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Molecular markers reconstruct the invasion history of variable leaf watermilfoil (*Myriophyllum heterophyllum*) and distinguish it from closely related species

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Abstract Genetic variation is increasingly recognized as an important factor influencing the establishment and spread of introduced species, and depends on both the introduction history and partitioning of genetic variation within and among potential source populations. We examine patterns of genetic variation in native and introduced populations of variable leaf watermilfoil, *Myriophyllum heterophyllum*, using chloroplast (*trnL-F*) and ribosomal (ITS) DNA sequences, as well as amplified fragment length polymorphisms (AFLPs). We identify a strong phylogeographic break distinguishing populations located on the Atlantic Coastal Plain (ACP) versus other ("Continental") portions of the native range. Within these distinct biogeographic regions, we also find genetic variation to be strongly partitioned among populations as analysis of molecular variance (AMOVA) partitioned 91 and 75% of cpDNA and ITS diversity among populations, respectively. We demonstrate that the introduced ranges of variable leaf watermilfoil (northeastern and western US) result from multiple independent introductions from a variety of source populations, including lineages from both the ACP and Continental portions of the native range. In addition, we used our molecular markers to demonstrate that variable leaf watermilfoil is genetically distinct from three closely-related species that it is morphologically similar to. In particular, we demonstrate that *M. heterophyllum* is clearly distinct from a morphologically similar native species in the western US, *M. hippuroides*—whose distinctiveness from *M. heterophyllum* has been questioned—and therefore confirm the introduction of *M. heterophyllum* in the western US. Furthermore, we provide the first evidence for hybridization between these two species. Finally, our molecular markers identify previously unrecognized genetic variation in these four species, and therefore demonstrate the need for further taxonomic investigation.

Keywords Cryptic species · Biotypes · Hybrid vigor · Clonal reproduction · Hybridization

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Introduction

The study of molecular genetic variation in introduced species has become a central focus of invasion biology. Molecular analyses of native and introduced populations can be used to infer the introduction history, such as distinguishing single versus multiple introduction events and identifying the geographic source(s) and size(s) of founding populations (e.g., Novak and Mack 2005; Taylor and Keller 2007). In addition, molecular analyses can provide insight into the potential for adaptive evolution in introduced ranges that might facilitate their establishment and spread. For example, an increasing number of studies demonstrate that introduced species harbor a surprising amount of genetic variation, either from introductions of a large number of propagules that alleviate the effects of bottlenecks in founding populations or through multiple introductions (e.g., Roman and Darling 2007), or result in novel genetic variation in the introduced range that facilitates rapid adaptation (e.g., Kolbe et al. 2004). In cases where recognized species are morphologically similar, or where species boundaries are not clear, molecular analyses may also shed important insight on the taxonomic composition of invasions (e.g., Folino-Rorem et al. 2009). Molecular studies can therefore make direct contributions to invasive species management including the identification of potential introduction pathways (e.g., Novak and Mack 2001), potential biological control agents (e.g., Roderick and Navajas 2003), and distinguishing between different introduced taxa (e.g., Folino-Rorem et al. 2009) or native versus introduced taxa (e.g., Thum et al. 2006). Yet, the introduction history, and in some cases the taxonomy, remain poorly understood for many biological invasions.

Variable leaf watermilfoil, *Myriophyllum heterophyllum* Michx., has become a high-profile invasive aquatic plant species in the northeastern portion of the United States, and has more recently been identified in several locations in the western US states of Washington, Oregon, and California. Unlike transcontinental invaders, *M. heterophyllum* is native to eastern North America. Aiken (1981) described its native range as occurring from Florida eastward along the Atlantic Coastal Plain to Virginia, and also along the Gulf Coastal states northward to Michigan and Ontario, but is not considered native to either the northeastern or western US (see Figs. 3, 4).

The earliest recorded introduced specimen of *M. heterophyllum* in the northeastern US is from Bridgeport, Connecticut in 1932, and it is thought to have escaped from cultivation and subsequently spread across the region through vegetative propagules (Les and Mehrhoff 1999). The species now occurs extensively throughout the northeastern US, and has spread especially rapidly over the past 30 years (Thum and Lennon 2010). Earlier genetic investigations of a limited number of accessions revealed some genetic variation in the northeastern US invasion of *M. heterophyllum*. Most notably, Moody and Les (2002) distinguished a hybrid invasive lineage (*M. heterophyllum* × *M. laxum*) from pure *M. heterophyllum* in several Connecticut populations, and proposed the hybrid lineage as a unique biotype that is more invasive than non-hybrid populations in that region. However, Thum and Lennon (2006) did not identify hybrid genotypes in their survey of several New Hampshire populations, where *M. heterophyllum* is the most common nuisance aquatic plant. Based on these results, Thum and Lennon (2006) hypothesized that the northeastern US invasion may consist of two or more genetically distinct lineages that exhibit spatial structure across the region. Such spatial structure might reflect different independent introductions of unique genotypes. However, multiple accessions from a large number of populations from within both the native and invasive ranges have not yet been sampled to evaluate the possibility that genetic variation results from a single introduction from a genetically diverse ancestral source population. The introduction history in the western US is also unknown, but presumably has occurred more recently than the original northeastern US introduction(s), as the number of western populations is considerably lower than in the northeastern US, and historical records of *M. heterophyllum* are lacking.

One complication involved in reconstructing introduction histories on the basis of historical records is the difficulty in distinguishing *M. heterophyllum* from several closely related species. While species of *Myriophyllum* can be distinguished on the basis of morphology, morphological identifications rely primarily on flower and fruit traits, which are often reduced or altogether unavailable from collected material since many aquatic plants invest more heavily in asexual versus sexual reproduction (Moody et al. 2008). Thus, in practical terms, distinguishing species of *Myriophyllum* can be difficult, and in some cases invasive species can

be mistaken for native species, and vice versa, when only vegetative material is available for morphological identification (e.g., Thum et al. 2006). For example, *M. heterophyllum* is morphologically similar to *Myriophyllum hippuroides* Nutt. ex Torr. & A. Gray. Historically, these two species have been readily identified by a geographic disjunction, as *M. hippuroides* occurs in the western part of North America whereas *M. heterophyllum* occurs in the east. Although the two species can be told apart morphologically (Aiken 1981), Reed (1970) questioned whether the latter should be considered as a separate species given their similarity. In addition, although they exhibited distinct cpDNA and ITS profiles, a recent phylogenetic analysis demonstrated that *M. heterophyllum* was paraphyletic with respect to *M. hippuroides*, further drawing into question their species boundaries (Moody and Les 2010). Given the recent identification of putative introduced populations of *M. heterophyllum* in the western US, it is important to determine whether these represent distinct species, and if so, to develop reliable and efficient identification methods to distinguish them. Similarly, *M. heterophyllum* is morphologically similar to the closely related *Myriophyllum laxum* Schuttl. Ex Chapm. and *Myriophyllum pinnatum* Britton, Sterns & Pogenb., and it overlaps its geographic range with both species in the southeastern portion of the US. Interspecific hybridization may further complicate morphological identifications (e.g., Moody and Les 2007). *Myriophyllum heterophyllum* has also been shown to hybridize with *M. laxum* (Moody and Les 2002), but it is not known whether *M. heterophyllum* hybridizes with other closely related species. As such, although taxonomists have not questioned morphological species boundaries among recognized species of milfoils, the practical difficulties encountered by lake managers associated with their identification warrants the development and evaluation of molecular tools to provide an efficient DNA based identification system for milfoils.

Here, we utilize DNA sequences from the chloroplast DNA (cpDNA) *trnL-F* region and the nuclear ribosomal (nrDNA) internal transcribed spacer regions (ITS) and amplified fragment length polymorphisms (AFLPs) to investigate genetic variation in *M. heterophyllum* in its native range and two disjunct introduced ranges in the US (northeastern versus western US). Specifically, we ask whether variable leaf watermilfoil invasions result from single versus multiple introductions, and what the likely geographic origin(s) of introduced genotypes are. In addition, we use our molecular markers to answer the specific question of whether putative populations of introduced *M. heterophyllum* are distinct from native *M. hippuroides* in the western US. Finally, we further evaluate the potential of molecular markers for identifying and distinguishing distinct taxa within the North American milfoils.

Materials and methods

Sample collection and identification

We obtained samples of *M. heterophyllum* from populations throughout its native range (252 individuals from 84 different populations) and from populations in the northeastern US (231 individuals from 86 populations) and western US (30 individuals from 8 populations) where it is considered introduced and invasive. In addition, we collected a more limited sampling from three closely related species: *M. hippuroides* (21 individuals from eight populations), *M. laxum* (36 individuals from 10 populations), and *M. pinnatum* (11 individuals from five populations). Many of the samples included in our analyses were obtained from state or lake management agencies seeking to confirm taxonomic identifications of plants with molecular genetic data. Most samples received from agencies for taxonomic identification consisted of only a single plant and as such roughly half of our study populations are represented by a single individual (44 introduced populations, 41 native populations). However, the remaining populations are represented by multiple accessions, from which we can examine within and among population genetic variation in both the native and introduced ranges. Details of sampling locations and sample sizes are provided in "Appendix".

Specimens were initially identified to species using morphological characters. However, *Myriophyllum* species can be very difficult to differentiate on the basis of morphological characters when only vegetative material is available, which is often the case for natural populations (Aiken 1981; Moody and Les 2007; Thum et al. 2006). For each species, we had at least one specimen identified on the basis of flowering material, which allowed us to match our genotypes to morphological identifications. For many

of the samples where flowering material was lacking on our specimens, historical records from those same locations were based on flowering material, and for these sites we assumed the taxonomic composition of the population was unchanged. For some sites, only vegetative material was available, and we gave these samples a putative identification based solely on cpDNA and ITS by comparing them to previously published accessions from Moody and Les (2002, 2010). Finally, we further evaluated the morphological and cpDNA/ITS identifications with amplified fragment length polymorphisms (AFLPs) on a subset of samples that were representative of the different cpDNA + ITS genotypes identified (see "Appendix").

For each plant, we extracted DNA from one apical meristem (fresh or freeze-dried) using DNEasy Plant Mini Kits (Qiagen) or a modified CTAB protocol (Gustinich et al. 1991).

DNA sequencing analysis

We amplified and sequenced two genes: the nrDNA internal transcribed spacers 1 and 2 and the intervening 5.8S ribosomal subunit (hereafter referred to collectively as ITS) and a segment of cpDNA including the 5′ and 3′ exons of *trnL*, the intron between them, and the intron between the 3′ exon of *trnL* and *trnF*, including *trnF* (hereafter referred to collectively as cpDNA). In most cases, we amplified ITS using the universal primers ITS1 and ITS4 (Soltis and Kuzoff 1995), but in some cases we substituted the ITS1 primer with a *Myriophyllum* specific internal primer (5′-GCGGAAGGATCATTGTCGAA-3′) to eliminate potential contaminants. For cpDNA, we used primers c and f from Taberlet et al. (1991). All PCR reactions contained the following: 1 μl of 10× PCR buffer (Invitrogen), 2 mM $MgCl_2$, 2 pmol each primer, 0.2 mM each dNTP, 1 unit of *Taq* DNA polymerase (Invitrogen), 1 μl template DNA and brought to a total volume of 10 μl with sterile, distilled and deionized water. Thermal cycling consisted of the following: One cycle at 94°C for 2 min followed by 25 cycles of 94°C for 1 min, 53°C for 30 s, 72°C for 1 min and a final extension at 72°C for 8 min. We visualized 2–5 μl of PCR products on an agarose gel (~1.5%) to check for size and purity, the remaining was treated with the enzymes Exonuclease I (New England Biolabs) and Antarctic Phosphatase (New England Biolabs) to eliminate unincorporated primers and dNTPs before sequencing. PCR products were sequenced using BigDye terminator chemistry (Applied Biosystems) on ABI 3130xl and 3730xl DNA sequencers. In most cases, direct sequencing of PCR products produced clean and unambiguous sequence. However, in several cases for ITS we found more than one ambiguous base pair. In these cases, we cloned the PCR products using the TOPO TA cloning kit (Invitrogen) and sequenced 5–10 positive inserts. DNA sequences were edited using Sequencher, version 4.2 (Gene Codes Corporation) and aligned using ClustalW, as implemented in MEGA version 3.1 (Kumar et al. 2004).

We constructed phylogenies and haplotype networks for both cpDNA and ITS. We constructed maximum parsimony trees in PAUP*, version 4.0 (Swofford 2002) using heuristic searches with simple sequence addition to obtain starting trees, and the tree-bisection-reconnection branch swapping algorithm. We constructed neighbor-joining trees in MEGA version 3.1 (Kumar et al. 2004) using uncorrected genetic distances as our model of sequence evolution because of the very low average sequence divergence among samples, as recommended by Nei and Kumar (2000). For both analyses, we used 1000 bootstrap replicates to assess nodal support under the same parameters as initial analyses. In both phylogenetic analyses, we used *M. laxum* as the outgroup based on the recent genus-wide phylogeny by Moody and Les (2010). We constructed haplotype networks using statistical parsimony (Templeton et al. 1992), as implemented in the program TCS version 1.21 (Clement et al. 2000), using a 95% connection limit. We treated indels consisting of multiple base pairs as single transformations. For individuals that exhibited ITS sequence polymorphisms (e.g., the previously identified hybrids), we considered both sequences in the haplotype network.

We examined geographic patterns of genetic variation in two ways. First, we mapped the geographic distributions of cpDNA haplotypes and ITS genotypes/alleles to identify any apparent biogeographic affinities and to identify the potential geographic origin(s) of invasive lineages. Second, we used Analysis of Molecular Variance (AMOVA), as implemented in Arlequin v. 3.11 (Excoffier et al. 2005) to partition genetic variation within and among

populations in the invaded and native ranges. If genetic variation is partitioned primarily within populations in the native range, a single introduction from a genetically variable source population may explain the diversity of genotypes in the invaded range(s). On the other hand, if genetic variation is partitioned primarily among populations in the native range, such that native populations harbor little genetic variation, then the diversity of genotypes in the introduced range(s) would be best explained by multiple introductions from genetically distinct sources.

We performed four separate AMOVAs for both cpDNA and ITS data. First, we compared within versus among population genetic variation in all native populations combined. Second, we compared within versus among population genetic variation in all introduced populations combined. Third, we compared genetic variation among native and introduced regions, including only the northeastern US introduced range as we did not have enough within-population sampling in the western US to conduct a meaningful analysis. Fourth, we compared two different geographic regions within the native range that showed an obvious phylogeographic break with our molecular markers (see "Results" and "Discussion"). We restricted our AMOVA analyses to those populations where we had genetic data from four or more individuals (44 populations).

AFLP analysis

We conducted an AFLP analysis on a subset of our sampled populations in order to evaluate species and hybrid identifications on the basis of morphology and/or DNA sequences. We were especially interested in determining whether there was strong support for genetic differentiation between putative populations of introduced *M. heterophyllum* and native populations of *M. hippuroides* in the western US, as the proper delimitation of this species pair has strong implications for management in that region. However, we also used AFLPs to (1) determine the extent to which the recognized species *M. pinnatum*, *M. laxum*, and *M. heterophyllum* were differentiated from one another, (2) identify potential hybridization among species (or confirm hybridization in the case of *M. heterophyllum* and *M. laxum*), and (3) corroborate patterns of genetic variation found in native and introduced populations of *M. heterophyllum*. We stress that it was not our intent to evaluate the potential for gene flow among introduced genotypes of *M. heterophyllum*, as we did not have sufficient DNA to perform AFLP genotyping on a large number of individuals from a large number of populations. However, we did include samples from all of the major genetic groups identified with cpDNA and ITS sequences; in total, we analyzed 125 individuals from 49 populations (1–6 individuals per population; see "Appendix").

AFLPs were prepared as described in Vos et al. (1995) with some modifications. We digested ~100 ng of total genomic DNA with EcoRI and MseI restriction enzymes and ligated EcoRI and MseI adaptors (Applied Biosystems). PCR amplification was performed in two steps—"preselective" versus "selective" amplification). Preselective reactions employed the EcoRI-A and MseI-C primers (Applied Biosystems) and selective reactions employed the EcoRI-ACA and MseI-CAT primers (Applied Biosystems) using the manufacturer's protocol. Additional primer pairs also yielded consistent and reliable profiles, but a pilot study on a subset of our samples showed that the qualitative results presented in this paper did not change when adding additional primer pairs and we therefore present results from the single primer pair. Selective amplification products were run on an ABI 3130xl automated DNA sequencer at AWRI using the internal size standard MapMarker1000 ROX (BioVentures, Inc.).

We scored the AFLP data with GeneMapper v4.0 (Applied Biosystems). We limited our analysis to fragments between 80 and 500 bp in length. In an initial analysis, we determined allele bins using a peak height threshold (PHT) of 200 relative fluorescence units (RFU) and a bin width of 0.75 bp. This ensured that only strong bands were included in the binset. We then automatically scored the data using the bin set using a PHT of 30 RFU, but we visually checked and edited all allele calls. Scoring in this manner led to highly reproducible genotypes with low scoring error rates (Jaccard Index = 98–100% similarity for 15 re-genotyped individuals).

We evaluated species boundaries and potential hybridization among recognized species using Structure v2.3.2 (Pritchard et al. 2000; Falush et al. 2007). We used an admixture model with no priors, correlated allele frequencies, and a single α. However, we

evaluated models employing all possible combinations of the above parameters, and our results were robust to different combinations. We initially ran Structure for values of K from one to ten, and evaluated the number of distinct genetic clusters (K) by examining the plot of the likelihood of the data against K, and using Evanno et al.'s (2005) ΔK statistic (see Supplementary Web Appendix 3). Although the ΔK method indicated K = 2, the likelihood scores continued to rise across values of K. Moreover, ΔK is indicative only of the highest level of structure in the dataset (e.g., minimum value of K for which structure could be explained). Therefore, we used the hierarchical approach of Coulon et al. (2008) to identify further genetic structuring; we repeated the analysis of ΔK for each group of the K groups identified in the previous step until no further sub-structuring was evident. For each analysis, we ran the MCMC for 250,000 generations, preceded by a burn-in period of 50,000 generations. For each analysis, we assigned each individual to the group for which its inferred ancestry was highest, and we assigned each population to a single group provided that >50% of the individuals were assigned to that group.

Results

Characterization of molecular markers

cpDNA

We found 19 unique cpDNA haplotypes among our samples. Sequences ranged in size from 900 to 925 bp. The final cpDNA alignment was 935 bp, with 907 positions constant, 12 positions that were parsimony informative, seven singleton substitutions, and fourteen indels ranging from 1 to 11 bp were also present (Supplementary Web Appendix 1). All unique cpDNA sequences were deposited in GenBank (accessions HQ605725-HQ605743).

In our phylogenetic analyses, we found 10 equally parsimonious trees of 23 steps, and support for most nodes in both the maximum parsimony and neighbor-joining analyses were low due to the low genetic distances. As such, the relationships among cpDNA haplotypes were best represented as networks. We found one loop in our cpDNA haplotype network (haplotypes C, G, H, and I; Fig. 1).

ITS

Direct sequencing of ITS PCR products yielded a total of 32 unique genotypes. Fifteen of these lacked ambiguities, indicating homogenization of ITS sequences across the genomes of these individuals. However, there were obvious polymorphisms in ITS sequence at one or more sites in many individuals, indicating the presence of multiple distinct copies of ITS. We refer to ITS sequences as homozygous (only one allele detected in direct sequencing) or heterozygous (two unique alleles detected), but recognize that distinct copies of ITS may occur as paralogs since ITS is found in tandem repeats (Alvarez and Wendel 2003). For sequences with only a single polymorphic site, we could easily distinguish two alleles without the need for molecular cloning. However, we cloned PCR products from at least one individual for each of the ITS direct sequences with more than one ambiguous base position, or when direct sequences were poor due to the presence of indels. In all cases, we recovered only two alleles and one or both of the alleles recovered from cloning corresponded to alleles found in individuals with homogeneous ITS sequences.

After decomposing polymorphic ITS sequences into their composite alleles, we found 24 unique ITS alleles among our samples. Alleles ranged in size from 709 to 711 bp and the final ITS alignment of all sequences was 712 bp (Supplementary Web Appendix 2) of which 672 were conserved, 12 were parsimony informative, 26 were singleton substitutions, and four were indels. All unique ITS sequences were deposited in GenBank (accessions HQ605744-HQ605767).

In our phylogenetic analyses, we found 6 equally parsimonious trees of 41 steps, and support for most nodes in both the maximum parsimony and neighbor-joining analyses were low due to the low genetic distances. As with cpDNA, the relationships among ITS alleles were best represented as networks. We found one loop in our ITS network (alleles 1, 5, 16, and 17; Fig. 2).

AFLPs

In total, we identified 116 polymorphic AFLP loci in our dataset. AFLP profiles had an average of 35.6 bands per individual (range 22–55). Our hierarchical

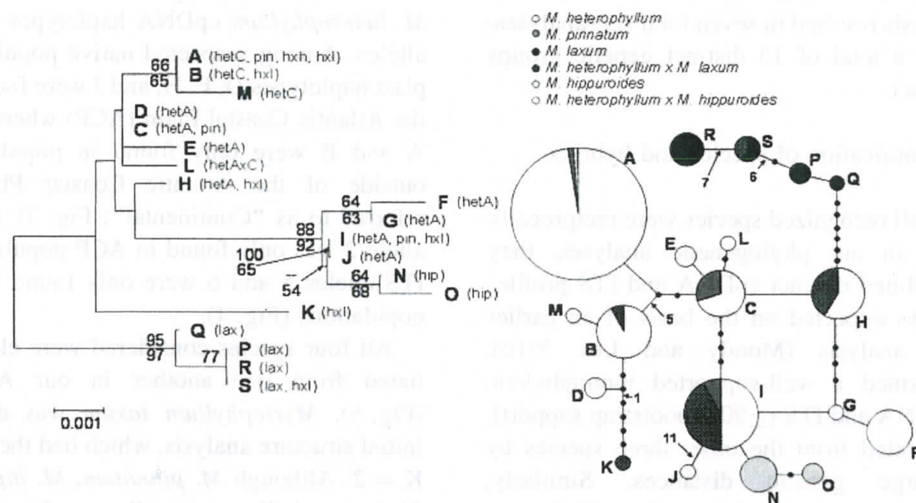

Fig. 1 trnL-F phylogeny (*left*) and haplotype network (*right*). Identical letters in the phylogeny and haplotype network correspond to identical haplotypes. Numbers above and below branches at nodes in the phylogeny are bootstrap values from maximum parsimony and neighbor-joining analyses, respectively (1,000 replicates). In the haplotype network, circle sizes are proportional to the number of populations where each haplotype was found (because many populations are represented by a single individual), and arrows indicate the size of indels (treated as single evolutionary transitions regardless of size). hetA = ACP *M. heterophyllum*, hetC = Continental *M. heterophyllum*, hip = *M. hippuroides*, lax = *M. laxum*, pin = *M. pinnatum*, h × h = *M. heterophyllum* × *M. hippuroides* hybrid, h × l = *M. heterophyllum* × *M. laxum* hybrid

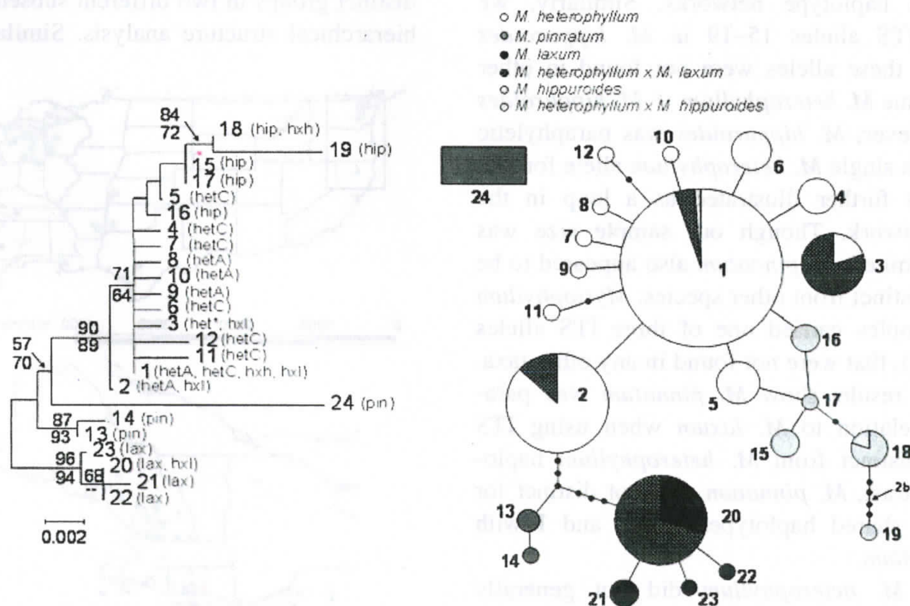

Fig. 2 ITS phylogeny (*left*) and haplotype network (*right*). Identical alleles in the phylogeny and haplotype are indicated by identical numbers. Bootstrap values from maximum parsimony and neighbor-joining analyses (1,000 replicates) are indicated above and below branches at nodes in the phylogeny, respectively. In the haplotype network, circle sizes are proportional to the number of populations where each haplotype was found (because many populations are represented by a single sample), and arrows indicate the size of indels (treated as single evolutionary transitions regardless of size). hetA = ACP *M. heterophyllum*, hetC = Continental *M. heterophyllum*, hip = *M. hippuroides*, lax = *M. laxum*, pin = *M. pinnatum*, hxh = *M. heterophyllum* × *M. hippuroides* hybrid, hxl = *M. heterophyllum* × *M. laxum* hybrid, het* represents a unique *M. heterophyllum* genotype (i.e., populations FL001, FL002 and CT 2.56) that was identified as distinct from both ACP and Continental forms using AFLPs

structure analysis resulted in seven total sub-analyses, and identified a total of 13 distinct genetic groups (detailed below).

Molecular identification of species and hybrids

Although not all recognized species were reciprocally monophyletic in our phylogenetic analyses, they generally exhibited distinct cpDNA and ITS profiles (Figs. 1, 2). As expected on the basis of an earlier phylogenetic analysis (Moody and Les 2010), *M. laxum* formed a well-supported monophyletic group for cpDNA and ITS (≥90% bootstrap support), and was separated from the other three species by relatively large genetic distances. Similarly, *M. hippuroides* was clearly distinct from *M. heterophyllum* at cpDNA and ITS (although the two species were not reciprocally monophyletic for either marker). All *M. hippuroides* samples carried either the N or O cpDNA whereas these haplotypes were not found in *M. heterophyllum* (or any other species), and these two haplotypes formed a clade in phylogenetic analyses and haplotype networks. Similarly, we found only ITS alleles 15–19 in *M. hippuroides* samples, and these alleles were not found in other taxa (except one *M. heterophyllum* × *M. hippuroides* hybrid). However, *M. hippuroides* was paraphyletic in relation to a single *M. heterophyllum* allele for ITS (5), which is further illustrated as a loop in the haplotype network. Though our sample size was much more limited, *M. pinnatum* also appeared to be genetically distinct from other species. *Myriophyllum pinnatum* samples carried one of three ITS alleles (13, 14, or 24), that were not found in any other taxa. Phylogenetic results show *M. pinnatum* was paraphyletic in relation to *M. laxum* when using ITS but clearly distinct from *M. heterophyllum* haplotypes. In contrast, *M. pinnatum* was not distinct for cpDNA, but shared haplotypes A, C, and I with *M. heterophyllum*.

Although *M. heterophyllum* did not generally share alleles with other recognized species, except for cases of interspecific hybridization (see below) and sharing of cpDNA haplotypes with *M. pinnatum*, it was not monophyletic. *Myriophyllum heterophyllum* was paraphyletic in relation to *M. pinnatum* and *M. hippuroides* for ITS and paraphyletic in relation to *M. hippuroides* using cpDNA (Figs. 1, 2). Moreover, we found clear differences in geographic affinities for *M. heterophyllum* cpDNA haplotypes and three ITS alleles. Among suspected native populations chloroplast haplotypes D, E, H, and J were found only along the Atlantic Coastal Plain (ACP) whereas haplotypes A and B were only found in populations located outside of the Atlantic Coastal Plain (hereafter referred to as "Continental"; Fig. 3). Similarly, ITS allele 2 was only found in ACP populations whereas ITS alleles 5 and 6 were only found in Continental populations (Fig. 4).

All four species considered were clearly differentiated from one another in our AFLP analysis (Fig. 5). *Myriophyllum laxum* was distinct in the initial structure analysis, which had the highest ΔK at K = 2. Although *M. pinnatum*, *M. hippuroides*, and *M. heterophyllum* were all part of a single group in the initial structure analysis at K = 2, they did form distinct genetic clusters in subsequent analyses using the hierarchical structure analysis, although not all individuals within a recognized species were always included in the same data partitions. For example, individuals identified as *M. pinnatum* formed two distinct groups in two different subsets of data in the hierarchical structure analysis. Similarly, subgroups

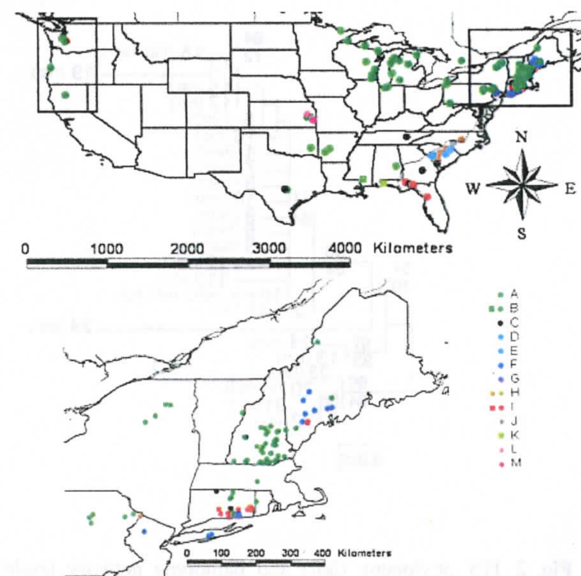

Fig. 3 Geographic distributions of *M. heterophyllum* cpDNA haplotypes throughout the native and introduced ranges (*boxed areas*). *Bottom portion* shows the northeastern invasive range (*boxed region in top*) in more detail. Approximate location of the Atlantic Coastal Plain is indicated by *light shading*. *Squares* represent those haplotypes found in *M. heterophyllum* × *M. laxum* hybrids

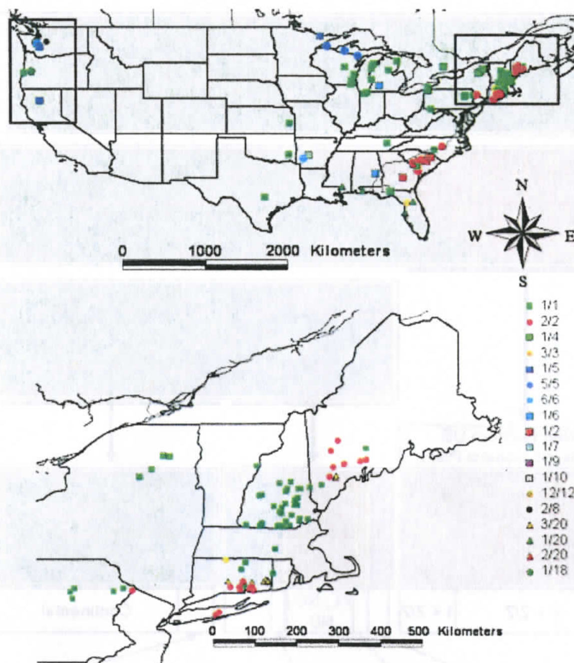

Fig. 4 Geographic distribution of *M. heterophyllum* ITS genotypes in native and introduced ranges (*boxed areas*). *Bottom portion* shows the northeastern invasive range in more detail. Approximate location of the Atlantic Coastal Plain is indicated by *light shading*. *Note*: *Squares* are ITS genotypes that were heterogeneous with the most common ITS allele (1); *triangles* indicate *M. heterophyllum* × *M. laxum* hybrids, all of which carried the *M. laxum* allele (20) in combination with a *M. heterophyllum* allele (indicated by *color*); *M. heterophyllum* × *M. hippuroides* hybrid is indicated with a *pentagon*

of *M. heterophyllum* were identified from different levels of the hierarchical analysis.

We found clear evidence for hybridization among different species, and several other cases where hybridization is suggested but will require additional sampling to confirm. We found clear evidence for hybrid *M. heterophyllum* × *M. hippuroides* in one western population (OR102). These individuals exhibited the ITS genotype 1/18, strongly suggesting biparental inheritance of ITS from *M. heterophyllum* (ITS allele 1) and *M. hippuroides* (ITS allele 18; see "Appendix"), and were also revealed as hybrids with AFLPs. We also identified clear evidence for hybridization between *M. heterophyllum* and *M. laxum* (see also Moody and Les 2002) using both ITS and AFLPs (Fig. 5; see "Appendix"). In addition to these clear cases of hybridization, our hierarchical AFLP analysis suggested hybridization among taxa at each sub-level of analysis. However, a detailed treatment of population structure and hybridization among distinct groups is beyond the scope of this paper.

Genetic differentiation of native *M. heterophyllum*

At the broadest spatial scale, native populations of *M. heterophyllum* formed two clearly distinct groups (ACP versus Continental). These two groups were strongly differentiated for cpDNA and ITS; AMOVA attributed 21.8 and 30.2% of the genetic variation to differences between these regions for cpDNA and ITS, respectively (Table 1). As mentioned in the preceding section, the strong genetic differentiation between ACP and Continental *M. heterophyllum* was evident in geographic affinities of cpDNA and ITS (Figs. 3, 4, respectively) and distinct genetic clusters in AFLP analyses (Fig. 5). In addition, we found evidence for further structuring of populations within the ACP, as populations located along southern portions of the ACP were clearly differentiated from more northerly populations in the introduced northeastern range with AFLPs (Fig. 5).

In addition to the ACP/Continental phylogeographic break, native populations of *M. heterophyllum* were characterized by very high among- and very low within-population genetic differentiation. When all native *M. heterophyllum* populations were considered as a single group, AMOVA partitioned 90.6 and 74.8% of the genetic variation among populations for cpDNA and ITS, respectively (Table 1). The lower among-population genetic variation for ITS as compared to cpDNA reflects the within-individual heterozygosity, as ITS is a nuclear gene. However, we rarely found more than one unique ITS genotype in a native population; only four native populations (of 51 where two or more individuals were sampled) had more than one ITS genotype (maximum of two unique ITS genotypes; "Appendix"). Similarly, only four of the native populations had more than one cpDNA haplotype (maximum of three haplotypes; "Appendix"). The strong phylogeographic break between ACP and Continental populations likely influences the among-population component of variation. However, even when we treated ACP and Continental populations as separate groups, AMOVA still partitioned the majority of genetic variation among- rather than within-populations within regions

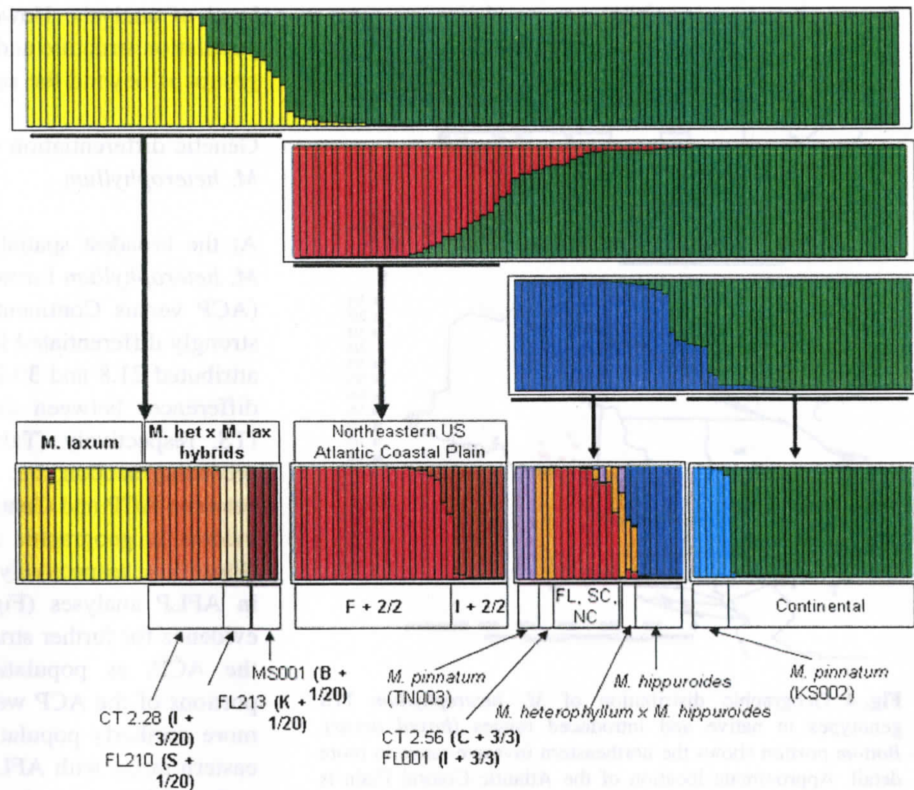

Fig. 5 Results from the hierarchical structure analysis. Initial analysis including all samples at K = 2 shown at *top*, with subsequent analyses from the hierarchical analysis indicated by connections with *arrows*. For each box, each *vertical bar* represents an individual, and its proportion of membership to each cluster is denoted by *different colors*. Select taxa, populations, and cpDNA and ITS genotypes referred to in text are highlighted along the *top* and *bottom* of the bottom panel. "M. het × M. lax hybrids" refer to individuals identified as putative *M. heterophyllum* × *M. laxum* hybrids with ITS sequences. "FL, SC, and NC" refers to populations located in the US states of Florida, South Carolina, and North Carolina on the Atlantic Coastal Plain

($P < 0.0001$; 65.5 and 48.6% for cpDNA and ITS, respectively; Table 1).

Genetic composition of introduced populations

Northeastern US

In the northeastern US, we found nine distinct cpDNA + ITS genotypes representing independent introductions of several distinct taxa from distinct sources (Table 2). Hereafter, we describe cpDNA + ITS genotypes in the form $i + j/k$, where 'i' denotes the cpDNA haplotype and 'j/k' denotes the two ITS alleles. Two genotypes (A + 1/1 and A + 1/4) clearly originate from Continental populations of *M. heterophyllum* as their cpDNA haplotype was only found in Continental populations, and representative individuals from these genotypes were assigned >95% posterior probability of belonging to Continental *M. heterophyllum* in our AFLP analysis. A + 1/1 was the most common and widespread introduced genotype in the northeastern US, whereas A + 1/4 was found only in the Adirondack Region of New York (Supplementary Web Appendix 4). Similarly, four genotypes showed clear evidence for ACP origins: F + 2/2 was found in several populations from Connecticut, Maine, and New York and formed its own group in our AFLP analysis (Fig. 5); I + 2/2 was found only in several Connecticut populations and formed a separate group from F + 2/2 in our AFLP analysis; genotypes C + 2/2 and H + 2/2 also likely originate from the ACP, but we were unable to collect AFLP information for these samples. In addition to the genotypes above, we found the same hybrid *M. heterophyllum* × *M. laxum* genotype (I + 3/20) identified in an earlier study by Moody and Les (2002) in ten Connecticut populations and two in Maine. One of these genotypes (CT2.28) formed a distinct genetic cluster with a hybrid population identified from Florida (FL210) in our AFLP analysis (Fig. 5), which may indicate its origin from a genetically distinct population of hybrids in Florida. We also identified a distinct and previously-unrecognized genotype (C + 3/3) from one Connecticut population (CT 2.56) that formed a distinct cluster in our AFLP analysis with a sample from one Florida population (FL001), suggesting it may also originate from Florida (Fig. 5). Finally, we identified the C + 1/1 genotype from a single

Table 1 Analyses of molecular variance (AMOVA) for cpDNA and ITS

	cpDNA			ITS		
	df	% Variation	*P*	*df*	% Variation	*P*
Native range						
Atlantic Coastal Plain versus Continental						
Among regions	1	21.8	0.0059	1	30.2	0.0235
Among pop. within region	22	65.5	<0.0001	20	48.6	<0.0001
Within pops	98	12.7		220	21.2	
All populations						
Among pop.	23	90.6	<0.0001	21	74.8	<0.0001
Within pop.	98	9.4		220	25.2	
Introduced range						
Among pop.	21	97.7	<0.0001	21	59	<0.0001
Within pop.	104	2.3		224	41	
Native versus introduced ranges						
Among regions	1	14.6	0.0020	1	32.3	0.0020
Among pop. within region	44	78	<0.0001	42	44	<0.0001
Within pops	202	7.4		444	23.7	

Four separate analyses for each marker were conducted: "ACP versus continental" grouped native populations into the Atlantic Coastal Plain (ACP) versus those located outside the ACP whereas "all populations" treated all native populations as a single group; "introduced range" included only those populations considered as introduced in the northeastern US (introduced western populations had samples sizes that were too low to conduct a meaningful AMOVA), "native versus introduced ranges" grouped populations into those that were native (ACP + continental) versus those considered as introduced in the northeastern US

df degrees of freedom

Table 2 Invasive genotypes (cpDNA + ITS) identified in the northeastern and western US

Introduced range	cpDNA haplotype	ITS allele 1	ITS allele 2	Putative taxon
Northeastern/Western US	A	1	1	Continental *M. heterophyllum*
Northeastern US	A	1	4	Continental *M. heterophyllum*
Northeastern US	F	2	2	ACP *M. heterophyllum*
Northeastern US	I	2	2	ACP *M. heterophyllum*
Northeastern US	C	2	2	ACP *M. heterophyllum*[a]
Northeastern US	H	2	2	ACP *M. heterophyllum*[a]
Northeastern US	I	3	20	*M. heterophyllum* × *M. laxum*
Northeastern US	C	1	1	*M. heterophyllum or M. pinnatum*[b]
Northeastern US	C	3	3	*M. heterophyllum*
Western US	A	1	5	Continental *M. heterophyllum*[a]
Western US	A	5	5	Continental *M. heterophyllum*
Western US	B	1	6	Continental *M. heterophyllum*
Western US	B	6	6	Continental *M. heterophyllum*[a]
Western US	I	2	8	ACP *M. heterophyllum*[a]
Western US	A	1	18	*M. heterophyllum* × *M. hippuroides*

[a] Genotype not included in AFLP analysis

[b] Taxon unclear because the cpDNA haplotype for this genotype was found in both *M. pinnatum* and *M. heterophyllum*

population in New Hampshire (NH034), and its geographic origin is unclear presently.

As in the native range, northeastern populations showed very high among-population variation. Analysis of Molecular Variance partitioned 98 and 59% of the genetic variation among introduced northeastern populations for cpDNA and ITS, respectively (Table 1). In fact, all of the northeastern populations where we examined two or more individuals, except for CT 2.54, had only a single cpDNA + ITS genotype indicating that introduced genotypes rarely co-occur within a single lake ("Appendix"). In addition, we did not find evidence for extensive gene flow among introduced genotypes. Although a comprehensive study of gene flow among populations using AFLPs was beyond the scope of this study, gene flow among the three most common ITS genotypes (1/1, 2/2, and 3/20) would likely be revealed through ITS sequences alone given that none of them shared any ITS alleles in common.

Western US

We identified seven distinct cpDNA + ITS genotypes in the western US, at least four of which represent independent introductions from distinct sources (Table 2). All genotypes identified as introduced *M. heterophyllum* were clearly distinct from native populations of *M. hippuroides* for cpDNA, ITS, and AFLPs, demonstrating a lack of gene flow with *M. heterophyllum* genotypes excepting one genotype demonstrating clear evidence of recent hybridization (see below). Five *M. heterophyllum* genotypes found in the west (A + 1/1, A + 5/5, A + 1/6, A + 1/5, B + 6/6) originate from Continental *M. heterophyllum* populations. Representative individuals from the first three genotypes listed above were included in the AFLP analysis and had posterior probabilities of belonging to Continental *M. heterophyllum* exceeding 98% (Fig. 5). The remaining two genotypes had cpDNA and ITS alleles that were only found in Continental *M. heterophyllum* populations. Although we did not have sufficient material to conduct a finer scale study of gene flow among introduced Continental *M. heterophyllum* in the western US, based on ITS genotypes alone it is possible that the two heterogeneous ITS genotypes (1/5 and 1/6) represent hybridization among independently introduced genotypes (1/1 and 5/5, 1/1 and 6/6). One genotype (I + 2/2) shows clear genetic affinity with ACP *M. heterophyllum*; this genotype was not available for AFLP analysis, but this ITS allele was only found in ACP populations. Finally, one population (OR102) with genotype (A + 1/18) was unequivocally identified as hybrid *M. heterophyllum* × *M. hippuroides* on the basis of ITS and AFLPs (see "Appendix"; Fig. 5).

Discussion

Introduction history and invasion dynamics

The earliest known record of *M. heterophyllum* in southern New England is from Bridgeport, Connecticut in 1932, indicating that this species has been present in this region since at least this time. In contrast, the timing of introduction to the western US is less clear, but presumably occurred much more recently given the lack of historical records from the region. Our study demonstrates that these two introduced ranges have been colonized by different source populations for the most part. For example, cpDNA haplotype B and ITS haplotypes 5, 6, and 8 were found in introduced populations in the western US but not in the northeastern US. In contrast, cpDNA haplotypes C, F, and H and ITS alleles 3, 4, and 23 were found in the northeastern US but not in the western US. The most common introduced cpDNA + ITS genotype (A + 1/1) was found in both ranges, but given how common this genotype is throughout the native range, it is not clear whether this genotype originates from the same source population in both introduced ranges.

In addition, both introduced ranges have been independently colonized by distinct genotypes that represent independent introductions. In both introduced ranges, we found clear evidence of introduced genotypes originating from both of the distinct biogeographic regions identified in the native range (ACP and Continental), as evidenced by the geographic affinities of cpDNA haplotypes and ITS alleles and the clear phylogeographic structure between ACP and Continental *M. heterophyllum* in the AFLP analysis (see Table 2). The strong partitioning of genetic variation among native populations, even within biogeographic regions, strongly suggests that distinct introduced genotypes within the

ACP and Continental *M. heterophyllum* groups reflect independent introductions from distinct local source populations within these biogeographic regions. For example, there were two introduced genotypes in the northeastern US, and four in the western US, that can be confidently assigned to originating from Continental *M. heterophyllum* populations. It is unlikely that these distinct Continental *M. heterophyllum* genotypes arose from a single source population because native populations are characterized by high among- and very low within-population genetic variation. Thus, introduced genotypes from any single source population would likely be limited to one or a few closely related clones. In the northeastern US, we found three additional distinct introduced genotypes (I + 3/20, C + 1/1, and C + 3/3) whose geographic origins could not be confidently determined, but most likely represent introductions from distinct source populations given that we rarely found genetic variation within native populations.

Our study therefore sheds light on the invasion dynamics of *M. heterophyllum*. Although *M. heterophyllum* is capable of clonal spread, invasion in both the northeastern and western US cannot be explained by a simple model of subsequent vegetative spread from a single introduced source population, but rather the geographic extent of both introduced ranges must be explained by a history of independent introductions of different genotypes to different locations with subsequent spread. Thus, understanding invasion dynamics in this system requires understanding the pathway(s) for introducing new genotypes in addition to understanding the mechanisms that facilitate establishment and spread of genotypes after their initial introduction.

The mechanism(s) for the initial introductions of the distinct genotypes identified here is unclear, but is likely related to human activities. For example, *M. heterophyllum* has been recommended as an aquarium and water garden plant, and has been available in the aquatic plant trade under a variety of names (Moody et al. 2008; Thum, personal observation) for over a century (Bissett 1907; Samuel 1894; Tricker 1897). It is therefore possible that a variety of genotypes are readily available in the aquarium trade, and that intentional or unintentional release of aquarium trade specimens serve as consistent sources for *M. heterophyllum* introductions (e.g., Cohen et al. 2007). Alternatively, or in addition to, it is possible that variable leaf watermilfoil has spread via man-made water vectors, or through animal vectors such as waterfowl (Les and Mehrhoff 1999).

While the genetic variation identified here clearly demonstrates multiple introductions, whether this genetic variation plays an important role in facilitating the establishment and spread of variable leaf watermilfoil is unclear and warrants further investigation. For example, it is possible that the distinct introduced genotypes evolved in distinct abiotic and biotic habitats, and they may therefore invade distinct habitats in their introduced ranges. Comparative ecological studies could therefore be used to construct ecological niche models to predict the spread of in individual genotypes (e.g., Thum and Lennon 2010) or search for lineage-specific biocontrol agents. In addition, gene flow among introduced genotypes has the potential to stimulate the evolution of invasiveness or facilitate adaptation in invaded ranges (e.g., Kolbe et al. 2004; Lavergne and Molofsky 2007; Ellstrand and Schierenbeck 2000). It is unclear at the present time whether or not gene flow among distinct introduced lineages has occurred in the northeastern and western US (although unique combinations of ITS and cpDNA alleles in both ranges are suggestive), and we are currently collecting additional data to evaluate the extent of gene flow among introduced *M. heterophyllum* genotypes.

Species boundaries and molecular identification

Our expanded survey of the four species examined here (*M. laxum*, *M. heterophyllum*, *M. hippuroides*, and *M. pinnatum*) unambiguously identifies them as distinct using both ITS DNA sequences and AFLP markers. This result is especially important in the case of distinguishing putative introduced *M. heterophyllum* from native *M. hippuroides* in the western US. The distinctiveness of *M. hippuroides* has been debated based on morphology alone (Reed 1970; Aiken 1981), and a recent phylogenetic analysis demonstrated that *M. hippuroides* and *M. heterophyllum* were not reciprocally monophyletic (Moody and Les 2010), which therefore draws into question their species status. Here we show that populations in the western US identified as *M. heterophyllum* versus *M. hippuroides* did not share cpDNA or ITS haplotypes in common, and formed two clearly distinct groups using AFLPs. Therefore, these two should be

considered as separate species, which confirms the designation of *M. heterophyllum* as an introduced species in the western US. In addition, we found clear evidence for hybridization between *M. heterophyllum* and *M. hippuroides* in one population in the western US. This finding is also significant, as hybridization is thought to play an important role in the evolution of invasiveness in milfoils (Moody and Les 2002), and may also lead to genetic pollution of native gene pools (Rhymer and Simberloff 1996). We therefore recommend that hybrid *M. heterophyllum* × *M. hippuroides* populations be either carefully monitored or targeted for control or eradication.

In addition, our expanded genetic and geographic survey of our four focal species suggests that their diversification history is more complicated than revealed by earlier analyses of ITS and cpDNA sequences based on a more a limited number of accessions (Moody and Les 2002, 2010). Specifically, several new observations indicate that these four species—as currently recognized—may harbor additional taxonomic diversity that is uncharacterized and/or may have histories of hybridization and introgression that are not revealed by ITS and cpDNA sequences.

First, *M. heterophyllum* exhibited a strong phylogeographic break between populations located on the Atlantic Coast Plain versus other portions of eastern North America. This phylogeographic break reflects a general pattern of differentiation exhibited by many taxa in unglaciated eastern North America (see Soltis et al. 2006). However, it is important to note that this genetic split between ACP and Continental *M. heterophyllum* was apparent in our AFLP data at levels of the hierarchical structure analysis before two other recognized species (*M. hippuroides* and *M. pinnatum*) were identified as distinct genetic groups (see Fig. 5). This suggests that the divergence of ACP and Continental *M. heterophyllum* preceded the divergence of *M. hippuroides* or *M. pinnatum*, and that *M. heterophyllum* may be composed of two or more distinct cryptic species.

Second, another genotype that was considered as *M. heterophyllum* on the basis of morphology and ITS genotype (3/3) was distinct from other ACP and Continental *M. heterophyllum*. This genotype was recognized as its own distinct cluster in our hierarchical structure analysis in the same subset of data where *M. hippuroides* formed its own distinct group. That it was not closely related to other *M. heterophyllum* suggests it is an inadequately characterized lineage of *M. heterophyllum*, and further taxonomic investigation of this lineage is therefore warranted.

Third, our overall AFLP analysis did not recognize an introduced New England genotype previously identified as *M. heterophyllum* × *M. laxum* hybrids on the basis of heterogeneous ITS sequences (3/20; see also Moody and Les 2002) as interspecific hybrids. Instead, these genotypes were identified as pure *M. laxum* in our initial structure analysis of $K = 2$ whereas other populations identified as *M. heterophyllum* × *M. laxum* hybrids on the basis of ITS showed clear evidence of genetic admixture at $K = 2$. However, the putative New England hybrids were identified as a distinct genetic cluster that included samples from one Florida population (FL210) in a subsequent hierarchical structure analysis of the *M. laxum* + *M heterophyllum* × *M. laxum* samples. It is therefore unclear whether the putative New England hybrids represent a highly introgressed hybrid lineage that has retained ITS heterozygosity, a distinct lineage of *M. laxum* (or a closely related taxon) that has retained divergent copies of ancestral ITS sequences, or whether our sampling of parental *M. heterophyllum* was insufficient to identify a significant fraction of the genome attributable to *M. heterophyllum*. Regardless of the reason(s), the discrepancy between ITS and AFLPs for these genotypes illustrates that potentially important variation has not been adequately captured in this group and that further taxonomic study is critical to evaluate the hypothesis that hybridization has preceded the evolution of invasiveness in this lineage, as these genotypes have been cited as one such example (Moody and Les 2002; Schierenbeck and Ellstrand 2009).

Fourth, we found evidence for potentially divergent lineages of *M. pinnatum* as well as potential instances of interspecific hybridization. Most strikingly, the ITS sequence (24) identified in one population of *M. pinnatum* (TN003) was comparatively divergent from other ITS sequences, and did not form a monophyletic group with ITS sequences found in other accessions of *M. pinnatum* (ITS alleles 13 and 14). Moreover, this population formed a distinct genetic cluster from other *M. pinnatum* (KS002) in our AFLP analysis. The specimen with the divergent ITS did not have flowering material available for identification, but was collected from a location where *M. pinnatum* had been identified on

the basis of flowering material; it is possible therefore that this lineage represents an undescribed form of North American *Myriophyllum*. In addition, we found evidence for hybridization in one *M. pinnatum* population (TN002); the single individual from this population appeared admixed between the unique TN003 *M. pinnatum* cluster and a cluster representing *M. heterophyllum* from the southeastern ACP in our hierarchical structure analysis. In addition, we identified cpDNA haplotype sharing between *M. heterophyllum* and *M. pinnatum* (haplotypes C and I). We recognize that the signature of admixture in the AFLP analysis may result from insufficient sampling across the geographic ranges of these taxa, especially considering the low sample size of *M. pinnatum*. However, these observations demonstrate that further sampling of *M. pinnatum* across its geographic range and additional taxonomic treatment is warranted.

Finally, we found evidence for additional genetic structuring within the ACP *M. heterophyllum*. Although all ACP populations shared ITS allele 2, our AFLP analysis distinguished populations from the southern portion of the ACP (Florida, Georgia, South Carolina, and North Carolina) from those considered as introduced in the northeastern US. In addition, we found two distinct cpDNA + ITS genotypes in the northeastern US (F + 2/2 and I + 2/2) that also formed two distinct groups in the AFLP analysis. Interestingly, we did not find either the F + 2/2 or I + 2/2 genotype in the southern ACP where *M. heterophyllum* is considered native, nor did we find any southern ACP individuals that showed similar posterior probabilities of group membership with northeastern ACP genotypes in the AFLP analysis. It is possible that the genetic diversity and structure of ACP *M. heterophyllum* has not been sufficiently characterized to determine the geographic origin(s) of northeastern ACP *M. heterophyllum*. Alternatively, we wish to acknowledge the possibility that the northeastern ACP *M. heterophyllum* genotypes may be historically native to the region that went unrecognized until surveying efforts increased over the past two decades in order to monitor for invasive aquatic plants. Early distribution records show that *M. heterophyllum* was present on Long Island, NY (Les and Mehrhoff 1999), which indicates that its native distribution extended northward along the ACP at least that far. Thus, it is possible that *M. heterophyllum* occurred in one or more Pleistocene refugia located in more northern areas of the ACP (e.g., Georges Bank or mid-Atlantic Coastal Plain refuges; e.g., Stemberger 1995). Given the importance of distinguishing native versus introduced populations, we recommend that additional detailed sampling of ACP populations be conducted to further evaluate the status of northeastern ACP lineages.

Overall, the recognition that many native and introduced aquatic plants can be difficult to identify on the basis of vegetative characters alone has led to an increasing interest in the use of molecular markers for identification of species and hybrids (e.g., Moody et al. 2008). In fact, US state agencies increasingly use molecular methods to verify identifications of putative introduced populations (e.g., New Hampshire Department of Environmental Services, Washington Department of Ecology, Wisconsin Department of Natural Resources). In general, species in our study exhibited unique cpDNA and ITS profiles, and were distinct for AFLPs, demonstrating the high potential utility of a DNA-based identification tool. However, we urge institutions utilizing DNA-based identifications to recognize the potential for misidentifications with ITS alone, especially given our observations suggesting that hybridization and introgression or cryptic diversity may be more prominent than currently recognized in this system. We recommend continued taxonomic survey of North American milfoils, including the integration of geographic surveys with extensive morphological and molecular study.

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Appendix

See Table 3.

Table 3 Sampling locations, cpDNA haplotypes, ITS genotypes, taxonomic identifications, and status of populations

Lake code	Lake name	Lat.	Long.	cpDNA haplotype(s)	ITS genotype(s)	Taxonomic identification	Putative status
KS001 (1)	Welda Prairie Area Unit 3	38.1834	−95.254	A (2), L (2), M (1)	1/1 (5)	Cont. *M. heterophyllum*	Native (Cont)
MI005 (1)	Fiddler's Pond	44.9142	−85.9659	A (3)	1/1 (3)	Cont. *M. heterophyllum*[a]	Native (Cont)
MI051 (2)	Lake Lancer	44.141	−84.4368	A (5)	1/1 (5)	Cont. *M. heterophyllum*	Native (Cont)
PA001 (1)	Ridge Pond	42.1649	−80.1007	A (4)	1/1 (3)	Cont. *M. heterophyllum*[a]	Native (Cont)
PA004 (1)	Cheat River	39.7321	−79.8793	A (2)	1/1 (1)	Cont. *M. heterophyllum*	Native (Cont)
IN002	Wauhob/Round Lakes	41.5336	−87.0404	A (5)	1/1 (5)	Cont. *M. heterophyllum*[a]	Native (Cont)
MI001	Bass Lake	44.7347	−86.0643	A (1)	1/1 (1)	Cont. *M. heterophyllum*	Native (Cont)
MI002	Long Lake	45.2	−83.4833	A (1)	1/1 (1)	Cont. *M. heterophyllum*	Native (Cont)
MI003	Lake Michigan (Platte River Point)	44.7317	−86.1528	A (1)	1/1 (1)	Cont. *M. heterophyllum*	Native (Cont)
MI012	Bar Lake	44.2958	−86.3034	A (2)	1/1 (1)	Cont. *M. heterophyllum*	Native (Cont)
WI001	Nagawicka Lake	43.0667	−88.3833	A (1)	1/1 (1)	Cont. *M. heterophyllum*	Native (Cont)
WI002	Spring Lake	42.85	−88.4333	A (1)	1/1 (1)	Cont. *M. heterophyllum*	Native (Cont)
WI012	Long Lake Outlet	43.6493	−88.1775	A (4)	1/1 (4)	Cont. *M. heterophyllum*	Native (Cont)
WI123	Lake Beulah	42.8342	−88.369	A (1)	1/1 (1)	Cont. *M. heterophyllum*	Native (Cont)
WI127	Lake Geneva	42.5833	−88.5333	A (1)	1/1 (1)	Cont. *M. heterophyllum*	Native (Cont)
CT 2.55 (1)	Crystal Lake	41.9404	−72.3758	A (4)	1/1 (6)	Cont. *M. heterophyllum*	Introduced (NE)
ME008 (3)	Lake Arrowhead	43.664	−70.722	A (6)	1/1 (6)	Cont. *M. heterophyllum*	Introduced (NE)
OR001 (1)	Siltcoos Lake	43.8835	−124.1024	A (1)	1/1 (1)	Cont. *M. heterophyllum*	Introduced (W)
CT001	Bolton Notch Pond	41.7886	−72.4536	A (1)	1/1 (1)	Cont. *M. heterophyllum*	Introduced (NE)
CT003	Lower Pond	41.3936	−72.3792	A (1)	1/1 (1)	Cont. *M. heterophyllum*	Introduced (NE)
MA001	Lake Quinsigamond	42.2433	−71.74	A (1)	1/1 (1)	Cont. *M. heterophyllum*	Introduced (NE)
ME006	Messalonskee Lake	44.479	−69.789	A (2)	1/1 (2)	Cont. *M. heterophyllum*	Introduced (NE)
NH004	Lake Winnipesaukee (Back Bay)	43.5764	−71.2183	A (1)	1/1 (1)	Cont. *M. heterophyllum*	Introduced (NE)
NH005	Balch Lake	43.6167	−70.9834	A (5)	1/1 (5)	Cont. *M. heterophyllum*	Introduced (NE)
NH010	Big Turkey Pond	43.1668	−71.5834	A (1)	1/1 (1)	Cont. *M. heterophyllum*	Introduced (NE)
NH011	Bixby Pond	43.223	−71.317	A (1)	1/1 (1)	Cont. *M. heterophyllum*	Introduced (NE)
NH012	Brindle Pond	43.3667	−71.2335	A (1)	1/1 (1)	Cont. *M. heterophyllum*	Introduced (NE)
NH013	Cheshire Pond	42.8167	−72.0002	A (1)	1/1 (1)	Cont. *M. heterophyllum*	Introduced (NE)
NH014	Crescent Lake	43.5834	−71.1835	A (1)	1/1 (1)	Cont. *M. heterophyllum*	Introduced (NE)
NH015	Forest Lake	42.7835	−72.3503	A (1)	1/1 (1)	Cont. *M. heterophyllum*	Introduced (NE)

Table 3 continued

Lake code	Lake name	Lat.	Long.	cpDNA haplotype(s)	ITS genotype(s)	Taxonomic identification	Putative status
NH016	Hopkinton Lake	43.188	−71.748	A (2)	1/1 (2)	Cont. *M. heterophyllum*	Introduced (NE)
NH018	Horseshoe Pond	42.852	−71.489	A (1)	1/1 (1)	Cont. *M. heterophyllum*	Introduced (NE)
NH019	Lake Winnisquam	43.5169	−71.5	A (1)	1/1 (1)	Cont. *M. heterophyllum*	Introduced (NE)
NH020	Lees Pond	43.7334	−71.3836	A (1)	1/1 (1)	Cont. *M. heterophyllum*	Introduced (NE)
NH021	Locke Lake	43.38	−71.248	A (1)	1/1 (1)	Cont. *M. heterophyllum*	Introduced (NE)
NH022	Massabesic Lake	42.9972	−71.3736	A (1)	1/1 (1)	Cont. *M. heterophyllum*	Introduced (NE)
NH023	Melendy Pond	42.7669	−71.6502	A (1)	1/1 (1)	Cont. *M. heterophyllum*	Introduced (NE)
NH024	Northwood Lake	43.2167	−71.2502	A (1)	1/1 (1)	Cont. *M. heterophyllum*	Introduced (NE)
NH025	Paugus Bay	43.576	−71.459	A (1)	1/1 (1)	Cont. *M. heterophyllum*	Introduced (NE)
NH026	Pearly Pond	42.7668	−72.0502	A (1)	1/1 (1)	Cont. *M. heterophyllum*	Introduced (NE)
NH027	Phillips Pond	42.919	−71.19	A (1)	1/1 (1)	Cont. *M. heterophyllum*	Introduced (NE)
NH028	Potanipo Pond	42.736	−71.679	A (1)	1/1 (1)	Cont. *M. heterophyllum*	Introduced (NE)
NH029	Squam River	43.695	−71.631	A (1)	1/1 (1)	Cont. *M. heterophyllum*	Introduced (NE)
NH031	Suncook River	43.3339	−71.2933	A (3)	1/1 (3)	Cont. *M. heterophyllum*	Introduced (NE)
NH032	Suncook Lake	43.3339	−71.2933	A (2)	1/1 (2)	Cont. *M. heterophyllum*	Introduced (NE)
NH033	Turtle Pond	43.2501	−71.5167	A (1)	1/1 (1)	Cont. *M. heterophyllum*	Introduced (NE)
NH101	Big Island Pond	42.8573	−71.2123	A (1)	1/1 (1)	Cont. *M. heterophyllum*	Introduced (NE)
NH102	Sunapee Lake	43.432	−72.067	A (2)	1/1 (1)	Cont. *M. heterophyllum*	Introduced (NE)
NH303	Contoocook Lake	42.7915	−72.0098	A (1)	1/1 (1)	Cont. *M. heterophyllum*	Introduced (NE)
NH304	Flints Pond, Hollis	42.75	−71.55	A (1)	1/1 (1)	Cont. *M. heterophyllum*	Introduced (NE)
NH305	Glen Lake, Goffstown	43.02	−71.59	A (1)	1/1 (1)	Cont. *M. heterophyllum*	Introduced (NE)
NH306	Gorham Pond	43.07	−71.63	A (1)	1/1 (1)	Cont. *M. heterophyllum*	Introduced (NE)
NH314	Powwow Pond	42.9086	−71.0242	A (1)	1/1 (1)	Cont. *M. heterophyllum*	Introduced (NE)
NH318	Otternic Pond	42.7716	−71.4234	A (1)	1/1 (1)	Cont. *M. heterophyllum*	Introduced (NE)
PA101	Harvey's Lake	41.3626	−76.0413	A (2)	1/1 (1)	Cont. *M. heterophyllum*	Introduced (NE)
PA102	Harris Pond	41.295	−76.1317	A (2)	1/1 (2)	Cont. *M. heterophyllum*	Introduced (NE)
PA103	Lily Lake	41.142	−76.0805	A (2)	1/1 (2)	Cont. *M. heterophyllum*	Introduced (NE)
PA104	Promised Land Lower Lake	41.3087	−75.1976	A (2)	1/1 (2)	Cont. *M. heterophyllum*	Introduced (NE)
PA105	Shohola Lake	41.3762	−74.9888	A (2)	1/1 (2)	Cont. *M. heterophyllum*	Introduced (NE)
RI001	Carbuncle Pond	41.6986	−71.7747	A (1)	1/1 (1)	Cont. *M. heterophyllum*	Introduced (NE)
VT007	Halls Pond	44.0906	−72.1217	A (2)	1/1 (1)	Cont. *M. heterophyllum*	Introduced (NE)

Table 3 continued

Lake code	Lake name	Lat.	Long.	cpDNA haplotype(s)	ITS genotype(s)	Taxonomic identification	Putative status
ONT102	Pigeon Lake	44.5614	−78.4795	A (5)	1/4 (5)	Cont. *M. heterophyllum*	Native (Cont)
NY101 (1)	Lake Placid, New York	44.2962	−73.992	A (5)	1/4 (5)	Cont. *M. heterophyllum*	Introduced (NE)
NY001	Long Lake	44.015	−74.385	A (1)	1/4 (1)	Cont. *M. heterophyllum*	Introduced (NE)
NY002	Raquette Lake	43.841	−74.643	A (3)	1/4 (3)	Cont. *M. heterophyllum*	Introduced (NE)
NY102	Lake Flower	44.3161	−74.1203	A (5)	1/4 (5)	Cont. *M. heterophyllum*	Introduced (NE)
MI031	Bass Lake	46.305	−89.173	A (5)	5/5 (5)	Cont. *M. heterophyllum*	Native (Cont)
MI122	Hamilton Lake	45.755	−87.785	A (5)	5/5 (4)	Cont. *M. heterophyllum*	Native (Cont)
WI004	Lake Superior (Siskiwit Bay)	46.85	−91.1333	A (1)	5/5 (1)	Cont. *M. heterophyllum*	Native (Cont)
WA020 (4)	Clear Lake (Thurston, Co.)	46.8283	−122.4756	A (8)	5/5 (6)	Cont. *M. heterophyllum*	Introduced (W)
CA005 (2)	Lake Prairie	40.786	−122.47	A (5)	1/5 (5)	Cont. *M. heterophyllum*	Introduced (W)
WA002	Blue Lake	46.8178	−122.47	A (4)	1/5 (3)	Cont. *M. heterophyllum*	Introduced (W)
MI004 (1)	Gull Lake, MI	42.4122	−85.4122	A (6)	1/6 (6)	Cont. *M. heterophyllum*[a]	Native
CT004	Upper Bolton Lake	41.8235	−72.4213	A (2)	–	Cont. *M. heterophyllum*	Introduced (NE)
ME014	Spaulding Pond	45.9856	−70.1523	A (1)	–	Cont. *M. heterophyllum*	Introduced (NE)
MI025	Fife Lake	44.5667	−85.3333	A (1)	–	Cont. *M. heterophyllum*	Native (Cont)
NH302	Cobbetts Pond	42.7931	−71.2916	A (1)	–	Cont. *M. heterophyllum*	Introduced (NE)
NH310	Lake Waukewan	43.6587	−71.5238	A (1)	–	Cont. *M. heterophyllum*	Introduced (NE)
IN004	Fishtrap Lake	41.6318	−86.7294	B (5)	1/1 (5)	Cont. *M. heterophyllum*[a]	Native (Cont)
MI015	Little Black Lake	43.1274	−86.2368	B (8)	1/1 (6)	Cont. *M. heterophyllum*[a]	Native (Cont)
OK002	Unnamed pond in SE Oklahoma	34.599	−95.3032	B (1)	1/1 (1)	Cont. *M. heterophyllum*[a]	Native (Cont)
PA002	Edinboro Lake Fen	41.8846	−80.1366	B (2)	1/1 (1), 1/6 (1)	Cont. *M. heterophyllum*	Native (Cont)
WI011	Iola Lake (Iola Millpond)	44.5105	−89.1258	B (5)	1/1 (5)	Cont. *M. heterophyllum*	Native (Cont)
AL001	Thurlow Reservoir	32.54	−85.89	B (1)	1/6 (1)	Cont. *M. heterophyllum*	Native (Cont)
AR002 (4)	Lake Hamilton	34.5699	−93.1918	B (7)	1/7 (7)	Cont. *M. heterophyllum*[a]	Native (Cont)
AR010	Lake Greeson	34.1435	−93.7116	B (5)	6/6 (5)	Cont. *M. heterophyllum*[a]	Native (Cont)
MN001	Low Lake	47.9667	−91.8333	B (1)	1/5 (1)	Cont. *M. heterophyllum*	Native (Cont)
OK001	Unnamed pond in SE Oklahoma	34.599	−95.3032	B (1)	12/12 (1)	Cont. *M. heterophyllum*[a]	Native (Cont)
WA006 (4)	Florence Lake	47.1658	−122.6869	B (7)	1/6 (6)	Cont. *M. heterophyllum*	Introduced (W)

Table 3 continued

Lake code	Lake name	Lat.	Long.	cpDNA 2haplotype(s)	ITS genotype(s)	Taxonomic identification	Putative status
WA008	Lake Josephine	47.1536	−122.6781	B (1)	6/6 (1)	Cont. *M. heterophyllum*	Introduced (W)
TX001 (3)	San Marcos River	29.8881	−97.9343	C (4), A (1)	1/1 (5)	Cont. *M. heterophyllum*	Native (Cont)
FL201 (5)	Lake Jackson	30.5238	−84.3525	C (7), I (4)	1/1 (10), 1/9 (1)	ACP *M. heterophyllum*[a]	Native
GA001	Little Ocmulgee Lake	32.1022	−82.9367	C (1)	1/2 (1)	ACP *M. heterophyllum*	Native
NH034	Hilltop Pond	43.441	−72.011	C (2)	1/1 (2)	ACP *M. heterophyllum*	Introduced (NE)
SC010	Greene's Pond	32.7903	−81.2425	C (1)	2/2 (1)	ACP *M. heterophyllum*[a]	Native
TN001	Highway 40 pond	35.8998	−84.6384	C (5)	1/1 (5)	ACP *M. heterophyllum*[a]	Native
SC002	Airport Road Pond	33.7833	−81.75	D (1)	1/2 (1)	ACP *M. heterophyllum*[a]	Native
SC009	John's Pond	33.6644	−81.84	D (1)	2/2 (1)	ACP *M. heterophyllum*[a]	Native
SC016	Carter's Pond	33.9196	−80.351	E (1)	1/2 (1)	ACP *M. heterophyllum*[a]	Native
SC014 (1)	Baumann's Pond	34.3542	−79.8189	E (6)	1/2 (6)	ACP *M. heterophyllum*[a]	Native
ME002 (2)	Shagg Pond	44.423	−70.532	F (7)	2/2 (6)	ACP *M. heterophyllum*	Introduced (NE)
ME010 (5)	Lake Auburn (The Basin)	44.144	−70.248	F (7)	2/2 (5)	ACP *M. heterophyllum*	Introduced (NE)
ME015 (3)	Sebago Lake	43.8503	−70.5665	F (5)	2/2 (5)	ACP *M. heterophyllum*	Introduced (NE)
ME109 (5)	Horseshoe Pond	44.2008	−69.8973	F (5)	2/2 (5)	ACP *M. heterophyllum*	Introduced (NE)
ME110 (5)	Pleasant Pond	44.22	−69.789	F (7)	2/2 (7)	ACP *M. heterophyllum*	Introduced (NE)
NY004 (1)	Yaphank Lakes	40.842	−72.9359	F (4)	2/2 (4)	ACP *M. heterophyllum*[a]	Introduced (NE)[c]
CT 2.18	Rogers Lake	41.3494	−72.2988	F (6)	2/2 (6)	ACP *M. heterophyllum*	Introduced (NE)
CT 2.25	Pataganset Lake	41.3748	−72.2338	F (2)	2/2 (2)	ACP *M. heterophyllum*	Introduced (NE)
CT 2.47	Gorton Pond	41.3402	−72.2099	F (3)	2/2 (3)	ACP *M. heterophyllum*	Introduced (NE)
CT 2.57	Powers Lake	41.3934	−72.2563	F (7)	2/2 (6)	ACP *M. heterophyllum*	Introduced (NE)
ME005	Thompson Lake	44.066	−70.488	F (1)	2/2 (1)	ACP *M. heterophyllum*	Introduced (NE)
ME107	Bryant Pond (Lake Christopher)	44.6476	−70.378	F (5)	2/2 (5)	ACP *M. heterophyllum*	Introduced (NE)
NY005	Canaan Lake	40.7876	−73.0209	F (3)	2/2 (3)	ACP *M. heterophyllum*	Introduced (NE)[c]
NJ001	Lake Musconetcong	40.9144	−74.6931	G (1)	–	ACP *M. heterophyllum*	Introduced (NE)[c]
NC004 (1)	Carroll's Pond	35.6017	−78.6075	H (1)	2/2 (1)	ACP *M. heterophyllum*[a]	Native
SC015 (1)	Unnamed pond in Kline, SC	33.4067	−81.3319	H (6)	2/2 (6)	ACP *M. heterophyllum*[a]	Native
SC018 (1)	Upper (Big) Wood Creek Farms Pond	34.1178	−80.8356	H (6)	2/2 (6)	ACP *M. heterophyllum*[a]	Native

Table 3 continued

Lake code	Lake name	Lat.	Long.	cpDNA haplotype(s)	ITS genotype(s)	Taxonomic identification	Putative status
SC001	Unnamed pond near Allendale, SC	32.9994	−81.3408	H (2)	1/2 (2)	ACP *M. heterophyllum*[a]	Native
PA107	Ditch by Abbott's Pond	41.3223	−74.8033	H (2)	2/2 (1)	ACP *M. heterophyllum*	Introduced (NE)[c]
SC012	Airport Borrow Pit Pond	33.9506	−81.125	H (1)	1/2 (1)	ACP *M. heterophyllum*[a]	Native
GA002	Lake Seminole	30.7764	−84.7996	I (1)	1/10 (1)	ACP *M. heterophyllum*	Native
WA004	Clear Lake (Pierce Co.)	46.9258	−122.2761	I (1)	2/8 (1)	ACP *M. heterophyllum*	Introduced (W)
FL002	Bubbling Springs	29.1012	−82.4348	I (1)	3/3 (1)	ACP *M. heterophyllum*[a]	Native
SC011 (1)	Unnamed pond in Kline, SC	33.4017	−81.3297	I (5), H (1)	1/2 (5), 2/2 (1)	ACP *M. heterophyllum*[a]	Native
SC019	Edisto Research and Education Center	33.3512	−81.3115	I (5)	1/1 (3), 2/2 (2)	ACP *M. heterophyllum*[a]	Native
CT 2.50 (2)	Cedar Lake	41.4052	−72.5018	I (5)	2/2 (5)	ACP *M. heterophyllum*	Introduced (NE)
CT 2.6 (5)	Lake Quonnipaug	41.3964	−72.6959	I (9)	2/2 (10)	ACP *M. heterophyllum*	Introduced (NE)
CT 2.51	Messerschmidt Pond	41.3385	−72.4901	I (2)	2/2 (2)	ACP *M. heterophyllum*	Introduced (NE)
CT 2.54	Moodus Reservoir	41.5139	−72.4267	I (6), C (1)	2/2 (5)	ACP *M. heterophyllum*	Introduced (NE)
NC001	Unnamed pond near Pinehurst, NC	35.195	−79.469	J (2)	1/1 (2)	ACP *M. heterophyllum*	Native
OR102 (2)	Fern Ridge Lake	44.0944	−123.2983	A (2)	1/18 (2)	*M. heterophyllum* × *M. hippuroides*	Introduced (W)
FL210 (6)	Lake Kerr	29.362	−81.7887	S (5)	1/20 (4)	*M. heterophyllum* × *M. laxum*	Native
FL213 (5)	Escambia River	30.596	−87.2357	K (5)	1/20 (5)	*M. heterophyllum* × *M. laxum*	Native
MS001 (4)	Black Creek Water Park	31.0869	−89.4853	B (5)	1/20 (5)	*M. heterophyllum* × *M. laxum*	Native
CT 2.28 (2)	Amos Lake	41.5203	−71.9803	I (3)	3/20 (3)	*M. heterophyllum* × *M. laxum*	Introduced (NE)
CT 2.14	Bashan Lake	41.4963	−72.4205	I (2)	3/20 (3)	*M. heterophyllum* × *M. laxum*	Introduced (NE)
CT 2.26	Gardner Lake	41.5141	−72.2329	I (1)	3/20 (1)	*M. heterophyllum* × *M. laxum*	Introduced (NE)
CT 2.44	Black Pond	41.5275	−72.7433	I (6)	3/20 (8)	*M. heterophyllum* × *M. laxum*	Introduced (NE)
CT 2.48	Billings Lake	41.5056	−71.8732	I (5)	3/20 (5)	*M. heterophyllum* × *M. laxum*	Introduced (NE)
CT 2.49	Anderson Lake	41.5077	−71.8943	I (6)	3/20 (5)	*M. heterophyllum* × *M. laxum*	Introduced (NE)
CT 2.5	Pickerel Lake	41.5334	−72.421	I (5)	3/20 (4)	*M. heterophyllum* × *M. laxum*	Introduced (NE)
CT021	Glasgo Pond	41.5569	−71.8892	I (1)	3/20 (1)	*M. heterophyllum* × *M. laxum*	Introduced (NE)
CT022	Pachaug Pond	41.5817	−71.93	I (1)	3/20 (1)	*M. heterophyllum* × *M. laxum*	Introduced (NE)
ME001	Collins Pond	43.8303	−70.4267	I (1)	3/20 (1)	*M. heterophyllum* × *M. laxum*	Introduced (NE)
ME007	Little Sebago Lake	43.8786	−70.4114	I (2)	3/20 (2)	*M. heterophyllum* × *M. laxum*	Introduced (NE)
CT024	Day Pond	41.556	−72.418	–	3/20 (1)	*M. heterophyllum* × *M. laxum*	Introduced (NE)

Table 3 continued

Lake code	Lake name	Lat.	Long.	cpDNA haplotype(s)	ITS genotype(s)	Taxonomic identification	Putative status
FL202	Lake Taylor	28.137	−82.6146	S (3)	1/20 (3)	*M. heterophyllum* × *M. laxum*	Native
SC003	Oxpen Lake	34.6	−80.25	H (1)	2/20 (1)	*M. heterophyllum* × *M. laxum*	Native
SC004	Sandhill W'life Refuge, pond B	34.55	−80.2333	H (1)	2/20 (1)	*M. heterophyllum* × *M. laxum*	Native
SC005	Sandhill W'life Refuge, pond C	34.55	−80.2333	H (1)	2/20 (1)	*M. heterophyllum* × *M. laxum*	Native
SC006	Unnamed pond on Rt.145	34.55	−80.2167	H (1)	2/20 (1)	*M. heterophyllum* × *M. laxum*	Native
SC013	Coontail Lagoon Pond	33.59	−81.7614	H (1)	2/20 (1)	*M. heterophyllum* × *M. laxum*	Native
CA002 (1)	Indian Creek	40.147	−120.6489	N (5)	15/16 (2), 15/15 (3)	*M. hippuroides*[a]	Native
CA003 (2)	Lookout	41.2109	−121.1537	O (5)	16/16 (5)	*M. hippuroides*[a]	Native
CA004 (3)	Sierra Valley	39.7935	−120.3771	N (4)	15/16 (1), 16/17 (2), 15/15 (2)	*M. hippuroides*[a]	Native
OR002 (1)	McFadden's Marsh	44.3833	−123.2833	N (1)	18/18 (1)	*M. hippuroides*	Native
CA001	Friant Kern Canal	37.0008	−119.7039	N (1)	15/15 (1)	*M. hippuroides*	Native
WA003	Chamber Lake	47.0181	−122.8322	N (2)	19/19 (2)	*M. hippuroides*	Native
WA011	Lake Terrell	48.8694	−122.6886	O (1)	18/18 (1)	*M. hippuroides*	Native
WA012	Loma Lake	48.1342	−122.2542	N (1)	18/18 (1)	*M. hippuroides*	Native
FL203 (4)	Beakman Lake	29.1263	−81.6207	P (7)	21/21 (2), 20/21 (2), 20/20 (2)	*M. laxum*	Native
FL204 (4)	Wildcat Lake	29.1702	−81.6263	S (7)	21/21 (1), 20/20 (7)	*M. laxum*	Native
FL211 (5)	Juniper Lake	30.7704	−86.1286	S (5)	20/23 (5)	*M. laxum*	Native
FL212 (4)	Eglin Lake/Duck Pond	30.6706	−86.6339	S (4)	20/22 (3)	*M. laxum*[a]	Native
FL004	Puddinhead Lake	30.4983	−86.425	–	20/20 (1)	*M. laxum*	Native
FL005	Silver Lake	30.4067	−84.41	R (1)	20/20 (1)	*M. laxum*	Native
FL006	Lake Annie	27.2097	−81.3492	P (1)	20/20 (1)	*M. laxum*	Native
FL007	Moore Lake	30.3975	−84.4119	Q (1)	20/20 (1)	*M. laxum*	Native
NC003	Boiling Springs Lake	34.0333	−78.05	–	20/20 (1)	*M. laxum*	Native
SC007	Sexton Lake	34.4667	−80.3	R (1)	20/20 (1)	*M. laxum*	Native
SC008	Lower Wood Creek Farms Pond	34.1153	−80.8381	R (4)	20/20 (5)	*M. laxum*	Native
CT 2.56 (2)	Manitook Lake	41.9796	−72.7951	C (5)	3/3 (5)	*M. heterophyllum*[d]	Introduced (NE)
FL001 (1)	[b]	[b]	[b]	I (1)	3/3 (1)	*M. heterophyllum*[d]	Native

Table 3 continued

Lake code	Lake name	Lat.	Long.	cpDNA haplotype(s)	ITS genotype(s)	Taxonomic identification	Putative status
KS002 (5)	Mined Land Wildlife Area No. 1	37.473	−94.692	C (4)	14/14 (4)	*M. pinnatum*[a]	Native
TN002 (1)	Unnamed pond	36.484	−88.3293	C (1)	–	*M. pinnatum*[a]	Native
TN003 (3)	Unnamed farm wetland	35.6067	−85.9292	A (3)	24/24 (3)	*M. pinnatum*[a]	Native
CT101	Bluff Point	41.3142	−72.0358	I (1)	13/13 (1)	*M. pinnatum*[a]	Native
OK003	[b]	35.0046	−97.1838	I (1)	13/13 (1)	*M. pinnatum*[a]	Native

Samples for the AFLP analysis are indicated in bold (sample sizes for AFLPs in parentheses). Sample sizes for cpDNA haplotypes and ITS genotypes in parentheses in their respective columns

Cont continental

[a] Populations where morphological identifications of species were made

[b] Samples where the exact location or water body is unknown other than the state of origin

[c] Samples in the northeastern US that are considered introduced but that may be native to the region (see text for details)

[d] One genotype that was identified as a unique (possibly hybrid) type with AFLPs that was previously identified as *M. heterophyllum* using ITS alone (see text for details)

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