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Phylogenetic Congruence and Discordance Among One Morphological and Three Molecular Data Sets from Pontederiaceae

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Abstract.—A morphological data set and three sources of data from the chloroplast genome (two genes and a restriction site survey) were used to reconstruct the phylogenetic history of the pickerelweed family Pontederiaceae. The chloroplast data converged towards a single tree, presumably the true chloroplast phylogeny of the family. Unrooted trees estimated from each of the three chloroplast data sets were identical or extremely similar in shape to each other and mostly robustly supported. There was no evidence of significant heterogeneity among the data sets, and the few topological differences seen among unrooted trees from each chloroplast data set are probably artifacts of sampling error on short branches. Despite well-documented differences in rates of evolution for different characters in individual data sets, equally weighted parsimony permits accurate reconstructions of chloroplast relationships in Pontederiaceae. A separate morphology-based data set yielded trees that were very different from the chloroplast trees. Although there was substantial support from the morphological evidence for several major clades supported by chloroplast trees, most of the conflicting phylogenetic structure on the morphology trees was not robust. Nonetheless, several statistical tests of incongruence indicate significant heterogeneity between molecules and morphology. The source of this apparent incongruence appears to be a low ratio of phylogenetic signal to noise in the morphological data. [Chloroplast DNA; congruence tests; incongruence; morphology; *ndh*F; noise; Pontederiaceae; *rbc*L.]

Good correspondence among trees derived from independent sources of data constitutes strong reciprocal corroboration of accurate phylogenetic inference (reviewed by de Queiroz et al., 1995; Hillis, 1995; Miyamoto and Fitch, 1995). This is the principle of congruence in phylogenetic systematics. Conversely, discordance can serve to highlight instances where subsets of the available evidence have distinctly different rates or modes of evolution (Bull et al., 1993; Huelsenbeck et al., 1994, 1996), or even different phylogenetic histories (reviewed by Doyle, 1992). However, if trees from different data sets are sampling estimates of the true phylogeny, then differences among them may instead reflect sampling error (Rodrigo et al., 1993; Page, 1996). Avoidance of sampling error is a prime motive for using a large amount of data from diverse sources during phylogenetic reconstruction. With the growing trend towards collecting and combining multiple sources of data in individual phylogenetic studies, there is an increasingly urgent need to distinguish between tree conflicts attributable to sampling error or other sources of noise, and those attributable to divergent historical signal.

Four phylogenetic data sets are now available for the pickerelweed family Pontederiaceae. A study of chloroplast DNA restriction site variation has recently been published (Kohn et al., 1996), and surveys of nucleotide variation in portions of two chloroplast genes, *ndh*F and *rbc*L, are detailed here for the first time. The *ndh*F survey was performed with primers designed specifically for this study. Evidence from *rbc*L provides strong support for the monophyly of the family (Graham and Barrett, 1995). The fourth phylogenetic study of Pontederiaceae, performed by Eckenwalder and Barrett (1986), was based solely on morphological evidence. The most-parsimonious trees inferred from those data are very different from those inferred from the chloroplast data (Graham and Barrett, 1995; Kohn et al., 1996).

The morphology-based trees of the family are poorly supported. Trees only a few

steps longer than the most-parsimonious trees are strikingly different in shape from the shortest trees (Eckenwalder and Barrett, 1986). The morphological data set is by far the smallest in size of all the available data sets, raising the possibility that differences in trees inferred from molecules versus morphology reflect sampling error in the latter data set rather than conflicting signals. Chloroplast genes are linked with each other, and so all parts of individual chloroplast genomes have the same pedigree within species and the same phylogenetic history among species. Different genealogical histories can therefore be ruled out as a cause of any incongruence among the chloroplast data sets. However, rates and modes of evolution are known to vary substantially in different parts of the chloroplast genome. If these processes are sufficiently strong, they may be a source of the tangible differences in trees that are inferred from separate parts of the chloroplast genome. If differences among trees from individual chloroplast data sets are not well supported, then they too may reflect sampling error alone.

Until recently, assessment of the degree of congruence among data sets rested almost solely on qualitative comparisons of tree topology ("taxonomic congruence"; Mickevich, 1978). Such visual comparisons of tree shape can still be rewarding, particularly where estimates of the robustness of tree structure are available. Several statistical tests have been used to gauge incongruence among data sets (Templeton, 1983; Kishino and Hasegawa, 1989; Rodrigo et al., 1993; Farris et al., 1994). The relative superiority of individual tests of incongruence was addressed by Lutzoni and Vilgalys (1995) and Cunningham (1997). They found that related tests of incongruence derived from the work of Templeton (1983) and Kishino and Hasegawa (1989) are oversensitive because they can be easily misled by tree structure that reflects sampling error instead of phylogenetic signal. However, Mason-Gamer and Kellogg (1996) suggested how modified versions of these tests can be used to avoid comparing poorly supported tree structures from different data sets, and hence permit discrimination between the effects of noise and robustly conflicting signal on differences in tree shape.

In this study, we examined the extent and source of incongruence between three chloroplast data sets and between the chloroplast genome and morphology. The taxonomic, biogeographic, and evolutionary implications of trees inferred from these different data sets have been addressed elsewhere (Eckenwalder and Barrett, 1986; Graham and Barrett, 1995; Kohn et al., 1996; Barrett and Graham, 1997). By examining the robustness and congruence of trees inferred from each data set, we determined whether the differences among them were a consequence of noise in the data, such as that due to sampling error, or instead reflected divergent evolutionary processes or histories among the data.

Materials and Methods

Twenty-four taxa (22 species, with 3 varieties of one, *Pontederia cordata*) from five genera of Pontederiaceae were considered in this study. These taxa represent a substantial fraction of the 35–40 species of the family and include representatives from four major genera (*Eichhornia, Heteranthera, Monochoria*, and *Pontederia*), two segregate genera (*Reussia* and *Zosterella*) not recognized here, and the monotypic genus *Hydrothrix*. Apart from two monotypic genera (*Scholleropsis* and *Eurystemon*), all taxa not considered here belong taxonomically within the other genera. A single outgroup taxon (*Philydrum lanuginosum* from Philydraceae) was included. Several lines of morphological and molecular evidence indicate that this family is closely related to Pontederiaceae and may be its sister group (Chase et al., 1993; Davis, 1995; Tillich, 1995).

Four sources of phylogenetic evidence were considered, three from the chloroplast genome. The chloroplast data sets comprise results of a survey of restriction site variation in the chloroplast genome (Kohn et al., 1996) and DNA sequence variation in two chloroplast genes, *ndh*F and *rbc*L. Voucher information for the chloroplast data is provided in Kohn et al. (1996). GenBank ac-

cession numbers for the DNA sequences presented here are U41573–U41597 (*rbc*L partial sequences) and U41598–U41622 (*ndh*F partial sequences). The *rbc*L locus codes for the large subunit of the photosynthetic protein ribulosebisphosphate carboxylase/oxygenase (EC 4.1.1.39). The *ndh*F locus codes for a subunit of an NADH dehydrogenase that may function in the chlororespiratory chain (Rochaix, 1997). The fourth data set is a revision (see Appendix) of a morphological data set of Eckenwalder and Barrett (1986).

Polymerase chain reaction (PCR) amplifications of these genes were performed with use of standard reagents and reaction conditions. Two strands were sequenced for all taxa. With some exceptions, these represent both forward and reverse strands. Single-stranded templates for sequencing were generated in asymmetric PCR reactions by using double-stranded PCR product. Single-stranded DNAs were purified by precipitation with three-fifths volume of 20% PEG 8000, 2.5M NaCl, and were sequenced manually by use of internally situated forward- and reverse-sequencing primers. A 490-bp segment of *ndh*F was amplified and sequenced by using forward and reverse primers designed for this study (Table 1). This region of *ndh*F is part of the highly variable 3′-end of the gene (Olmstead and Sweere, 1994; Kim and Jansen, 1995). Primers used for amplifying and sequencing *rbc*L were designed by Zurawski et al. (1984). A 1,343-bp internal region at the 5′-end of *rbc*L was sequenced for all taxa, except for *Hydrothrix*, for which a 1,169-bp region was sequenced. Length differences were not encountered in the region of *rbc*L examined, but two short (6-bp) insertions/deletions (indels) were observed in *ndh*F, one of which was informative. Each entire indel was coded as a single additional character. Two informative characters in the restriction site data may correspond to DNA sequence variation in the *ndh*F data. Nucleotide characters involved in gain or loss of these restriction sites (three variable nucleotide sites, two of which are informative) were therefore excluded from the *ndh*F data set in combinations involving this set and the restriction site data.

Maximum parsimony analyses were conducted by using PAUP 3.1.1 (Swofford, 1993). The three chloroplast data sets were each analyzed individually and in all possible combinations. The morphological data were analyzed individually and in combination with the chloroplast data. Heuristic searches were performed by using tree bisection–reconnection (TBR) branch-swapping, with MULPARS and "Steepest descent" options activated. All character and character-state changes were equally weighted (cf. Eckenwalder and Barrett, 1986, in which some morphological characters were ordered). Multiple random-addition replicates were used in all searches to reduce the risk of finding only local optima (Maddison, 1991). Because the outgroup was by far the most-divergent taxon in the study (see below), basic searches were repeated both with and without the outgroup included. Prerelease versions of PAUP* 4.0 (d055–d061, kindly provided by

TABLE 1. Oligonucleotides used to amplify and sequence a 3′-portion of the chloroplast gene *ndh*F.

Primer name[a]	Primer sequence	Base pair in *ndh*F[b]
ndh2F	5′-ACTCATGCTTATTCGAAAGC	1,042–1,061
ndh3F[c]	5′-TATTCAATATCGTTATGGGG	1,420–1,439
ndh4F[c]	5′-CTTTATTCATTGGATCAATAGGAAT	1,655–1,679
ndh4R[c]	5′-GAGTTAACCATTTTGATAATA	1,712–1,732
ndh2R[c]	5′-CTATATAACCGCGATTATATGACC	1,961–1,984
ndh1.6R	5′-CCTACTCCATTGGTAATTCCAT	2,066–2,087
ndh1R	5′-AATAAATAAGACGAAATTCGACC	2,134–2,156

[a] F = forward strand, R = reverse strand.
[b] Reference sequence = *Oryza sativa*.
[c] Used as a sequencing primer.

David Swofford) were used in several of the congruence tests described below.

Tree resolution was measured as the number of fully resolved nodes and the number of nonterminal branches (taxon partitions; clades on rooted trees) retained in strict consensuses. Nonparametric bootstrap analyses (Felsenstein, 1985) were performed to estimate branch robustness. Tree support was taken as the average bootstrap support of branches retained in the strict consensus of the shortest trees from each maximum parsimony analysis (Olmstead and Sweere, 1994). Hillis and Bull (1993) demonstrated that bootstrap analysis provides biased, but usually conservative, estimates of the "accuracy" of individual clades, which they defined as the probability that a result represents the true phylogeny. Accurate branches tend to be those supported by 70% or more of the replicates, so long as rates of change are not very high or very unequal among lineages. While recognizing that there is an element of arbitrariness in defining classes of robustness for different levels of bootstrap support, we refer to branches with $< 50\%$, between 50% and 70%, and $> 70\%$ bootstrap support as poorly, moderately, and robustly supported, respectively. Other methods have been used to assess clade support. One nonstatistical method is to derive indices for branches that describe their stability (persistence) in trees less optimal than the shortest ones (Bremer, 1988). Empirical comparisons of bootstrapping versus decay indices suggest that these measures are correlated (Olmstead and Sweere, 1994), and so we used only one of them in this study.

Inference of Congruence and Incongruence among Data Sets

Taxonomic congruence.—A variety of metrics can be used to gauge the dissimilarity of different trees to each other (reviewed by Penny and Hendy, 1985; Page, 1993; Steel and Penny, 1993). One of these, the partition metric (d_s), measures the total number of unique taxon partitions observed in pairwise comparisons of trees. A taxon partition describes the two sets of taxa split by a single branch on a rooted or unrooted tree. The distribution of this metric is highly skewed towards a maximum distance of $2n-6$ symmetric differences for n taxa (Steel and Penny, 1993), so random pairs of trees are extremely unlikely to have a low partition distance. Such distributions of tree-to-tree distances can be used to assess whether or not trees inferred from different data sets are more similar to each other than would be expected by chance (Penny et al., 1982). The distribution for 24-taxon trees was estimated from 999 random trees. We used all three modes of random-tree generation (equiprobable trees, random joining, and random partitioning) in MacClade 3.0 (Maddison and Maddison, 1992), each of which generated one-third of the 999 trees. All three modes were considered since each can result in somewhat different distributions of tree shape (Maddison and Maddison, 1992).

Pairwise tree-to-tree distances were determined in PAUP for all of the unrooted trees from the parsimony analyses. Large matrices summarizing tree-to-tree distances are hard to digest. They can be converted into a "tree of trees" by using the neighbor-joining algorithm in PHYLIP 3.5c (Felsenstein, 1995) to summarize graphically the overall similarity among the shortest trees. Using phenograms to summarize shapes of trees is not new (reviewed by Podani and Dickinson, 1984), although its use in addressing congruence is (see Dickinson et al., 1988, for a multivariate approach). The summary phenogram is a synopsis of tree-to-tree dissimilarity; it can be used to assess at a glance whether there are trees in one data set that are more similar to trees in the same data set than those from other data sets (i.e., they cluster together) or vice versa (they form intermingled clusters).

Data congruence.—Farris et al. (1994) described a simple test, the incongruence length difference (ILD) test (also known as the partition-homogeneity test), for measuring the significance of incongruence among data sets. The test (implemented in PAUP*) randomly repartitions characters from two or more data sets into new data sets as large as each of the original data sets. Shortest trees are estimated for the shuffled data partitions. If the sum of tree lengths for the shuf-

fled data sets is greater than the sum for the original data sets for a critical number of replicates, the null hypothesis of congruence is rejected and one (or more) of the original data sets is taken to be significantly distinct from the others.

The penalty in parsimony steps required to find suboptimal trees with one data set that are optimal for another data set will tend to be small when the two data sets are highly congruent (Swofford, 1991). Several related parsimony-based tests (e.g., Templeton, 1983; Kishino and Hasegawa, 1989) can be used to assess, for a given data set, the significance of the difference in length between a pair of trees. Both tests are implemented in PAUP*. When the two trees compared are from rival data sets, the significance of the difference in length between the trees can be used to evaluate congruence (e.g., Larson, 1994; Paterson et al., 1995; Mason-Gamer and Kellogg, 1996; Cunningham, 1997).

In Templeton's (1983) method, differences in the number of steps required for individual characters between rival trees are used in a Wilcoxon's signed-ranks test to determine the significance of the total difference in length between the trees (see Larson, 1994). A method for estimating the variance (across characters) of the difference in the number of steps between two trees is described in Appendix C of Kishino and Hasegawa (1989). This estimate can be used to perform a paired *t*-test, to decide whether the trees are significantly different in length. When either test is used to assess congruence, reconstructions of character evolution on the rival trees are considered for one data set at a time. Reciprocal tests using both data sets should be performed, in case any incongruence is in one direction only. To avoid confusion, we refer to the data set being used to perform these tests as the "test" data set, designate its trees as the "test trees," and use the adjective "rival" (following Mason-Gamer and Kellogg, 1996) to describe the alternative data set or its trees.

More than one most-parsimonious tree is often inferred in phylogenetic analysis. The more shortest trees there are, the more pairwise tests are possible, and the greater the danger of making Type I errors and hence of finding false incongruence among trees. However, correcting the experiment-wise error rate when making such multiple tests is not desirable, because the multiple most-parsimonious trees from a single data set are not statistically independent of each other, given the large amount of topology they typically have in common. To minimize the number of tests, we chose a single representative test tree from among the most-parsimonious trees. The test tree chosen to represent each individual chloroplast data set was, to err on the conservative side, the one that was the most-distinct in shape from the trees in rival chloroplast data sets. For comparisons of the morphological and molecular trees, each test tree was picked arbitrarily, because trees inferred from each data set were all highly distinct from those from the other data set.

Branches that are weakly supported by particular data sets typically represent only ambiguously supported patterns within that data set. Significant but spurious conflict among data sets can be inferred with use of Templeton's test, or of the Kishino–Hasegawa test, if the rival most-parsimonious tree includes conflicting but poorly supported clades (Mason-Gamer and Kellogg, 1996). For each data set considered, Mason-Gamer and Kellogg therefore suggested comparing its most-parsimonious tree(s) with a single summary tree that incorporated only well-supported nodes on rival trees. The most-parsimonious tree(s) from a data set can then be compared only with phylogenetic structure that is well supported by the rival data set. We used MacClade to construct constraint trees that summarize the phylogenetic structure supported at various confidence levels in the bootstrap analysis of each data set. The cutoffs used were bootstrap values of 50% and greater, 60% and greater, and so on, up to 95% and greater support. Branches not supported at or above a particular level of bootstrap support were reduced to polytomies on the constraint tree.

Polytomies in PAUP are interpreted as multiple speciation events (hard polytomies) in calculations of tree length. A hard

polytomy tends to contribute to increased tree length, because each surrounding node is taken to be independently connected to the polytomy, and so changes in each character along descendant branches are counted as multiple independent events (Maddison, 1989). A constraint tree that has many polytomies, but otherwise is topologically consistent with the test tree, will consequently tend to be significantly different from the test tree. Because we interpret polytomies as reflecting a lack of resolving power by the rival data set (i.e., as soft polytomies), their effect on tree length can be misleading. This is another source of spurious incongruence between data sets. To avoid this effect, the polytomies may be resolved in a manner consistent with the test data set, to the maximum extent that this is possible while still maintaining the constraint tree's structure. In PAUP this can be achieved by using the rival constraint tree as a topological constraint in tree searches that use the test data set. The shortest tree(s) satisfying this constraint can then be used as the rival tree(s) in comparisons with the test tree.

To examine the behavior of extremely noisy data sets in tests of congruence with the molecular data, we constructed 20 small random data sets, using the "Fill random" function in MacClade. Each data set consisted of 30 four-state characters, with an equiprobable distribution of character states. The random data sets thus contained approximately the same number of informative characters as the morphological data. Heuristic searches were used to find shortest trees for these data sets, and bootstrap analyses were performed to determine how often one found robustly supported clades with such data. Each random data set was tested against the moderately to strongly supported structure on the molecular trees in the manner described above and was also assessed for incongruence with the molecular data by using the Incongruence-Length Difference (ILD) test.

RESULTS

Tree Resolution and Support

Basic tree statistics for unrooted trees from the analyses of the individual and combined data sets are presented in Table 2. Both chloroplast genes yielded approximately the same number of informative characters, although almost three times more sequence was examined for *rbc*L than for *ndh*F. Taken together, the two sequence data sets had approximately the same number of informative characters as the restriction site data. The morphological data set had the small-

TABLE 2. Summary statistics for phylogenetic trees of Pontederiaceae (ingroup taxa only) derived from individual and combined analyses of the chloroplast and morphological data sets. CI = consistency index; RI = retention index.

Analysis	No. informative characters	Length[a]	No. trees	No. nonterminal branches[b]	CI[c]	CI[a]	RI	Mean bootstrap value[d]
*ndh*F partial sequence data[e]	59	144	9	17 (14)	0.628	0.708	0.833	78
*rbc*L partial sequence data	61	168	48	15 (12)	0.576	0.685	0.820	77
Restriction site (RS) data	104	299	10	18 (16)	0.454	0.582	0.718	80
Combined *ndh*F + *rbc*L data[e]	120	313	4	19 (18)	0.598	0.693	0.824	84
Combined *ndh*F + RS data[e,f]	161	440	8	18 (16)	0.507	0.620	0.757	88
Combined *rbc*L + RS data	165	468	2	20 (20)	0.496	0.618	0.757	88
All molecular data combined [e,f]	222	609	4	19 (18)	0.525	0.637	0.775	90
Morphological data	33	121	5	19 (19)	0.474	0.496	0.715	52
All data combined[e,f]	255	748	12	17 (14)	0.499	0.599	0.749	94

[a] Including autapomorphies.

[b] In strict consensus trees (number of fully resolved nodes given in parentheses), of a maximum of 21 nonterminal branches and 22 fully resolved nodes for 24 taxa.

[c] Excluding autapomorphies.

[d] Based on branches observed in strict consensus trees (including those with < 50% bootstrap support).

[e] Including two indels in *ndh*F.

[f] Excluding two informative and one variable but noninformative character in *ndh*F.

est number of informative characters of any individual data set.

Tree resolution and support generally increased with increasing size of the uncombined or combined molecular data sets (Table 2; Figs. 1, 2). From 12 to 20 fully resolved nodes were retained in the strict consensuses of the molecular trees. The mean bootstrap support ranged from 77% for the *rbc*L data alone, to ~90% for several of the pairwise and fully combined chloroplast data sets. Despite the small number of characters in the morphological data set, its strict consensus tree was highly resolved (Table 2; Fig. 3a). However, its average bootstrap support (52%) was much lower than for any of the molecular data sets. When all the data were combined, the resulting strict consensus tree was somewhat poorer in resolution than the combined molecular tree (with 14 and 18 fully resolved nodes, respectively), and its average bootstrap support was marginally higher (94%, compared with 90%).

The strict consensuses and representative shortest trees from the phylogenetic analyses are a posteriori rooted on the same branch to emphasize the very high degree of topological similarity among all trees found in analyses of the molecular data (Figs. 1, 2, 3b) in comparison with the morphological data (Fig. 3a). This rooting is that found in the combined analysis of the sequence data (see Graham and Barrett, 1995), but different data sets and data-set combinations found different root locations. Bootstrap support is reported for each branch on the strict consensus trees (Figs. 1–3, left trees). Representative shortest trees (right trees) are included to illustrate branch lengths, computed by using ACCTRAN optimization with respect to this rooting. There were 6 fully resolved (dichotomous) nodes and 11 nonterminal branches in the strict consensus of all trees from separate and combined analyses of the chloroplast data sets, but only 1 fully resolved node and 5 nonterminal branches when shortest trees from morphology were included in the consensus (Fig. 4).

Bootstrap support for branches seen in strict consensus trees for individual or combined analyses of the three chloroplast data sets increased with combination of the data sets (summarized in Fig. 5a). Moderately to well-supported branches in the combined analysis were typically moderately to well supported by each of the individual chloroplast data sets, even in several instances where these branches were not seen on their strict consensus trees. In total, 11 of the 13 best-supported branches in the combined tree had > 50% bootstrap support from all three individual data sets. Bootstrap support of individual branches was typically greater for the combined data set than for any of the individual data sets. Support for a few branches unique to (and not well supported on) individual strict consensus trees decreased substantially or disappeared in the combined analysis of the chloroplast data.

The strict consensus tree from the analysis of the morphological data was the only unrooted tree in this study in which any branches had < 50% bootstrap support (10 of 19 branches; Fig. 3a). However, of nine branches with > 50% bootstrap support, six were also moderately to strongly supported by the combined chloroplast data (summarized in Fig. 5b). For all six clades, the level of bootstrap support was lower in the morphological than in the molecular analysis. Several of these clades are important taxonomic groups, including the genera *Monochoria* and *Pontederia*. Bootstrap support for a clade consisting of *Heteranthera sensu lato* (incorporating *Hydrothrix*) had just under 50% bootstrap support from the morphological data (Fig. 3a; branch o in Fig. 5b).

Most of the unique branches on the morphological strict consensus tree had < 50% bootstrap support from that data, and no bootstrap support from the molecular data or in the combined analysis of all the data (branches ac and ae–ak in Fig. 3a; omitted from Fig. 5b). The remaining unique branches on the morphological strict consensus tree (branches aa, ab, and ad) had little or no support when all the data were combined. Bootstrap support for most branches in the molecular trees changed very little upon combination with the morphological data (Fig. 5b). Only four branches showed even moderate changes in bootstrap support when the morphological data were added

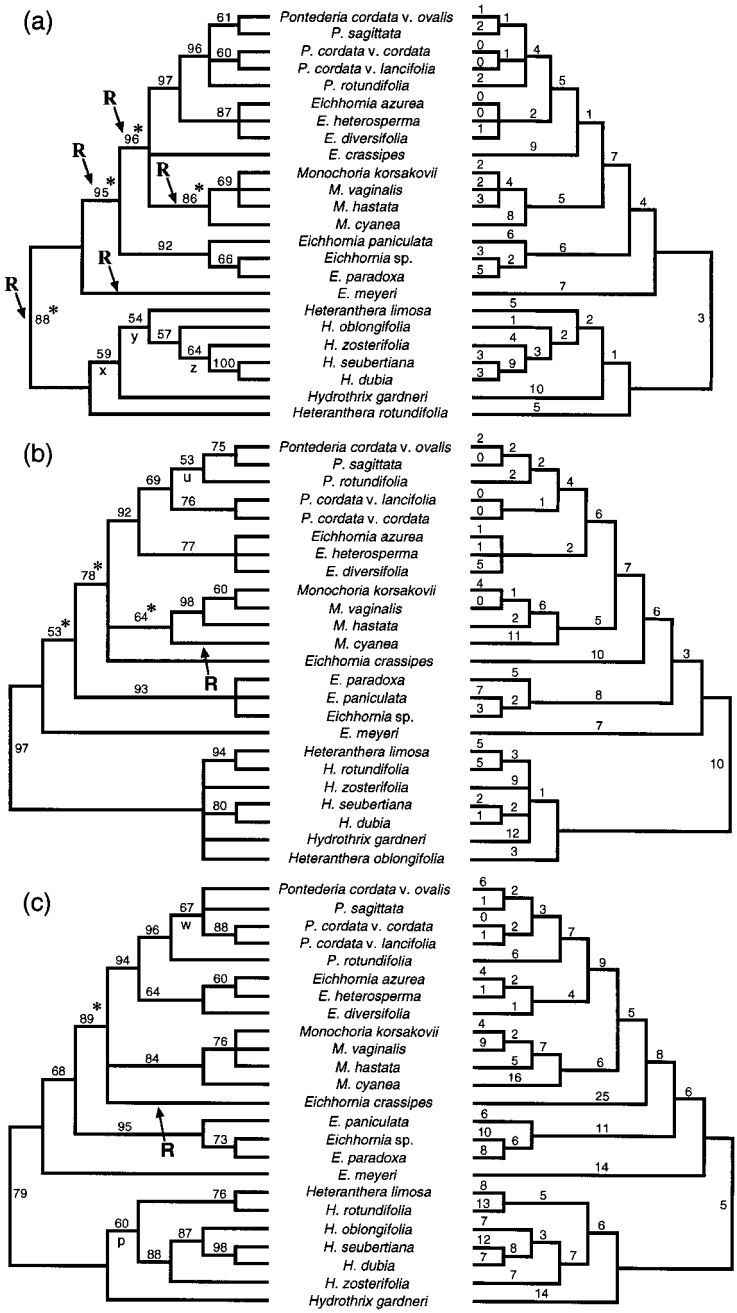

FIGURE 1. Results of parsimony analyses for three individual data sets from Pontederiaceae, based on variation in the chloroplast genes *ndh*F (a) and *rbc*L (b), and restriction-site variation in the chloroplast genome (c). Trees on the left side are strict consensus trees. Nonterminal branches marked with letters (p, u, x, y, z) are unique to that strict consensus tree; branch w (c) is also seen in Figure 3. Bootstrap values are indicated above branches. Asterisks indicate branches for which bootstrap support dropped by ≥ 20% when the outgroup (Philydraceae) was included in the analysis. R = root position(s) indicated by the outgroup. Each tree on the right side is one of the most-parsimonious trees for that data set and is used to demonstrate branch lengths.

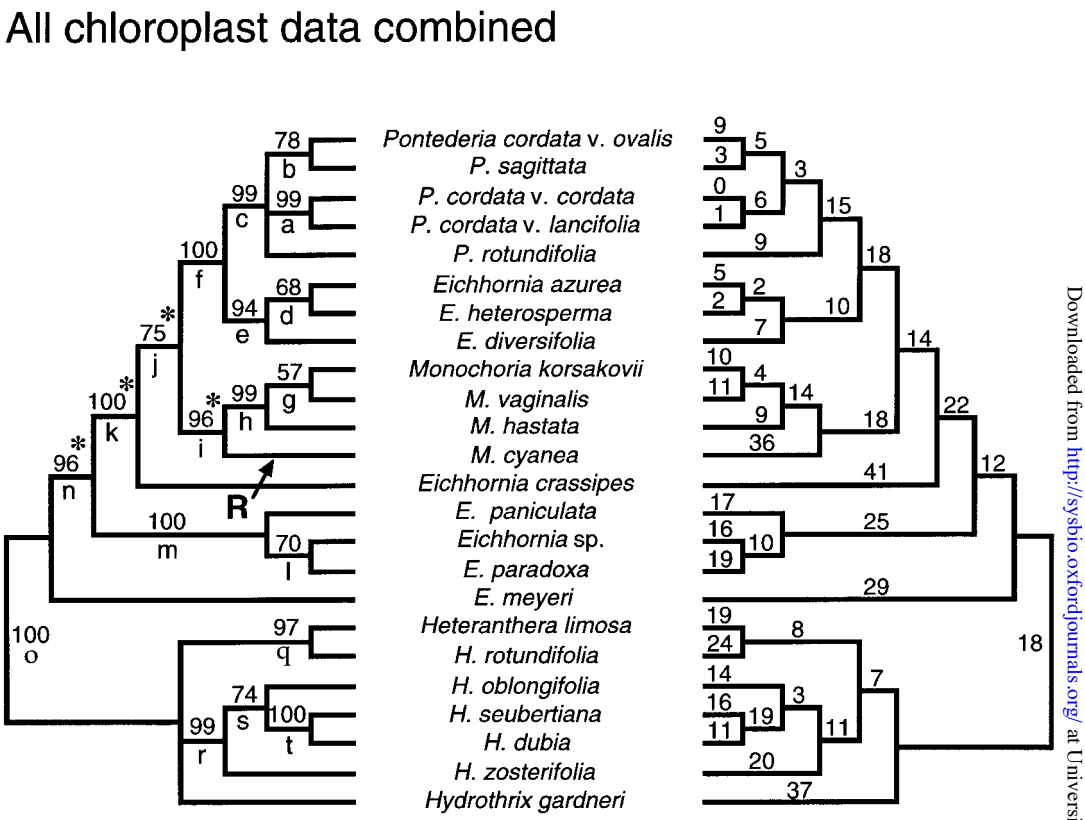

FIGURE 2. Results of parsimony analyses for a combined chloroplast data set comprising three chloroplast data sets from Pontederiaceae. The tree on the left side is a strict consensus tree. Nonterminal branches marked with letters (a–o, q–t) are also found on other strict consensus trees (Figs. 1, 3). Bootstrap values are indicated above branches. Asterisks indicate branches for which bootstrap support dropped by ≥ 20% when the outgroup was included in the analysis. R = root position indicated by the outgroup. The tree on the right side is one of the most-parsimonious trees and is used to demonstrate branch lengths.

to the three chloroplast data sets. Two of these represented improvements in bootstrap support (branches l and w). Support for a third branch (s), which was fairly robustly supported by the chloroplast data, decreased to < 50% upon addition of the morphological data. Support for an alternative arrangement of taxa in this part of the tree (branch z) rose to just under 50% (Fig. 5b). This branch was also moderately supported by the *ndh*F data. Support for two alternative arrangements at the base of *Heteranthera s. l.* (branches p and v) hovered around the 50% mark with or without inclusion of the morphological data.

All trees presented in the Figures and Tables include ingroup taxa only. Inclusion of the outgroup did not resolve where the root of the family is. In several cases, no single most-parsimonious root position was found (Figs. 1a, 3a; the combined analysis of *ndh*F and restriction site data, not shown here). In others, a single most-parsimonious root position was found, but this position differed among data sets (cf. Figs. 1–3). When the outgroup was included in analyses involving the molecular data, a decline of 20% or more in bootstrap support was always seen for one or more of the branches neighboring the root or roots and nowhere else on the

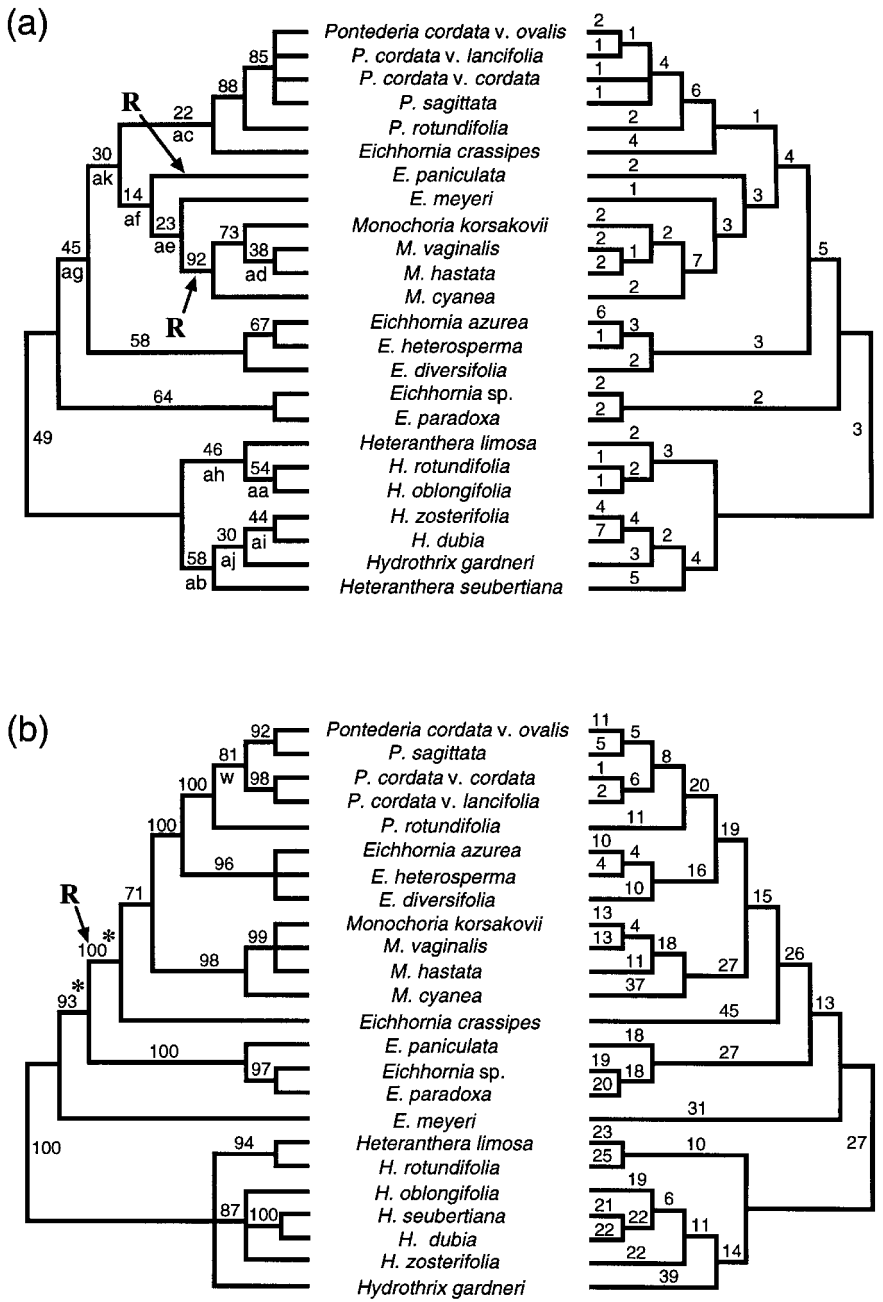

FIGURE 3. Results of parsimony analyses for the morphological data and all current data sets from Pontederiaceae combined. Trees on the left side are strict consensus trees. Bootstrap values are indicated above branches. Each tree on the right side is one of the most-parsimonious trees for that data set and is used to demonstrate branch lengths. R = root position(s) indicated by the outgroup. (a) The morphological data. Nonterminal branches marked with letters (aa–ak) are unique to this strict consensus tree. Several other rootings were found (not shown) that bisected branches not seen on trees that were inferred by using only taxa in Pontederiaceae. (b) All four data sets combined. Asterisks indicate branches for which bootstrap support dropped by ≥ 20% with the outgroup included in this analysis.

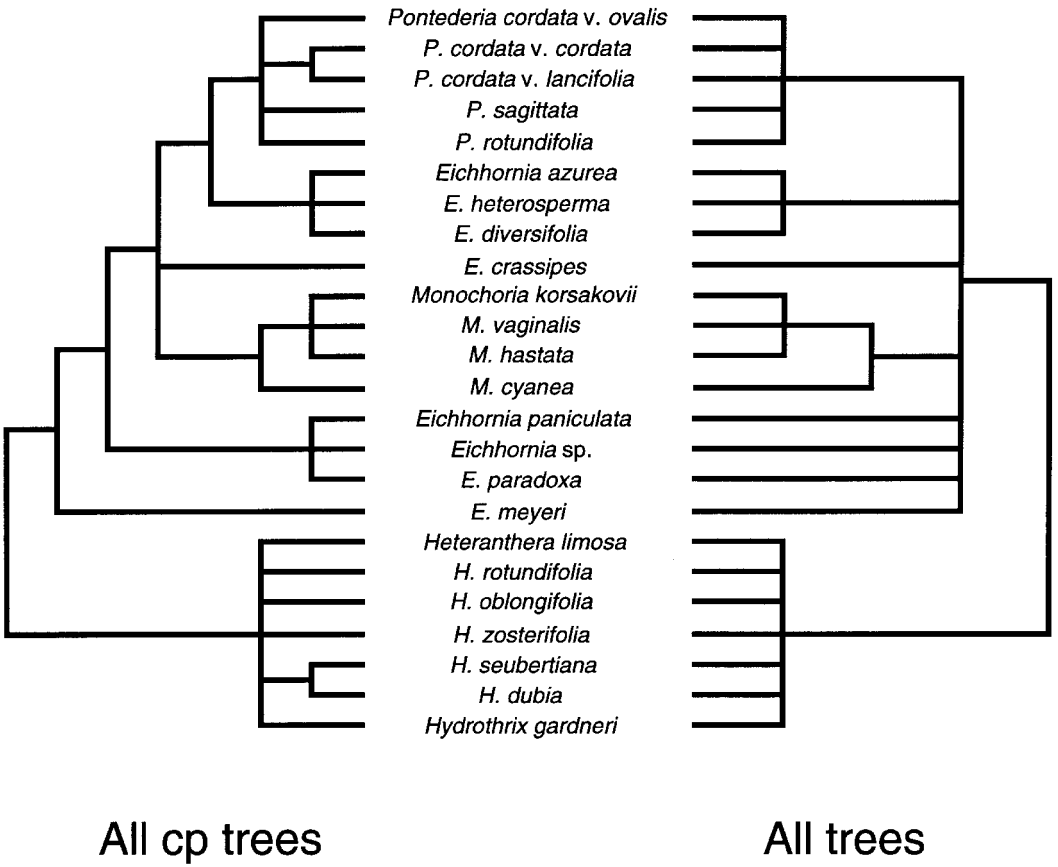

FIGURE 4. Strict consensus trees of all shortest unrooted trees from the single and various combined analyses of the three chloroplast (cp) data sets (left tree) and of all the data sets (right tree).

trees (asterisked branches in Figs. 1, 2, 3b). For the morphological data, the outgroup indicated multiple most-parsimonious roots, including several branches not seen in the unrooted analysis of this data set. For all data sets, the root was supported by no less than 56%, and usually < 50% of bootstrap replicates (results not shown). Inclusion of the outgroup taxon also resulted in loss of resolution, as measured by the number of nonterminal branches or fully resolved nodes in the strict consensus trees (results not shown). The single branch leading to the outgroup taxon accounted for 6–9% of total tree length for the morphological data, and 21–31% of tree length for the other cases, indicating that inference of root position is probably adversely affected by long-branch attraction for most or all of the data sets.

Taxonomic Congruence

A neighbor-joining tree summarizing the topological similarity of all most-parsimonious unrooted chloroplast-based trees is presented in Figure 6a. The summary tree demonstrates that all of the trees from the individual and combined chloroplast data sets are very similar, and in some cases, identical. Three distinct clusters on the summary tree involve trees from the *rbc*L data set, from the *ndh*F data set, and from these two data sets combined. For the restriction site data set and most other combinations of the chloroplast data sets, the individual shortest trees

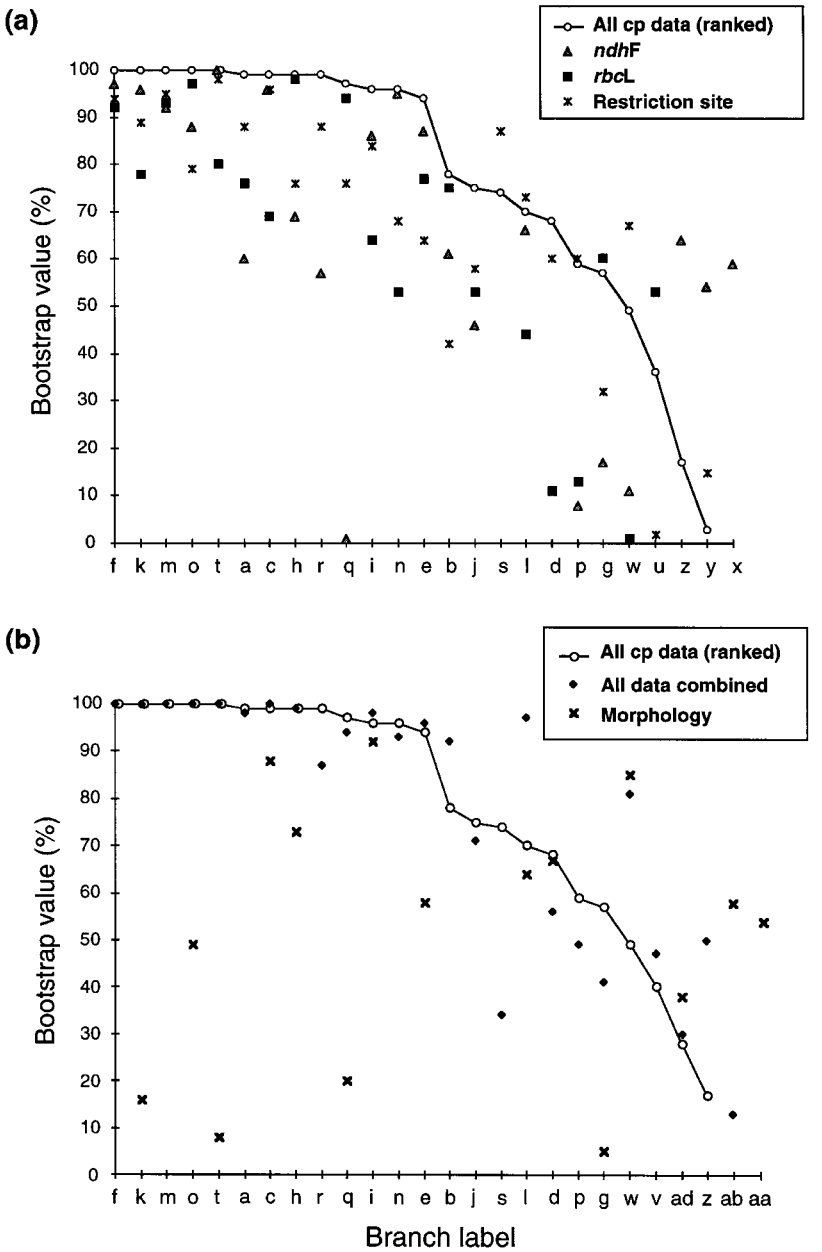

FIGURE 5. Spectrum of bootstrap support for branches found in strict consensus trees of Pontederiaceae. (a) The three individual chloroplast data sets (*ndh*F, *rbc*L, restriction site) and the three chloroplast data sets combined. (b) The morphological data, the combined chloroplast data, and all four data sets combined. Branches seen on at least one strict consensus tree (a–ad; Figs. 1–3) are ranked according to their support in the combined chloroplast analysis, where this exists. Most branches with < 50% support on the morphology-based tree (ac, ae–ak) have no support from the molecular or combined analysis and so are omitted. Bootstrap values < 1% are also omitted. Branch v is included in (b) even though it was not retained on any strict consensus tree, because it had just under 50% bootstrap support when all data were combined. It corresponds to the following taxon partition: (*Hydrothrix gardneri, Heteranthera oblongifolia, Heteranthera zosterifolia, Heteranthera seubertiana, Heteranthera dubia*).

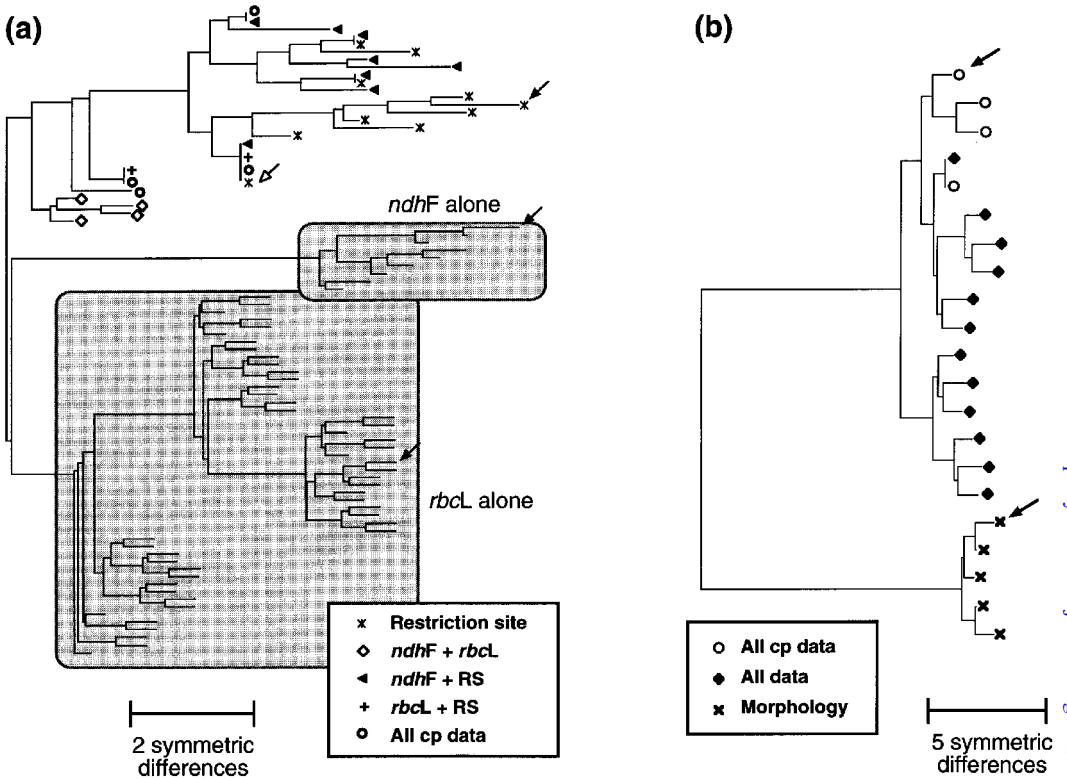

FIGURE 6. Neighbor-joining trees summarizing dissimilarity in tree shape as measured by the partition metric (number of symmetric differences). The phenograms are midpoint rooted for compactness. (a) A neighbor-joining tree of all shortest unrooted trees from analyses involving each single, and all possible two-way and three-way combinations of the three chloroplast data sets. The *ndh*F- and *rbc*L-derived trees group into distinct clusters and are highlighted in labeled boxes. Other most-parsimonious trees are individually labeled according to the data set or data-set combination from which they were derived. The three most distinct trees in each chloroplast data set (used as test trees in Table 4) are indicated with solid arrows. A restriction site tree converged on by three other data-set combinations that include this data set is indicated with an open arrow. (b) A neighbor-joining tree of all shortest unrooted trees from analyses involving the combined chloroplast data, the morphological data, and all the data combined. Individual trees are labeled according to the data set they were derived from. The test trees used in Table 5 are indicated with solid arrows.

were typically more similar to trees from other data sets than to other shortest trees from the same data set. This is reflected in intermingling of these trees across a large portion of the summary phenogram. The phenogram also indicates cases where various data-set combinations yielded shortest trees identical to some of the restriction site trees. In one case, three different data-set combinations yielded the same topology as one of the shortest restriction site trees (see Fig. 6a; open arrow).

This tree topology (Fig. 1c, 2; right tree) is also the closest in shape of the restriction site trees to the cluster of trees found by the combined *ndh*F and *rbc*L data. The distance between the former tree (Fig. 6a) and each of the four trees in this cluster is very small, approximately four symmetric differences. Two of the four trees found from the combined *ndh*F and *rbc*L data are fully bifurcated, and all of the restriction site trees are fully bifurcated. The smallest possible distance between two nonidentical but fully bi-

furcated trees is two symmetric difference units (scale bar in Fig. 6a; many trees on the phenogram are only one symmetric difference unit apart because they differ from each other in a single minor polytomy). The two data sources used to infer these trees, the restriction site data and the combined sequence data, should be completely independent from each other, apart from their shared history. A very conservative estimate of the upper bounds of the probability of finding a pair of fully bifurcated trees from different data sets that are this similar by chance can be estimated from Table 4 of Hendy et al. (1984). The distribution has been calculated for up to 16 taxa. For 16 taxa and four symmetric differences, the probability is 1.87×10^{-12}. The lower bound is the probability of finding two identical trees. For 24-taxon trees, this is 1.77×10^{-27} (see Cavalli-Sforza and Edwards, 1967).

A demonstration that the morphological data are overwhelmed in combination with the chloroplast data is the near or actual identity of chloroplast trees that include and exclude the morphological data (Fig. 6b). In contrast, trees inferred from morphology alone are the most-distinctive topologies found in any analysis, and a large distance (~26–27 symmetric differences) separates them from the trees inferred from the molecular data. However, even though they are highly distinct from the molecular trees, the morphological trees are still more similar to the molecular trees than would be expected by chance (Table 3). In comparison, the smallest distance observed among 999 random trees was 32 symmetric difference units, representing a very small fraction of all possible pairwise comparisons (Table 3).

The summary phenogram portrays relationships among different tree topologies in a hierarchical manner. The validity of using a hierarchical approach for summarizing tree dissimilarity was assessed from the correlation of the raw matrix of tree-to-tree distances to a secondary matrix of the tree-to-tree distances on the summary phenogram (cophenetic correlation, r_{CS}, of Sneath and Sokal, 1973; calculations performed with NYTSYS-pc version 1.80, Rohlf, 1993). For the phenogram in Figure 6a, r_{CS} was 0.940 ($P < 0.01$; approximate Mantel t-test). For the phenogram in Figure 6b, r_{CS} was 0.993 ($P < 0.01$). A large degree of the hierarchy present in the latter phenogram results from a single very long branch separating the morphological trees from the other trees since, with regard to the morphology-based trees alone, r_{CS} was 0.394 ($P > 0.10$). However, the low degree of hierarchy among the morphological trees in the phenogram is also suggested by the phenogram itself, since several branches in this part of the tree have nearly zero length. For the remaining trees considered in Figure 6b, r_{CS} was 0.809 ($P < 0.01$). These lines of evidence demonstrate substantial hierarchy in the distribution of tree-to-tree distances and validate the use of hierarchical (phenogram-based) summaries of taxonomic congruence among trees (see Rohlf and Fisher, 1968).

TABLE 3. Distribution of partition metric, d_S, for 24-taxon trees estimated by using 999 random trees.

d_S (symmetric differences)	% of comparisons at this distance
0–30	0
32	0.001
34	0.010
36	0.184
38	2.11
40	17.6
42	80.2

Character Congruence

The ILD test indicated no significant heterogeneity among the three individual chloroplast data sets ($P = 0.97$). The results of Templeton tests were very similar to the Kishino–Hasegawa tests, so only the latter are presented here (Tables 4–6). Instead of reporting results for cutoffs representing different levels of bootstrap support, only the lowest cutoff that yielded nonsignificant results (or failing that, the ≥95% cutoff) is reported (Tables 4–6). The lower the cutoff, the more phylogenetic structure is included in the rival constraint tree, and the greater the likelihood of a significant difference between the test tree and trees that satisfy the rival constraints. Thus, if the first cutoff with a nonsignificant result was at the 60% level,

TABLE 4. Assessment of congruence among chloroplast data sets with use of the Kishino–Hasegawa test. A most-parsimonious tree from each data set was tested against trees found by using constraints consistent with the phylogenetic structure supported by the rival chloroplast data sets.

	*ndh*F data Tree 1 of 9 (144 steps)		*rbc*L data Tree 27 of 48 (169 steps)		RS data[a] Tree 3 of 10 (299 steps)		
	*rbc*L	RS	*ndh*F	RS	*rbc*L	*ndh*F	*ndh*F
Support for structure considered in constraint tree (%)	≥50	≥50	≥50	≥50	≥50	≥50	≥60
No. branches in constraint tree[b]	16	19	17	19	16	17	14
No. trees found in constrained search	2	3	2	1	1	7	5
Length increase of constrained vs. test trees (steps)	1	3	3	1	1	8	2
SD of difference[c]	1.73	2.23	3.00	2.65	3.32	3.72–4.23	2.45–3.16
P[c]	0.564	0.180	0.318	0.706	0.764	0.032–0.059	0.415–0.528

[a]RS= Restriction site data.
[b]Nonterminal branches with this level of support on the bootstrap majority-rule consensus tree.
[c]Range in this value where different among trees found in constrained searches.

for example, cutoffs at higher levels of bootstrap support did not yield significant results (results not shown).

Each chloroplast data set was congruent with the rival data sets when the cutoff was set as low as 50–60% (Table 4). This is not surprising, given that most moderately to well-supported branches are supported by all three data sets, and only a few moderately supported branches are unique to individual chloroplast data sets (Fig. 5a). For the restriction site data, the test tree was significantly different from the *ndh*F constraints for the ≥50% cutoff but not for the ≥60% cut-

TABLE 5. Assessment of congruence between chloroplast (cp) data and morphological data determined with the Kishino–Hasegawa test. A most-parsimonious tree from each data set was tested against trees found by using constraints consistent with the phylogenetic structure supported by the rival data set.

	Combined cp data Tree 1 of 4 (609 steps) vs. morphology		Morphological data Tree 1 of 5 (121 steps) vs. combined cp	
Support for structure considered in constraint tree (%)	≥50	≥60	≥50	≥95
No. branches in constraint tree[a]	9	6	20	12
No. trees found in constrained search	1	2	1	6
Length increase of constrained vs. test trees (steps)	19	0	19	14
SD of difference[b]	4.98	0–2.00	6.59	5.67–6.02
P[b]	0.0001	1.00	0.006	0.018–0.025

[a]Nonterminal branches with this level of support on the bootstrap majority-rule consensus tree.
[b]Range in this value where different among trees found in constrained searches.

TABLE 6. Use of the Kishino–Hasegawa test to show that random data are incongruent with phylogenetic structure well supported by the chloroplast (cp) data. Results for two representative random data sets are shown here. In each case, a single most-parsimonious tree from the random data set was tested against trees found by using constraints consistent with the phylogenetic structure that is well supported by the molecular data.

	Random data set 8 Tree 1 of 1 (315 steps) vs. combined cp	Random data set 20 Tree 1 of 2 (320 steps) vs. combined cp
Support for structure considered in constraint tree (%)	≥95	≥95
No. branches in constraint tree[a]	12	12
No. trees found in constrained search	9	6
Length increase of constrained vs. test tree (steps)	54	53
SD of difference[b]	7.53–8.68	11.9–13.0
P[b]	< 0.0001	0.0001–0.0003

[a] Nonterminal branches with this level of support on the bootstrap majority-rule consensus tree.
[b] Range in this value where different among trees found in constrained searches.

off. Three branches on the *ndh*F tree have no or very poor support from the other chloroplast data sets (branches x, y, and z; Figs. 1a, 5a). These do not represent serious conflicts between the chloroplast data sets, if we take 70% as the cutoff for strongly supported branches.

The ILD test indicated that the morphological data were significantly distinct from the three combined chloroplast data sets ($P = 0.01$). However, for the chloroplast data, the Kishino–Hasegawa test indicated no serious conflict between the test tree and a tree structure that was moderately to strongly supported by bootstrap analysis of the morphological data (significant P values at the ≥50% cutoff, but not at the ≥60% cutoff; Table 5). Only two branches unique to the morphological data lay between these two bootstrap support values (branches aa and ab; Figs. 3a, 6b). These do not represent serious conflicts with the molecular data, if we take 70% as the cutoff for strongly supported branches. However, a strikingly different pattern was observed when the test was performed in the opposite direction. For the morphological data, the Kishino–Hasegawa test indicated significant conflict between the morphology test tree and moderately to strongly supported structure on the molecular trees (Table 5). Significant incongruence was indicated even at the ≥95% cutoff (only 12 nodes on the molecular tree have this much support).

Incongruence between Chloroplast Data and Random Data

The ILD test indicated significant incongruence of individual random data sets with the molecular data. Each of the 20 random data sets was significantly distinct from the molecular data, at $P < 0.01$. Only 2 of the 20 random data sets had some branches supported by > 50% of bootstrap replicates. Two branches on one tree had 51% and 59% bootstrap support, and one branch on the other tree was supported by 56% of bootstrap replicates. By the Kishino–Hasegawa test, the molecular data were incongruent with this structure ($P < 0.0001$ in both cases). The test tree was the same chloroplast tree as that used in Table 5. However, the molecular data set was not incongruent with any well-supported structure on the trees inferred from the random data sets, because there were no clades on the random trees supported by > 70% of the bootstrap replicates. In the reciprocal analysis, the 20 random data sets were incongruent with moderately to strongly supported structure on the molecular trees by the Kishino–Hasegawa test. Conflict was

significant for all 20 random data sets when each random data set and a test tree from it were tested against structure supported by 95% or more bootstrap replicates on the molecular constraint tree (P values all ≤ 0.0003; the results for two representative cases are summarized in Table 6).

Discussion

Accuracy of the Phylogenetic Inferences

Finding trees as similar as some of those inferred from different chloroplast data sets is extremely unlikely ($1.77 \times 10^{-27} < P < 1.87 \times 10^{-12}$). This seems to provide extremely strong evidence that the different data sets are rapidly converging towards a single estimate of the chloroplast's phylogenetic history in Pontederiaceae. Are there any other possible explanations for the near-identity of the trees inferred from different parts of the chloroplast genome?

One assumption of the parsimony criterion is that all characters evolved independently from each other. This is a potentially dangerous assumption to make when linked characters are used to infer organismal history (reviewed by Doyle, 1992). It is, however, a reasonable assumption for inferring the phylogeny of the linkage group in question (the chloroplast genome), unless there are strong functional correlations between many individual chloroplast characters, which is improbable. One conceivable source of spurious congruence is long-branch attraction (Hillis, 1995). Long branches tend to attract each other, if multiple changes on them are substantially more likely than single changes on neighboring short branches (Felsenstein, 1978, 1983; Hendy and Penny, 1989). Reconstructions of character evolution indicate that most informative characters change only once or twice across the entire tree (Table 7), and few branches in the chloroplast trees are especially long relative to other branches (Figs. 1, 2; right trees). This suggests a low probability of multiple change on most or all of the 45 branches on the unrooted trees, for most of the informative characters. The chloroplast data sets for Pontederiaceae therefore do not seem to be in the Felsenstein zone. Thus, the only convincing explanation for their congruence is the historical signal they share in common. The near-identity of trees inferred from the different data sets is strong evidence that these modest samples of the chloroplast genome are sufficient for inferring its phylogenetic history very accurately (see Hillis, 1995).

The unrooted chloroplast trees are highly congruent, despite different rates and modes of evolution in different parts of this genome. Our restriction site survey spans both single-copy and inverted-repeat regions, the latter region being known to evolve at a substantially slower rate than the rest of the chloroplast genome (Jansen and Palmer, 1987; Wolfe et al., 1987). Rates of restriction site change also are affected by variation among codon positions in the rates of nucleotide substitution and by differences in transition and transversion rates (Albert et al., 1992). These rates can vary from gene to gene. For example, the pattern of change among codon positions differs between *rbc*L and *ndh*F (Olmstead et al., 1998; and see Albert et al., 1993; Kim and Jansen, 1995). Combining data sources with substantially different rates or modes of evolution is potentially undesirable (Bull et al., 1993; Huelsenbeck et al., 1994, 1996; but see Chippendale and Weins, 1994; Nixon and Carpenter, 1996) unless weighting schemes can be identified to take account of the different evolutionary processes in different subsets of the data (e.g., Miyamoto et al., 1994; Allard and Carpenter, 1996; Cunningham, 1997).

However, if all characters change sufficiently slowly, they may be equally weighted during phylogenetic inference, even though they do not actually change with equal probability (Felsenstein 1981, 1983). The extreme congruence of the chloroplast data attests that equal weighting is sufficient (here at least) for accurate phylogenetic reconstruction. This should be reassuring to others using equally weighted parsimony to reconstruct phylogenies at this taxonomic level with chloroplast data. Whether or not the root of the family can be accurately inferred by using the chloroplast

TABLE 7. Distribution of character-state change classes on the 24-taxon tree (right side) in Figure 2.

Frequency of change	*ndh*F[a]	*rbc*L[a]	Restriction site
None	403	1,240	182
1	53	61	100
2	21	24	40
3	10	13	22
4	0	4	7
5	4	1	5

[a]DNA characters with > 1 change are not necessarily homoplastic because they have four possible states.

data is a more-complicated issue that will be addressed elsewhere.

Phylogenies inferred from molecules and morphology were more similar to each other than would be expected by chance, but there were also very substantial differences in the topology of the trees estimated from these different sources of evidence. Nonetheless, the few strongly supported branches on the morphology trees are consistent with clades inferred by the molecular data. No branches on the morphology trees that conflict with the molecular trees can be unambiguously ascribed to conflicting phylogenetic signals between molecules and morphology. Most of the remaining branches on the morphology-based tree are one or a few steps long (Fig. 3a) and consequently (Felsenstein, 1985) have very weak support from bootstrap analysis (Fig. 5b). There is well-supported phylogenetic signal in the morphological data, but it floats in a sea of noise. We should not be surprised, then, that the morphological data behave similarly to random data in tests of congruence with the molecular data. The low ratio of signal to noise in the morphological data set also means that using different weighting schemes to improve the congruence of the chloroplast and morphological data has little merit.

Source of Noise in the Morphological Data and its Consequences for Phylogenetic Inference

The low consistency indices for the morphological data (Table 2) indicate a high level of homoplasy in these characters. Eckenwalder and Barrett (1986) suggested that this homoplasy might be partly a function of extreme lability in vegetative characters in these aquatic plants. Despite evidence that vegetative characters are more homoplasious than the reproductive characters in the family, consistency indices for the different kinds of morphological characters are not unusually low (at least on the morphological trees), given the number of taxa in our study (Barrett and Graham, 1997).

A high overall level of homoplasy need not in itself result in less-robust or more-ambiguous phylogenetic estimation (e.g., Sanderson and Donoghue, 1989; Jansen et al., 1990). The level of homoplasy in the restriction site data set is very similar to the morphological data set (Table 2), yet the latter data set is overwhelmed by the former when these two data sources are combined (results not shown), and the former data yield robustly supported trees that are congruent with the other chloroplast evidence (Table 4). The lack of robustness of trees inferred from the morphological data set thus seems to be a consequence of its relatively small size, not its level of homoplasy. Indeed, there is a significant correlation ($r = 0.826$; $P < 0.01$) between the mean bootstrap support by a data set for its trees and the number of informative characters, across the nine data sets and data-set combinations considered in Table 2. This correlation should be treated cautiously, given that five of these nine data sets are combinations of the other four, but it does suggest that sampling error is a major source of noise in the morphological data. Increasing the number of morphological characters to match the number of characters in either single-gene data set ought to substantially improve both the robustness of a morphology-based phylogeny of the family and the ability to critically evaluate potential conflicts between morphological and molecular data sets.

In addition to sampling error and multiple substitutions on very long branches, common sources of noise in molecular data include poor alignment of nucleotide data and misscoring of gels or chromatograms because of sequencing artifacts or human error. The extreme congruence among the chloroplast data sets indicates that if such errors were made, their impact on phyloge-

netic analysis is extremely small. Other potential sources of noise in the morphological data include misscoring of characters (several errors in these are corrected in the Appendixes), large phenotypic variances in quantitative and qualitative character-state classes (Stevens, 1991), and a weak genetic basis to individual characters. Avoiding these sources of error is commonly left to the common sense and intuition of investigators. Regardless of its exact source, the impact of noise in the morphological data on phylogenetic estimation in Pontederiaceae is negligible, because this data set is almost completely overwhelmed by the chloroplast evidence when the two sets are combined, and this combination has little effect on chloroplast tree resolution or support. However, our study demonstrates that care should be taken in interpreting findings of significant incongruence among data sets, such as that found between molecules and morphology here. Our finding of incongruence between the robust chloroplast data and the random data sets demonstrates that the "heterogeneity" or incongruence detected by the ILD test of Farris et al. (1994) can result from a high amount of noise in one of the data sets, rather than a truly conflicting phylogenetic signal.

Very noisy data sets can also result in significant incongruence if their shortest trees are tested against a tree structure that is robustly supported by the chloroplast data (Table 5, 6). However, in such cases we are in effect comparing trees from different data sets that are both poorly supported by the noisy data and find ourselves in the "absurd position of proving that one bad tree is significantly worse than another" (Felsenstein, 1988:540). The apparent "badness" of the robust chloroplast trees is entirely from the perspective of the noisy data: Even completely random data have their preferred, if poorly supported, trees. In contrast, despite the lack of robustness of the morphological data from Pontederiaceae, the strong phylogenetic signal evident in the chloroplast data does not conflict with the few clades that are well supported by morphology (Table 5).

Cunningham (1997) suggested that Templeton's test and related tests are too conservative. More specifically, Lutzoni and Vilgalys (1995) suggested that these tests cannot address whether observed differences between trees inferred from different data sets are due to sampling error. These criticisms are valid if a tree from a test data set is compared with most-parsimonious trees from an unrobust rival data set. Such comparisons can misleadingly indicate incongruence between them, because they assess all branches on the rival tree, including those that may reflect sampling error (or other types of noise) instead of phylogenetic history. A better test is to assess a data set and its trees only against structure that is robustly supported by the rival data set (Mason-Gamer and Kellogg, 1996). Our study illustrates the critical importance of considering the level of support by each data set for its own trees before interpreting tests of congruence among data sets. Because sampling error and other sources of noise can lead to ambiguous and distorted phylogenetic inference on poorly supported branches, these branches should be excluded from consideration in tests of character congruence that consider tree shape.

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APPENDIX 1

Comments on the Morphological Characters

7. Leaf whorls ("Axillary dwarf shoots").—All but the first of the members of each leaf whorl in *H. gardneri* may arise from one or more short shoots with annular insertion on the long shoot (Goebel, 1913; Rutishauser, 1983) or alternatively, they may be intercalary leaves produced from a meristematic ring below the shoot apex (Rutishauser, 1983). By either interpretation, the leaf whorls of this species are a unique feature of this taxon, and the coding employed in Eckenwalder and Barrett (1986) is retained.

8. Stipules.—In Pontederiaceae, the structures variously described as stipules or ligules are outgrowths of the lower leaf zone above the insertion of the upper leaf zone (Richards, 1980). Confusion over whether to call these structures stipules or ligules appears to have arisen from an historical precedent set by de Candolle that monocotyledons do not have stipules (Richards, 1980). As defined by Richards, "stipule" includes the "ligule" of grasses, the tonguelike outgrowths between sheath and blade. Although less elaborate, such ligules are developmentally homologous with the stipule in Pontederiaceae and are therefore coded identically (state 0). *Heteranthera dubia* and *H. seubertiana* possess ligule-like structures, and the leaf whorl members of *Hydrothrix gardneri* have stipules (Rutishauser, 1983).

9. Petiole.—Character state 2 is a new state that indicates petiole absence.

11–15. Leaf-blade characters.—These refer to the blades of adult, petiolate leaves, where these are produced, or to the blades of adult, sessile leaves, where not. The boundary between petiole and lamina is not sharp in *Eichhornia* sp., but this did not unduly interfere with assignment of leaf-blade character states in this species. The filiform leaf of *Hydrothrix gardneri* has no structures that are obviously homologous to a petiole or leaf-blade, and so characters 9 and 11–15 are treated as missing data (?) for this taxon.

12. Maximum length of lamina ("maximum leaf size").—Character states: 0 = 5 – 10 cm; 1 = > 10 cm; 4 = < 5 cm. These are modifications of the ranges given in Eckenwalder and Barrett (1986).

16. Inflorescence type.—Character state 2 refers here to a spike or raceme, 3 to a subumbel or umbel, and 4 to a two-flowered pseudanthium.

20. Peduncle pubescence.—Taxa are coded as hairy (state 1) if any part of the inflorescence axis is pubescent.

21. Flower attachment.—The pedicellate coding (state 0) is used here if at least some of the flowers in the inflorescence have pedicels.

22. Flower number.—The flower count for *Philydrum-lanuginosum* refers to the entire panicle, not each spike.

33. Stamen diversity.—*Hydrothrix-gardneri* has a single fertile stamen and two staminodes; *Philydrum-lanuginosum* has only a single stamen. This character is coded as missing (?) for these taxa.

43. Anther dehiscence.—An additional character. State: 0 = regular; 1 = poricidal.

APPENDIX 2. Morphological data set for Pontederiaceae taken from Eckenwalder and Barrett (1986), with some revisions. Corrected entries are indicated with asterisks. *Philydrum lanuginosum* (Philydraceae) is the outgroup. Two taxa have been added to the matrix (*Eichhornia paradoxa* and *E. meyeri*). An undescribed species of *Eichhornia*, referred to here as *Eichhornia* sp., was incorrectly identified as *E. paradoxa* by Eckenwalder and Barrett (1986). One new character is added (number 43) and one deleted (character 19). The numbering of the remaining characters is retained for consistency. The character state 1′ in Eckenwalder and Barrett (1986) is replaced with 4 here.

	Characters																																							
Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
Philydrum lanuginosum	0	1	0	0	0	0	0	1	2*	0	2*	1*	0	0	0	0	0	0	1	0	4*	1	4*	0	0	1	1	1	0	0	2	?*	0	0	0*	1	0*	0	0	0	0	0
Pontederia cordata var. cordata	0	1	0	0	0	0	0	0	0	1	4	1	1	0	0	1	0	1	1*	1	4	1	0	0	2	1	0	0	0	0	0	0	0	0	0	0	2	2	1	2	2	0
Pontederia cordata var. lancifolia	0	1	0	0	0	0	0	0	0	1	0	1	0	0	0	1	0	1	1*	1	4	1	0	0	2	1	0	0	0	0	0	0	0	0	0	0	2	2	1	2	2	0
Pontederia cordata var. ovalis	0	1	0	0	0	0	0	0	0	1	4	0	0	0	0	1	0	1	1	1	4	1	0	0	2	1	4	0	0	0	0	0	0	0	0	0	2	2	1	2	2	0
Pontederia rotundifolia	0	1	0	1	0	0	0	0	0	0	4	1	2	1	0	2	0	1	1*	1	1	1	0	0	2	1	0	0	0	0	0	0	0	0	0	0	2	2	1	3	2	0
Pontederia sagittata	0	1	0	0	0	0	0	0	0	1	4	1	2	0	0	1	0	1	1*	1	4	1	0	0	2	1	0	0	0	0	0	0	0	0	0	0	2	2	1	1	2	0
Eichhornia azurea	0	1	0	1	0	1	0	0	0	0	4	1	0	2	1	2	0	1	1*	1	0	1	1	0	3	1	0	0	0	1	0	0	0	0	0	0	0	0*	0	0	1	0
Eichhornia crassipes	0	1	0	0	1	0	0	0	1	0	4	1	1	1	1	2	0	1	1*	1	1	1	1	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eichhornia diversifolia	1	1	0	1	0	1	0	0	0	0	4	4	1	1	1	2	1	1	1*	1	2	1	0	1	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eichhornia heterosperma	0*	1	0	1	0	1	0	0	0	0	0	0	0	2	1	2	1	1	1*	1	2	1	0	1	3	1	0	0	0	0	0	0	0	0	0	0	0	0*	0	0	1	0
Eichhornia meyeri	2	1	0	0	0	0	0	0	0	0	4	0	1	0	0	2	0	0	1	0	1	1	4	0	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eichhornia paniculata	2*	1	0	0	0	0	0	0	0	0	4	0	1	0	0	0	0	0	1	1	4	1	0	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eichhornia paradoxa	2	1	0	0	0	0	0	0	0	0	1	1	0	0	0	3	1	0	0	0	2	1	0	1	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eichhornia sp.	2	1	0	0	0	0	0	0	0	0	2*	0*	0	1	0	3*	1	0	0	0*	2	1	0	1	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Monochoria cyanea	1	1	0	0	0	0	0	0	0	0	4	1*	0*	0	0	2	0	1	0	0*	2	0	4*	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0*	1
Monochoria hastata	0*	1	0	0*	0	0	0	0	0	0	4	1*	2	0	0	2,3*	1	1	0	0	1	0	4*	0	1	1	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	1
Monochoria korsakovii	1*	1	0	0	0	0	0	0	0	0	4	1*	1	0	0	0*	0	1	0	0	1	0	4*	0	1	1	0	0	0	0	0	1	0	1	1	0	0	0	0	0	1*	1
Monochoria vaginalis	1	1	0	0	0	0	0	0	0	0	0*	1*	1	0	0	2,3*	1	1	0	0	2	0	4*	0	1	1	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	1
Heteranthera dubia	0	1	1	1	0	1	0	0*	2*	0	2*	0*	0	1	0	2	1	0	0	1	3	0	4	1	4	1	1	0	0	0	1	0	1	0	1	1	0	1	0	0	1	0
Heteranthera limosa	2	1	0	0	0	0	0	0	0	0	0	4*	1	1	0*	2	1	0	0	1	3	1*	0	1	4	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0
Heteranthera oblongifolia	2	1	0	1	0	0	0	0	0	0	0	0*	1	1	1*	2	1	0	0	1	2	1*	0	1	3	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0
Heteranthera rotundifolia	2	1	0	1	0	0	0	0	0	0	4	0	1	1	1	2	1	0	0	1	3	1	0	1	3	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0
Heteranthera seubertiana	2	1	0	0	0	1	0	0*	2*	0	1	1*	0	1	0	2	0	0	1	1	1	1	4	1	3	1	1	0	4	0	1	1	0	0	1	0	0	0	0	0	0	0
Heteranthera zosterifolia	1	1	1	1	0	1	0	0	0	0	1	4*	0	1	0	2	1	0	0	1	2*	1*	4	1	4	1	0	0	0	0	1	1	1*	0	1	0	0	0	0	0	0	0
Hydrothrix gardneri	2	1	1	0	0	1	1	0*	?*	0	?*	?*	?*	?*	?*	4*	1	0	0	1	2	1*	4	1	2*	1	1	0	0	0	2	?*	0	0	1	0	0*	0	0	0	0	0