Beyond what meets the eye:
DNA barcoding and aquatic plant identification

Ryan A. Thum, Gregory M. Chorak, Del Hannay, Zachary J. Kuzniar, and Ashley L. Wolfe

Successful aquatic plant management depends on the correct identification of the species slated for management. However, even for experienced botanists, aquatic plants can be difficult to identify based on appearance alone. Aquatic plants are especially prone to identification problems because many unrelated species have evolved similar growth forms. Whorls of finely divided leaves, for example, can be found in watermilfoils, hydrilla, coontails, and bladderworts, making reliable identification difficult. Further, introduced and invasive species can have closely related native species that can be difficult to distinguish based on morphology alone.

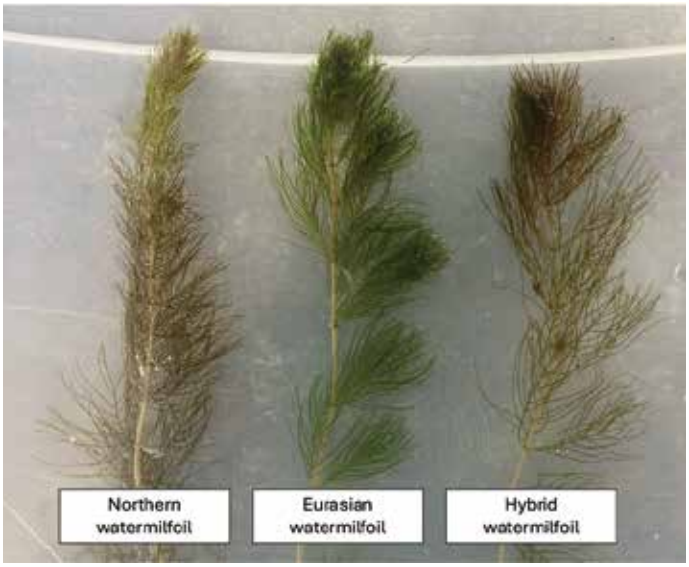


Figure 1. Visual comparison of (left to right) Northern, Eurasian, and hybrid watermilfoil. Photo courtesy of Hannah Hoff.

For example, several recently discovered invasive eelgrass species in the United States that are morphologically similar to ecologically important native species, *Vallisneria americana* and *V. neotropicalis*, highlights the critical need for additional species identification tools. Similarly, invasive Eurasian and hybrid watermilfoil are difficult to distinguish from native northern watermilfoil (Figure 1). Finally, even for morphologically distinct species, diagnostic characters such as flowers or fruits may be missing during life stages or times of the year, making visual identifications challenging.

The consequences of misidentification can be costly. On the one hand, misidentifying an invasive species as a native may fail to control a population that could cause economic or environmental harm locally and spread to other lakes. On the other hand, misidentifying a native species as an invasive species may result in unnecessary economic and environmental costs.

While traditional identification methods based on morphology, ecology, and the phenology of species remain valuable tools, the limitations described above have resulted in increasing interest in using DNA barcoding as an identification tool for aquatic plants. Understanding how DNA barcoding works, as well as its limitations, will allow users to develop and employ this technology to inform management decisions.

What is DNA barcoding?

DNA barcoding gets its name from analogy to how a barcode scanner matches a product at the grocery store to an inventory database. Using a short, standardized region of DNA, researchers can compare the DNA sequence of an unknown specimen to reference sequences from known species. Rather than a pattern of black and white bars, a DNA barcode consists of a short sequence of the four DNA building blocks – adenine (A), thymine (T), cytosine (C), and guanine (G). Barcode regions are selected because they can be compared across many species while still containing enough variation to distinguish among them.

Once a sample has been collected, DNA barcoding typically involves four steps: (1) DNA is extracted from the specimen, (2) a barcode region is amplified, (3) the amplified DNA is sequenced, and (4) the resulting sequence is compared to a reference database of known species (Figure 2). When a strong match is found, the unknown specimen can confidently be identified to species.

DNA barcoding has become a widely used tool for species identification because it can be applied to specimens that are difficult to identify using traditional morphological characteristics, including species that appear very similar to one another, or specimens that lack typical diagnostic characteristics such as flowers or fruits. Because many aquatic plant species look similar, and because they often reproduce primarily through vegetative propagation, DNA barcoding is an attractive method for aquatic plant identification. While DNA barcoding can be a powerful tool for species identification, the approach also has important limitations that users should understand.

Limitations and misconceptions of barcoding

One common misconception is that every species has a unique DNA bar-

code and that sequencing a barcode gene will automatically identify an unknown organism. While barcodes are often useful for species identification, successful DNA barcoding depends on both the barcode itself and the availability of reference sequences for comparison. Some genes are reliable barcodes for some groups of organisms, but not for others. Researchers build reference databases by sequencing barcode genes from many correctly identified specimens. These databases allow unknown samples to be compared against a catalog of known species. Then, they compare unknown samples to those records. Aquatic plants are underrepresented in many genetic databases compared to other groups of plants and animals. If a species is missing from the reference database, or if the diversity within that species has not been thoroughly documented, identification may be uncertain. This is similar to scanning a product barcode that does not exist in the store inventory system – the barcode is present, but there is no reference available to interpret it.

Another challenge is that species do not always possess unique barcode sequences. Closely related species may share identical DNA barcode sequences because they diverged relatively recently in evolutionary time. In addition, there may be genetic variation at the barcoding gene among individuals of the same species, and so an individual that is not an exact match to a sequence in the reference database may be uncertain as to whether it is a member of a closely related species or a species that has not been cataloged in the reference database. For these reasons, researchers often need to understand how much variation exists both within the target species and among its closest relatives before a barcode can be used confidently for species identification.

Hybridization can further complicate species identification. Many aquatic plants readily hybridize, creating offspring that contain genetic material from multiple species. When researchers examine many genes across the genome, these hybrid relationships can often be detected. However, DNA barcoding typically relies on a single gene. As a result, a hybrid individual may possess a barcode sequence inherited from one parent, while much of the rest of its genome comes from another species. In these cases, DNA barcoding may provide an incomplete picture of an organism's ancestry. This is especially relevant to aquatic plant management when there is hybridization between native and introduced species (e.g., between native northern watermilfoil and invasive Eurasian watermilfoil).

Finally, while correct species identification is essential for determining whether a population should be managed or preserved, that information alone may not be sufficient to determine the specific management strategies or actions. Individuals within a species can differ in traits that are important for management, including growth characteristics and susceptibility to specific control measures. For example, DNA barcodes for species like Eurasian watermilfoil and hydrilla alone would not distinguish known herbicide resistant versus susceptible varieties, which require more extensive genetic research and tools.

Looking ahead

DNA barcoding is helping transform aquatic plant identification from a process that relies on increasingly scarce, specialized taxonomic expertise to one that also incorporates standardized genetic evidence that can be reproduced, shared, and expanded as reference libraries continue to grow. As reference databases grow and additional barcode markers are developed, these tools will become increasingly useful for researchers and lake managers alike. While DNA barcoding is not a replacement for botanical expertise, it is an important

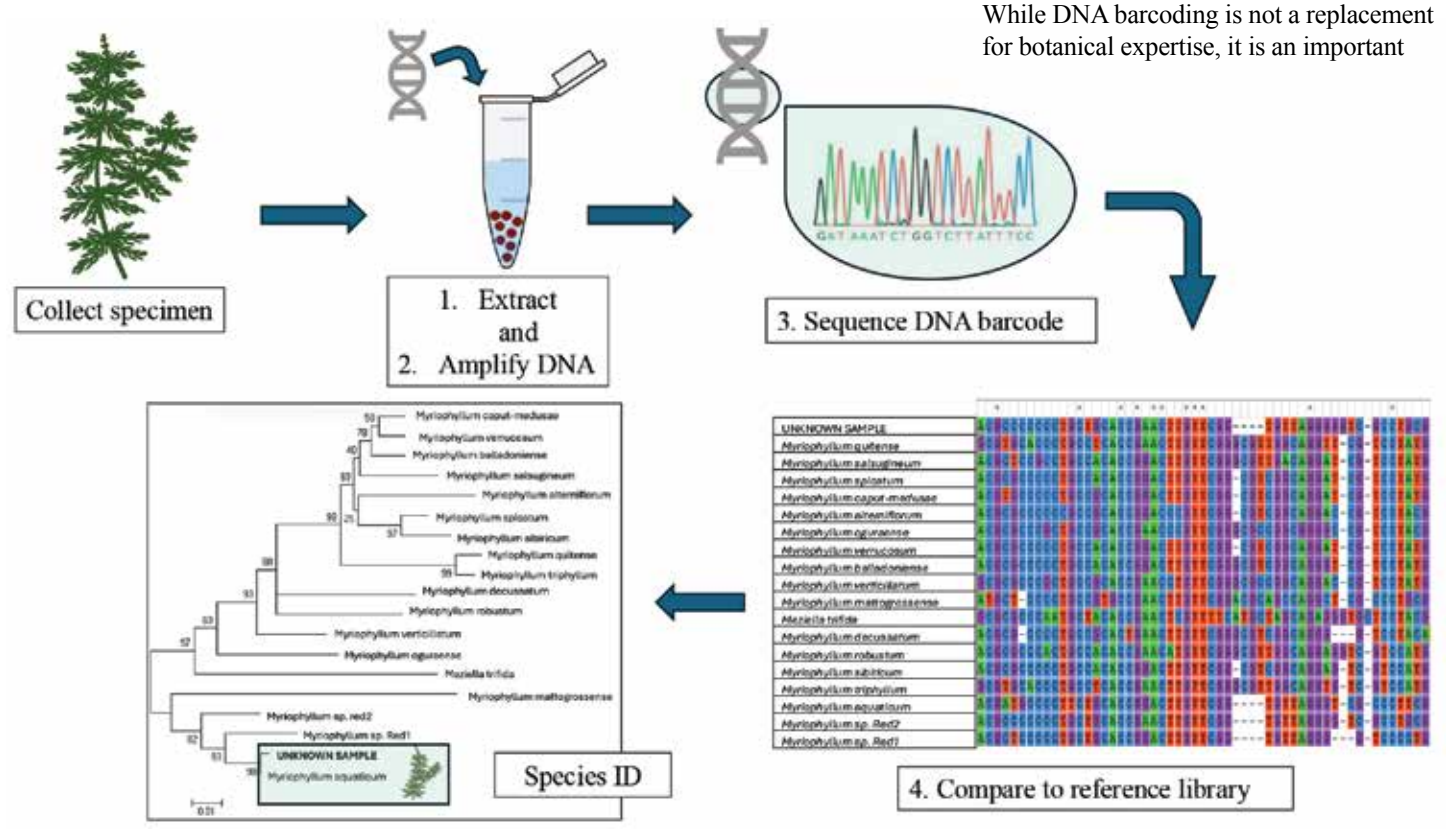


Figure 2. How DNA barcoding works.

addition to the aquatic plant management toolbox. Continued investment in developing and validating barcode resources for aquatic plants will improve species identification, support more informed management decisions, and help protect aquatic ecosystems.

Ryan Thum is a professor of ecological genetics and genomics of aquatic and invasive plants. His general fields of interest encompass evolutionary and molecular ecology, with a specific emphasis on invasive aquatic species. His research is rooted in conceptual evolutionary ecology, but aims to contribute conceptual knowledge and understanding to guide practical decision making and solutions regarding invasive species – one of the world's most pressing environmental problems. Most of his research is organized around the simple idea of how genetic variation influences invasive plant management outcomes.

Gregory M. Chorak, PhD, is research faculty in the Department of Plant Sciences and Plant Pathology at Montana State University. His research focuses on the genetics, genomics, and identification of aquatic plants, with an emphasis on invasive species, hybridization, and the application of molecular tools to support aquatic plant management.

Del Hannay is a graduate research assistant in Dr. Ryan Thum's lab at Montana State University. Their work focuses on the genetic basis of adaptation to herbicide treatment in invasive Eurasian watermilfoil. Broadly, their research interests include answering questions on what patterns of genetic diversity exist within and among species, and how this diversity contributes to adaptation. They can be contacted at del.hannay@student.montana.edu.

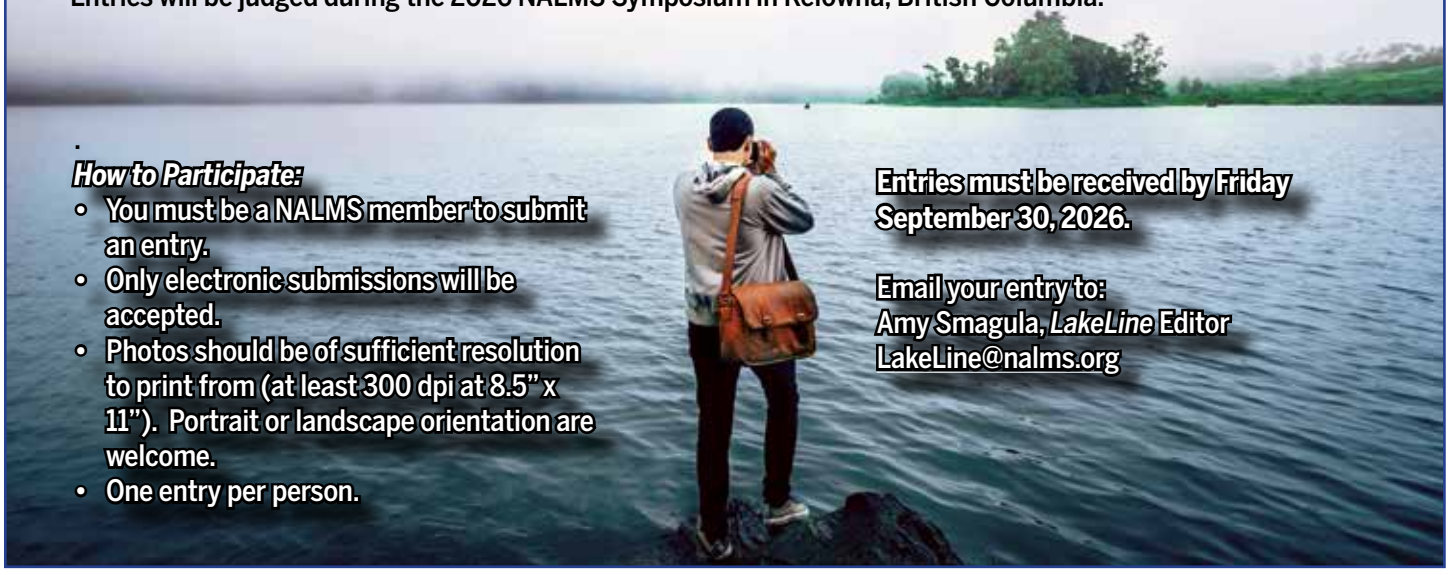
Zachary J. Kuzniar was an invasive plant scientist based in Boise, Idaho. He conducts research at Montana State University on the genetic diversity of aquatic plants and how those findings inform management strategies. He also works as a technical sales representative for Cygnet Enterprises, specializing in herbicide-based management of lakes, ponds, and irrigation canals.

Ashley Wolfe received both her BS and MS from Montana State University. She completed her master's degree in Plant Science in Dr. Thum's lab in 2024, during which she developed MilfoilMapper, an interactive database designed to support watermilfoil research and management. Ashley continues her work in Dr. Thum's lab, now serving as a research associate. Her research focuses on exploring the variation in Eurasian watermilfoil strain response to the herbicide florpyrauxifen-benzyl, as well as performing genetic identifications of various invasive aquatic plant species.

YOU could be the winner of the 2026 NALMS Annual Photo Contest!

Two winning images will be selected, a Member's Choice winner selected by Symposium attendees and an Editors' Choice winner selected by the editor and production editor for the entry that will make the best *LakeLine* cover. Prizes will be awarded to the contest winners, and your favorite lake or reservoir photo could grace a cover of *LakeLine*!

Entries will be judged during the 2026 NALMS Symposium in Kelowna, British Columbia.



How to Participate:

- **You must be a NALMS member to submit an entry.**
- **Only electronic submissions will be accepted.**
- **Photos should be of sufficient resolution to print from (at least 300 dpi at 8.5" x 11"). Portrait or landscape orientation are welcome.**
- **One entry per person.**

Entries must be received by Friday September 30, 2026.

Email your entry to:
Amy Smagula, *LakeLine* Editor
LakeLine@nalms.org