POTAMOGETON WRIGHTII (POTAMOGETONACEAE) NATURALIZED IN OHIO, U.S.A.

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ABSTRACT

In 2021, an unknown *Potamogeton* species was discovered in Alum Creek Lake in central Ohio. After we were unable to identify the plants as any known North American species, we expanded our search to include all pondweed species and incorporated molecular data to aid in the identification. DNA sequences were obtained for the nuclear internal transcribed spacer (ITS) region and the plastid *trnT-trnF* region and compared against previously reported sequences for *Potamogeton* species. Morphological and molecular data consistently identified the plants as *P. wrightii*, a species native to eastern Eurasia and Malesia and not previously reported to be naturalized in North America.

RESUMEN

En 2021, se descubrió una especie desconocida de *Potamogeton* en el lago Alum Creek en el centro de Ohio. Después de no poder identificar las plantas como ninguna especie conocida de América del Norte, ampliamos nuestra búsqueda para incluir todas las especies de espiga de agua e incorporamos datos moleculares para ayudar en la identificación. Se obtuvieron secuencias de ADN para la región nuclear espaciadora transcrita interna (ITS) y la región *trnT-trnF* del plástido y se compararon con secuencias informadas previamente para especies de *Potamogeton*. Los datos morfológicos y moleculares identificaron consistentemente las plantas como *P. wrightii*, una especie nativa del este de Eurasia y Malesia y de la que no se había informado previamente que estuviera naturalizada en América del Norte.

INTRODUCTION

Pondweeds (genus *Potamogeton* L.) are among the most widespread and diverse aquatic plants (Haynes & Hellquist 2000; Crow & Hellquist 2023). Species are predominantly submersed, although many have floating leaves, and pollination predominantly happens above water. There are 30 species native to temperate North America (Haynes & Hellquist 2000; Lindqvist et al. 2006; Les et al. 2009; POWO 2024), as well as one invasive species, *P. crispus* L. (curly-leaf pondweed) (Thayer et al. 2024). The northeastern U.S.A., ranging from approximately Illinois to Maine, boasts the greatest density of species, with 26 species native to that region (Crow & Hellquist 2023). In Ohio, U.S.A., there are 20 *Potamogeton* species, including the invasive *P. crispus* (Kartesz 2018).

There is a steady potential for exotic species to be newly introduced into North America (Les & Mehrhoff 1999), and aquatic plants are among the most impactful invaders (Macêdo et al. 2024). Additionally, many exotic aquatic species have been imported already and persist in the hands of private individuals or commercial sources (Maki & Galatowitsch 2004). Indeed, most aquatic invasive plants originally were introduced through the intentional or unintentional release of cultivated plants into natural habitats (Les & Mehrhoff 1999). Not all nonindigenous plants released into natural habitats become invasive, but these plants have the potential to become troublesome invasive weeds (Jeschke & Pyšek 2018). Among the most notorious and costly invasive species in the U.S.A. are several aquatic plants, such as *Hydrilla verticillata* (L.f.) Royle, *Myriophyllum spicatum* L., and *Pontederia crassipes* Mart. (Pimentel et al. 2000; Macêdo et al. 2024).

Novel invasive plants often go undetected for ten years or more before a taxonomic expert confirms that they are indeed a new element in the flora (e.g., Knowlton 1923; Fernald 1932; Weatherby 1932; Les & Mehrhoff 1999). It is important to make an initial identification quickly, because the potential for controlling invasives erodes over time as the species become more widespread and entrenched (Alvarez & Solís 2018).

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Molecular tools are a valuable option to achieve the objective of rapid identification, and there are many examples of invasive species whose molecular identification helped them to be recognized as problematic (e.g., Saltonstall 2003; Les et al. 2006; Tippery et al. 2020a).

Beginning in 2021, an unknown pondweed species was discovered to be scattered throughout Alum Creek Lake in Delaware County, Ohio. Similar-sized patches were located again in 2022 and 2023. Alum Creek Lake (also known as Alum Creek Reservoir) is a mesotrophic artificial reservoir that was constructed in 1974 (USACE 2022, 2024). The lake has a surface area of approximately 1,370 ha (3,390 acres) and a maximum depth 20.4 m (67 ft) (USACE 2022, 2024). Morphological identification was inconclusive using keys for North American pondweeds (Haynes & Hellquist 2000; Crow & Hellquist 2023). Consequently, we expanded our search to include *Potamogeton* taxa worldwide, and we employed DNA sequencing technology to facilitate identification.

MATERIALS AND METHODS

Plants were collected several times over the years 2021–2023 during annual vegetation surveys. Surveys were conducted by boat, using an apparatus consisting of two hard garden rake heads clamped together and attached to a 15 m rope. Rake tosses were conducted in every inlet and bay. The overall meander survey was supplemented with targeted surveys at points of entry such as boat ramps, fishing docks, inflows, and outflows. The survey methods were similar to what has been described previously (Johnson & Newman 2011). All data on plant locations were collected through the Fulcrum data collection app (Spatial Networks, Inc., San Francisco, California, U.S.A.). Morphological features were documented, and a preliminary morphological identification was made using available guides to North American plants (Haynes & Hellquist 2010; Crow & Hellquist 2023).

Molecular identification was conducted using plants that were collected on 27 Sep 2023 (*Warman s.n.*). A voucher specimen was deposited in the UW-Whitewater herbarium (UWW). DNA was extracted using the CTAB method (Doyle & Doyle 1987), modified as described by Tippery et al. (2020b). The ITS region (using the p5F, p3F, p2R, and p4R primers, Baldwin 1992; Cheng et al. 2016) and the *trnT-trnF* spacer (using a, b, c, d, e, and f primers, Taberlet et al. 1991) were amplified using polymerase chain reaction (PCR) with the Phire Hot Start II DNA Polymerase (Thermo Fisher Scientific, Waltham, Massachusetts, U.S.A.). PCR products were cleaned enzymatically using the *ExoI* and *FastAP* enzymes (Thermo Fisher Scientific). Sanger sequencing (Sanger et al. 1977) used the same primers that were used for PCR reactions and was conducted through Eurofins Genomics LLC (Louisville, Kentucky, U.S.A.).

Genetic similarity to previously reported DNA sequences was assessed initially by conducting a BLAST search with the newly obtained sequences (Altschul et al. 1990; Johnson et al. 2008). Additionally, the new sequences were combined with a set of reference sequences for *Potamogeton* species that were downloaded from GenBank (Benson et al. 2012; https://www.ncbi.nlm.nih.gov/genbank/). The sequences were aligned manually in Mesquite ver. 3.81 (Maddison & Maddison 2023). Phylogenetic analyses were conducted using IQ-TREE ver. 2.0.5 (Nguyen et al. 2015; Minh et al. 2020) with 1,000 ultrafast bootstrap replicates (Minh et al. 2013). Phylogenetic trees were rooted with *Stuckenia* Börner as the outgroup, then trimmed to depict a smaller number of taxa that were most related to the unknown species.

RESULTS

The unknown plants were located in shallow water (1–2 m), scattered in sheltered or exposed areas along the main channel (Fig. 1). Other shallow habitats in Alum Creek Lake were dominated by macrophytes including brittle naiad (*Najas minor* All.), *Hydrilla verticillata* (L.f.) Royle, and coontail (*Ceratophyllum demersum* L.). The unknown *Potamogeton* plants appeared in distinct clusters among other vegetation, most commonly in habitats with sago pondweed (*Stuckenia pectinata* (L.) Börner).

Morphologically, the unknown plants shared several features with the native *P. illinoensis* Morong and the non-native *P. crispus*, including leaves with undulate margins (Fig. 2). However, the unknown plants differed

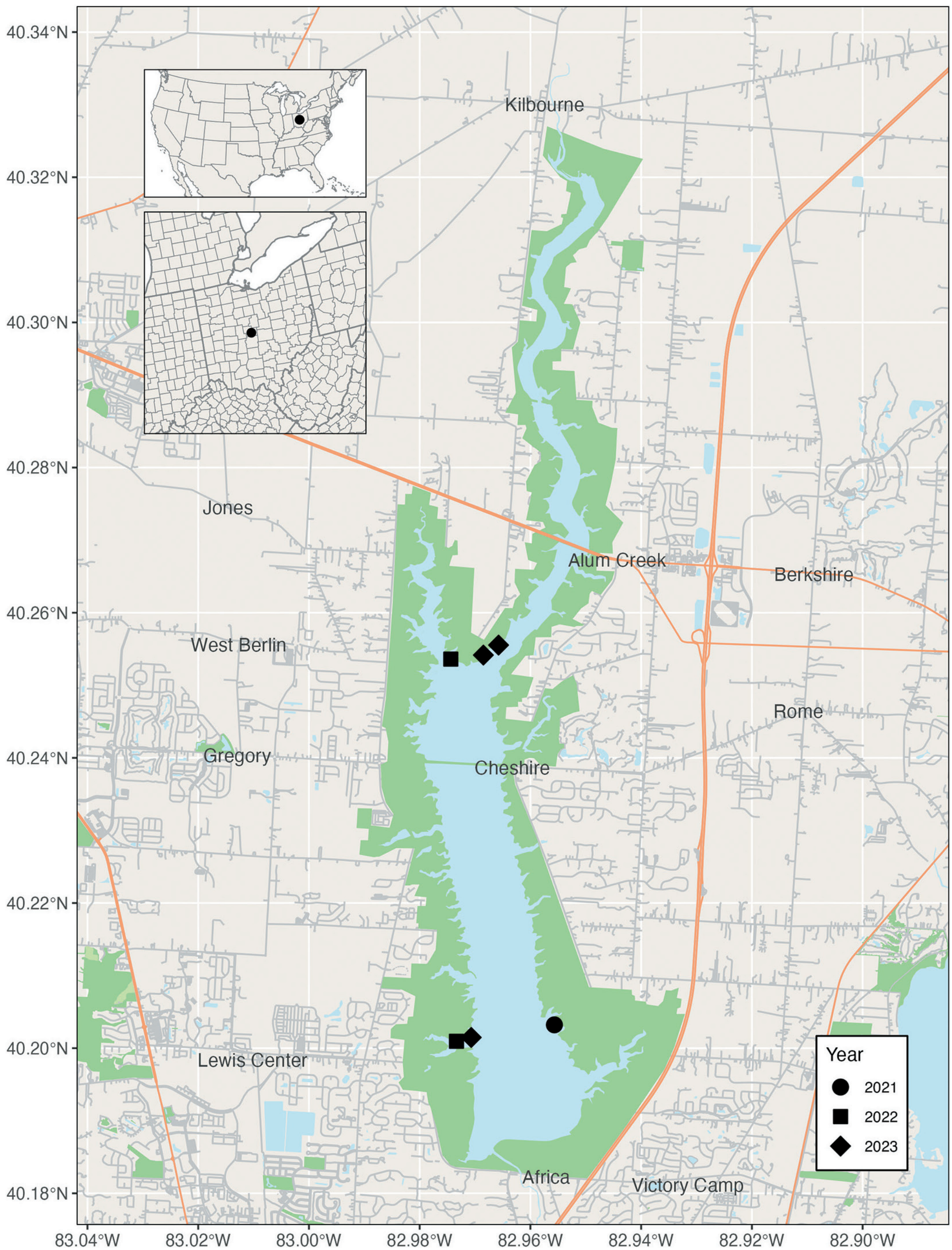

Fig. 1. Map of Alum Creek Lake and surrounding area, showing sites where *P. wrightii* was located during surveys over three years. Inset maps show the location of Alum Creek Lake in the U.S.A. and Ohio.

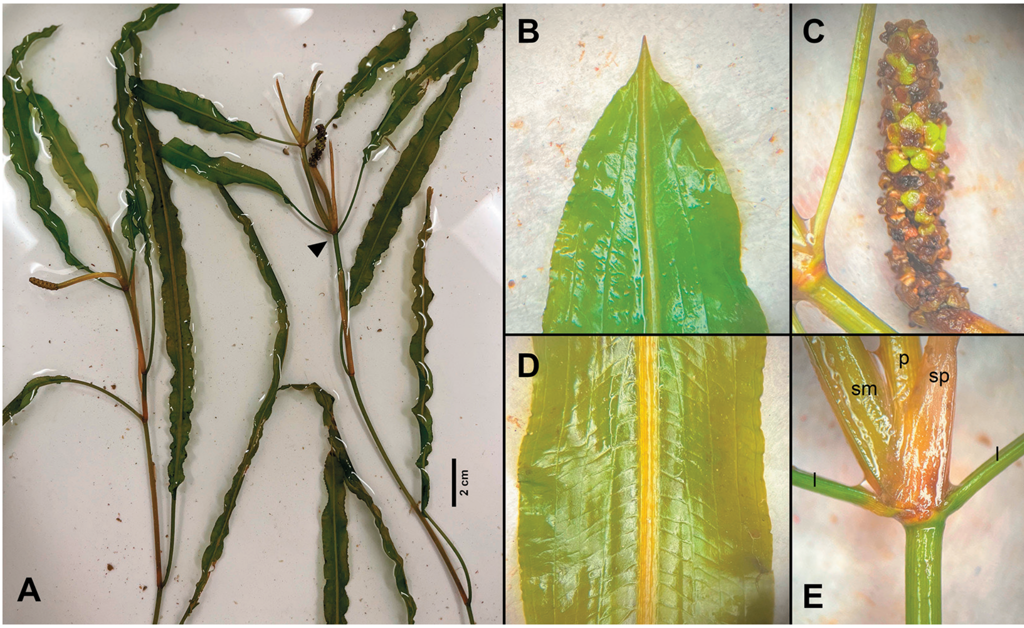

Fig. 2. Morphological features of the Alum Creek Lake plants. A. Shoot portions, showing vegetative and reproductive features. The arrow shows the region that is highlighted in E. **B.** Leaf apex. **C.** Infructescence. **D.** Leaf blade approximately at the midpoint. **E.** Reproductive node, bearing two opposite leaves (l), continuation of the stem (sm), which is covered by the stipule of the left-hand leaf, peduncle (p), and free stipule (sp).

from *P. crispus* by having petioles > 1 cm long (vs. sessile leaves in *P. crispus*), leaf blades exceeding 8 cm (< 8 cm in *P. crispus*), and opposite phyllotaxis at reproductive nodes (consistently alternate phyllotaxis in *P. crispus*) (Guo et al. 2010; Fig. 2). The unknown plants were similar to *P. illinoensis* in having petiolate submersed leaves with apiculate or mucronate apices, but they differed in having a denticulate margin (vs. entire in *P. illinoensis*), and the leaves of the unknown plants were consistently petiolate, whereas the leaves of *P. illinoensis* frequently are sessile (Haynes & Hellquist 2000; Crow & Hellquist 2023). Reevaluation of the plant specimens using the Flora of China floristic treatment (Guo & Li 1992; Guo et al. 2010) identified them as *P. wrightii* Morong, native to eastern Eurasia and Malesia (POWO 2024). Plants were observed to produce seeds, although seed viability and germination were not investigated.

We obtained DNA sequences for the unknown Alum Creek Lake plant that were 100% identical to previously published sequences for *P. wrightii* for the ITS region (GenBank accession number LC464129; G. Fukuoka, Y. Tokuoka, and H. Hayakawa, unpublished) and the *trnT-trnF* region (plastid genome accession number OQ561453; Choi et al. 2023). Newly obtained sequences were deposited to GenBank under accession numbers PP341466 (ITS) and PP352072 (*trnT-trnF*).

Phylogenetic analysis also implicated *P. wrightii* as the most similar taxon to the Alum Creek Lake plant. The ITS phylogeny (Fig. 3, left) recovered a strongly supported clade that included two *P. wrightii* accessions, as well as two hybrid species that have *P. wrightii* as a parent: *P.* × *anguillanus* Koidz. (= *P. perfoliatus* L. × *P. wrightii*) and *P.* × *malainoides* Miki (= *P. distinctus* A. Benn. × *P. wrightii*) (Ito et al. 2014; Haraguchi & Hamaguchi 2020). Apart from the hybrids, the ITS clade containing *P. wrightii* was differentiated from all other species including *P. distinctus*. The *trnT-trnF* phylogeny (Fig. 3, right) showed several species to be close relatives of the Alum Creek Lake plant, including *P. wrightii* but also *P. distinctus* and *P. nodosus* Poir. Both phylogenies indicated that neither *P. crispus* nor *P. illinoensis* were closely related to the Alum Creek Lake plant (Fig. 3).

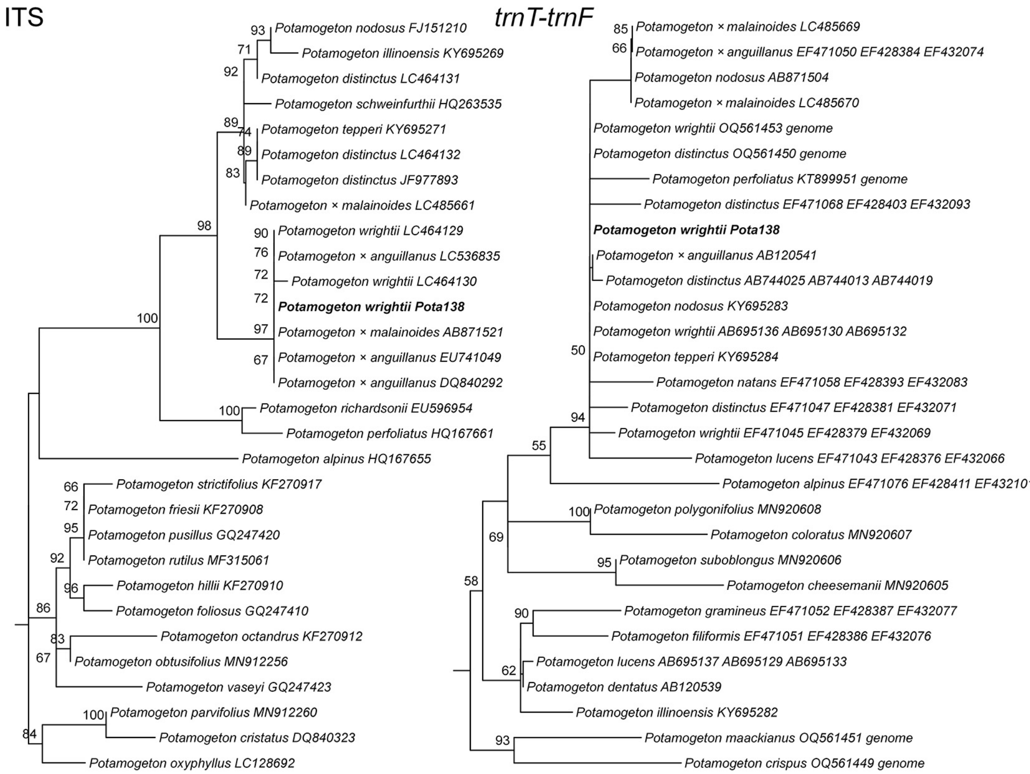

Fig. 3. Phylogeny of *Potamogeton wrightii* and related species, constructed using the nuclear internal transcribed spacer (ITS) (left) region (left) or the plastid *trnT-trnF* region (right). Species names are followed by their GenBank accession numbers. Bootstrap support values > 50% are shown at nodes. *Potamogeton crispus* was included in the ITS phylogenetic analysis but resolved to a position more distantly related than the other taxa shown. Sequences obtained from the newly collected plant (identified as 'Pota138') are highlighted in boldface.

DISCUSSION

Alum Creek Lake is a popular destination for fishing, boating, and other recreation (USACE 2024). The high level of human activity at such a destination approximately 32 km (20 mi) north of the Columbus, Ohio metropolitan center would be expected to increase the chance of depositing aquarium water containing *P. wrightii* plant or propagules, or if this species is already present and undetected in another lake, increase the chance of transferring the plants from one water body to another. Now that *P. wrightii* is known from Alum Creek Lake, recreation activities also present a secondary challenge, namely that this species could be transported to new water bodies in the area. The fact that *P. wrightii* exists across a wide latitudinal range in its native habitat might make it an effective invader across much of North America, and we recommend that the Alum Creek Lake plants should be eradicated as soon as possible.

The ITS region proved to be more effective than the *trnT-trnF* region for identifying the Alum Creek Lake plants. The relatively high sequence variability in ITS allowed for near (but inexact) matches to be ruled out more effectively, whereas on the *trnT-trnF* phylogeny there were insufficient sequence differences to rule out several species (e.g., *P. distinctus*, *P. nodosus*). Nevertheless, the combination of exact DNA sequence matches for both the ITS and *trnT-trnF* regions, combined with the morphological evidence, leave no doubt about the identification of the Alum Creek Lake plants as *P. wrightii*.

There has been some confusion regarding the correct application of names for eastern Eurasian pondweed species related to *P. wrightii*. For example, the name *P. malaianus* Miq. continues to be used by some authors, despite the fact that this name has been synonymized with *P. nodosus* (Wiegleb 1990; Guo et al. 2010). In some cases, the name "*P. malaianus*" has been misapplied to hybrids between *P. perfoliatus* and *P. wrightii* (e.g., Ito et al. 2014), but such plants are more correctly placed under the hybrid name *P.* × *anguillanus* (Iida et al. 2013). The orthographically similar name *P.* × *malainoides* refers to a hybrid between *P. distinctus* and *P. wrightii* (Ito et al. 2014). Another Eurasian species, *P. intortusifolius* J.B.He, L.Y.Zhou & H.Q.Wang, is considered by POWO (2024) to be a synonym of *P.* × *anguillanus*, whereas Guo et al. (2010) regard it to be synonymous with *P. wrightii*. The close molecular similarity of GenBank species corresponding to these taxa could indicate that their taxonomy has not been resolved conclusively and deserves further attention.

Potamogeton wrightii was not previously known to be invasive or even adventive outside of its native range (POWO 2024). It will be important to raise awareness about this species and its potential to become a new invasive plant in North America. The species likely is cultivated in aquaria, although there are few mentions of its commercial availability (e.g., https://www.flowgrow.de/db/aquaticplants/potamogeton-wrightii). A wide variety of aquatic plant species are available commercially and are obtained relatively easily (Maki & Galatowitsch 2004), and such plants can be introduced intentionally or unintentionally into natural habitats. Commercially available plants are frequently labeled or identified incorrectly, either to evade the detection of prohibited species, to increase the commercial appeal by applying a more attractive name, or simply through apathy or ignorance of correct nomenclature (Van den Neucker & Scheers 2022). Thus, it continues to be important to spread awareness about potential noxious weeds.

We found no evidence for hybridization, which would be expected to manifest as polymorphic DNA sequences for the nuclear ITS region (Moody & Les 2002; Wang et al. 2007; Kaplan et al. 2009; Ito et al. 2014). Nevertheless, the propensity for hybridization among pondweed species should inspire some caution, and we recommend continuous surveillance not only for *P. wrightii* plants but also for any natural hybrids that might form. Additionally, the discovery of *P. wrightii* should promote increased vigilance for detecting other populations of this species, which may have been misidentified as *P. crispus* or another species. Additional populations may have been unreported for lack of evidence, both morphological and genetic. The discovery of *Potamogeton wrightii* in Ohio highlights the importance of using DNA in plant surveys, and we are hopeful that the early detection of this species will enable it to be eradicated swiftly and relatively inexpensively.

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