

# The Role of Plant Systematics in Invasive Aquatic Plant Management

MICHAEL L. MOODY<sup>1</sup>, D. H. LES<sup>2</sup> AND J. M. DITOMASO<sup>3</sup>

## ABSTRACT

The correct identification of both invasive and native aquatic plants is crucial for effective aquatic plant management. Incorrect identification of the plants in our aquatic systems can lead to inadvertent outcomes such as eradicating native species or applying ineffective control strategies. While this practice may appear straightforward, the correct identification of invasive aquatic plant taxa has proven to be complex. Many aquatic plants are particularly difficult to identify correctly using morphology alone due to their often reduced floral characters and convergent vegetative morphology. The past two decades have given us the technological tools through molecular (DNA) sequence data and computational methods to more accurately identify nonnative plants in North America and have been particularly useful for early detection. Molecular tools have also revealed unique “genetic types” (i.e., genotypes), including newly formed hybrids among the most problematic of our invasive and wetland plants. Unique genotypes often correlate with different physical types that respond in unique ways to the environment around them. Given the body of literature describing variable responses of invasive taxa to their environment and well-documented herbicide resistant weed “biotypes”, managers of invasive plants must discern the genetic identity to the finest relevant scale. We recommend: (1) creating a government-funded, centralized molecular lab for the purpose of quick and accurate aquatic plant identification, also referred to as

DNA “barcoding” (this lab could immediately be used for early detection of invasive taxa with the intention of stopping invasive aquatic plants before they become a problem); (2) incorporating our growing knowledge of genotypes with studies of invasive taxa emphasizing ecological preferences, growth habits, reproductive ability, and herbicide and/or bio-control resistance; and (3) integrating current internet sites that emphasize invasive aquatic plants into a single existing interactive site that will include not only known invasive plants, but all aquatic plant genera to facilitate early detection. Time and money can be used more effectively by aquatic plant management agencies if we develop reliable methods to correctly identify aquatic plant taxa before management is implemented.

*Key words:* evolution, genotype, molecular data, species, taxonomy.

## INTRODUCTION

Fundamental to the effective management of invasive aquatic plants is the correct application of taxonomic identifications to both invasive and native species in an aquatic system. Incorrect identifications can lead to the inadvertent eradication of rare or nontarget species, the application of ineffective control strategies, and the failure to recognize newly introduced invasive species. Plant systematics, the field of study concerned with plant diversity, encompasses the taxonomy, classification, and evolution of plant taxa. Botanists have relied on past systematic studies to provide a source of recognition for North American plants as well as a reference for determining their native or nonnative status. Generally, the native status of North American aquatic plants is determined by literature and museum (herbarium) records that have been collected, recognized and/or recorded by early plant taxonomists (Les and Mehrhoff 1999). These written records and specimen collections are essential for defining

---

<sup>1</sup>University of Western Australia, Dept. of Environment and Conservation, Plant Biology, Crawley, WA, 6009 Australia; Michael.Moody@dec.wa.gov.au

<sup>2</sup>University of Connecticut, Department of Ecology & Evolutionary Biology, 75 North Eagleville Road, Storrs, CT 06269-3043.

<sup>3</sup>University of California, Davis, Plant Sciences, 210 Robbins Hall, Davis, CA 95616. Received for publication June 2006 and in revised form January 2007.

the native flora in a historical sense. Modern plant systematists, paleontologists, and ecologists continue to interpret the native ranges of North American plants with respect to changing climate and fossil records of ancient floras. Although biologists from varied disciplines continue to debate the historical time frame most appropriate for categorizing specific taxa as native or invasive to a particular geographical region, plant systematists are responsible for interpreting the taxonomic identity of suspected plant invaders.

Aquatic plants are particularly difficult to identify properly due to their often reduced floral characters and convergent vegetative morphology. This problem is evident in the commonly used early classification schemes proposed by Cronquist (1968) and Takhtajan (1969) that assumed close relationships among what are now known to be quite distantly related aquatic plant groups, such as the water lilies (Nymphaeaceae) and lotus (Nelumbonaceae). Plant taxonomy has undergone incredible advances due to the advent of phylogenetic studies, including both morphological and molecular data. Molecular data have been especially useful given they provide a relatively simple means of comparing distantly related species by a common set of characters (DNA) and overcome many of the interpretive problems (e.g., convergence, character losses) that arise when evaluating morphological characters. Systematic relationships identified through phylogenetic analysis of molecular data have provided a more effective means of recognizing distinct invasive aquatic taxa. The application of molecular data is especially useful for early detection of invasions and provides plant systematists with an effective tool for clarifying traditional "species" limits and also for determining more specific "biotypes" or "genetic-types" (i.e., genotypes), which often exhibit little or no detectable morphological variation.

Our goal is two-fold. First, we outline the advances in methodology made over the past two decades that more accurately describe invasive aquatic plant taxa and that communicate this knowledge to the general public. Second, we describe the future role of plant systematics research relative to invasive aquatic plant management and specify our perceived research priorities. Although much state and federal funding has been deployed toward management of some of our worst aquatic plant invaders (Pimentel et al. 2000, Cronk and Fennessy 2001), we intend to emphasize that time and money can be better appropriated by aquatic plant management agencies if more refined techniques of identification for target aquatic invasive plant taxa are incorporated in management plans.

## TWO DECADES OF PROGRESS

Unprecedented technological advances the past two decades have redefined the role of plant systematics in invasive aquatic plant management. New molecular methods make it possible to quickly and precisely define invasive aquatic taxa and to more closely examine their population level dynamics. Information technology has facilitated the rapid dissemination of information regarding invasive aquatic plants (including descriptive profiles, distributions and recommended control techniques) to federal and local management agencies as well as to the general public.

## Legislation

Legislation over the past decade has provided a working definition of invasive plants. The standard definition of invasive plants now accepted by the United States Department of Agriculture (USDA), and United States Geological Survey (USGS) is "non-native (or alien) to the ecosystem under consideration and whose introduction is likely to cause economic or environmental harm or harm to human health..." (Executive Order 13112, 1999). Both organizations recognize invasive plants as a major concern ([biology.usgs.gov/invasive/](http://biology.usgs.gov/invasive/); [www.invasivespeciesinfo.gov/](http://www.invasivespeciesinfo.gov/)). Although this definition leaves room for some interpretation, it provides a baseline to frame research proposals related to management of invasive aquatic plants. The direct "economic harm" caused by invasive aquatic plants may not be as conspicuous as that for terrestrial invasive plants, which damage crops or grazing land, but "environmental harm" also can be argued under this definition, and in many cases definitively linked to long term economic harm. As one example, the United States spends more than \$100 million annually to manage just two aquatic species, hydrilla (*Hydrilla verticillata* L.) and water hyacinth (*Eichhornia crassipes* [Mart.] Solms; Les 2002). Certainly, the official recognition of invasive plants as a stated concern by government agencies is an important development for invasive plant research.

## Information Technology

Early detection of nonnative aquatic plants in a waterbody is one of the most important goals of plant systematics in invasive aquatic plant management. Early detection and eradication of exotic taxa before they become established is the most effective deterrent to biological invasions (Rejmanek and Pitcairn 2002, Simberloff 2003) but is only possible if exotic taxa are recognized. One way to facilitate early detection is to establish an interactive network of scientists and other management personnel who communicate new discoveries (or potentially new discoveries) of invasive aquatic plants.

In recent years, information technology via the World Wide Web has been important in describing invasive aquatic plants to the public and local management groups and also in advising them of management options (Table 1). These Internet sites generally focus attention on taxa already known to be invasive and often include both terrestrial and aquatic plants. Although these sites are informative, they often are redundant or provide conflicting information. Most invasive plant sites require the user to know the name of the plant before initiating a search or to scroll through a long list of plants and select common or taxonomic names to access a description, which does not always include a visual representation. However, improvements are available (including character based interactive search engines for aquatic plant taxa) that are compatible with all computer systems, making these sites more accessible to both aquatic plant management personnel and the general public. These sites can assist with the identification of invasive plants as well as provide important distributional, biological, ecological, and management information toward development of an early detection system. More specific recommendations are described in Future Priorities and Research Objectives.

TABLE 1. MOST ACCESSIBLE INTERNET SITES AND SPONSORS WITH DESCRIPTIONS OF INVASIVE AQUATIC PLANTS.

Organization	World Wide Web address
USGS	<a href="http://www.biology.usgs.gov/invasive/">www.biology.usgs.gov/invasive/</a>
USDA	<a href="http://plants.usda.gov/index.html">plants.usda.gov/index.html</a>
IFAS	<a href="http://plants.ifas.ufl.edu/">plants.ifas.ufl.edu/</a>
USDA-APHIS	<a href="http://lucidcentral.org/keys/FNW/FNW%20Disseminules%20Key/html/">lucidcentral.org/keys/FNW/FNW%20Disseminules%20Key/html/</a>
Invasive Plant Atlas of New England	<a href="http://nbii-nin.ciesin.columbia.edu/ipane/">http://nbii-nin.ciesin.columbia.edu/ipane/</a>
USDA- Forest Service	<a href="http://www.invasive.org/weeds.cfm">www.invasive.org/weeds.cfm</a>
National Park Service	<a href="http://www.nps.gov/plants/alien/factmain.htm#p1lists">www.nps.gov/plants/alien/factmain.htm#p1lists</a>
Washington State	<a href="http://www.ecy.wa.gov/programs/wq/plants/weeds/index.html">www.ecy.wa.gov/programs/wq/plants/weeds/index.html</a>
The World Conservation Union	<a href="http://www.issg.org/database/welcome/">www.issg.org/database/welcome/</a>
Nature Conservancy	<a href="http://tncweeds.ucdavis.edu/index.html">tncweeds.ucdavis.edu/index.html</a>
Florida exotic Pest Plant Council	<a href="http://www.fleppc.org/">www.fleppc.org/</a>
U.S. Army Corp of Engineers	<a href="http://el.erdc.usace.army.mil/aqua/apis/">el.erdc.usace.army.mil/aqua/apis/</a>
Southeast exotic Pest Plant Council	<a href="http://www.se-eppc.org/weeds.cfm">www.se-eppc.org/weeds.cfm</a>
Western aquatic Plant Management	<a href="http://www.wapms.org/plants/">www.wapms.org/plants/</a>
California Invasive Plant Council	<a href="http://www.cal-ipc.org/">www.cal-ipc.org/</a>
Weed Research and Information Center	<a href="http://wric.ucdavis.edu/">wric.ucdavis.edu/</a>
Univ. California Statewide IPM Program	<a href="http://www.ipm.ucdavis.edu/PMG/weeds_common.html">www.ipm.ucdavis.edu/PMG/weeds_common.html</a>

The Internet has become a venue for buying and selling invasive plants as well as an avenue of importation for exotic plants (from overseas sources) whose invasive nature has not yet been assessed. Kay and Hoyle (2001) conducted a systematic search of 12 invasive aquatic weeds on the Internet, including five aquatic plant taxa found on the national noxious weed list (*Azolla*, *Lagarosiphon*, *Limnophila*, *Salvinia* and *Sparganium*), all for sale by multiple dealers. Many states now prohibit the sale of these plants within their political boundaries; unfortunately, the prohibition of aquatic plant sales in one state does not preclude their sale in a neighboring state. In the southeastern United States, water lettuce (*Pistia stratiotes* L.) is prohibited for sale in Florida but not in adjacent Georgia; likewise in the Pacific Northwest, parrotfeather watermilfoil (*Myriophyllum aquaticum* [Vell.] Verdc.) is prohibited for sale in Washington but not in neighboring Oregon.

Also problematic is the lack of any regulations requiring proper plant identification by on-line aquatic plant sellers. Many sellers list *Anacharis*-*Elodea*-*Egeria* interchangeably so that it is virtually impossible to identify the plants until they are delivered. Also, the invasive parrotfeather watermilfoil and variable-leaf watermilfoil (*M. heterophyllum* Michx.) have been listed and sold as *M. mattogrossense* Hoehne (Moody, unpublished data); thus, even buyers who consciously try to avoid purchasing invasive species can unknowingly be misled into purchasing mislabeled invasive species. Although advances in information technology have improved the reporting means for early detection of invasive aquatic plants, the dangers of unfettered access to plants worldwide via the Internet represents a daunting problem.

### Molecular Markers and Invasive Aquatic Plant Studies

Perhaps the greatest advance in plant systematics over the past two decades is the development of DNA-based molecular data (Avisé 2000, Judd et al. 2002), which have been highly successful at resolving many notoriously difficult taxonomic questions. Molecular data have been utilized in phylogenetic reconstruction, classification, hybrid identifica-

tion, species delimitation, and population-level studies of aquatic plant taxa to provide a better understanding of the invasive aquatic plants we are attempting to manage.

Evolutionary (phylogenetic) tree reconstruction studies have proliferated over the past 20 years. DNA sequence data have been particularly useful for clarifying phylogenetic relationships among aquatic taxa, many invasive in North America, such as *Glossostigma* (Les et al. 2006); Haloragaceae (*Myriophyllum*; Moody 2004); Hydrocharitaceae (*Butomus*, *Egeria*, *Hydrilla*, *Hydrocharis*, *Lagarosiphon*, *Najas*; Les et al. 2005); Lemnaceae (*Landoltia*, *Spirodela*; Les et al. 2002); Lythraceae (*Lythrum*, *Trapa*; Graham et al. 2005); and *Spartina* (Baumel et al. 2002). An important byproduct of these phylogenetic studies is the development of species-specific molecular markers, which usually associate the markers directly with voucher specimens to provide a morphological reference for verification of species identities.

Given the growing number of phylogenetic studies of aquatic plant groups, species-level identification using molecular markers is becoming possible for many invasive aquatic plant taxa. Several state agencies (e.g., Maine, Minnesota and New Hampshire Depts. of Natural Resources; Washington Dept. of Ecology) currently are using nrDNA internal transcribed spacer (ITS) sequence data (see Moody and Les 2002) to make positive identifications of invasive watermilfoil species and hybrids. This approach has been particularly useful for facilitating early detection when only small plant fragments with inconspicuous vegetative characteristics are available. In cases where a new invasion of a waterbody by an aquatic plant is suspected, a rapid molecular assay to effectively determine the identity of the plant species would be particularly useful. This form of identification, referred to as DNA barcoding, has been effective in identifying both plants and animals in broad surveys (Hebert et al. 2003, Kress et al. 2005) and also has been suggested as an ideal way to screen plant imports (Armstrong and Ball 2005).

Molecular methods also have been useful for understanding among- and within-population-level variation in invasive aquatic taxa (Madeira et al. 1997, 2004, Carter and Sytsma

2001, Gifford et al. 2002, Saltonstall 2002). More sensitive molecular markers (e.g., intron sequences, microsatellites, restriction fragment length polymorphisms [RFLPs], random amplified polymorphic DNA [RAPD]) have made it possible to identify subspecific “biotypes” or genotypes, which can express different invasive characteristics than other members of a particular species. For example, the common reed (*Phragmites australis* [Cav.] Trin. ex Steud.), a native North American species with a circumboreal distribution, has become aggressive in North America and is now found in habitats where it once had not occurred. Saltonstall (2002) used molecular markers to identify a genotype introduced to North America from Europe that is now the most common and aggressive “biotype” in North America; a native biotype also exists, which has become less frequent and may even warrant conservation efforts.

The case of hydrilla also demonstrates the utility of molecular markers in identifying specific genotypes. Hydrilla is recognized as monotypic worldwide (i.e., comprising the single species *Hydrilla verticillata*) and has become an invasive nuisance across North America, yet both monoecious and dioecious plants have been reported in the United States. Molecular markers have made it possible to identify at least two different genotypes among the invasive populations in North America (Madeira et al. 1997, 2004 [cpDNA]; Moody and Les unpubl. data [nrDNA-ITS]). Often, these genotypes are assumed to represent the monoecious versus dioecious “strains”, but this assumption has yet to be verified and published using molecular analyses of actual flowering specimens (which occur rarely), although Madeira et al. (2004) have sampled specimens from the native range of hydrilla having the two purported “strains”. In addition, Albrecht et al. (2004) isolated a fluridone-resistant biotype in hydrilla that can be traced to a specific gene. A relatively simple molecular assay can now be performed to determine whether an invasive hydrilla population is resistant to fluridone before an herbicide application is performed. With resistance to fluridone and other herbicides now known to occur in several aquatic plant species (Albrecht et al. 2004, Koschnick et al. 2006) such a screening measure would prevent the use of chemicals in cases where they would not only be ineffective, but where their application could enhance the evolution of resistant genotypes. Without such information, continued

herbicide application to resistant plants eventually would select them to become dominant in a system, presenting an even more desperate situation for managers.

One of the more compelling results to emerge from population-level studies using molecular data is the definitive evidence of hybridization in several invasive aquatic plant groups (Table 2). The majority of these cases reveal the hybrid to be the dominant invasive type. These hybridization events commonly involve a cross between a nonnative and native species, but also may involve multiple nonnative species that have come in contact with each other, as is the case with saltcedar (*Tamarix* spp.; Gaskin and Schaal 2002, 2003). The link between hybridization and invasiveness in plants is well-documented (Ellstrand and Schierenbeck 2000, Vilà et al. 2000, Sakai et al. 2001), and evidence for hybridization among invasive aquatic plants is growing (Table 2).

## Future Priorities and Research Objectives

The primary concern to systematists studying invasive aquatic plants is the correct identification of plant taxa, which enables early detection of invasive (or potentially invasive) taxa, quick assessment of taxonomic identity at the species level, and identification of hybrids. Once invasive plants have been accurately identified, a researcher can properly access and incorporate other published data on ecological preferences, growth habits, reproductive ability, and herbicide and/or biocontrol resistance. Much time and money can be wasted on aquatic plant management if a target plant has not been correctly identified. To these ends we recommend the following priorities for the role of plant systematics in invasive aquatic plant management.

## An Invasive Aquatic Plant Database

The distribution of information describing invasive aquatic taxa for use by aquatic plant management agencies nationwide is essential. The Internet-accessible databases currently available vary widely in quality, accuracy, type of information provided, and specificity (invasive aquatic plants vs. all invasive plants). Most lack any type of interactive key for identifying aquatic plants. As more aquatic invasive plants are recognized and more supplementary information becomes

TABLE 2. INTER- AND INTRA-SPECIFIC HYBRID INVASIVE AQUATIC PLANTS AND TYPE OF DATA USED FOR IDENTIFICATION. NR = NUCLEAR RIBOSOMAL; N = NUCLEAR; CP = CHLOROPLAST; RFLP = RESTRICTION FRAGMENT LENGTH POLYMORPHISM; RAPD = RANDOMLY AMPLIFIED POLYMORPHIC DNA; SSR = SIMPLE SEQUENCE REPEATS.

Parent taxa	Common name	Evidence	Authors
<i>Caulerpa racemosa</i> var. <i>tubinata-wivifera</i> × <i>C.</i> sp.	Grape caulerpa	nrDNA sequence RFLP	Durand et al. 2002
<i>Cardamine rivularis</i> × <i>C. amara</i>	Bittercress	nDNA and cpDNA	Urbanska et al. 1997
<i>Lythrum salicaria</i> × <i>L. alatum</i>	Purple loosestrife	isozyme	Strefeler et al. 1996
<i>Myriophyllum heterophyllum</i> × <i>M. laxum</i>	Variable-leaf watermilfoil	nrDNA sequence	Moody and Les 2002
<i>Myriophyllum spicatum</i> × <i>M. sibiricum</i>	Eurasian watermilfoil	nrDNA sequence	Moody and Les 2002
<i>Nasturtium microphyllum</i> × <i>N. officianale</i>	Watercress	cytological	Bleeker et al. 1999
<i>Sarcocornia fruticosa</i> × <i>S. perennis</i>	Glasswort, chickenclaws	RAPD	Figueroa et al. 2003
<i>Schinus terebinthifolius</i> ×	Brazilian Peppertree	cpDNA and SSR	Williams et al. 2005
<i>Spartina alterniflora</i> × <i>S. foliosa</i>	Cordgrass	RAPD	Daehler and Strong 1997
<i>Spartina alterniflora</i> × <i>S. maritima</i>	Cordgrass	isozymes	Gray et al. 1991
<i>Tamarix</i> spp.	Saltcedar	nDNA intron sequence	Gaskin and Schaal 2002
<i>Typha latifolia</i> × <i>T. angustifolia</i>	Cattails	RAPD	Kuehn et al. 1999

known, Internet web sites can be edited promptly, a distinct advantage over the lengthy process of book revision and publication. One solution would be to integrate the present databases into a single location to consolidate information and facilitate interactions among site directors. The exchange of questions, answers, and ideas stimulated by such a forum would ultimately lead to more accurate and consistent data among sites, thereby providing an extremely helpful service to management personnel and the general public. Rather than initiate yet another database, it seems more practical to begin with a high-quality existing database, such as the IFAS (<http://plants.ifas.ufl.edu/photos.html>) or USGS (<http://nas.er.usgs.gov/taxgroup/plants/>) site, and consolidate information from the other sources into one database specifically addressing issues on invasive aquatic plants. Such a database should include not only those taxa currently known to be invasive to North America, but also incorporate information to help in early detection of new introductions. With this goal in mind, the database should include information on all known aquatic genera such as the inventory provided by Cook's (1996) Aquatic Plant Book. The database should be freely accessible to anyone. The specific contents of such a database would include the following information:

#### A. Taxonomic inclusion

- Complete taxonomic data (described below) for at least one species from each aquatic genus (long-term goal will include all species)
- All currently recognized aquatic invasive species
- All aquatic taxa with available phylogenetic or DNA data
- Plants sold in the trade in North America

#### B. An interactive key (using a character-based interface)

- Begin with a simple format with easy to identify characters
- Base on vegetative characters when possible
- Include link to definition of technical characters
- Format with a narrowing list of taxa as a compound-list of characters is defined

#### C. Plant taxon descriptions

- Scanned voucher specimen
- Photographs and line drawings
- DNA sequence data (link to NCBI [GenBank])
- Native distribution (genus and species)
- Mapped distributional data (GPS coordinate-based)
- Current invasive distribution (if applicable)
- Life history information (what is known)
- Description of closely related native aquatic taxa
- Hybrid potential (overlap of non- native to closely related native species)
- Expert reviews of taxonomic descriptions (when possible)

A database with these basic parameters would provide an excellent initial source that would only improve over years of continuous development. New information and recommendations would be encouraged. It also would be advisable to have an ongoing editorial group that could incorporate updates and manage new technology for improving the website content.

## Plant Identification

*Traditional Taxonomy.* Many aquatic plant groups still are in need of basic taxonomic treatments that include keys to species, provide comprehensive descriptions and accurately specify geographical distributions. Several of the invasive taxa being managed in North America come from developing regions of the world where they have not been well-examined taxonomically, even in their native range. Although recent molecular studies have uncovered genotypic variation in taxa such as *Hydrilla* (Madeira et al. 1997, 2004; Moody and Les, unpubl. data) and *Tamarix* (Gaskin and Schaal 2002) that could be interpreted as species level variation, the taxonomic treatment of these groups is inadequate to assist with such determinations and would benefit from a thorough reevaluation. Priorities include detailed systematic studies of *Hydrilla* (currently underway by L. Benoit, University of Connecticut), *Trapa* (to determine whether the species introduced to North America is *Trapa natans*), and *Azolla* (to determine the number of taxa in North America and their distributions). As secondary concerns, taxonomic work is needed to clarify the taxonomy and invasive nature of North American *Cabomba* (currently underway by A. Weiss, University of Connecticut), *Lagarosiphon*, *Limnophila*, *Hydrocharis* and *Egeria*.

*Molecular Methods.* To achieve more efficient management, early detection, and prevention, we advocate the development of a centralized laboratory where molecular facilities and appropriate expertise combine to facilitate the rapid identification of aquatic plants. Although this function could be performed by individual molecular labs (as is now being done), a centralized molecular lab would be necessary to accommodate any system designed to achieve large-scale identifications of invasive aquatic plants. Such a facility could best be maintained by a government agency (e.g., USDA or USGS) with a significant interest in invasive aquatic plant management. Alternatively, the facility could be maintained through a United States university as long as a reliable source of government funding could be identified.

To be successful, a centralized molecular facility of this type would require supervision by a trained molecular systematist possessing not only a thorough knowledge of modern (molecular) technology, but also a broad understanding of the biology, ecology and management of invasive aquatic plants. Full time lab technicians would be necessary to maintain efficient turnaround times for plant identifications. The facility should be developed to interact with other groups (e.g., state and local management agencies) who are involved in the management of invasive aquatic plants.

When fully operational, molecular markers would be used for early detection by identifying taxa from even small plant fragments and/or specimens that lack reproductive parts

(especially important for submerged plant taxa). Genetic screening using specific DNA markers to detect species, hybrids and genotypic variants (see previous section) would greatly expedite identification of invasive aquatic taxa (e.g., *Hydrilla*, *Landoltia*, *Myriophyllum*) as well as provide exceptionally high levels of reliability. Molecular identification of aquatic plants and development of new markers should be prioritized for those taxa most difficult to identify because some species (e.g., water hyacinth, water lettuce) are readily identifiable by their distinctive morphology. Currently, many molecular markers already exist. An assortment of previously generated DNA sequences can be downloaded from GenBank ([www.ncbi.nlm.nih.gov/](http://www.ncbi.nlm.nih.gov/)) by any organization (private, governmental or academic) for comparative purposes in conjunction with a molecular sequencing lab capable of generating a DNA sequence from a specimen of unknown plant material. This DNA “barcoding” approach is being recommended for use in many biological systems (Kress et al. 2005) and is facilitated by technological advances that allow many analyses to be completed within 24 hours. Ideally, a specific or “universal” DNA sequence region should be emphasized to simplify the identification of taxa. Kress et al. (2005) recommended the nrDNA internal transcribed spacer (ITS) region as a good candidate gene for barcoding in plants. The ITS locus is recommended predominantly because of the breadth of data available (>36,000 plant sequences currently in GenBank). Eventually, combining the nrDNA region with regions of DNA originating from chloroplast DNA (which usually is maternally inherited) will likely be necessary to better address instances of hybridization and/or function when the ITS region is not easily interpreted. Also, as more specific molecular data become available, subspecific identification could be developed to provide greater resolution among individual plants of a species. The logistics of how such a lab would receive and verify comparative samples and where voucher specimens would be preserved are issues that need to be addressed. Certainly, a centralized molecular facility would require a sophisticated computer system capable of analyzing, organizing, and disseminating data rapidly and efficiently. Interaction with similar facilities will also be important. For example, the University of Guelph, Canada, currently is running a barcoding facility to identify plant taxa ([www.uoguelph.ca/foibis/barcoding.htm](http://www.uoguelph.ca/foibis/barcoding.htm)).

It is critical that specific molecular markers for invasive taxa are developed with reference to verified (vouchered) specimens from which the DNA originated. These specimens should possess the identifying traits of the given species, including floral characters if possible. In addition, multiple specimens for each taxon should be verified (using morphology) to determine the accuracy and consistency of the DNA data. This objective can be achieved using information provided in previously published systematic studies and/or further verified by taxonomic experts affiliated with the centralized lab. The applicability of a molecular marker (barcode) must be defined by the taxonomist; thus, the marker can be designed to function at the species, genotype, or bio-type level. The availability of such hierarchical genetic information would be pertinent to a diverse pool of management objectives.

## General Life History Studies

While technological advances make it increasingly easier to obtain genetic data, research on general life-history traits of aquatic plant taxa have been neglected. Yet, an understanding of the basic biology of nonnative taxa is essential information to any aquatic plant management program. Today it is easier to obtain DNA sequence within a few hours than to spend months, or even years, conducting field studies to obtain comprehensive life history information on aquatic plants. Despite the voluminous literature on some notoriously weedy species, there remains a surprising dearth of information regarding such basic elements as habitat requirements, pollinators, dispersal mechanisms, breeding systems, and population structure. A good example is the well-known hydrilla, which had been regarded as a tropical species incapable of spread into northward latitudes, despite the fact that native populations are known to occur within a few degrees latitude of the Arctic Circle (Les et al. 1997).

As studies of aquatic plant invasions in North America reveal more complex issues (e.g., different genotypes, hybridization, herbicide resistance), a basic understanding of the biology of these plants in their native range becomes critical to effectively formulate management plans for North America. Specific information on environmental tolerances (e.g., pH, salinity, sediment types), reproduction (e.g., selfing, outcrossing, pollinators), natural predators, and natural plant competition is essential. Better life history information for invasive species can sometimes identify habitat characteristics that help determine which types of communities are most susceptible to establishment of a particular species or genotype (Remjanek 2000, Sakai et al. 2001, Les et al. 2006). Also, given the complex interactions of hybridization and invasiveness (Table 2), controlled breeding experiments involving North American species and closely related invasive taxa (as determined from phylogenetic analyses) might better reveal the potential for hybridization, thus providing additional insight into the invasive potential of particular nonnative species.

In addition, a prehistorical component (i.e., the fossil record) should be incorporated into the study of aquatic plant distributions. If the long-term vegetational history of a regional flora is thoroughly understood, it may be possible to better ascertain where native North American plants have occurred in the past and where they may migrate in the future. The flora of any geographical region is not permanent, thus, a better understanding of the prehistorical climate and flora of a region would help to evaluate the logic of protecting the biological status quo. Can we be certain that the expansion of native North American taxa such as variable-leaf watermilfoil and cabomba from the southeastern to the northeastern United States represents a serious threat, or is it the consequence of what inevitably would have been a natural expansion? Answers to such questions could help focus resources on the most significant threats, or at least on those that are most preventable or manageable.

## Integrating Molecular Methods and Management

*Genotype Resistance.* An important contemporary lesson in invasive aquatic plant management is the realization that

unique genotypes of a single species potentially can respond in novel ways to their environment. Therefore, identifications of potentially invasive aquatic plants (using molecular data) should be made to the finest level of resolution available before conducting research or applying any management treatment (e.g., herbicide application or establishment of biocontrol agents).

For example, hydrilla has at least two distinct genotypes (Madeira et al. 2004), and several genotypes that vary with respect to fluridone tolerance (Albrecht et al. 2004), yet these are almost never determined before initiating management practices such as herbicide treatments. Likewise, researchers conducting studies on the effects of herbicides and biocontrol agents, for instance, seldom determine whether different genotypes may occur, a possibility that could significantly bias the outcome of their experiments and result in improper management recommendations. In addition, detailed herbicide or biocontrol treatment results should be public record. Anecdotal evidence suggests that conspecific populations often exhibit differential responses to herbicides, but such variability is impossible to quantify, much less link to genotype or any other cause without access to records that detail prior treatment history and outcomes.

Herbicide resistance in weedy terrestrial plants is a well-recognized problem with 307 resistant biotypes known among 183 species (<http://www.weedscience.org/in.asp>). In several cases, the specific gene(s) involved with resistance have been identified (Gressel 2002, Tranel and Wright 2002, Basu et al. 2004). Among invasive aquatic taxa, the recent discovery of a fluridone-resistant hydrilla biotype (Albrecht et al. 2004) through isolation of the phytoene desaturase (PDS) gene first discovered in cyanobacteria (Chamovitz et al. 1993) provides a cautionary paradigm for aquatic plant managers who rely primarily on herbicides to control invasive populations. Continued research in this area and further refinement of associated molecular techniques should become a priority for aquatic plant scientists. As more information is disclosed regarding the extent and diversity of genotypic variation within and among invasive aquatic populations, specific genotypes may be identified that are more or less susceptible to control methods. Long-term costs could be reduced tremendously by eliminating ineffective treatments for populations with genotypes known to be resistant to certain herbicides or biocontrol agents. More refined methods to effectively target resistant genotypes also should be developed.

**Hybridization.** Aquatic plant managers must recognize that hybrids have unique genetic profiles and generally respond differently to chemicals, predators, and general environmental conditions compared to their parental taxa (Fritz 1999, Fritz et al. 1999, Rieseberg et al. 2003, Gross and Rieseberg 2005), and many invasive plant populations have hybrid origins (Table 2; Ellstrand and Schierenbeck 2001). A notable example is the hybrid origin of invasive saltcedar populations in western North America (Gaskin and Schaal 2002). Variation in the efficacy of biocontrol agents among populations (DeLoach and Tracy 1997) indicates a possible link to hybridization (Gaskin and Schaal 2002). Another example is found in watermilfoil, where Eurasian watermilfoil is known to hybridize with the native northern watermilfoil

(*Myriophyllum sibiricum* Kom.). The hybrid populations are most often monotypic (i.e., lacking Eurasian watermilfoil; Moody and Les 2002, 2007). The native milfoil weevil (*Euhrychiopsis lecontei* Dietz) has been used as an experimental biocontrol agent for Eurasian watermilfoil, and its effectiveness differs for Eurasian watermilfoil and the invasive hybrid (Newman et al. 2001, Newman 2004, Roley and Newman 2006). Data indicating that herbicide efficacy (biomass reduction) ranges from 10% to 90% among populations currently recognized as "Eurasian watermilfoil" (Cronk and Fennessy 2001) provide an impetus for further research. These systems are more complex than currently known. Increasing examples of invasive hybrid taxa are being discovered (Table 2), but remarkably little is known about the underlying biology that leads to their invasive nature. Studies incorporating both ecological parameters and molecular methods should be prioritized to better understand the link between hybridization and invasiveness.

**Among- and Within-Population Variation.** Future research should continue to develop molecular tools applicable to the study of genetic structure in invasive aquatic plants, from species to genotypes. Genotype specific molecular markers (e.g., microsatellites, RFLPs, AFLPs) are well established as valuable tools in crop weed research on genetic diversity and population dynamics (O'Hanlon et al. 2000, Gressel 2002, Basu et al. 2004). Similar studies are just beginning among invasive aquatic taxa (Baumel et al. 2001, Carter and Sytsma 2001, Gaskin and Schaal 2002, Saltonstall 2003, Williams et al. 2005), which will lead to a much better understanding of population structure and may eventually help to disclose why specific genotypes appear to be more invasive in specific environments. Another long-term goal should be to integrate the growing body of plant genomic research with studies directed toward elucidating the genetic mechanisms of invasiveness in plants (Lee 2002, Basu et al. 2004). Naturally, this objective will require integrated research projects between ecologists and molecular biologists. Due to the complex issues within such disparate areas as molecular genetics and ecology, an approach that emphasizes interdisciplinary integration may best address issues of invasive aquatic plant management.

In conclusion, regardless of whether a centralized facility for the study of invasive aquatic plants ever becomes a reality, many molecular markers already exist for aquatic invasive plants that can be immediately incorporated into management plans. Appropriate interactions must be established between scientists capable of performing the genetic analyses and aquatic plant managers. Screening should be conducted to determine the genetic composition of aquatic plant populations both before and after the treatment of lakes (or other aquatic habitats) with herbicides and/or biocontrol agents.

Ultimately, a fully integrated approach to aquatic plant management, as we have described, should enable managers to answer basic questions to determine whether their programs are successful, such as: (1) does one genotype consistently return following treatments that seem to be effective in the short term, and (2) do currently treated populations exhibit greater resistance than when previously treated? Effective management practices would be indicated by negative responses to both questions.

## LITERATURE CITED

- Albrecht, M., R. S. Arias, B. E. Scheffler, S. O. Duke, M. Netherland and F. E. Dayan. 2004. Somatic mutation-mediated evolution of herbicide resistance in the nonindigenous invasive plant hydrilla (*Hydrilla verticillata*). *Mol. Ecol.* 13:3229-3237.
- Armstrong, K. F. and S. L. Ball. 2005. DNA barcodes for biosecurity: invasive species identity. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 360:1813-1823.
- Avise, J. C. 2000. *Molecular markers, natural history and evolution*, 4th. edition. Kluwer Academic Publishers, Norwell. 684 pp.
- Basu, C., M. D. Halfhill, T. C. Mueller and C. N. Stewart Jr. 2004. Weed genomics: new tools to understand weed biology. *Trends Ecol. Evol.* 8:391-398.
- Baumel, A., M. L. Ainouche and J. E. Levasseur. 2001. Molecular investigations in populations of *Spartina anglica* C. E. Hubbard (Poaceae) invading coastal Brittany (France). *Mol. Ecol.* 10:1689-1701.
- Baumel, A., M. L. Ainouche, R. J. Bayer, A. K. Ainouche and M. T. Misset. 2002. Molecular phylogeny of hybridizing species from the genus *Spartina* Schreb. (Poaceae). *Mol. Phylogenet. Evol.* 22:303-314.
- Bleeker, W., H. Huthmann and H. Hurka 1999. Evolution of hybrid taxa in *Nasturtium* R. Br. (Brassicaceae). *Folia Geobot.* 34:421-433.
- Carter, M. C. and M. D. Sytsma. 2001. Comparison of the genetic structure of North and South American populations of a clonal aquatic plant. *Biol. Invasions* 3:113-118.
- Chamovitz, D., G. Sandmann and J. Hirschberg. 1993. Molecular and biochemical characterization of herbicide-resistant mutants of cyanobacteria reveals that phytoene desaturation is a rate-limiting step in carotenoid biosynthesis. *J. Biol. Chem.* 268:17348-17353.
- Cronk, J. K. and M. S. Fennessy. 2001. *Wetland plants biology and ecology*. CRC Press LLC, Washington, DC. 462 pp.
- Cronquist, A. 1968. The evolution and classification of flowering plants. Houghton Mifflin Co., Boston. 1244 pp.
- Cook, C. D. K. 1996. *Aquatic plant book*. SPB Academic Publishing, Amsterdam. 228 pp.
- Daehler, C. C. and D. R. Strong. 1997. Hybridization between introduced smooth Cordgrass (*Spartina alterniflora*; Poaceae) and native California cordgrass (*S. foliosa*) in San Francisco Bay, California, USA. *Am. J. Bot.* 84:607-611.
- DeLoach, C. J. and J. L. Tracy. 1997. The effects of biological control of saltcedar (*Tamarix ramosissima*) on endangered species: biological assessment draft. U.S. Department of Agriculture-Agricultural Research Service. 524 pp.
- Durand, C., M. Manuel, C. F. Boudouresque, A. Meinesz, M. Verlaque and Y. Le Parco. 2002. Molecular data suggest a hybrid origin for the invasive *Caulerpa racemosa* (Caulerpaceae, Chlorophyta) in the Mediterranean Sea. *J. Evolut. Biol.* 15:122-133.
- Ellstrand, N. C. and K. A. Schierenbeck. 2001. Hybridization as a stimulus for the evolution of invasiveness in plants? *Proc. Natl. Acad. Sci. USA.* 97:7043-7050.
- Figueroa, M. E., J. M. Castillo, S. Redondo, T. Luque, E. M. Castellanos, F. J. Nieva, C. J. Luque, A. E. Rubio-Casal and A. J. Davy. 2003. Facilitated invasion by hybridization of *Sarcocornia* species in a salt-marsh succession. *J. Ecol.* 91:616-626.
- Fritz, R. S. 1999. Resistance of hybrid plants to herbivores: genes, environment, or both? *Ecology* 80:382-91.
- Fritz, R. S., C. Moulia and G. Newcombe. 1999. Resistance of hybrid plants and animals to herbivores, pathogens, and parasites. *Ann. Rev. Ecol. Syst.* 30:565-591.
- Gaskin, J. F. and B. A. Schaal. 2002. Hybrid *Tamarix* widespread in U.S. invasion and undetected in native Asian range. *Proc. Natl. Acad. Sci. USA.* 99:11256-11259.
- Gaskin, J. F. and B. A. Schaal. 2003. Molecular phylogenetic investigation of U.S. invasive *Tamarix*. *Syst. Bot.* 28:86-95.
- Gifford, A. L. S., J.-P. Ferdy and J. Molofsky. 2002. Genetic composition and morphological variation among populations of the invasive grass, *Phalaris arundinacea*. *Can. J. Bot.* 80:779-785.
- Graham, S. A., J. Hall, K. Sytsma and S. Shi. 2005. Phylogenetic analysis of the Lythraceae based on four gene regions and morphology. *Int. J. Plant Sci.* 166:995-1017.
- Gray, A. J., D. F. Marshall and A. F. Raybould. 1991. A century of evolution in *Spartina anglica*. *Adv. Ecol. Res.* 21:1-61.
- Gressel, J. 2002. *Molecular biology of weed control*. Taylor & Francis Inc., NY. 456 pp.
- Gross, B. L. and L. H. Rieseberg. 2005. The ecological genetics of homoploid hybrid speciation. *J. Hered.* 96:241-252.
- Hebert, P. D. N., A. Cywinska, S. L. Ball and J. R. deWaard. 2003. Biological identifications through DNA barcodes. *Proc. R. Soc. Lond. B. Bio. Sci.* 270:313-322.
- Judd, W. S., C. S. Campbell, E. A. Kellogg, P. F. Stevens and M. J. Donoghue. 2002. *Plant systematics a phylogenetic approach*, 2<sup>nd</sup> ed. Sinauer Associates, Inc. Sunderland, Boston. 576 pp.
- Kay, S. H. and S. T. Hoyle. 2001. Mail Order, the internet and invasive aquatic weeds. *J. Aquat. Plant Manage.* 39:88-91.
- Kress, W. J., K. J. Wurdack, E. A. Zimmer, L. A. Weigt and D. H. Janzen. 2005. Use of DNA barcodes to identify flowering plants. *Proc. Natl. Acad. Sci. USA.* 102:8369-8374.
- Kuehn, M. M., J. E. Minor and B. N. White. 1999. An examination of hybridization between the cattail species *Typha latifolia* and *Typha angustifolia* using random amplified polymorphic DNA and chloroplast DNA markers. *Mol. Ecol.* 8:1981-1990.
- Lee, C. E. 2002. Evolutionary genetics of invasive species. *Trends Ecol. Evol.* 17:386-391.
- Les, D. H., L. J. Mehrhoff, M. A. Cleland and J. D. Gabel. 1997. *Hydrilla verticillata* (Hydrocharitaceae) threatens Connecticut. *J. Aquat. Plant Manage.* 35:10-14.
- Les, D. H. and L. J. Mehrhoff. 1999. Introduction of nonindigenous aquatic vascular plants in southern New England: a historical perspective. *Biol. Invasions* 1:281-300.
- Les, D. H. 2002. Nonindigenous aquatic plants: a garden of earthly delight. *Lakeline* 22:20-24.
- Les, D. H., D. J. Crawford, E. Landolt, J. D. Gabel and R. T. Kimball. 2002. Phylogeny and systematics of Lemnaceae, the duckweed family. *Syst. Bot.* 27:221-240.
- Les, D. H., M. L. Moody and C. L. Soros. 2005. A reappraisal of phylogenetic relationships in the monocotyledon family Hydrocharitaceae. *Aliso* 22:209-228.
- Les, D. H., R. S. Capers and N. P. Tippery. 2006. Introduction of *Glossostigma* (Phrymaceae) to North America: a taxonomic and ecological overview. *Am. J. Bot.* 93:927-939.
- Madeira, P. T., T. K. Van, K. K. Steward and R. J. Schnell. 1997. Random amplified polymorphic DNA analysis of the phenetic relationships among world-wide accessions of *Hydrilla verticillata*. *Aquat. Bot.* 59: 21 7-236.
- Madeira, P. T., T. K. Van and T. D. Center. 2004. An improved molecular tool for distinguishing monoecious and dioecious Hydrilla. *J. Aquat. Plant Manage.* 42:28-32.
- Moody, M. L. and D. H. Les. 2002. Evidence of hybridity in invasive watermilfoil (*Myriophyllum*) populations. *Proc. Natl. Acad. Sci. USA* 99:14867-14871.
- Moody, M. L. 2004. Systematics of the angiosperm family Haloragaceae R. Br. emphasizing the aquatic genus *Myriophyllum*: phylogeny, hybridization and character evolution. Ph.D. dissertation, University of Connecticut. 246 pp.
- Moody, M. L. and D. H. Les. 2007. Distribution and composition of invasive hybrid watermilfoil (*Myriophyllum spicatum* × *M. sibiricum*) populations in North America. *Biol. Invasions* (in press).
- Newman, R. M., D. W. Ragsdale and D. D. Biesboer. 2001. Factors Influencing the Control of Eurasian Watermilfoil With Native or Naturalized Insects. Status report submitted for 1999-2001 to the Minnesota Department of Natural Resources, Ecological Services, St. Paul, MN. 47 pp.
- Newman, R.M. 2004. Biological control of Eurasian watermilfoil: Completion report for 2001-2004 to the Minnesota Department of Natural Resources, Ecological Services, St. Paul, MN. 80 pp.
- O'Hanlon P. C., R. Peakall and D. T. Briese. 2000. A review of new PCR-based genetic markers and their utility to weed ecology. *Weed Res.* 40:239-254.
- Pimentel, D., L. Lack, R. Zuniga and D. Morrison. 2000. Environmental and economic costs of nonindigenous species in the United States. *BioScience* 50:53-65.
- Rejmanek, M. 2000. Invasive plants: approaches and predictions. *Aust. Ecol.* 25:497-506.
- Rejmanek, M. and M. J. Pitcairn. 2002. When is eradication of exotic pest plants a realistic goal, pp. 249-253. *In:* C. R. Veitch and M. N. Clout (eds.). *Turning the tide: the eradication of invasive species*. IUCN SSC Invasive Species Specialist Group. Gland.
- Rieseberg, L. H., O. Raymond, D. M. Rosenthal, Z. Lai, K. Livingstone, T. Nakazato, J. L. Durphy, A. E. Schwarzbach, L. A. Donovan and C. Lexer. 2003. Major ecological transitions in wild sunflowers facilitated by hybridization. *Science* 301:1211-1216.

- Roley, S. S. and R. M. Newman. 2006. Developmental performance of the milfoil weevil, *Euhrychiopsis lecontei* (Coleoptera: Curculionidae), on northern watermilfoil, eurasian watermilfoil, and hybrid (northern x eurasian) watermilfoil. *Environ. Entomol.* 35:121-126.
- Sakai, A. K., F. W. Allendorf, J. S. Holt, D. M. Lodge, J. Molofsky, K. A. With, S. Baughman, R. J. Cabin, J. E. Cohen, N. C. Ellstrand, D. E. McCauley, P. O'Neil, I. M. Parker, J. N. Thompson and S. G. Weller. 2001. The population biology of invasive species. *Ann. Rev. Ecol. Syst.* 32:305-332.
- Saltonstall, K. 2002. Cryptic invasion by a non-native genotype of the common reed, *Phragmites australis*, into North America. *Proc. Natl. Acad. Sci. USA* 99:2445-2449.
- Saltonstall, K. 2003. A rapid method for identifying the origin of North American *Phragmites* populations using RFLP analysis. *Wetlands* 23:1043-1047.
- Simberloff, D. 2003. Eradication—preventing invasion at the outset. *Weed Sci.* 51:247-253.
- Strefeler, M. S., E. Darms, R. L. Becker and E. J. Katovich. 1996. Isozyme characterization of genetic diversity in Minnesota populations of purple loosestrife, *Lythrum salicaria* (Lythraceae). *Am. J. Bot.* 83:265-273.
- Takhtajan, A. L. 1969. Flowering plants. Smithsonian Institution Press, Washington DC. 510 pp.
- Tranel, P. J. and T. R. Wright. 2002. Resistance of weeds to ALS-inhibiting herbicides: what have we learned? *Weed Sci.* 50:700-712.
- Urbanska, K. M., H. Hurka, E. Landolt, B. Neuffer and K. Mummenhoff. 1997. *Plant Syst. Evol.* 204:233-256.
- Vilà, M., E. Weber and C. M. D'Antonio. 2000. Conservation implications of invasion by plant hybridization. *Biol. Invasions* 2:207-217.
- Williams, D. A., W. A. Overholt, J. P. Cuda and C. R. Hughes. 2005. Chloroplast and microsatellite DNA diversities reveal the introduction history of Brazilian peppertree (*Schinus terebinthifolius*) in Florida. *Mol. Ecol.* 14:3643-3656.