



Molecular detection of a non-native hybrid eelgrass, *Vallisneria spiralis* Linnaeus (1753) × *V. denseserrulata* Makino (1921), in the southeastern United States

Siobhan B. Gorham^{a,*}, Seifu Seyoum^a, Bradley T. Furman^a, Kelly M. Darnell^b, Laura K. Reynolds^c, Michael D. Tringali^a

^a Florida Fish and Wildlife Conservation Commission, Fish and Wildlife Research Institute, 100 8th Avenue SE, St. Petersburg, FL 33701, USA

^b Division of Coastal Sciences, School of Ocean Science and Engineering, The University of Southern Mississippi, Ocean Springs, MS 39564, USA

^c Soil and Water Sciences Department, University of Florida, Gainesville, FL 32611, USA

ARTICLE INFO

Keywords:

NrITS
Vallisneria americana Michaux
Vallisneria neotropicalis Marie-Victorin
Exotic species
Invasive species management
Submerged aquatic vegetation (SAV)

ABSTRACT

During a genetic survey of *Vallisneria americana* in the southeastern United States, a non-native hybrid between *V. spiralis* Linnaeus and *V. denseserrulata* Makino was detected in eight locations across Florida and Alabama. Although previously reported in Japan, Hungary, and Australia, this is the first documented occurrence of the hybrid in North America. Because natural ranges of both parent species are not sympatric in the wild, it is thought that the hybridization event occurred in aquaria. The presence of both species in the aquarium pet trade also makes the industry a potential source of the introduction in the United States. More extensive sampling will be necessary to determine the full distribution of the hybrid, as well as to evaluate its potential impact on native *Vallisneria* populations and the aquatic ecosystems they support.

1. Introduction

In North America, *Vallisneria americana* Michaux (1803) provides important environmental structure for finfish, invertebrates, and epiphytic communities (Diehl and Kornijów, 1998), as well as vital forage for diverse resident and migratory waterfowl (Korschgen and Green, 1988) and the Florida manatee (*Trichechus manatus latirostris*) (Reich and Worthy, 2006). Recent anthropogenic and environmental stressors have threatened or degraded *V. americana* populations throughout the United States, and an increasing number of restoration efforts have been attempted to mitigate accelerating losses (Kraemer et al., 1999; Schloesser and Manny, 2007; Lloyd et al., 2011; Engelhardt et al., 2014). In Florida, restoration typically involves the transplant of cultured or wild stock, the selection of which has been guided by the idea that certain natural ecotypes or specifically cultivated genets (i.e., cultivars) have become locally adapted to environmental stressors found at recipient sites (e.g., salinity fluctuations) or exhibit growth characteristics (e.g., branching rate, foliar productivity) that enhance performance in restoration settings (Gettys and Haller, 2013). To that end, cultivars of *V. americana* have been developed by researchers

(Tootoonchi et al., 2020) and practitioners (SWFWMD, 2000) and are now regularly outplanted in Florida.

Implicit in this practice is the assumption that *V. americana* is the only species of *Vallisneria* found in Florida and that statewide outplanting does not disrupt population structure or otherwise cause outbreeding depression. However, the taxonomy of *Vallisneria* in North America has been debated for more than 200 years (Michaux, 1803; Rydberg, 1909; Marie-Victorin, 1943; Godfrey and Wooten, 1979). Over time, *V. americana* Michaux was distinguished from then *V. spiralis* Linnaeus (Michaux, 1803) and further divided into northern (*V. americana* Michaux) and southern (*V. neotropicalis* Marie-Victorin) populations (Marie-Victorin, 1943) before being consolidated under *V. americana* Michaux (Godfrey and Wooten, 1979; Lowden, 1982), with trait differences interpreted as phenotypic plasticity. Recently, phylogenetic work by Les et al. (2008) reinvigorated the debate, supporting the existence of three species in North America (*V. americana*, *V. neotropicalis*, and an unnamed umbellate species from Texas); however, the study lacked the spatial resolution to resolve the issue regionally. Additional evidence for latitudinal species richness in *Vallisneria* was found during microsatellite primer development for mid-Atlantic

* Corresponding author.

E-mail address: siobhan.gorham@myfwc.com (S.B. Gorham).

<https://doi.org/10.1016/j.aquabot.2021.103445>

Received 11 May 2021; Received in revised form 9 August 2021; Accepted 24 August 2021

Available online 28 August 2021

0304-3770/© 2021 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

populations of *V. americana* (Burnett et al., 2009), which were ineffective in Florida-sourced material (B. Marsden, personal communication), suggesting genetic divergence of northern and southern populations (Selkoe and Toonen, 2006).

To address the long-standing question of *Vallisneria* species diversity in the southeastern United States, we conducted a limited genetic survey of leaf material from 46 locations across four states – Florida, Alabama, Mississippi, and Kentucky – using sequence data from the nuclear ribosomal internal transcribed spacer (nrITS) gene, which includes the internal transcribed spacer 1 (partial sequence), 5.8S ribosomal RNA gene (complete sequence), and internal transcribed spacer 2 (complete sequence). Here, we report the occurrence of a credible hybrid between two non-native species, *V. spiralis* Linnaeus (Europe and southwestern Asia) and *V. denseserrulata* Makino (Africa and Asia) (Lowden, 1982) at several locations in southern and central Florida and one in Alabama, marking the first known observations of the hybrid in the continental United States.

2. Materials and methods

2.1. Field collection

During 2018 and 2019, we collected a total of 46 shoots of *Vallisneria* from 39 sites in Florida ($n = 35$), Mississippi ($n = 9$), Kentucky ($n = 1$) and Alabama ($n = 1$). Sites in Florida were chosen based on prior knowledge of extant populations and a review of herbarium records, while samples in Mississippi, Kentucky, and Alabama were samples of opportunity. After preliminary analysis revealed an unusual nrITS sequence from a site in Boynton Beach, Florida, we collected an additional 8 shoots from the area, giving us a total of 54 shoots. All samples were chosen haphazardly within sites and carefully excavated from surficial sediments to maintain intact roots, rhizomes, and leaves. Reproductive structures, when present, were retained for further study. When possible, multiple unconnected ramets were taken per site. Specimens were stored in ambient water, transported to greenhouse facilities at Eckerd College (St. Petersburg, FL), and maintained in labeled aquaria.

2.2. DNA extraction

Upon collection, tissue specimens were preserved at room temperature in 90% non-denatured absolute ethyl alcohol (EtOH, 99.5% purity) until DNA extractions were performed. Approximately 2-cm lengths of leaf material were excised and blotted with lint-free Kimwipes. Any remaining EtOH was evaporated by incubation at 37 °C for one hour. Tissues were homogenized with a high-speed, drill-driven pestle in 1.5-µl microtubes containing 350 µl of chilled cell lysis solution (Qiagen, Germantown, MD); bouts of grinding were done briefly but repeatedly to avoid overheating the tissues. Total genomic DNA was then extracted using the PureGene DNA isolation kit according to the manufacturer's instructions (Gentra Systems Inc., Minneapolis, MN). DNA pellets were rehydrated in 100 µl of deionized water and quantified using a Nanodrop ND-1000 UV-vis spectrophotometer (Thermo Fisher Scientific, Waltham, MA). DNA concentrations were adjusted to 150 ng/µl and stored at 4 °C for use prior to analysis.

From the genomic DNA, we amplified DNA fragments of nrITS via the polymerase chain reaction (PCR) by using the ITS5/ITS4 primers of Baldwin (1992). Amplifications were conducted in total reaction volumes of 50 µl, each containing 200–300 ng of total DNA, 50 µM of dNTP mix, 0.25 µl of 0.1 mg/ml BSA; 5 µl of GoTaq® DNA Polymerase buffer (5×), 2.5 mM MgCl₂ (Promega Corporation, Madison, WI, final concentration), 0.125 µl of a 100-mM solution of each primer, and 2.5 units of Go Taq DNA polymerase (Promega Corporation, Madison, WI). Reactions were performed using a step-down thermal profile: 94 °C for 2 min, 5 × (45 s at 94 °C, 55 °C, and 72 °C), 8 × (40 s at 94 °C, 53 °C, and 72 °C), 10 × (35 s at 94 °C, 47 °C, and 72 °C), 12 × (30 s at 94 °C, 46 °C,

and 72 °C), with a final extension at 72 °C for 15 min. Amplicons were gel purified by using the StrataPrep DNA Gel Extraction Kit (Agilent Technologies, Santa Clara, CA) and cycle sequenced from both directions with the same primers using BigDye (version 3.1; Applied Biosystems Inc. Waltham, MA). Sequencing products were visualized on an Applied Biosystems Prism 3130XL Genetic Analyzer and aligned and edited using Sequencher (Version 4.9; Gene Codes Corporation, Ann Arbor, MI).

We used nrITS sequences to reconstruct a maximum-likelihood phylogenetic tree using MEGA7 (Tamura et al., 2013), based on the Tamura 3-parameter substitution model assuming uniform rates among sites and using the Nearest-Neighbor heuristic method of inference. The reliabilities of inferred branch lengths were assessed with 1000 bootstrap replicates. We chose four sequences observed in the initial reconstruction (two sequences from each of the two major clades) to perform a GenBank BLAST search; 19 previously accessioned sequences identified in the search as having both high and intermediate sequence similarities to the four sequences were retained for a final phylogenetic reconstruction, which was performed after realignment (644 bp total sequence length) using ClusallW and default options as implemented in MEGA7.

3. Results

For the study sequences and homologous reference sequences, four well-supported major clades were recovered in the phylogenetic reconstruction (Fig. 1). One clade contained the majority of study sequences, which clustered closely with reference sequences from GenBank (EF142990, EF142991, and EF142992) of *V. neotropalis* (Les et al., 2008). The compressed cluster labeled as the *V. americana* clade contained six reference sequences from GenBank (EF142958, EF142959, EF142960, EF142961, EF526407, and AY335964). However, sequences for nine study specimens unexpectedly clustered within a clade comprised of previously accessioned *V. spiralis* and *V. denseserrulata*, occupying an intermediate position, characteristic of hybrids in this topology, between the two taxa (Fig. 1). Of the nine hybrid shoots, one was collected in Lake Guntersville, Alabama, and the rest were from various sites in Florida (Fig. 2). Two of the reference sequences from GenBank (EF142978 and EF142979), which matched closely to the nine Florida specimens, occurred within the *V. spiralis* and *V. denseserrulata* clade and occupied intermediate positions with respect to the known species. The origins of these two specimens are unknown; they were identified in Les et al. (2008) as cultivars of *V. spiralis*.

We identified the presence of the hybrid at 8 of 46 sample sites. Hybrid specimens were found in a cluster of four locations in the Boynton Beach region of South Florida, in and around the Lake Osbourne and Lake Ida system (two lakes connected by a constructed canal). The other hybrid specimens were found in Alabama (Lake Guntersville) and lakes in southeastern Florida (Winding Waters), central Florida (Lake Mariana), and west central Florida (Lake Maggiore).

4. Discussion

In this study we report, for the first time, the presence of a putative non-native hybrid, *V. spiralis* × *V. denseserrulata*, within the southeastern United States. The phylogenetic reconstructions of Les et al. (2008) depicted *V. spiralis* and *V. denseserrulata* as reciprocally monophyletic but closely related sibling taxa. Inspection of chromatograms for the intermediately positioned sequences in our study confirmed the presence of numerous doublet peaks occurring at the parsimony informative sites for *V. spiralis* and *V. denseserrulata*. We observed variation among the intermediate genotypes, with some reflecting parental genotypes at various parsimony informative sites, while overall maintaining a plurality of variable genotypes at those sites. This observation is consistent with a post hoc hypothesis that 9 out of 54 total samples were derived from offspring/cultivars of multiple hybrid origins of the African and

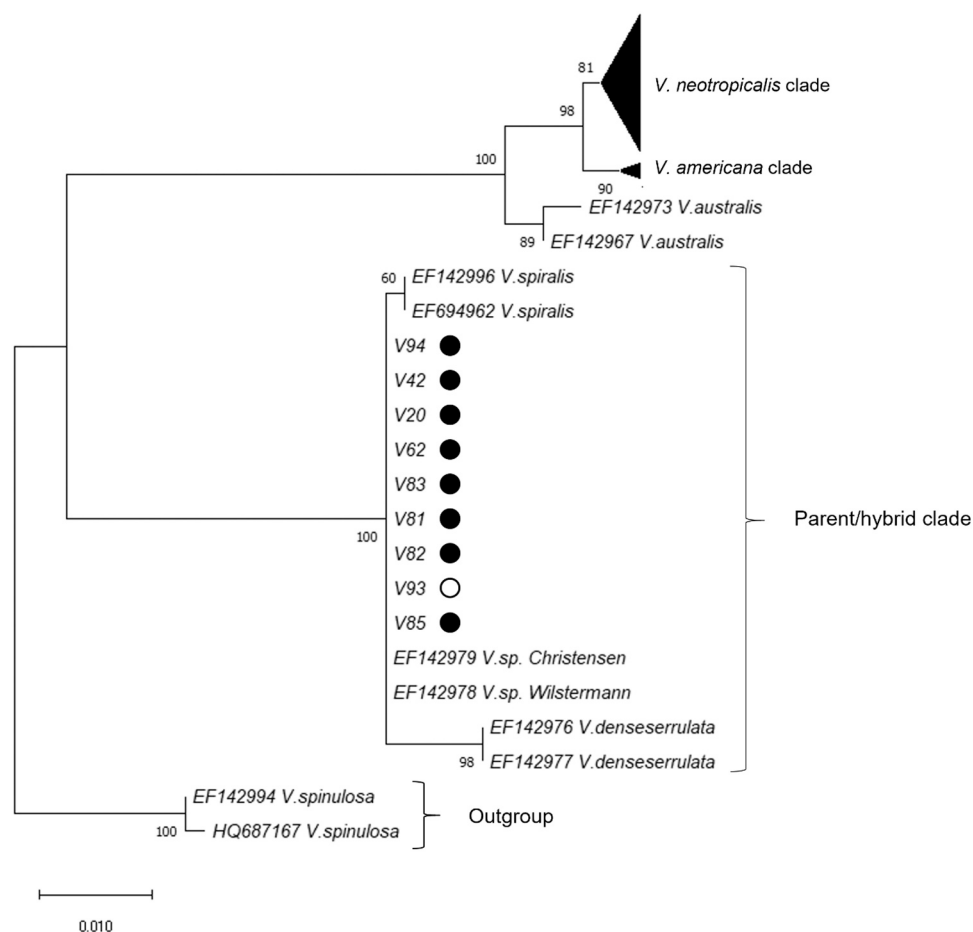


Fig. 1. Phylogenetic reconstruction of study and reference sequences of *Vallisneria* based on the Maximum Likelihood method as implemented in MEGA7. The Tamura 3-parameter substitution model was selected on the basis of the lowest BIC score in the model test function. The tree with the highest log likelihood (−1206.16) is depicted, rooted with *V. spinulosa* as the outgroup. The percentage of trees for which associated taxa clustered together in a bootstrap analysis (1000 replicates) is shown next to the branches. Branch lengths are scaled to the number of substitutions per site. The compressed cluster labeled as the *V. neotropica* clade contains 41 study sequences and three reference sequences from GenBank (EF142990, EF142991, and EF142992); the compressed cluster labeled as the *V. americana* clade contains six reference sequences from GenBank (EF142958, EF142959, EF142960, EF142961, EF526407, and AY335964). "Hybrid" sequences marked with closed circles were collected from Florida; the sequence marked with an open circle was collected from Alabama.

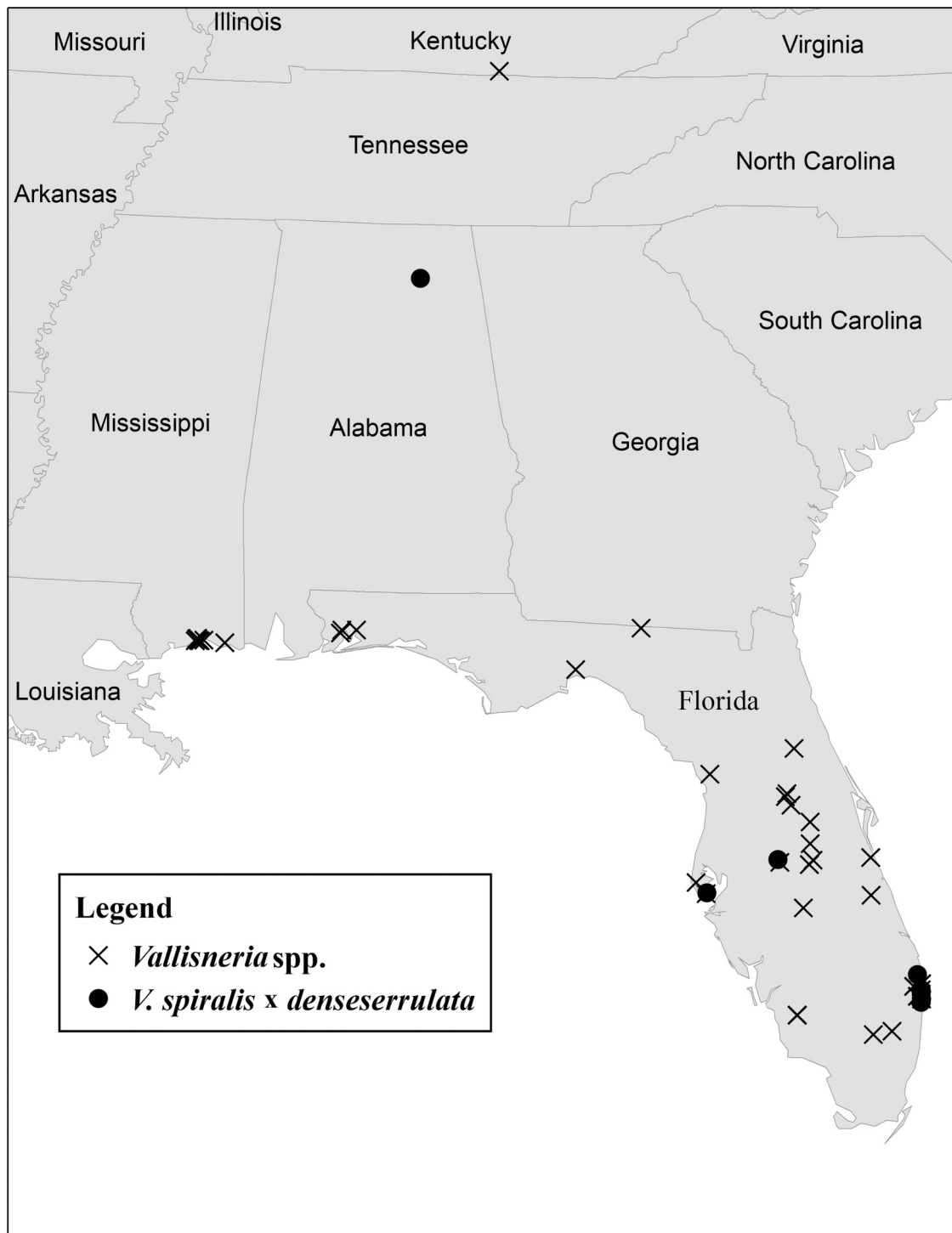


Fig. 2. Sample collection sites (n = 46). Black circles denote locations where the hybrid was detected.

Eurasian taxa (Lowden, 1982). While it is far less likely that autopolyploidy is responsible for the observed genotypes, it remains possible that the 9 samples originated through historical allopolyploidation events (in which case the observed differences would be reflected across more than 2n chromosome sets).

Contemporaneous hybrid forms of these two species, confirmed by subcloning of the nrITS gene, have previously been identified in Japan, Hungary, and Australia (Wasekura et al., 2016). Because both parental species are not sympatric in the wild, and both are common in the commercial aquarium trade, it is thought that the hybridization event occurred in aquaria (Wasekura et al., 2016). While our study does not

allow for inferences regarding the time, location, or frequency of introduction to the United States, the absence of either component species and the frequency of hybrid occurrence in our limited dataset, 4 of 39 sites visited in preliminary sampling (i.e., prior to preliminary nrITS data and targeted resampling) and 8 out of 46 total sites, suggests that either it was introduced multiple times or has gone unnoticed in native populations for decades. Samples were limited to 1–2 collections per site, so we do not yet know whether the hybrid is dominant in the systems where it is present or whether multiple species coexist. Indeed, both could be true. Phenotypic plasticity in *Vallisneria* spp. is particularly high and dependable diagnostic traits, when available, tend to be

minute staminate characters that are themselves ephemeral, making reliable field-based identifications difficult (McFarland, 2006).

Aquarium trade-based introductions of non-native aquatic species are well documented worldwide (Padilla and Williams, 2004; Martin and Coetzee, 2011; Reid et al., 2019; West et al., 2019) and in Florida (Schmitz et al., 1991). It was not until 1969 that Florida Statute 369.25 (previously 403.271) regulated aquatic plant introductions. Prior to that time, it was common to propagate non-native plant species in Florida's canals and rivers prior to harvest and resale (Schmitz et al., 1991). This practice is thought to have been responsible for the introductions of Eurasian watermilfoil (*Myriophyllum spicatum* Linnaeus), parrots-feather (*Myriophyllum aquaticum* Vell.), and hygrophila (*Hygrophila polysperma* Roxb.) (Schmitz et al., 1991). While the details of *V. spiralis* × *V. denseserrulata* hybridization and/or introduction to the United States remain unknown, the variable success of past submerged aquatic vegetation introductions and a long history of *Vallisneria* aquaculture and commercial trade in the region make the industry and its customers likely vectors for introduction and dispersal.

The impact of widespread non-native hybrids on native *V. americana* populations is not yet known. In some cases, hybridization of non-native plants has been found to increase the success of invasive species in wetlands (Moody and Les, 2002; Wasekura et al., 2016). In others, non-native species have had little to no effect on ecosystem function (Blackburn et al., 2011; Shackelford et al., 2013). Interspecific hybridization is frequently introgressive and, when it is, negative impacts can arise from the effects of outbreeding depression and elevated drift-loads or from hybrid swarming/species assimilation (Allendorf et al., 2001; Edmands, 1999, 2002; Bomblies and Weigel, 2007). All plants sampled in hybrid populations in Japan were female, indicating that the hybrid may be an asexual, dominant genotype (Wasekura et al., 2016). However, it is imperative to understand the biology and ecology of this new exotic species prior to expending management resources on control or eradication, particularly given large recent declines in *V. americana* habitat and population size. Nevertheless, the assumption of monospecific regional populations of *V. americana* as evidenced by the restoration approach must be abandoned. Our study provides an initial, though incomplete, view of hybrid distribution, but its commonness in our sample set should be of concern to resource managers and calls into question the transplanting methods used by recent restoration efforts (e.g., the movement of individuals from wild populations and/or aquaculture throughout the state of Florida and the region). The detection of a non-native species in potential source stock and the unknown status of outplanted cultivars highlights the need for a greater understanding of *Vallisneria* species distribution and phylogeography, population structure, and ecology. Further research needs include more rigorous sampling to determine the locations and range of the hybrid, evaluation of the potential for hybridization between the hybrid and native populations, assessment of the habitat quality provided by the hybrid, determination of whether the hybrid has a competitive advantage over native populations, and development of a protocol for field identification.

CRediT authorship contribution statement

Siobhan B. Gorham: Conceptualization, Visualization, Investigation, Data curation, Writing – original draft. **Seifu Seyoum:** Software, Investigation, Formal analysis, Resources, Writing – review & editing. **Bradley T. Furman:** Visualization, Investigation, Validation, Writing – review & editing. **Kelly M. Darnell:** Writing – review & editing. **Laura K. Reynolds:** Writing – review & editing. **Michael D. Tringali:** Software, Formal analysis, Resources, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

the work reported in this paper.

Acknowledgements

We thank Jaime Richardson, Amanda Christiansen, Maggie Bass, Kyle Miller, Greg Knothe, Taylor Hayhurst, Dr. Patrick Biber, Christian Hayes, Samantha Smith, Eric Sparks, and the FWC Division of Habitat and Species Conservation for collection and transportation of biological material. We are grateful to Dr. Liza Conrad for the use of the Eckerd College greenhouse and to Drs. Carrie Reinhardt, Christine Rohal, and Charles Martin for discussion and helpful contributions. We also thank Craig Mallison and Caroline Gorga for their guidance during the project. Equally, we thank two anonymous reviewers for comments on an earlier draft of this manuscript. This research was partially funded by SJRWMD Contract 31945.

References

- Allendorf, F.W., Leary, R.F., Spruell, P., Wenburg, J.K., 2001. The problems with hybrids: setting conservation guidelines. *Trends Ecol. Evol.* 16, 613–622.
- Baldwin, B.G., 1992. Phylogenetic utility of the internal transcribed spacers of ribosomal DNA in plants: an example from the Compositae. *Mol. Phylogenet. Evol.* 1, 3–16.
- Blackburn, T.M., Pyšek, P., Bacher, S., Carlton, J.T., Duncan, R.P., Jarošík, V., Wilson, J.R.U., Richardson, D.M., 2011. A proposed unified framework for biological invasions. *Trends Ecol. Evol.* 26, 333–339.
- Bomblies, K., Weigel, D., 2007. Hybrid necrosis: autoimmunity as a potential gene-flow barrier in plant species. *Nat. Rev. Genet.* 8, 382–393.
- Burnett, R.K., Lloyd, Michael W., Englehardt, Katharina A.M., Neel, Maile C., 2009. Development of 11 polymorphic microsatellite markers in a macrophyte of conservation concern, *Vallisneria americana* Michaux (Hydrocharitaceae). *Mol. Ecol. Resour.* 9, 1427–1429.
- Diehl, S., Kornijów, R., 1998. Influence of submerged macrophytes on trophic interactions among fish and macroinvertebrates. In: Jeppesen, E., Søndergaard, M., Søndergaard, M., Christoffersen, K. (Eds.), *The Structuring Role of Submerged Macrophytes in Lakes*. Springer, New York, NY.
- Edmands, S., 1999. Heterosis and outbreeding depression in interpopulation crosses spanning a wide range of divergence. *Evolution* 53, 1757–1768.
- Edmands, S., 2002. Does parental divergence predict reproductive compatibility? *Trends Ecol. Evol.* 17 (11), 520–527.
- Engelhardt, K.A.M., Lloyd, M.W., Neel, M.C., 2014. Effects of genetic diversity on conservation and restoration potential at individual, population, and regional scales. *Biol. Conserv.* 179, 6–16.
- Gettys, L., Haller, W.T., 2013. Effect of ecotype, sediment composition, and fertility level on productivity of eight Florida ecotypes of American eelgrass (*Vallisneria spiralis*). *J. Aquat. Plant Manag.* 51, 127–131.
- Godfrey, R.K., Wooten, J.W., 1979. *Aquatic and Wetland Plants of Southeastern United States: Monocotyledons*. University of Georgia Press, Athens.
- Korschgen, C.E., Green, W.L., 1988. American wild celery (*Vallisneria spiralis*): ecological considerations for restoration. Technical Report. U.S. Department of Interior Fish and Wildlife Service, Washington, DC, 0–24.
- Kraemer, G.P., Chamberlain, R.H., Doering, P.H., Steinman, A.D., Hanisak, M.D., 1999. Physiological responses of transplants of the freshwater angiosperm *Vallisneria spiralis* along a salinity gradient in the Caloosahatchee Estuary (Southwestern Florida). *Estuaries* 22, 138–148.
- Les, D.H., Jacobs, S.W.L., Tippery, N.P., Chen, L., Moody, M.L., Wilstermann-Hildebrand, M., 2008. Systematics of *Vallisneria* (Hydrocharitaceae). *Syst. Bot.* 33, 49–65.
- Lloyd, M.W., Burnett, R.K., Engelhardt, K.A.M., Neel, M.C., 2011. The structure of population genetic diversity in *Vallisneria spiralis* in the Chesapeake Bay: implications for restoration. *Conserv. Genet.* 12, 1269–1285.
- Lowden, R.M., 1982. An approach to the taxonomy of *Vallisneria* L. (Hydrocharitaceae). *Aquat. Bot.* 13, 269–298.
- Marie-Victorin, K., 1943. *Les Vallisnères Américaines*. Institut botanique de l'Université de Montréal, Montréal, Can.
- Martin, C.D., Coetzee, J.A., 2011. Pet stores, aquarists and the internet trade as modes of introduction and spread of invasive macrophytes in South Africa. *Water SA* 37.
- McFarland, D., 2006. Reproductive Ecology of *Vallisneria spiralis* Michaux. U.S. Army Engineer Research and Development Center, Vicksburg, MS.
- Michaux, A., 1803. *Flora boreali-Americana*. Crapelet, Parisiis et Argentorati.
- Moody, M.L., Les, D.H., 2002. Evidence of hybridity in invasive watermilfoil (*Myriophyllum*) populations. *Proc. Natl. Acad. Sci. USA* 99, 14867–14871.
- Padilla, D.K., Williams, S.L., 2004. Beyond ballast water: aquarium and ornamental trades as sources of invasive species in aquatic ecosystems. *Front. Ecol. Environ.* 2, 131–138.
- Reich, K., Worthy, G., 2006. An isotopic assessment of the feeding habits of free-ranging manatees. *Mar. Ecol. Prog. Ser.* 322, 303–309.
- Reid, A.J., Carlson, A.K., Creed, I.F., Eliason, E.J., Gell, P.A., Johnson, P.T.J., Kidd, K.A., McCormack, T.J., Olden, J.D., Ormerod, S.J., Smol, J.P., Taylor, W.W., Tockner, K., Vermaire, J.C., Dudgeon, D., Cooke, S.J., 2019. Emerging threats and persistent conservation challenges for freshwater biodiversity. *Biol. Rev.* 94, 849–873.

- Rydberg, P.A., 1909. Elodaceae. North American Flora, New York Botanical Garden, 67–71.
- Schloesser, D.W., Manny, B.A., 2007. Restoration of Wildcelery, *Vallisneria americana* Michx., in the Lower Detroit River of the Lake Huron-Lake Erie Corridor. *J. Gt. Lakes Res.* 33, 8–19.
- Schmitz, D.C., Nelson, B. V., Nall, L. E., Schardt, J. D., 1991. Exotic aquatic plants in Florida: a historical perspective and review of the present aquatic plant regulation program. In: *Proceedings of the Symposium on Exotic Pest Plants*, University of Miami, Miami, Florida, 303–323.
- Selkoe, K.A., Toonen, R.J., 2006. Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. *Ecol. Lett.* 9, 615–629.
- Shackelford, N., Hobbs, R.J., Heller, N.E., Hallett, L.M., Seastedt, T.R., 2013. Finding a middle-ground: the native/non-native debate. *Biol. Conserv.* 158, 55–62.
- Southwest Florida Water Management District (SWFWMD), 2000. Crystal River/Kings Bay Surface Water Improvement and Management (SWIM) Plan, Brooksville, FL. (www.swfwmd.state.fl.us/documents/plans/crystal_river-kings_bay_2000.pdf).
- Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA7: molecular evolutionary genetics analysis version 7.0. *Mol. Biol. Evol.* 30, 2725–2729.
- Tootoonchi, M., Gettys, L.A., Thayer, K.L., Markovich, I.J., Sigmon, J.W., Sadeghibaniani, S., 2020. Ecotypes of aquatic plant *Vallisneria americana* tolerate different salinity concentrations. *Diversity* 12, 65–81.
- Wasekura, H., Horie, S., Fujii, S., Maki, M., 2016. Molecular identification of alien species of *Vallisneria* (Hydrocharitaceae) species in Japan with a special emphasis on the commercially traded accessions and the discovery of hybrid between nonindigenous *V. spiralis* and native *V. denseserrulata*. *Aquat. Bot.* 128, 1–6.
- West, A.M., Jarnevich, C.S., Young, N.E., Fuller, P.L., 2019. Evaluating potential distribution of high-risk aquatic invasive species in the water garden and aquarium trade at a global scale based on current established populations. *Risk Anal.* 39, 1169–1191.