

Molecular confirmation of hybridization with invasive curly-leaf pondweed (*Potamogeton crispus*) in the Sacramento–San Joaquin Delta, California

AJAY R. JONES AND RYAN A. THUM*

ABSTRACT

Weed managers recognize that hybridization can influence invasiveness in target weeds. As such, the identification of hybridization in target weeds has become of fundamental interest. Curly-leaf pondweed (*Potamogeton crispus*) is a heavily managed invasive aquatic weed in the United States. The genus is known for extensive interspecific hybridization, but the extent to which invasive *P. crispus* in the United States hybridizes is unknown. In October 2018, an aquatic vegetation survey in the California Sacramento–San Joaquin river delta identified plants that were suspected as *P. crispus* hybrids. These plants closely resembled *P. crispus* but differed in several ways, including having smaller, finer leaves and lacking the presence of true turions. We performed genetic analysis on these plants by comparing the internal transcribed spacer (ITS) DNA sequences from the putative hybrids to those identified as pure *P. crispus* and to *Potamogeton* accessions retrieved from GenBank. The putative hybrids had two divergent ITS sequences, one of which corresponded to sequences from *P. crispus*, and the other of which corresponded to sequences from *P. pusillus*, providing strong evidence of interspecific hybridization between these two species. Further, we identified genetic diversity even among pure *P. crispus* in North America. The extent of genetic diversity and the relevance to *P. crispus* ecology or management are currently unknown. Given the extent of management of *P. crispus* in North America, and the recognition that hybridization and genetic diversity can impact management outcomes, a geographic survey of genetic diversity and hybridization in *P. crispus* is warranted.

Key words: GenBank, Pondweed hybrid, *Potamogeton berchtoldi*, *Potamogeton pusillus*, putative hybrid.

INTRODUCTION

Aquatic plant managers increasingly recognize that genetic variation can impact aquatic plant management outcomes. For example, fluridone efficacy in Florida

*First author: SePRO Corporation, 11550 North Meridian Street, Suite 600, Carmel, IN 46032-4565. Second Author: Assistant Professor, Department of Plant Science and Plant Pathology, Montana State University, Plant Bioscience Building, PO Box 173150, Bozeman, MT 59717. Corresponding author's E-mail: ajones@sepro.com. Received for publication March 31, 2020 and in revised form October 7, 2020.

populations of hydrilla (*Hydrilla verticillata*) is influenced by DNA substitutions in the phytoene desaturase gene (Michel et al. 2004), which can be detected by genetic screening (Benoit and Les 2013). Similarly, different genotypes of Eurasian (*Myriophyllum spicatum*) and hybrid watermilfoil (*M. spicatum* × *M. sibiricum*) vary in their growth and response to several herbicides (e.g., Glomski and Netherland 2009, Berger et al. 2012, Thum et al. 2012, LaRue et al. 2013, Taylor et al. 2017, Netherland and Willey 2018), and distinct phenotypes of fanwort (*Cabomba caroliniana*) differ in their response to several herbicides (Bultemeier et al. 2009).

Hybridization between invasive species and their native relatives is one source of genetic variation that can influence invasiveness (Ellstrand and Schierenbeck 2000). For example, introduced Eurasian watermilfoil frequently hybridizes with native northern watermilfoil (*M. sibiricum*) in North America (Moody and Les 2002, 2007, Zuellig and Thum 2012). Hybridization is associated with increased invasiveness in milfoils (Moody and Les 2002, LaRue et al. 2012), and different hybrid genotypes exhibit different responses to several commonly used herbicides (Poovey et al. 2007, Glomski and Netherland 2009, Berger et al. 2012, 2015, Thum et al. 2012, LaRue et al. 2013, Taylor et al. 2017, Netherland and Willey 2018). The identification of hybrids frequently requires molecular genetic data because hybrids can be difficult to distinguish from parental species, especially when parental species themselves are difficult to distinguish from closely related species.

Potamogeton crispus is a frequently managed invasive aquatic plant in the United States and Canada, causing a multitude of problems such as outcompeting native plants, nutrient loading after senescence, and reducing recreation by blocking waterways (Jones et al. 2012, James et al. 2009, Parkinson and Mangold, 2016). Hybridization among *Potamogeton* pondweeds is common in their native range. For example, Zalewska (2002) identified 78 hybrids out of 5,000 herbarium specimens of *Potamogeton*. Invasive *P. crispus* has been documented on the basis of morphological analysis in many places; however, genetic data collection has not been routinely incorporated into management projects for *P. crispus*, and to our knowledge, no genetic survey of the species has been conducted in North America. Therefore, the extent to which *P. crispus* may hybridize is unknown.

In the Sacramento–San Joaquin River Delta, *P. crispus* is typically managed by morphological assessment using point

TABLE 1. SAMPLES AND GENBANK ACCESSION NUMBERS USED IN THIS ANALYSIS.

Species/sample	GenBank accession number	Reference
<i>P. alpinus</i>	FJ151201	Kaplan et al. 2009
<i>P. amplifolius</i>	EF526388	Direct submission
<i>P. berchtoldi</i> ¹	GQ247388	Les et al. 2009
<i>P. bicupulatus</i>	EF526391	Direct submission
<i>P. crispus</i>	DQ840287	Wang et al. 2007
<i>P. crispus</i>	EF526369	Direct submission
<i>P. crispus</i>	EF526372	Direct submission
<i>P. crispus</i> x <i>P. ochreatus</i>	GU814246	Kaplan et al. 2011
<i>P. epiphydrus</i>	FJ151206	Kaplan et al. 2009
<i>P. foliosus</i>	GQ247410	Les et al. 2009
<i>P. maackianus</i>	DQ840271	Wang et al. 2007
<i>P. malaiamus</i>	EU741050	Du et al. 2009
<i>P. natans</i>	FJ151208	Kaplan et al. 2009
<i>P. nodosus</i>	AF102273	Direct submission
<i>P. nodosus</i>	FJ151210	Kaplan et al. 2009
<i>P. oakesianus</i>	FJ151212	Kaplan et al. 2009
<i>P. ochreatus</i>	GU814250	Kaplan et al. 2011
<i>P. octandrus</i>	JF977909	Li et al. 2011
<i>P. perfoliatus</i>	EU596953	Kaplan et al. 2009
<i>P. praelongus</i>	JX012092	Zalewska-Galosz and Ronikier 2012
<i>P. pulcher</i>	EF526400	Direct submission
<i>P. pusillus</i>	GQ247420	Les et al. 2009
<i>P. richardsonii</i>	EU596954	Kaplan et al. 2009
<i>P. robbinsii</i>	EF526390	Direct submission
<i>P. vaseyi</i>	GQ247422	Les et al. 2009
<i>P. zosteriformis</i>	GQ247438	Les et al. 2009

¹We have kept the name *P. berchtoldi* when referencing the specific GenBank accession. However, we refer to the species as *P. pusillus*, as this is the currently accepted official taxonomic name at this time.

intercept surveys (Madsen 1999) along with sonar/gps Biobase® (Howell and Richardson 2017). Following species identification and confirmation of biovolume, infested areas of *P. crispus* are treated with fluridone pellets (Sonar Q®, Sonar One®, and Sonar PR®), dipotassium salt of endothall (Aquathol K®), or diquat. Follow up surveys of biovolume are done in the fall to ensure reduction of the invasive plant. These monitoring and treatment actions are largely conducted by the California Division of Boating and Waterways (California Department of Parks and Recreation-Division of Boating and Water Ways 2017).

In this article, we document hybrid *P. crispus* in a California population using molecular methods. During an aquatic vegetation survey as part of a collaboration of California Division of Boating and Waterways and SePRO Corporation in October 2018, we identified a *Potamogeton* with an unusual phenotype in several locations, which we suspected as hybrid *P. crispus*. The stems of the putative hybrid were thinner and darker green than that of *P. crispus*, with a more fusiform cross section around 2 mm in diameter. The leaves of the plant were dark green, softer, lorate, and alternating, whereas *P. crispus* generally has lighter green or olive leaves with a crispy texture. The width of the leaves was 4–7 mm with a total length of 15–50 mm and around 75% less curly compared to *P. crispus*. The margins of the leaves were also lacking the serrate quality of *P. crispus*. Turions typical of *P. crispus* were not found on the plant, but the apical meristems bore a resemblance of a pseudo turion by being thicker on two sides with leaves coming from each apex.

MATERIALS AND METHODS

On October 3, 2018, an annual macrophyte survey was completed on Franks Tract, the largest waterbody in the Sacramento–San Joaquin Delta. We sampled 100 points that were generated by evenly spacing them over the study area using GIS. We sampled each point using a weighted, double-headed, 0.33-m-wide rake, which was dragged for ~3 m along the bottom and then pulled up to the boat for analysis. We recorded each species of submerged macrophyte that was present on the rake. We identified the putative hybrid *P. crispus* from four sample points.

We extracted total genomic DNA using DNeasy Plant Mini Kits (Qiagen)¹ from dried meristem tissue that was preserved in the field with silica gel. We extracted from six putative hybrids from the Frank Tract populations, two putative pure *P. crispus* individuals from Frank's Tract, and one *P. crispus* collected in a creek located near Montana State University in Bozeman.

We performed molecular identifications of samples by comparing internal transcribed spacer (ITS) sequences from our samples to those from accessions retrieved from GenBank (Table 1). In particular, for suspected hybrid *P. crispus*, we predicted to see two unique ITS sequences for each individual that corresponded to sequences from *P. crispus* and another species.

We amplified ITS using the universal primers ITS4 and ITS 5 (Soltis and Kuzoff 1995). All PCR reactions contained the following: 1× GoTaq Hot Start PCR buffer (Promega), 2 mM MgCl₂, 2 pmol each primer, 0.2 mM each dNTP, 1 unit of GoTaq Hot Start DNA polymerase (Promega), 2 μl template DNA, and brought to a total volume of 25 μl with molecular biology-grade water. Thermal cycling consisted of the following: one cycle at 94 C for 2 min followed by 25 cycles of 94 C for 1 min, 53 C for 30 sec, 72 C for 1 min, and a final extension at 72 C for 5 min. We visualized 2 μl of PCR products on an agarose gel (~1.5%) to check for size and purity.

We treated PCR products with the enzymes Exonuclease I (New England Biolabs)² and Antarctic Phosphatase (New England Biolabs)³ to eliminate unincorporated primers and dNTPs before sequencing. PCR products were sent for sequencing to the University of Illinois at Urbana-Champaign's Core Sequencing Facility on an ABI 3730xl DNA sequencer.

In some cases, direct sequencing of PCR products produced clean and unambiguous sequence. However, in the case of putative hybrids, we found more than one ambiguous base pair, and sequence quality was poor due to insertions and deletions of base pairs (indels). We selected one representative individual to clone the PCR product using the TOPO TA cloning kit (ThermoFisher)⁴ and sequenced eight positive inserts).

DNA sequences were edited using Sequencher, version 4.2 (Gene Codes Corporation) and aligned using ClustalW, as implemented in MEGA X (Kumar et al. 2018). The final alignment size was 640 bp after trimming. We constructed a neighbor-joining tree in MEGA using a Kimura two-parameter model of DNA sequence evolution with uniform rates among sites, complete deletion of gaps and missing data, and 1,000 bootstrap replicates.



Figure 1. Photograph of putative hybrid *Potamogeton crispus* identified in four sites at Frank's Tract. The stem of the putative hybrid is thinner and darker green than that of *P. crispus*, with a more fusiform cross section around 2 mm in diameter. The leaves of the plant were dark green, softer, lorate, and alternating. The width of the leaves was 4 to 7 mm with a total length of 15 to 50 mm and around 75% less curly compared to *P. crispus*. The margins of the leaves were also lacking the serrate quality of *P. crispus*.

RESULTS AND DISCUSSION

As suspected on the basis of morphology, we found clear evidence for hybridization of *P. crispus* in Franks Tract in the Sacramento–San Joaquin Delta. Putative hybrids contained two divergent ITS sequences, one of which corresponded to GenBank accessions for *P. crispus*, and the other ITS sequence corresponded to GenBank accessions for *P. berchtoldii* (Figure 1) (Les et al. 2009). Although GenBank accessions label the species *P. berchtoldii*, *P. berchtoldii* is not recognized as its own species and is widely referred to as *P. pusillus*. Therefore, we have kept the name *P. berchtoldii* when referencing the specific GenBank accession, but we refer to the species as *P. pusillus* throughout the text, as this is the currently accepted official taxonomic name at this time (ITIS 2020). Out of 640 total bp, *P. crispus* differed from *P. pusillus* at 36 nucleotide positions. At each of these positions, putative hybrid individuals exhibited clear biparental polymorphisms that corresponded to the two parental ITS sequences (Table 2). Therefore, our data provide strong evidence for hybridization between these two species.

Hybridization in *P. crispus* has been documented with several species in its native range including crosses with *P. praelongus* and *P. perfoliatus* (Kaplan and Fehrer 2013). In its invasive range, hybridization has been documented in New South Wales, Australia, with *P. ochreatus* (Kaplan et al. 2011). To our knowledge, this is the first documentation of hybridization between *P. crispus* and *P. pusillus*.

Generally, hybrid pondweed species have not been recorded to pose management risks. However, hybridization has been widely documented to precede the evolution of invasiveness in many plants (Ellstrand and Schierenbeck

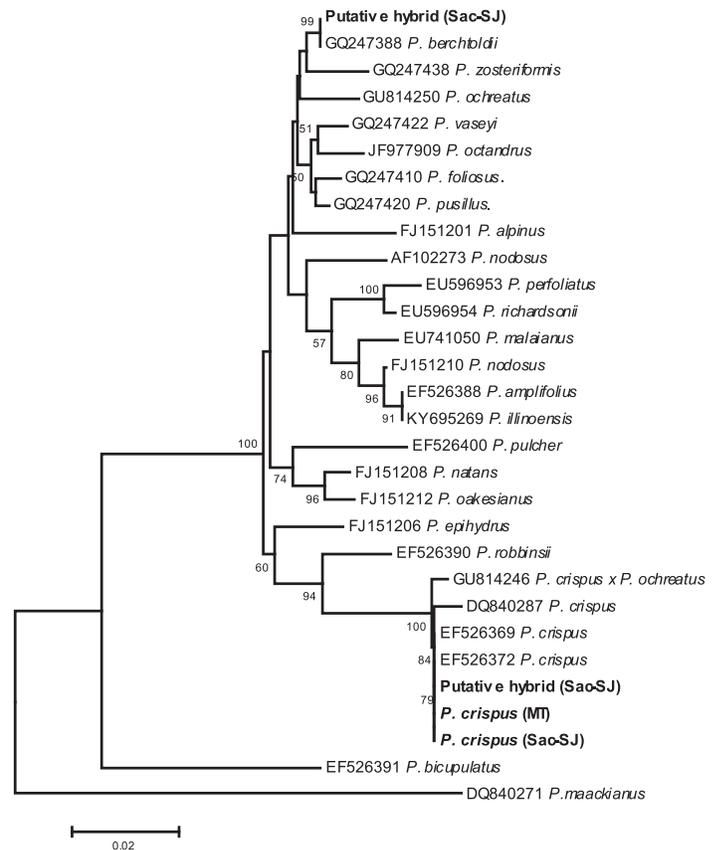


Figure 2. Neighbor-joining tree of samples and accessions used in this analysis. Samples sequenced as part of this study are in bold and are named as in Table 2 (U.S. postal codes; “Sac-SJ” = Sacramento–San Joaquin Delta, CA). Putative hybrids possessed two clearly distinct ITS alleles corresponding to those most closely related to *P. crispus* and *P. berchtoldii*. The tree was constructed using a Kimura two-parameter model of DNA sequence evolution with uniform rates among sites, complete deletion of gaps and missing data, and 1,000 bootstrap replicates. Only branches supported by greater than 50% bootstrap replicates are labeled. Samples obtained from GenBank are prefixed with their accession number. Note that we have kept the name *P. berchtoldii* when referencing the specific GenBank accession. However, we refer to the species as *P. pusillus*, as this is the currently accepted official taxonomic name at this time.

2000). For aquatic plants specifically, hybridization between invasive Eurasian watermilfoil and native northern watermilfoil has concerned managers, as some hybrid genotypes have been found to exhibit faster growth and/or resistance to some commonly used herbicides (Berger et al. 2012, 2015, LaRue et al. 2012, Thum et al. 2012, Netherland and Willey 2018). There is no indication at this time that this is indeed a viable population, and it may be the case that the single observation of hybridization may be an interim event. It may be of interest to continue to search and track the population with annual surveys and to monitor the increase or potential decline of the hybrid if it is indeed found again.

In addition to *P. pusillus*, there are five other species in the *Potamogetonacae* family found in Franks tract including *P. richardsonii*, *P. nodosus*, *P. illinoensis*, *Stuckenia pectinata*, and *S. filiformis* (Caudill et al. 2019). It is unclear if *P. crispus*

TABLE 2. VARIABLE POSITIONS WITHIN AND AMONG *P. CRISPUS* AND *P. BERCHTOLDII* IN THE 640 BASE PAIR DNA ALIGNMENT OF SAMPLES AND GENBANK ACCESSIONS USED IN THIS STUDY. POSITIONS THAT ARE ITALICIZED REPRESENT POSITIONS THAT WERE VARIABLE WITHIN *P. CRISPUS* SAMPLES AND ACCESSIONS. ALL OTHER POSITIONS SEPARATE *P. BERCHTOLDII* FROM *P. CRISPUS* AND WERE POLYMORPHIC IN PUTATIVE HYBRIDS. GENBANK ACCESSION NUMBERS ARE PRECEDED BY GEOGRAPHIC LOCATIONS WHEN THEY WERE AVAILABLE; U.S. POSTAL CODES ARE USED FOR U.S. LOCATIONS. "SAC-SJ" REFERS TO SAMPLES COLLECTED FROM THE SACRAMENTO-SAN JOAQUIN Delta, CA. WE HAVE KEPT THE NAME *P. BERCHTOLDII* WHEN REFERENCING THE SPECIFIC GENBANK ACCESSION. HOWEVER, WE REFER TO THE SPECIES AS *P. PUSILLUS*, AS THIS IS THE CURRENTLY ACCEPTED OFFICIAL TAXONOMIC NAME AT THIS TIME.

Species name	5	11	20	43	72	90	159	163	183	208
<i>P. berchtoldii</i> (GQ247388)	T	C	A	A	T	C	A	G	G	G
<i>P. crispus</i> (China DQ840287)	C	T	T	A	C	T	T	A	C	T
<i>P. crispus</i> (Czech GU814246)	C	T	T	G	T	T	T	A	C	T
<i>P. crispus</i> (CT EF526369)	C	T	T	A	T	T	A	A	C	T
<i>P. crispus</i> (CT EF526372)	C	T	T	A	T	T	A/T	A	C	T
<i>P. crispus</i> (Sac-SJ)	C	T	T	A	T	T	A/T	A	C	T
<i>P. crispus</i> (MT)	C	T	T	A	T	T	T	A	C	T
Putative hybrid (Sac-SJ)	C/T	C/T	A/T	A	T	C/T	A	A/G	C/G	G/T
	211	212	213	220	237	366	411	416	422	424
<i>P. berchtoldii</i> (GQ247388)	A	C	T	A	A	C	C	C	C	A
<i>P. crispus</i> (China DQ840287)	T	T	A	—	G	T	A	T	T	T
<i>P. crispus</i> (Czech GU814246)	T	T	A	—	G	T	A	T	T	T
<i>P. crispus</i> (CT EF526369)	T	T	A	—	G	T	A	T	T	T
<i>P. crispus</i> (CT EF526372)	T	T	A	—	G	T	A	T	T	T
<i>P. crispus</i> (Sac-SJ)	T	T	A	—	G	T	A	T	T	T
<i>P. crispus</i> (MT)	T	T	A	—	G	T	A	T	T	T
Putative hybrid (Sac-SJ)	A/T	C/T	A/T	A/—	A/G	C/T	A/C	C/T	C/T	A/T
	427	428	433	445	479	481	482	488	491	492
<i>P. berchtoldii</i> (GQ247388)	T	C	T	G	A	C	C	T	C	T
<i>P. crispus</i> (China DQ840287)	C	T	T	A	A	T	T	C	T	C
<i>P. crispus</i> (Czech GU814246)	C	T	A	A	A	T	T	T	T	C
<i>P. crispus</i> (CT EF526369)	C	T	T	A	A	T	T	T	T	C
<i>P. crispus</i> (CT EF526372)	C	T	T	A	A/G	T	T	T	T	C
<i>P. crispus</i> (Sac-SJ)	C	T	T	A	A/G	T	T	T	T	C
<i>P. crispus</i> (MT)	C	T	T	A	G	T	T	T	T	C
Putative hybrid (Sac-SJ)	C/T	C/T	T	A/G	A	C/T	C/T	T	C/T	C/T
	495	496	499	520	532	536	571	575	582	624
<i>P. berchtoldii</i> (GQ247388)	C	—	C	A	G	T	C	G	C	G
<i>P. crispus</i> (China DQ840287)	T	T	G	T	A	—	T	A	T	C
<i>P. crispus</i> (Czech GU814246)	T	T	G	T	G	—	T	A	T	C
<i>P. crispus</i> (CT EF526369)	T	T	G	T	G	—	C	A	T	C
<i>P. crispus</i> (CT EF526372)	T	T	G	T	G	—	C/T	A	T	C
<i>P. crispus</i> (Sac-SJ)	T	T	G	T	G	—	C/T	A	T	C
<i>P. crispus</i> (MT)	T	T	G	T	G	—	T	A	T	C
Putative hybrid (Sac-SJ)	C/T	T/—	C/G	A/T	G	T/—	C	A/G	C/T	C/G
	627	629	631	632						
<i>P. berchtoldii</i> (GQ247388)	C	C	—	—						
<i>P. crispus</i> (China DQ840287)	A	—	C	A						
<i>P. crispus</i> (Czech GU814246)	A	—	C	A						
<i>P. crispus</i> (CT EF526369)	A	—	C	A						
<i>P. crispus</i> (CT EF526372)	A	—	C	A						
<i>P. crispus</i> (Sac-SJ)	A	—	C	A						
<i>P. crispus</i> (MT)	A	—	C	A						
Putative hybrid (Sac-SJ)	A/C	C/—	C/—	A/—						

is capable of hybridizing with these other species. The hybrid did possess pseudo-turions at the apical meristem (Figure 2), which may indicate that it can reproduce both sexually and asexually, as in the case of *P. crispus*. Asexual reproduction through the formation and sprouting of turions is one of the aspects that makes *P. crispus* such a successful invasive species. Newman et al. (pers. comm.) found that turions can stay viable for 7 yr in benthic substrate. If the turion-like structures on the hybrid are

viable, this means that the hybrid could have the potential for invasive qualities as well. The fact that for *P. crispus* to hybridize it has to cross-pollinate with other Potamogeton species may make this find somewhat of a rarity given that the plants primary reproductive strategy is largely asexual. The rarity of cross-pollination may be offset by the profuseness of *P. crispus* in an infestation event, however; *P. crispus* was found at only 10% of sites and *P. bechtoldii* was found at 27% of sites. Moreover, both parent species were

found at only 3% of sites together. The following year, in October 2019, *P. crispus* was found at 7% of sites and *P. pusillus* was found at 13% of sites. The hybrid was not found in 2019, leading us to believe that the high frequency of occurrence of *P. pusillus* in 2018 led to an increased likelihood of hybridization with *P. crispus*. Considering that we did not find the hybrid in 2019, it may be possible that the hybrid was incapable of reproduction and likely not of management concern. Despite the lack of management concerns the site where the hybrid was found will continue to be monitored on an annual basis for other macrophyte management goals.

In addition to documenting hybridization between *P. crispus* and *P. pusillus*, it is clear from our data that there is at least some genetic diversity even among pure *P. crispus* in North America. We found three nucleotide positions that exhibited variation among accessions of *P. crispus* (Table 2). Two *P. crispus* accessions from North America exhibited heterozygosity at these nucleotide positions (Table 2). The extent of genetic diversity, and any implications for *P. crispus* ecology or management, are currently unknown. It is not known if treatment methodologies used to control *P. crispus* would also be successful on the hybrid; however, there is no indication that the hybrid would be more or less resilient to certain herbicides. Given the extent of management of *P. crispus* in North America, and the recognition that genetic diversity (including hybridization) can impact management outcomes, a geographic survey of genetic diversity for *P. crispus* is warranted.

SOURCES OF MATERIALS

¹DNeasy Plant Mini Kit, Qiagen Corp., 27220 Turnberry Lane, Suite 200, Valencia, CA 91355.

²Exonuclease I, New England Biolabs, 240 County Rd, Ipswich, MA 01938.

³Antarctic phosphatase, New England Biolabs, 240 County Rd, Ipswich, MA 01938.

⁴TOPO™ TA Cloning™ Kit, Life Technologies Corp., 5791 Van Allen Way Carlsbad, CA 92008.

ACKNOWLEDGEMENTS

We thank Leah Simantel and Emma Rice for assistance with the molecular work. We also would like to thank Patricia Gilbert, Mark Heilman, Scott Shuler, and the California Department of Boating and Waterways for helping find the plant. Support for the project was provided in part by the Montana Agricultural Experiment Station (Project MONB00249).

LITERATURE CITED

Benoit LK, Les DH. 2013. Rapid identification and molecular characterization of phytoene desaturase mutations in fluridone-resistant Hydrilla (*Hydrilla verticillata*). *Weed Sci.* 61(1):32–40.

Berger ST, Netherland MD, MacDonald GE. 2012. Evaluating fluridone sensitivity of multiple hybrid and Eurasian watermilfoil accessions under mesocosm conditions. *J. Aquat. Plant Manage.* 50:135–141.

Berger ST, Netherland MD, MacDonald GE. 2015. Laboratory documentation of multiple-herbicide tolerance to fluridone, norflurazon, and topramazine in a hybrid watermilfoil (*Myriophyllum spicatum* × *M. sibiricum*) population. *Weed Sci.* 63(1):235–241.

Bultemeier BW, Netherland MD, Ferrell JA, Haller WT. 2009. Differential herbicide response among three phenotypes of *Cabomba caroliniana*. *Invasive Plant Sci. Manage.* 2:352–359.

California Department of Parks and Recreation—Division of Boating and Waterways. 2017. Submerged Aquatic Vegetation Control Program [*Egeria densa*, Curlyleaf Pondweed, Eurasian Watermilfoil, Fanwort, and Coontail]. Submerged Aquatic Vegetation Control Program Annual 2017 Monitoring Report. Sacramento. 298 pp.

Caudill J, Jones AR, Gilbert T, Madsen JD, Heilman MA, Shuler S. 2019. Aquatic plant community restoration following the long-term management of invasive *Egeria densa* with fluridone treatments. *Manage. Biol. Inv.* 10(3):473–485.

Du ZY, Yang CF, Chen JM, Guo YH. 2009. Nuclear and chloroplast DNA sequences data support the origin of *Potamogeton intortusifolius* J.B. He in China as a hybrid between *P. perfoliatus* Linn. and *P. wrightii* Morong. *Aquat. Bot.* 91:47–50.

Ellstrand NC, Schierenbeck KA. 2000. Hybridization as a stimulus for the evolution of invasiveness in plants? *Proc. Natl. Acad. Sci. U.S.A.* 97:7043–7050.

Glomski LM, Netherland MD. 2009. Response of Eurasian and hybrid watermilfoil to low use rates and extended exposures of 2,4-D and triclopyr. *J. Aquat. Plant Manage.* 48:12–14.

Howell, AW, Richardson RJ. 2017. Correlations between Ecosound Biovolume and Aquatic Plant Biomass [Blog post]. blog.biobasemaps.com/2017/07/27/guest-blog-correlations-between-ecosound-biovolume-and-aquatic-plant-biomass/. Accessed Jan 1, 2019.

Integrated Taxonomic Information System. 2020. *Potamogeton bertholdii* Fieber: Taxonomic Serial Number 39006. https://www.itis.gov/servlet/SingleRpt/SingleRpt?search__topic=TSN&search__value=39006#null. Accessed Jan 8th, 2019.

James WF, Barko JW, Eakin HL, Sorge PW. 2002. Phosphorus budget and management strategies for an urban Wisconsin lake. *Lake Reservoir Manage.* 18(2):149–163.

Jones AR, Johnson JA, Newman RM. 2012. Effects of repeated, early season, herbicide treatments of curlyleaf pondweed on native macrophyte assemblages in Minnesota lakes. *Lake Reservoir Manage.* 28(4):364–374.

Kaplan Z, Fehrer J. 2013. Molecular identification of hybrids from a former hot spot of *Potamogeton* hybrid diversity. *Aquat. Bot.* 105:34–40.

Kaplan Z, Uotila P. 2011. *Potamogeton x exilis* (*P. alpinus* x *P. natans*), a new hybrid pondweed from Finland Nordic J. Bot. 29:477–483.

Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018 MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.* 35:1547–1549.

LaRue EA, Zuellig MP, Netherland MD, Heilman MA, Thum RA. 2013 Hybrid watermilfoil lineages are more invasive and less sensitive to a commonly used herbicide than their exotic parent (Eurasian watermilfoil). *Evol. Appl.* 6:462–471.

Les DH, Murray NM, Tippery NP. 2009 Systematics of two imperiled pondweeds (*Potamogeton vaseyi*, *P. gemmiparus*) and taxonomic ramifications for subsection Pusilli (Potamogetonaceae). *Syst. Bot.* 34:643–651.

Li DZ, Gao LM, Li HT, Wang H, Ge XJ, Liu JQ, Chen ZD, Zhou SL, Chen SL, Yang JB, Fu CX, Zeng CX, Yan HF, Zhu YJ, Sun YS, Chen SY, Zhao L, Wang K, Yang T, Duan GW. 2011. Comparative analysis of a large dataset indicates that internal transcribed spacer (ITS) should be incorporated into the core barcode for seed plants. *Proc. Natl. Acad. Sci. U.S.A.* 108:19641–19646.

Madsen JD. 1999 Point intercept and line intercept methods for aquatic plant management. APCRP Technical Notes Collection (ERDC/TN APCRP-MI-02) (February), pp. 1–16, <https://doi.org/10.21236/ADA361270>.

Michel AR, Arias RS, Scheffler BE, Duke SO, Netherland MD, Dayan FE. 2004. Somatic mutation-mediated evolution of herbicide resistance in the nonindigenous invasive plant hydrilla (*Hydrilla verticillata*). *Mol. Ecol.* 13:3229–3237.

Moody ML, Les DH. 2002. Evidence of hybridity in invasive watermilfoil (*Myriophyllum*) populations. *Proc. Natl. Acad. Sci. U.S.A.* 99:14867–14871.

Moody ML, Les DH. 2007. Geographic distribution and genotypic composition of invasive hybrid watermilfoil (*Myriophyllum spicatum* x *M. sibiricum*) populations in North America. *Biol. Inv.* 9:559–570.

Parkinson H, Mangold J, McLane C. 2016. Curlyleaf Pondweed. Montana State University Extension Publications. 12 pp.

- Poovey AG, Slade JG, Netherland MD. 2007. Susceptibility of Eurasian watermilfoil (*Myriophyllum spicatum*) and a milfoil hybrid (*M. spicatum* x *M. sibiricum*) to triclopyr and 2, 4-D amine. *J. Aquat. Plant Manage.* 45:111–115.
- Taylor LA, McNair JN, Guastello P, Pashnick J, Thum RA. 2017. Heritable variation for vegetative growth rate in ten distinct genotypes of hybrid watermilfoil. *J. Aquat. Plant Manage.* 55:51–57.
- Thum RA, Weisel DJ, Zuellig MP, Heilman M, Hausler P, Tynning P, Huberty L, Netherland MD. 2012. Field documentation of decreased herbicide response by a hybrid watermilfoil population. *J. Aquat. Plant Manage.* 50:141–146.
- Woolf TE, Madsen JD. 2003. Seasonal biomass and carbohydrate allocation patterns in southern Minnesota curlyleaf pondweed populations. *J. Aquat. Plant Manage.* 41:113–118.
- Zalewska-Gaosz J. 2002. Occurrence and distribution of Potamogeton hybrids (Potamogetonaceae) in Poland. *Feddes Repertorium: Z. botan. Tax. Geobot.* 113(5-6):380–393.
- Zuellig MP, Thum RA. 2012. Multiple introductions of invasive Eurasian watermilfoil and recurrent hybridization with native northern watermilfoil in North America. *J. Aquat. Plant Manage.* 50:1–19.