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# Systematics of the Aquatic Angiosperm Genus *Myriophyllum* (Haloragaceae)

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***Abstract*—**The angiosperm genus *Myriophyllum* (Haloragaceae) is among the most species-rich genera of aquatic core-eudicots. *Myriophyllum* has a cosmopolitan distribution with its center of diversity in Australia (> 37 endemics). The widespread invasive species of the genus (*M. aquaticum*, *M. heterophyllum*, and *M. spicatum*) have drawn attention from international natural resource managers. *Myriophyllum* species are notoriously difficult to identify using vegetative morphology alone, which commonly is all that is available for these highly clonal plants. The relationships among taxa have been difficult to determine with suspected parallelisms in sex expression, sepal and petal loss, and reduced stamen number. A molecular phylogenetic approach was taken to examine relationships among taxa and to employ molecular markers for the reliable identification of *Myriophyllum* species. This study included ≈ 80% of the known *Myriophyllum* species. Both nrDNA ITS and cpDNA *matK* and *trnK* data were used to examine phylogenetic relationships among species. The nrDNA ITS data proved highly variable and could differentiate between all but one species pair examined. These analyses also uncovered multiple cryptic species among Australian complexes. Phylogenetic results support major realignments in the subgeneric classification including a recombination for the rare monotypic genus *Meziella*, which was nested within *Myriophyllum*. Here we present the new combinations and taxa *Myriophyllum* subgenus ***Meziella,*** sections ***Pectinatum*** and ***Pelonastes***, subsections ***Isophylleae*** and ***Nudiflorum*** with the new combination ***Myriophyllum trifidum*** to accommodate the former monospecific genus *Meziella*.

***Keywords*—**aquatic plant, Bayesian analysis, cryptic species, molecular phylogeny, *matK*, nrDNA ITS.

The angiosperm genus *Myriophyllum* L. (Haloragaceae R. Br.) is among the most species-rich (≈ 68 spp.) of the aquatic core-eudicots (as defined by APG II 2003). These "watermilfoils" have a world-wide distribution (except Antarctica) with a center of diversity in Australia (42 spp.; 37 endemic). North America (14 spp.; seven endemic) and Asia (16 spp.; eight endemic) also have high continental diversity and share seven common species (at least four due to introductions). *Myriophyllum* is well-known for its invasive species. The aggressive Eurasian *M. spicatum* L. (Eurasian watermilfoil) and South American *M. aquaticum* (Vell.) Verdc. (parrotfeather) are now established on most continents and listed as noxious weeds in several U. S. A. states. The North American endemic *M. heterophyllum* Michx. reportedly is naturalized in Europe (Cirujano and Medina 1997; Wimmer 1997) and Asia (Yu et al. 2002), and also is considered to be invasive outside its endemic range in the northeast and northwest United States (Les and Mehrhoff 1999; Moody and Les 2002). Hybridization also has been shown to play a role in North American invasions with two hybrid lineages now recognized (*M. spicatum* × *M. sibiricum* and *M. heterophyllum* × *M. laxum*; Moody and Les 2002).

Infrageneric relationships in *Myriophyllum* have proven to be particularly frustrating using morphology alone, as stated by Meijden (1969, p. 303), "…. the species show a reticulate affinity by parallelism, especially as regards reduction in both vegetative and sexual organs, the usefulness of distinguishing infrageneric taxa is debatable and not advisable." Reliable morphological identification of *Myriophyllum* is particularly difficult in the field. Plant identification is most complex when reproductive structures are lacking, as is common among many aquatic taxa (Sculthorpe 1967; Cronk and Fennesy 2001). Even *Myriophyllum* species that presumably are distantly related are not easily differentiated when only submerged vegetative characters are available, as often is the case (Aiken 1981; Orchard 1986). Furthermore, common vegetative plasticity in *Myriophyllum* (e.g. submerged and emergent vegetative forms) compounds the problem.

The characteristic morphology of *Myriophyllum* is a submerged stem with whorled or alternate, pectinate leaves. The submerged stems have vascular lacunae (air chambers) that allow the plants to transfer oxygen to the submerged roots as well as to confer buoyancy. Stem size varies widely from compact mud-flat forms to some plants surfacing from a rooted base at water depths of > 10 m. Most *Myriophyllum* have an emergent inflorescence with flowers borne individually in the axils of emergent leaves (bracts) and most species are monoecious with female flowers basal, male flowers distal, and hermaphrodite flowers often intermediate.

In such a large complex genus, infrageneric delimitation has emphasized departures from this common morphological theme (Schindler 1905; Orchard 1986). Some species display dioecy (e.g. *M. aquaticum*, *M. implicatum* Orchard), whereas others are monoecious but with separate male and female emergent inflorescences on the same individual (e.g. "*M. propinquum* Alliance" species [Table 1], *M. lophatum* Orchard). Strictly hermaphroditic flowers also are found (e.g. *M. callitrichoides* Orchard, *M. mattogrossense* Hoehne, *M. muricatum* Orchard) as are submerged flowers (e.g. *M. dicoccum* F. Muell., *M. farwellii* Morong, *M. humile* (Raf.) Morong). Some species are highly diminutive, possess linear leaves, often lack differentiated submerged leaves and grow only in shallow water or on mudflats (e.g. *M. drummondii* Benth., *M. lophatum*, *M. pedunculatum* Hook. f., *M. votschii* Schindl.), whereas several display both the common submerged morphology as well as a "mud flat form" (e.g. *M. heterophyllum*, *M. humile*, *M. pinnatum* Britton, Sterns & Poggenb.).

***Taxonomic History*—***Myriophyllum* has been hypothesized as distinct within Haloragaceae due to a combination of characteristics including its aquatic habit (also found in *Meionectes* R. Br., *Meziella* Schindl., and *Proserpinaca* L.), propensity towards monoecy (also found in *Laurembergia* P. J. Bergius) and a fruit that splits at maturity into two or four individual nutlets (not found elsewhere in the family; Schindler 1905; Orchard 1986). *Myriophyllum* was found to be paraphyletic in

TABLE 1. *Myriophyllum* classification systems as interpreted from Schindler (1905; column 1) and Orchard (1986; column 2). Orchard did not present a formal classification at the subgeneric level. The *Myriophyllum* "Alliances" follow the terminology of Orchard (1986). *Taxa sampled for our analyses. Numbers following a species name refer to the number of individuals sampled for these analyses (usually in column two), when taxa only present in column 1 then included there. Not all taxa sampled are included here (See Appendix 1 for further sampling details).

| Genus *Myriophyllum* (Schindler 1905) | Genus *Myriophyllum* L. (Orchard 1986) |
|---|---|
| subgenus ***Eumyriophyllum*** | *M. aquaticum* Alliance |
| section ***Pentapteris*** | **M. aquaticum* (Vell.) Verdc. (3) |
| subsection ***Spirophyllum*** | **M. robustum* Hook. f. (1) |
| *M. gracile* | *M. aquaticum* Alliance Associates |
| **M. trachycarpum* | **M. verticillatum* L. (4) |
| **M. filiforme* | **M. heterophyllum* Michx. (9) |
| subsection ***Pelonastes*** | **M. hippuroides* Nutt. (1) |
| **M. tillaeoides* | *M. salsugineum* Alliance |
| **M. longibracteolatum* | **M. salsugineum* Orchard (2) |
| **M. pedunculatum* | **M. quitense* Kunth (4) |
| **M. votschii* | **M. triphyllum* Orchard (1) |
| **M. amphibium* | **M. caput-medusae* Orchard (2) |
| subsection ***Spondylophyllum*** | *M. porcatum* Orchard |
| **M. ussuriense* | **M. verrucosum* Lindl. (3) |
| **M. robustum* | *M. salsugineum* Alliance Associates |
| **M. verticillatum* | **M. spicatum* L. (7) |
| *M. propinquum* | *M. muelleri* Sonder |
| **M. brasiliense* | **M. decussatum* Orchard (1) |
| **M. spicatum* | *M. indicum* Willd. |
| **M. verrucosum* | *M. tetrandrum* Roxb. |
| **M. elatinoides* | *M. propinquum* Alliance |
| *M. indicum* | *M. propinquum* A. Cunn. |
| **M. latifolium* | **M. variifolium* Hook. f. (8) |
| subsection ***Leiocarpium*** | **M. simulans* Orchard (4) |
| **M. alterniflorum* | **M. alpinum* Orchard (2) |
| *M. muelleri* | **M. crispatum* Orchard (6) |
| section ***Tessaronia*** | **M. ussuriense* Maxim. (2) |
| subsection ***Trachycarpaeum*** | *M. propinquum* Alliance Associates |
| *M. tetrandrum* | **M. papillosum* Orchard (2) |
| *M. tuberculatum* | **M. latifolium* F. Muell. (2) |
| *M. intermedium* | *M. muricatum* Alliance |
| *M. axilliflorum* | **M. muricatum* Orchard (3) |
| subsection ***Spondylastrum*** | *M. tuberculatum* Roxb. |
| **M. heterophyllum* | *M. muricatum* Alliance Associates |
| **M. hippuroides* | **M. dicoccum* F. Muell. (2) |
| **M. pinnatum* (2) | **M. balladoniense* Orchard (1) |
| *M. sparsiflorum* | *M. striatum* Alliance |
| subsection ***Ptilophyllum*** | *M. implicatum* Orchard |
| **M. humile* (3) | *M. striatum* Orchard |
| **M. laxum* (8) | *M. costatum* Orchard |
| **M. tenellum* (3) | *M. striatum* Alliance Associates |
| subgenus ***Brachytheca*** | *M. gracile* Benth. |
| *M. integrifolium* | **M. trachycarpum* F. Muell. (2) |
| **M. drummondii* | **M. filiforme* Benth. (2) |
| *M. glomeratum* | **M. petraeum* Orchard (2) |
| subgenus ***Dicarpum*** | *M. mezianum* Alliance |
| *M. mezianum* | **M. coronatum* Meijden (1) |
| **M. dicoccum* | *M. mezianum* Schindl. |
| | *M. siamense* (Craib) Tardieu |
| | *M. bonii* Tardieu |
| | *M. integrifolium* Alliance |
| | **M. limnophilum* Orchard (1) |
| | *M. integrifolium* Hook. f. |
| | **M. drummondii* Benth. (1) |
| | **M. echinatum* Orchard (1) |
| | *M. integrifolium* Alliance Associates |
| | *M. glomeratum* Schindl. |
| | *M. callitrichoides* Alliance |
| | *M. callitrichoides* Orchard |
| | *M. pedunculatum* Alliance |
| | **M. amphibium* Labill. (1) |
| | **M. pedunculatum* Hook. f. (4) |
| | **M. tillaeoides* Diels (1) |
| | *M. pedunculatum* Alliance Associates |
| | **M. lophatum* Orchard (2) |
| | *M. austropygmaeum* Orchard |
| | **M. votschii* Schindl. (1) |
| | *M. pygmaeum* Mattf. |

regard to the monotypic *Meziella*, in recent phylogenetic analyses (Moody and Les 2007a), but the sampling of *Myriophyllum* was not comprehensive and ITS data alone proved ambiguous. *Meziella* is similar in habit to *Myriophyllum* but possesses hermaphrodite flowers (although described as functionally monoecious) and while forming four nutlets, they do not split at maturity due to a persistent exocarp (Orchard and Keighery 1993). The other aquatic Haloragaceae (*Proserpinaca* and *Meionectes*) are distinct, having perfect, two- or three-merous flowers with a nut and are only distantly related (Moody and Les 2007a).

A tribal status for *Myriophyllum* within Haloragaceae (Myriophylleae) was applied by Schindler (1905). Orchard (1975) supported this ranking and suggested that an elevation to subfamily might be warranted. Schindler's treatment of *Myriophyllum* recognized only 36 species, whereas recent treatments of the genus now distinguish about 68 (Orchard 1980, 1981, 1986; Aiken 1981; Yu et al. 2002). Schindler's (1905) classification included three subgenera, two sections, seven subsections and two series. Both Meijden (1969) and Orchard (1986) suspected Schindler's classification to be artificial. Orchard (1986) proposed seven distinct "Alliances" based on a range of characters for each group (Table 1). None of these treatments had an explicit phylogenetic basis.

Here we use Bayesian and parsimony analyses of multiple molecular data sets (nrDNA ITS and cpDNA *trnK* and *matK*) to: 1) examine phylogenetic relationships among *Myriophyllum* and evaluate species limits and subgeneric "Alliances" as currently proposed (Table 1); 2) establish a clade-based infrageneric classification scheme; 3) define morphological character states that delimit taxonomic groups within *Myriophyllum* and evaluate the potential parallelisms pertaining to sexual dimorphism and reductions in floral morphology; and 4) determine the utility of ITS as a de facto "barcode" among the often difficult-to-identify watermilfoils.

## MATERIALS AND METHODS

***Taxon Sampling***—Forty-three known species and multiple unknown or undescribed *Myriophyllum* taxa were sampled; *Meziella trifida* (Nees) Schindl., *Laurembergia repens* (L.) Berg., *Trihaloragis hexandrus* (F. Muell.) M. L. Moody & D. H. Les, *Gonocarpus montanus* (Hook. f.) Orchard, and *Haloragis digyna* Labill. also were sampled, the latter four as outgroup taxa (Appendix 1). Sampling included all *Myriophyllum* species known to Europe, North America, and South America, eight of 16 Asian species, 28 of 42 Australian species (mostly lacking narrow endemics), and lacked the two African endemic species. Most taxa were sampled from multiple accessions and, when possible, from across a wide geographic range (Appendix 1). Most taxa were collected in the field and NaCl-CTAB preserved (Rogstad 1992) while some were sampled from herbarium specimens (Appendix 1). The sampling covers most alliances proposed by Orchard (1986) and all of Schindler's (1905) subgenera and sections (Table 1).

We examined sequence data from the nrDNA ITS (here forward referred to as 'ITS') and cpDNA *matK* + *trnK* region (here forward referred to as 'cpDNA'). Three data sets were constructed: (1) ITS had 71 accessions (several not included in cpDNA data sets) including: a) multiple cloned copies from *M. papillosum* Orchard and *M. sibiricum* Komarov, which had polymorphisms, making direct sequencing techniques impractical and cloning techniques necessary (as described in Moody and Les 2002); b) multiple accessions of *M. crispatum* Orchard, *M. filiforme* Benth., *M. heterophyllum*, *M. laxum* Schuttlw. ex Chapm., *M. muricatum*, *M. pedunculatum*, *M. quitense* Kunth, *M. sibiricum*, *M. simulans* Orchard, and *M. variifolium* Hook. f., which showed variation in ITS, but not cpDNA, across their geographic range; and c) accessions of *M. amphibium* Labill. and *M. echinatum* Orchard, which did not amplify for cpDNA; (2) cpDNA included 60 accessions; (3) combined data included 60 accessions that were represented in both ITS and cpDNA data sets, sometimes using only a single

copy of ITS when multiple were found in a single taxon (in these cases ITS haplotypes formed an exclusive lineage; see Fig. 1).

***DNA Extraction, PCR, and Sequencing***—Total genomic DNA was extracted from fresh, NaCl-CTAB preserved, and herbarium specimen leaf material using a modified CTAB miniprep procedure (Doyle and Doyle 1987) or the Qiagen DNeasy Plant Minikit (Qiagen Inc., Valencia, California). Double-stranded DNAs were amplified using PCR following the protocols and conditions of Moody et al. (2001) to amplify the ITS-1, ITS-2, and 5.8S region of nuclear ribosomal DNA using the ITS4 and ITS5 primers or, in the case of several herbarium specimens, amplification was conducted on smaller segments using ITS2 and ITS5 to amplify the ITS-1 region and ITS3 and ITS4 to amplify the ITS-2 region (White et al. 1990). The cpDNA *trnK* introns and *matK* coding region were amplified using the primers trnK-3914F and trnK-2R (Johnson and Soltis 1994). Several additional primers were used to amplify *trnK* and *matK* from DNA of herbarium leaf material including matK68F, matK1872R (Johnson and Soltis 1994), matK900F (Moody and Les 2002), and trnKR and matK70R. Cycle sequencing of ITS used combinations of the ITS2, ITS3, ITS4, and ITS5 (White et al. 1990) primers. The *trnK* introns and *matK* region were sequenced using trnK3914F, matK68F, matK1872R, matK900F, trnK360F, trnK2R, matK70R, trnKR, and the Haloragaceae specific primer "trnk3F" (Moody and Les 2007a). Sequences were obtained using Big Dye terminator technology on an ABI 3100 automated sequencer (Applied Biosystems, Foster City, California).

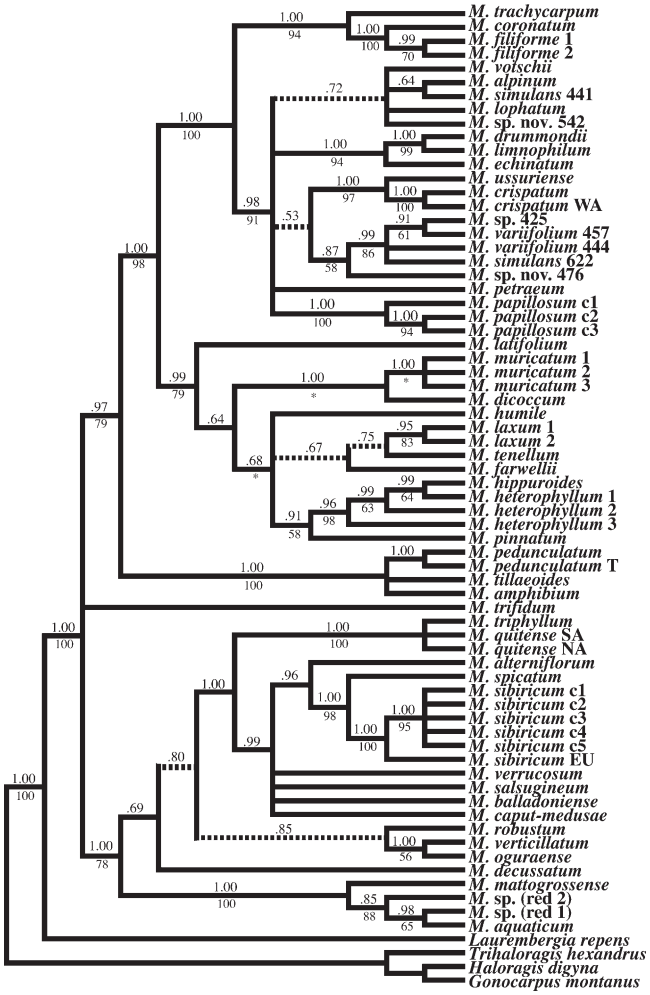


FIG. 1. Phylogenetic relationships in *Myriophyllum* as indicated by a majority rule consensus of 29,000 trees (after discarding burn-in) from Bayesian analysis of ITS sequence data analyzed under a GTR + I + Γ model. Harmonic mean –ln likelihood = 6,956.49. Dashed lines refer to clades not resolved in the strict consensus of the parsimony analysis. Numbers above branches refer to posterior probabilities and numbers below branches are bootstrap support from parsimony analyses. When numbers are lacking below branches parsimony did not resolve clades or had bootstrap values < 50% supporting that node. Species accessions followed by c1, c2, etc. refer to multiple copies cloned from individuals. Species accessions followed by numbers refer to divergent genotypes. EU = Europe, NA = North America, SA = South America, T = Tasmania, WA = Western Australia. [* *M. muricatum* and *M. dicoccum* not included in bootstrap analyses of ITS data, as discussed in text, thus bootstrap not relevant].

***Phylogenetic Analyses***—Sequences were edited for polymorphisms and/or variable sites using Sequencher 4.1.2 (Gene Code Corp., Ann Arbor, Michigan) and aligned manually using a similarity (Simmons 2004) and event-based approach (Morrison 2006). Alignment minimized indel events and sequence similarity was used to identify gap boundaries and optimize multiple alignment possibilities within gaps when necessary using MacClade 4.06 (Maddison and Maddison 2003). Combined data had 1.42% (cpDNA 1.13%; ITS 2.53%) of sites coded as missing data. The *trnK*3 intron could not be amplified for *M. dicoccum* and *M.* sp. nov. 542 and ITS 1 could not be amplified for *M. dicoccum*. Parsimony analyses were performed with PAUP* 4.0b10 (Swofford 2002) using heuristic searches with random taxon addition sequences and tree bisection-reconnection with unordered, equally weighted characters and 1,000 analysis replicates. Indels were treated as missing data and invariant indel regions were removed. Standard measures of homoplasy, ensemble consistency index (CI; Kluge and Farris 1969), ensemble rescaled consistency index (RC), ensemble retention index (RI; Farris 1989), and level of internal support (bootstrap values; Felsenstein 1985) were calculated using PAUP* 4.0b10. Bootstrap analyses were conducted using 1,000 replicate heuristic searches as above except for analyses of ITS where saved trees were limited to 500 for tree scores > 1,000.

Bayesian MCMC analyses (Yang and Rannala 1997) were performed on each data set using MrBayes v3.1.2 (Huelsenbeck and Ronquist 2001). The ITS and cpDNA *trnK* and *matK* regions (*matK* was treated with each codon position as a separate data set using an individual best-fit model for each) were each initially examined to determine the best-fit model using MrModeltest v1.1b (Nylander 2003) as determined by the likelihood ratio test (Felsenstein 1988). Bayesian analyses were performed with the best-fit model for each character partition twice for $3.0 \times 10^7$ generations. Markov Chain Monte Carlo was implemented with four heated chains and trees were sampled every 1,000 generations. Trace plots of likelihood scores were used to determine when stationarity was reached and compared from independent runs to assess convergence. Consensus trees recovered from each individual run were visually compared for topology and posterior probability and we also compared split frequencies using AWTY (Nylander et al. 2008) to further determine consensus among duplicate analyses of each of the three data sets analyzed. The first 1,000 trees were discarded as burn-in, with the remaining trees used to generate a 50% majority rule consensus tree where the percentage of the nodes recovered represented each node's posterior probability (PP). Nodal support was determined using Bayesian PP ≥ 0.95 as the criterion for strong support.

***Incongruence***—Although data were combined regardless of outcome (see Discussion Below) incongruence was examined between ITS (reduced to include only taxa in cpDNA) and cpDNA. Congruence of data was tested using the incongruence length difference test (ILD; Farris et al. 1995) as implemented in PAUP* 4.0b10. ITS and cpDNA data were analyzed using 1,000 homogeneity replicates with heuristic searches as described above under parsimony analysis. Incongruence also was determined visually by comparing tree topologies. Where incongruence was detected, the conflicting branches were evaluated for relative support given parsimony bootstrap and Bayesian posterior probabilities and were discussed individually.

***Analysis of Character Evolution***—Character states were optimized on the phylogenetic hypothesis resulting from our Bayesian analysis of combined data so relative branch lengths, as determined by Bayesian analysis, could be incorporated for ML analysis of ancestral states. Two unidentifiable taxa (*M.* sp. "red 1" and *M.* sp. "red 2") were trimmed from the tree given our lack of floral characters for these taxa. Ancestral state optimization was performed using likelihood methodologies implemented in the program Mesquite 1.04 (Maddison and Maddison 2004). We chose to use a one-rate model following the observations of Mooers and Schluter (1999). The ML model used for the analysis of the morphological data was Mk1 (Lewis 2001). Key characters traditionally associated with *Myriophyllum* taxonomy (stamen number, sepals present/absent, and degree of sexual dimorphism) were optimized. Character states were compiled from several literature sources (Schindler 1905; Meijden 1969; Orchard 1975, 1980, 1981, 1986; Aiken 1981; Yu et al. 2002) and herbarium specimens. In some cases sexual dimorphism was reported to be somewhat labile with occasional cases of dioecy in primarily monoecious taxa or the reverse as well as some inconsistency in the reporting of presence/absence of hermaphrodite flowers. In these cases taxa were coded for their primary character

state based on the most current treatments and observations from specimens used in these analyses.

## Results

The ITS data set (71 accessions) consisted of 644 bp of aligned sequence. There were 337 variable characters with 260 parsimony informative (including outgroup taxa). The cpDNA data set (60 accessions) *trnK* 5′ intron had 735 bp aligned sequence of which 203 sites were variable and 103 parsimony informative. The *matK* data set had 1,503 bp aligned of which 414 sites were variable and 194 parsimony informative. The *trnK* 3′ data set had 146 bp aligned of which 61 sites were variable and 33 parsimony informative. Data sets are available on TreeBASE (study number S2323).

***Parsimony Results*—**Parsimony analysis of the ITS data resulted in 9,949 equally parsimonious trees in three islands of 1,196 steps (CI = 0.492, RC = 0.386, RI = 0.785). When *M. dicoccum* and *M. muricatum* were removed ITS data resulted in 15,674 equally parsimonious trees of 1,110 steps (CI = 0.512, RC = 0.405, RI = 0.791). Parsimony analysis of the cpDNA data resulted in 520 equally parsimonious trees of 1,095 steps (CI = 0.742, RC = 0.658, RI = 0.886). The combined analysis resulted in 128 equally parsimonious trees of 2,265 steps (CI = 0.612; RC = 0.498, RI = 0.813). For cpDNA and combined analyses parsimony results were comparable to Bayesian analyses, but with less resolution. Bootstrap is not directly comparable to Bayesian PP, but in general bootstrap support provides lower values than for Bayesian PP (Cummings et al. 2003; Douady et al. 2003) as was the case here. For ITS a long branch for the clade including *M. dicoccum* and *M. muricatum* was resolved and the placement of the clade was incongruent with results from Bayesian analyses. The clade was placed sister to *M*. subg. *Brachytheca* rather than within the clade (BS < 50%; not shown). This is in disagreement with Bayesian analysis of ITS and all other analyses of all data sets which agreed on the position of this clade (Figs. 1–3). We attributed this incongruent result to long-branch attraction (Felsenstein 1978). Given that most other relationships among taxa were not incongruent between parsimony and Bayesian analyses these taxa were removed from the data set to perform bootstrap analyses of the ITS data. In general, ITS data provided lower resolution under parsimony than Bayesian analyses (Figs. 1–3).

***Bayesian Results*—**Posterior probability distributions of 29,000 sampled trees were obtained for each Bayesian analysis using a best-fit ML models with defined parameters (Table 2). Visual comparison of the majority consensus trees from the two separate runs for each data set disclosed no major discrepancies between tree topologies or PP nodal support. Comparisons of split frequencies using AWTY (Nylander et al. 2008) also supported consensus among multiple runs. Final trees represented the majority rule consensus of 29,000 trees, conservatively discarding the first 1,000 (one million generations) as burn in (Figs. 1–3). ML parameters and likelihood scores are presented in Table 2. Bayesian estimated branch lengths are represented on phylograms based on consensus results from analyses of each data set (Fig. 4). Bayesian results for ITS were incongruent with parsimony analyses in the placement of *M. dicoccum* and *M. muricatum* as discussed above.

***Phylogenetic Relationships*—**All Bayesian analyses resolved two major clades within *Myriophyllum*, *M*. subg. *Brachytheca*, and *M*. subg. *Myriophyllum* (Figs. 1–3). *Myriophyllum* subg. *Meziella* was resolved as sister to *M*. subg. *Brachytheca* in the combined and cpDNA analyses (Figs. 2, 3), whereas *M*. subg. *Meziella* was part of a polytomy with *M*. subg. *Brachytheca* and *M*. subg. *Myriophyllum* in ITS analyses (Fig. 1).

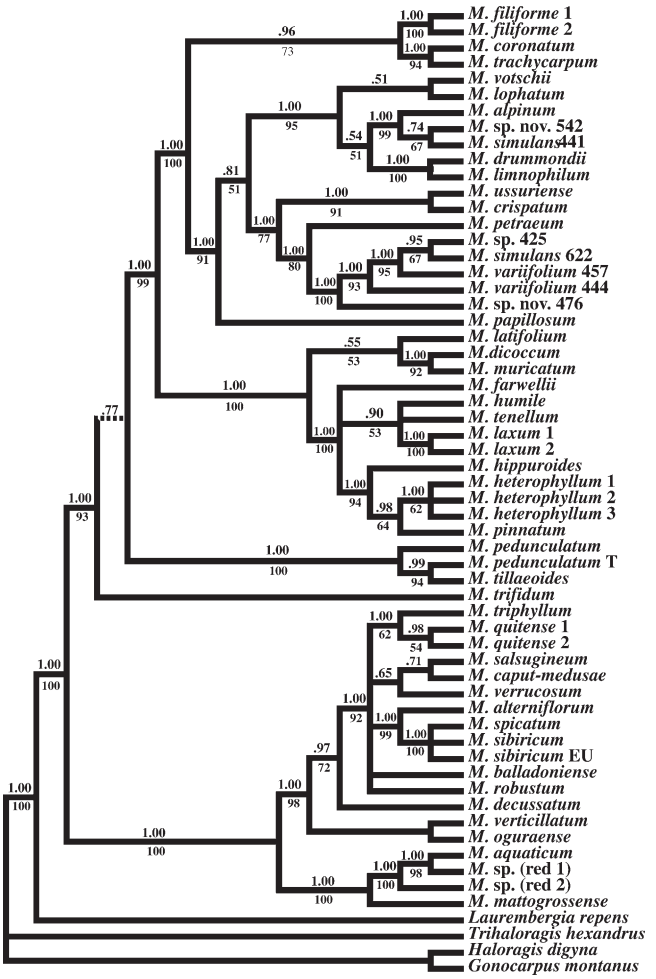


Fig. 2. Phylogenetic relationships in *Myriophyllum* as indicated by a majority rule consensus of 29,000 trees (after discarding burn-in) from Bayesian analysis of cpDNA (*trnK* 5′ intron, *trnK* 3′ intron and *matK*) sequence data analyzed under models defined in Table 2. Harmonic mean –ln likelihood = 10,634.40. Dashed lines refer to clades not resolved in the strict consensus of the parsimony analysis. Numbers above branches refer to posterior probabilities and numbers below branches are bootstrap support from parsimony analyses. When numbers are lacking below branches parsimony did not resolve clades or had bootstrap values < 50% supporting that node. Numbers after species refer to divergent genotypes among accessions fitting species descriptions. EU = Europe, NA = North America, SA = South America, T = Tasmania.

*Myriophyllum* subg. *Myriophyllum*—This subgenus had two well-supported sister clades using cpDNA and combined data: 1) *M*. sect. *Pectinatum* had two South American species (*M. aquaticum* and *M. mattogrossense*) and two undescribed taxa of unknown geographic origin and 2) *M*. sect. *Myriophyllum* contained several geographically diverse species. ITS data provided only weak support for *M*. sect. *Myriophyllum* and resolved *M. decussatum* Orchard as sister to the rest of *M*. sect. *Myriophyllum* (Fig. 1), whereas cpDNA and combined analyses provide an alternative hypothesis regarding this taxon (Figs. 2, 3).

All analyses supported *M. verticillatum* L. and *M. oguraense* Miki as a clade (Figs. 1–3) within *M*. sect. *Myriophyllum*. ITS supported *M. robustum* Hook. f. as sister to this clade only

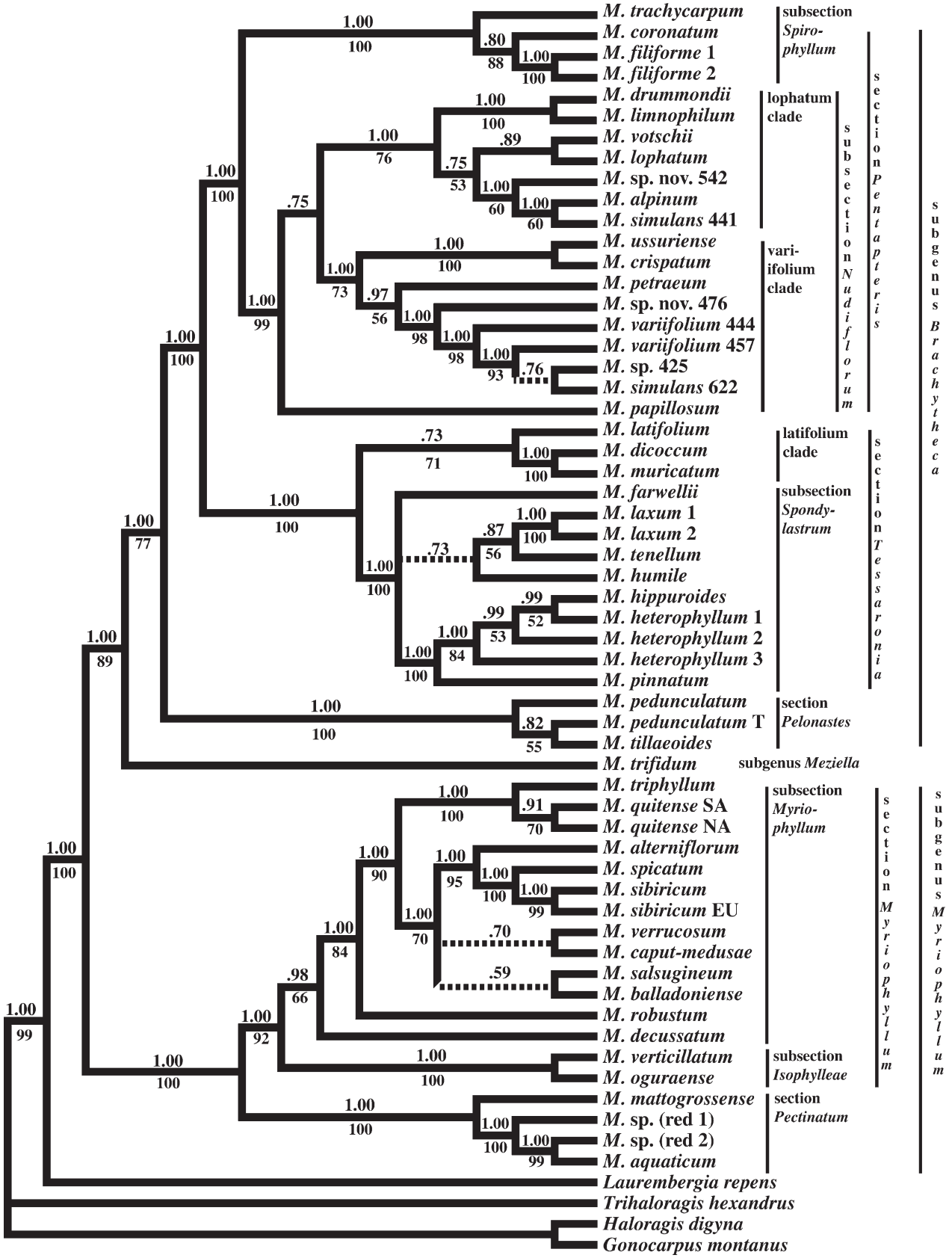


FIG. 3. Phylogenetic relationships in *Myriophyllum* as indicated by a majority rule consensus tree of 29,000 trees (after discarding burn-in) from Bayesian analysis of combined ITS and cpDNA sequence data analyzed under models as defined in Table 2. Harmonic mean –ln Likelihood = 17,532.97. Dashed lines refer to clades not resolved in the strict consensus of the parsimony analysis. Numbers above branches refer to posterior probabilities and numbers below branches are bootstrap support from parsimony analyses. When numbers are lacking below branches parsimony did not resolve clades or had bootstrap values < 50% supporting that node. Numbers after species refer to unique genotypes among accessions fitting species descriptions. Clade-based classification system is represented along the right margin. EU = Europe, NA = North America, SA = South America, T = Tasmania.

TABLE 2. Average likelihood model parameters estimated for Bayesian analyses of each of the 6 data partitions in the combined data (columns 2–6) and the ITS data with additional taxa (column 7) after the first 1,000 trees were discarded as burn in. Harmonic mean –ln likelihoods are: combined–17532.97; cpDNA–10634.40; ITS–6956.49. The *matK* data were partitioned into first, second and third codon positions.

| | *trnK5* | *trnK3* | *matK* pos1 | *matK* pos2 | *matK* pos3 | ITS | ITS |
|---|---|---|---|---|---|---|---|
| **model** | GTR + Γ | GTR + Γ | GTR + Γ | GTR + I + Γ | GTR + Γ | GTR + I + Γ | GTR + I + Γ |
| **C > T** | 1.260 | 1.713 | 1.496 | 1.203 | 1.169 | 4.174 | 4.271 |
| **C > G** | 0.625 | 1.317 | 2.156 | 1.202 | 0.849 | 0.598 | 0.410 |
| **A > T** | 0.299 | 0.155 | 0.141 | 0.286 | 0.326 | 2.077 | 1.953 |
| **A > G** | 0.815 | 1.605 | 1.381 | 1.445 | 1.081 | 2.163 | 1.614 |
| **A > C** | 0.683 | 1.464 | 1.619 | 1.538 | 1.104 | 0.996 | 0.723 |
| **A** | 0.347 | 0.311 | 0.327 | 0.301 | 0.295 | 0.207 | 0.212 |
| **C** | 0.145 | 0.156 | 0.185 | 0.152 | 0.216 | 0.305 | 0.325 |
| **G** | 0.195 | 0.184 | 0.143 | 0.140 | 0.173 | 0.285 | 0.269 |
| **T** | 0.313 | 0.349 | 0.345 | 0.407 | 0.317 | 0.204 | 0.194 |
| α | 0.467 | 1.201 | 0.350 | 1.264 | 0.455 | 0.848 | 1.097 |
| **inv.** | ——– | ——— | ——– | 0.142 | —— | 0.597 | 0.291 |

under Bayesian analyses with weak support, whereas cpDNA and combined data included *M. robustum* within *M.* subsect. *Myriophyllum*. Relationships among members of *M.* subsect. *Myriophyllum* generally were not well-supported. There was strong support in all analyses for a clade including *M. quitense* and *M. triphyllum* Orchard and a clade with three northern hemisphere taxa (*M. alterniflorum* DC. sister to *M. spicatum* and *M. sibiricum*).

*MYRIOPHYLLUM* SUBG. *BRACHYTHECA*—This subgenus was resolved in all analyses with *M.* sect. *Pelonastes* well-supported as sister to the rest of the subgenus (Figs. 1–3). *Myriophyllum* sect. *Tessaronia* branched next and was well-supported in all Bayesian analyses including the Austral-Asian species (*M. dicoccum*), Australian species (*M. latifolium* F. Muell., *M. muricatum*) and the North American endemics, *M.* subsect. *Spondylastrum*. The North American endemics formed a well-supported clade in all analyses (Figs. 1–3), but relationships among most of these taxa were generally not well-supported.

Sister to *M.* sect. *Tessaronia* was *M.* sect. *Pentapteris*, which was well-supported in all analyses (Figs. 1–3) with two subsections *M.* subsect. *Spirophyllum* and *M.* subsect. *Nudiflorum*. Combined and cpDNA resolved a sister relationship of *M. papillosum* to the rest of *M.* subsect. *Nudiflorum* with weak support, whereas ITS included *M. papillosum* as part of a polytomy with other members of *M.* subsect. *Nudiflorum*. Resolution within the "variifolium + lophatum" clade was not well-supported and in some cases there was well-supported incongruence between ITS and cpDNA. Taxa with characteristics of *M. simulans* Orchard were polyphyletic and *M. variifolium* paraphyletic. Within *M.* sect. *Pentapteris* there is consensus for three major clades using cpDNA and combined data: 1) the "variifolium" clade; 2) the "lophatum" clade; and 3)

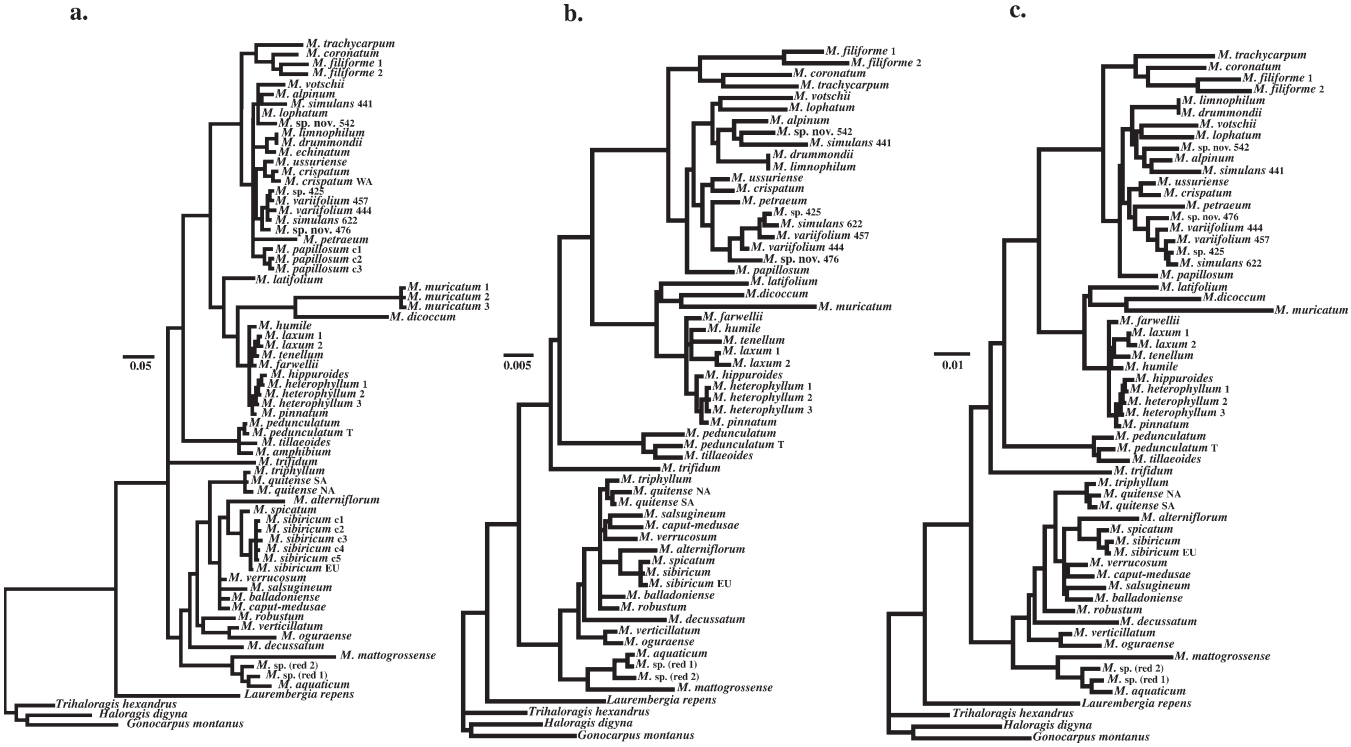


FIG. 4. Phylogenetic relationships in *Myriophyllum* as indicated by phylograms of majority rule consensus tree of 29,000 trees (after discarding burn-in) from Bayesian analyses of: a) ITS data; b) cpDNA data; c) Combined ITS and cpDNA data. Branch lengths are proportional.

*M.* subsect. *Spirophyllum* (Fig. 3). ITS data alone did not support all of these clades (Fig. 1).

Emphasis was placed on widespread sampling of accessions from the Australian taxa in Orchard's (1986) "*M. propinquum* Alliance" (including *M. alpinum*, *M. crispatum*, *M. simulans*, and *M. variifolium*) due to the high morphological variability among most of these taxa. Divergent ITS and/or cpDNA genotypes were found among all these species, except *M. alpinum*. Some taxa not strictly conforming to the morphological description of previously recognized species were recognized to have unique genotypes. In cases where there is strong support for independent lineages (thus supporting a hypothesis of reproductive isolation) coupled with unique morphology we make recommendations for new species.

## Discussion

***Data and Phylogenetic Analyses***—Incongruence length difference (ILD) tests found significant incongruence between the ITS and cpDNA data. Both empirical and simulated data (Dolphin et al. 2000; Yoder et al. 2001; Barker and Lutzoni 2002) have demonstrated that this test has suggested noncombinability of data when data performed better if partitions were combined. The ILD test does appear to be conservative with low susceptibility to type II error, thus a simple way to initially examine data partitions if congruence is not rejected. When congruence is rejected there can be a number of reasons involved with rates of molecular evolution between data sets (Dolphin et al. 2000; Darlu and Lecointre 2002; Barker and Lutzoni 2002). Bayesian analyses with case appropriate ML models for individual partitions of data can help lessen some problems associated with combining molecular data sets that evolve under different evolutionary models (Nylander et al. 2004). This approach was used in these combined data analyses.

Incongruence may also exist between the nuclear and plastid genomes as a consequence of hybridization or lineage sorting. Recent hybridization among *Myriophyllum* species has been documented genetically (Moody and Les 2002) and has been suggested to occur among members of Orchard's (1986) "*M. propinquum* Alliance". Ploidy level is not known for most *Myriophyllum*, but some chromosome counts have been completed (*M. alterniflorum* [$2n = 14$], *M. quitense* [$2n = 42$], *M. sibiricum* [$2n = 42$], *M. spicatum* [$2n = 42$], *M. ussuriense* [$2n = 14, 21$], and *M. verticillatum* [$2n = 28$]; Löve and Löve 1958; Löve and Ritchie 1966; Löve 1978; Ceska et al. 1986) with an apparent base number of $n = 7$ and polyploids not uncommon. However, we do not find incongruence between data sets regarding the polyploid taxa ($2n = 28, 42$) that would suggest an allopolyploid history nor did we find many instances in which multiple divergent copies of ITS were identified, but where they were found (e.g. *M. sibiricum*, *M. papillosum*) they formed a monophyletic lineage. This does not preclude the potential of allopolyploid origins for these taxa, as ITS can homogenize over time in allopolyploids to one parental genotype (Wendel et al. 1995), but evidence of an allopolyploid history does not manifest itself through incongruence regarding these known polyploid taxa.

Where incongruence was found in phylogenetic hypotheses between plastid and nrDNA data sets regarding specific taxa (i.e. *M. robustum*, *M. decussatum*, *M. petraeum*) nodal support for the relationship in one or both data sets was generally weak, thus making the result of incongruence ambiguous. Some cases of incongruence had strong support for alternative hypotheses provided by each data set and these cases usually involved relationships at tips of the phylogeny (i.e. *M. tillaeoides*, *M. hippuroides*) among closely related species. These cases may represent hybrid origins, but the data do not discount the alternative hypotheses of lineage sorting, thus additional data to test a hybrid hypothesis will be needed. Where there was incongruence between nuclear and plastid DNA phylogenies, each case will be discussed individually. Long-branch attraction (Felsenstein 1978) involving *M. dicoccum* and *M. muricatum* (see Fig. 4) also was likely to be associated with at least some of the incongruence observed between ITS and cpDNA data sets detected using ILD, a parsimony based approach.

***Myriophyllum Systematics***—Neither Meijden and Caspers (1971) nor Orchard (1986) adopted a formal subgeneric classification system for *Myriophyllum* and both authors regarded Schindler's (1905) subgeneric system to be largely artificial (Meijden and Caspers 1971; Orchard 1986). Our findings also often contradict Schindler's classification. Although Orchard (1986) recognized "Alliances," his system was informal and not based explicitly on phylogenetic hypotheses. Despite the historical difficulties with subgeneric classification of *Myriophyllum*, we believe that our phylogenetic results, which strongly support several nested clades, allow us to formally recognize three subgenera, five sections, and five subsections (see Table 3 and taxonomic discussion below). Thus we propose a formal subgeneric classification, which conserves most of Schindler's (1905) nomenclature (with some major realignments) and we recognize types, absent from Schindler's (1905) treatment. The subgeneric names (Table 3) are used in our ensuing discussion of phylogenetic relationships. In some cases, where relationships among clades remained unresolved (or required further sampling), we have used clade-based terminology instead of applying formal taxonomic categories.

***Taxonomic Status of Meziella***—The status of the monotypic Western Australian Declared Rare Flora (DRF) *Meziella* was ambiguous until recently. Prior to its recent rediscovery by Orchard and Keighery (1993) this taxon was known from a single specimen collected in 1901 in southwest Western Australia and thought to be extinct. Schindler (1905) erected the genus, but prior to that the taxon had been included in *Gonocarpus* (1844) and *Haloragis* (1846). Given their access to limited plant material, both Schindler (1905) and Orchard (1975) tentatively placed *Meziella* in a position near *Haloragis*. With fresh flowering and fruiting material Orchard and Keighery (1993) assessed a close relationship of *Meziella* with *Myriophyllum*. Moody and Les (2007a) found *Myriophyllum* to be paraphyletic in regard to *Meziella* and recommended a more thorough sampling of *Myriophyllum* to further evaluate the status of the former genus.

*Meziella* and *Myriophyllum* share a four-loculed ovary forming four nutlets (pyrenes), which is distinctive among Haloragaceae. In *Myriophyllum* the nutlets usually dehisce at fruit maturity, while in *Meziella* they are indehiscent due to a persistent hardened exocarp (Orchard and Keighery 1993); although the hardened exocarp is similar to that of some *Myriophyllum* species (e.g. *M. decussatum*, *M. muricatum*) described as having "tardy" dehiscence (Orchard 1986, personal observation). *Meziella* fruits also are distinctively ornamented with the persistent sepals becoming long woody spines and elongate spines forming on the exocarp; however, extreme fruit ornamentation in the form of spines also is found

Table 3. Subgeneric classification for *Myriophyllum* using phylogenetic hypotheses from combined nrDNA ITS and cpDNA *trnK* + *matK* data (unless otherwise denoted) and species continental distribution. Unknown taxa are those not sampled for molecular data and whose placement remains unclear given morphology alone. All known *Myriophyllum* species are included. [* not included in molecular analyses but likely placement based on morphological evidence and/or affinities suggested by Orchard (1986)]. Afr = Africa, Aus = Australia, Eu = Europe, NA = North America, SA = South America. This comprehensive list is based on multiple sources (Australian Plant Names Index [APNI], http://www.anbg.gov.au/cgi-bin/apni; Meijden 1969; Meijden and Caspers 1971; Orchard 1975, 1980, 1981, 1986; Aiken 1981; Yu et al. 2002).

| Subgeneric classification | Continental distribution |
|---|---|
| **1) *Myriophyllum* subgenus *Myriophyllum*** | |
| **A. *M.* section *Myriophyllum*** | |
| **1. *M.* subsection *Myriophyllum*** | |
| *M. alterniflorum* DC. | Northern Hemisphere: Asia, EU, NA |
| *M. balladoniense* Orchard | Aus |
| *M. caput-medusae* Orchard | Aus |
| *M. decussatum* Orchard | Aus |
| *M. porcatum* Orchard* | Aus |
| *M. quitense* Kunth | NA, SA |
| *M. robustum* Hook. f. | Aus |
| *M. salsugineum* Orchard | Aus |
| *M. sibiricum* Komarov | Northern Hemisphere: Asia, EU, NA |
| *M. spicatum* L. | All continents (except Antarctica); endemic Asia, EU |
| *M. triphyllum* Orchard | Aus |
| *M. verrucosum* Lindl. | Aus |
| **2. *M.* subsection *Isophylleae*** | |
| *M. oguraense* Miki | Asia |
| *M. verticillatum* L. | Northern Hemisphere: Asia, EU, NA |
| **B. *M.* section *Pectinatum*** | |
| *M. aquaticum* (Vell.) Verdc. | All continents (except Antarctica); endemic SA |
| *M. mattogrossense* Hoehne | SA |
| **2) *M.* subgenus *Meziella*** | |
| *M. trifidum* (Nees) M.L. Moody & D.H. Les | Aus |
| **3) *M.* subgenus *Brachytheca*** | |
| **A. *M.* section *Pelonastes*** | |
| *M. amphibium* Labill. | Aus |
| *M. pedunculatum* Hook. f. | Aus |
| *M. tillaeoides* Diels | Aus |
| **B. *M.* section *Tessaronia*** | |
| *M. dicoccum* F. Muell. | Asia, Aus |
| *M. exasperatum* D. Wang, D. Yu & Z. Yu Li* | Asia |
| *M. latifolium* F. Muell. | Aus |
| *M. muricatum* Orchard | Aus |
| *M. tuberculatum* Roxb.* | Asia |
| **1. *M.* subsection *Spondylastrum*** | |
| *M. farwellii* Morong | NA |
| *M. heterophyllum* Michx. | Asia, Eu, NA; endemic NA |
| *M. hippuroides* Nutt. ex Torr. & A. Gray | NA |
| *M. humile* (Raf.) Morong | NA |
| *M. laxum* Schuttl. ex Chapm. | NA |
| *M. pinnatum* Britton, Sterns & Poggenb. | NA |
| *M. tenellum* Bigelow | NA |
| **C. *M.* section *Pentapteris*** | |
| **1. *M.* subsection *Spirophyllum*** | |
| *M. bonii* Tardieu* | Asia |
| *M. coronatum* Meijden | Aus |
| *M. costatum* Orchard* | Aus |
| *M. filiforme* Benth. | Aus |
| *M. implicatum* Orchard* | Aus |
| *M. mezianum* Schindl.* | Afr |
| *M. siamense* (Craib) Tardieu* | Asia |
| *M. striatum* Orchard* | Aus |
| *M. trachycarpum* F. Muell. | Aus |
| **2. *M.* subsection *Nudiflorum*** | |
| *M. alpinum* Orchard | Aus |
| *M. austropygmaeum* Orchard* | Aus |
| *M. crispatum* Orchard | Aus |
| *M. drummondii* Benth. | Aus |
| *M. echinatum* Orchard | Aus |
| *M. gracile* Benth.* | Aus |
| *M. integrifolium* Hook. f.* | Aus |
| *M. lapidicola* Orchard* | Aus |
| *M. limnophilum* Orchard | Aus |
| *M. lophatum* Orchard | Aus |
| *M. papillosum* Orchard | Aus |
| *M. petraeum* Orchard | Aus |
| *M. propinquum* A. Cunn.* | Aus |
| *M. pygmaeum* Mattf.* | Aus |
| *M. simulans* Orchard | Aus |
| *M. ussuriense* Maxim. | Asia, NA |
| *M. variifolium* Hook. f. | Aus |
| *M. votschii* Schindl. | Aus |
| **Unknown (not sampled)** | |
| *M. artesium* Halford & Hensham | Aus |
| *M. axilliflorum* Baker | Afr. |
| *M. callitrichoides* Orchard | Aus |
| *M. glomeratum* Schindl. | Aus |
| *M. indicum* Willd. | Asia |
| *M. muelleri* Sonder | Aus |
| *M. oliganthum* (Wight & Arn.) F. Muell. | Asia |
| *M. tetrandrum* Roxb. | Asia |

among *Myriophyllum* species (e.g. *M. coronatum* Meijden, *M. muricatum*). The characteristic trifid submerged leaves of *Meziella* also occur in members of *M.* sect. *Pelonastes*, which branches subsequent to *Meziella* in our molecular phylogeny (Fig. 3). Both *Myriophyllum pedunculatum* and *M. tillaeoides* primarily have linear leaves but also have some submerged leaves that become trifid, a trait uncommon in *Myriophyllum*, but shared with *Meziella*. Although other character states found in *Meziella* are not common among Haloragaceae (e.g. apiculate stamens, four stamens) or within *Myriophyllum* (e.g. all hermaphrodite flowers), they all occur (but only simultaneously in *M. mattogrossense*) within some *Myriophyllum* species (Orchard and Keighery 1993).

Our data strongly support not only a close relationship of *Meziella* and *Myriophyllum* but further indicate that *Myriophyllum* is paraphyletic with regard to *Meziella*. Despite its lack of dehiscent nutlets, *Meziella* is resolved with strong support by cpDNA and combined analyses as sister to *M.* subg. *Brachytheca* (PP = 1.00; BS = 93, 89 respectively). ITS data alone remain ambiguous as to the placement of *Meziella* within *Myriophyllum*. Using ITS alone, Moody and Les (2007a) found a weakly supported sister group relationship of *Meziella* with *Myriophyllum* using Bayesian analysis, a relationship not supported by parsimony analysis, which placed *Meziella* as part of a polytomy with *Myriophyllum* species. That study included a larger family wide sampling, but a less inclusive sampling of *Myriophyllum*. When the expanded *Myriophyllum* sampling is added to the Moody and Les (2007a) ITS data set the results remain ambiguous (not shown). Given the strong

phylogenetic evidence (including shared morphology) for the transfer of *Meziella* to *Myriophyllum*, we accommodate this taxon by the new combination *Myriophyllum trifidum* (Nees) M. L. Moody and D. H. Les (=*Meziella trifida* (Nees) Schindler) and recognition of a monotypic subgenus: *Myriophyllum* subg. *Meziella* (Schindl.) M. L. Moody and D. H. Les (see taxonomic revision below).

***Myriophyllum (Higher Level Relationships)***—A fundamental split of *Myriophyllum* into distinct clades is evident here (Fig. 3) and we recognize three subgenera to accommodate them (Table 3). Schindler's (1905) treatment also divided *Myriophyllum* into three subgenera (Table 1), but the taxonomic alignments within his subgenera are not entirely supported by our analyses. *Myriophyllum* subg. *Myriophyllum* (Fig. 3; Table 3) corresponds well to Schindler's (1905) *M.* subsect. *Spondylophyllum* + *M.* subsect. *Leiocarpium* (Table 1), but his inclusion of *M. propinquum* A. Cunn. (then much more broadly defined to include Australian taxa [in part *M. crispatum*, *M. simulans*, and *M. variifolium*]) and *M. ussuriense* Maxim. as allied with the other taxa in *M.* subsect. *Spondylophyllum*, is strongly discordant with our phylogenetic results. Our realigned *M.* subg. *Brachytheca* (Fig. 3; Table 3) includes species distributed throughout the major sections of Schindler's (1905) *Myriophyllum* classification. Schindler's subgeneric classification heavily emphasized the presence or absence of hermaphrodite flowers to distinguish his two most species-rich sections, and relied primarily on floral morphology to further delimit segregates. However, our phylogenetic results coupled with ancestral character state optimization indicate that several of the reproductive characteristics shared among disparate *Myriophyllum* are a consequence of parallelism, convergence, or plesiomorphy rather than synapomorphy (Fig. 5). This observation supports Meijden (1969) who concluded that infrageneric classification of *Myriophyllum* has likely been confounded by misleading patterns of character evolution.

Although Orchard (1986) also did not recognize the same fundamental groupings of species within *Myriophyllum* indicated by our phylogenetic analysis, his "*M. aquaticum* Alliance" and "*M. salsugineum* Alliance" taken together encompass all the taxa in *M.* subg. *Myriophyllum*. A notable exception is that he recognized *M. heterophyllum* and *M. hippuroides* Nutt. ex Torr. & A. Gray as loosely allied to the "*M. aquaticum* Alliance", whereas our phylogenetic analyses clearly support their inclusion in *M.* subg. *Brachytheca*, which encompasses Orchard's (1986) other seven "Alliances" (Table 1). So far we have been unable to identify morphological attributes that are exclusive to either of these clades, a factor that helps to explain their lack of recognition in earlier treatments of the genus (Schindler 1905; Meijden 1969; Meijden and Caspers 1971; Orchard 1986).

***Myriophyllum Subgenus Myriophyllum***—This subgenus has a world-wide distribution. Most species are widespread or relatively common in their range, with only few maintaining a narrow endemism (e.g. *M. balladoniense* Orchard, *M. decussatum*, *M. porcatum* Orchard). This clade includes all the naturally widespread northern hemisphere species (*M. alterniflorum*, *M. sibiricum*, *M. verticillatum*) and all the South American species (*M. aquaticum*, *M. mattogrossense*, *M. quitense*). Also included are two wide-spread invasive species (*M. aquaticum*, *M. spicatum*) whose natural distributions are limited, but which are widespread outside their native range. Although no one morphological feature has been identified that distinguishes all members of this clade, most have strictly whorled submerged leaves (opposite in *M. decussatum*), and the linear emergent leaves that are common among the other subgenera are found only in *M. balladoniense*.

***Myriophyllum Section Pectinatum***—This clade is sister to the rest of *M.* subg. *Myriophyllum* and is supported in all analyses. Morphologically, *M. mattogrossense* and *M. aquaticum* share an emergent inflorescence with all leaves pectinate. This characteristic is uncommon in *Myriophyllum,* although strictly pectinate emergent leaves also are found in the next branching *M.* subsect. *Isophylleae*, as well as in *M. robustum* and the distantly related *M. alpinum*. *Myriophyllum aquaticum* and *M. mattogrossense* also are the only species of *Myriophyllum* endemic to South America (although there is some debate concerning the status of *M. quitense* in North America; see below).

*Myriophyllum mattogrossense* has been poorly collected, but is known to range widely across South America and likely is more common than currently known (Orchard 1981; Orchard and Kasselman 1992). Outside of some collections from Ecuador (Orchard and Kasselman 1992), *M. mattogrossense* has been described as a submerged species with only its inflorescence becoming emergent. In contrast, *Myriophyllum aquaticum* is dioecious and frequently found in an emergent form producing dense populations (the submersed leaves often deteriorate when the plants grow in standing water). This species, which has been sold commonly in the ornamental aquatic plant trade, is invasive on most continents.

*Myriophyllum* sect. *Pectinatum* also includes two taxa (*M.* sp. "red 1" and *M.* sp. "red 2") that cannot be identified using any current taxonomic resource. These taxa were acquired from the aquatic plant trade in the U. S. A. (Maine and Washington) and Australia (Queensland) as "*M. mattogrossense*" or "*M. propinquum*", often with the common name "feather plant". The taxa are most closely related to *M. aquaticum* but are highly divergent in both ITS and cpDNA (Figs. 1–4). All nonindigenous *Myriophyllum aquaticum* specimens collected from geographically diverse locations in North America (Appendix 1) have identical ITS genotypes (they are strictly female plants) that are unique from these two taxa.

Although these *M. aquaticum*-like taxa clearly are distinct at the molecular level, defining them morphologically has remained elusive given that no sexual structures have yet been observed for either. Unfortunately, attempts to induce flowering so far have failed and the original locality data are not available for either taxon. These plants can survive in inundated soils with all leaves emergent, rigid, and pectinate. Some emergent forms of *Myriophyllum* sp. "red 1" were examined in cultivation growing adjacent to *M. aquaticum*. These cultivars closely resembled *M. aquaticum* but maintained phenotypes that were distinctly red and more compact in habit than *M. aquaticum*, whereas submerged forms of these plants were common to many *Myriophyllum* species and, unlike *M. aquaticum*, they do not appear to shed leaves with inundation. The emergent forms of these plants resemble those described for Ecuadorian *Myriophyllum* that have emergent vegetative forms, but were included under *M. mattogrossense* with a more inclusive definition of the species (Orchard and Kasselman 1992). Further examination of these cultivated plants in flower will be needed to compare to Ecuadorian taxa and also to exclude the possibility that their identity might lay among the more poorly known Asian species not included in these analyses (e.g. *M. tetrandrum* Roxb. and *M. exasperatum* D. Wang, D. Yu & Z. Yu Li), given that the Australian cultivar originated from the Asian plant trade. Although none

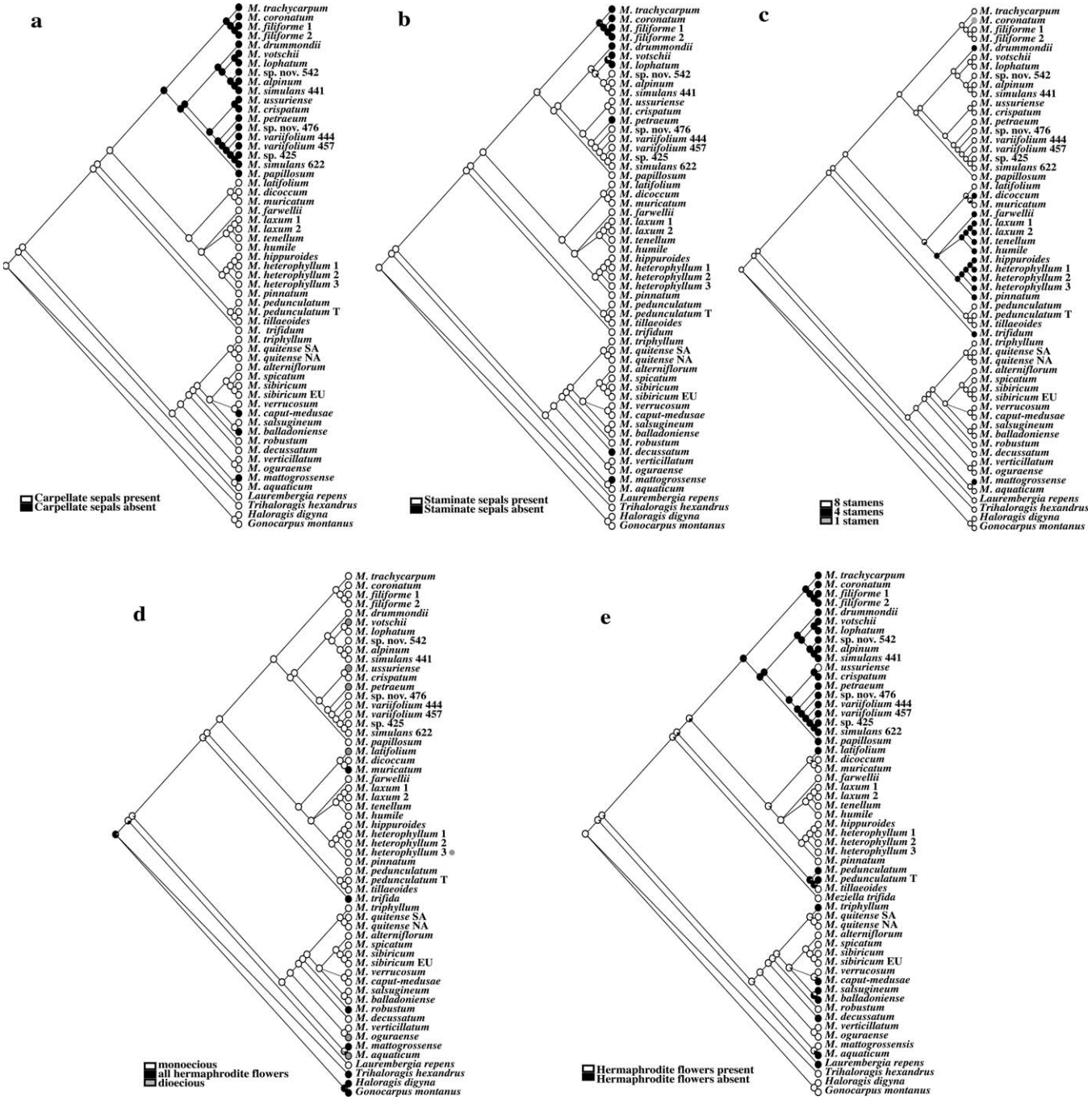


FIG. 5. Maximum likelihood ancestral character state reconstructions for *Myriophyllum* using the combined ITS and cpDNA phylogram from Fig. 4 (above) to account for branch lengths. Two taxa (*M.* sp. (red 1) and *M.* sp. (red 2)) were trimmed from trees due to lack of information regarding reproductive structures. The ancestral character state reconstructions are as follows: a) carpellate sepals: present = white, absent = black; b) staminate sepals: present = white, absent = black; c) stamen number: 8 = white, 4 = black, ≤ 2 = grey; d) sexual dimorphism: monoecious = white, dioecious = grey, morphological hermaphrodite = black; e) sexual dimorphism: some hermaphrodite flowers present = white, hermaphrodite flowers usually absent = black. Coloration of circles at nodes is proportional to the likelihood of representative character states being ancestral at the given node.

of the Asian species has been described to have the emergent vegetative form found in these taxa (Dong et al. 2002; Yu et al. 2002).

***Myriophyllum* Section *Myriophyllum***—This clade is strongly supported in combined and cpDNA analyses. All members were part of the "*M. salsugineum* Alliance" of Orchard (1986) except *M. balladoniense* for which the placement was considered uncertain and *M. robustum*, which was suspected to have an affinity with *M. aquaticum*. *Myriophyllum* sect. *Myriophyllum* includes two sister clades, *M.* subsect. *Myriophyllum* and *M.* subsect. *Isophylleae*. Although *M.* subsect. *Isophylleae* is supported by all analyses, Bayesian analyses of ITS resolve a sister group relationship with *M. robustum* (with a short branch and weak support), which is not resolved by parsimony or any analyses of cpDNA and combined data. Its affinity is most likely with *M.* subsect. *Myriophyllum* (discussed below). Kadono (1988) suspected a close relationship between the two taxa of *M.* subsect. *Isophylleae* (*M. oguraense* and *M. verticillatum*), which share the characteristic of having pectinate emergent leaves that are similar in shape to, but much smaller

than, the submerged leaves. Also, both have an elongate turion (vegetative overwintering bud). The primary characteristic that differentiates these taxa is the turion shape (club shaped in *M. verticillatum* and linear in *M. oguraense*; Weber and Nooden 1974; Kadono 1988). Until recently *M. oguraense* was believed endemic to Japan (Miki 1934; Kadono 1988); however, its recent discovery in China confirms a wider distribution in sympatry with its sister species *M. verticillatum*, found at the limits of its range in China and Japan (Yu et al. 2002).

*Myriophyllum verticillatum* is found throughout the northern temperate zone. Several varieties of *Myriophyllum verticillatum* have been proposed in North America (Fassett 1940; Fernald 1950) but these taxa later were regarded as artificial (Aiken 1979, 1981) and no molecular variation was found among a wide geographic sampling in the U. S. A. (Appendix 1). Vegetative forms of *M. verticillatum* have been mistaken for *M. heterophyllum*, *M. hippuroides*, and *M. spicatum* in North America, but its pectinate bracts and turion shape readily distinguish this species from all other North American taxa.

*Myriophyllum* subsect. *Myriophyllum* is here defined broadly to include *M. robustum* and *M. decussatum* which conflicts with results from Bayesian analyses of ITS (parsimony results are ambiguous). In general, ITS provides poor support for higher-level relationships among *M.* subg. *Myriophyllum* and the conflict observed in Bayesian analyses is not strongly supported. In cpDNA and combined analyses *M. decussatum* is sister to the rest of the members of this subsection. Orchard (1986) considered *M. decussatum* aberrant within the genus in many characteristics, but suggested it most likely was loosely allied with *M. salsugineum* due to its emergent leaf shape. Our results do not contradict that hypothesis. Morphologically, the species of this subsection have distinctive ovate emergent leaves that are entire, lobed, or toothed with the notable exceptions of *M. robustum* (pectinate) and *M. balladoniense* (linear). Many of the taxa in this group had been part of a broadly defined *M. elatinoides* Gaudich. until Orchard's (1980, 1986) revisions recognized several new species and clarified the priority of *M. quitense*.

Although many relationships among species of *M.* subsect. *Myriophyllum* were not well-supported, two small clades were resolved consistently (Figs. 1–3). A clade including *M. quitense* (New World) and *M. triphyllum* (New Zealand) was well-supported by all analyses. *Myriophyllum quitense* is the only other formally recognized South American species besides *M. aquaticum* and *M. mattogrossense* (Orchard 1981). It ranges into Mexico (Retana 1983) with disjunct populations in the Deschutes River, Oregon (Fernald 1919) and recently has been recognized from New Brunswick and British Columbia, Canada (Ceska et al. 1986; McAlpine et al. 2007). Both ITS and cpDNA provide evidence of molecular differentiation between the North American and South American populations of *M. quitense* (Fig. 4) but morphological variation is not evident (Orchard 1981; personal observations). The distinct molecular variation (Fig. 4) between South American and North American taxa does lend support to the hypothesis that North American *M. quitense* might represent relict populations (Ceska et al. 1986) rather than an introduction from South America (Aiken 1981).

A clade of predominantly northern hemisphere temperate taxa (*M. alterniflorum*, *M. sibiricum*, and *M. spicatum*) also is well-supported (Figs. 1–3). These taxa have a northern hemisphere distribution, although *M. spicatum* has been introduced to North America (Couch and Nelson 1985) and has become notoriously invasive across the continent. The members of this clade were hypothesized to be part of a polyploid complex including *M. verticillatum* (Aiken 1981). Although our phylogenetic evidence excludes *M. verticillatum* from such a scenario, the chromosome counts of *M. alterniflorum* ($2n = 14$), *M. sibiricum* ($2n = 42$) and *M. spicatum* ($2n = 42$) support the possibility that *M. sibiricum* and *M. spicatum* could have originated from a hexaploid descendent of *M. alterniflorum*, which is sister to the latter two taxa.

The status of *M. sibiricum* (ex. *M. exalbescens*) has been of some contention. Some viewed the taxon as distinct from *M. spicatum* (Fasset 1940; Sculthorpe 1967; Aiken 1981), whereas others have recognized it as merely a subspecies (Patten 1954) or variety (Jepson 1925; Nichols 1975). Much of the debate over taxonomic rank was clarified with the establishment that *M. spicatum* was introduced to North America around 1942 (Couch and Nelson 1985), which occurred well after Fernald's (1919) description of *M. exalbescens* (= *M. sibiricum*). Work describing growth and turion formation in *M. sibiricum* helped to identify morphological characters that differentiated *M. sibiricum* from *M. spicatum* (Aiken 1979, 1981; Moody and Les 2007b) and the typification of *M. sibiricum* (Aiken and Cronquist 1988) has clarified the nomenclature.

A geographically diverse sampling (Appendix 1; Moody and Les 2002, 2007b) has indicated consistent molecular divergence between *M. spicatum* and *M. sibiricum* and this observation is supported morphologically by their leaf length/pinnae ratios (Moody and Les 2007b). European specimens of *M. sibiricum* also were examined for molecular and morphological variation compared to North American taxa. The DNA sampled from three *M. sibiricum* accessions from Europe (Appendix 1) all shared three point mutations in ITS that were unique from North American *M. sibiricum*; however, the plants lacked clear morphological differentiation (e.g. in leaf length/pinnae ratio) from North American *M. sibiricum*. Segregation of North American and European *M. sibiricum* at the species or infraspecific level might be appropriate if the genetic variation remains consistent through a larger sampling across the species range.

All other species of *M.* subsect. *Myriophyllum* are Australian endemics. Relationships among these taxa are not strongly supported, despite their distinctness both morphologically (Orchard 1980, 1981, 1986) and genetically (Fig. 4). These species have adapted to a wide range of aquatic habitats from ephemeral water bodies (*M. verrucosum* Lindl., *M. balladoniense*) to slow moving streams (*M. caput-medusae* Orchard) and permanent water bodies with high salinity (*M. salsugineum*).

***Myriophyllum* Subgenus *Brachytheca*—**This clade is highly diverse with many species retaining narrow endemism in comparison to *M.* subg. *Myriophyllum*. This subgenus is greatly expanded from Schindler's (1905) treatment including many species from his *M.* subg. *Pentapteris*. The majority of species in this clade are restricted to Australia, and several are considered to be of conservation concern (e.g. *M. latifolium*, *M. petraeum* Orchard, etc.). There is a clade of seven North American endemics (Fig. 3) and some species that range into Asia, Papua New Guinea, and New Zealand. There is a conspicuous absence of species from South America and Europe in this clade. The diversity of vegetative forms found in *M.* subg. *Brachytheca* is much greater than that found in *M.* subg. *Myriophyllum* (i.e. minute linear-leaved plants to robust plants displaying various degrees of heterophylly), a factor that

correlates with the diversity of habitats where these plants have evolved (i.e. deep-water lakes to shallow ephemeral pools with a highly labile wet-season duration).

***Myriophyllum* Section *Pelonastes*—***Myriophyllum pedunculatum* and *M. tillaeoides* form a clade sister to the rest of *M.* subg. *Brachytheca* (Fig. 3). These species along with *M. amphibium* (only sampled for ITS) and *M. austropygmaeum* Orchard (not sampled) make up the core species of Orchard's (1986) "*M. pedunculatum* Alliance." However, *M. austropygmaeum* lacks sepals on its carpellate flowers (discussed later) and likely does not belong here. The plants of this clade are small, opposite-leaved perennials that usually form mats in ephemeral water holes. They retain sepals on their carpellate flowers, a characteristic found elsewhere in *M.* subg. *Brachytheca* only among *M.* sect. *Tessaronia* (Fig. 5a). Orchard (1986) suggested a loose alliance of these taxa with other vegetatively similar *Myriophyllum* (*M. lophatum*, *M. pygmaeum* Mattf., and *M. votschii*), some of which are only distantly related in *M.* subsect. *Nudiflorum* (Fig. 3).

Currently there are three described subspecies of *M. pedunculatum*. Two genotypes of *M. pedunculatum* were recognized in these analyses, here referred to as *M. pedunculatum* (T) [*Les 643*, Tasmania] and *M. pedunculatum* subsp. *pedunculatum* all collected from New South Wales (Appendix 1). The Tasmanian accession is sister to *M. tillaeoides* using cpDNA but sister to other *M. pedunculatum* subsp. *pedunculatum* using ITS. The smooth fruits of *M. pedunculatum* (T) are similar to *M. pedunculatum* subsp. *novae-zelandiae* Orchard, whereas the deep red stigmas and vegetative features are more common to other *M. pedunculatum* subspecies (subsp. *longibracteolatum*, subsp. *pedunculatum*). Besides its geographic isolation (endemic to Western Australia), *M. tillaeoides* differs from *M. pedunculatum* (including *M. pedunculatum* [T]) in lacking pedunculate male flowers and by having sessile trifid submerged leaves. The pattern of molecular incongruence and morphological variation could have taxonomic implications regarding species delimitation in this group and potential hybrid origins of species. The diminutive nature of the members in this clade has made their morphological assessment difficult and it is evident from these data that further sampling of *M. pedunculatum* populations will be necessary to assess the taxonomic status of Tasmanian *M. pedunculatum* as well as phylogenetic patterns throughout this clade.

***Myriophyllum* Section *Tessaronia*—**This is a well-supported clade that includes a geographically diverse assemblage of Australian taxa and a subclade of endemic North American species. The Australian taxa include *M. latifolium*, *M. muricatum*, and *M. dicoccum*, the latter two found primarily in northern Australia. *Myriophyllum dicoccum* also ranges into India and Vietnam (Meijden and Caspers 1971; Yu et al. 2002) while *M. muricatum* was split only recently from *M. tuberculatum* Roxb. (Orchard 1986), a species known primarily from India and Malaysia. Schindler (1905) implied a close link between *M. tuberculatum* and North American endemics by placing them in the same section (*Tessaronia*) and Meijden (1969) noted the strong similarities of *M. dicoccum* and the North American endemic *M. humile*, most notably the unusual submerged flowers also shared with *M. farwellii*. A better sampling of the Asian taxa from Schindler's (1905) *M.* subsect. *Trachycarpaeum* (Table 1) will be necessary to further explore the link between Asian and North American taxa.

*Myriophyllum dicoccum* and *M. muricatum* form a well-supported clade in all analyses but are highly divergent at the molecular level with long branches based on both ITS and cpDNA (Fig. 4). As discussed earlier, the long branch of this clade may be responsible for its anomalous placement by parsimony analysis of ITS data. Orchard (1986) considered these two taxa closely allied (Table 1) based on a host of vegetative characteristics including a robust habit, irregularly arranged (i.e. both alternate and whorled) submerged leaves, and elongate, mostly entire leaves. These features also are characteristic of *M. latifolium*, some North American endemics, and Asian species of Schindler's (1905) *M.* sect. *Tessaronia*. The relationship of *Myriophyllum latifolium* sister to *M. dicoccum* and *M. muricatum* is tentative due to relatively weak branch support and ambiguity given ITS alone (Figs. 1–3). This rare, narrow endemic of the coastal region of central and northern New South Wales is distinct among members of this clade in being dioecious, having carpellate flowers in fascicles, and leaves that are strictly whorled. Orchard suggested that *M. latifolium* was loosely allied with *M. papillosum* based mostly on their shared fascicled flowers and large, flattened emergent leaves; however, he did not consider the retention of sepals on carpellate flowers in *M. latifolium* (Fig. 5), whose loss apparently is synapomorphic for *M.* sect. *Pentapteris*, which includes *M. papillosum*.

*Myriophyllum* subsect. *Spondylastrum*, including all the North American endemic taxa, is a well-supported but heteromorphic clade where relations among most taxa remain poorly resolved (Figs. 1–3). One clade including *M. heterophyllum*, *M. hippuroides*, and *M. pinnatum* is supported in all analyses although relationships among these species are incongruent between ITS and cpDNA. These taxa have the characteristic *Myriophyllum* submerged morphology. *Myriophyllum heterophyllum* and *M. hippuroides* both can become robust plants with wide diameter stems and their submerged and emergent leaves mostly appear to be whorled (but see England and Tolbert [1964] regarding leaf initiation). Generally, these taxa are delimited by the degree of dissection of their emergent leaves and by their geographical (east-west North America) disjunction. *Myriophyllum heterophyllum* also is vegetatively plastic, whereas *M. hippuroides* is not.

*Myriophyllum heterophyllum* has three ITS genotypes defined by point mutations and these are paraphyletic with respect to *M. hippuroides*. Our cpDNA data revealed no variability in *M. heterophyllum*, but a recent study indicated at least two major chloroplast lineages based on *trnL–trnF* data (R. Thum et al. pers. comm.) that appear to have geographic structure (midwest vs. southeast U. S. A.). Our cpDNA results are incongruent in relation to ITS with *M. hippuroides* sister to *M. pinnatum-M. heterophyllum*. *Myriophyllum pinnatum* is distinct from each of these taxa in vegetative form with scattered leaves (rather than whorled) as is common to other endemic North American taxa. *Myriophyllum heterophyllum* is known to hybridize with *M. laxum* (Moody and Les 2002) and the incongruence between data sets could be due to hybrid origins of species in this clade, although lineage sorting also is a possibility.

*Myriophyllum heterophyllum*, *M. laxum*, and *M. pinnatum* are sympatric in the southeastern U. S. A. The latter two are similar vegetatively, with submerged and emergent leaves that usually are scattered or irregularly whorled. When reproductive structures are lacking, *M. laxum* can be mistaken for *M. pinnatum* and hybrid *M. heterophyllum* × *M. laxum*. Although the more robust stems of *M. pinnatum* generally differ from the more "lax" stems of *M. laxum*, this characteristic can be

plastic and difficult to evaluate. Moody and Les (2002) initially described the invasive hybrids *M. heterophyllum* × *M. laxum* as involving *M. heterophyllum* and *M. pinnatum*. However, collections of flowering *M. laxum* specimens in Florida [*Moody 173, 174*; Appendix 1] provided molecular evidence that *M. laxum* (not *M. pinnatum*) was in fact the taxon involved in the hybrid formed with *M. heterophyllum*. Several specimens from Florida [*Moody 53, 57, 58, 67, 69, 77*; Appendix 1], were believed to be *M. pinnatum* based on vegetative morphology, herbarium specimen location data, and distribution; however, they proved to have the *M. laxum* genotype as confirmed by DNA extracted from reproductive material. *Myriophyllum pinnatum* has emergent, deeply-lobed bracts and fruits with prominent tuberculate ridges, whereas *M. laxum* has much smaller, often spatulate or obovate, unlobed bracts and fruits lacking prominent tuberculate ridges. The phylogenetic placement of the three remaining North American endemics is ambiguous within *M.* subsect. *Spondylastrum*. These taxa (*M. farwellii*, *M. humile*, and *M. tenellum* Bigelow) all lack the differentiated emergent growth form of the rest of *M.* sect. *Tessaronia* and two are unusual in having submerged flowers (*M. farwellii*, *M. humile*).

Although relationships among many of the North American endemics remain unclear given these data, a comparably high level of molecular differentiation is evident among the species (Fig. 4). All species have a unique ITS and cpDNA profile. Although genotypic variation is found within some species across their range (e.g. *M. laxum* and *M. heterophyllum*; Fig. 4), the genotypes are distinct from other species in the clade. This extent of variation has proven to be particularly helpful for plant identification. Given that many of the taxa in this clade display high plasticity and similarity in vegetative form, a DNA marker (ITS) has been used to differentiate the northeast and northwest invasive *M. heterophyllum* and its hybrid *M. heterophyllum* × *M. laxum* from native taxa for early detection of invasive species for state management agencies (e.g. Maine and New Hampshire Department of Natural Resources and Washington Department of Ecology).

***Myriophyllum* Section *Pentapteris*—**This group is well supported by both ITS and cpDNA. It is much reduced from Schindler's (1905) treatment and includes all members of Schindler's *M.* subsect. *Spirophyllum* but only few species from *M.* subsect. *Pelonastes* and *M.* subsect. *Spondylophyllum*. The loss of sepals on carpellate flowers is a synapomorphy for *M.* sect. *Pentapteris* (Fig. 5a) within *M.* subg. *Brachytheca*. This reduction also occurs in parallel among a few members of *M.* subg. *Myriophyllum* (Fig. 5a). The loss of sepals in staminate flowers is uncommon in the genus but is found among several members of *M.* sect. *Pentapteris*, including *M.* subsect, *Spirophyllum* and some members of the "lophatum" clade (Fig. 5b).

*Myriophyllum* subsect. *Spirophyllum* is inclusive of at least three species from northern Australia, all of which have asepalous staminate flowers (Figs. 3, 5b). This subsection also likely includes the other members of Orchard's (1986) "*M. striatum* Alliance" and "*M. mezianum* Alliance" given their shared morphology. The relationship between *M. filiforme* and *M. trachycarpum* F. Muell. was expected (Schindler 1905; Orchard 1986). Two accessions of *M. filiforme* with distinct cpDNA and ITS genotypes (Fig. 4) have been identified. The individual accessions are geographically disjunct, but further sampling will be needed to identify if this variation is consistent and whether distinct morphological features can be identified to correspond with these relatively highly divergent genotypes.

*Myriophyllum coronatum* also is part of *M.* subsect. *Spirophyllum*. It is known only from a single Australian locality (Bronto Lake at the Northern-most tip in the Cape York peninsula, Queensland), but also occurs in Papua New Guinea. This species has been allied to several east Asian species not sampled here (*M. mezianum* Schindl., *M. bonii* Tardieu, and *M. siamense* (Craib) Tardieu), all share reduced staminate flowers (two petals, lack sepals, one stamen), dicarpic carpellate flowers (except the tetracarpic *M. bonii*), and a similar, emergent, mat-like habit, which lacks pinnate leaves (Meijden 1969; Orchard 1986), a combination of characteristics that is unique in the genus. Given the well-supported placement of *M. coronatum* in *M.* subsect. *Spirophyllum*, these morphologically and geographically similar species likely belong here as well. Meijden (1969) and Schindler (1905) hypothesized a close relationship of *M. coronatum* with *M. dicoccum* given their shared dicarpic carpellate flowers, but *M. dicoccum* differs substantially from *M. coronatum* in the presence of hermaphrodite flowers, tetramerous staminate flowers and the retention of sepals on all flowers.

*Myriophyllum* subsect. *Nudiflorum* is well-supported. It has an Austral-Asian distribution and its center of diversity is in Australia including at least 12 endemics. It encompasses a diverse assemblage of vegetative forms and includes all the species sampled here from Orchard's (1986) "*M. propinquum* Alliance" (except *M. latifolium*), "*M. integrifolium* Alliance", and "*M. pedunculatum* Alliance Associates." There are two major clades within *M.* subsect. *Nudiflorum* and a weakly supported sister taxon relationship of *M. papillosum* resulting from cpDNA and combined analyses. ITS provided little resolution at higher levels in this clade, although some small clades were supported.

In comparison to other *Myriophyllum* subsect. *Nudiflorum* taxa, *M. papillosum* has robust vegetative features with wide diameter stems and leaves frequently in whorls > 6. It also has unusual reproductive features for the genus with male flowers in fascicles and carpellate flowers often densely clustered in the axils of emergent leaves. Multiple copies of ITS were found in *M. papillosum*, which were identified using cloning techniques and together formed a clade (Fig. 1). The robust nature and odd floral arrangements in combination with multiple copies of the nrDNA ITS could indicate a polyploid origin of this taxon. Chromosome counts have not yet been performed for most *Myriophyllum* taxa and will be necessary to evaluate this hypothesis.

The "lophatum" clade is well-supported under cpDNA and combined analyses, whereas ITS does not resolve the clade, rather the taxa form a polytomy with members of the "variifolium" clade. The "lophatum" clade includes species ranging widely in habit. There are small, mat-forming, opposite, and linear-leaved species of shallow ephemeral pools (*M. lophatum*, *M. votschii*) and robust plants with pinnate leaves that sometimes grow in deep waters (*M. simulans* 441, *M. alpinum* Orchard).

*Myriophyllum lophatum* and *M. votschii* were proposed as allies to Orchard's (1986) "*M. pedunculatum* Alliance," here recognized as part of *M.* sect. *Pelonastes*. *Myriophyllum lophatum* grows in sympatry with *M. pedunculatum* in southeast Australia while *M. votschii* grows in sympatry with *M. pedunculatum* in New Zealand. While the vegetative form of these three species is nearly identical, as are most reproductive

features, the lack of sepals along with molecular data in *M. lophatum* and *M. votschii* allies them with *M.* sect. *Pentapteris*. The other group of diminutive taxa, Orchard's (1986) "*M. integrifolium* Alliance," are closely allied to *M. lophatum* and *M. votschii* and share floral features, but have alternate rather than opposite leaves. The three endemic Western Australian species of the alliance (*M. drummondii*, *M. echinatum*, and *M. limnophilum*) were sampled for these analyses and found to be monophyletic. *Myriophyllum limnophilum* and *M. drummondii* are the only species sampled that could not be distinguished from each other using ITS. Given limited morphological variation (degree of leaf dimorphism and fruit ornamentation) the status of these species will need further evaluation.

*Myriophyllum simulans* 441 is well-supported as part of the "lophatum clade" while *M. simulans* 622 is not (Fig. 3; Appendix 1). Both taxa fit the broad description attributed to this species, but clearly are distinct from each other in our analyses. Orchard (1986, 1990) described *M. simulans* as highly variable with affinities to *M. gracile* Benth. and *M. variifolium*. Only the latter has been sampled here, as the former is much less common; living specimens could not be collected and herbarium specimens provided poor quality DNA. The shape and size of the staminate flower sepals and carpellate flower bracteoles as well as emergent leaf shape (linear vs. lanceolate) readily distinguish the *M. simulans* genotypes sampled here; however, a much broader sampling of these taxa will be necessary to determine the consistency of these features. Orchard (1986) regarded *M. simulans* as being an extremely variable species with some "genetically fixed" isolates, a hypothesis supported by our observations.

Another "lophatum" clade member, *Myriophyllum* sp. nov. 542 [*Les 542*; Appendix 1] also would fit broadly into the definition of *M. simulans*, but is divergent in emergent leaf features, with extremely long (> 28mm), many toothed, linear emergent leaves that are strictly whorled, a combination of features not found among other closely allied *Myriophyllum* and will be formally recognized (Moody, pers. obs.). Combined and cpDNA data strongly support the affinity of *M. simulans* 441 and *Myriophyllum* sp. nov. 542 to *M. alpinum*, but also provide evidence that all three taxa are divergent genetically (Fig. 4).

The "variifolium" clade is well-supported in both cpDNA and combined analyses, but not using ITS alone in which members of the group are part of a polytomy within the "lophatum" clade. The Australian members of this clade were sampled more intensively given their notoriously challenging taxonomic history. Orchard (1986) described two new species from this group (*M. crispatum* and *M. simulans*) suggesting that local "genetically fixed" lineages were still possible within *M. simulans* and *M. variifolium*. Without reproductive features the plants in this group are often impossible to distinguish with certainty. However, our analyses have distinguished unique genetic lineages, and in some cases, corresponding morphotypes.

The Australian *M. crispatum* and Asian *M. ussuriense* were well-supported sister taxa in all analyses. The cpDNA and combined analyses resolved this clade as the sister group to the rest of the "variifolium" clade. *Myriophyllum ussuriense* is the only known Asian member of the "variifolium" clade. Lability in sexual expression appears to be common among the taxa of this clade, with the normally monoecious *M. crispatum*, *M. simulans*, and *M. variifolium* also having some individuals with either strictly carpellate or staminate-flowered stems (Orchard 1986; personal observation) and the normally dioecious *M. ussuriense* recently described as having some monoecious individuals (Ueno and Kadono 2001). Meijden and Caspers (1971) described *M. ussuriense* as being nearly indistinguishable from *M. propinquum*, but their evaluation occurred before Orchard's revisions (1981, 1986) in which the new Australian species were recognized (including *M. crispatum*) and *M. propinquum* no longer was considered to occur naturally in Australia. *Myriophyllum crispatum* is unique in this clade by its dense indumentum (crisped hairs) on the stem and base of some of the leaves. This characteristic is common on emergent stems of plants in southeast Australia, but notably absent in the disjunct Western Australia and Queensland taxa. *Myriophyllum crispatum* was resolved as a single lineage when multiple accessions were examined, although ITS distinguished the disjunct west and southeast populations.

Inclusion of the poorly known *M. petraeum*, a declared priority species (Department of Environment and Conservation [DEC], Washington), in the "variifolium" clade is supported by cpDNA and combined data. The alternate, linear leaves and lack of sepals on staminate flowers in *M. petraeum* also characterize members of the "lophatum" clade, but its columnar fruits are similar to those found in *M. variifolium* and *M. propinquum*. Orchard (1986) associated *M. petraeum* with his "*M. striatum* Alliance" whose members sampled here are far removed in *M.* subsect. *Spirophyllum*.

All other taxa in the "variifolium" clade, which includes only the members of Orchard's (1986) "*M. propinquum* Alliance" in exclusion of *M. alpinum*, are similar morphologically and have strongly supported relationships in all analyses. Orchard (1986) regarded "*M. propinquum* s. s." as occurring only in New Zealand, and transferred all Australian taxa described formerly under the broader definition of *M. propinquum* to *M. crispatum*, *M. simulans*, or *M. variifolium*. *Myriophyllum simulans* and *M. crispatum* differ from *M. propinquum* and *M. variifolium* in their fruit structure; however, the distinction between *M. variifolium* and *M. propinquum* is based on their geographic disjunction and differences in morphology, specifically "greater size in all parts" for the former (Orchard 1986, pg. 203). All accessions sampled here were collected in Australia and fell within the size range of *M. variifolium*. We were not able to attain specimens of *M. propinquum* from New Zealand for these analyses, and have followed the geographic and morphological distinctions of Orchard (1980, 1986) until sampling of New Zealand material can be conducted.

*Myriophyllum propinquum* and *M. variifolium* are unique from other members of the "variifolium" clade in having distinctive green/yellow columnar-shaped fruits, whereas other members of the clade have fruits with a distinctly rounded base, red coloration and tuberculate ornamentation. We detected two distinct genotypes (*M. variifolium* 444 and 457) having the *M. propinquum*/*M. variifolium* fruit type (Fig. 3; Appendix 1) in both ITS and cpDNA corresponding to multiple accessions (Appendix 1). They do not form a clade under any analyses, but are paraphyletic regarding *M. simulans* 622 and *M.* sp. 425 (discussed below). Neither genotype was consistent morphologically with the species description of *M. propinquum* and no morphological synapomorphies have been identified to distinguish the unique genotypes. Additional sampling across the geographic distribution of *M. variifolium* will be necessary to evaluate the need for further taxonomic revisions.

Two other taxa included here are allied closely to *M. variifolium* (*M. simulans* 622 and *M.* sp. 425). *Myriophyllum simulans* 622 was discussed in relation to *M. simulans* 441 above. *Myriophyllum* sp. 425 has characteristics of both *M. gracile* and *M. simulans* but does not strictly follow the description of either. Its position within the clade is incongruent with respect to ITS and cpDNA data analyses, aligning as sister to either *M. simulans* 622 (cpDNA) or *M. variifolium* 457 (ITS), but distinct genotypically from each. Thus, it likely is not a recent hybrid between the two, although a deeper hybrid history is possible. *Myriophyllum* sp. 425 was collected well outside the documented range of *M. gracile* but given the morphological similarities between these taxa it will be necessary to sample the latter to establish if the taxa are conspecific. Two individuals with genotypes matching *Myriophyllum* sp. nov. 476 [*Moody 476, Les 653*] also have been identified (Appendix 1). This taxon is sister to the four species of the "variifolium" clade discussed above (Fig. 3) and is morphologically distinct from all. It will be described in a forthcoming publication.

Our molecular analyses clearly show that substantial taxonomic revision is necessary among the "variifolium" clade species. Although several genotypes are consistent across multiple accessions that can be defined under current species definitions (e.g. *M. crispatum*, *M. variifolium* 444), some taxa with unique genotypes were detected, which in some cases also possessed distinctive morphological characteristics that were not previously documented. Thus, we suspect these taxa to represent multiple cryptic species. Further DNA sampling of accessions of previously described species (e.g. *M. gracile*, *M. propinquum*) along with wider geographic sampling of highly variable species (*M. simulans*, *M. variifolium*) will be necessary to clarify the extent of taxonomically significant variation among the "variifolium" clade.

***Character Evolution***—Meijden's (1969) suggestion that infrageneric relationships in *Myriophyllum* cannot be elucidated by morphology alone has some merit. Molecular data have proven informative in this regard by helping to identify plesiomorphy and/or parallelism as well as homology among *Myriophyllum* character states. Our phylogenetic results indicate that some character states, which are homoplasious when all *Myriophyllum* taxa are considered, can also be useful to delimit some clades. For example, the loss of sepals defines *M.* sect. *Pentapteris* within *M.* subg. *Brachytheca*, despite being of labile occurrence in *M.* subg. *Myriophyllum* (Fig. 5b). Similarly, reduction in stamen number is diagnostic for *M.* subsect. *Spondylastrum*, even though other reductions are found sporadically throughout the genus (Fig. 5c). However, this character needs to be evaluated further as some taxa described as having four stamens have also been reported to have eight (e.g. *M. callitrichoides* [Orchard 1986], *M. farwellii* [Aiken 1981]).

Sexual dimorphism has been described as somewhat labile within *Myriophyllum* species, and some species described as monoecious also have sporadically occurring dioecious individuals (e.g. *M. crispatum*, *M. variifolium*, *M. simulans*, etc.) or, as is the case with *M. ussuriense*, a dioecious species with sporadic occurrence of monoecious individuals (Ueno and Kadono 2001; Ceska et al. 1986; Orchard 1981, 1986). Many monoecious species also have intermediate hermaphrodite flowers and reporting of hermaphrodite flowers in monoecious taxa has been inconsistent. For example Schindler (1905) described *M. trachycarpum* and *M. filiforme* as having first hermaphrodite flowers then becoming unisexual, whereas Orchard (1986) described these species as lacking hermaphrodite flowers. Our ancestral character state optimization supports the hypothesis that degree of sexual dimorphism is particularly labile in the genus (Fig. 5d, e) and the use of this character for subgeneric classification (e.g. Schindler 1905) has given rise to artificial groups. Reduction in floral features is particularly common among aquatic plants as is the tendency towards monoecy or dioecy (Sculthorpe 1967). The high level of homoplasy associated with such features in our phylogenetic analyses helps to explain why it has been so difficult to define subgeneric relationships in *Myriophyllum* strictly on the basis of morphology.

***DNA "Barcoding" Myriophyllum***—We have shown that ITS sequence data can provide reliable markers (i.e. "DNA barcoding"; Chase et al. 2005) capable of distinguishing among all *Myriophyllum* species sampled so far (except *M. drummondii* vs. *M. limnophilum* of southwest Western Australia) as well as invasive hybrid taxa in North America (Moody and Les 2002, 2007b). Particular caution must be exercised when hybrid taxa are concerned, because ITS can become homogenized to either parent of a hybrid with long term introgression (Wendel et al. 1995). Given the lack of information regarding fertility, the degree of introgression is not yet known among the invasive hybrid watermilfoil populations. Thus, relying strictly on ITS data could fail to differentiate such hybrids from "pure" parental genotypes.

The reliability of ITS to effectively discern among North American taxa has been confirmed by sampling across a wide geographic area for most species (Appendix 1; unpublished data). In *Myriophyllum,* where identification on the basis of morphology can be particularly difficult (especially when reproductive characters are lacking), DNA-based identification can provide a useful resource for aquatic plant management programs (Moody et al. 2008). For example, this approach is able to distinguish native from invasive taxa for early detection (and removal before establishment) because DNA methods allow identification of even small vegetative fragments. Several North American agencies with aquatic plant management concerns (i.e. Maine, Minnesota, New Hampshire, and Wisconsin Departments of Natural Resources and Washington State Department of Ecology) already have incorporated these methods into their management programs (Moody, unpublished data).

## Taxonomic Revision

**Myriophyllum trifidum** (Nees) M. L. Moody & D. H. Les, comb. nov., *Gonocarpus trifidus* Nees, Plantae Preissianae 1: 159. 1844. *Haloragis trifida* (Nees) Walpers, Repertorium Botanices Systematicae 5: 672. 1846. *Meziella trifida* (Nees) Schindl. Das Pflanzenreich 23: 61. 1905.—TYPE: AUSTRALIA. "In turfosis humidis ad lacum haud procul ab oppidulo Albany (Plantagenet) m. Octobri 1840 Herb. *Preiss No. 2401*" (holotype: LE!; isotype MEL!).

**Myriophyllum** subgenus **Meziella** (Schindl.) M. L. Moody & D. H. Les, comb. et stat. nov. *Meziella* Schindl. Das Pflanzenreich 23: 61. 1905.—TYPE: *M. trifidum* (Nees) M. L. Moody & D. H. Les.

Diagnosis: Leaves alternate. Emergent leaves linear, submerged leaves often trifid. Hermaphrodite flowers. Sepals persistent on fruit becoming elongate spines. Mericarps not separating freely at maturity.

Myriophyllum subgenus Myriophyllum—TYPE: *M. spicatum* L. Species Plantarum 2: 992. 1753.

This subgenus includes in part members of Schindler's (1905) *M.* subg. *Eumyriophyllum* [nom. inval.]. This includes eight of 19 members of *M.* sect. *Pentapteris* Schindl. and all species from *M.* subsect. *Spondyllophyllum* Schindl. and *M.* subsect. *Leiocarpium* Schindl. All the core species of Orchard's "*M. salsugineum* Alliance" and "*M. aquaticum* Alliance" are in this group.

Diagnosis: This is a heteromorphic group in both vegetative and reproductive morphology. In general species have submerged leaves pectinate and whorled (if present). Emergent leaves are usually whorled (sometimes becoming alternate or subwhorled only towards the apex), but sometimes opposite (*M. decussatum*) or mostly alternate (*M. alterniflorum*, *M. balladoniense*). Emergent leaves pectinate, entire, or toothed, generally ovate, obovate (linear for *M. balladoniense*). Monoecious, dioecious, or all flowers hermaphrodite.

Myriophyllum section Myriophyllum—TYPE: *M. spicatum* L.

*Myriophyllum* subsect. *Spondylophyllum* Schindl. Das Pflanzenreich 23: 86. 1905.

This section includes six of the ten members of *Myriophyllum* subsect. *Spondylophyllum* and both species from *M.* subsect. *Leiocarpium*.

Diagnosis: Same as for *M.* subgenus *Myriophyllum*.

Myriophyllum subsection Myriophyllum—TYPE: *M. spicatum* L.

*Myriophyllum* series *Anisophylleae* Schindl. Das Pflanzenreich 23: 89. 1905.

This subsection closely follows *M.* series *Anisophylleae*, but does not include *M. propinquum*.

Diagnosis: Submerged leaves, if present, all whorled. Emergent leaves usually entire or toothed at least towards apex (pectinate in *M. robustum*). Monoecious (all hermaphrodite flowers in *M. robustum*).

**Myriophyllum** subsection **Isophylleae** (Schindl.) M. L. Moody & D. H. Les, comb. et stat. nov. *Myriophyllum* series *Isophylleae* Schindl. Das Pflanzenreich 23: 86. 1905.—TYPE: *M. verticillatum* L. Species Plantarum 2: 992. 1753.

This subsection closely follows *M.* series *Isophylleae* Schindl., but does not include *M. aquaticum* (ex. *M. brasiliense*).

Diagnosis: Submerged leaves whorled and pectinate, emergent leaves pectinate becoming distinctively smaller towards apex. Elongate turions. Monoecious with intermediate hermaphrodite flowers.

**Myriophyllum** section **Pectinatum** M. L. Moody & D. H. Les, sect. nov.—TYPE: *M. aquaticum* (Vell.) Verdc. Kew Bulletin 28: 36. 1973.

Folia omnia verticillata; difert a *M.* subsect. *Myriophyllum* folia omnia emersa pectinata et difert a *M.* subsect. *Isophylleae* folia emersa comparate non redacta de foliis submersis.

Diagnosis: Submerged and emergent leaves whorled and pectinate. Emergent leaves pectinate and not highly reduced in relation to submerged leaves. Plants dioecious or flowers all hermaphrodite.

Myriophyllum subgenus Brachytheca Schindl. Das Pflanzenreich. 23: 102. 1905.—TYPE: *M. variifolium* Hook. f. Hooker's Icones Plantarum 3: 289. 1840.

This subgenus is more broadly defined than the original circumscription (three spp.) now including all species from *M.* subsect. *Spirophyllum* Schindl. and *M.* sect. *Tessaronia* Schindl.

Diagnosis: This is a heteromorphic group in both vegetative and reproductive morphology. Submerged leaves alternate, opposite, or whorled; pectinate, trifid, linear, or lacking. Emergent leaves linear, terete, lanceolate, oblanceolate, ovate, obovate, or pectinate (*M. alpinum*). Monoecious or dioecious (all hermaphrodite flowers in *M. muricatum*).

Myriophyllum section Pentapteris DC. Prodromus 3: 68. 1828.—TYPE: *M. variifolium* Hook. f.

This section is much reduced from Schindler's (1905) treatment and includes *M.* subsect. *Spirophyllum* but only few species from *M.* subsect. *Pelonastes* and *M.* subsect. *Spondylophyllum*.

Diagnosis: Monoecious or dioecious, lacking hermaphrodite flowers. Lacking sepals in carpellate flowers and often in staminate flowers. Usually eight stamens (also four, two, or one).

Myriophyllum subsection Spirophyllum Schindl. Das Pflanzenreich 23: 82. 1905.—TYPE: *M. trachycarpum* F. Muell. Fragmenta Phytographiae Australiae 2: 87. 1861.

This subsection varies from Schindler's (1905) description by the inclusion of *M. coronatum*. The placement of *M. gracile* is as yet unknown.

Diagnosis: Leaves alternate or opposite becoming distally alternate. Leaves linear, lanceolate, oblanceolate, ovate, obovate, lower-most submerged leaves becoming pectinate in some species. Monoecious. Carpellate and staminate flowers lack sepals. Stamens eight or one.

**Myriophyllum** subsection **Nudiflorum** M. L. Moody & D. H. Les, subsect. nov.—TYPE: *M. variifolium* Hook. f. Hooker's Icones Plantarum 3: 289. 1840.

Folia verticillata, alterna, vel sparsa. Folia submersa linearia ad pectinata. Flores unisexuales, plantae monoeciae vel dioeciae. Flores feminei sepalis, petalis et staminibus nullis.

This subsection includes most members of Orchard's *M. propinquum* Alliance and *M. integrifolium* Alliance and elements from across Schindler's classification.

Diagnosis: Vegetatively heteromorphic with leaves whorled, opposite or alternate. Submerged leaves linear or pectinate. Emergent leaves linear, ovate, obovate, lanceolate, oblanceolate, or pectinate. Plants monoecious or dioecious, lacking hermaphrodite flowers. Carpellate flowers lack a perianth. Staminate flowers with perianth present (sepals sometimes lacking) with (2, 4) or 8 stamens.

Myriophyllum section Tessaronia Schindl. Das Pflanzenreich 23: 95. 1905.—TYPE: *M. heterophyllum* Michx. Flora Boreali-Americana 2: 191. 1803.

This section closely follows that of Schindler (1905).

Diagnosis: Submerged leaves whorled (subwhorled) or alternate, pectinate or scale-like (*M. tenellum*). Emergent leaves (when present) ovate, obovate, lanceolate, oblanceolate; often lobed, toothed, or serrate. Plants monoecious usually with hermaphrodite flowers intermediate on the inflorescence. Sepals present on carpellate and staminate flowers. Stamens four (eight).

Myriophyllum subsection Spondylastrum Schindl. Das Pflanzenreich 23: 98. 1905.—TYPE: *M. heterophyllum* Michx.

This subsection follows Schindler (1905) with the addition of all members of *M.* subsect. *Ptilophylllum* Schindl.

Diagnosis: Plants monoecious with hermaphrodite flowers intermediate on the inflorescence. Sepals present on carpellate

and staminate flowers. Stamens four. Endemic to North America.

**Myriophyllum** section **Pelonastes** (Hook. f.) M. L. Moody & D. H. Les, comb. et stat. nov. *Pelonastes* Hook. f. Hooker's Journal of Botany and Kew Garden Miscellany 474. 1847.—TYPE: *M. tillaeoides* Diels, Botanische Jahrbucher fur Systematik 35: 448. 1904. *Myriophyllum* subsect. *Pelonastes* (Hook. f.) Schindl. Das Pflanzenreich 23: 83. 1905.

This section closely follows *M.* subsect. *Pelonastes* but excludes *M. votschii*.

Diagnosis: Small plants. Leaves opposite, linear, lanceolate, ovate with lower-most submerged leaves sometimes trifid. Sepals present on staminate and carpellate flowers. Stamens eight.

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Appendix 1. Accession data for taxa included in these phylogenetic analyses. When multiple collection numbers are listed multiple specimens were sampled and duplicate sequences for ITS were recovered (if numbers follow taxon name, multiple genotypes were recovered from different populations). Multiple collection numbers after a name refer to multiple locations within the general geographic location listed. GenBank numbers follow consecutively: ITS, *trnK* 5′ intron, *matK*, *trnK* 3′ intron. When GenBank number is replaced with "—-", then no sequence was generated. When a single GenBank number is listed after a taxon it is for ITS. * refers to DNA extracted directly from an herbarium specimen. HPBG = Harold Porter Botanical Garden, RBGC = Royal Botanical Garden Canberra.

*Gonocarpus montanus*, *Moody 448* (CONN), Australia, New South Wales; *Moody 449b* (CONN), Australia, New South Wales, EF178770, EF178952, EF179044, EF178860; *Haloragis digyna*, *Moody 411* (CONN), Australia, Western Australia, EF178747, EF178929, EF179021, EF178838; *Laurembergia repens*, *Rourke* (Cult.) HPBG, South Africa; **Williams 113* (GHPG), South Africa, EF178735, EF178917, EF179009, EF178827; *Myriophyllum alpinum*, *Moody 449*, *453* (CONN), Australia, New South Wales, EF178720, EF178902, EF178994, EF178812; *M. alterniflorum*, *Moody 109a*, *111* (CONN), U. S. A., Wisconsin, EF178704, EF178886, EF178978, EF178797; *M. amphibium*, **Orchard 5295* (NSW), Australia, Tasmania, FJ870941; *M. aquaticum*, *Moody* (cult.), U. S. A., University of Connecticut; *Moody 51* (CONN), U. S. A., California; *Moody 56* (CONN), U. S. A., Florida, EF178727, EF178909, EF179001, EF178819; *M. balladoniense*, *Moody 389* (CONN), Australia, Western Australia, EF178708, EF178890, EF178982, EF178801; *M. caput-medusae*, *Moody 443*, *446* (CONN), Australia, New South Wales, EF178703, EF178885, EF178977, EF178796; *M. coronatum*, **Paijmans 3039* (CANB), Australia, Queensland, EF178717, EF178899, EF178991, EF178809; *M. crispatum*, *Moody 433*, *437*, *445* (CONN), Australia, New South Wales; *Les 660* (CONN), Australia, New South Wales, EF178721, EF178903, EF178995, EF178813; *M. crispatum* (WA), *Moody 413*, *418* (CONN), Australia, Western Australia, FJ870942; *M. decussatum*, **Stretch s. n.* (PERTH), Australia, Western Australia, FJ870943, FJ861354, FJ870930, FJ861337; *M. dicoccum*, *Jacobs 8252, 8259* (NSW), Australia, Northern Territory, AY335976, AY336006, AY335982,———; *M. drummondii*, *Moody 409* (CONN), Australia, Western Australia, EF178725, EF178907, EF178999, EF178817; *M. echinatum*, **Keighery 689* (PERTH), Australia, Western Australia, FJ870944; *M. farwellii*, *Moody 97* (CONN), U. S. A., Minnesota; *Moody 106* (CONN), U. S. A., Wisconsin; *Callahan s. n.* (CONN), U. S. A., New Hampshire, EF178731, EF178913, EF179005, EF178823; *M. filiforme* (1), **Wilson 1810* (NSW), Australia, Northern Territory, EF178716, EF178898, EF178990, EF178808; *M. filiforme* (2), *Jacobs 8021* (CONN), Australia, Western Australia, FJ870945, FJ861351, EF178990, FJ861334; *M. heterophyllum* (1), *Moody 101* (CONN), U. S. A., Minnesota; *Moody 150* (CONN), U. S. A., Maine, AF513824, EF178915, EF179007, EF178825; *M. heterophyllum* (2), *Moody H2* (CONN), U. S. A., Connecticut; *Moody 149* (CONN), U. S. A., Maine, AF513823; *M. heterophyllum* (3), *Moody 105* (CONN), U. S. A., Wisconsin; *Moody 141* (CONN), U. S. A., Rhode Island; *Moody 143* (CONN), U. S. A., Massachusetts; *Moody 176* (CONN), U. S. A., South Carolina; *Moody 178* (CONN), U. S. A., Oregon, AF513822; *M. hippuroides*, *Moody 179* (CONN), U. S. A., Oregon, FJ870946, FJ861364, FJ870939, FJ861347; *M. humile*, *Moody 141* (CONN), U. S. A., Rhode Island; *Moody CT10* (CONN), U. S. A., Connecticut; *Gerber s. n.* (CONN), U. S. A., Wisconsin, FJ870947, FJ861363, FJ870938, FJ861346; *M. latifolium*, **Orchard 4793* (NSW), Australia, New South Wales; **Jacobs 6706* (NSW), Australia, New South Wales, EF178729, EF178911, EF179003, EF178821; *M. laxum* (1), *Moody 57*, *58*, *67*, *77*, *173*, *174* (CONN), U. S. A., Florida, EF178732, EF178914, EF179006, EF178824; *M. laxum* (2), *Moody 170* (CONN), U. S. A., South Carolina; *Moody 172* (CONN), U. S. A., North Carolina, FJ870948, FJ861365, FJ870940, FJ861348; *M. limnophilum*, *Moody 417* (CONN), Australia, Western Australia, FJ870949, FJ861358, FJ870933, FJ861341; *M. lophatum*, *Moody 455*, *456* (CONN), Australia, New South Wales, EF178718, EF178900, EF178992, EF178810; *M. mattogrossense*, *Ritter 2314* (LPB), Bolivia, Carrasco, EF178728, EF178910, EF179002, EF178820; *M. muricatum* (1), *Jacobs 8557* (NSW), Australia, Queensland, FJ870963, FJ861362, FJ870937, FJ861345; *M. muricatum* (2), *Jacobs 8577* (NSW), Australia, Queensland, FJ870964; *M. muricatum* (3), *Jacobs 8604* (NSW), Australia, Queensland, FJ870965; *M. oguraense*, *Kadono s. n.* (HYO), Japan, Hyogo, EF178705, EF178887, EF178979, EF178798; *M. papillosum*, *Moody 424* (CONN), Australia, New South Wales; *Les 614* (CONN), Australia, New South Wales, (FJ870950, FJ870951, FJ870952**)**, EF178906, EF178998, EF178816; *M. pedunculatum* subsp. *pedunculatum*, *Moody 452*, *467* (CONN), Australia, New South Wales; *Les 652* (CONN), Australia, New South Wales, EF178711, EF178893, EF178985, EF178804; *M. pedunculatum* (T), *Les 643* (CONN), Australia, Tasmania, FJ870953, FJ861357, EF178985, FJ861340; *M. petraeum*, **Archer 1564* (NSW), Australia, Western Australia; **Brown 1123* (PERTH), Australia, Western Australia, EF178712, EF178894, EF178986, EF178805; *M. pinnatum*, *Moody 511* (CONN), U. S. A., Connecticut; *NASC* (GH), U. S. A., Missouri, FJ870966, FJ890500, FJ890498, FJ890499; *M. quitense* (NA), *Moody 180*, *183* (CONN), U. S. A., Oregon,

EF178700, EF178882, EF178974, EF178793; *M. quitense* (SA), **Goodall 83* (C), Chile; *Ritter 3939* (LPB), Bolivia, FJ870954, FJ861352, EF178974, FJ861335; *M. robustum*, *Schwarz s. n.* (CONN), New Zealand (Cult.), FJ870955, FJ861353, FJ870929, FJ861336; *M. salsugineum*, *Moody 412* (CONN), Australia, Victoria; Les (Cult.), Australia, University of Tasmania, EF178701, EF178883, EF178975, EF178794; *M. sibiricum*, *Moody 82* (CONN), U. S. A., California; *Moody 99* (CONN), U. S. A., Minnesota; *Moody 181* (CONN), U. S. A., Oregon; *Moody 125* (CONN), U. S. A., Wisconsin; *Moody 212* (CONN), U. S. A., Colorado, DQ786014-DQ86018, EF178706, EF178888, EF178980; *M. sibiricum* (Eu.), **Ulvinen 6124* (C), Finland; **Fenskild 8291* (C), Finland; **Hamen 61* (C), Denmark, FJ870956, FJ861350, FJ870928, FJ861333; *M. simulans* 441, *Moody 440, 441* (CONN), Australia, Victoria, EF178719, EF178901, EF178993, EF178811; *M. simulans* 622, *Les 622, 646* (CONN), Australia, New South Wales, EF178722, EF178904, EF178996, EF178814; *M. spicatum*, *Moody 79* (CONN), U. S. A., Florida; *Moody 86* (CONN), U. S. A., California; *Moody 117* (CONN), U. S. A., Wisconsin; *Moody 134* (CONN), U. S. A., Connecticut; *Moody 159* (CONN), U. S. A., Indiana; *Moody 164* (CONN), U. S. A., Minnesota; *Moody 185* (CONN), U. S. A., Oregon; EF178702, EF178884, EF178976, EF178795; *M. tenellum*, *Moody 110* (CONN), U. S. A., Wisconsin; *Moody 93* (CONN), U. S. A., Minnesota; *Callahan s.n.* (CONN), U. S. A., New Hampshire, EF178730, EF178912, EF179004, EF178822; *M. tillaeoides*, *Moody 415* (CONN), Australia, Western Australia, EF178710, EF178892, EF178984, EF178803; *M. trachycarpum*, *Jacobs 8843* (NSW), Australia, Northern Territory; *Martine 863* (CONN), Australia, Western Australia, EF178715, EF178897, EF178989, EF178807; *M. trifidum*, *Moody 404, 405, 410* (CONN), Australia, Western Australia, EF178734, EF178916, EF179008, EF178826; *M. triphyllum*, *Glenny 7455* (PDD), New Zealand, FJ870957, FJ861349, FJ870927, FJ861332; *M. ussuriense*, *Kadono "Kasai-1"* (HYO), Japan, Hyogo; *Kadono "Kasai-2"* (HYO), Japan, Hyogo, EF178726, EF178908, EF179000, EF178818; *M. variifolium* 444, *Moody 444, 469, 473* (CONN), Australia, New South Wales; *Les 628* (CONN), Australia, FJ870958, FJ861360, FJ870935, FJ861343; *M. variifolium* 457, *Moody 450, 457, 460* (CONN), Australia, Victoria, FJ870959, FJ861361, FJ870936, FJ861344; *M. verrucosum*, *Moody 427* (CONN), Australia, New South Wales; *Les 601* (CONN), Australia, New South Wales, *Jacobs 8777* (NSW), Australia, Northern Territory, EF178707, EF178889, EF178981, EF178800; *M. verticillatum*, *Moody 83* (CONN), U. S. A., California; *Moody 100* (CONN), U. S. A., Minnesota; *Moody 147* (CONN), U. S. A., Maine; *Moody 177* (CONN), U. S. A., Oregon, EF178709, EF178891, EF178983, EF178802; *M. votschii*, **Rixon 31* (NSW), New Zealand EF178714, EF178896, EF178988, EF178806; *M.* sp. 425, *Moody 425* (CONN), Australia, New South Wales, FJ870960, FJ861359, FJ870934, FJ861342; *M.* sp. (red 1), (Cultivated in U. S. A., Maine pet store as 'M. mattogrossense'); (Cultivated in U. S. A., Washington pet store as 'M. propinquum'), FJ870962, FJ861356, FJ870932, FJ861339; *M.* sp. (red 2), *Jacobs 8547* (CONN) Cultivated in Australia, FJ870961, FJ861355, FJ870931, FJ861338; *M.* sp. nov. *476*, *Moody 476* (CONN), Australia, New South Wales, EF178723, EF178905, EF178997, EF178815; *M.* sp. nov. *542*, *Les 542* (CONN), Australia, New South Wales, EF178713, EF178895, EF178987, **—.** *Trihaloragis hexandrus*, **Bright 93* (PERTH), Australia, WA; **Lepschi 3360* (PERTH), Australia, WA EF178759, EF178941, EF179033, EF178849.