



## Chloroplast DNA Sequencing Reveals the Presence of Two Exotic *Phragmites* Haplotypes in Northeast Wisconsin

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### Introduction

*Phragmites australis*, known as common reed, is an invasive reed grass found across much of North America north of Mexico (Colin and Eguiarte 2016). In Wisconsin, notably along Green Bay and Lake Michigan (Tulbure et al. 2007), invasive *Phragmites* overtakes valuable wetlands, reducing native wetland biodiversity, especially along major rivers, shorelines, and Great Lakes coastal areas. At least 14 haplotypes based on sequences of two non-coding chloroplast loci (*trnT-trnL* and *rbcL-psaI*) have been described from native North American *Phragmites* populations, with haplotype E being the most common and widespread throughout North America, including Wisconsin and the Great Lakes region (Saltonstall 2002, Meadows and Saltonstall 2007; Lambert et al. 2016). Invasive haplotype M is the most prevalent introduced haplotype in North America including the Great Lakes (Saltonstall 2002, 2003).

While invasive haplotype M has been documented across much of North America (Saltonstall 2002, 2003), substantial evidence now supports the hypothesis of multiple introductions of invasive *Phragmites* from Europe and Asia into North America. Secondary and multiple introductions of invasive species such as *Phragmites* provide increased opportunities for hybridization with native populations, which can improve the invasiveness of a plant (Ellstrand and Schierenbeck 2006).

Since *Phragmites* populations in Wisconsin have been largely uncharacterized by chloroplast haplotype, we sought to provide a more detailed study of *Phragmites* populations in Northeast Wisconsin. The objective of our study was to test the hypothesis of multiple introductions of invasive *Phragmites* in the Great Lakes by evaluating chloroplast haplotypes in populations of *Phragmites* in Northeast Wisconsin (Figure 2).

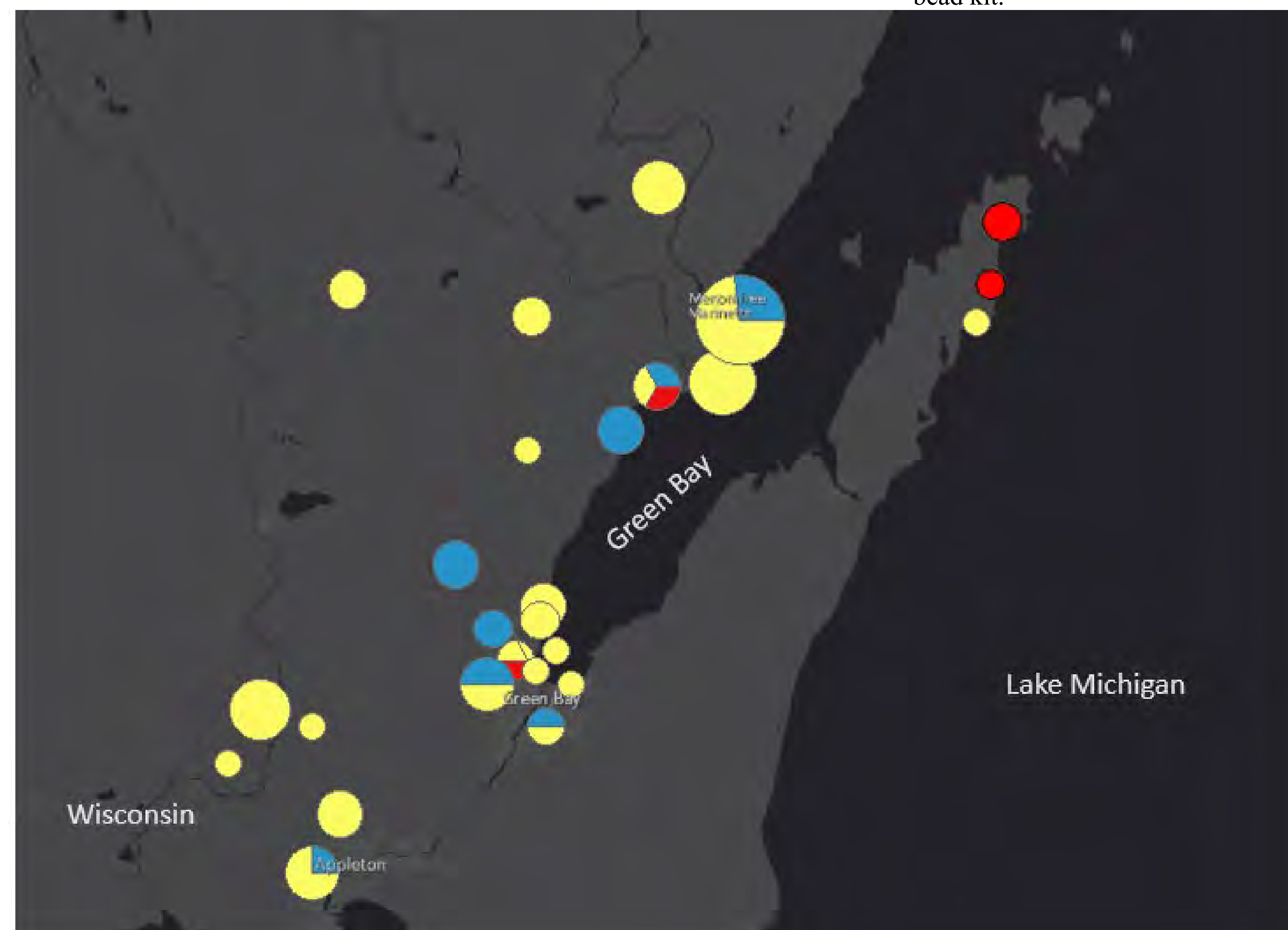
### Materials & Methods

*Phragmites* leaves were collected as part of a larger study investigating the impact of chemical treatment of *Phragmites* on wetland restoration. Leaves were collected from transects monitored at inland and coastal sites in Brown, Oconto, Marinette, Door, and Outagamie Counties (Figure 2) in July and August of 2017 and 2018. Leaves were dried at 4°C in Ziploc bags using silica beads. Dried samples were portioned out into 10 milligram samples from which chloroplast DNA was isolated using a MagJET Genomic DNA Kit (Fischer Thermo Scientific, Waltham, MA, USA) using the manufacturer's protocol. Two noncoding chloroplast loci (*trnT-trnL* and *rbcL-psaI*) were amplified by polymerase chain reaction (PCR) following Saltonstall (2002). Sanger sequencing of cleaned PCR products was conducted at the University of Kentucky HealthCare Genomics Core Laboratory. Electropherograms were reviewed and consensus sequences of forward and reverse sequences were assembled using Geneious version R8.1 (Biomatters Inc., Newark, New Jersey, USA). Consensus sequences were run through BLAST to assess similarity to Genbank *Phragmites trnT-trnL* and *rbcL-psaI* sequences. Naming of haplotypes followed Lambertini et al. (2012) and Saltonstall (2016).

### Results



**Figure 1:** Project overview and data collection. (A & B) Field data collection featuring research assistants Jennifer Bahling and Rebecca Malcore, Dr. Lisa Grubisha, and ecologist Bobbie Webster. (C) A typical stand of native *Phragmites*. (D & E) Rebecca Malcore performing chloroplast DNA isolations using a magnetic bead kit.



**Figure 2:** Distribution of *Phragmites* haplotypes in Northeast Wisconsin. Pie charts are sized proportionate to the number of leaf samples collected from each site (ranging from 1 to 11). Yellow indicates exotic haplotype M, blue indicates exotic haplotype O, and red indicates native haplotype E.

### Conclusions

- ❖ We report the first widespread records of exotic haplotype O in Wisconsin, and the Great Lakes, from eight different inland and coastal sites monitored in the 2017 and 2018 field seasons.
- ❖ The pervasive North American exotic haplotype M was common at both inland and coastal plots.
- ❖ Exotic haplotype O was found in the same transect as the other exotic haplotype M or native haplotype E in five of the eight transects it was identified in.
- ❖ These results have implications for land managers as hybridization between both native (E) and invasive (M, O) and invasive M with invasive O haplotypes may result in increased invasive ability or have other ecological impacts to Wisconsin wetlands.
- ❖ Further research is needed to determine if these two haplotypes have distinct phenotypic differences (i.e., Inflorescence height) that could be used for field identification.
- ❖ We are currently evaluating these plants for potential hybridization (exotic-exotic, exotic-native) using seven simple sequence repeat loci (SSRs).

### Literature Cited

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### Acknowledgements/Sponsorship

This study was supported by a University of Wisconsin – Green Bay Department of Natural and Applied Sciences Heirloom Plant Undergraduate Research Grant to R.M.M. and a Wisconsin Department of Natural Resources Aquatic Invasive Species Research & Development Grant AIRD11117 to R.W.H. and B.J.W.