EURASIAN WATERMILFOIL SURVEY OF THREE RESERVOIRS IN THE LOWER CLARKS FORK RIVER, MONTANA: II. TAXONOMIC ANALYSIS OF NATIVE AND NONNATIVE WATERMILFOILS

John D. Madsen[1], Joshua C. Cheshier[1], Vipaporn Phuntumart[2], Ryan Thum[3], and Mark Welch[4]
[1]Geosystems Research Institute, Mississippi State University, Mississippi State, MS
[2]Department of Biological Sciences, Bowling Green State University, Bowling Green, KY
[3]Annis Water Resources Institute, Grand Valley State University, Grand Rapids, MI
[4]Department of Biological Sciences, Mississippi State University, Mississippi State, MS

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[2]Department of Biological Sciences, Bowling Green State University, Bowling Green, KY
[3]Annis Water Resources Institute, Grand Valley State University, Grand Rapids, MI
[4]Department of Biological Sciences, Mississippi State University, Mississippi State, MS

Introduction

In 2008, the Lower Clark Fork Eurasian Watermilfoil Task Force issued a request for proposals to survey the Lower Clark Fork River reservoirs of Cabinet Gorge, Noxon Rapids, and Thompson Falls for the presence of Eurasian watermilfoil. Our proposal was selected from those submitted.

We divided the project into three tasks:

Task 1. Survey Current Aquatic Vegetation Community

We generated a grid of points over the entire system from Cabinet Gorge Dam to Thompson Falls Dam. These points were displayed using software on a notebook computer or handheld computer allowing navigation to each point. At each point, the depth was recorded. If the depth was less than 40', we recorded the plant species present from one toss of an aquatic plant sample rake. If no plants were found on the first rake toss, then one more rake toss was made to ensure that plants were not present at that site. Estimates of acreage for species of interest were based on the number of points at which the species were present and the size of the sample grid for each reservoir.

At each reservoir, 6 samples of each species of *Myriophyllum* were collected for genetic analysis. Samples were photographed digitally with the sample number, and subsamples shipped to Dr. Ryan Thum of Grand Valley State University, Dr. Vipaporn Phuntumart of Bowling Green State University, and Dr. Mark Welch of Mississippi State University for analysis. Three separate analyses were performed to independently verify or corroborate results as subcontractors used different approaches genetically identify samples. Each sample also had pressed specimens and a digital photo for future analysis. In addition to the three reservoirs in the Lower Clark Fork River system (Thompson Falls, Noxon Rapids, and Cabinet Gorge), we collected six samples of each species from Pend Oreille Lake and River, which technically makes four reservoirs. However, the Pend Oreille Lake and River sites are a reference for the other three locations. Results of the quantitative survey are reported in Report I (Madsen and Cheshier 2009).

Task 2. Eurasian Watermilfoil Littoral Survey

Eurasian watermilfoil was mapped using a combination of hydroacoustic sensing, visual observation, and rake throws to locate plant beds. This technique offers finer-resolution point mapping to outline beds of where Eurasian watermilfoil was located. While outlining beds sounds simple, particularly considering the ease with which terrestrial weeds can be mapped; this technique is in fact extremely difficult to do with submersed plants growing in 20 to 30 feet of water depth. The entire shoreline circumference was patrolled to find Eurasian watermilfoil infestations. Locations of curlyleaf pondweed (*Potamogeton crispus* L.) and flowering rush (*Butomus umbellatus* L.) were also mapped as part of this task.

Boat launches are areas of particular concern. Boat launches were monitored closely for the presence of Eurasian watermilfoil, and plants mapped by location using a GPS. Results of the littoral survey are reported in Report I (Madsen and Cheshier 2009). This report presents the results of morphological and genetic analysis of milfoil samples collected during the quantitative survey (Task 1).

Task 3. Morphological and Genetic Analyses of *Myriophyllum* species.

Samples collected as part of Task 1 were analyzed by three labs discussed in Task 1 above. The purpose of the morphological and genetic analyses were to determine whether 1) Eurasian watermilfoil can be readily and consistently separated from northern watermilfoil by simple morphological characteristics, 2) there is any evidence of hybridization between *Myriophyllum* species, and 3) the various genetic analytical techniques consistently separate samples between Eurasian and northern watermilfoil. While morphological characteristics were used to identify Eurasian watermilfoil and northern watermilfoil samples in the field, we wanted to verify the ability to identify between these two species in the field using genetic analysis, and identify simple morphological factors that can be used to consistently and reliably differentiate between Eurasian watermilfoil and northern watermilfoil by citizens and volunteers trained in plant identification. Hybridization has been reported to occur elsewhere in North America (Moody and Les 2002); we also wanted to determine if there was any evidence of hybridization in this region, and if hybrids can be separated from pure strains of each species using morphological traits.

Proper identification between a desirable native and an invasive species is of paramount importance in an invasive species management program; it is particularly important that simple traits be identified for use in separating similar-appearing species if volunteers are used in surveying. Eurasian watermilfoil and northern watermilfoil have a long history of confusion, often by professional taxonomists (Hellquist 1993).

Species Analyzed

Eurasian watermilfoil. Eurasian watermilfoil (*Myriophyllum spicatum* L.) is as invasive submersed aquatic plant that is not native to the U.S. Eurasian watermilfoil can grow submersed in 20 or more feet of water, is rooted to the bottom yet forms a dense surface canopy, and is an evergreen perennial that overwinters as a green shoot. Although the plant produces viable seed, reproduction and spread is almost entirely clonal by stem fragment (Madsen and Welling 2002, Madsen 2005). The plant forms fragments ("autofragments") in the fall through stem abscission, and the resulting fragments have abundant starch reserves. This is a key form of spread within and between lakes. Fragments formed by mechanical breakage are also viable. A native of Eurasia, it was introduced in multiple locations in North America in the 1940's (Madsen 2005). Management is predominantly by the herbicides diquat, endothall, 2,4-D, triclopyr, and fluridone; though other control techniques are available (Madsen 2005). Management with herbicides can be done selectively with a number of products (Getsinger et al. 1997, Parsons et al. 2001, Madsen et al. 2002). Overwinter drawdown has also been demonstrated to be effective. Several native species of *Myriophyllum* are also found in North America that may be easily confused with Eurasian watermilfoil, including northern watermilfoil (*Myriophyllum sibiricum* Komarov) and whorled watermilfoil (*Myriophyllum verticillatum* L).

Northern Watermilfoil. Northern watermilfoil (*Myriophyllum sibiricum* Komarov) is a submersed aquatic plant native to the U.S. Synonyms used in the past include *M. exalbescens* and *M. spicatum* var. *exalbescens*; the latter further exacerbating the confusion between the two species. The plant overwinters as a turion formed in the fall (Aiken and Walz 1979), though the plant also forms seeds. The distribution of northern watermilfoil is circumboreal, with plants found throughout North America, Europe and Siberia (Aiken 1981). Northern watermilfoil is a common component of permanent waters in the temperate zone of North America. While it may occasionally form nuisance problems in North America, it rarely causes the widespread problems associated with Eurasian watermilfoil. Northern watermilfoil is susceptible to many of the same herbicides as Eurasian watermilfoil, including 2,4-D, triclopyr, and fluridone; but is often more tolerant to the concentration or exposure time of these herbicides than the nonnative species.

Methods and Materials

We sampled four lakes in the Lower Clark Fork River system, from upstream to downstream: Thompson Falls Reservoir, Noxon Rapids Reservoir, Cabinet Gorge Reservoir (these three in Montana) and Pend Oreille Lake and River (Idaho) (Figure 1a,b). At each lake, we collected samples of Eurasian watermilfoil and northern watermilfoil from six locations; both species sampled from the same location if possible. At each site and for each species, five portions were collected. One portion was kept on ice and shipped to each of the three university laboratories (a total of three portions), one portion was photographed for future reference, and one portion was pressed as a herbarium specimen. Herbarium specimens were used for morphological analysis, and then prepared for mounting and storage in the Mississippi State University herbarium. Each specimen was labeled for the reservoir, nominal species identification (as identified by J. Madsen on site), and sample number for the reservoir. Samples for genetic analysis were stored on ice (to keep cool, not frozen) until shipped by courier service to the three laboratories.

Thompson Falls Reservoir

Six samples of northern watermilfoil were collected from Thompson Falls Reservoir (Figure 2). Eurasian watermilfoil was not present in this reservoir, so only samples of the native northern milfoil were collected.

Noxon Rapids Reservoir

Six samples of northern watermilfoil and six samples of Eurasian watermilfoil were collected from Noxon Rapids Reservoir (Figure 3a, 3b, and 3c).

Cabinet Gorge Reservoir

Six samples of northern watermilfoil and six samples of Eurasian watermilfoil were collected from Cabinet Gorge Reservoir (Figure 4a, b).

Pend Oreille Lake and River

Two samples of Eurasian watermilfoil and one sample of northern watermilfoil were collected from Pend Oreille Lake (Figure 5a). The remaining four samples of Eurasian watermilfoil and five samples of northern watermilfoil were collected from the Pend Oreille River upstream of the Albeni Falls Dam, in Idaho (Figure 5b). Pend Oreille Lake and River were sampled as a reference to the upstream reservoirs, to determine if these plants were genetically similar. Also, as a more widely infested lake with more visitors, we hypothesized that this lake may be more likely to have hybridized *Myriophyllum*.

Morphological Analysis

Pressed herbarium specimens were analyzed in the lab after returning to the Geosystems Research Institute. For each pressed specimen, the stem color was coded as green or red. In addition, the apical meristem was recorded as rounded or flat. The leaf tips were recorded as rounded or flat. For each sample, six node/internode combinations were selected beginning 210 mm below the apex. For six internodes, the internode length was measured, the number of leaflets from one leaf per node counted, the length of the leaf measured, and the length of the leaflet measured. Stem thickness was measured at the middle of the internode for those internode intervals. All measurements were made in mm. Data was analyzed using Statistix 9.0 (Analytical Software 2008).

Genetic Analysis

Genetic analysis of all 42 samples was performed at three laboratories (Vipaporn Phuntumart, Bowling Green University; Ryan Thum, Grand Valley State University; and Mark Welch, Mississippi State University). A total of 42 plants samples (24 of *Myriophyllum sibiricum* (Northern watermilfoil) and 18 of *Myriophyllum spicatum* (Eurasian watermilfoil)) collected from Thompson Falls (*M. sibiricum* only), Noxon Rapids, Cabinet Gorge and Pend Oreille Lake and River were sent to each laboratory for analysis. The methods each lab used are as follows:

Bowling Green University. A total of 42 plants samples (24 of *Myriophyllum sibiricum* (Northern watermilfoil) and 18 of *Myriophyllum spicatum* (Eurasian watermilfoil)) collected from Thompson Falls (*M. sibiricum* only), Noxon Rapids, Cabinet Gorge and Pend Oreille Lake and River were received in August 2009. Total genomic DNA was extracted from these plant tissues using DNeasy kit (Qiagen, CA). Measurement of DNA concentration was performed by spectrophotometer (NanoDrop ND-1000, Thermoscientific). PCR amplification of the nuclear ribosomal DNA of the 3' end of the 18S-like gene to the 5' end of the 28S-like gene were performed using primers ITS4: 5'TCCTCCGCTTATTGATATGC3' and ITS5: 5'GGAAGTAAAAGTCGTAACAAGG3' (White et al. 1990), 50-100 ng genomic DNA and Phusion™ High-Fidelity DNA Polymerase (NEB, MA). The amplified products were subjected to electrophoresis on 1.5% agarose gels containing 5.0 ug/ml of ethidium bromide. The amplicons were purified using a QIAquick PCR purification kit (QIAGEN, CA). DNA sequencing was performed by Geneway, CA.

Grand Valley State University. We used phylogenetic analysis of DNA sequence data to identify *Myriophyllum spicatum, M. sibiricum*, and to identify potential hybrids. Specifically, we sequenced the internal transcribed spacers 1 and 2 (ITS) and compared them to samples of known identity from Moody and Les (2002), available on GenBank. In addition, we sequenced a chloroplast gene, trnL-F, using the protocol of Taberlet and others (1991) and compared them to known sequences from GenBank as well as sequences from our own collections.

Mississippi State University. In addition to confirming genetic results supplied by two other molecular laboratories, we developed a cost effective and high-throughput analysis that should aid in identifying invasive and native watermilfoils. The approach we used was that of polymerase chain reaction restriction fragment length polymorphisms (PCR-RFLPs). A PCR-

RFLP analysis involves the PCR amplification of DNA that is variable across individuals of interest. The PCR product is then digested with a restriction enzyme that targets polymorphisms. That is, genetic divergence between target taxa has resulted in a gain or loss of a restriction enzyme cut site. The digested PCR product was run on agarose gels, and different restriction enzyme profiles became apparent. Ribosomal ITS sequence for both *Myriophyllum sibiricum* and *M. spicatum* were downloaded from NCBI's Genbank (Accession #'s DQ786012- DQ786027). Sequences were analyzed and compared. Restriction enzyme cut sites that were present in representative sequences of one species, and absent in representative sequences of the other were targeted for marker development. One restriction enzyme presented itself as particularly useful for the purpose of PCR-RFLPs; *HhaI* was found to cut specific segments that differentiate between Eurasian and northern watermilfoil. *HhaI* cut ribosomal ITS from *M. spicatum* at three restriction cut sites absent in *M. sibiricum* (Figure 6). As a result a 239 bp fragment results when digesting *M. sibiricum* ribosomal ITS with *HhaI*. This 239 bp fragment was absent after digesting *M. spicatum* ribosomal ITS with *HhaI*. We PCR amplified ribosomal ITS from samples collected from Montana lakes, and digested these amplicons with *HhaI*. Digests were then run on 4% metaphor agarose gels, and restriction fragment profiles were scored visually.

Results and Discussion

Morphological Analysis

The first three characteristics assessed are evaluated in Table 1. The first characteristic was whether the leaf end was flat or rounded. All but one of the northern watermilfoil leaf ends were rounded (Figure 8), while all but one of the Eurasian watermilfoil leaves were classed as flattened – a significant difference at the $p<0.0001$ level (Figure 7, Table 1). All but one of the Eurasian watermilfoil had a flattened meristem, while all but four of the northern watermilfoil stems had rounded apical meristems; a significant difference at the $p<0.0001$ level (Table 1). These two characteristics were useful in differentiating the species when used in concert with other characteristics. Stem color, on the other hand, was not diagnostic (Table 1).

An analysis of the number of leaflets, leaf length, leaflet length, stem thickness and internode length are compared in Table 2. Stem thickness and internode length were not diagnostic tests (Table 2). The average leaflet number of Eurasian watermilfoil was more than twice that of northern watermilfoil (Table 2). The distribution of leaflet pair number is largely nonoverlapping (Figure 9), so this characteristic is useful for differentiating these two species in the Lower Clark Fork River system. The leaf length of northern watermilfoil was significantly greater than Eurasian watermilfoil, but this was not marked enough to be a useful character (Table 2). Leaf length in northern watermilfoil and Eurasian watermilfoil were significantly overlapping (Figure 10). Leaflet length was significantly longer in northern watermilfoil than in Eurasian watermilfoil (Table 2). The distribution of leaflet lengths between the two species, however, overlapped completely, which renders this not a useful characteristic (Figure 11).

Therefore, the three useful characteristics for differentiating northern watermilfoil and Eurasian watermilfoil were the rounded apical meristem, rounded leaf tip, and fewer than 12 leaflet pairs for northern watermilfoil; and flattened apical meristem, flattened leaf tip, and more than 12

leaflet pairs for Eurasian watermilfoil. The identifier, however, should look at all three characteristics on more than one node, and on more than one plant stem (Table 3).

Genetic Analysis

Bowling Green University. The rDNA sequences were successfully amplified from all the samples (Figure 12). All the sequences were further analyzed by BLAST (Basic Local Alignment Search Tool) analysis using the E-value of 10-3. As expected, the sequences of rDNA of *M. sibiricum* and *M. spicatum* showed strong homology to the rDNA sequences of known sequences of *M. sibiricum* and *M. spicatum* that have been deposited in the National Center for Biotechnology Information (NCBI) database, respectively (Table 4). The accession numbers of the obtained sequences have been submitted to the NCBI DNA database.

A DNA methodology was successfully developed to differentiate *M. spicatum* and *M. sibiricum* found in the Thompson Falls (northern only), Noxon Rapids, Cabinet Gorge and Pend Oreille Lake and River. PCR amplification of the rDNA was used for identification of 42 samples in total. The PCR products of rDNA sequences of both species were ranging from 700-1000 nucleotide-long. Subsequent sequencing and BLAST analyses revealed that the sequences exhibited 94-99% identities to those of *Myriophyllum* sp. that have been reported and publicly available via NCBI. Our results corresponded to the results obtained from traditional taxonomic analyses.

Grand Valley State University. The genetic identifications based on both ITS and trnL-F for all samples from the four MT populations were identical to their identifications based on morphology (Figures 13, 14). Furthermore, there was no evidence for hybridization among the two species, which would have been suggested by any of the following: 1) presence of one copy of *M. spicatum*-like ITS and one copy of *M. sibiricum*-like ITS DNA sequences in an individual, 2) different genetic identifications for the two markers, or 3) different genetic identification from morphology. However, the analysis of two molecular markers cannot rule out a history of hybridization for any of the given individuals. For example, it is possible that some of the lineages sampled have a pedigree that includes member(s) from the other species. Subsequent and repeated backcrossing to one parental species would over time lead to a low probability of observing 1-3 above in a two-locus system. Thus, while there is no evidence for any of the samples being F1 hybrids, analysis of a large number of independent molecular markers would be required to determine whether any genetic admixture has occurred between the two species along the pedigrees of the analyzed samples.

Mississippi State University. Results are presented in Figure 15. In all cases, we confirmed the identification of watermilfoil samples presented by specialists in the field. We also observed no definitive evidence for hybridization in this sample.

Conclusions

1. Morphological analysis indicated that the number of leaflet pairs, the shape of the apical meristem, and the shape of the leaf tip were diagnostic characteristics between northern

watermilfoil and Eurasian watermilfoil (see Table 3). These characteristics should be used together on multiple samples from the population, not alone on a single sample.

2. Genetic analysis indicated that the species were correctly identified in all instances. Visible characteristics are sufficient to differentiate northern watermilfoil from Eurasian watermilfoil.

3. For these samples in this system, there was no evidence of hybridization between Eurasian watermilfoil and northern watermilfoil.

Recommendations

1. As of 2008, northern watermilfoil and Eurasian watermilfoil populations in the Lower Clark Fork River can be differentiated using three morphological characteristics, as indicated in Table 3. Volunteer monitors should be trained to differentiate northern watermilfoil from Eurasian watermilfoil by using all three characteristics (leaflet number, flattened leaf end, and flattened apical meristem) as indicated.

Acknowledgements

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Contact Information:

Dr. John D. Madsen
Geosystems Research Institute
Box 9652
Mississippi State University
Mississippi State, MS 39762-9652
Ph. 662-325-2428
e-mail jmadsen@gri.msstate.edu
Web http://www.gri.msstate.edu

Table 1. Comparison of Boolean characteristics of northern watermilfoil and Eurasian watermilfoil, with a comparison by Fisher's exact test.

Characteristic	Northern watermilfoil		Eurasian watermilfoil		Fisher's Exact Test P-value
Flat Leaf End	No	Yes	No	Yes	<0.0001
	95.8% (23)	4.2% (1)	5.6% (1)	94.4% (17)	
Flat Apical Meristem	No	Yes	No	Yes	<0.001
	83.3% (20)	16.7% (4)	5.6% (1)	94.4% (17)	
Stem Color	Green	Red	Green	Red	0.256
	87.5% (21)	12.5% (3)	72.2% (13)	27.8% (5)	

Table 2. Comparison of morphological characteristics of northern watermilfoil and Eurasian watermilfoil, with a comparison by T-test

	Northern watermilfoil		Eurasian watermilfoil		T-test
Variable	Mean	SE Mean	Mean	SE Mean	p-value
Leaflet Number	8.10	0.123	16.32	0.253	<0.0001
Leaf Length (mm)	20.0	0.420	18.07	0.415	0.001
Leaflet Length (mm)	13.8	0.438	8.556	0.244	<0.0001
Stem thickness (mm)	1.03	0.015	1.056	0.022	0.44
Internode Length (mm)	15.6	0.916	13.06	0.701	0.0256

Table 3. Characteristics for differentiating northern watermilfoil and Eurasian watermilfoil: Diagnostic characteristic, criteria for northern watermilfoil, criteria for Eurasian watermilfoil, and whether it is a valid characteristic for field identification. Shaded blocks are not recommended for field identification. Use more than one characteristic to consistently identify between watermilfoil species. Reliance on only one characteristic may lead to a false conclusion.

Diagnostic characteristic (units)	Northern watermilfoil M. sibiricum	Eurasian watermilfoil *M. spicatum*	Recommended Characteristic for Field Identification
Leaf end	Rounded	Flat	Yes
Apical meristem	Rounded	Flat	Yes
Stem Color	Green (88%)	Red (28%)	No
Leaflet number	Less than 12 pair	More than 12 pair	Yes
Leaf length (mm)	20	18	No
Leaflet length (mm)	14	9	No
Stem thickness (mm)	1	1	No
Internode length (mm)	16	13	No

Table 4. Genetic identification of *Myriophyllum sibiricum* and *M. spicatum.*

Samples[a]	Accession number[b]
CG-Spi-01	EF526362.1 *Myriophyllum spicatum*
CG-Spi-02	EF526362.1 *Myriophyllum spicatum*
CG-Spi-03	DQ786013.1 *Myriophyllum spicatum*
CG-Spi-04	EF526362.1 *Myriophyllum spicatum*
CG-Spi-05	EF526362.1 *Myriophyllum spicatum*
CG-Spi-06	EF526362.1 *Myriophyllum spicatum*
NR-Spi-01	EF526362.1 *Myriophyllum spicatum*
NR-Spi-02	EF526362.1 *Myriophyllum spicatum*
NR-Spi-03	EF526362.1 *Myriophyllum spicatum*
NR-Spi-04	EF526362.1 *Myriophyllum spicatum*
NR-Spi-05	EF526362.1 *Myriophyllum spicatum*
NR-Spi-06	EF526362.1 *Myriophyllum spicatum*
PO-Spi-01	EF526362.1 *Myriophyllum spicatum*
PO-Spi-02	EF526362.1 *Myriophyllum spicatum*
PO-Spi-03	EF526362.1 *Myriophyllum spicatum*
PO-Spi-04	EF526362.1 *Myriophyllum spicatum*
PO-Spi-05	EF526362.1 *Myriophyllum spicatum*
PO-Spi-06	EF526362.1 *Myriophyllum spicatum*
TF-Sib-01	DQ786018.1 *Myriophyllum sibiricum*
TF-Sib-02	DQ786018.1 *Myriophyllum sibiricum*
TF-Sib-03	EF178706.1 *Myriophyllum sibiricum*
TF-Sib-04	DQ786018.1 *Myriophyllum sibiricum*
TF-Sib-05	DQ786018.1 *Myriophyllum sibiricum*
TF-Sib-06	DQ786018.1 *Myriophyllum sibiricum*
CG-Sib-01	AF513838.1 *Myriophyllum sibiricum*
CG-Sib-02	AF513838.1 *Myriophyllum sibiricum*
CG-Sib-03	AF513838.1 *Myriophyllum sibiricum*
CG-Sib-04	DQ786024 *Myriophyllum sibiricum*
CG-Sib-05	AF513838.1 *Myriophyllum sibiricum*
CG-Sib-06	AF513838.1 *Myriophyllum sibiricum*
NR-Sib-01	AF513838.1 *Myriophyllum sibiricum*
NR-Sib-02	AF513838.1 *Myriophyllum sibiricum*

Table 4. Genetic identification of *Myriophyllum sibiricum* and *M. spicatum.*

Samples[a]	Accession number[b]
NR-Sib-03	AF513838.1 *Myriophyllum sibiricum*
NR-Sib-04	EF526362.1 *Myriophyllum spicatum*
NR-Sib-05	AF513838.1 *Myriophyllum sibiricum*
NR-Sib-06	EF526362.1 *Myriophyllum spicatum*
PO-Sib-01	AF513838.1 *Myriophyllum sibiricum*
PO-Sib-02	AF513838.1 *Myriophyllum sibiricum*
PO-Sib-03	AF513838.1 *Myriophyllum sibiricum*
PO-Sib-04	EF526362.1 *Myriophyllum spicatum*
PO-Sib-05	AF513838.1 *Myriophyllum sibiricum*
PO-Sib-06	AF513838.1 *Myriophyllum sibiricum*

[a]CG=Cabinet Gorge, NR= Noxon Rapids, PO=Pend Oreille Lake and River and TF=Thompson Falls

[b]Accession numbers of closest species as determined by BLAST

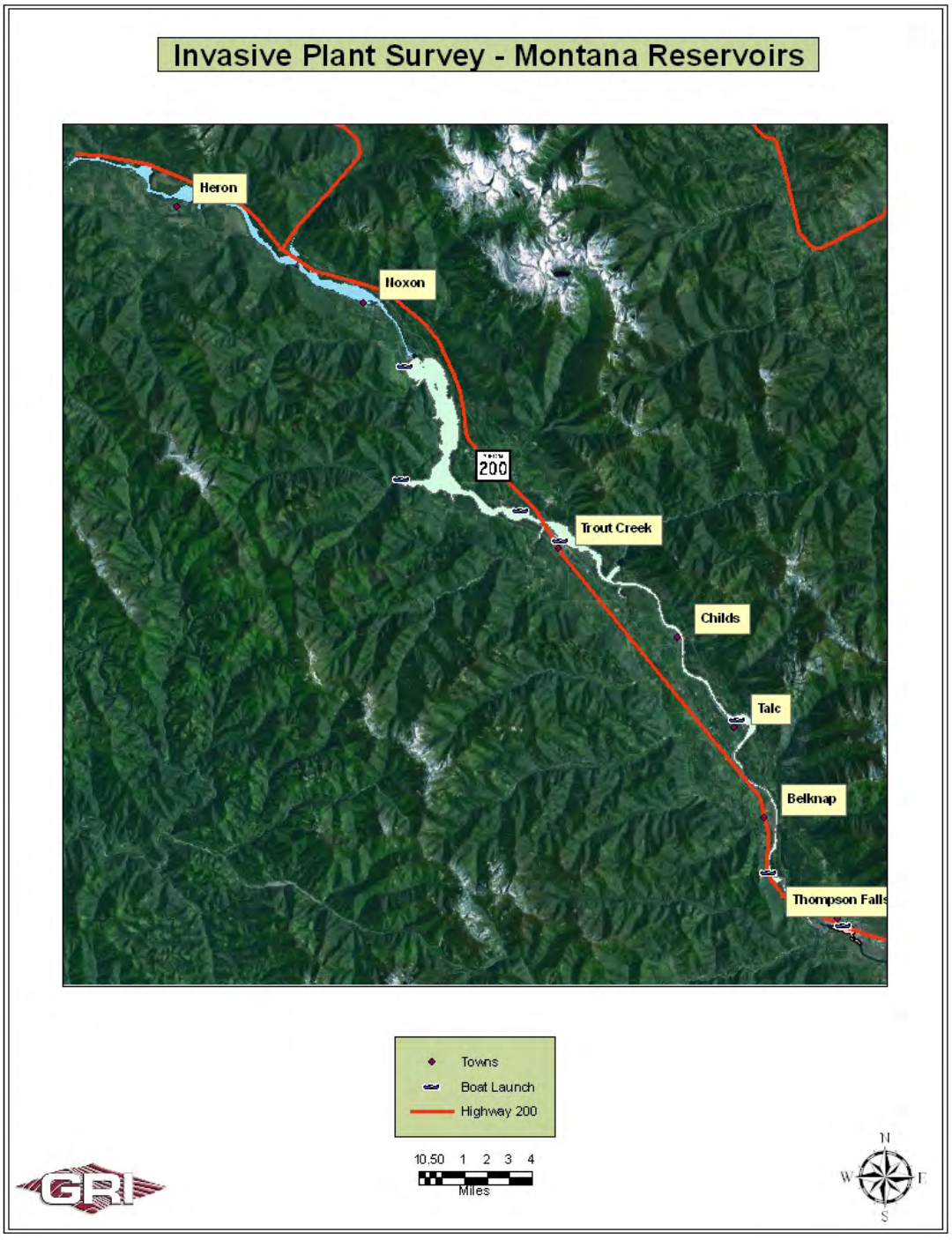

Figure 1a. Map showing the relative location of Thompson Falls Reservoir, Noxon Rapids Reservoir, and Cabinet Gorge Reservoir to Heron, MT.

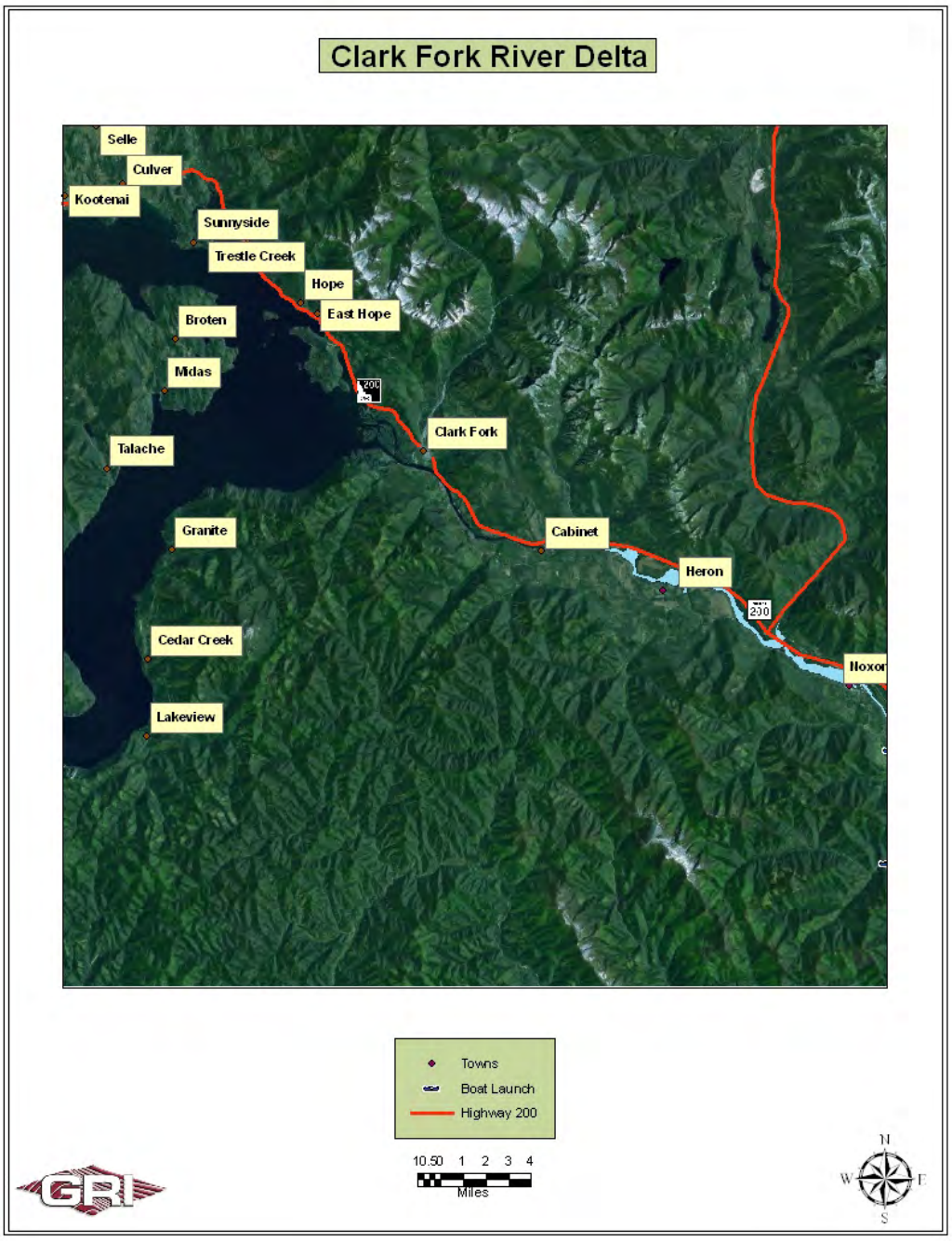

Figure 1b. Map of Noxon Reservoir from Noxon, MT, Cabinet Gorge Reservoir, to Pend Oreille Lake (Idaho). Pend Oreille River continues downstream from the upper left of Pend Oreille Lake.

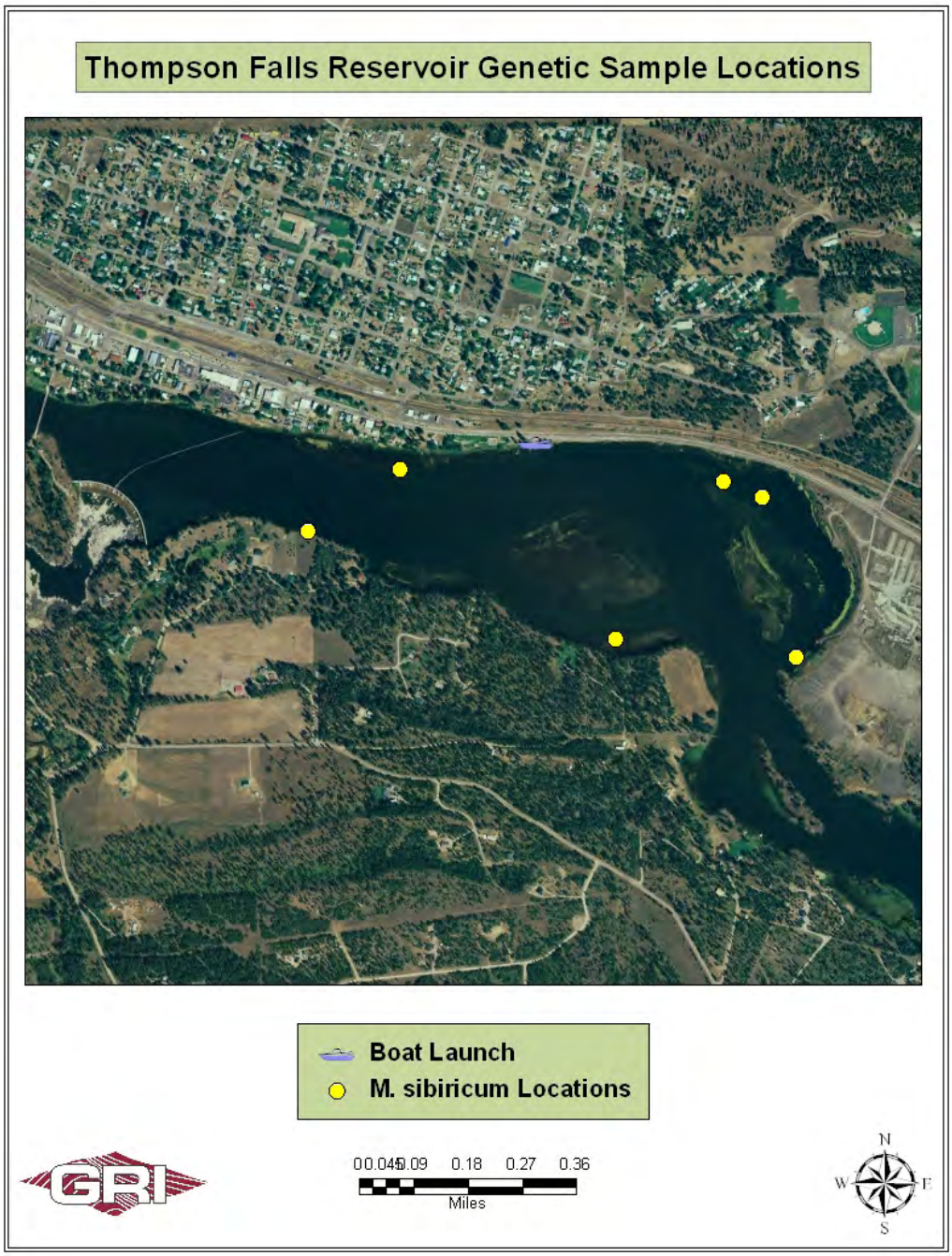

Figure 2. Northern watermilfoil sample locations in Thompson Falls Reservoir, Montana. No Eurasian watermilfoil was found in Thompson Falls Reservoir.

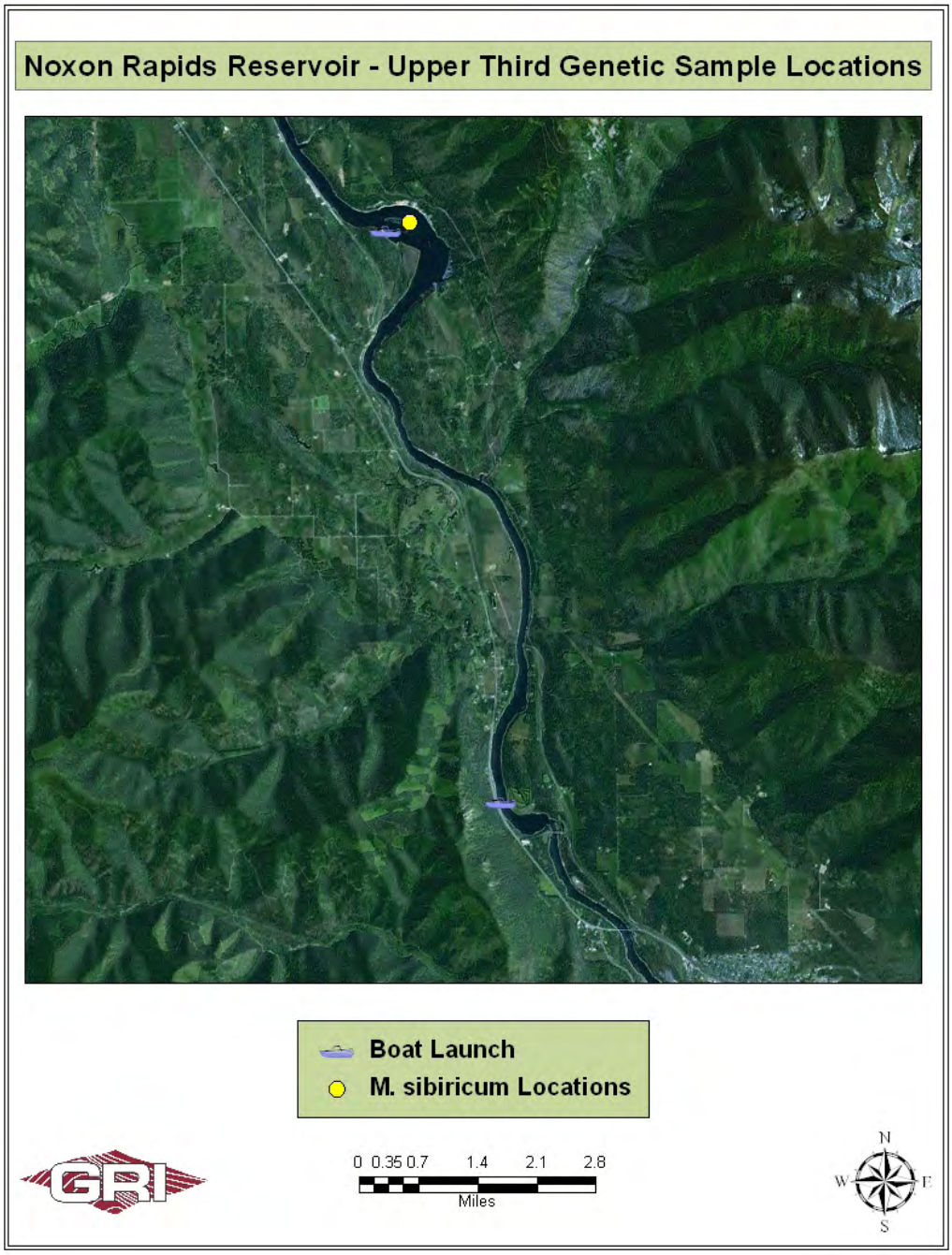

Figure 3a. One sample of northern watermilfoil was taken from the upper third section of Noxon Rapids Reservoir.

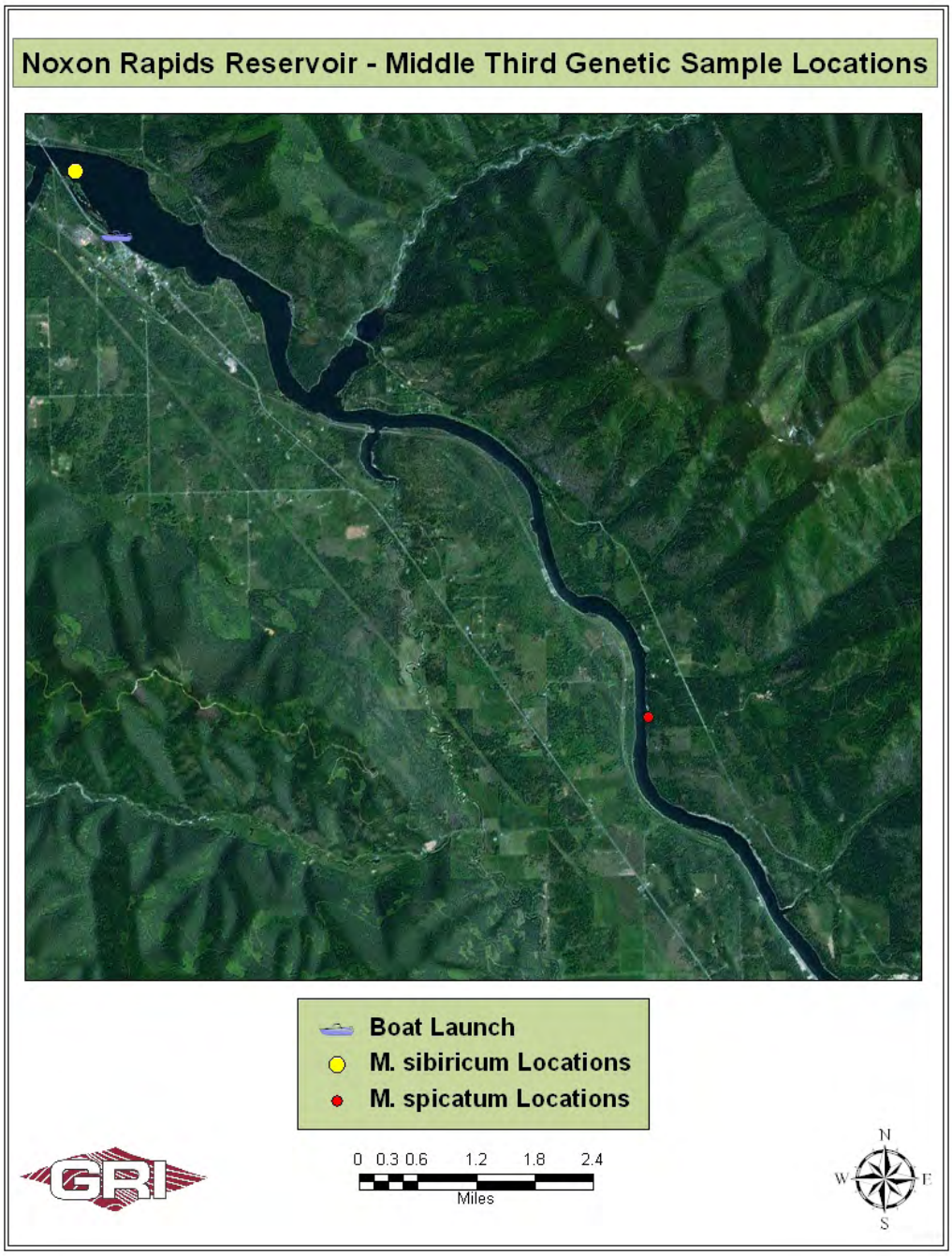

Figure 3b. One sample of northern watermilfoil and one sample of Eurasian watermilfoil were taken from the middle third of Noxon Rapids Reservoir.

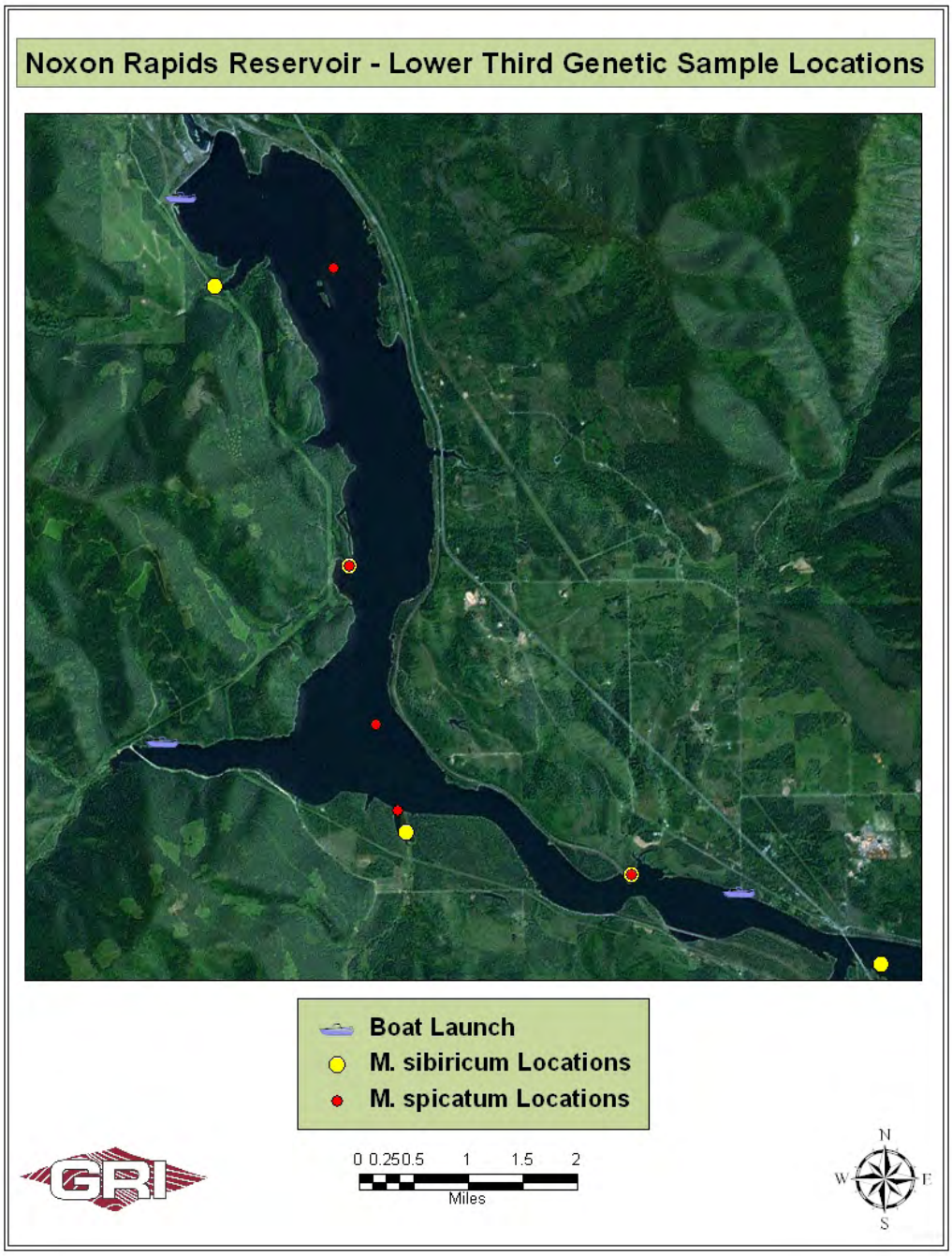

Figure 3c. Five samples of Eurasian watermilfoil and four samples of northern watermilfoil were taken from the lower third of Noxon Rapids Reservoir.

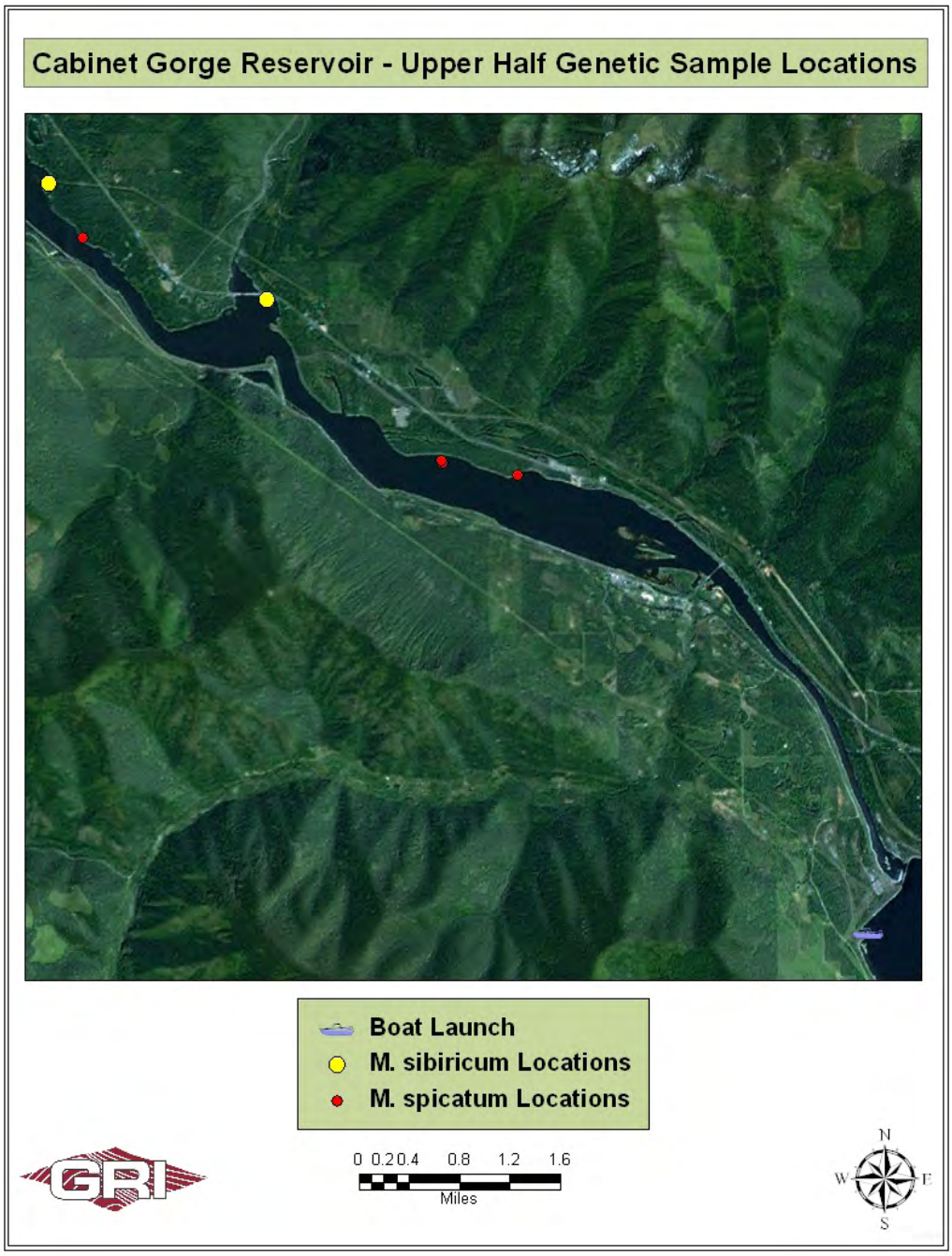

Figure 4a. Two samples of northern watermilfoil and three samples of Eurasian watermilfoil were collected in the upper half of Cabinet Gorge Reservoir.

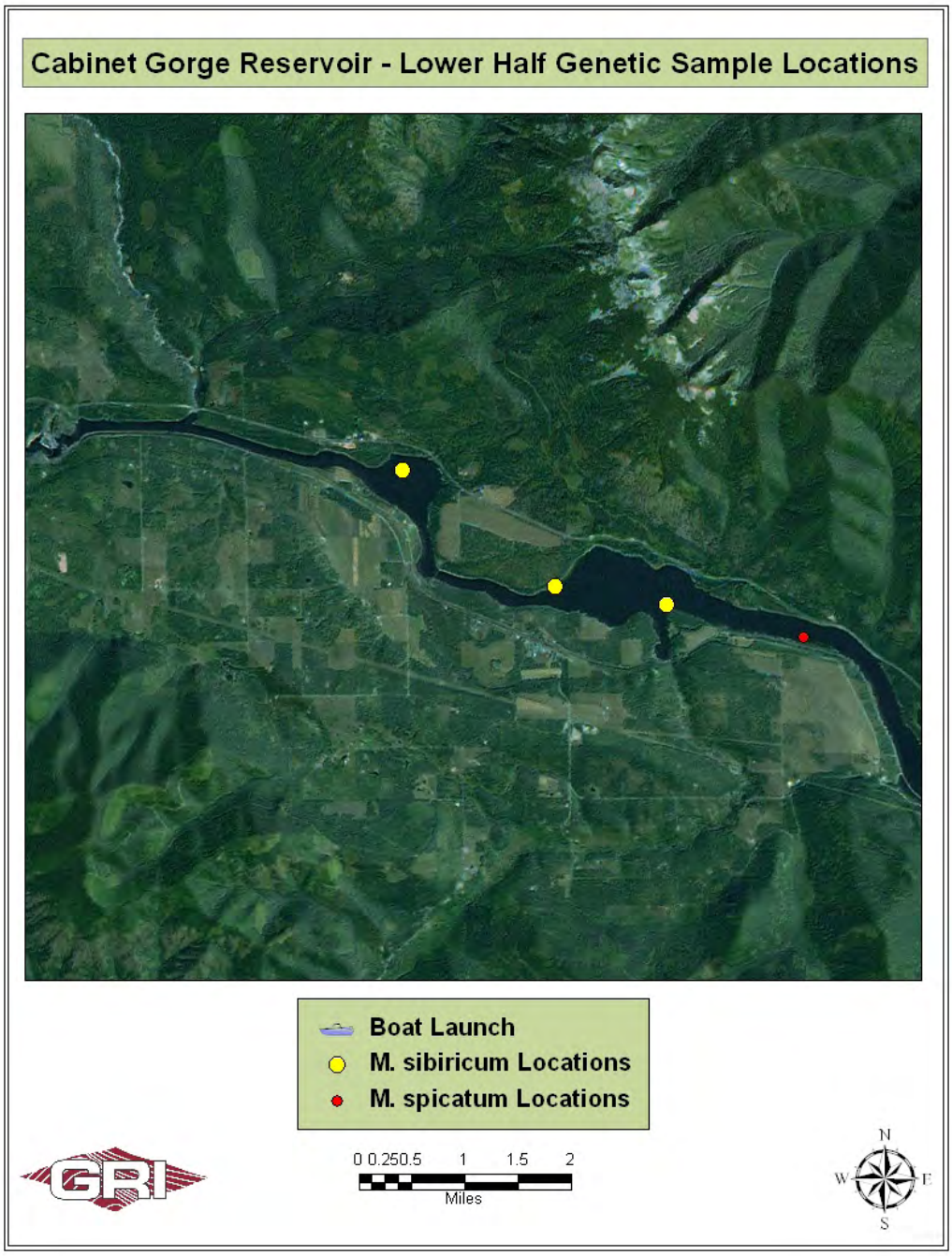

Figure 4b. Four samples of northern watermilfoil and three samples of Eurasian watermilfoil were collected in the upper half of Cabinet Gorge Reservoir.

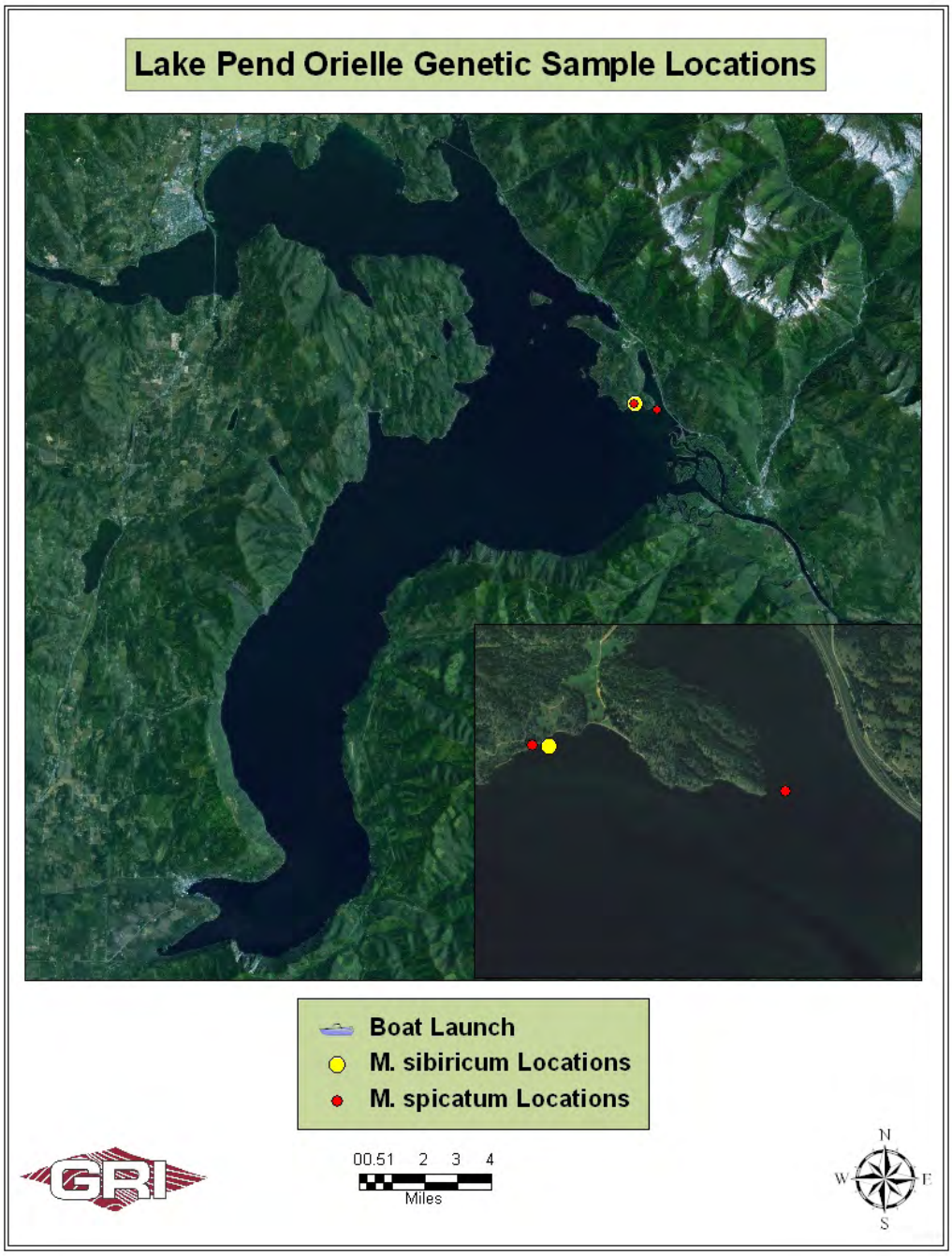

Figure 5a. Two samples of Eurasian watermilfoil and one sample of northern watermilfoil were collected from Pend Oreille Lake.

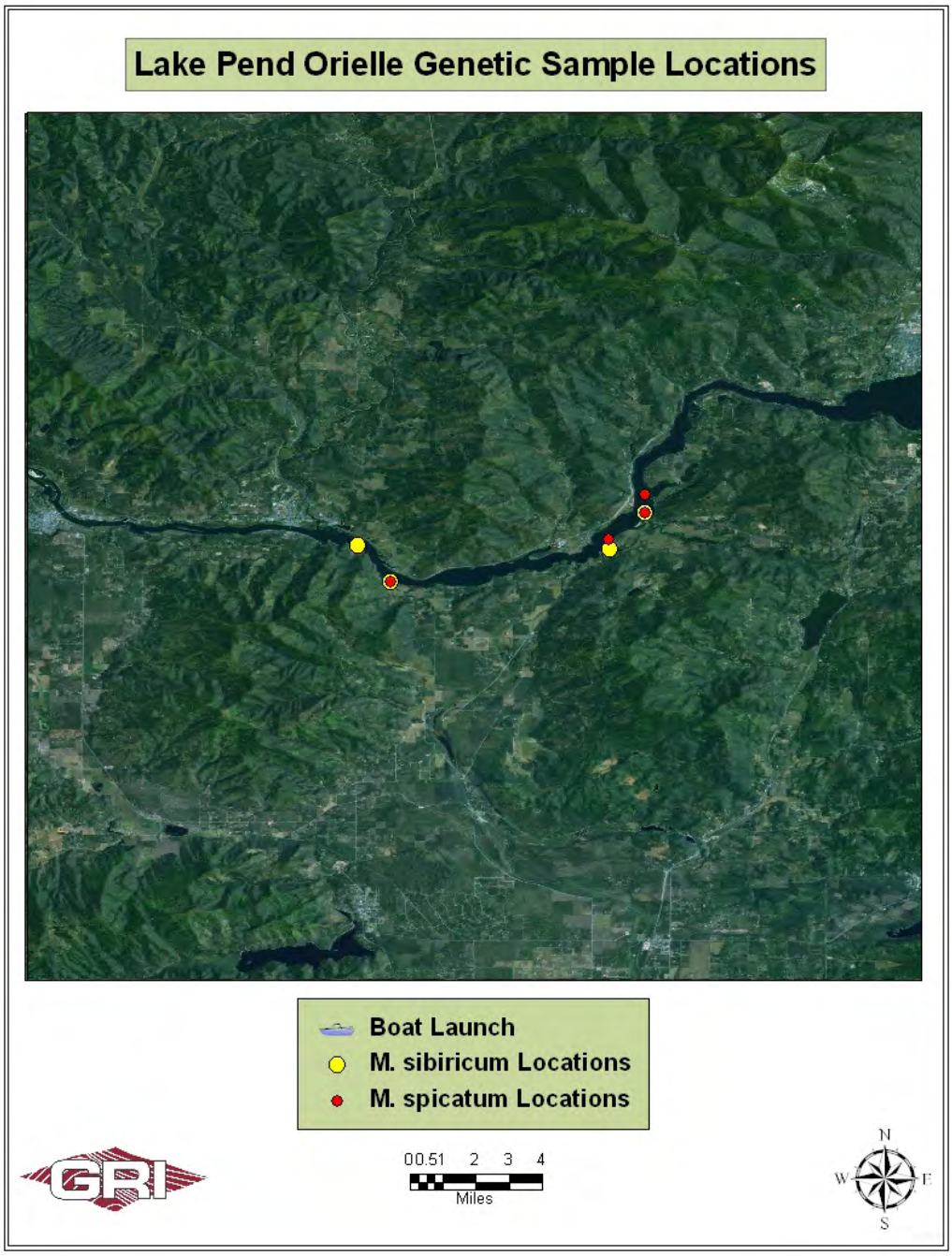

Figure 5b. Four samples of Eurasian watermilfoil and five samples of northern watermilfoil were collected from the Pend Oreille River.

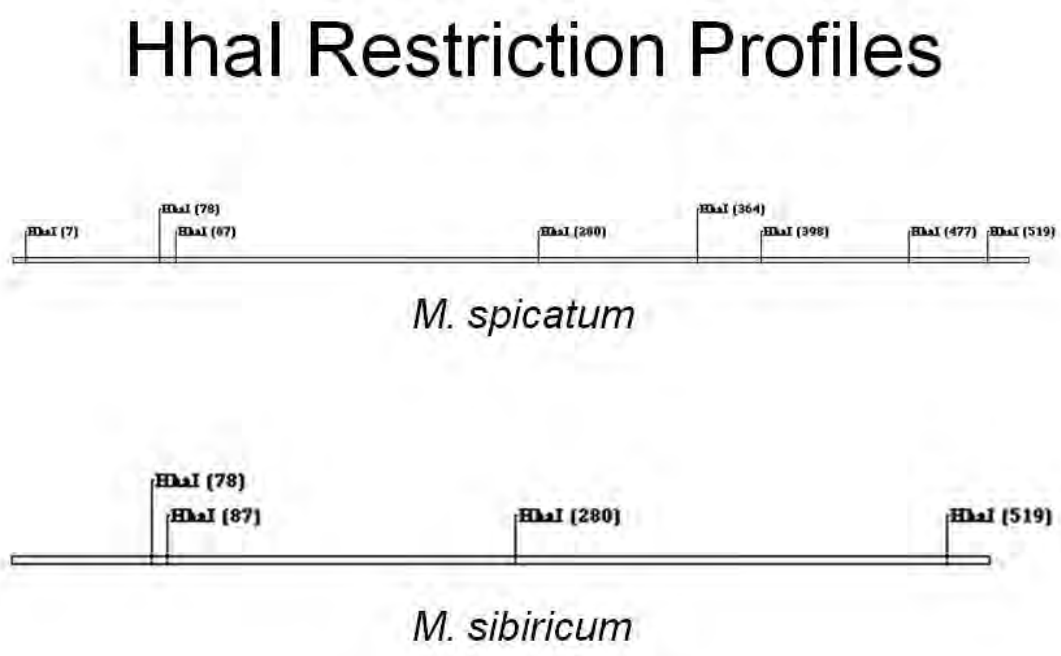

Figure 6. *Hhal* restriction profiles for Eurasian watermilfoil (top) and northern watermilfoil (bottom).

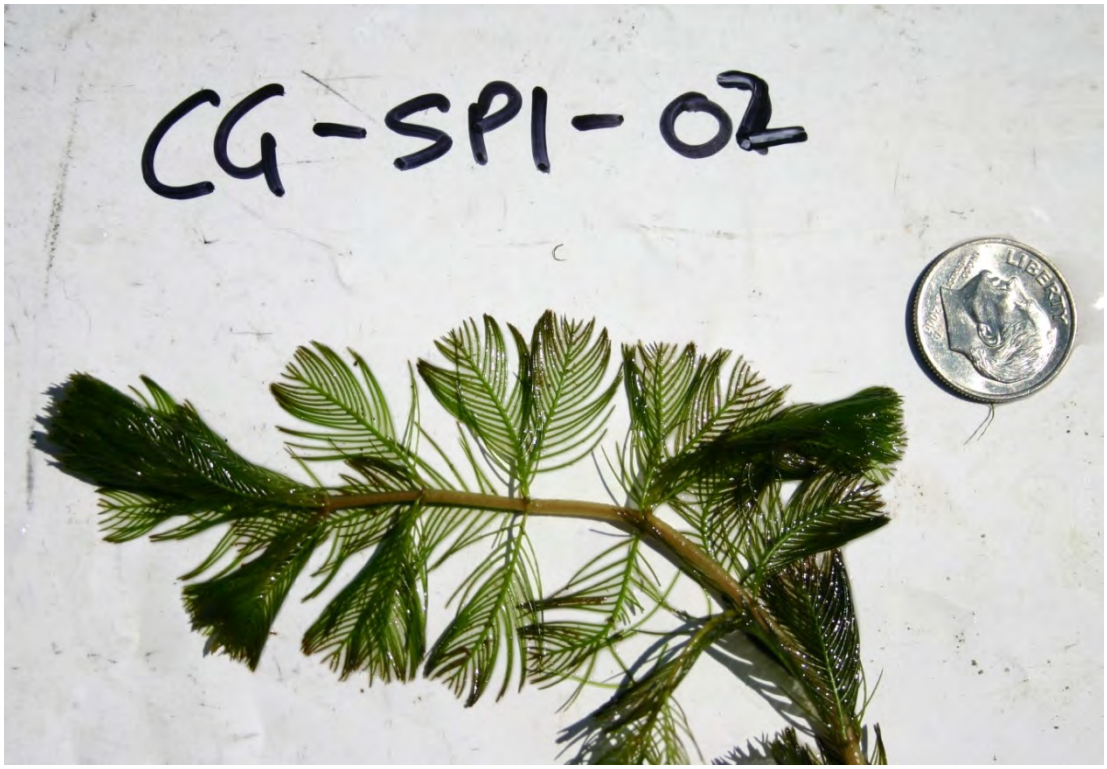

Figure 7. Eurasian watermilfoil specimen from Cabinet Gorge Reservoir. Leaf tips are flattened, stem color is reddish. Apical meristem is flattened. Leaves have approximately sixteen pairs of leaflets.

Figure 8. Northern watermilfoil specimen From Pend Oreille River displaying rounded leaf tips, rounded apical meristem, and greenish stem color. Leaves have approximately eight pairs of leaflets.

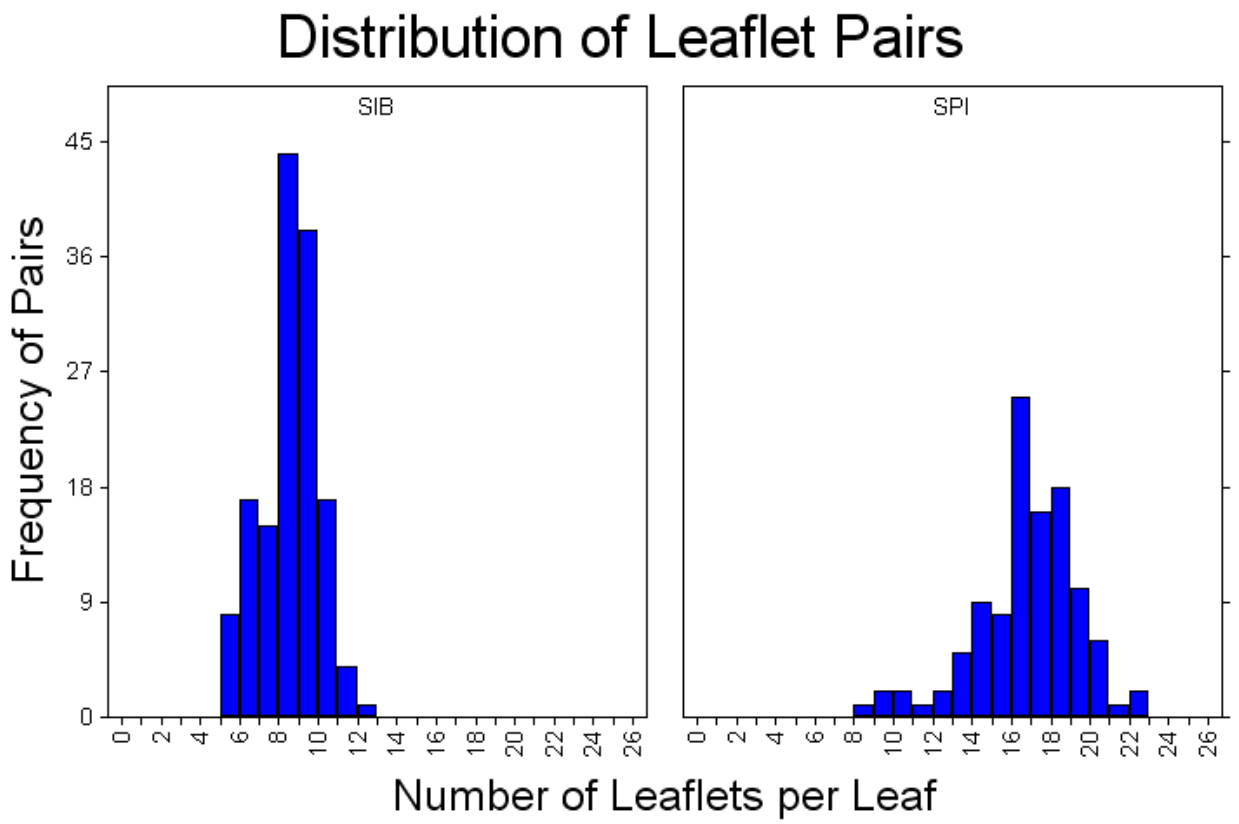

Figure 9. Frequency of number of leaflet pairs per leaf for northern watermilfoil (SIB, left) and Eurasian watermilfoil (SPI, right).

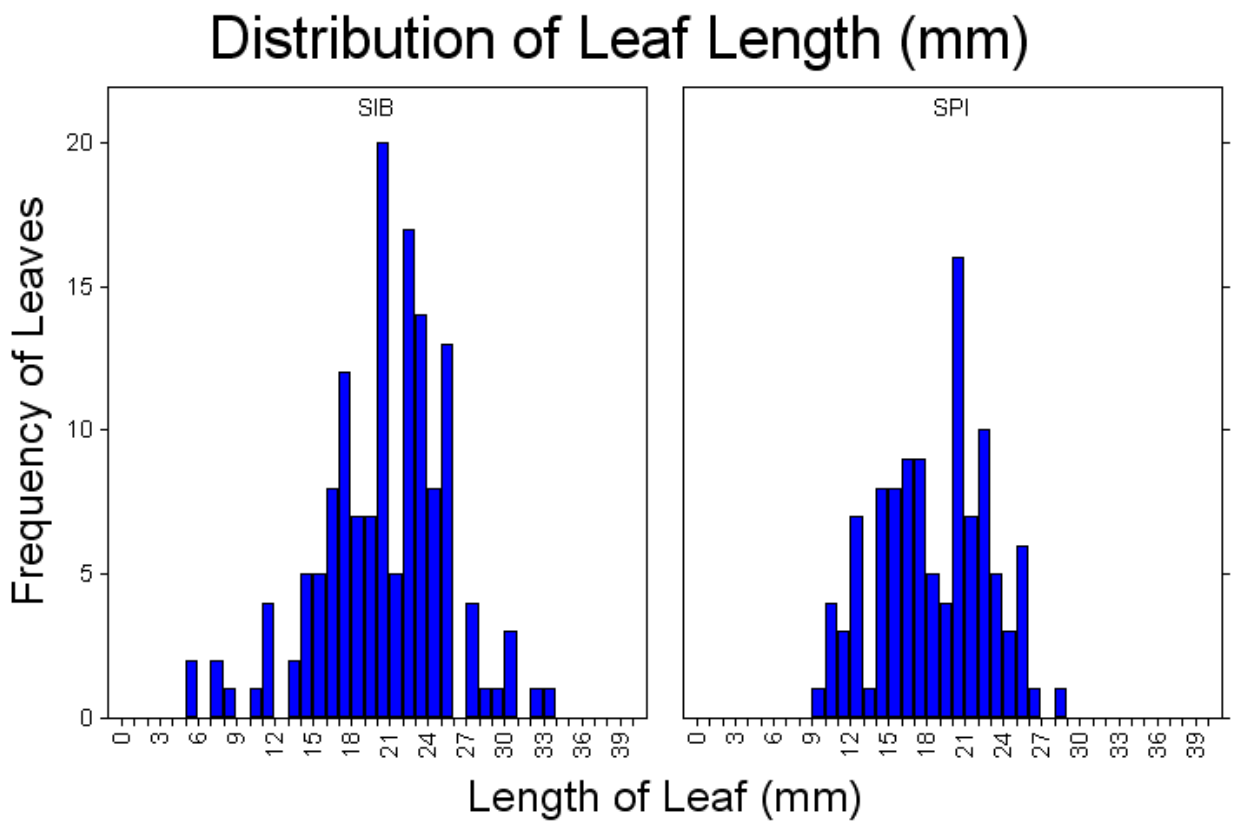

Figure 10. Distribution of leaf lengths (in mm) for northern watermilfoil (SIB, left) and Eurasian watermilfoil (SPI, right).

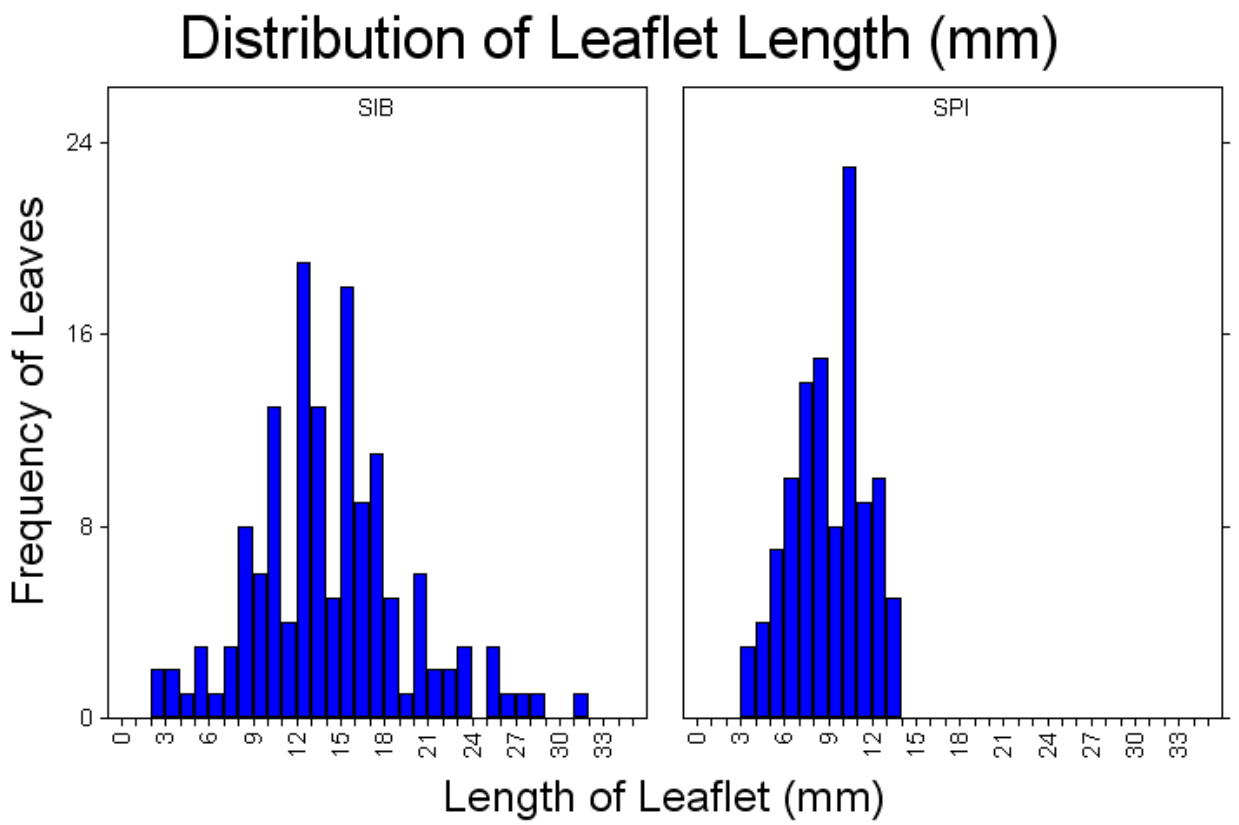

Figure 11. Frequency of the length of leaflets on leaves of northern watermilfoil (SIB, left) and Eurasian watermilfoil (SPI, right).

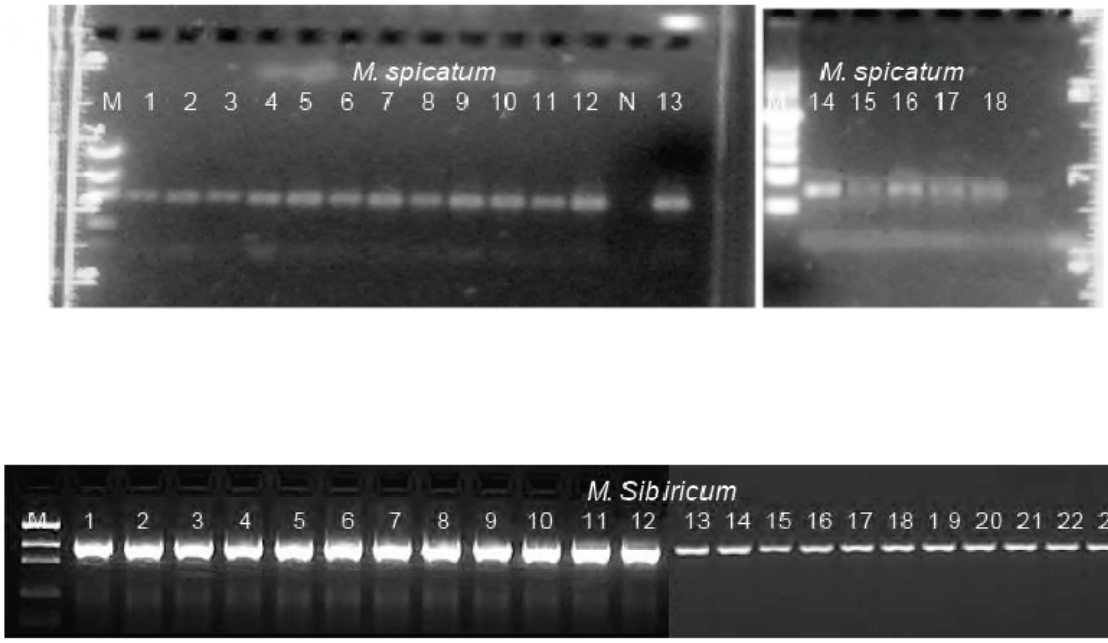

Figure 12. Agarose gel electrophoresis of the PCR products of the ITS region of *M. spicatum* and *M. sibiricum* amplified with primer pair IT4/ITS5. M=molecular marker, N= negative control. Analysis by V. Phuntumart, Bowling Green University.

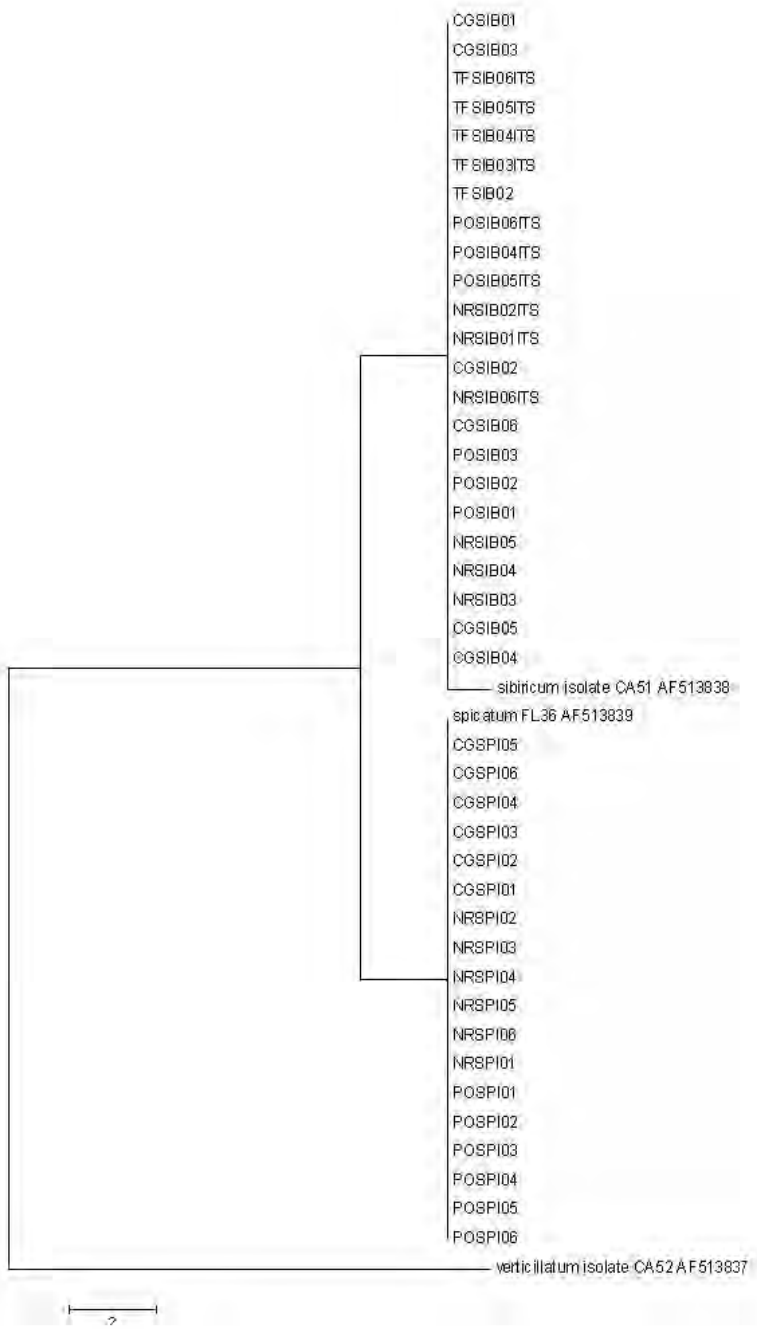

Figure 13. Phylogenetic comparison of samples to GenBank ITS DNA sequence data (from Moody and Les 2002). All individuals identified as *M. spicatum* (SPI) group with the GenBank spicatum and all individuals identified as *M. sibiricum* (SIB) group with GenBank sibiricum. Phylogeny constructed in MEGA 4.0 using the neighbor-joining algorithm and number of nucleotide differences in a 354 bp alignment of ITS. Analysis by R. Thum, Grand Valley State University.

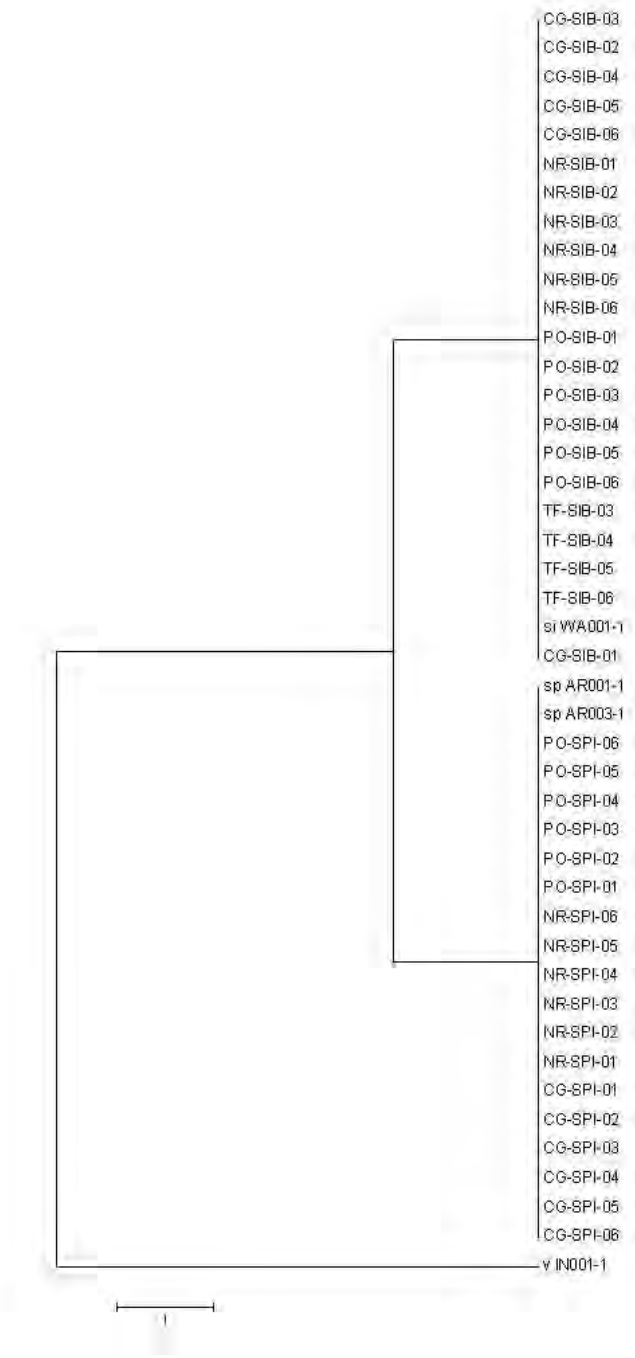

Figure 14. Phylogenetic comparison of samples to trnL DNA sequence data (from R. Thum's collections). All individuals identified as *M. spicatum* (SPI) group with the other spicatum and all individuals identified as *M. sibiricum* (SIB) group with other sibiricum. Phylogeny constructed in MEGA 4.0 using the neighbor-joining algorithm and number of nucleotide differences in a 955 bp alignment of trnL. Analysis by R. Thum, Grand Valley State University.

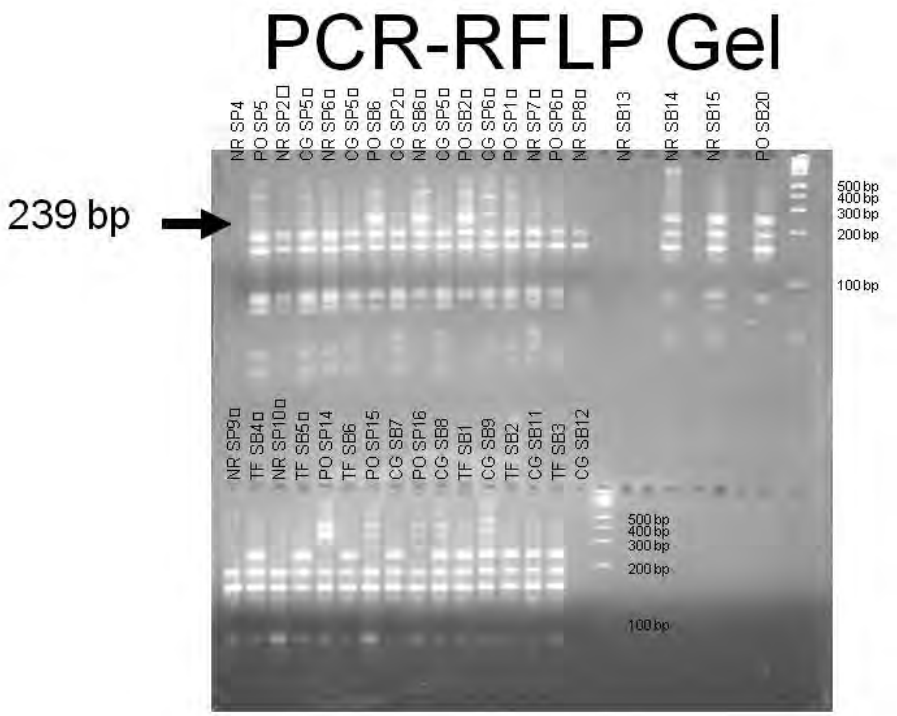

Figure 15. PCR-RFLP of the *M. sibiricum* (SB) and *M. spicatum* (SP) samples from the Lower Clark Fork River system. Analyses by M. Welch, Mississippi State University.