Aquatic Botany 151 (2018) 38–42

Contents lists available at ScienceDirect

Aquatic Botany

journal homepage: www.elsevier.com/locate/aquabot

Genomic variation of introduced *Salvinia minima* in southeastern United States

Carol A. Rowe[a], Donald P. Hauber[b], Paul G. Wolf[a,c,*]

[a] Department of Biology, Utah State University, Logan, UT 84322, USA
[b] Department of Biological Sciences, Loyola University, New Orleans, LA 70118, USA
[c] Ecology Center, Utah State University, Logan, UT 84322, USA

ARTICLE INFO

Keywords:
Fern
RAD-seq
Genetics
Population genetics
Mating system
Invasive species

ABSTRACT

Common salvinia, *Salvinia minima* Baker (Salviniaceae), is a small, floating aquatic fern native to Central and South America that has invaded fresh water bodies in southeastern United States since the 1930s. We examined genetic variation across much of the introduced range of this species in the United States using codominant RAD-seq markers. Data from over 600 variable loci showed a reduction in heterozygosity from east to west in addition to a corresponding trend in assignment of samples to one of two genetic groups. Our data are consistent with previous published work and with the hypothesis that common salvinia had a single introduction on the east end of its current range in the United States. From there it migrated westward, losing genetic diversity during this spread. The data are also consistent with sexual reproduction, although we are unable to estimate the extent of this relative to asexual spreading. Future genetic work should include sampling from the native range to help determine the original sources of North American common salvinia.

1. Introduction

Common salvinia, *Salvinia minima* Baker (Salviniaceae), is a small, floating aquatic fern native to Central and South America and is believed to be first introduced into wetland habitats in the southeastern United States in the late 1920s (Jacono et al., 2001). According to collection records, *S. minima* was probably first introduced into widely separated river drainages across the southeastern US starting in the 1930s (Jacono et al., 2001). *Salvinia minima* has been cultivated in greenhouse aquaria, backyard ponds, and pools as an ornamental plant since the late 1880s (Weatherby, 1921, 1937; Fernald, 1950). Thus, introduction into natural areas likely occurred due to accidental (i.e. flooding) or intentional release. Since its initial introductions in drainages west of the Florida panhandle, it has colonized rapidly into suitable habitats likely assisted by human movement including recreational watercrafts, but the spread is not as aggressive in the rest of Florida (Jacono et al., 2001). This reduced invasiveness was attributed to the presence of the salvinia weevil, *Cyrtobagous salvineae* Calder & Sands which was documented in 68% of the Florida collections (Jacono et al., 2001). However, there is some question as to whether this is the same species as the *C. salvineae* that is found on the giant salvinia (*S. molesta*) in Brazil (Calder and Sands, 1985; Madeira et al., 2006; Russell et al., 2017). *Salvinia minima* can grow and spread rapidly asexually by fragmentation but can also reproduce sexually (De La Sota and Cassá De Pazos, 2001). The base chromosome number in the genus is n = 9. *Salvinia minima* is believed to be tetraploid (2n = 36) but hexaploids (possibly hybrid derivatives involving *S. minima* and *S. sprucei* Kuhn) have been identified near Manaus, Brazil (De La Sota and Cassá De Pazos, 2001). Two previous genetic studies from more than 10 years ago considered population variation along the Gulf Coast resulting in contrasting findings. An isozyme study (Hauber and Lingam, unpub) found no variation among populations from Texas to Florida, whereas a study using dominant RAPD markers (Madeira et al., 2003) found considerable within and between population variation across the Southeast. Here we used a DNA sequencing approach that potentially captures thousands of codominant loci to examine genetic variation of introduced populations of *S. minima*. Our objectives are: 1) To test for multiple introductions of *S. minima* in thesSoutheastern United States, 2) To examine east -west patterns of genetic variation, 3) To determine whether introduced populations are propagating asexually or if within-population variation suggests significant levels of sexual reproduction, and 4) To examine genetic evidence for recent or historical hybridization. We also attempt to explain why different methods detect different levels of genetic variation.

* Corresponding author at: Ecology Center, Utah State University, Logan, UT 84322, USA.
E-mail address: paul.wolf@usu.edu (P.G. Wolf).

https://doi.org/10.1016/j.aquabot.2018.07.011
Received 22 March 2018; Received in revised form 17 July 2018; Accepted 21 July 2018
Available online 27 July 2018

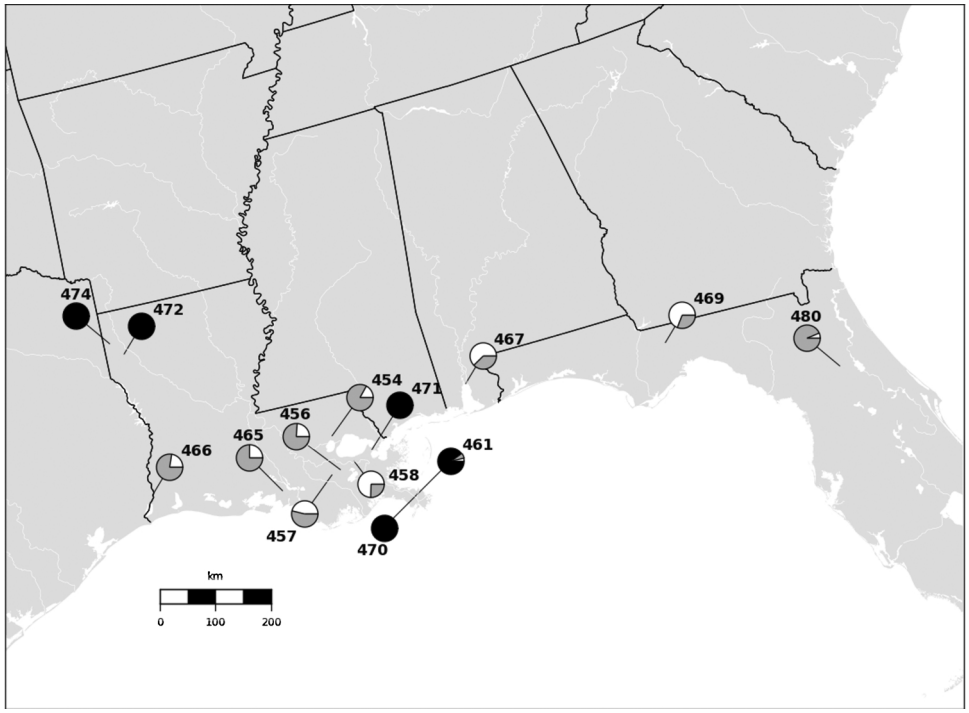

Fig. 1. Map of sampling locations for *Salvinia minima* and *S. molesta* in southeastern United States. Colours denote STRUCTURE assignment at K = 3: white = cluster 1; gray = cluster 2; black = cluster 3 (*S. molesta*). More details on localities in Supplementary Table 1.

2. Methods

2.1. Sampling

Salvinia minima samples were collected from southeastern (Orange County) Texas east along the US Gulf Coast to central (Marion County) Florida (Fig. 1). Efforts were made to include sites where introductions were reported (Jacono et al., 2001). *Salvinia molesta* was also collected at four sites, in part, to control for possible adulterated samples of *S. minima* because immature *S. molesta* can be confused with *S. minima* in the field. Every attempt was made to collect individuals that appeared healthy and separated by > 10 m from subsequent samples. This was true for both *S. minima* and *S. molesta*. Most collections were made fresh and kept in in situ water until DNA extractions. The exceptions were populations 475–481 (Supplementary Table 1), which were collected and stored in silica. Fresh specimens were rinsed in deionized water and forceps were used when needed to remove occasional invertebrates, duckweed and biofilm. Approximately 0.1 g of the healthiest appearing leaflets were excised from the leaf axis for extraction.

2.2. DNA extraction and genomic DNA library preparation

Genomic DNA was extracted using the CTAB method from Neubig et al. (2014). Extractions were assessed for quality and quantity by visualization on a 1% agarose gel and a NanoDrop instrument (Thermo Scientific, Wilmington, MA). The genomic library was made with a double digestion restriction site-associated DNA sequencing (ddRADseq) protocol (Gompert et al., 2012; Parchman et al., 2012), using EcoR1 and Mse1 to fragment the genomic DNA. Fragments were ligated to barcoded (indexed) oligonucleotides (with barcodes unique to each individual) on the EcoRI ends of the fragments. Samples were then PCR-amplified using iproof high-fidelity DNA polymerase (New England Biolabs) with primers that overlap the ligated oligonucleotides. All fragments were first mixed with only one other (barcoded) individual, which then were further amplified in duplicate to reduce stochastic variation in PCR amplification, before final pooling of all barcoded samples. The library was then reduced to fragments in the size range of 250–350 bp using a Blue Pippin (Sage Science, Beverly, MA). Quality and quantity were further verified using TapeStation 2200 (Agilent Technologies). The size-selected, multiplexed samples were run on a single lane of Illumina HiSeq 2500 with 100bp single-end sequencing at Genomic Sequencing and Analysis Facility at the University of Texas at Austin (GSAF).

2.3. Data processing

Raw Illumina reads were processed with ipyrad v.0.5.15 (Eaton, 2014). This process was carried out twice, with the first round using the entire dataset of 96 samples (including *S. molesta* samples) to identify low-coverage and failed reads that should be removed from further analyses, as well as to verify replication for quality control. The second round of analysis was performed on a reduced dataset of 63 *S. minima* individuals and 15 *S. molesta*, and included a more stringent filtering to remove possible duplicated loci, loci with less than 6× coverage, and loci that were not in at least 70% of samples. All raw DNA sequence data plus every detail of the data processing steps and parameters used are available on Digital Commons (https://doi.org/10.15142/T3VK80). Using ipyrad, samples are first demultiplexed and quality filtered. Within-sample clusters are generated using USEARCH (Edgar, 2010), and reads are aligned using MUSCLE (Edgar, 2004). Error rate and heterozygosity are then estimated, and consensus bases are called and filtered. Finally, clusters were generated across samples, and filters are applied to the resulting data, generating a number of genotype output formats. Due to the lack of a reference genome, ipyrad assembled the data de novo using vsearch (Enns et al., 1990). The clustering threshold was set to 90% sequence similarity.

2.4. Analyses

We confirmed genetic differentiation of *S. minima* from *S. molesta* using a neighbor-joining (NJ) tree using adegenet (Jombart, 2008). Because introduced species often have reduced genetic variation, we next used two different methods to identify natural genetic clusters. If both methods converge, we can have more confidence in our

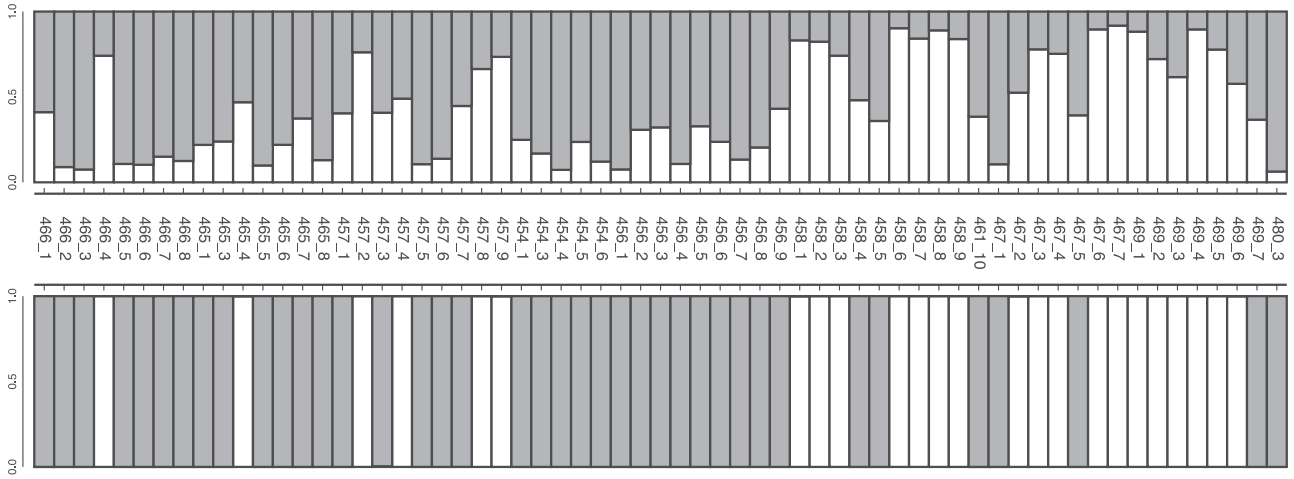

Fig. 2. Results of Structure analysis (top panel) and DAPC (bottom panel) of *S. minima*, for two genetic clusters (K = 2). White = cluster 1; gray = cluster 2. Refer to Fig. 1 and Supplementary Table 1 for localities of samples denoted by numbers.

inferences. These methods used are Discriminant Analysis of Principal Components (DAPC; Jombart and Collins, 2015) using *adegenet* 2.0.1 in R, and STRUCTURE 2.3.4 (Pritchard et al., 2000; Falush et al., 2003, 2007; Hubisz et al., 2009). In both analyses, individuals were treated independently from each other, without any predefined associations due to geographic proximity.

DAPC is a multivariate analysis that builds on the strengths of Principal Component Analysis (PCA) and Discriminant Analysis (DA), where genetic structure among individuals is determined such that within-group variation is minimized while between-group variation is maximized. A k-means algorithm is also used to infer genetic clusters and a statistical measure of goodness of fit using Bayesian Information Criterion (BIC) taken across the number of clusters to infer the optimal number of clusters and individual assignment to each cluster.

STRUCTURE uses a Bayesian clustering approach that first randomly assigns individuals to pre-determined groups (K groups): *S. minima* was tested against 2 through 6 groups, while *S. molesta* was tested across 1 through 4 groups. A Markov Chain Monte Carlo (MCMC) estimation is applied wherein individuals are re-assigned to each group based upon variant frequency estimates. In our analyses, we used a burn-in of 500,000 followed by 1,000,000 iterations with 50 replicates for each of the tested K groups. For each K group tested, a population Q-matrix is formed for each of the 50 replicates, which shows the average individual membership coefficient to each cluster. To determine the "optimal" K value from the STRUCTURE output, we implemented STRUCTURE HARVESTER (Earl and vonHoldt, 2012). This web-based program processes STRUCTURE results across all tested K groups, and performs the Evanno method (Evanno et al., 2005) for detecting the optimal number of K groups that best fit the dataset. Note that the Evanno method does not always select the optimal K groups, most notably if the best group is K = 1. Therefore, we also assessed the effect of number groups (K) using a graphical representation resulting from CLUMPAK (Kopelman et al., 2015), which implements STRUCTURE HARVESTER (Earl and vonHoldt, 2012), CLUMPP (Jakobsson and Rosenberg, 2007), and DISTRUCT (Rosenberg, 2004). Detailed output is shown in our data analyses on Digital Commons (https://doi.org/10.15142/T3VK80). Observing the STRUCTURE patterns as K groups increases, one can assess whether adding each group provides additional, meaningful structure to the data rather than mirroring the structure that is already there. After selecting the optimal K group, we visualized both the DAPC and STRUCTURE results using a custom python script.

We further examined the relationship of longitude with both cluster assignment and heterozygosity using linear regression. Levels of genetic variation were explored by examining patterns of heterozygosity across loci and individuals. Locus heterozygosity was calculated by counting the number of heterozygous individuals, dividing by the total number of individuals, for each locus. Individual heterozygosity was calculated by counting the number of heterozygous loci, dividing by the total number of loci, for each individual. Details for all analyses are available on Digital Commons (https://doi.org/10.15142/T3VK80)

3. Results

Initial analyses with both *S. molesta* and *S. minima* samples were quality-filtered with very low stringency to maintain as many loci as possible that are shared across the two species. Eight samples were considered failures due to extremely low sample coverage. Unfortunately, most of these were from herbarium specimens from the native range of *S. minima*. After filtering, we retained 88 samples with 21,059 loci. Distinct genetic differentiation between *S. minima* and *S. molesta* was confirmed using the NJ tree (not shown). Collection population 461 included 9 individuals of *S. molesta*, and a single *of S. minima*, confirmed by both the NJ tree and DAPC cluster assignment. Individual 465_2 was a far outlier as observed in a scatter plot of the DAPC results. Subsequent analyses focused on *S. minima*, with select comparisons to *S. molesta*, but always treating the species separate and with their own loci.

After data quality filtering the *S. minima* dataset, we retained 687 SNP loci across 63 individuals. DAPC results showed two genetic groups across *S. minima* with 100%, or nearly so, assignment to one group or the other (Fig. 2). From STRUCTURE, plots of the optimal alignment(s) from CLUMPP for each K group clearly show that there is either a single genetic cluster, or two. Where K = 2, CLUMPP resulted in two main alignments in which 23 of the 50 STRUCTURE replicates resulted in two distinctly separated genetic groups which were similar to that observed in DAPC. Whereas DAPC assigned individuals exclusively, or nearly so, to one cluster or the other, STRUCTURE revealed samples with lower individual assignment to each of the two clusters. However, the majority assignment in STRUCTURE was to the same cluster as in DAPC (Fig. 2). This probability cluster assignment from STRUCTURE/CLUMPP was used to further examine their cluster relationship with geography. The proportion of individuals assigned to cluster 1 was regressed against the longitude positions. We detected a positive relationship with a slope of 0.04218, an intercept of 4.21, r = 0.3895, and p = 0.0016 (Fig. 3), indicating that a null hypothesis of no relationship should probably be rejected, but the evidence for such a relationship remains weak. Furthermore, we estimated heterozygosity for *S. minima*, across loci (mean 0.153; S.E. 0.006; Fig. 4) and across individuals (mean 0.144; S.E. 0.022; Fig. 5). The general patterns are consistent with typical distributions for neutral loci (Nei et al., 1976).

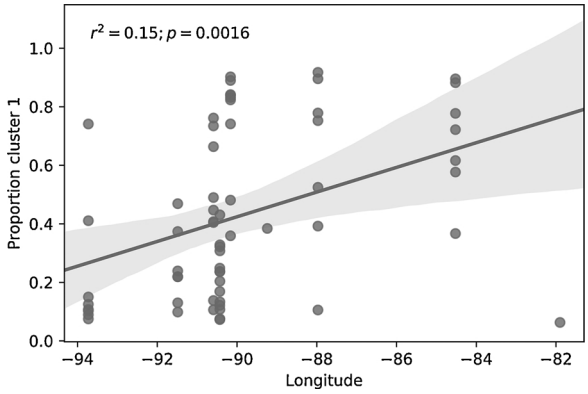

Fig. 3. Scatter plot of proportion of sample clustering with cluster 1 for individual plants as a function of longitude. Shaded area denotes 95% confidence interval of regression line. Numbers on plot refer to site number (at that longitude) as shown on Fig. 1 and in Supplementary Table 1.

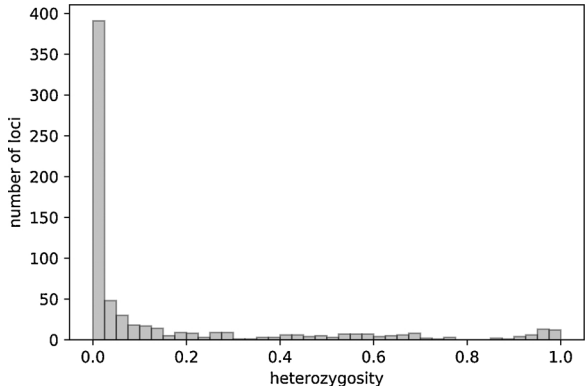

Fig. 4. The distribution of mean heterozygosity across loci for *S. minima*. Locus heterozygosity was calculated by counting the number of heterozygous individuals, dividing by the total number of individuals, for each locus.

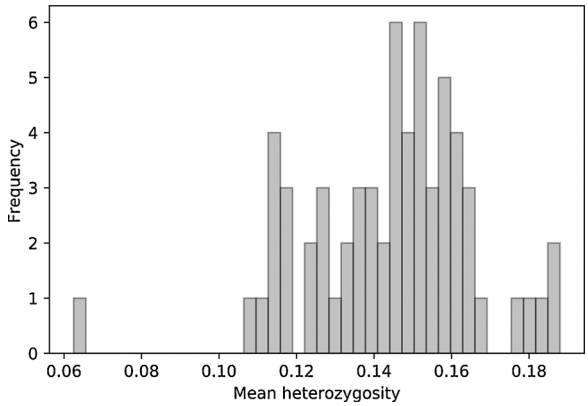

Fig. 5. The distribution of mean heterozygosity across individuals for *S. minima*. Individual heterozygosity was calculated by counting the number of heterozygous loci, dividing by the total number of loci, for each individual.

We also performed a linear regression analysis of heterozygosity (of individuals) on longitude (Fig. 6). The relationship was significant, with a slope of 0.002, intercept of 0.334, r = 0.258, and p = 0.041. This provides some, albeit weak, evidence that heterozygosity is higher in the eastern end of the range. We do not have data from native range, but the levels of heterozygosity detected here are consistent with sexual reproduction in the introduced range of *S. minima*. If populations were spreading only via asexual cloning, then heterozygosity would remain the same. With asexual reproduction of related individuals,

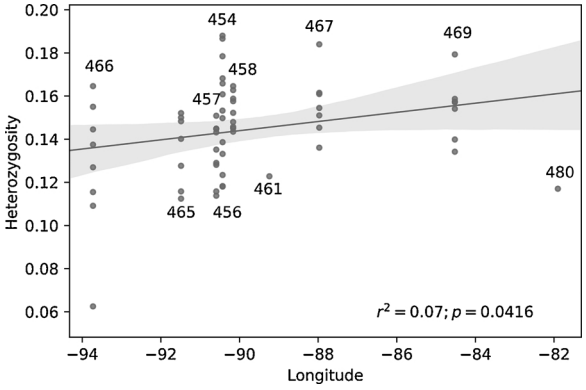

Fig. 6. Linear regression of heterozygosity (of individuals) on longitude. Shaded area denotes 95% confidence interval of regression line. Numbers on plot refer to site number (at that longitude) as shown on Fig. 1 and in Supplementary Table 1.

heterozygosity is expected to drop each generation. Thus, the east-to-west drop in heterozygosity is consistent with sexual reproduction at some point since introduction.

The *S. molesta* dataset was filtered to 15 individuals and 461 loci. Both DAPC and STRUCTURE resulted in a single genetic cluster. Estimated heterozygosity across loci for *S. molesta* (mean 0.1669; S.E. 0.0078) was similar to that observed in *S. minima*.

4. Discussion

Our analysis of over 600 co-dominant genetic markers revealed more variation in *Salvinia* minima than in a previous (unpublished) isozyme study. Our results are consistent with the patterns of variation detected using dominant RAPD markers (Madeira et al., 2003). The two analyses (DAPC and STRUCTURE) we used to examine genetic structure appear to have an optimal number of "source population" at K = 2. This is consistent with two scenarios: one involving two introductions from the native range into southern United States, the alternative with a single introduction (probably in the east) and the second source population represents recombinant genotypes resulting from sexual reproduction of the introduced genotype. Currently, we are not aware of a way to distinguish support for these alternatives, but we are able to say with some confidence is likely that only one or two introductions occurred, and we find no evidence for several independent invasions of *S. minima*. This hypothesis is also supported by the reduction in heterozygosity in the west end of the range of *S. minima*.

Because we have no strong evidence for more than one or two introductions, we cannot fully address our initial goal of determining if there has been hybridization between diverse introduced populations. During some plant invasions, multuple introductions can result in an increase in genotypic variation by hybridization and recombination between invasive genotypes (Lavergne and Molofsky, 2007). Such hybridization can overcome the effects of genetic bottlenecks associated with invasions. We detected no such patterns for *S. minima*.

Depicting relationships among individuals based on variation at polymorphic markers is inherently challenging. Tree figures and even 2- and 3-dimentional PCA depictions can miss underlying genetic structure within genomes. These problems are exacerbated when comparing samples that are in the introduced range of a species in which levels of variation are likely reduced. Our approach, involving large numbers of codominant markers, was aimed to increase the chances of detecting multiple origins, and we find no strong evidence for more than a single origin of *S. minima* in the regions where we sampled.

We suspect that the lack of variation in the isozyme study is a function of reduced variation at the protein level compared to DNA in

general (Kreitman, 1983; Casillas and Barbadilla, 2017) and because isozymes target important metabolic genes, whereas RAPD and ddRAD-seq use nonspecific genomic regions.

Variation in heterozygosity across individuals provides further evidence that sexual reproduction is occurring in the introduced range. This contrasts with the assumption that *S. minima* is sterile and spreads only via clonal reproduction (Jacono et al., 2001; Morgan, 2009). Still, sexual reproduction may indeed be rare, and, if we are examining the descendants of a single introduction then even sexual reproduction is not going to generate much additional variation; only new combinations of standing genetic variation. We did not perform genotype frequency analysis because sample sizes are small and because failure to fit random mating patterns of genotypes can be confounded in populations of an introduced species (where matings would likely be among relatives) and a plant that spreads clonally. Some of the interesting questions that have emerged from this study are: 1) Is there more support for sexual propagation and, if so, how common is it? 2) How is it that, except for the east to west migration, there does not appear to be any evidence of distribution of genetic variation by distance? 3) How does variation in the native range compare with that in the introduced populations? Selecting a few populations from both the western and eastern ends of the introduced range, and collecting larger samples at these sites would allow for a genotype frequency analysis to test for sexual reproduction and better assess population genetic structure. Future work on common salvinia in the native range and comparison with the complete introduced range populations would enable inference as to the origin of introduced populations. Samples we obtained from the native range unfortunately exhibited low sample coverage and could not be included in the analysis. This might be due to poor preservation of herbarium specimens. Aquatic species require special attention to higher than normal silica to specimen ratio. Extracting DNA from fresh specimens collected across the native range of South America would be a more reliable strategy. Here we find that *S. minima* had a single origin in southeastern part of its introduced range in USA, and subsequently moved westward. We find evidence of sexual reproduction and maintenance of moderate levels of genetic variation.

Contributions of authors

DPH and PGW conceived and planned the project.
CAR and DPH performed the molecular work.
CAR performed the data processing, exploration, and analysis.
All three authors wrote the manuscript.

Declarations of interest

None.

Acknowledgements

Thanks to Zach Gompert, Aaron Duffy, and Deepthi Lingam for assistance with this project. We thank the Rev. J. H. Mullahy Endowment for funding this research, and Kathlene Pryer, Alan Smith, Colette Jacono, and Larry Raymond for access to herbarium specimens.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.aquabot.2018.07.011.

References

Calder, A.A., Sands, D.P.A., 1985. A new Brazilian *Cyrtobagous* hustache (Coleoptera: curculionidae) introduced into Australia to control *salvinia*. J. Aust. Entomol. Soc. 24, 57–64.
Casillas, S., Barbadilla, A., 2017. Molecular population genetics. Genetics 205, 1003–1035.
De La Sota, E.R., Cassá De Pazos, L.A., 2001. Two cytotypes and a new hybrid in *Salvinia* séguier. Acta Amazon. 31 557–557.
Earl, D.A., vonHoldt, B.M., 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv. Genet. Resour. 4, 359–361.
Eaton, D.A., 2014. PyRAD: assembly of de novo RADseq loci for phylogenetic analyses. Bioinformatics 30, 1844–1849.
Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32, 1792–1797.
Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26, 2460–2461.
Enns, J.T., Ochs, E.P., Rensink, R.A., 1990. VSearch: macintosh software for experiments in visual search. Behav. Res. Methods 22, 118–122.
Evanno, G., Regnaut, S., Goudet, J., 2005. Detecting the number of clusters of individuals using the software structure: a simulation study. Mol. Ecol. 14, 2611–2620.
Falush, D., Stephens, M., Pritchard, J.K., 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics 164, 1567–1587.
Falush, D., Stephens, M., Pritchard, J.K., 2007. Inference of population structure using multilocus genotype data: dominant markers and null alleles. Mol. Ecol. Notes 7, 574–578.
Fernald, M.L., 1950. Gray's Manual of Botany: A Handbook of the Flowering Plants and Ferns of the Central and Northeastern United States and Adjacent Canada. American Book Company, New York.
Gompert, Z., Lucas, L.K., Nice, C.C., Fordyce, J.A., Forister, M.L., Buerkle, C.A., 2012. Genomic regions with a history of divergent selection affect fitness of hybrids between two butterfly species. Evolution 66, 2167–2181.
Hubisz, M.J., Falush, D., Stephens, M., Pritchard, J.K., 2009. Inferring weak population structure with the assistance of sample group information. Mol. Ecol. Resour. 9, 1322–1332.
Jacono, C.C., Davern, T.R., Center, T.D., 2001. The adventive status of *Salvinia minima* and *S. Molesta* in the southern United States and the related distribution of the weevil *Cyrtobagous salviniae*. Castanea 66, 214–226.
Jakobsson, M., Rosenberg, N.A., 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics 23, 1801–1806.
Jombart, T., 2008. Adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 24, 1403–1405.
Jombart, T., Collins, C., 2015. A Tutorial for Discriminant Analysis of Principle Components (dapc) Using Adegenet 2.0.0.
Kopelman, N.M., Mayzel, J., Jakobsson, M., Rosenberg, N.A., Mayrose, I., 2015. CLUMPAK: a program for identifying clustering modes and packaging population structure inferences across K. Mol. Ecol. Resour. 15, 1179–1191.
Kreitman, M., 1983. Nucleotide polymorphism at the alcohol dehydrogenase locus of *Drosophila melanogaster*. Nature 304, 412–417.
Lavergne, S., Molofsky, J., 2007. Increased genetic variation and evolutionary potential drive the success of an invasive grass. Proc. Natl. Acad. Sci. (U. S. A.) 104, 3883–3888.
Madeira, P.T., Jacono, C.C., Tipping, P., Van, T.K., Center, T.D., 2003. A genetic survey of *Salvinia minima* in the southern United States. Aquat. Bot. 76, 127–139.
Madeira, P.T., Tipping, P.W., Gandolfo, D.E., Center, T.D., Van, T.K., O'brien, C.W., 2006. Molecular and morphological examination of *Cyrtobagous* sp. collected from Argentina, Paraguay, Brazil, Australia, and Florida. BioContol 51, 679–701.
Morgan, V.H., 2009. Salvinia minima. Nonindigenous Aquatic Species Database. USGS, Gainesville, FL USGS: Nonindigenous Aquatic Species Database, Gainesville, FL.
Nei, M., Fuerst, P.A., Chakraborty, R., 1976. Testing the neutral mutation hypothesis by distribution of single locus heterozygosity. Nature 262, 491.
Neubig, K.M., Whitten, W.M., Abbott, J.R., Elliott, S., Soltis, D.E., Soltis, P.S., 2014. Variables affecting DNA preservation in archival DNA specimens. In: Applequist, W.L., Campbell, L.M. (Eds.), DNA Banking for the 21st Century: Proceedings of the U.S. Workshop on DNA Banking. The William L. Brown Center at the Missouri Botanical Garden, St. Louis, MO, USA.
Parchman, T.L., Gompert, Z., Mudge, J., Schilkey, F.D., Benkman, C.W., Buerkle, C.A., 2012. Genome-wide association genetics of an adaptive trait in lodgepole pine. Mol. Ecol. 21, 2991–3005.
Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. Genetics 155, 945–959.
Rosenberg, N.A., 2004. DISTRUCT: a program for the graphical display of population structure. Mol. Ecol. 4, 137–138.
Russell, A., McKay, F., Moshman, L., Madeira, P.T., Diaz, R., Johnson, S., Cibils, X., Blair, Z., 2017. Surveys in Argentina and Uruguay Reveal *Cyrtobagous salviniae* (Coleoptera: Curculionidae) populations Adapted to Survive Temperate Climates in Southeastern USA. V. 107.
Weatherby, C.A., 1921. Other records of *Salvinia natans* in the United States. Am. Fern J. 11, 50–53.
Weatherby, C.A., 1937. A further note on *Salvinia*. Am. Fern J. 27, 98–102.