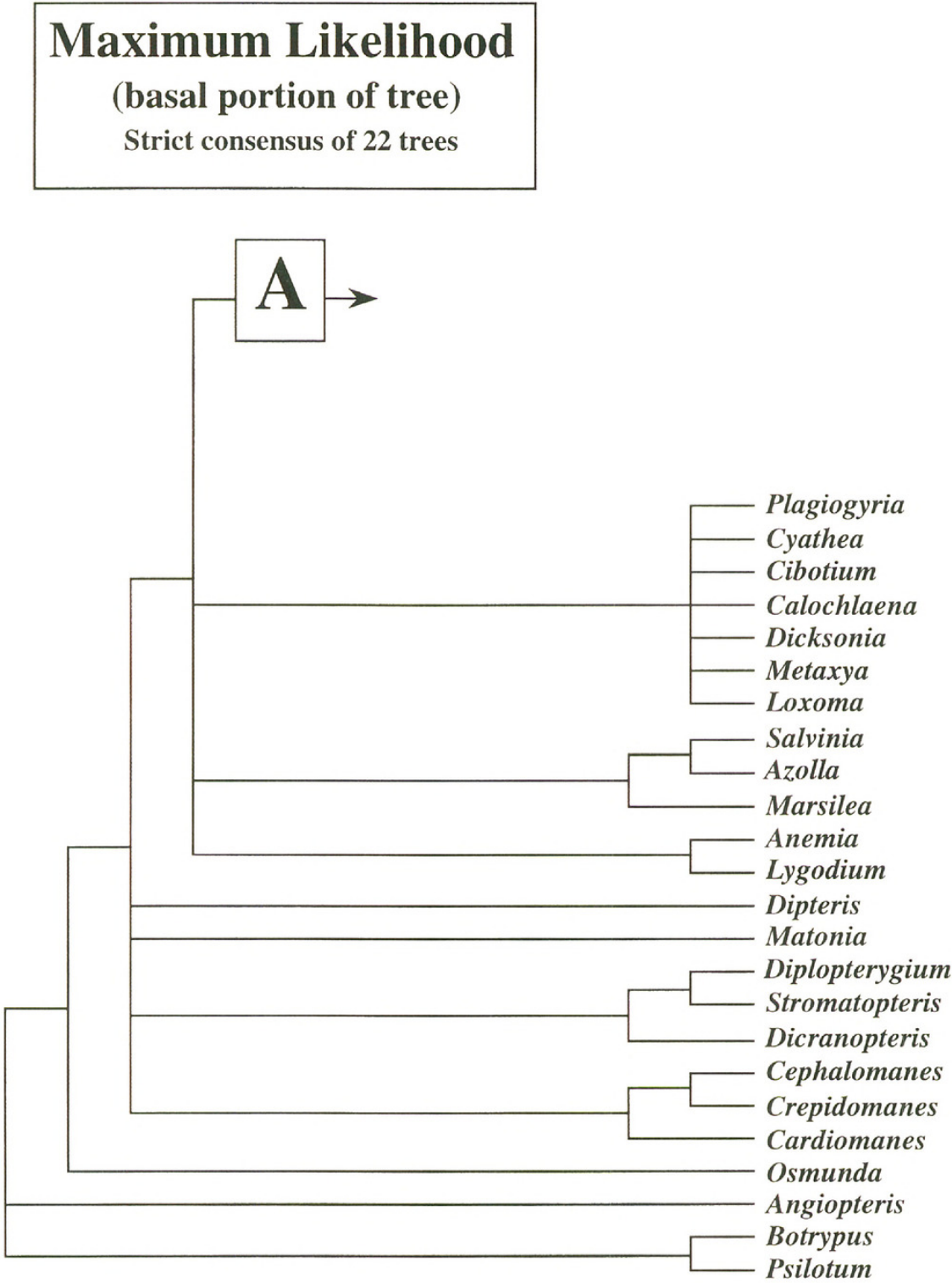


FIG. 6. Strict consensus tree of the best ML tree and the 21 trees whose log-likelihoods were not significantly worse than the best ML tree. Taxa shown in the tree are indicated in the Appendix. The tree was rooted by the eusporangiate ferns and Psilotaceae. The basal and upper portions of the tree are connected along the branch labeled "A".

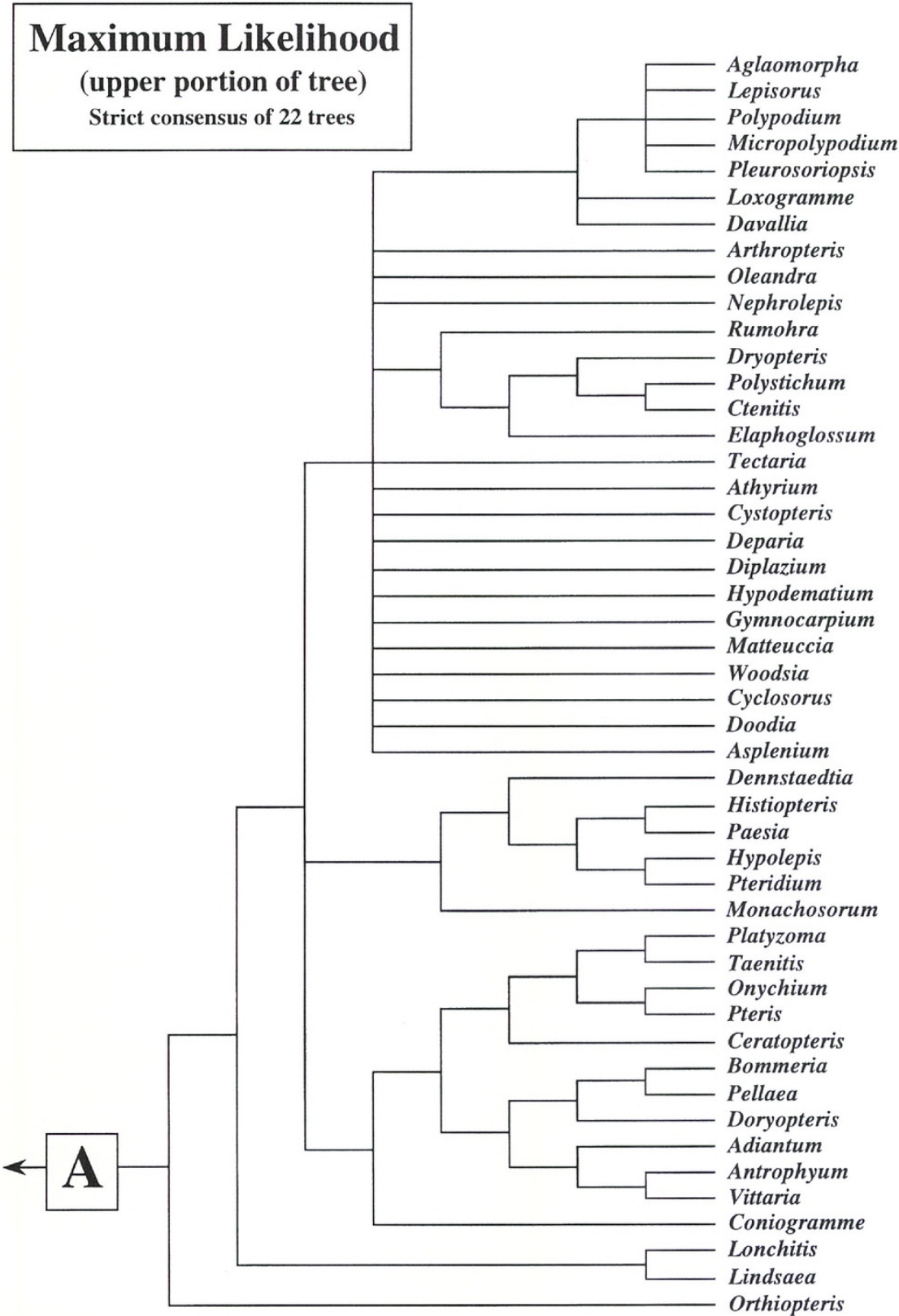


FIG. 6. Continued.

TABLE 1. Mean and standard error (SE) of synonymous (Ks) and nonsynonymous (Ka) nucleotide substitutions per nucleotide site in ferns and angiosperms. The t-test is based on the difference between the mean values of fern and angiosperm families.

		Ferns		Angiosperms		t-test	
		Mean	SE	Mean	SE	t	P
Interfamilial	Ks	1.170	0.461	0.372	0.111	1789	< 0.0001
	Ka	0.028	0.008	0.025	0.010	351	< 0.0001
Intergeneric	Ks	0.411	0.258	0.104	0.074	29	< 0.0001
	Ka	0.018	0.011	0.013	0.006	8	< 0.0001

sus tree of these eight equally most parsimonious trees is shown in Fig. 4. All of these equally most parsimonious trees obtained by the weighted MP analysis corresponded in topology to the trees 2 steps longer (5980 steps) than the equally most parsimonious trees obtained using the equal weighting criterion.

The ML analysis produced 25 different trees, because some of the 30 random addition sequence searches resulted in the same trees. The ML tree with the best log-likelihood (−21540.3479) is shown in Fig. 5. Three of the 30 random addition sequence searches resulted in finding the same best tree. A statistical test of the variance of log-likelihood differences (Kishino and Hasegawa, 1989) between each of the 24 less likely trees and the best ML tree was carried out to determine whether any of the trees were significantly worse than the best ML tree. Three trees had a significantly lower log-likelihood than that of the best ML tree, whereas the log-likelihoods of the other 21 trees were not significantly worse than that of the best ML tree. The strict consensus tree of these 21 trees and the best ML tree is shown in Fig. 6. Although global branch swapping was executed on all of the 30 trees, no trees with a higher log-likelihood than the best ML tree (Fig. 5) were obtained.

The average interfamilial and intergeneric Ks and Ka values for ferns and angiosperms are shown in Table 1. A t-test shows that these values are significantly higher in ferns than in angiosperms at the 1% level. The intrafamilial Ks and Ka values among fern and angiosperm genera are shown in Table 2.

We also attempted to calculate Ks and Ka values using the Li et al. (1985) method, however, the “LWL91” program (Li, 1993) kindly provided by Li (The University of Texas, Houston) could do the calculations for only up to 30 species-pairs. We were unable to expand the taxon number limitation of the program. Preliminary indications were that Ks and Ka values were very similar using both methods.

DISCUSSION

PHYLOGENETIC ANALYSES.—The results of computer simulation studies have shown that ML tends to be the most robust method to infer phylogenetic relationships (Kobayashi-Fukami and Tateno, 1991; Hasegawa et al., 1991), although the method takes relatively more time than other methods. MP may give erroneous results under some conditions (Hendy and Penny, 1989; DeBry,

TABLE 2. Average estimated number and standard error of nucleotide substitutions per nucleotide site between genera in a family. Data for angiosperms are drawn from DNA databases, and are available from K. Ueda upon request. For families, the number in parentheses indicates the number of genera used for this calculation. Ks = the estimated number of synonymous nucleotide substitutions per nucleotide site and standard error. Ka = the estimated number of nonsynonymous nucleotide substitutions per nucleotide site and standard error.

Fern family	Ks	Ka	Angiosperm family	Ks	Ka
Aspleniaceae (3)	0.18 ± 0.012	0.014 ± 0.0046	Acanthaceae (10)	0.14 ± 0.037	0.011 ± 0.0033
Blechnaceae (4)	0.16 ± 0.035	0.010 ± 0.0069	Araceae (8)	0.17 ± 0.088	0.015 ± 0.0052
Cyatheaceae (2)	0.027	0.0051	Areaceae (6)	0.034 ± 0.0074	0.0098 ± 0.0030
Dennstaedtiaceae (4)	0.24 ± 0.10	0.011 ± 0.0040	Asteraceae (26)	0.088 ± 0.034	0.016 ± 0.0054
Dicksoniaceae (3)	0.23 ± 0.068	0.016 ± 0.0071	Betulaceae (5)	0.043 ± 0.019	0.0035 ± 0.0014
Gleicheniaceae (2)	0.29	0.0098	Bromeliaceae (7)	0.035 ± 0.010	0.0044 ± 0.0021
Hymenophyllaceae (4)	0.42 ± 0.086	0.038 ± 0.0079	Campanulaceae (12)	0.23 ± 0.086	0.017 ± 0.0070
Marsileaceae (3)	0.27 ± 0.048	0.012 ± 0.0033	Caryophyllaceae (5)	0.15 ± 0.040	0.0076 ± 0.0028
Oleandraceae (2)	0.21	0.013	Epacridaceae (5)	0.15 ± 0.048	0.016 ± 0.0040
Ophioglossaceae (3)	0.35 ± 0.24	0.017 ± 0.0057	Ericaceae (12)	0.14 ± 0.042	0.016 ± 0.0053
Osmundaceae (2)	0.14	0.02	Fagaceae (5)	0.11 ± 0.10	0.012 ± 0.0086
Polypodiaceae (6)	0.22 ± 0.058	0.013 ± 0.0032	Geraniaceae (5)	0.15 ± 0.057	0.0040 ± 0.0017
Pteridaceae (11)	0.51 ± 0.15	0.020 ± 0.012	Lamiaceae (7)	0.12 ± 0.029	0.017 ± 0.0049
Schizaeaceae (3)	1.40 ± 0.36	0.029 ± 0.0078	Loganiaceae (7)	0.16 ± 0.052	0.017 ± 0.0048
Thelypteridaceae (2)	0.21	0.0087	Magnoliaceae (5)	0.014 ± 0.011	0.0085 ± 0.0070
Vittariaceae (2)	0.37	0.03	Nymphaeaceae (7)	0.061 ± 0.037	0.0054 ± 0.0021
			Onagraceae (7)	0.12 ± 0.047	0.011 ± 0.0037
			Poaceae (10)	0.13 ± 0.049	0.017 ± 0.0074
			Saxifragaceae (22)	0.093 ± 0.096	0.0078 ± 0.0039
			Solanaceae (16)	0.045 ± 0.024	0.010 ± 0.0056

1992; Zharkikh and Li, 1993; Takezaki and Nei, 1993) especially when the rate of nucleotide substitution is quite high or not constant (Felsenstein and Sober, 1986). However, those studies are based on the simulation of a small number of data sets using simple models, and more studies are necessary to reveal the applicability of each method for real data sets. In this study we therefore used the three different methods (NJ, MP, and ML) to infer phylogenetic relationships, and incorporated all of the results in the following discussion.

Optimal trees recovered from all three methods of tree construction had a similar overall topology. The trees we recovered were not completely congruent with any formerly proposed phylogenetic scheme (e. g., Bower, 1928; Holttum, 1949; Wagner, 1969; Nayar, 1970; Bierhorst, 1971; Mickel, 1974; Lovis, 1977), although each scheme is at least in part consistent with our results. The phylogenetic patterns observed in our analyses are discussed here in order, from the most basal lineages to the uppermost branches.

Osmundaceae (*Osmunda* and *Plenasium*) tended to be a sister taxon to all other leptosporangiate ferns on the *rbcL* trees. This phylogenetic position was supported with less than 50% bootstrap probability in both the NJ and equally weighted MP analyses. The distinctness of the Osmundaceae from other leptosporangiate ferns has been documented using both morphological and molecular data (e.g., Gifford and Foster, 1988; Stein et al., 1992). The basal branching of the Osmundaceae is concordant with the fossil record (Stewart and Rothwell, 1993). Although a phylogenetic relationship of Osmundaceae to *Plagiogyria* has been suggested (e. g., Mickel, 1974), *rbcL* trees do not support this hypothesis. The placement of Plagiogyriaceae as a sister group to Osmundaceae in the MP tree (Fig. 2) would require 78 additional steps.

The gleichenioid ferns (Cheiropleuriaceae, Dipteridaceae, Gleicheniaceae, and Matoniaceae) and Hymenophyllaceae were basally situated in all trees (Figs. 1–6). The relationships were nearly identical to those inferred by Hasebe et al. (1994). Our NJ tree (Fig. 1) weakly supported monophyly of the gleichenioids, and intergeneric relationships were not well resolved. A relationship between the gleichenioid and polypodioid (Grammitidaceae and Polypodiaceae) ferns has been suggested (e.g., Bierhorst, 1971; Holttum, 1973; Gifford and Foster, 1988), but detailed morphological analyses by Jarrett (1980) did not support this hypothesis, suggesting instead that Polypodiaceae are among the most derived, mainly indusiate fern families. The *rbcL* analyses here (Figs. 1–6) and in Haufler and Ranker (1995) clearly support the latter hypothesis.

Stromatopteris is usually treated as a subfamily of the Gleicheniaceae (e.g., Kramer, 1990a) or as a separate family (Bierhorst, 1971). The *rbcL* analyses strongly support the monophyly of *Dicranopteris*, *Diplopterygium*, and *Stromatopteris*. Although generic relationships within the family were not inferred with high statistical confidence (Figs. 1 and 3), it appears from the *rbcL* results (Figs. 1–6) that the segregation of *Stromatopteris* as a separate family would result in a paraphyletic Gleicheniaceae *sensu stricto*. Furthermore, as shown in Tables 1 and 2, the Ks value between *Diplopterygium* and *Stromatopteris* (0.29) was much smaller than the average interfamilial Ks value (1.17 ± 0.46)

TABLE 3. Differences in the estimated numbers of synonymous nucleotide substitutions per nucleotide site (Ks) between two fern species, labeled A and B, where species C is a reference species for each Ks value. This difference for each species pair is in the column labeled Ks(A,C)–Ks(B,C), and those values indicated with an asterisk (*) are significantly different from the null hypothesis: Ks(A,C)–Ks(B,C) = 0 at the 5% level. The column labeled SpA/SpB contains the ratio of the differences in the estimated number of nucleotide substitutions between species A and species B.

Species A	Species B	Species C	Ks(A,C)–Ks(B,C)	SpA/SpB
<i>Anemia</i>	<i>Salvinia</i>	<i>Dipteris</i>	0.011 ± 0.28	1.0
<i>Actinostachys</i>	<i>Salvinia</i>	<i>Dipteris</i>	–0.084 ± 0.28	0.94
<i>Lygodium</i>	<i>Salvinia</i>	<i>Dipteris</i>	–0.023 ± 0.29	0.98
<i>Ceratopteris</i>	<i>Acrostichum</i>	<i>Doryopteris</i>	0.048 ± 0.071	1.2
<i>Ceratopteris</i>	<i>Pteris</i>	<i>Doryopteris</i>	0.15 ± 0.073*	1.6
<i>Ceratopteris</i>	<i>Taenitis</i>	<i>Doryopteris</i>	0.12 ± 0.071	1.5
<i>Ceratopteris</i>	<i>Onychium</i>	<i>Doryopteris</i>	0.17 ± 0.071*	1.7
<i>Ceratopteris</i>	<i>Platyzoma</i>	<i>Doryopteris</i>	0.10 ± 0.074	1.5

for ferns and closer to the mean intergeneric Ks value (0.41 ± 0.26). Our results are therefore consistent with Kramer’s (1990a) treatment of these genera in one family.

Cardiomanes has been treated in a separate subfamily from other genera in Hymenophyllaceae because it has a thicker lamina than other filmy ferns (Iwatsuki, 1990). The *rbcL* trees (Figs. 1–6) suggest that this segregation of *Cardiomanes* results in the paraphyly of the remaining genera (subfamily Hymenophylloideae). Thus, revision of subfamilial classification in Hymenophyllaceae may be necessary after more sequence data for this family are accumulated.

Schizaeaceae were the next clade to diverge in all three methods of analysis (Figs. 1, 2, and 5). As shown in Tables 1 and 2, the average Ks value among the three genera in Schizaeaceae (*Actinostachys*, *Anemia*, and *Lygodium*) was 1.4 ± 0.36 , which is higher than the average intergeneric Ks value (0.41 ± 0.26) and higher than the average interfamilial Ks value (1.17 ± 0.46) for ferns. This suggests that the genera in Schizaeaceae diverged from each other at an earlier time than did most families and genera of ferns. An alternative hypothesis is that *rbcL* has an accelerated rate of base substitution in the Schizaeaceae. However, the latter hypothesis is not supported, because the results of the relative rate tests (Wu and Li, 1985) using *Dipteris* as a reference species did not show any significant rate variation between each of the schizaeaceous lineages and *Salvinia* (Table 3). This result was also supported when we used *Calochlaena* or *Lindsaea* instead of *Salvinia* (data not shown). Thus, we interpret the high sequence divergence among schizaeaceous genera as evidence of a long evolutionary history. This is consistent with the substantial morphological divergence among these genera (Kramer, 1990b) and a long fossil history (Stewart and Rothwell, 1993). This result supports the classification in which the four genera are treated as separate families (e.g., Pichi Sermolli, 1977). Pteridaceae (Wagner, 1969; Mickel, 1974; Holttum, 1973; Lovis, 1977; Kramer, 1990c) and Marsileaceae (Bierhorst, 1971; Pichi Sermolli, 1977; Lovis, 1977) have been

postulated to have a close relationship to Schizaeaceae, but our analyses do not support these hypotheses.

Monophyly of the heterosporous water ferns was supported by morphological and fossil evidence (Rothwell and Stockey, 1994), by a previous molecular study that included *Marsilea* and *Salvinia* (Hasebe et al., 1994), and by this study, where we used all extant water fern genera (*Azolla*, *Marsilea*, *Pilularia*, *Regnellidium*, and *Salvinia*). The trees in this study (Figs. 1–4) show the same intergeneric relationships among the water ferns as those inferred by Rothwell and Stockey (1994).

The tree ferns (Cyatheaceae and Dicksoniaceae) diverged after the water ferns in most of the *rbcL* analyses (Figs. 1, 2, 4, and 5), although this order was not well supported by the bootstrap analyses for either NJ or MP analyses (Figs. 1 and 3). Monophyly of the tree ferns was also inferred by Hasebe et al. (1994) and Wolf et al. (1994). The incorporation of *rbcL* data for additional tree fern taxa in the present study provided some fresh insights into relationships within this clade. For example, *Loxoma* (Loxomataceae) and *Plagiogyria* (Plagiogyriaceae) are depicted as sister taxa within the tree fern clade in the NJ, MP and ML analyses, although bootstrap support for the monophyletic relationship between *Loxoma* and *Plagiogyria* is not strong, especially with MP. Morphological evidence that supports *Loxoma* and *Plagiogyria* as sister taxa is lacking; therefore this hypothesis requires further testing. Nevertheless, *rbcL* data provide strong support for inclusion of both *Loxoma* and *Plagiogyria* within the tree fern clade. Some morphological traits that support this relationship are sporangial annuli, spore wall, and gametophyte morphology (Atkinson, 1973; Tryon and Tryon, 1982; Tryon and Lugardon, 1990). Overall generic relationships among tree ferns, resolved by the different algorithms, were not concordant. Monophyly of *Dicksonia* and *Calochlaena* was supported by the NJ and ML methods, but not by the MP method.

Pteridaceae are a large family, sometimes subdivided into six groups (Tryon and Tryon, 1982; Tryon et al., 1990; Table 4). As in a previous study (Hasebe et al., 1994), *rbcL* data suggest that members of the Pteridaceae share a recent common ancestor. However, the Pteridaceae are not a monophyletic group because the Vittariaceae are placed in the Pteridaceae as a sister group to *Adiantum* (Figs. 1–6). This relationship did not change, even when the number of taxa in Vittariaceae was increased (Crane et al., 1995). The following five monophyletic groups were recognized with a high degree of confidence in all three tree construction methods: Group 1 = *Onychium*, *Platyzoma*, *Pteris*, and *Taenitis*; Group 2 = *Acrostichum* and *Ceratopteris*; Group 3 = *Argyrochosma*, *Bommeria*, *Cheilanthes*, *Doryopteris*, *Hemionitis*, *Notholaena*, and *Pellaea*; Group 4 = *Coniogramme*; and Group 5 = *Adiantum* and the Vittariaceae. These five monophyletic groups were partly congruent with traditional classifications (Table 4). A more detailed analysis of relationships in Group 3 is provided by Gastony and Rollo (1995).

A relationship between evolutionary rate and life history factors was demonstrated in seed plants by Bousquet et al. (1992), with *rbcL* evolving more rapidly in annual plants than in perennials. We used the relative rate test (Wu

TABLE 4. Comparison of classification systems of the Pteridaceae.

	Tryon and Tryon (1982)	Tryon et al. (1990)	This <i>rbcL</i> study (Figs. 1–4)
<i>Platyzoma</i>	Platyzomateae	Platyzomatoideae	Group 1
<i>Taenitis</i>	Taenitideae	Taenitidoideae	Group 1
<i>Onychium</i>	Cheilantheae	Taenitidoideae	Group 1
<i>Pteris</i>	Pterideae	Pteridoideae	Group 1
<i>Ceratopteris</i>	Ceratopterideae	Ceratopteridoideae	Group 2
<i>Acrostichum</i>	Pterideae	Pteridoideae	Group 2
<i>Argyrochosma</i>	Cheilantheae	Cheilanthoideae	Group 3
<i>Pellaea</i>	Cheilantheae	Cheilanthoideae	Group 3
<i>Cheilanthes</i>	Cheilantheae	Cheilanthoideae	Group 3
<i>Doryopteris</i>	Cheilanthese	Cheilanthoideae	Group 3
<i>Hemionitis</i>	Cheilantheae	Cheilanthoideae	Group 3
<i>Notholaena</i>	Cheilantheae	Cheilanthoideae	Group 3
<i>Bommeria</i>	Cheilantheae	Cheilanthoideae	Group 3
<i>Coniogramme</i>	Taenitideae	Cheilanthoideae	Group 4
<i>Adiantum</i>	Adiantaeae	Adiantoideae	Group 5

and Li, 1985) to compare *Ceratopteris* (an annual fern) to some perennial species in the Pteridaceae, using *Doryopteris* as a reference species (Table 3). When we compared the evolutionary rate of *Ceratopteris* to *Onychium* or *Pteris*, the null hypothesis of constancy in evolutionary rate in both lineages was rejected at the 5% significance level. However, the null hypothesis was not rejected for other perennial pteroid ferns (Table 3). More data from other gene sequences are necessary to determine whether the evolutionary rate of *rbcL* is accelerated in annual ferns as in annual angiosperms.

A detailed analysis of phylogenetic relationships in Dennstaedtiaceae sensu lato was provided by Wolf et al. (1994) and Wolf (1995). The taxa of Dennstaedtiaceae sampled here formed four distinct groups, but the relationships among the groups were weakly resolved. The four groups are: Group 1 = Dennstaedtiaceae sensu stricto (*Blotiella*, *Dennstaedtia*, *Histiopteris*, *Hypolepis*, *Microlepia*, *Paesia*, and *Pteridium*) and Monachosoraceae; Group 2 = *Lonchitis*; Group 3 = *Lindsaea* and *Sphenomeris*; and Group 4 = *Orthiopteris*.

The most derived indusiate ferns (Aspleniaceae, Blechnaceae, Davalliaceae, Dryopteridaceae, Grammitidaceae, Lomariopsidaceae, Nephrolepidaceae, Oleandraceae, Polypodiaceae, Thelypteridaceae) diverged after the dennstaedtioid (Dennstaedtiaceae and Monachosoraceae) and pteroid (Pteridaceae and Vittariaceae) ferns. All of our analyses are consistent with the branching of the Aspleniaceae at the base of this group. The relationships among the Blechnaceae, Thelypteridaceae, and Dryopteridaceae subfamily Athyrioideae sensu Kramer and Kato (1990) were different in the three tree construction methods.

The *rbcL* results indicate that Dryopteridaceae sensu Kramer et al. (1990) are polyphyletic, as inferred by Hasebe et al. (1994) and Wolf et al. (1994). For example, *Tectaria* does not cluster with other members of the Dryopteridaceae (also reported by Wolf et al., 1994), but shows a close relationship to the Oleandraceae. Although *Tectaria* and *Ctenitis* are usually included in the same sub-

group of Dryopteridaceae, they appear on divergent branches in all the *rbcL* trees (Figs. 1–5). This result was confirmed even when we included other species of *Tectaria* and *Ctenitis*: *T. gaudichaudii* (Wolf et al., 1994), *T. fauriei*, and *C. sinii* (Hasebe et al., unpublished data) in our analyses.

RbcL data show that the Polypodiaceae, Davalliaceae, Grammitidaceae, Oleandraceae, and Nephrolepidaceae share a recent common ancestor with some members of the Dryopteridaceae. Within the polypodioids (Grammitidaceae and Polypodiaceae), *Loxogramme* is the most basal. Additional details of relationships within the Polypodiaceae are provided in Haufler and Ranker (1995). *Loxogramme* shares several morphological characters with Grammitidaceae and Polypodiaceae, and Tryon and Tryon (1982) suggested that *Loxogramme* was derived from the common ancestor of both groups, which is supported by the *rbcL* trees (Figs. 1–5).

Pleurosoriopsis is a small epilithic fern distributed only in eastern Asia. Phylogenetic relationships of this genus are controversial and *Pleurosoriopsis* has been placed in Pteridaceae (Copeland, 1947), Aspleniaceae (Löve et al., 1977), Grammitidaceae (Tryon and Tryon, 1982) and Pleurosoriopsidaceae (Kuriita and Ikebe, 1977). Our results suggest a close affinity of *Pleurosoriopsis* with the Polypodiaceae and Grammitidaceae, and support the hypothesis of Tryon and Tryon (1982) based on spore germination and morphological characters of the petiolar trichomes, spores, and gametophytes.

REPLY TO SMITH (1995).—Sixteen questions concerning fern relationships from the standpoint of non-molecular data were posed by Smith (1995, this volume). The global analyses of *rbcL* nucleotide sequence data provide responses for all of them except one. Below we list each question and the answer suggested by *rbcL* data. 1) Where goes *Saccoloma*? We analyzed *Orthiopteris*, which is often segregated from *Saccoloma* in the paleotropics. The MP and ML *rbcL* trees (Figs. 2–6) placed *Orthiopteris* basal to Dennstaedtiaceae sensu lato. Wolf (1995) added two species of *Saccoloma* to his analysis and found them to emerge within the *Hypolepis* clade (*Blotiella* to *Pteridium* in Fig. 2), but with *Orthiopteris* in the same position as we found it in the global analysis. 2) What are the relationships of *Monachosorum*? This genus is often included in the Dennstaedtiaceae and the *rbcL* analyses support this treatment. Wolf et al. (1994) and Wolf (1995) further resolved the basal position of *Monachosorum* to the *Hypolepis* clade. 3) What are the relationships of *Ceratopteris*? *Ceratopteris* is in the Pteridaceae and is most closely related to *Acrostichum*. 4) What are the affinities of *Pleurosoriopsis*? *Pleurosoriopsis* is closely allied to Grammitidaceae and Polypodiaceae. 5) What are the relationships of Hymenophyllopsidaceae? We have been unable to get living material of this family, which is endemic to the highlands of the Roraima formation in South America. 6) What are the relationships of the Hymenophyllaceae? Hymenophyllaceae are basally situated in *rbcL* trees. 7) What is the relationship, or lack thereof, between Polypodiaceae and Grammitidaceae? The members of these families used in the present study form a monophyletic group. 8) What are the relationships of Plagiogyriaceae? Plagiogyriaceae are closely related to

the tree ferns (Cyatheaceae, Dicksoniaceae, and Metaxiaceae) and appear to have an affinity with *Loxoma*. 9) What are the relationships and origin of the heterosporous fern families? The heterosporous ferns are more closely related to the most derived leptosporangiate ferns (including the tree fern group) than they are to the Schizaeaceae. Azollaceae, Salviniaceae, and Marsileaceae form a strongly supported, monophyletic group. 10) What are the relationships of Psilotaceae? *Psilotum* and *Tmesipteris* form a monophyletic group in the NJ tree (Fig. 1; tree rooted by *Angiopteris* only) and do not have a close relationship with any leptosporangiate ferns including *Stromatopteris* (contrary to Bierhorst, 1968, p.266), which is a sister taxon to *Diplopterygium*. This result also was supported when we assigned only *Angiopteris* as an outgroup in the MP and ML analyses (analysis not shown). *Psilotum* and *Tmesipteris* grouped with Ophioglossaceae in trees produced from plastid 16S (Manhart, 1995) and nuclear 18S ribosomal RNA sequences (Wolf et al., unpublished). 11) What are the relationships of Thelypteridaceae? Thelypteridaceae do not form a sister group with Cyatheaceae (Holttum and Sen, 1961) or Aspleniaceae (Nayar, 1976; Pichi Sermolli, 1977), but are closely related to the athyrioids (*Athyrium* to *Woodsia* in Fig. 1) in our analyses. 12) What are the relationships among the Schizaeaceae? Schizaeaceae form a monophyletic group. In Schizaeaceae, *Lygodium* is sister to *Actinostachys* and *Anemia*. 13) Are the Pteridaceae (and Vittariaceae) most closely related to or derived from the Schizaeaceae, or are they more closely allied to the higher leptosporangiate ferns? Pteridaceae diverged from Dennstaedtiaceae sensu lato and are not among the most derived clades of indusiate, leptosporangiate ferns; nor are they closely related to Schizaeaceae. 14) Are the Dennstaedtiaceae allied with the Dicksoniaceae or are they part of a higher leptosporangiate clade, ancestral to, or an early offshoot from the line leading to the dryopteroids, asplenioids, and blechnoids? Dennstaedtiaceae are polyphyletic with some taxa diverging after the tree ferns, and the rest forming a monophyletic clade closely related to the most derived indusiate ferns. 15) What is a reasonable circumscription of Dryopteridaceae? Dryopteridaceae are polyphyletic and share a common ancestor with the Aspleniaceae and Oleandraceae (Fig. 1). Dryopteridaceae sensu lato perhaps should be divided after more detailed analyses. 16) Do the higher leptosporangiate families, including Aspleniaceae, Blechnaceae, Davalliaceae, Dennstaedtiaceae sensu lato, Dryopteridaceae, Grammitidaceae, Pteridaceae, and Polypodiaceae form a clade? All together, these taxa form a well-supported, monophyletic group.

KS AND KA VALUES.—Ks and Ka values are useful for comparing the variation in nucleotide substitutions per nucleotide site among lineages. Average Ks and Ka values between families and between genera within families were significantly higher in ferns than in angiosperms (Table 1). There are at least two explanations for this: 1) fern genera and families are older than those of angiosperms, on average, and have therefore accumulated more nucleotide substitutions, or 2) nucleotide substitution rates are accelerated in ferns (Hasebe et al., 1993). To choose between these will require inferring the correct phy-

logenetic relationships among land plants, identifying an appropriate outgroup for the ferns, and then applying the relative rate test (Wu and Li, 1985; Muse and Weir, 1992).

FUTURE PROSPECTS.—Global analyses of *rbcL* sequences in leptosporangiate ferns have provided possible answers to many questions in fern systematics. So far, we have extensive data for only one plastid gene. Inferences from this study must be tested with non-molecular data and also with more conserved or more rapidly evolving regions of the fern genome. A comparison of trees obtained from both *rbcL* and non-molecular data is reported elsewhere in this volume (Pryer et al., 1995). Some fern plastid genomes have been shown to be maternally inherited (Gastony and Yatskievych, 1992). This may lead to conflicts between *rbcL* phylogenies and those based on biparentally inherited, nuclear-encoded, gene sequence data, although the possibility of conflicts at the interfamilial level, where hybridization has not been reported in ferns, is much lower than at the infrageneric or intergeneric levels.

One of the most limiting factors in this type of study is data analysis. Computational speed is unlikely to be improved by many orders of magnitude in the near future and thus a more efficient approach may be to examine smaller data sets, although this can lead to the problem of long branch attraction, especially when using maximum parsimony analysis (Felsenstein, 1978; Hendy and Penny, 1989; Huelsenbeck and Hillis, 1993). Selection of tree construction methods is another problem that has not been fully studied. In our analyses, the results of three different methods gave almost concordant results for relationships among ferns, giving us confidence in our results.

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LITERATURE CITED

- ADACHI, J., and M. HASEGAWA. 1994. *MOLPHY: Programs for molecular phylogenetics, ver. 2.2*. Institute of Statistical Mathematics, Tokyo.

- ALBERT, V. A., M. W. CHASE, and B. D. MISHLER. 1993. Character-state weighting for cladistic analysis of protein-coding DNA sequences. *Ann. Missouri Bot. Gard.* 80:752–766.
- ATKINSON, L. R. 1973. The gametophyte and family relationships. *Bot. J. Linn. Soc.* 67(Supplement):73–90.
- BIERHORST, D. W. 1968. On the Stromatopteridaceae (fam. nov.) and on the Psilotaceae. *Phytomorphology* 18:232–268.
- . 1971. *Morphology of vascular plants*. Macmillan, New York.
- BOUSQUET, J., S. H. STRAUSS, A. H. DOERKSEN, and R. A. PRICE. 1992. Extensive variation in evolutionary rate of *rbcL* gene sequences among seed plants. *Proc. Natl. Acad. Sci. U.S.A.* 89:7844–7848.
- BOWER, F. O. 1928. *The ferns (Filicales)*, volume III. *The leptosporangiate ferns*. Cambridge University Press, Cambridge.
- BREMER, K. 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. *Evolution* 42:795–803.
- CHASE, M. W., D. E. SOLTIS, R. G. OLMSTEAD, D. MORGAN, D. H. LES, B. D. MISHLER, M. R. DUVAL, R. A. PRICE, H. G. HILLS, Y.-L. QIU, K. A. KRON, J. H. RETTIG, E. CONTI, J. D. PALMER, J. R. MANHART, K. J. SYTSMA, H. J. MICHAELS, W. J. KRESS, K. G. KAROL, W. D. CLARK, M. HEDRÉN, B. S. GAUT, R. K. JANSEN, K.-J. KIM, C. F. WIMPEE, J. F. SMITH, G. R. FURNIER, S. H. STRAUSS, Q.-Y. XIANG, G. M. PLUNKETT, P. S. SOLTIS, S. M. SWENSEN, S. E. WILLIAMS, P. A. GADEK, C. J. QUINN, L. E. EGUIARTE, E. GOLENBERG, G. H. LEARN, JR., S. W. GRAHAM, S. C. H. BARRETT, S. DAYANANDAN, and V. A. ALBERT. 1993. Phylogenetics of seed plants: An analysis of nucleotide sequences from the plastid gene *rbcL*. *Ann. Missouri Bot. Gard.* 80:528–580.
- COPELAND, E. B. 1947. *Genera Filicum*. H. Hagerup, Copenhagen.
- CRANE, E. H., D. R. FARRAR, and J. F. WENDEL. 1995. Phylogeny of the Vittariaceae: Convergent simplification leads to a polyphyletic *Vittaria*. *Amer. Fern J.* 85:283–305.
- DEBRY, R. W. 1992. The consistency of several phylogeny-inference methods under varying evolutionary rates. *Molec. Biol. Evol.* 9:537–551.
- FELSENSTEIN, J. 1978. Cases in which parsimony or compatibility methods will be positively misleading. *Syst. Zool.* 27:401–410.
- . 1981. Evolutionary trees from DNA sequences: A maximum likelihood approach. *J. Molec. Evol.* 17:368–376.
- . 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39:783–791.
- . 1993. *PHYLIP (Phylogeny Inference Package) version 3.5c*. Distributed by the author. Department of Genetics, University of Washington, Seattle.
- FELSENSTEIN, J., and H. KISHINO. 1993. Is there something wrong with the bootstrap on phylogenies? A reply to Hillis and Bull. *Syst. Biol.* 42:193–200.
- FELSENSTEIN, J., and E. SOBER. 1986. Parsimony and likelihood: An exchange. *Syst. Zool.* 35:617–626.
- FUKAMI-KOBAYASHI, K., and Y. TATENO. 1991. Robustness of maximum likelihood tree estimation against different patterns of base substitution. *J. Molec. Evol.* 32:79–91.
- GASTONY, G. J., and D. R. ROLLO. 1995. Phylogeny and generic circumscriptions of cheilanthoid ferns (Pteridaceae: Cheilanthesoideae) inferred from *rbcL* nucleotide sequences. *Amer. Fern J.* 85:341–360.
- GASTONY, G. J., and G. YATSKIEVYCH. 1992. Maternal inheritance of the chloroplast and mitochondrial genomes in cheilanthoid ferns. *Amer. J. Bot.* 78:716–722.
- GIFFORD, E. M., and A. S. FOSTER. 1988. *Morphology and evolution of vascular plants*, 3rd ed. Freeman, New York.
- HASEBE, M., M. ITO, R. KOFUJI, K. UEDA, and K. IWATSUKI. 1993. Phylogenetic relationships of ferns deduced from *rbcL* gene sequence. *J. Molec. Evol.* 37:476–482.
- HASEBE, M., T. OMORI, M. NAKAZAWA, T. SANO, M. KATO, and K. IWATSUKI. 1994. *RbcL* gene sequences provide evidence for the evolutionary lineages of leptosporangiate ferns. *Proc. Natl. Acad. Sci. U.S.A.* 91:5730–5734.
- HASEGAWA, M., T. HASHIMOTO, J. ADACHI, N. IWABE, and T. MIYATA. 1993. Early branchings in

- the evolution of eukaryotes: Ancient divergence of *Entamoeba* that lacks mitochondria revealed by protein sequence data. *J. Molec. Evol.* 36:380–388.
- HASEGAWA, M., H. KISHINO, and N. SAITOU. 1991. On the maximum likelihood method in molecular phylogenetics. *J. Molec. Evol.* 32:443–445.
- HASEGAWA, M., H. KISHINO, and T. YANO. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *J. Molec. Evol.* 22:160–174.
- HAUFLER, C. H., and T. A. RANKER. 1995. *RbcL* sequences provide phylogenetic insights among sister species of the fern genus *Polypodium*. *Amer. Fern J.* 85:361–374.
- HENDY, M. D., and D. PENNY. 1989. A framework for the quantitative study of evolutionary trees. *Syst. Zool.* 38:297–309.
- HIESEL, R., A. VON HAESELER, and A. BRENNICKE. 1994. Plant mitochondrial nucleic acid sequences as a tool for phylogenetic analysis. *Proc. Natl. Acad. Sci. U.S.A.* 91:634–638.
- HILLIS, D. M., and J. J. BULL. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Syst. Biol.* 42:182–192.
- HOLTUM, R. E. 1949. The classification of ferns. *Biol. Rev.* 24:267–296.
- . 1973. Posing the problems. Pp. 1–10 in A. C. Jermy, J. A. Crabbe, and B. A. Thomas, eds. *The phylogeny and classification of the ferns*. *Bot. J. Linn. Soc.* 67 (suppl. 1):i–xiv, 1–284.
- HOLTUM, R. E., and U. SEN. 1961. Morphology and classification of the tree ferns. *Phytomorphology* 11:406–420.
- HUELSENBECK, J. P., and D. M. HILLIS. 1993. Success of phylogenetic methods in the four-taxon case. *Syst. Biol.* 42:247–264.
- IWATSUKI, K. 1990. Hymenophyllaceae. Pp. 157–163 in K. Kubitzki, ed. 1990. *The families and genera of vascular plants. Vol. 1. Pteridophytes and gymnosperms*. Vol. eds. K. U. Kramer and P. S. Green. Springer-Verlag, Berlin.
- JARRETT, F. M. 1980. Studies in the classification of the leptosporangiate ferns: I. The affinities of the Polypodiaceae *sensu stricto* and the Grammitidaceae. *Kew Bull.* 34:825–833.
- JUKES, T. H., and C. R. CANTOR. 1969. Evolution of protein molecules. Pp. 21–132, in H. N. Munro, ed., *Mammalian protein metabolism*. Academic Press, New York.
- KIMURA, M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J. Molec. Evol.* 16:111–120.
- KISHINO, H., and M. HASEGAWA. 1989. Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea. *J. Molec. Evol.* 29:170–179.
- KRAMER, K. U. 1990a. Gleicheniaceae. Pp. 145–152 in K. Kubitzki, ed. 1990. *The families and genera of vascular plants. Vol. 1. Pteridophytes and gymnosperms*. Vol. eds. K. U. Kramer and P. S. Green. Springer-Verlag, Berlin.
- . 1990b. Schizaeaceae. Pp. 258–263 in K. Kubitzki, ed. 1990. *The families and genera of vascular plants. Vol. 1. Pteridophytes and gymnosperms*. Vol. eds. K. U. Kramer and P. S. Green. Springer-Verlag, Berlin.
- . 1990c. Notes on the higher level classification of the recent ferns. Pp. 49–52 in K. Kubitzki, ed. 1990. *The families and genera of vascular plants. Vol. 1. Pteridophytes and gymnosperms*. Vol. eds. K. U. Kramer and P. S. Green. Springer-Verlag, Berlin.
- KRAMER, K. U., R. E. HOLTUM, R. C. MORAN, and A. R. SMITH. 1990. Dryopteridaceae. Pp. 101–144, in K. Kubitzki, ed. 1990. *The families and genera of vascular plants. Vol. 1. Pteridophytes and gymnosperms*. Vol. eds. K. U. Kramer and P. S. Green. Springer-Verlag, Berlin.
- KRAMER, K. U., and P. S. GREEN, VOL. EDS. 1990. *Pteridophytes and gymnosperms*. Vol 1, in K. Kubitzki, ed., *The families and genera of vascular plants*. Springer-Verlag, Berlin.
- KRAMER, K. U., and M. KATO. 1990. Dryopteridaceae subfamily Athyrioideae. Pp. 130–144 in K. Kubitzki, ed. 1990. *The families and genera of vascular plants. Vol. 1. Pteridophytes and gymnosperms*. Vol. eds. K. U. Kramer and P. S. Green. Springer-Verlag, Berlin.
- KURITA, S., and C. IKEBE. 1977. On the systematic position of *Pleurosoriopsis makinoi* (Maxim.) Fomin. *J. Jap. Bot.* 52:39–48.
- LI, W.-H. 1993. Unbiased estimation of the rates of synonymous and non-synonymous substitution. *J. Molec. Evol.* 36:96–99.
- LI, W.-H., C.-I. WU, and C.-C. LUO. 1985. A new method for estimating synonymous and nonsy-

- nonymous rates of nucleotide substitution considering the relative likelihood of nucleotide and codon changes. *Molec. Biol. Evol.* 2:150–174.
- LÖVE, Á., D. LÖVE, and R. E. G. PICHI SERMOLLI. 1977. *Cytotaxonomical atlas of the Pteridophyta*. Cramer, Vaduz.
- LOVIS, J. D. 1977. Evolutionary patterns and processes in ferns. *Adv. Bot. Res.* 4:230–415.
- MADDISON, D. R. 1991. Discovery and importance of multiple islands of most-parsimonious trees. *Syst. Zool.* 40:315–328.
- MADDISON, W. P., and D. R. MADDISON. 1992. *MacClade: Analysis of phylogeny and character evolution*. Version 3. Sinauer Associates, Sunderland, MA.
- MANHART, J. R. 1994. Phylogenetic analysis of green plant *rbcL* sequences. *Molec. Phyl. Evol.* 3: 114–127.
- . 1995. Chloroplast 16S rDNA sequences and phylogenetic relationships of fern allies and ferns. *Amer. Fern J.* 85:182–192.
- MICKEL, J. T. 1974. Phyletic lines in the modern ferns. *Ann. Missouri Bot. Gard.* 61:474–482.
- MISHLER, B. D., L. A. LEWIS, M. A. BUCHHEIM, K. S. RENZAGLIA, D. J. GARBARY, C. F. DELWICHE, F. W. ZECHMAN, T. S. KANTZ, and R. L. CHAPMAN. 1994. Phylogenetic relationships of the “green algae” and “bryophytes.” *Ann. Missouri Bot. Gard.* 81:451–483.
- MUSE, S. V., and B. S. WEIR. 1992. Testing for equality of evolutionary rates. *Genetics* 132:269–276.
- NAYAR, B. K. 1970. A phylogenetic classification of the homosporous ferns. *Taxon* 19:229–236.
- . 1976. The classification of ferns. Pp. 145–204, in P. Kachroo, ed., *Recent advances in botany*. Bishen Singh and Mahendra Pal Singh, Dehra Dun, India.
- NEI, M., and T. GOJOBORI. 1986. Simple methods for estimating the numbers of synonymous and nonsynonymous nucleotide substitutions. *Molec. Biol. Evol.* 3:418–426.
- OHYAMA, K., H. FUKAZAWA, T. KOHCHI, H. SHIRAI, T. SANO, S. SANO, K. UMESONO, Y. SHIKI, M. TAKEUCHI, Z. CHANG, S. AOTA, H. INOKUCHI, and H. OZEKI. 1986. Chloroplast gene organization deduced from complete sequence of liverwort *Marchantia polymorpha* chloroplast DNA. *Nature* 322:572–574.
- OLMSTEAD, R. G., and J. D. PALMER. 1994. Chloroplast DNA systematics: A review of methods and data analysis. *Amer. J. Bot.* 81:1205–1224.
- OLSEN, G. J., H. MATSUDA, R. HAGSTROM, and R. OVERBEEK. 1992. *FastDNAm1*. Version 1.0.6. University of Illinois, Urbana and Argonne National Laboratory, Argonne, Illinois.
- . 1994. *FastDNAm1*: A tool for construction of phylogenetic trees of DNA sequences using maximum likelihood. *Comput. Appl. Biosci.* 10:41–48.
- PICHI SERMOLLI, R. E. G. 1977. Tentamen pteridophytorum genera in taxonomicum ordinem redigendi. *Webbia* 31:313–512.
- PRYER, K. M., A. R. SMITH, and J. E. SKOG. 1995. Phylogenetic relationships of extant pteridophytes based on evidence from morphology and *rbcL* sequences. *Amer. Fern J.* 85:205–282.
- RANKER, T. A. 1995. DNA sequence data in phylogeny reconstruction: An overview of techniques and analyses. *Amer. Fern J.* 85:123–133.
- ROTHWELL, G. W., and R. A. STOCKEY. 1994. The role of *Hydropteris pinnata* gen. et sp. nov. in reconstructing the cladistics of heterosporous ferns. *Amer. J. Bot.* 81:479–492.
- SANDERSON, M. J. 1989. Confidence limits on phylogenies: The bootstrap revisited. *Cladistics* 5: 113–129.
- SMITH, A. R. 1995. Non-molecular phylogenetic hypotheses for ferns. *Amer. Fern J.* 85:104–122.
- STEIN, D. B., D. S. CONANT, M. E. AHEARN, E. T. JORDAN, S. A. KIRCH, M. HASEBE, K. IWATSUKI, M. K. TAN, and J. A. THOMSON. 1992. Structural rearrangements of the chloroplast genome provide an important phylogenetic link in ferns. *Proc. Natl. Acad. Sci. U.S.A.* 89:1856–1860.
- STEWART, W. N. and G. W. ROTHWELL. 1993. *Paleobotany and the evolution of plants*, 2nd ed. Cambridge University Press, Cambridge.
- SWOFFORD, D. L. 1993. *Phylogenetic analysis using parsimony, version 3.1. User's manual*. Illinois Natural History Survey, Champaign, IL.
- TAKEZAKI, N., and M. NEI. 1995. Inconsistency of the maximum parsimony method when the rate of nucleotide substitution is constant. *J. Molec. Evol.* 39:210–218.
- TRYON, A. F., and B. LUGARDON. 1990. *Spores of the pteridophyta*. Springer-Verlag, New York.

- TRYON, R. M., and A. F. TRYON. 1982. *Ferns and allied plants, with special reference to tropical America*. Springer-Verlag, New York.
- TRYON, R. M., A. F. TRYON, and K. U. KRAMER. 1990. Pteridaceae. Pp. 230–256 in K. Kubitzki, ed. 1990. *The families and genera of vascular plants. Vol. 1. Pteridophytes and gymnosperms*. Vol. eds. K. U. Kramer and P. S. Green. Springer-Verlag, Berlin.
- WAGNER, W. H., JR. 1969. The construction of a classification. Pp. 67–90 in U.S. National Academy of Science. *Systematic Biology*. U.S. Natl. Acad. Sci. Publ. No. 1692. National Academy Press, Washington DC.
- WOLF, P. G. 1995. Phylogenetic analyses of *rbcL* and nuclear ribosomal RNA gene sequences in Dennstaedtiaceae. *Amer. Fern J.* 85:306–327.
- WOLF, P. G., P. S. SOLTIS, and D. E. SOLTIS. 1994. Phylogenetic relationships of dennstaedtioid ferns: Evidence from *rbcL* sequence variation. *Molec. Phyl. Evol.* 3:383–392.
- WU, C.-I., and W.-H. LI. 1985. Evidence for higher rates of nucleotide substitution in rodents than in man. *Proc. Natl. Acad. Sci. U.S.A.* 82:1741–1745.
- YOSHINAGA, K., Y. KUBOTA, T. ISHII, and K. WADA. 1992. Nucleotide sequence of *atpB*, *rbcL*, *trnR*, *dedB* and *psaI* chloroplast genes from *Angiopteris lygodiiifolia*: A possible emergence of Spermatophyta lineage before the separation of Bryophyta and Pteridophyta. *Pl. Molec. Biol.* 18: 79–82.
- ZHARKIKH, A., and W.-H. LI. 1993. Inconsistency of the maximum-parsimony method: The case of five taxa with a molecular clock. *Syst Biol.* 42:113–125.

APPENDIX. Pteridophyte *rbcL* sequence data compiled for studies in this issue of the American Fern Journal. These are arranged alphabetically by family, subfamily, and genus. Taxonomy of families follows Kramer and Green (1990). Taxa used for the calculation of the expected number of synonymous and nonsynonymous nucleotide substitutions per nucleotide site (Ks and Ka, respectively) are indicated in a column labeled Ks/Ka.

Family (subfamily)	Species	Locality (country, state, or province)	Source/Voucher	Data- base acces- sion	Literature citation	Ks/ Ka	Figures in this study
Aspleniaceae	<i>Asplenium antiquum</i> Makino	Japan, Tokyo	Murakami 596901 (TI)	U30596	This study (Murakami)	*	1,2,3,4,5,6
Aspleniaceae	<i>Asplenium filipes</i> Copel.	Japan, Kagoshima	Murakami 596902 (TI)	U30605	Hasebe et al., 1994		
Aspleniaceae	<i>Asplenium griffithianum</i> Hook.	Japan, Kagoshima	Murakami 596903 (TI)		Murakami et al., unpubl.		
Aspleniaceae	<i>Asplenium incisum</i> Thunb.	Japan, Mie	Murakami 596904 (TI)		Murakami et al., unpubl.		
Aspleniaceae	<i>Asplenium nidus</i> L.	Laos, Vientiane	Murakami 596905 (TI)		Murakami et al., unpubl.		
Aspleniaceae	<i>Asplenium nidus</i> L.	U.S.A., Guam	MBG 896449 (UTC)	U05907	Wolf et al., 1994		
Aspleniaceae	<i>Asplenium normale</i> D. Don	Japan, Mie	Murakami 596906 (TI)		Murakami et al., unpubl.		
Aspleniaceae	<i>Asplenium normale</i> D. Don var. <i>boreale</i> Ohwi ex Kurata	Japan, Wakayama	Murakami 596907 (TI)		Murakami et al., unpubl.		
Aspleniaceae	<i>Asplenium normale</i> D. Don var. <i>shimurae</i> H. Ito	Japan, Mie	Murakami 596908 (TI)		Murakami et al., unpubl.		
Aspleniaceae	<i>Asplenium oligophlebium</i> Baker	Japan, Mie	Murakami 596909 (TI)		Murakami et al., unpubl.		
Aspleniaceae	<i>Asplenium pekinense</i> Hance	Japan, Wakayama	Murakami 596910 (TI)		Murakami et al., unpubl.		
Aspleniaceae	<i>Asplenium prolongatum</i> Hook.	Japan, Mie	Murakami 596911 (TI)		Murakami et al., unpubl.		
Aspleniaceae	<i>Asplenium ritoense</i> Hayata	Japan, Wakayama	Murakami 596912 (TI)		Murakami et al., unpubl.		
Aspleniaceae	<i>Asplenium ruprechtii</i> Kurata (= <i>Camptosorus sibiricus</i> Rupr.)	Japan, Toyama	Murakami 596918 (TI)	U30606	This study (Murakami)	*	1,2,3,4

Family (subfamily)	Species	Locality (country, state, or province)	Source/Voucher	Data- base acces- sion	Literature citation	Ks/ Ka	Figures in this study
Aspleniaceae	<i>Asplenium serellii</i> Hook.	Japan, Mie	Murakami 596913 (TI)		Murakami et al., un- publ.		
Aspleniaceae	<i>Asplenium trichomanes</i> L.	Japan, Mie	Murakami 596914 (TI)		Murakami et al., un- publ.		
Aspleniaceae	<i>Asplenium tripteropus</i> Nakai	Japan, Wakayama	Murakami 596915 (TI)		Murakami et al., un- publ.		
Aspleniaceae	<i>Asplenium wilfordii</i> Mett. ex Kuhn	Japan, Mie	Murakami 596916 (TI)		Murakami et al., un- publ.		
Aspleniaceae	<i>Asplenium wrightii</i> D.C. Ea- ton ex Hook.	Japan, Mie	Murakami 596917 (TI)		Murakami et al., un- publ.		
Aspleniaceae	<i>Phyllitis scolopendrium</i> (L.) Newn.	Japan, Fukushima	Hasebe 26544 (TI)	U30607	Hasebe et al., 1994	*	1,2,3,4
Azollaceae	<i>Azolla caroliniana</i> Willd.	U.S.A., cultivated	Pryer 959a (DUKE)	U24185	This study (Pryer)	*	1,2,3,4,5,6
Blechnaceae (Blechnoideae)	<i>Blechnum orientale</i> L.	Japan, Kagoshima	TI698 (TI)	U05606	Hasebe et al., 1994	*	1,2,3,4
Blechnaceae (Blechnoideae)	<i>Blechnum occidentale</i> L.	U.S.A., Hawaii	Wolf & Anderson-Wong 242 (UTC)	U05909	Wolf et al., 1994		
Blechnaceae (Blechnoideae)	<i>Blechnum brasiliense</i> Desv.	Unknown	MBG 801368 (UTC)	U05910	Wolf et al., 1994		
Blechnaceae (Blechnoideae)	<i>Doodia maxima</i> J. Sm.	Unknown	MBG U508 (UTC)	U05921	Wolf et al., 1994	*	1,2,3,4,5,6
Blechnaceae (Blechnoideae)	<i>Doodia media</i> R. Br.	Unknown	MBG 801282 (UTC)	U05922	Wolf et al., 1994		
Blechnaceae (Blechnoideae)	<i>Sadleria pallida</i> Hook. & Arm.	U.S.A., Hawaii	Ranker 1287 (COLO)	U05943	Wolf et al., 1994	*	1,2,3,4
Blechnaceae (Blechnoideae)	<i>Woodwardia fimbriata</i> Sm.	U.S.A., California	MBG 897131 (UTC)	U05950	Wolf et al., 1994		
Blechnaceae (Stenochlaeno- ideae)	<i>Stenochlaena tenuifolia</i> (Desv.) Moore	Unknown	MBG 840392 (UTC)	U05945	Wolf et al., 1994		

APPENDIX. Continued.

Family (subfamily)	Species	Locality (country, state, or province)	Source/Voucher	Data- base acces- sion	Literature citation	Ks/ Ka	Figures in this study
Blechnaceae (Stenochlaeno- ideae)	<i>Stenochlaena palustris</i> (Burm.) Bedd.	Singapore, Singapore	Hasebe 27701 (TI)	U05652	Hasebe et al., 1994	*	1,2,3,4
Cheiropleuriaceae	<i>Cheiropleuria bicuspid</i> (Blu- me) C. Presl	Japan, Okinawa	Yokoyama 27619 (TI)	U05607	Hasebe et al., 1994	*	1,2,3,4
Cyatheaceae	<i>Cyathea lepifera</i> (J. Sm. ex Hook.) Copel. (= <i>Sphaer- opteris lepifera</i> (J. Sm. ex Hook.) R. Tryon)	Japan, Okinawa	Yokoyama 5176 (TI)	U05616	Hasebe et al., 1994	*	1,2,3,4,5,6
Cyatheaceae	<i>Cyathea</i> sp.	U.S.A., Hawaii, cultivat- ed	Wolf & Anderson-Wong 256 (UTC)	U05914	Wolf et al., 1994		
Cyatheaceae	<i>Sphaeropteris cooperi</i> (F. Muell.) R. Tryon	Australia	MBG 894468 (UTC)	U05944	Wolf et al., 1994	*	1,2,3,4
Davalliaceae	<i>Davallia denticulata</i> (Burm. f.) Mett.	Unknown	MBG 896474 (UTC)	U05917	Wolf et al., 1994	*	1,2,3,4,5,6
Davalliaceae	<i>Davallia mariesii</i> T. Moore ex Baker	Japan, Jukushima	Hasebe 26557 (TI)	U05617	Hasebe et al., 1994		
Dennstaedtiaceae (Dennstaedtioideae)	<i>Blotiella pubescens</i> (Kaulf.) R. Tryon	La Réunion	Strasberg s.n. (REU, UTC)	U05911	Wolf et al., 1994		1,2,3,4
Dennstaedtiaceae (Dennstaedtioideae)	<i>Coptodipteris wilfordii</i> (Moore) Nakai & Momose	Japan, Mt. Fuji	Togashi 360 (UC)	U18635	Wolf, 1995		
Dennstaedtiaceae (Dennstaedtioideae)	<i>Dennstaedtia punctilobula</i> (Michx.) Moore	U.S.A., Vermont	Paris s.n. (UTC)	U05918	Wolf et al., 1994	*	1,2,3,4,5,6
Dennstaedtiaceae (Dennstaedtioideae)	<i>Dennstaedtia samoensis</i> (Brack.) Moore	Vauatu	Kew 1971-5068 (UTC)	U18637	Wolf, 1995		
Dennstaedtiaceae (Dennstaedtioideae)	<i>Dennstaedtia auriculata</i> Nalvarrete	Ecuador	Navarrete s.n. (QCA)	U18636	Wolf, 1995		
Dennstaedtiaceae (Dennstaedtioideae)	<i>Histiopteris incisa</i> (Thunb.) J. Sm.	Australia, Queensland	Kato et al. 237 (TI)	U05627	Hasebe et al., 1994	*	1,2,3,4,5,6

Family (subfamily)	Species	Locality (country, state, or province)	Source/Voucher	Data- base acces- sion	Literature citation	Ks/ Ka	Figures in this study
Dennstaedtiaceae (Dennstaedtioidae)	<i>Histiopteris incisa</i> (Thunb.) J. Sm.	New Caledonia	UCBG 57.0776 (UTC)	U05926	Wolf, 1995		
Dennstaedtiaceae (Dennstaedtioidae)	<i>Hypolepis aspidoides</i> Christ	Western Samoa	Kew #1965-58402 (UTC)	U18634	Wolf, 1995		
Dennstaedtiaceae (Dennstaedtioidae)	<i>Hypolepis hostilis</i> (Kunze) C. Presl	Ecuador	Navarrete s.n. (QCA)	U18638	Wolf, 1995		
Dennstaedtiaceae (Dennstaedtioidae)	<i>Hypolepis muelleri</i> Wakef.	Australia	Thomson 189 (UTC)	U05927	Wolf, 1995		
Dennstaedtiaceae (Dennstaedtioidae)	<i>Hypolepis punctata</i> (Thunb.) Mett.	Japan, Nara	Hasebe 27605 (TI)	U05628	Hasebe et al., 1994	*	1,2,3,4,5,6
Dennstaedtiaceae (Dennstaedtioidae)	<i>Leptolepia novae-zelandiae</i> (Col.) Mett. ex Diels	New Zealand	Parris 11713 (UTC)	U18639	Wolf, 1995		
Dennstaedtiaceae (Dennstaedtioidae)	<i>Lonchitis hirsuta</i> L.	Puerto Rico	Axelrod 4221 (UPRRP, UTC)	U05929	Wolf et al., 1994	*	1,2,3,4,5,6
Dennstaedtiaceae (Dennstaedtioidae)	<i>Lonchitis mannii</i> (Baker) Al- ston	Cameroon	Thomas 2764 (UC)	U18641	Wolf, 1995		
Dennstaedtiaceae (Dennstaedtioidae)	<i>Microlepia strigosa</i> (Thunb.) C. Presl	U.S.A., Hawaii	Wolf & Anderson-Wong 249 (UTC)	U05931	Wolf, 1995		1,2,3,4
Dennstaedtiaceae (Dennstaedtioidae)	<i>Microlepia szechuanica</i> Ching	Unknown	From spores (UTC)	U18643	Wolf, 1995		
Dennstaedtiaceae (Dennstaedtioidae)	<i>Microlepia platyphylla</i> (Don) J. Sm.	China	From spores (UTC)	U18642	Wolf, 1995		
Dennstaedtiaceae (Dennstaedtioidae)	<i>Paesia scaberula</i> (Rich.) Kuhn	Unknown	From spores (UTC)	U05937	Wolf et al., 1994		1,2,3,4,5,6
Dennstaedtiaceae (Dennstaedtioidae)	<i>Peridium aquilinum</i> (L.) Kuhn	U.S.A., Idaho	Wolf 237 (UTC)	U05939	Wolf, 1995		
Dennstaedtiaceae (Dennstaedtioidae)	<i>Peridium aquilinum</i> (L.) Kuhn	Japan, Tochigi	Hasebe 27606 (TI)	U05646	Hasebe et al., 1994	*	1,2,3,4,5,6
Dennstaedtiaceae (Dennstaedtioidae)	<i>Peridium esculentum</i> (G. Forst.) Cockayne	Australia	UCBG 56.0657 (UTC)	U05940	Wolf, 1995		

APPENDIX. Continued.

Family (subfamily)	Species	Locality (country, state, or province)	Source/Voucher	Data- base acces- sion	Literature citation	Ks/ Ka	Figures in this study
Dennstaedtiaceae (Lindsaeoideae)	<i>Lindsaea ensifolia</i> Sw.	U.S.A., Hawaii	Palmer s.n. (UTC)	U05928	Wolf, 1995		
Dennstaedtiaceae (Lindsaeoideae)	<i>Lindsaea parasitica</i> (Roxb. ex Griffith) Hieron.	Brunei	Kew #1991-1934 (UTC)	U18640	Wolf, 1995		
Dennstaedtiaceae (Lindsaeoideae)	<i>Lindsaea odorata</i> Roxb.	Japan, Okinawa	Hasebe & Lin C106 (TI)	U05630	Hasebe et al., 1994	*	1,2,3,4,5,6
Dennstaedtiaceae (Lindsaeoideae)	<i>Odontosoria chinensis</i> (L.) J. Sm. (= <i>Sphenomeris chinensis</i> (L.) Maxon)	U.S.A., Hawaii	Ranker 1231 (COLO)	U05934	Wolf, 1995		1,2,3,4
Dennstaedtiaceae (Lindsaeoideae)	<i>Odontosoria chinensis</i> (L.) J. Sm. (= <i>Sphenomeris chinensis</i> (L.) Maxon)	Japan, Okinawa	Hasebe 245B (TI)	U05651	Hasebe et al., 1994	*	
Dennstaedtiaceae (Lindsaeoideae)	<i>Odontosoria scandens</i> (Desv.) C. Chr.	Puerto Rico	Axelrod 5353 (UPRRP)	U05935	Wolf, 1995		
Dennstaedtiaceae (Lindsaeoideae)	<i>Tapeinidium melanesicum</i> Kramer	Fiji	Smith 9382 (UC)	U18647	Wolf, 1995		
Dennstaedtiaceae (Lindsaeoideae)	<i>Tapeinidium luzonicum</i> (Hook.) Kramer	Taiwan	Huang & Kao 7525 (UC)	U18646	Wolf, 1995		
Dennstaedtiaceae (Saccolomatoideae)	<i>Orthiopteris kingii</i> (Bedd.) Holtum	Indonesia, Bogor	Hasebe 27604 (TI)	U18644	Wolf, 1995		1,2,3,4,5,6
Dennstaedtiaceae (Saccolomatoideae)	<i>Saccoloma moluccanum</i> (Bl.) Mett.	Philippines	Emer 9067 (VT)	U18649	Wolf, 1995		
Dennstaedtiaceae (Saccolomatoideae)	<i>Saccoloma elegans</i> Kaulf.	Ecuador	Barrington 1064 (VT)	U18645	Wolf, 1995		
Dicksoniaceae	<i>Calochlaena dubia</i> (R.Br.) M.D. Turner & R.A. White	Australia, Queensland	Kato et al. 201 (TI)	U05615	Hasebe et al., 1994	*	1,2,3,4,5,6

Family (subfamily)	Species	Locality (country, state, or province)	Source/Voucher	Data- base acces- sion	Literature citation	Ks/ Ka	Figures in this study
Dicksoniaceae	<i>Calochlaena villosa</i> (C. Chr.) M.D. Turner & R.A. White	Papua N.G.	Kew #1974-94 (UTC)	U05912	Wolf et al., 1994		
Dicksoniaceae	<i>Culcita coniifolia</i> (Hook.) Maxon	Costa Rica	Barrington s.n. (UTC)	U18648	Wolf, 1995		
Dicksoniaceae	<i>Cibotium barometz</i> (L.) J. Sm.	Japan, Kagoshima	TI1288 (TI)	U05610	Hasebe et al., 1994	*	1,2,3,4,5,6
Dicksoniaceae	<i>Cibotium glaucum</i> (J. Sm.) Hook. & Arn.	U.S.A., Hawaii	SA 82-159 (UTC)	U05913	Wolf et al., 1994		
Dicksoniaceae	<i>Dicksonia antarctica</i> Labill.	Australia, Queensland	Hasebe 27615 (TI)	U05618	Hasebe et al., 1994	*	1,2,3,4,5,6
Dicksoniaceae	<i>Dicksonia antarctica</i> Labill.	Australia	MBG 830474 (-2) (UTC)	U05919	Wolf et al., 1994		
Dipteridaceae	<i>Dipteris conjugata</i> Reinw.	Japan, Okinawa	Hasebe 27618 (TI)	U05620	Hasebe et al., 1994	*	1,2,3,4,5,6
Dryopteridaceae (Dryopterideae)	<i>Arachniodes aristata</i> (G. Forst.) Tindale	Japan, Mie	Yokoyama 5170 (TI)	U30608	This study (Yokoya- ma)	*	1,2,3,4
Dryopteridaceae (Dryopterideae)	<i>Cyrtomium hookerianum</i> (Pr.) C. Chr.	Japan, Oh-ita	Yokoyama 5169 (TI)		Hasebe et al., unpubl.		
Dryopteridaceae (Dryopterideae)	<i>Dryopteris cristata</i> (L.) A. Gray	U.S.A.	MBG 791348 (UTC)	U05923	Wolf et al., 1994	*	1,2,3,4,5,6
Dryopteridaceae (Dryopterideae)	<i>Dryopteris dickinsii</i> (Fr. & Sav.) C. Chr.	Japan, Kumamoto	Hasebe 26555 (TI)	U05622	Hasebe et al., 1994		
Dryopteridaceae (Dryopterideae)	<i>Polystichum munitum</i> (Kaulf.) C. Presl	U.S.A., Idaho	Soltis & Soltis s.n. (WS)	U05938	Wolf et al., 1994		
Dryopteridaceae (Dryopterideae)	<i>Polystichum tripterum</i> (Kun- ze) C. Presl	Japan, Mie	Yokoyama 5171 (TI)	U30832	This study (Yokoya- ma)		1,2,3,4,5,6
Dryopteridaceae (Onocleaceae)	<i>Matteuccia struthiopteris</i> (L.) Todaro	U.S.A., cultivated	Wolf 464 (UTC)	U05930	Wolf et al., 1994	*	1,2,3,4,5,6

APPENDIX. Continued.

Family (subfamily)	Species	Locality (country, state, or province)	Source/Voucher	Data- base acces- sion	Literature citation	Ks/ Ka	Figures in this study
Dryopteridaceae (Onocleaceae)	<i>Matteuccia struthiopteris</i> (L.) Todaro	Japan, Fukushima	<i>Sano 16</i> (CBM)	D43917	Sano et al., unpubl.		
Dryopteridaceae (Onocleaceae)	<i>Onoclea sensibilis</i> L.	Japan, Fukushima	<i>Hasebe 26565</i> (TI)	U05640	Hasebe et al., 1994	*	1,2,3,4
Dryopteridaceae (Onocleaceae)	<i>Onoclea sensibilis</i> L.	U.S.A.	MBG 791216 (UTC)	U05936	Wolf et al., 1994		
Dryopteridaceae (Onocleaceae)	<i>Woodsia obtusa</i> (Spreng.) Torr.	U.S.A., Missouri	<i>Raven 27640</i> (UTC)	U05949	Wolf et al., 1994		
Dryopteridaceae (Onocleaceae)	<i>Woodsia polystichoides</i> D.C. Eaton	Japan, Fukushima	<i>Hasebe 27602</i> (TI)	U05657	Hasebe et al., 1994	*	1,2,3,4,5,6
Dryopteridaceae (Physematieae)	<i>Athyrium filix-femina</i> (L.) Roth ex Mertens	unknown	MBG 871825 (UTC)	U05908	Wolf et al., 1994	*	1,2,3,4,5,6
Dryopteridaceae (Physematieae)	<i>Athyrium niponicum</i> (Mett.) Hance	Japan, Tokyo	<i>Sano 38</i> (CBM)	D43891	Sano et al., unpubl.		
Dryopteridaceae (Physematieae)	<i>Athyrium sheareri</i> (Bak.) Ching	Japan, Aichi	<i>Sano 41</i> (CBM)	D43892	Sano et al., unpubl.		
Dryopteridaceae (Physematieae)	<i>Athyrium vidalii</i> (Miq.) Koidz.	Japan, Chiba	<i>Sano 25</i> (CBM)	D43893	Sano et al., unpubl.		
Dryopteridaceae (Physematieae)	<i>Athyrium yokoscense</i> (Fr. & Sav.) H. Christ	Chiba, Japan	<i>Sano 22</i> (CBM)	D43894	Sano et al., unpubl.		
Dryopteridaceae (Physematieae)	<i>Cornopteris crenulatoserru- lata</i> (Makino) Nakai	Japan, Fukushima	<i>Sano 19</i> (CBM)	D43896	This study (Sano)	*	1,2,3,4
Dryopteridaceae (Physematieae)	<i>Cornopteris decurrenti-alata</i> (Hook.) Nakai	Japan, Nagasaki	<i>Sano 18</i> (CBM)	D43897	Sano et al., unpubl.		
Dryopteridaceae (Physematieae)	<i>Cystopteris fragilis</i> (L.) Bernh.	U.S.A., Utah	<i>Taye 2755</i> (UTC)	U05916	Wolf et al., 1994	*	1,2,3,4,5,6
Dryopteridaceae (Physematieae)	<i>Deparia bonincola</i> (Nakai) M. Kato	Japan, Tokyo	<i>Sano 12</i> (CBM)	D43899	This study (Sano)	*	1,2,3,4,5,6
Dryopteridaceae (Physematieae)	<i>Deparia conilii</i> (Fr. & Sav.) M. Kato	Japan, Kagoshima	<i>Sano 32</i> (CBM)	D43901	Sano et al., unpubl.		

Family (subfamily)	Species	Locality (country, state, or province)	Source/Voucher	Data- base acces- sion	Literature citation	Ks/ Ka	Figures in this study
Dryopteridaceae (Physematieae)	<i>Deparia fenziiana</i> (Luerss.) M. Kato	U.S.A., Hawaii	<i>Sano 14</i> (CBM)	D43900	Sano et al., unpubl.		
Dryopteridaceae (Physematieae)	<i>Deparia kiusiana</i> (Koidz.) M. Kato	Japan, Oh-ita	<i>Sano 13</i> (CBM)	D43902	Sano et al., unpubl.		
Dryopteridaceae (Physematieae)	<i>Deparia okuboana</i> (Makino) M. Kato	China, Yun-nan	<i>Sano 21</i> (CBM)	D43903	Sano et al., unpubl.		
Dryopteridaceae (Physematieae)	<i>Deparia otomasui</i> (Kurata) Serizawa	Japan, Kumamoto	<i>Sano 28</i> (CBM)	D43904	Sano et al., unpubl.		
Dryopteridaceae (Physematieae)	<i>Deparia petersenii</i> (Kunze) M. Kato	New Zealand, Auckland	<i>Sano 10</i> (CBM)	D43905	Sano et al., unpubl.		
Dryopteridaceae (Physematieae)	<i>Deparia prolifera</i> (Kaulf.) Hook. & Grev.	U.S.A., Hawaii	<i>Sano 15</i> (CBM)	D43906	Sano et al., unpubl.		
Dryopteridaceae (Physematieae)	<i>Deparia pycnosora</i> (H. Christ) M. Kato	Japan, Tochigi	<i>Sano 26</i> (CBM)	D43907	Sano et al., unpubl.		
Dryopteridaceae (Physematieae)	<i>Deparia viridifrons</i> (Maki- no) M. Kato	Japan, Chiba	<i>Sano 24</i> (CBM)	D43908	Sano et al., unpubl.		
Dryopteridaceae (Physematieae)	<i>Diplazium cavalerianum</i> (Christ.) M. Kato	Japan, Chiba	<i>Sano 11</i> (CBM)	D43909	Sano et al., unpubl.		
Dryopteridaceae (Physematieae)	<i>Diplazium donianum</i> (Mett.) Tard.	Japan, Kagoshima	<i>Sano 29</i> (CBM)	D43911	Sano et al., unpubl.		
Dryopteridaceae (Physematieae)	<i>Diplazium esculentum</i> (Retz.) Sw.	Japan, Kagoshima	<i>Hasebe 276001</i> (TI)	U05619	Hasebe et al., 1994	*	1,2,3,4,5,6
Dryopteridaceae (Physematieae)	<i>Diplazium lonchophyllum</i> Kunze	Unknown	MBG 903385 (UTC)	U05920	Wolf et al., 1994		
Dryopteridaceae (Physematieae)	<i>Diplazium mesosorum</i> (Ma- kino) Koidz.	Japan, Tochigi	<i>Sano 44</i> (CBM)	D43910	Sano et al., unpubl.		
Dryopteridaceae (Physematieae)	<i>Diplazium squamigerum</i> (Mett.) Matsum.	Japan, Saitama	<i>Sano 20</i> (CBM)	D43912	Sano et al., unpubl.		

APPENDIX. Continued.

Family (subfamily)	Species	Locality (country, state, or province)	Source/Voucher	Data- base acces- sion	Literature citation	Ks/ Ka	Figures in this study
Dryopteridaceae (Physematiaceae)	<i>Diplazium subsinuatum</i> (Wall. ex Hook. & Grev.) Tagawa	Japan, Kagoshima	<i>Sano 30</i> (CBM)	D43913	Sano et al., unpubl.		
Dryopteridaceae (Physematiaceae)	<i>Diplazium tomitaroanum</i> Masam.	Japan, Kagoshima	<i>Sano 43</i> (CBM)	D43914	Sano et al., unpubl.		
Dryopteridaceae (Physematiaceae)	<i>Diplazium wichurae</i> (Mett.) Diels	Japan, Shizuoka	<i>Sano 13</i> (CBM)	D43915	Sano et al., unpubl.		
Dryopteridaceae (Physematiaceae)	<i>Gymnocarpium dryopteris</i> (L.) Newman	U.S.A., Idaho	<i>Wolf 238</i> (UTC)	U05925	Wolf et al., 1994	*	1,2,3,4,5,6
Dryopteridaceae (Physematiaceae)	<i>Gymnocarpium oyamense</i> (Baker) Ching	Japan, Saitama	<i>Hasebe 483</i> (TI)	U05626	Hasebe et al., 1994		
Dryopteridaceae (Physematiaceae)	<i>Hypodematium crenatum</i> (Forssk.) Kuhn subsp. <i>fau- riei</i> (Kodama) K. Iwats.	Japan, Saitama	<i>Sano 17</i> (CBM)	D43916	This study (Sano)	*	1,2,3,4,5,6
Dryopteridaceae (Rumohreaceae)	<i>Rumohra adiantiformis</i> (G. Forst.) Ching	Australia, Victoria	<i>Hasebe & Nishida 5200</i> (TI)	U05648	Hasebe et al., 1994	*	1,2,3,4,5,6
Dryopteridaceae (Rumohreaceae)	<i>Rumohra adiantiformis</i> (G. Forst.) Ching	La Réunion	<i>Strasberg s.n.</i> (REU)	U05942	Wolf et al., 1994		
Dryopteridaceae (Tectariaceae)	<i>Ataxipteris sinii</i> (Ching) Holtum	Japan, Kagoshima	<i>Sano 40</i> (CBM)	D43898	Hasebe et al., unpubl.		
Dryopteridaceae (Tectariaceae)	<i>Ctenitis eatonii</i> (Baker) Ching	Taiwan, Nantoh	<i>Hasebe 27600</i> (TI)	U05614	Hasebe et al., 1994	*	1,2,3,4,5,6
Dryopteridaceae (Tectariaceae)	<i>Lastreopsis munita</i> (Mett.) Tindale	Australia, New South Wales	<i>Kato et al. 244</i> (TI)		Hasebe et al., unpubl.		
Dryopteridaceae (Tectariaceae)	<i>Tectaria devexa</i> (Kunze) Co- pel.	Japan, Okinawa	<i>Sano 50</i> (CBM)	D43918	This study (Yokoya- ma)	*	1,2,3,4,5,6
Dryopteridaceae (Tectariaceae)	<i>Tectaria gaudichaudii</i> (Mett.) Maxon	U.S.A., Hawaii	<i>Wolf 268 & Anderson- Wong</i> (UTC)	U05946	Wolf et al., 1994		

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Equisetaceae	<i>Equisetum arvense</i> L.	U.S.A., Michigan	<i>Manhart 05/29/87-1</i> (TAMU)	L11053	Manhart, 1994		
Gleicheniaceae (Gleichenioideae)	<i>Dicranopteris linearis</i> (Burm. f.) Underw.	U.S.A., Hawaii	<i>Wolf 255 & Anderson- Wong</i> (UTC)	U18626	Wolf, 1995		1,2,3,4,5,6
Gleicheniaceae (Gleichenioideae)	<i>Diplopterygium glaucum</i> (Houtt.) Nakai	China, Yun-nan	<i>Murakami 27621</i> (TI)	U05624	Hasebe et al., 1994	*	1,2,3,4,5,6
Gleicheniaceae (Stromatopteridoideae)	<i>Stromatopteris moniliformis</i> Mett.	New Caledonia	<i>Hasebe 27621</i> (TI)	U05653	Hasebe et al., 1994	*	1,2,3,4,5,6
Grammitidaceae	<i>Micropolypodium okuboi</i> (Yatabe) Hayata	Japan, Kagoshima	<i>Hasebe 26575</i> (TI)	U05658	Hasebe et al., 1994	*	1,2,3,4,5,6
Hymenophyllaceae (Cardiomanioideae)	<i>Cardiomanes reniforme</i> (G. Forst.) C. Presl	New Zealand, Waitomo	<i>Yokoyama 5174</i> (TI)	U30833	This study (Yokoyama)	*	1,2,3,4,5,6
Hymenophyllaceae (Hymenophylloideae)	<i>Cephalomanes thysanostomum</i> (Makino) K. Iwats.	Japan, Okinawa	<i>Hasebe 26549</i> (TI)	U05608	Hasebe et al., 1994	*	1,2,3,4,5,6
Hymenophyllaceae (Hymenophylloideae)	<i>Crepidomanes birmanicum</i> (Bedd.) K. Iwats.	Japan, Tokyo	<i>Hasebe 27622</i> (TI)	U05613	Hasebe et al., 1994	*	1,2,3,4,5,6
Hymenophyllaceae (Hymenophylloideae)	<i>Gonocormus minutus</i> van den Bosch (= <i>Crepidomanes minutus</i> (Blume) K. Iwats.)	Japan, Tokyo	<i>Hasebe 27623</i> (TI)	U05625	Hasebe et al., 1994	*	1,2,3,4
Hymenophyllaceae (Hymenophylloideae)	<i>Hymenophyllum fucoides</i> (Sw.) Sw.	Costa Rica	<i>Crane 930613-10</i> (ISC)	U20933	Crane et al., 1995		1,2,3,4
Hymenophyllaceae (Hymenophylloideae)	<i>Vandenboschia davallioides</i> (Gaud.) Copel.	U.S.A., Hawaii	<i>Wolf 248 & Anderson- Wong</i> (UTC)	U05948	Wolf, 1995		1,2,3,4

APPENDIX. Continued.

Family (subfamily)	Species	Locality (country, state, or province)	Source/Voucher	Data- base access- sion	Literature citation	Ks/ Ka	Figures in this study
Isoetaceae	<i>Isoetes melanopoda</i> Gay & Durieu	U.S.A., Texas	Manhart 03/10/88-1 (TAMU)	L11054	Manhart, 1994		
Lomariopsidaceae	<i>Elaphoglossum hybridum</i> (Bory) Moore	La Réunion	T. Ranker 1375 (COLO)	U05924	Wolf et al., 1994	*	1,2,3,4,5,6
Lomariopsidaceae	<i>Elaphoglossum yoshinagae</i> (Yatabe) Makino	Japan, Kagoshima	Hasebe 26559 (TI)	U05623	Hasebe et al., 1994		
Loxomataceae	<i>Loxoma cunninghamii</i> Cunn.	New Zealand, New Plymouth	Hasebe 939 (TI)	U30834	This study (Yokoyama)	*	1,2,3,4,5,6
Lycopodiaceae	<i>Lycopodium digitatum</i> Dill. ex A. Braun	U.S.A., Michigan	Manhart 06/12/88 (TAMU)	L11055	Manhart, 1994		
Marattiaceae	<i>Angiopteris evecta</i> (G. Forst.) Hoffm.	U.S.A., Hawaii	Nagata 12/20/88 (TAMU)	L11052	Manhart, 1994		1,2,3,4,5,6
Marattiaceae	<i>Angiopteris lygodifolia</i> Ros.	Japan, Shizuoka	no information	X58429	Yoshinaga et al., 1992		
Marsileaceae	<i>Marsilea macropoda</i> Engelm. ex A. Braun	U.S.A., Alabama	Pryer 959 (DUKE)	U24263	This study (Pryer)	*	1,2,3,4,5,6
Marsileaceae	<i>Marsilea quadrifolia</i> L.	Japan, Yamaguchi	Hasebe 26546 (TI)	U05633	Hasebe et al., 1994		
Marsileaceae	<i>Marsilea quadrifolia</i> L.	U.S.A., cultivated	Manhart 07/31/91-2 (TAMU)	L13480	Manhart, 1994		
Marsileaceae	<i>Pilularia americana</i> A. Br.	U.S.A., Georgia	Pryer 978 (DUKE)	U24263	This study (Pryer)	*	1,2,3,4
Marsileaceae	<i>Regnellidium diphyllum</i> Lindm.	U.S.A., cultivated	Pryer 977 (DUKE)	U24262	This study (Pryer)	*	1,2,3,4
Matoniaceae	<i>Matonia pectinata</i> R. Br.	Malaysia, Selangor	Hasebe 27620 (TI)	U05634	Hasebe et al., 1994	*	1,2,3,4,5,6
Metaxyaceae	<i>Metaxya rostrata</i> (Kunth) C. Presl	Costa Rica	Conant s.n. (LSC)	U05635	Hasebe et al., 1994	*	1,2,3,4,5,6
Monachosoraceae	<i>Monachosorum arakii</i> Tagawa	Japan, Mie	Hasebe 590 (TI)	U05636	Hasebe et al., 1994	*	1,2,3,4,5,6
Monachosoraceae	<i>Monachosorum henryi</i> H. Christ	Taiwan	Moran 5461 (HAST, MO, F)	U05932	Wolf et al., 1994		

Family (subfamily)	Species	Locality (country, state, or province)	Source/Voucher	Data- base access- ion	Literature citation	Ks/ Ka	Figures in this study
Nephrolepidaceae	<i>Nephrolepis cordifolia</i> (L.) C. Presl	Japan (TI), cultivated	<i>Hasebe 26556</i> (TI)	U05637	Hasebe et al., 1994	*	1,2,3,4,5,6
Nephrolepidaceae	<i>Nephrolepis cordifolia</i> (L.) C. Presl	cultivated	MBG 834344 (UTC)	U05933	Wolf et al., 1994		
Nephrolepidaceae	<i>Nephrolepis hirsutula</i> (G. Forst.) C. Presl	Japan, Tokyo	<i>Yokoyama 2000</i> (TI)	U05638	Hasebe et al., 1994		
Oleandraceae	<i>Arthropteris beckeri</i> (Hook.) Mett.	Australia, Queensland	<i>Kato et al. 205</i> (TI)	U05605	Hasebe et al., 1994	*	1,2,3,4,5,6
Oleandraceae	<i>Oleandra pistillaris</i> (Sw.) C. Chr.	Malay Peninsula	<i>Hasebe 27603</i> (TI)	U05639	Hasebe et al., 1994	*	1,2,3,4,5,6
Ophioglossaceae	<i>Botrychium bitematum</i> (Sav.) Underw.	U.S.A., Tennessee	<i>Wilson 5979</i> (TAMU)	L13474	Manhart, 1994	*	1,2,3,4
Ophioglossaceae	<i>Botrychium strictum</i> Un- derw. (= <i>Botrypus strictus</i> (Underw.) Holub)	Japan, Kanagawa	<i>Hasebe 27626</i> (TI)	B14881	Hasebe et al., 1993	*	1,2,3,4,5,6
Ophioglossaceae	<i>Helminthostachys zeylanica</i> (L.) Hook.	Japan	<i>Sahashi s.n.</i> (UNC)	L40907	This study (Hauk)		1,2,3,4
Ophioglossaceae	<i>Ophioglossum engelmannii</i> Prantl	U.S.A., Texas	<i>Manhart 04/20/89-1</i> (TAMU)	L11058	Manhart, 1994	*	1,2,3,4
Ophioglossaceae	<i>Botrychium multifidum</i> (S. G. Gmelin) Rupr. (= <i>Sceptridium multifidum</i> (Gmel.) Nishida ex Taga- wa)	U.S.A., Michigan	<i>Hauk 579</i> (UNC)	L40906	This study (Hauk)		1,2,3,4
Osmundaceae	<i>Osmunda cinnamomea</i> L. var. <i>fokiensis</i> Copel.	Japan, Tochigi	<i>Hasebe 27624</i> (TI)	D14882	Hasebe et al., 1993	*	1,2,3,4,5,6
Osmundaceae	<i>Plenasium bromelifolium</i> C. Presl (= <i>Osmunda banksi- folia</i> (C. Presl) Kuhn)	Indonesia, East Kali- mantan	<i>Kato et al. 23273</i> (TI)	U05645	Hasebe et al., 1994	*	1,2,3,4

APPENDIX. Continued.

Family (subfamily)	Species	Locality (country, state, or province)	Source/Voucher	Data- base acces- sion	Literature citation	Ks/ Ka	Figures in this study
Plagiogyriaceae	<i>Plagiogyria japonica</i> Nakai	Japan, Mie	<i>Hasebe 27614</i> (TI)	U05643	Hasebe et al., 1994	*	1,2,3,4,5,6
Polypodiaceae (Drynariaceae)	<i>Aglaomorpha speciosa</i> (Blume) Roos	cultivated (TI) 90-690	<i>Hasebe 26660</i> (TI)	U05642	Hasebe et al., 1994	*	1,2,3,4,5,6
Polypodiaceae (Lepidoreae)	<i>Lepisorus thunbergianus</i> (Kaulf.) Ching	Japan, Tokyo	<i>Hasebe 512</i> (TI)	U05629	Hasebe et al., 1994	*	1,2,3,4,5,6
Polypodiaceae (Loxogrammeae)	<i>Loxogramme graminoides</i> (Baker) C. Chr.	Japan, Fukuoka	<i>Hasebe 26661</i> (TI)	U05631	Hasebe et al., 1994	*	1,2,3,4,5,6
Polypodiaceae (Microsoreae)	<i>Colysis shintenensis</i> (Hayata) H. Ito	Japan, Kagoshima	<i>Hasebe 26551</i> (TI)	U05612	Hasebe et al., 1994	*	1,2,3,4
Polypodiaceae (Polypodiaceae)	<i>Pleopeltis macrocarpa</i> (Bory de V. ex Willd.) Kaulf.	Costa Rica, Alajuela	<i>Andrews 456</i> (KANU)	U21152	Haufler and Ranker, 1995	*	1,2,3,4
Polypodiaceae (Polypodiaceae)	<i>Pleopeltis thyssanolepis</i> (A. Braun ex Klotzsch) Andrews and Windham	Mexico, Oaxaca	<i>Andrews 1000</i> (KANU)	U21153	Haufler and Ranker, 1995		
Polypodiaceae (Polypodiaceae)	<i>Polypodium amorphum</i> Sukdorsf	U.S.A., Washington	<i>Haufler s.n.</i> (KANU)	U21142	Haufler and Ranker, 1995		
Polypodiaceae (Polypodiaceae)	<i>Polypodium appalachianum</i> Haufler and Windham	U.S.A., New Hampshire	<i>Haufler s.n.</i> (KANU)	U21141	Haufler and Ranker, 1995		
Polypodiaceae (Polypodiaceae)	<i>Polypodium australe</i> Fée	England, Cornwall	<i>Haufler s.n.</i> (KANU)	U21140	Haufler and Ranker, 1995	*	1,2,3,4,5,6
Polypodiaceae (Polypodiaceae)	<i>Polypodium fauriei</i> H. Christ	Japan, Tochigi	<i>Yahara s.n.</i> (TI)	U21148	Haufler and Ranker, 1995		
Polypodiaceae (Polypodiaceae)	<i>Polypodium glaberulum</i> Mickel and Beitel	Mexico, Oaxaca	<i>Barrington 922</i> (KANU)	U21143	Haufler and Ranker, 1995		
Polypodiaceae (Polypodiaceae)	<i>Polypodium glycyrrhiza</i> D.C. Eaton	U.S.A., California	<i>Haufler s.n.</i> (KANU)	U21146	Haufler and Ranker, 1995		
Polypodiaceae (Polypodiaceae)	<i>Polypodium macaronesicum</i> A. Bobrov	Canary Islands, Tenerife	<i>Hennipman FS 082</i> (Hennipman's personal herbarium)	U21151	Haufler and Ranker, 1995		

Family (subfamily)	Species	Locality (country, state, or province)	Source/Voucher	Data- base acces- sion	Literature citation	Ks/ Ka	Figures in this study
Polypodiaceae (Polypodieae)	<i>Polypodium pellucidum</i> Kaulfuss	U.S.A., Hawaii	Haufler s.n. (KANU)	U21149	Haufler and Ranker, 1995		
Polypodiaceae (Polypodieae)	<i>Polypodium plesiosorum</i> Kunze	Mexico, Chiapas	Barrington 925 (KANU)	U21144	Haufler and Ranker, 1995		
Polypodiaceae (Polypodieae)	<i>Polypodium rhodopleuron</i> Kunze	Mexico, Vera Cruz	Barrington 926 (KANU)	U21145	Haufler and Ranker, 1995		
Polypodiaceae (Polypodieae)	<i>Polypodium scolieri</i> Hook. & Grev.	U.S.A., California	Haufler s.n. (KANU)	U21150	Haufler and Ranker, 1995		
Psilotaceae	<i>Psilotum nudum</i> L.	Japan (TI), cultivated	Hasebe 27627 (TI)	U30835	This study (Hasebe)		1,2,3,4,5,6
Psilotaceae	<i>Psilotum nudum</i> L.	U.S.A., cultivated	Manhart 04/06/88-1 (TAMU)	L11059	Manhart, 1994		
Psilotaceae	<i>Tmesipteris oblancoolata</i> Copel.	Indonesia, Anbon	Hasebe 27628 (TI)	U30836	This study (Hasebe)		1,2,3,4
Pteridaceae (Adiantoidae)	<i>Adiantum capillus-veneris</i> L.	Japan (TI), cultivated	Hasebe 26558 (TI)	D14880	Hasebe et al., 1993		
Pteridaceae (Adiantoidae)	<i>Adiantum pedatum</i> L.	Japan, Fukushima	Hasebe 26547 (TI)	U05602	Hasebe et al., 1994	*	1,2,3,4,5,6
Pteridaceae (Adiantoidae)	<i>Adiantum raddianum</i> C. Presl	U.S.A., Hawaii	Wolf 244 & Anderson- Wong (UTC)	U05906	Wolf et al., 1994		
Pteridaceae (Ceratopteridoi- deae)	<i>Ceratopteris thalictroides</i> (L.) Brongn.	Japan (TI), cultivated	Hasebe 27607 (TI)	U05609	Hasebe et al., 1994	*	1,2,3,4,5,6
Pteridaceae (Cheilantheoideae)	<i>Argyrochosma delicatula</i> (Maxon & Weath.) Wind- ham (= <i>Notholaena deli- catula</i> Maxon & Weath.)	Mexico, Nuevo León	Yatskievych & Gastony 89-229 (IND)	U19500	Gastony and Rollo, 1995		1,2,3,4
Pteridaceae (Cheilantheoideae)	<i>Argyrochosma fendleri</i> (Kunze) Windham (= <i>Notholaena fendleri</i> Kunze)	U.S.A., New Mexico	Sullivan et al. s.n. (IND)	U27727	Gastony and Rollo, 1995		

APPENDIX. Continued.

Family (subfamily)	Species	Locality (country, state, or province)	Source/Voucher	Data- base acces- sion	Literature citation	Ks/ Ka	Figures in this study
Pteridaceae (Cheilantheoideae)	<i>Bommeria ehrenbergiana</i> (Klotzsch) Underw.	Mexico, Hidalgo	<i>Yatskievych & Gastony</i> 89-203 (IND)	U19497	Gastony and Rollo, 1995		1,2,3,4,5,6
Pteridaceae (Cheilantheoideae)	<i>Cheilanthes albofusca</i> Baker	China, Yunnan	<i>Li & Xiang S-4L</i> (IND)	U19498	Gastony and Rollo, 1995		
Pteridaceae (Cheilantheoideae)	<i>Cheilanthes allosuroides</i> Mett.	Mexico, Jalisco	<i>Yatskievych & Gastony</i> 89-237 (IND)	U27239	Gastony and Rollo, 1995		1,2,3,4
Pteridaceae (Cheilantheoideae)	<i>Cheilanthes aurea</i> Baker	Mexico, Oaxaca	<i>Yatskievych & Gastony</i> 89-256 (IND)	U28786	Gastony and Rollo, 1995		
Pteridaceae (Cheilantheoideae)	<i>Cheilanthes bonariensis</i> (Willd.) Proctor	Mexico, Michoacan	<i>Yatskievych & Gastony</i> 89-246 (IND)	U19499	Gastony and Rollo, 1995		
Pteridaceae (Cheilantheoideae)	<i>Cheilanthes decora</i> (Brack.) R. Tryon & A. Tryon	U.S.A., Hawaii	<i>Flynn s.n.</i> (IND)	U27446	Gastony and Rollo, 1995		
Pteridaceae (Cheilantheoideae)	<i>Cheilanthes duclouxii</i> (H. Christ) Ching	China, Yunnan	<i>Li & Xiang S-18L</i> (IND)	U27447	Gastony and Rollo, 1995		
Pteridaceae (Cheilantheoideae)	<i>Cheilanthes horridula</i> Max- on	Mexico, Nuevo León	<i>Gastony 90-10-1</i> (IND)	U27448	Gastony and Rollo, 1995		
Pteridaceae (Cheilantheoideae)	<i>Cheilanthes intramarginalis</i> (Kaulf. ex Link) Hook. (= <i>Mildella intramarginalis</i> (Kaulf. ex Link) Trev.)	Mexico, Hidalgo	<i>Yatskievych & Gastony</i> 89-207 (IND)	U27449	Gastony and Rollo, 1995		
Pteridaceae (Cheilantheoideae)	<i>Cheilanthes lanosa</i> (Michx.) D. C. Eaton	U.S.A., Indiana	<i>Hegeman s.n.</i> (IND)	U27205	Gastony and Rollo, 1995		
Pteridaceae (Cheilantheoideae)	<i>Cheilanthes rigida</i> (Sw.) Mett. (= <i>Cheiloplecton rigidum</i> (Sw.) Fée)	Mexico, Puebla	<i>Yatskievych & Gastony</i> 89-284 (IND)	U29133	Gastony and Rollo, 1995		
Pteridaceae (Cheilantheoideae)	<i>Coniogramme japonica</i> (Thunb.) Diels	Japan, Saga	<i>Hasebe 26662</i> (TI)	U05611	Hasebe et al., 1994	*	1,2,3,4,5,6

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Pteridaceae (Cheilantheoideae)	<i>Doryopteris concolor</i> (Langsd. & Fisch.) Kuhn (= <i>Cheilanthes concolor</i> (Langsd. & Fisch.) R. Tryon & A. Tryon	Taiwan, Nantoh	Hasebe 27609 (TI)	U05621	Hasebe et al., 1994	*	1,2,3,4,5,6
Pteridaceae (Cheilantheoideae)	<i>Doryopteris pedata</i> (L.) Fée var. <i>palmata</i> (Willd.) Hicken	Mexico, Hidalgo	Riba 1746 (IND)	U27206	Gastony and Rollo, 1995		
Pteridaceae (Cheilantheoideae)	<i>Hemionitis elegans</i> Davenp.	Mexico, Oaxaca	Yatskievych & Gastony 89-258 (IND)	U27729	Gastony and Rollo, 1995		
Pteridaceae (Cheilantheoideae)	<i>Hemionitis levyi</i> Fourn.	Mexico, Oaxaca	Yatskievych & Gastony 89-253 (IND)	U27725	Gastony and Rollo, 1995		1,2,3,4
Pteridaceae (Cheilantheoideae)	<i>Llavea cordifolia</i> Lag.	Mexico, Nuevo León	Yatskievych & Gastony 89-224A (IND)	U27726	Gastony and Rollo, 1995		
Pteridaceae (Cheilantheoideae)	<i>Notholaena rosei</i> Maxon	Mexico, Oaxaca	Yatskievych et al. 83- 453 (IND)	U27728	Gastony and Rollo, 1995	*	1,2,3,4
Pteridaceae (Cheilantheoideae)	<i>Notholaena sulphurea</i> (Cav.) J. Sm.	Mexico, Puebla	Yatskievych & Gastony 89-248 (IND)	U28254	Gastony and Rollo, 1995		
Pteridaceae (Cheilantheoideae)	<i>Pellaea andromedifolia</i> (Kaulf.) Fée	U.S.A., California	Gastony 86-8 (IND)	U19501	Gastony and Rollo, 1995	*	1,2,3,4,5,6
Pteridaceae (Cheilantheoideae)	<i>Pellaea boivinii</i> Hook.	Madagascar, Fianarantsoa	Liede & Conrad 2626 (IND)	U29132	Gastony and Rollo, 1995		
Pteridaceae (Cheilantheoideae)	<i>Pellaea cordifolia</i> (Sessé & Moc.) A.R. Sm.	U.S.A., Texas	Gastony 87-3 (IND)	U28253	Gastony and Rollo, 1995		
Pteridaceae (Cheilantheoideae)	<i>Pellaea pringlei</i> Davenp.	Mexico, Morelos	Gastony 87-11-16 (IND)	U28787	Gastony and Rollo, 1995		
Pteridaceae (Cheilantheoideae)	<i>Pellaea rotundifolia</i> (G. Forst.) Hook.	cultivated, Indiana Univ.	Gastony s.n. (IND)	U28788	Gastony and Rollo, 1995		

APPENDIX. Continued.

Family (subfamily)	Species	Locality (country, state, or province)	Source/Voucher	Data- base acces- sion	Literature citation	Ks/ Ka	Figures in this study
Pteridaceae (Cheilanthesaceae)	<i>Trachipteris pinnata</i> (Hook. f.) C. Chr.	Bolivia, Santa Cruz	Windisch 6088 (SJRP)	U27450	Gastony and Rollo, 1995		
Pteridaceae (Platyzomatoideae)	<i>Platyzoma microphyllum</i> R. Br.	Australia, Queensland	Kato et al. 303 (TI)	U05644	Hasebe et al., 1994	*	1,2,3,4,5,6
Pteridaceae (Pteridoideae)	<i>Acrostichum aureum</i> L.	Indonesia, Java	Hasebe 27613 (TI)	U05601	Hasebe et al., 1994	*	1,2,3,4
Pteridaceae (Pteridoideae)	<i>Pleurosoriopsis makinoi</i> (Maxim.) Fomin	Japan, Sugadaira	Yokoyama 5175 (TI)	U30837	This study (Yokoya- ma)		1,2,3,4,5,6
Pteridaceae (Pteridoideae)	<i>Pteris fauriei</i> Hieron	Japan, Tokyo	Hasebe 26553 (TI)	U05647	Hasebe et al., 1994	*	1,2,3,4,5,6
Pteridaceae (Pteridoideae)	<i>Pteris vittata</i> L.	U.S.A., Hawaii	Wolf 257 & Anderson- Wong (UTC)	U05941	Wolf et al., 1994		
Pteridaceae (Taenitidoideae)	<i>Onychium japonicum</i> (Thunb.) Kunze	Japan, Tokyo	Hasebe 422 (TI)	U05641	Hasebe et al., 1994	*	1,2,3,4,5,6
Pteridaceae (Taenitidoideae)	<i>Taenitis blechnoides</i> (Willd.) Sw.	Singapore, Singapore	Hasebe 27608 (TI)	U05654	Hasebe et al., 1994	*	1,2,3,4,5,6
Salvinaceae	<i>Salvinia cucullata</i> Roxb. ex Bory	cultivated (TI)	Hasebe 26550 (TI)	U05649	Hasebe et al., 1994	*	1,2,3,4,5,6
Schizaeaceae	<i>Actinostachys digitata</i> (L.) Wall.	Japan, Tokyo	Yokoyama 27617 (TI)	U05650	Hasebe et al., 1994	*	1,2,3,4
Schizaeaceae	<i>Anemia mexicana</i> Klotzsch	Australia, cultivated	Hasebe 26543 (TI)	U05603	Hasebe et al., 1994	*	1,2,3,4,5,6
Schizaeaceae	<i>Lygodium japonicum</i> (Thunb.) Sw.	Japan, Kanagawa	Hasebe 27616 (TI)	U05632	Hasebe et al., 1994	*	1,2,3,4,5,6
Schizaeaceae	<i>Lygodium japonicum</i> (Thunb.) Sw.	U.S.A., cultivated	Manhart 07/31/91-1 (TAMU)	L13479	Manhart, 1994		
Selaginellaceae	<i>Selaginella apoda</i> (L.) Spring	U.S.A., cultivated	Manhart 04/06/88-2 (TAMU)	L11280	Manhart, 1994		
Thelypteridaceae	<i>Cyclosorus opulentus</i> (Kaulf.) Nakaike	Ecuador	MBG 920036 (UTC)	U05915	Wolf et al., 1994	*	1,2,3,4,5,6

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Thelypteridaceae	<i>Thelypteris acuminata</i> (Houtt.) Morton	Japan, Chiba	<i>Sano 39</i> (CBM)	D43919	Sano et al., unpubl.	*	
Thelypteridaceae	<i>Thelypteris beddomei</i> (Ba- ker) Ching	Japan, Saga	<i>Hasebe 26564</i> (TI)	U05655	Hasebe et al., 1994	*	1,2,3,4
Thelypteridaceae	<i>Thelypteris palustris</i> (Sal- isb.) Schott var. <i>pubescens</i> (Laws.) Pernald	U.S.A.	MBG 810566 (UTC)	U05947	Wolf et al., 1994		
Vittariaceae	<i>Ananthacorus angustifolius</i> Underw. & Maxon	Costa Rica	<i>Farrar CR289</i> (ISC)	U20932	Crane et al., 1995		1,2,3,4
Vittariaceae	<i>Anetium citrifolium</i> Splitg.	Costa Rica	<i>Crane 930626-26</i> (ISC)	U21284	Crane et al., 1995		
Vittariaceae	<i>Antrophyum boryanum</i> (Willd.) Spreng.	La Réunion	<i>Ranker 1538</i> (ISC)	U20930	Crane et al., 1995		
Vittariaceae	<i>Antrophyum ensiforme</i> Hook.	Mexico	<i>Farrar 1994 (Mexico)</i> (ISC)	U20931	Crane et al., 1995		
Vittariaceae	<i>Antrophyum plantagineum</i> (Cav.) Kaulf.	Fiji	<i>Drake s.n.</i> (ISC)	U21285	Crane et al., 1995		
Vittariaceae	<i>Antrophyum reticulatum</i> (G. Forst.) Kaulf.	Australia, Queensland	<i>Kato et al. 431</i> (TI)	U05604	Hasebe et al., 1994	*	1,2,3,4,5,6
Vittariaceae	<i>Hecistopteris pumila</i> (Spreng.) J. Sm.	Costa Rica	<i>Crane 930626-25</i> (ISC)	U21286	Crane et al., 1995		
Vittariaceae	<i>Polytaenium cajanense</i> (Desv.) Benedict	Costa Rica	<i>Crane 930617-5</i> (ISC)	U20934	Crane et al., 1995		
Vittariaceae	<i>Polytaenium lanceolatum</i> (L.) Benedict	Costa Rica	<i>Crane 930626-37</i> (ISC)	U21287	Crane et al., 1995		
Vittariaceae	<i>Polytaenium lineatum</i> (Sw.) J. Sm.	Costa Rica	<i>Clark s.n.</i> Not vouch- ered	U20935	Crane et al., 1995		1,2,3,4
Vittariaceae	<i>Vittaria anguste-elongata</i> Hayata	Taiwan	<i>Chiou 920722</i> (ISC)	U21291	Crane et al., 1995		
Vittariaceae	<i>Vittaria dimorpha</i> K. Müll.	Costa Rica	<i>McAlpin, NYBG</i> (ISC)	U21292	Crane et al., 1995		

APPENDIX. Continued.

Family (subfamily)	Species	Locality (country, state, or province)	Source/Voucher	Data- base acces- sion	Literature citation	Ks/ Ka	Figures in this study
Vittariaceae	<i>Vittaria ensiformis</i> Sw.	La Réunion	<i>Ranker 1542</i> (ISC)	U21290	Crane et al., 1995		
Vittariaceae	<i>Vittaria flexuosa</i> Fée	Japan, Kagoshima	<i>Hasebe 27613</i> (TI)	U05656	Hasebe et al., 1994	*	1,2,3,4,5,6
Vittariaceae	<i>Vittaria gardneriana</i> Fée	Ecuador	<i>Clark 1130</i> (ISC)	U21294	Crane et al., 1995		
Vittariaceae	<i>Vittaria graminifolia</i> Kaulf.	Costa Rica	<i>Farrar CR312</i> (ISC)	U21295	Crane et al., 1995		
Vittariaceae	<i>Vittaria isoetifolia</i> Bory	La Réunion	<i>Ranker 1533</i> (ISC)	U20936	Crane et al., 1995		
Vittariaceae	<i>Vittaria lineata</i> (L.) J.E. Sm.	U.S.A.	<i>Lassiter 84-09-15B</i> (ISC)	U20937	Crane et al., 1995		
Vittariaceae	<i>Vittaria minima</i> (Baker) Benedict	Costa Rica	<i>Moran 3180</i> (ISC)	U21288	Crane et al., 1995		
Vittariaceae	<i>Vittaria remota</i> Fée	Costa Rica	<i>Moran 3180</i> (ISC)	U21289	Crane et al., 1995		
Vittariaceae	<i>Vittaria stipitata</i> Fée	Costa Rica	<i>Crane 930611-10</i> (ISC)	U21293	Crane et al., 1995		
Vittariaceae	<i>Vittaria zosterifolia</i> Willd.	Taiwan	<i>Chiou 920723</i> (ISC)	U21296	Crane et al., 1995		



Hasebe, Mitsuyasu et al. 1995. "Fern Phylogeny Based on rbcL Nucleotide Sequences." *American fern journal* 85, 134–181.

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