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

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Declarations of interest:

None

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

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.

Keywords: fern, RAD-seq, genetics, population genetics, mating system, invasive species

35 **1. Introduction**

36 Common salvinia, *Salvinia minima* Baker (Salviniaceae), is a small, floating aquatic fern native to
37 Central and South America and is believed to be first introduced into wetland habitats in the
38 southeastern United States in the late 1920s (Jacono et al., 2001). According to collection
39 records, *S. minima* was probably first introduced into widely separated river drainages across
40 the southeastern US starting in the 1930s (Jacono et al., 2001). *Salvinia minima* has been
41 cultivated in greenhouse aquaria, backyard ponds, and pools as an ornamental plant since the
42 late 1880s (Weatherby, 1921, 1937; Fernald, 1950). Thus, introduction into natural areas likely
43 occurred due to accidental (i.e. flooding) or intentional release. Since its initial introductions in
44 drainages west of the Florida panhandle, it has colonized rapidly into suitable habitats likely
45 assisted by human movement including recreational watercrafts, but the spread is not as
46 aggressive in the rest of Florida (Jacono et al., 2001). This reduced invasiveness was attributed
47 to the presence of the salvinia weevil, *Cyrtobagous salvineae* Calder & Sands which was
48 documented in 68% of the Florida collections (Jacono et al., 2001). However, there is some
49 question as to whether this is the same species as the *C. salvineae* that is found on the giant
50 salvinia (*S. molesta*) in Brazil (Calder and Sands, 1985; Madeira et al., 2006; Russell et al., 2017).
51 *Salvinia minima* can grow and spread rapidly asexually by fragmentation but can also reproduce
52 sexually (De La Sota and Cassá De Pazos, 2001). The base chromosome number in the genus is
53 n = 9. *Salvinia minima* is believed to be tetraploid (2n = 36) but hexaploids (possibly hybrid
54 derivatives involving *S. minima* and *S. sprucei* Kuhn) have been identified near Manaus, Brazil
55 (De La Sota and Cassá De Pazos, 2001). Two previous genetic studies from more than 10 years
56 ago considered population variation along the Gulf Coast resulting in contrasting findings. An

57 isozyme study (Hauber and Lingam, unpub) found no variation among populations from Texas
58 to Florida, whereas a study using dominant RAPD markers (Madeira et al., 2003) found
59 considerable within and between population variation across the Southeast. Here we used a
60 DNA sequencing approach that potentially captures thousands of codominant loci to examine
61 genetic variation of introduced populations of *S. minima*. Our objectives are: 1) To test for
62 multiple introductions of *S. minima* in thesSoutheastern United States, 2) To examine east -
63 west patterns of genetic variation, 3) To determine whether introduced populations are
64 propagating asexually or if within-population variation suggests significant levels of sexual
65 reproduction, and 4) To examine genetic evidence for recent or historical hybridization. We
66 also attempt to explain why different methods detect different levels of genetic variation.
67
68 **2. Methods**
69 *2.1 Sampling*
70 *Salvinia minima* samples were collected from southeastern (Orange County) Texas east along
71 the US Gulf Coast to central (Marion County) Florida (Fig. 1). Efforts were made to include sites
72 where introductions were reported (Jacono et al., 2001). *Salvinia molesta* was also collected at
73 four sites, in part, to control for possible adulterated samples of *S. minima* because immature *S.*
74 *molesta* can be confused with *S. minima* in the field. Every attempt was made to collect
75 individuals that appeared healthy and separated by >10 m from subsequent samples. This was
76 true for both *S. minima* and *S. molesta*. Most collections were made fresh and kept in *in situ*
77 water until DNA extractions. The exceptions were populations 475-481 (Supplementary Table
78 1), which were collected and stored in silica. Fresh specimens were rinsed in deionized water

79 and forceps were used when needed to remove occasional invertebrates, duckweed and
80 biofilm. Approximately 0.1g of the healthiest appearing leaflets were excised from the leaf axis
81 for extraction.
82
83 *2. 2 DNA extraction and Genomic DNA library preparation*
84 Genomic DNA was extracted using the CTAB method from Neubig et al. (2014). Extractions
85 were assessed for quality and quantity by visualization on a 1% agarose gel and a NanoDrop
86 instrument (Thermo Scientific, Wilmington, MA). The genomic library was made with a double
87 digestion restriction site-associated DNA sequencing (ddRADseq) protocol (Gompert et al.,
88 2012; Parchman et al., 2012), using EcoR1 and Mse1 to fragment the genomic DNA. Fragments
89 were ligated to barcoded (indexed) oligonucleotides (with barcodes unique to each individual)
90 on the EcoRI ends of the fragments. Samples were then PCR-amplified using iproof high-fidelity
91 DNA polymerase (New England Biolabs) with primers that overlap the ligated oligonucleotides.
92 All fragments were first mixed with only one other (barcoded) individual, which then were
93 further amplified in duplicate to reduce stochastic variation in PCR amplification, before final
94 pooling of all barcoded samples. The library was then reduced to fragments in the size range of
95 250-350 bp using a Blue Pippin (Sage Science, Beverly, MA). Quality and quantity were further
96 verified using TapeStation 2200 (Agilent Technologies). The size-selected, multiplexed samples
97 were run on a single lane of Illumina HiSeq 2500 with 100bp single-end sequencing at Genomic
98 Sequencing and Analysis Facility at the University of Texas at Austin (GSAF).
99
100 *2. 3 Data processing*

101 Raw Illumina reads were processed with ipyrad v.0.5.15 (Eaton, 2014). This process was carried
102 out twice, with the first round using the entire dataset of 96 samples (including *S. molesta*
103 samples) to identify low-coverage and failed reads that should be removed from further
104 analyses, as well as to verify replication for quality control. The second round of analysis was
105 performed on a reduced dataset of 63 *S. minima* individuals and 15 *S. molesta*, and included a
106 more stringent filtering to remove possible duplicated loci, loci with low coverage, and loci that
107 were not in at least 70% of samples. All raw DNA sequence data plus every detail of the data
108 processing steps and parameters used are available on Digital Commons
109 (https://doi.org/10.15142/T3VK80). Using ipyrad, samples are first demultiplexed and quality
110 filtered. Within-sample clusters are generated using USEARCH (Edgar, 2010), and reads are
111 aligned using MUSCLE (Edgar, 2004). Error rate and heterozygosity are then estimated, and
112 consensus bases are called and filtered. Finally, clusters were generated across samples, and
113 filters are applied to the resulting data, generating a number of genotype output formats. Due
114 to the lack of a reference genome, ipyrad assembled the data de novo using vsearch (Enns et
115 al., 1990). The clustering threshold was set to 90% sequence similarity.
116
117 *2. 4 Analyses*
118 We confirmed genetic differentiation of *S. minima* from *S. molesta* using a neighbor-joining (NJ)
119 tree using adegenet (Jombart, 2008). Because introduced species often have reduced genetic
120 variation, we next used two different methods to identify natural genetic clusters. If both
121 methods converge, we can have more confidence in our inferences. These methods used are
122 Discriminant Analysis of Principal Components (DAPC; Jombart and Collins, 2015) using

123 *adegenet* 2.0.1 in R, and STRUCTURE 2.3.4 (Pritchard et al., 2000; Falush et al., 2003; Falush et
124 al., 2007; Hubisz et al., 2009). In both analyses, individuals were treated independently from
125 each other, without any predefined associations due to geographic proximity.
126 DAPC is a multivariate analysis that builds on the strengths of Principal Component
127 Analysis (PCA) and Discriminant Analysis (DA), where genetic structure among individuals is
128 determined such that within-group variation is minimized while between-group variation is
129 maximized. A k-means algorithm is also used to infer genetic clusters and a statistical measure
130 of goodness of fit using Bayesian Information Criterion (BIC) taken across the number of
131 clusters to infer the optimal number of clusters and individual assignment to each cluster.
132 STRUCTURE uses a Bayesian clustering approach that first randomly assigns individuals
133 to pre-determined groups (K groups): *S. minima* was tested against 2 through 6 groups, while *S.*
134 *molesta* was tested across 1 through 4 groups. A Markov Chain Monte Carlo (MCMC)
135 estimation is applied wherein individuals are re-assigned to each group based upon variant
136 frequency estimates. In our analyses, we used a burn-in of 500,000 followed by 1,000,000
137 iterations with 50 replicates for each of the tested K groups. For each K group tested, a
138 population Q-matrix is formed for each of the 50 replicates, which shows the average individual
139 membership coefficient to each cluster. To determine the "optimal" K value from the
140 STRUCTURE output, we implemented STRUCTURE HARVESTER (Earl and vonHoldt, 2012). This
141 web-based program processes STRUCTURE results across all tested K groups, and performs the
142 Evanno method (Evanno et al., 2005) for detecting the optimal number of K groups that best fit
143 the dataset. Note that the Evanno method does not always select the optimal K groups, most
144 notably if the best group is K=1. Therefore, we also assessed the effect of number groups (K)

145 using a graphical representation resulting from CLUMPAK (Kopelman et al., 2015), which
146 implemnts STRUCTURE HARVESTER (Earl and vonHoldt, 2012), CLUMPP (Jakobsson and
147 Rosenberg, 2007), and DISTRUCT (Rosenberg, 2004). Detailed output is shown in our data
148 analyses on Digital Commons (https://doi.org/10.15142/T3VK80). Observing the STRUCTURE
149 patterns as K groups increases, one can assess whether adding each group provides additional,
150 meaningful structure to the data rather than mirroring the structure that is already there. After
151 selecting the optimal K group, we visualized both the DAPC and STRUCTURE results using a
152 custom python script.
153 We further examined the relationship of longitude with both cluster assignment and
154 heterozygosity using linear regression. Levels of genetic variation were explored by examining
155 patterns of heterozygosity across loci and individuals. Details for all analyses are available on
156 Digital Commons (https://doi.org/10.15142/T3VK80)
157
158 **3. Results**
159 Initial analyses with both *S. molesta* and *S. minima* samples were quality-filtered with very low
160 stringency to maintain as many loci as possible that are shared across the two species. Eight
161 samples were considered failures due to extremely low sample coverage. Unfortunately, most
162 of these were from herbarium specimens from the native range of *S. minima*. After filtering, we
163 retained 88 samples with 21,059 loci. Distinct genetic differentiation between *S. minima* and *S.*
164 *molesta* was confirmed using the NJ tree (not shown). Collection population 461 included 9
165 individuals of *S. molesta*, and a single *of S. minima*, confirmed by both the NJ tree and DAPC
166 cluster assignment. Individual 465_2 was a far outlier as observed in a scatter plot of the DAPC

167 results. Subsequent analyses focused on *S. minima*, with select comparisons to *S. molesta*, but
168 always treating the species separate and with their own loci.
169 After data quality filtering the *S. minima* dataset, we retained 687 SNP loci across 63
170 individuals. DAPC results showed two genetic groups across *S. minima* with 100%, or nearly so,
171 assignment to one group or the other (Fig. 2). From STRUCTURE, plots of the optimal
172 alignment(s) from CLUMPP for each K group clearly show that there is either a single genetic
173 cluster, or two. Where K=2, CLUMPP resulted in two main alignments in which 23 of the 50
174 STRUCTURE replicates resulted in two distinctly separated genetic groups which were similar to
175 that observed in DAPC. Whereas DAPC assigned individuals exclusively, or nearly so, to one
176 cluster or the other, STRUCTURE revealed samples with lower individual assignment to each of
177 the two clusters. However, the majority assignment in STRUCTURE was to the same cluster as in
178 DAPC (Fig. 2). This probability cluster assignment from STRUCTURE/CLUMPP was used to
179 further examine their cluster relationship with geography. The proportion of individuals
180 assigned to cluster 1 was regressed against the longitude positions. We detected a positive
181 relationship with a slope of 0.04218, an intercept of 4.21, $r = 0.3895$, and $p = 0.0016$ (Fig. 3),
182 indicating that a null hypothesis of no relationship should probably be rejected. Furthermore,
183 we estimated heterozygosity for *S. minima*, across loci (mean 0.153; S.E. 0.006; Fig. 4) and
184 across individuals (mean 0.144; S.E. 0.022; Fig. 5). The general patterns are consistent with
185 typical distributions for neutral loci (Nei et al., 1976). We also performed a linear regression
186 analysis of heterozygosity (of individuals) on longitude (Fig 6). The relationship was significant,
187 with a slope of 0.002, intercept of 0.334, $r = 0.258$, and $p = 0.041$. This provides some evidence
188 that heterozygosity is higher in the eastern end of the range. We do not have data from native

189 range, but the levels of heterozygosity detected here are consistent with sexual reproduction in
190 the introduced range of *S. minima*. If populations were spreading only via asexual cloning, then
191 heterozygosity would remain the same. With asexual reproduction of related individuals,
192 heterozygosity is expected to drop each generation. Thus, the east-to-west drop in
193 heterozygosity is consistent with sexual reproduction at some point since introduction.
194 We also provide a neighbor-joining tree of individuals based on genetic distance (Figure
195 7) depicting relationships among samples in this study.
196 The *S. molesta* dataset was filtered to 15 individuals and 461 loci. Both DAPC and
197 STRUCTURE resulted in a single genetic cluster. Estimated heterozygosity across loci for *S.*
198 *molesta* (mean 0.1669; S.E. 0.0078) was similar to that observed in *S. minima*.
199

200 **4. Discussion**

201 Our analysis of over 600 co-dominant genetic markers revealed more variation in *Salvinia*
202 minima than in a previous (unpublished) isozyme study. Our results are consistent with the
203 patterns of variation detected using dominant RAPD markers (Madeira et al., 2003). The two
204 analyses (DAPC and STRUCTURE) we used to examine genetic structure indicated two genetic
205 clusters and it is possible that these represent separate introductions. However, it is probably
206 more likely that the pattern is caused by introduction in the eastern end of the range with loss
207 of variation during westward migration. This hypothesis is also supported by the reduction in
208 heterozygosity in the west end of the range of *S. minima*.
209 Because we have no strong evidence for more than one introduction, we cannot fully
210 address our initial goal of determining if there has been hybridization between diverse

211 introduced populations. During some plant invasions, multuple introductions can result in an
212 increase in genotypic variation by hybridization and recombination between invasive genotypes
213 (Lavergne and Molofsky, 2007). Such hybridization can overcome the effects of genetic
214 bottlenecks associated with invasions. We detected no such patterns for *S. minima*.

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

222 We suspect that the lack of variation in the isozyme study is a function of reduced
223 variation at the protein level compared to DNA in general (Kreitman, 1983; Casillas and
224 Barbadilla, 2017) and because isozymes target important metabolic genes, whereas RAPD and
225 ddRAD-seq use nonspecific genomic regions.

226 We detected moderate levels of heterozygosity and nucleotide variation that are
227 consistent with some sexual reproduction. Furthermore, variation in heterozygosity across
228 individuals provides further evidence that sexual reproduction is occurring in the introduced
229 range. This contrasts with the assumption that *S. minima* is sterile and spreads only via clonal
230 reproduction (Jacono et al., 2001; Morgan, 2009). Still, sexual reproduction may indeed be rare,
231 and, if we are examining the descendants of a single introduction then even sexual
232 reproduction is not going to generate much additional variation, only new combinations of

233 standing genetic variation. We did not perform genotype frequency analysis because sample
234 sizes are small and because failure to fit random mating patterns of genotypes can be
235 confounded in populations of an introduced species (where matings would likely be among
236 relatives) and a plant that spreads clonally. Some of the interesting questions that have
237 emerged from this study are: 1) Is there more support for sexual propagation and, if so, how
238 common is it? 2) How is it that, except for the east to west migration, there does not appear to
239 be any evidence of distribution of genetic variation by distance? 3) How does variation in the
240 native range compare with that in the introduced populations? Selecting a few populations
241 from both the western and eastern ends of the introduced range, and collecting larger samples
242 at these sites would allow for a genotype frequency analysis to test for sexual reproduction and
243 better assess population genetic structure. Future work on common salvinia in the native range
244 and comparison with the complete introduced range populations would enable inference as to
245 the origin of introduced populations. Samples we obtained from the native range unfortunately
246 exhibited low sample coverage and could not be included in the analysis. This might be due to
247 poor preservation of herbarium specimens. Aquatic species require special attention to higher
248 than normal silica to specimen ratio. Extracting DNA from fresh specimens collected across the
249 native range of South America would be a more reliable strategy. Here we find that *S. minima*
250 had a single origin in southeastern part of its introduced range in USA, and subsequently moved
251 westward. We find evidence of sexual reproduction and maintenance of moderate levels of
252 genetic variation.
253
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258

259

260 **References**

261 Calder, A.A., Sands, D.P.A., 1985. A new Brazilian *Cyrtobagous* Hustache (Coleoptera: Curculionidae)
262 introduced into Australia to control *salvinia*. Journal of the Australian Entomological Society 24,
263 57-64.
264 Casillas, S., Barbadilla, A., 2017. Molecular population genetics. Genetics 205, 1003-1035.
265 De La Sota, E.R., Cassá De Pazos, L.A., 2001. Two cytotypes and a new hybrid in *Salvinia* Séguier. Acta
266 Amazonica 31, 557-557.
267 Earl, D.A., vonHoldt, B.M., 2012. STRUCTURE HARVESTER: a website and program for visualizing
268 STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources 4,
269 359-361.
270 Eaton, D.A., 2014. PyRAD: assembly of de novo RADseq loci for phylogenetic analyses. Bioinformatics 30,
271 1844-1849.
272 Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput.
273 Nucleic Acids Research 32, 1792-1797.
274 Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26, 2460-
275 2461.
276 Enns, J.T., Ochs, E.P., Rensink, R.A., 1990. VSearch: Macintosh software for experiments in visual search.
277 Behavior Research Methods 22, 118-122.
278 Evanno, G., Regnaut, S., Goudet, J., 2005. Detecting the number of clusters of individuals using the
279 software structure: a simulation study. Molecular Ecology 14, 2611-2620.
280 Falush, D., Stephens, M., Pritchard, J.K., 2003. Inference of population structure using multilocus
281 genotype data: Linked loci and correlated allele frequencies. Genetics 164, 1567–1587.
282 Falush, D., Stephens, M., Pritchard, J.K., 2007. Inference of population structure using multilocus
283 genotype data: Dominant markers and null alleles. Molecular Ecology Notes 7, 574–578.

284 Fernald, M.L., 1950, Gray's Manual of Botany: a handbook of the flowering plants and ferns of the
285 central and northeastern United States and adjacent Canada. American Book Company, New
286 York.
287 Gompert, Z., Lucas, L.K., Nice, C.C., Fordyce, J.A., Forister, M.L., Buerkle, C.A., 2012. Genomic regions
288 with a history of divergent selection affect fitness of hybrids between two butterfly species.
289 Evolution 66, 2167-2181.
290 Hubisz, M.J., Falush, D., Stephens, M., Pritchard, J.K., 2009. Inferring weak population structure with the
291 assistance of sample group information. Molecular Ecology Resources 9, 1322–1332.
292 Jacono, C.C., Davern, T.R., Center, T.D., 2001. The adventive status of *Salvinia minima* and *S. molesta* in
293 the southern United States and the related distribution of the weevil *Cyrtobagous salviniae*.
294 Castanea 66, 214-226.
295 Jakobsson, M., Rosenberg, N.A., 2007. CLUMPP: a cluster matching and permutation program for
296 dealing with label switching and multimodality in analysis of population structure.
297 Bioinformatics 23, 1801-1806.
298 Jombart, T., 2008. adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics
299 24, 1403-1405.
300 Jombart, T., Collins, C., 2015, A tutorial for discriminant analysis of principle components (dapc) using
301 adegenet 2.0.0.
302 Kopelman, N.M., Mayzel, J., Jakobsson, M., Rosenberg, N.A., Mayrose, I., 2015. CLUMPAK: a program for
303 identifying clustering modes and packaging population structure inferences across K. Molecular
304 Ecology Resources 15, 1179-1191.
305 Kreitman, M., 1983. Nucleotide polymorphism at the alcohol dehydrogenase locus of *Drosophila*
306 *melanogaster*. Nature 304, 412-417.

307 Lavergne, S., Molofsky, J., 2007. Increased genetic variation and evolutionary potential drive the success
308 of an invasive grass. Proceedings of the National Academy of Sciences (USA) 104, 3883-3888.
309 Madeira, P.T., Jacono, C.C., Tipping, P., Van, T.K., Center, T.D., 2003. A genetic survey of *Salvinia minima*
310 in the southern United States. Aquatic Botany 76, 127-139.
311 Madeira, P.T., Tipping, P.W., Gandolfo, D.E., Center, T.D., Van, T.K., O'brien, C.W., 2006. Molecular and
312 morphological examination of *Cyrtobagous* sp. collected from Argentina, Paraguay, Brazil,
313 Australia, and Florida. BioContol 51, 679-701.
314 Morgan, V.H., 2009, *Salvinia minima. USGS: Nonindigenous Aquatic Species Database.* Gainesville, FL. .
315 USGS: Nonindigenous Aquatic Species Database, Gainesville, FL.
316 Nei, M., Fuerst, P.A., Chakraborty, R., 1976. Testing the neutral mutation hypothesis by distribution of
317 single locus heterozygosity. Nature 262, 491.
318 Neubig, K.M., Whitten, W.M., Abbott, J.R., Elliott, S., Soltis, D.E., Soltis, P.S., 2014, Variables Affecting
319 DNA Preservation in Archival DNA Specimens. In: Applequist, W.L., Campbell, L.M. (Eds.), DNA
320 Banking for the 21st Century: Proceedings of the U.S. Workshop on DNA Banking. The William L.
321 Brown Center at the Missouri Botanical Garden, St. Louis, MO, USA.
322 Parchman, T.L., Gompert, Z., Mudge, J., Schilkey, F.D., Benkman, C.W., Buerkle, C.A., 2012. Genome-
323 wide association genetics of an adaptive trait in lodgepole pine. Molecular Ecology 21, 2991-
324 3005.
325 Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus
326 genotype data. Genetics 155, 945–959.
327 Rosenberg, N.A., 2004. DISTRUCT: a program for the graphical display of population structure. Molecular
328 Ecology 4, 137-138.

329 Russell, A., McKay, F., Moshman, L., Madeira, P.T., Diaz, R., Johnson, S., Cibils, X., Blair, Z., 2017. Surveys
330 in Argentina and Uruguay reveal *Cyrtobagous salviniae* (Coleoptera: Curculionidae) populations
331 adapted to survive temperate climates in southeastern USA. v. 107.
332 Weatherby, C.A., 1921. Other records of *Salvinia natans* in the United States. American Fern Journal 11,
333 50-53.
334 Weatherby, C.A., 1937. A further note on *Salvinia*. American Fern Journal 27, 98-102.

335

Figure legends

Figure 1. Map of sampling locations for *Salvinia minima* and *S. molesta* in southeastern United States. Colours denote STRUCTURE assignment at K=3: green = cluster 1; blue = cluster 2; magenta = cluster 3 (*S. molesta*).

Figure 2. Results of Structure analysis (top panel) and DAPC (bottom panel) of *S. minima*, for two genetic clusters (K = 2). Green = cluster 1; blue = cluster 2.

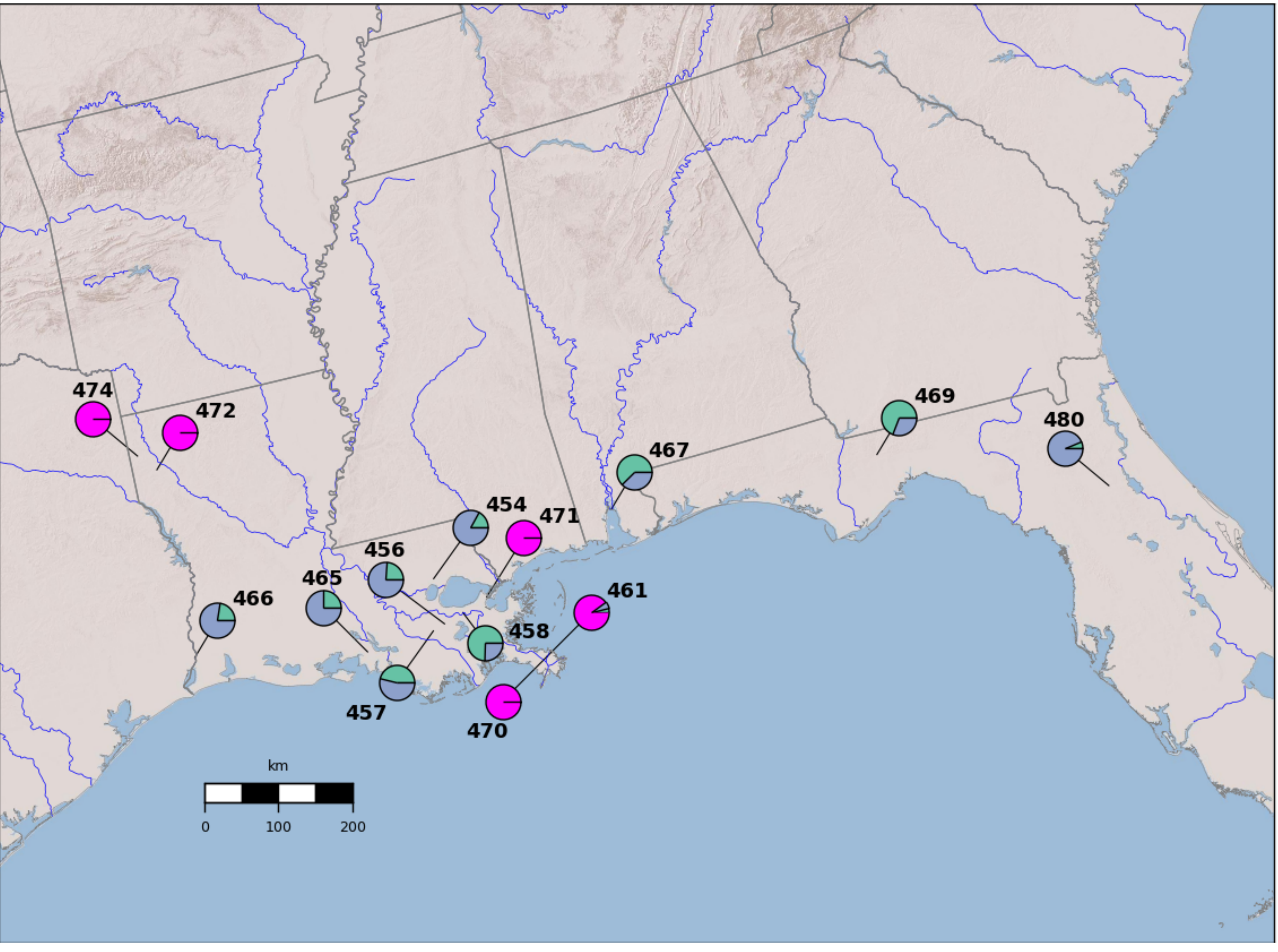
Figure 3. Scatter plot of proportion of sample clustering with cluster 1 as a function of longitude

Figure 4. The distribution of mean heterozygosity across loci for *S. minima*.

Figure 5. The distribution of mean heterozygosity across individuals for *S. minima*.

Figure 6. Linear regression analysis of heterozygosity (of individuals) on longitude.

Figure 7. Neighbor-joining tree of individuals of *S. minima*. Colours denote genetic assignment based on STRUCTURE at K = 2. Green = cluster 1; blue = cluster 2.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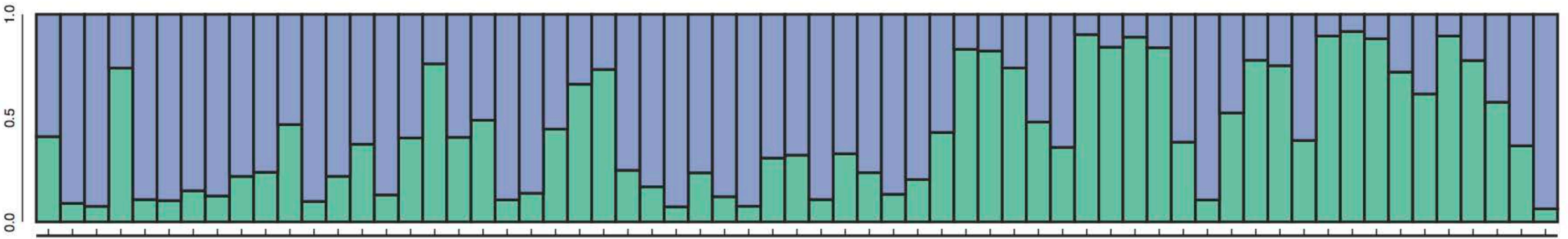

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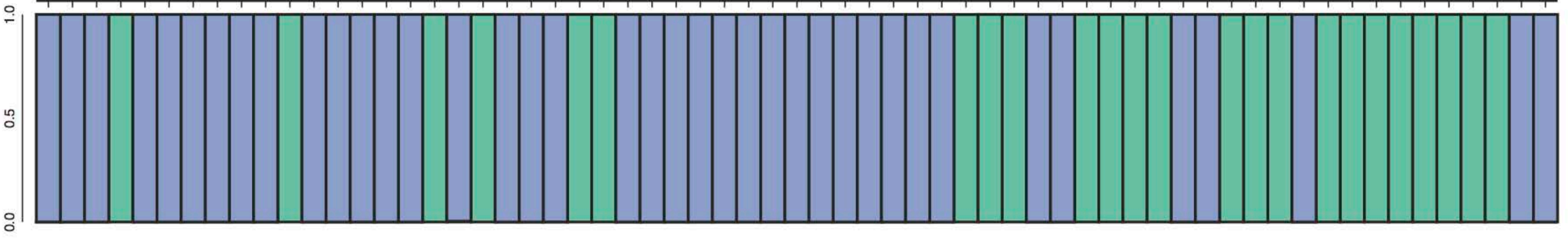
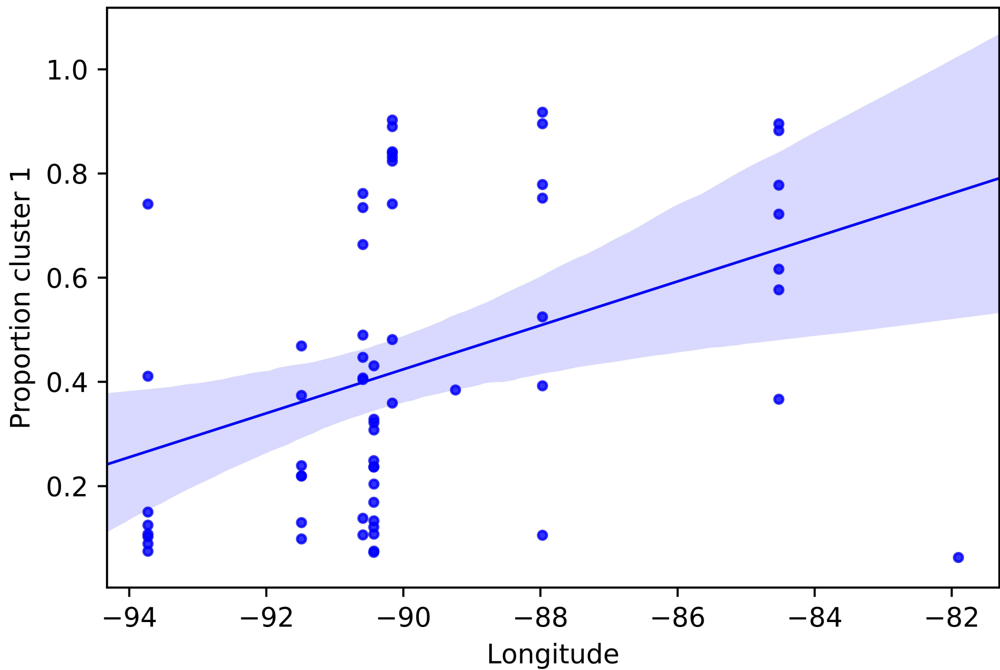
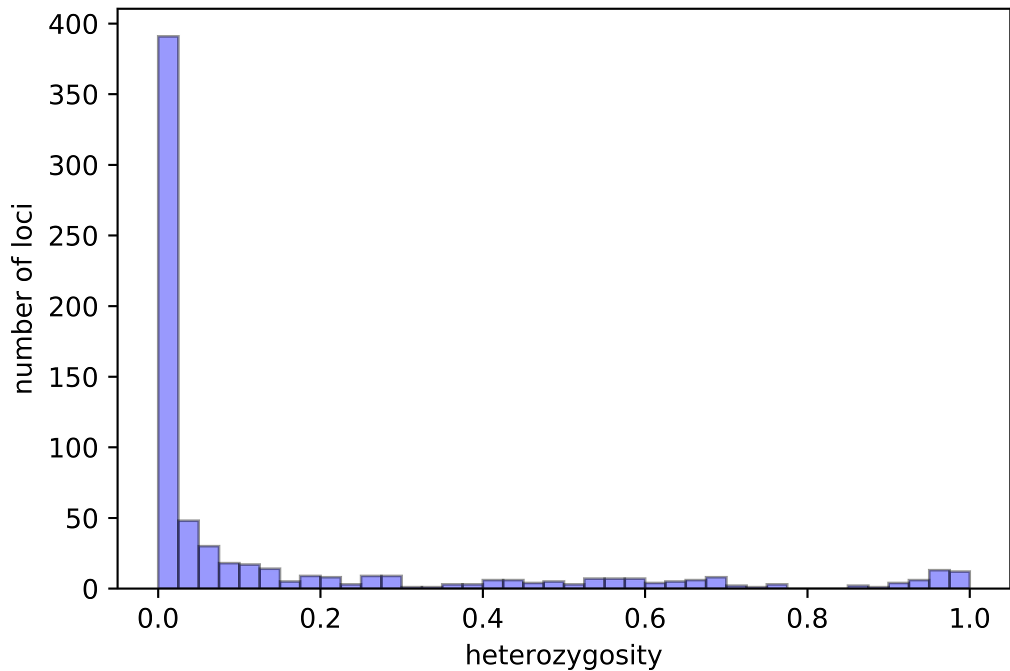
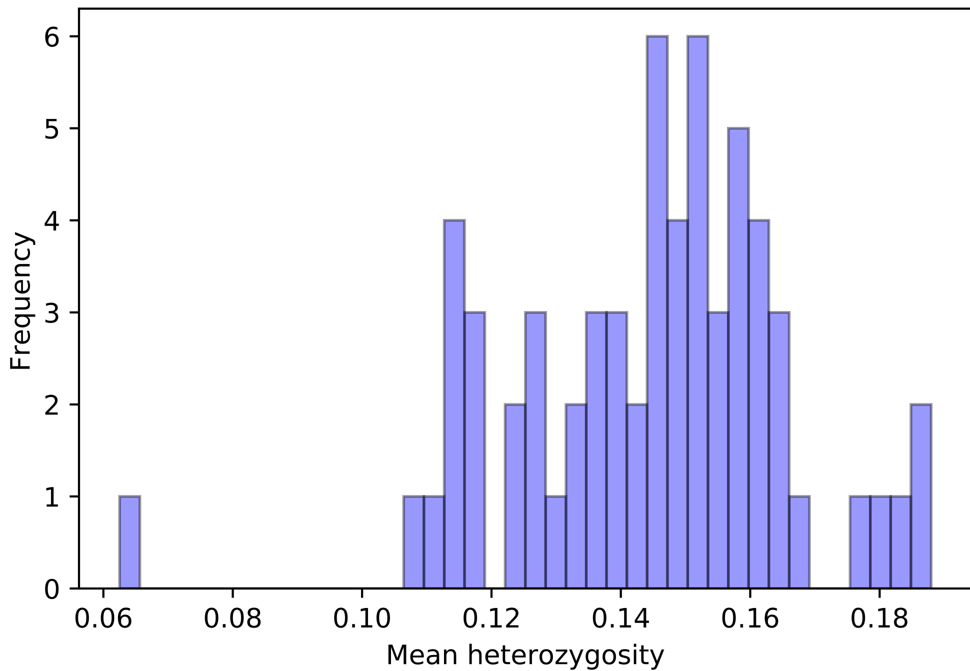
480_3



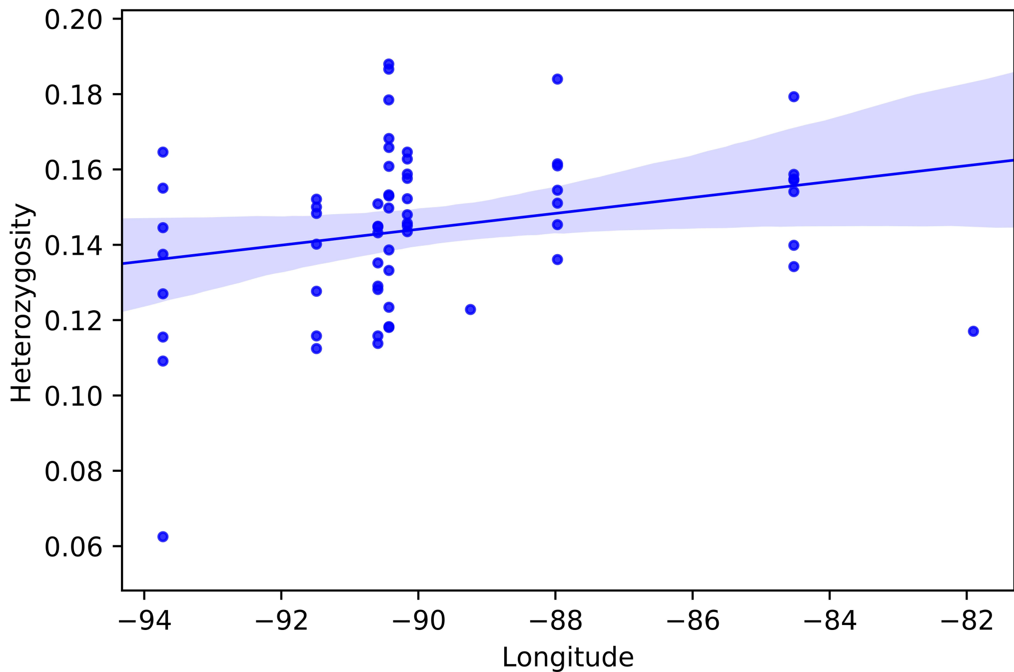
1.0
0.5
0.0


Proportion cluster 1
1.0
0.8
0.6
0.4
0.2
−94
−92
−90
−88
−86
−84
−82
Longitude

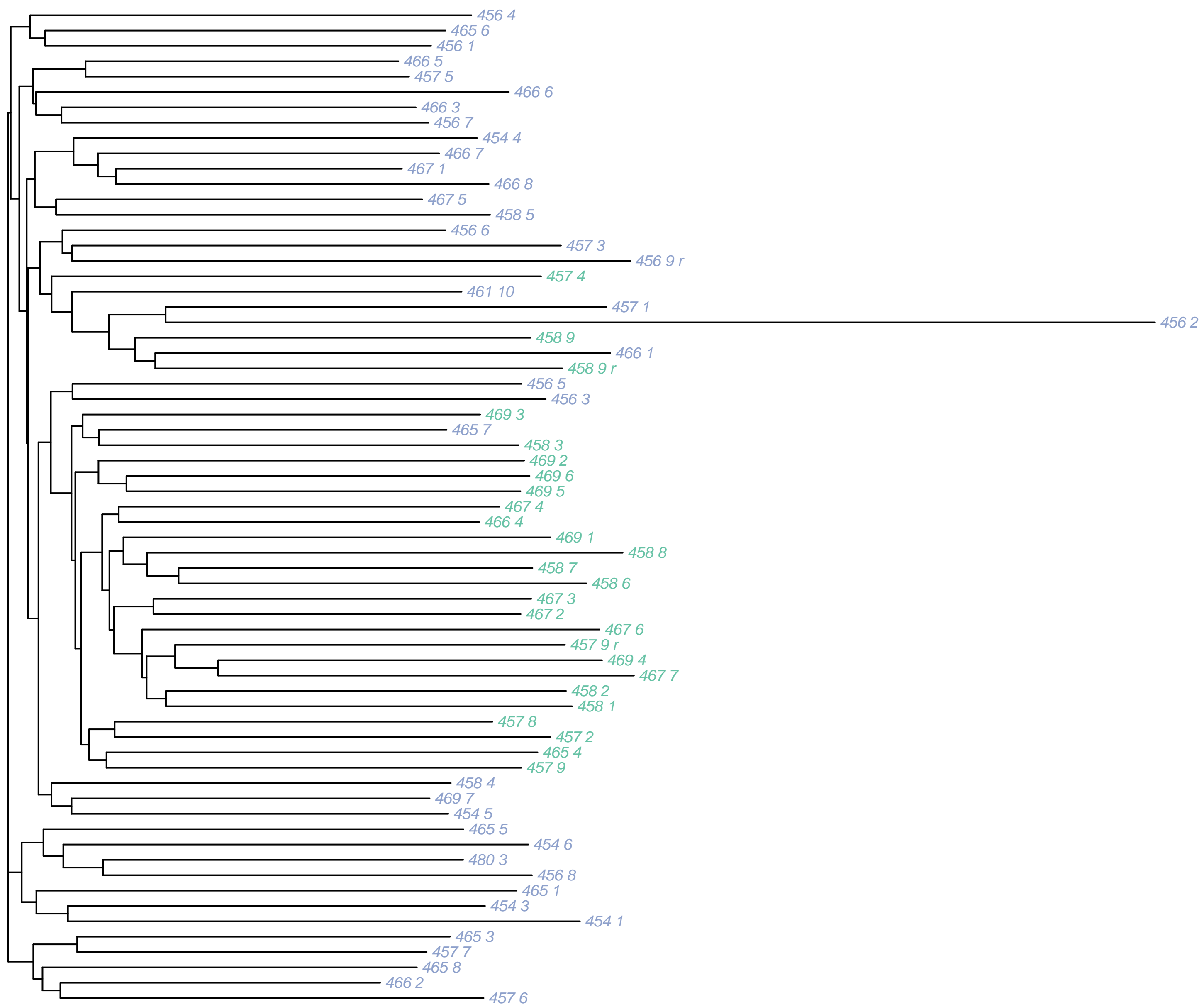

400
350
300
250
200
150
100
50
0
number of loci
0.0
0.2
0.4
0.6
0.8
1.0
heterozygosity



Frequency
Mean heterozygosity
0
1
2
3
4
5
6
0.06
0.08
0.10
0.12
0.14
0.16
0.18



0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
Heterozygosity
−94
−92
−90
−88
−86
−84
−82
Longitude



456 4
465 6
456 1
466 5
457 5
466 6
466 3
456 7
454 4
466 7
467 1
466 8
467 5
458 5
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457 3
456 9 r
457 4
461 10
457 1
456 2
458 9
466 1
458 9 r
456 5
456 3
469 3
465 7
458 3
469 2
469 6
469 5
467 4
466 4
469 1
458 8
458 7
458 6
467 3
467 2
467 6
457 9 r
469 4
467 7
458 2
458 1
457 8
457 2
465 4
457 9
458 4
469 7
454 5
465 5
454 6
480 3
456 8
465 1
454 3
454 1
465 3
457 7
465 8
466 2
457 6

Supplementary Table 1. Locality data for samples of *Salvinia* collected

No.	Species	Vicinity or collection	MGRS	Longitude	Latitude	County	State	Country
454	*S. minima*	Lafitte	15RYP4673966692	-90.43161	30.40705	Tangipahoa Parish	Louisiana	USA
456	*S. minima*	Paradis	15RYP4829508439	-90.42908	29.88157	Saint Charles Parish	Louisiana	USA
457	*S. minima*	Kraemer	15RYP3275602547	-90.59113	29.83148	Lafourche Parish	Louisiana	USA
458	*S. minima*	Jefferson	15RYP7384217364	-90.16254	29.9566	Jefferson Parish	Louisiana	USA
461	*S. molesta*	S. of Romere Pass	16RBT8225638232	-89.24077	29.25421	Plaquemines Parish	Louisiana	USA
465	*S. minima*	Franklin	15RXN4623093775	-91.48747	29.76564	Saint Mary Parish	Louisiana	USA
466	*S. minima*	Orange	15RVP2994231386	-93.72719	30.1117	Orange County	Texas	USA
467	*S. minima*	Mobile	16RDU0699094023	-87.97098	30.67533	Baldwin County	Alabama	USA
469	*S. minima*	Lake Tallequin	16RGU3799671081	-84.52154	30.44838	Leon County	Florida	USA
470	*S. molesta*	S. of Romere Pass	16RBT8225638232	-89.24077	29.25421	Plaquemines Parish	Louisiana	USA
471	*S. molesta*	Venetian Isles	16RBU2871329527	-89.81411	30.0668	Orleans Parish	Louisiana	USA
472	*S. molesta*	Wallace Lake	15SVR3293576352	-93.71251	32.32201	Caddo Parish	Louisiana	USA
474	*S. molesta*	Cross Lake	15SVR1243998972	-93.93234	32.52463	Caddo Parish	Louisiana	USA
475	*S. minima*	Pryer 5291	16PFS8179843964	-85.33963	10.3444		Guanacaste	Costa Rica
476	*S. minima*	Cult. Pryer 2364					Buenos Aires	Argentina

477	*S. minima*	Parana, Brazil;	Cordeiro & Pereira 1531					Brazil
478	*S. minima*	Sanders 9743	16QBF5431498765	-89.33333	18.96666		Quintana Roo	Mexico
479	*S. minima*	TJ Killeen 6824	20LNJ6663867789	-62.38084	-14.76306		Santa Cruz	Bolivia
480	*S. minima*	Jacono 88924	17RMN1248149838	-81.90181	29.3747	Marion County	Florida	USA
481	*S. minima*	Jacono 890	17RLN6000087613	-82.44731	29.71077	Alachua County	Florida	USA