A genetic survey of *Salvinia minima* in the southern United States

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Abstract

The genetic relationships among 68 samples of *Salvinia minima* (Salviniaceae) were investigated using RAPD analysis. Neighbor joining, principle components, and AMOVA analyses were used to detect differences among geographically referenced samples within and outside of Florida. Genetic distances (Nei and Li) range up to 0.48, although most are under 0.30, still relatively high levels for an introduced, clonally reproducing plant. Despite the diversity AMOVA analysis yielded no indication that the Florida plants, as a group, were significantly different from the plants sampled elsewhere in its adventive, North American range. A single, genetically dissimilar population probably exists in the recent (1998) horticultural introduction to Mississippi. When the samples were grouped into 10 regional (but artificial) units and analyzed using AMOVA the between region variance was only 7.7%. Genetic similarity among these regions may indicate introduction and dispersal from common sources. The reduced aggressiveness of Florida populations (compared to other states) may be due to herbivory. The weevil *Cyrtobagous salviniae*, a selective feeder, is found in Florida but not other states. The genetic similarity also suggests that there are no obvious genetic obstacles to the establishment or efficacy of *C. salviniae* as a biological control agent on *S. minima* outside of Florida. Published by Elsevier Science B.V.

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1. Introduction

The aquatic fern *Salvinia minima* Baker [Salviniaceae] originates in Meso and South America ranging naturally from south central Mexico to northern Argentina (Stoltze, 1983;

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Mickel and Beitel, 1988). Like other members of the genus, including the widely invasive *Salvinia molesta* D.S. Mitchell, its basic form is a ramet. The ramet consists of a floating rhizomal segment with three leaves in a whorl, two of which are entire, floating and green, and one which is submersed and deeply divided (resembling roots).

Local introductions of *S. minima* have been documented for Bermuda (Weatherby, 1937), Puerto Rico (Proctor, 1989) and Spain (Lawalree, 1964). First introduced to North America in the late 1920s (Small, 1931) *S. minima* has since exhibited extensive geographic spread. Jacono et al. (2001) mapped occurrence records from over 640 sites to demonstrate its distribution in 67 drainages of Florida, Georgia, South Carolina, Louisiana, Alabama, Texas and Mississippi. Following Small's (1931) description of *S. minima* in the St. Johns River drainage Jacono et al. (2001) detail early and separate introduction events in: Miami, Florida (1935); Savannah, Georgia (1936); Sarasota, Florida (1935); Gainesville and Bradenton, Florida (1937). They document the spread through peninsular Florida and eventually, by the late 1970s, the introduction to the Florida Panhandle. In subsequent introductions to Louisiana (Landry, 1981), Alabama (Haynes and Jacono, 2000), Texas, S. Carolina and Mississippi (Jacono et al., 2001), *S. minima* populations often exhibited the well-recognized invasive habit of excessive surface growth. In contrast, in much of Florida, *S. minima* is not as aggressive and constitutes a smaller proportion of the biomass. Jacono et al. (2001) attribute this to the presence of a herbivore, putatively *Cyrtobagous salviniae* Calder and Sands. They found this weevil in 68% of Florida drainages with documented populations of *S. minima* but not in the other southern states. Room (1990) describes the effect of herbivory by the weevil on *S. molesta* populations: "*C. salviniae* clearly regulates populations of salvinia in most situations . . . . Populations of *C. salviniae* increase until they destroy monocultures of salvinia, they crash due to starvation of larvae and emigration by adults, and then they persist in low-level equilibrium with the small plants scattered amongst fringing vegetation." Often, but not always, this description seems to fit the relationship between *S. minima* and *C. salviniae* in many water bodies of Florida.

Efforts are currently underway by several authors and collaborators to elucidate the ecology of *C. salviniae* on *S. minima* in Florida, to develop inexpensive rearing techniques, and to establish it in areas of the southern US infested with both *S. minima* and *S. molesta*. Questions have arisen regarding the use of the Florida weevil. *C. salviniae* from Florida, while morphologically identical to those on *S. molesta* in Brazil, are significantly smaller (Calder and Sands, 1985). Small size may be a beneficial adaptation to feeding on *S. minima*, a smaller plant, however, it raises a question as to whether Florida *Cyrtobagous* is actually the same species as the Brazilian weevil and whether it will be as effective controlling *S. molesta*. Goolsby et al. (2000) suggest from sequence evidence that Florida *Cyrtobagous* may differ sufficiently from the Brazilian weevil so as to constitute a different species. Despite the Florida weevil's establishment and control of *S. minima* in many Florida populations it should not be assumed that introduction to other southeastern populations will be successful. Potential genetic differences between the *S. minima* populations of Florida and those of the other states may reduce the efficacy of the Florida weevil. This study constitutes a survey of *S. minima* genetic variation throughout its southern U.S. range with an emphasis on Florida. It uses a technique called RAPD, or random amplified polymorphic DNA (Williams et al., 1990). RAPD has been increasingly utilized as a tool in studies involving biological control

(Nissen et al., 1995; Harry et al., 1998), and in the analysis of populations (Huff et al., 1993; Stewart and Excoffier, 1996).

The primary goal of this survey is to determine the degree of genetic divergence within Florida samples and between the Florida and other regional *S. minima* samples.

2. Materials and methods

2.1. Sampling and DNA extraction

A total of 68 samples were collected from seven southern states and a single outlier sample from Peru. These samples originate from 38 USGS cataloging units (a geographic area representing part of or all of a surface drainage basin, a combination of smaller drainage basins, or a distinct hydrologic feature). The number of cataloging units (drainages) by state where the samples were taken is as follows: Alabama—1, Florida—23, Georgia—2, Louisiana—7, S. Carolina—1, and Texas—3. A number of the drainages cross state lines. Most samples taken from the same drainage are from different sites. Where samples were taken from the same site collectors were directed to collect them from as far apart as practical and a minimum of 10 m apart. Samples (by drainage) from outside Florida taken from the same site include Atchafalaya (3 of 3 samples), Broad/St. Helena (2 of 2), E. Central Coast (2 of 3, one from second site), Lower Ochlockonee (3 of 3), Mobile/Tensaw (3 of 3), Sabine Lake (2 of 2), Mermentau (2 of 4, two from two other sites), and the Lower Angelina (2 of 2). In Florida five samples (5 of 6) from the Upper St. Johns were collected at Blue Spring State Park where the plant was observed in 1931. Three samples (3 of 3) were taken from Phillipee Creek of the Sarasota Bay drainage, the closest site to another early (1935) release where *Salvinia* could still be found.

As each sample was collected it was thoroughly rinsed in a jet of deionized water and blotted dry. Next the plant material was dessicated with silica gel in a 15 ml plastic centrifuge tube. Once back in the laboratory the desiccant was changed and the samples stored until DNA extraction. The submersed, dissected leaf was removed (to decrease the likelihood of microbial contamination) and the aerial leaves, lateral buds, and rhizome used for extraction. Up to 12 mg of dried plant material were used. Total DNA was extracted using the CTAB method as "micro" modified by Van and Madeira (1998). The DNA solution was quantified using fluorometry and stored at $-20\,^{\circ}$C.

2.2. Amplification, electrophoresis, and scoring

Amplification reactions were performed using 12 μl volumes (10 mM Tris–HCl pH 8.3, 50 mM KCl, 2.5 mM MgCl$_2$, 100 μM each of dATP, dCTP, dGTP and dTTP, 1.2 μM primer, 1.0 ng μl^{-1} genomic DNA, and 0.25 U μl^{-1} (Sigma) *Taq* DNA Polymerase in a PCT-100 Programmable Thermal Controller (MJ Research) with a Hot Bonnet. A thermal cycling "touchdown" program which begins annealing at 55 °C, steps down over 10 cycles to 45 °C, and uses fixed transition ramp times, was used to achieve greater pattern repeatability. One hundred twenty RAPD 10-mer primers (Operon Technologies: A1-A20, B1-B20, C1-C20, D1-D20, E1-E20, G1-G20) were screened with 84 of these producing amplification

products. Five primers (A3, A9, A13, C2, C5) were selected for the study based on the number and quality of polymorphic bands. The amplification products were electrophoresed in 20 cm, 2% agarose gels, visualized by ethidium bromide staining, and photographed using a FOTO/Analyst Minivisionary Benchtop Digital Documentation System (Fotodyne, Inc.). The extended length of 20 cm gels allowed the selection of primers that generated higher numbers of loci. Loci were identified by their nucleotide (bp) length and the primer used (e.g. 250B1). Nucleotide length was determined with reference to a 100 bp ladder (Gibco BRL). Three replicate amplifications of each genomic DNA/primer combination were utilized for analysis. An attempt was made to score all loci. Loci were scored as present (1), absent (0), or unclear (?). When replicates were analyzed two agreeing scores and an unclear (1,1,?; 0,0,?) were scored as the majority score (1; 0) however, when one replicate disagreed (1,1,0; 0,0,1) the band was scored as "missing data" (?; ?). Following scoring the data set was reduced to 88 loci by eliminating the dimmest loci, those with the highest number of "missing data" scores (lack of repeatability), and by selecting against loci which were too close to others (where homology was a concern). Since increasing the number of loci has a greater effect in reducing the variance of the distance estimates than eliminating scoring error (Skroch and Nienhuis, 1995) some ambiguities were accepted to achieve a higher number of loci. The percentage of ambiguities in the final data set was 7.5%.

2.3. Analysis

The binary data set was transformed to pairwise distances using the Nei and Li (1979) similarity matrix. Neighbor joining (Saitou and Nei, 1987) was then used to construct trees. Neighbor joining (NJ) is an algorithmic method (it constructs only one tree) which does not assume a constant evolutionary clock (ultrametric distances) although it does assume the data comes close to fitting an additive tree. Bootstrap Analysis (Felsenstein, 1985) of the NJ trees was carried out using the RAPDBOOT program (Black, 1995) in conjunction with the NEIGHBOR and CONSENSUS programs of PHYLIP. Principal components analysis (PCA) was conducted to extract the maximum variance possible onto three axes using the STAND, EIGEN, and PROJ routines of the NTSYS program (Rohlf, 1994). An analysis of molecular variance procedure was conducted using the Euclidean squared metric and the WINAMOVA program, version 1.55 (Excoffier et al., 1992). This analysis was first used for RAPDs by Huff et al. (1993) and has been described as essentially an analysis of phenotypic variance (Stewart and Excoffier, 1996) due to the dominant nature of RAPD markers. AMOVA computes molecular variance components at different hierarchical levels utilizing an analysis of variance (ANOVA) format. Significance testing is conducted utilizing permutation procedures overcoming both the testing limitations of normal theory and of the non-independence of distance measures. For each analysis 9999 permutations were carried out.

3. Results

Fig. 1 presents the U.S. distribution of *S. minima* as of November 1999 (Jacono et al., 2001). It lists the drainage names and displays the locations and the introduction dates
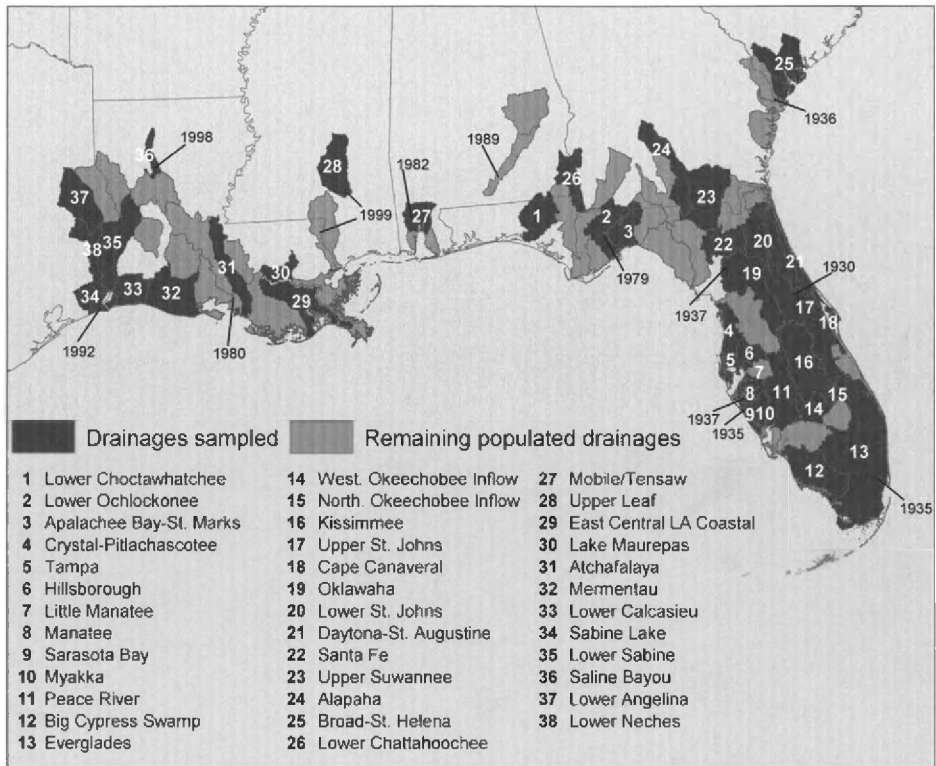
Fig. 1. Geographic distribution of *S. minima* in drainages of the southern United States as of November 2000 with the sampled drainages indicated. Dates mark earlier introduction sites.

for the earliest appearances of *S. minima* in each region. Reference numbers assigned in Fig. 1 are also displayed alongside drainages referred to in the text and figures to facilitate comparisons.

Fig. 2 presents the Nei and Li (1979) distances subjected to neighbor joining procedures. The Peru (Amazonian) sample, intended as an outlier, shows distances ranging from 0.45 to 0.69 from the U.S. samples. Two samples also appear as outliers for reasons that may be related to technique. These consist of one sample each from the Everglades (13) drainage and Little Manatee (7) drainage. In scoring the gels they displayed problems with either additional bands and/or poor amplification by some primers. These are displayed only in the neighbor joining analysis. The remaining samples show distances up to 0.48, however, the vast majority fall below 0.3. Bootstrap procedures show only two small clusters of possible statistical significance. Four Florida samples (found at the lower left of Fig. 2) from Blue Springs State Park on the St. John's River (17), in the area of the earliest Florida introduction, show a bootstrap value of 84%. Two samples (upper left of Fig. 2) from the same site in the East Central Louisiana Coastal drainage (29) have a bootstrap value of 83%. No other cluster exceeded significance figures of 70%. Other samples from the same sites often do cluster (13, 31, 32, 37) despite low bootstraps. Note the tree has short internal
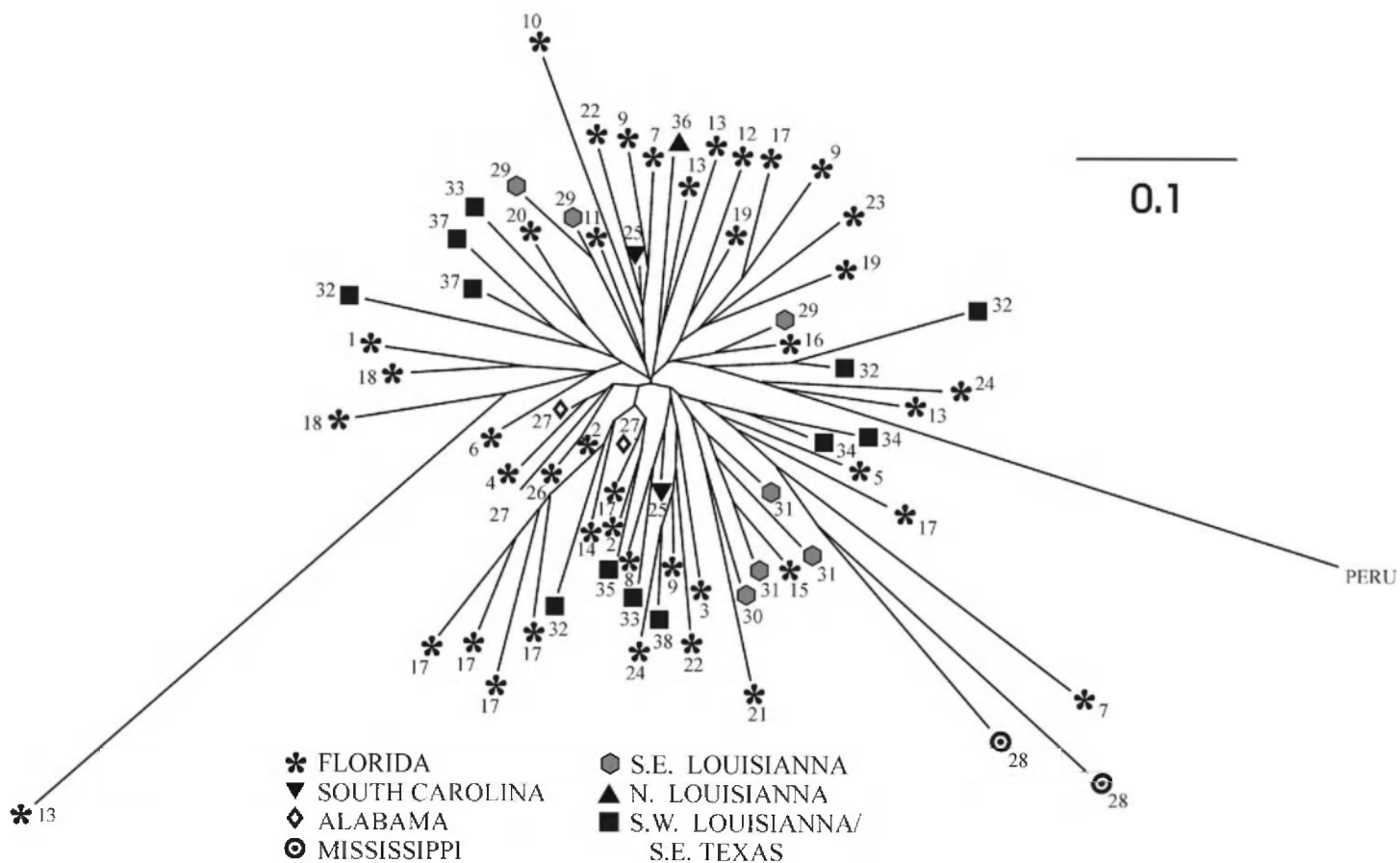
Fig. 2. Neighbor joining analysis using Nei and Li (1979) distances of *S. minima* samples from the southern United States. Symbols represent Florida and other regions of introduction. Numbers indicate the drainages as listed in Fig. 1.
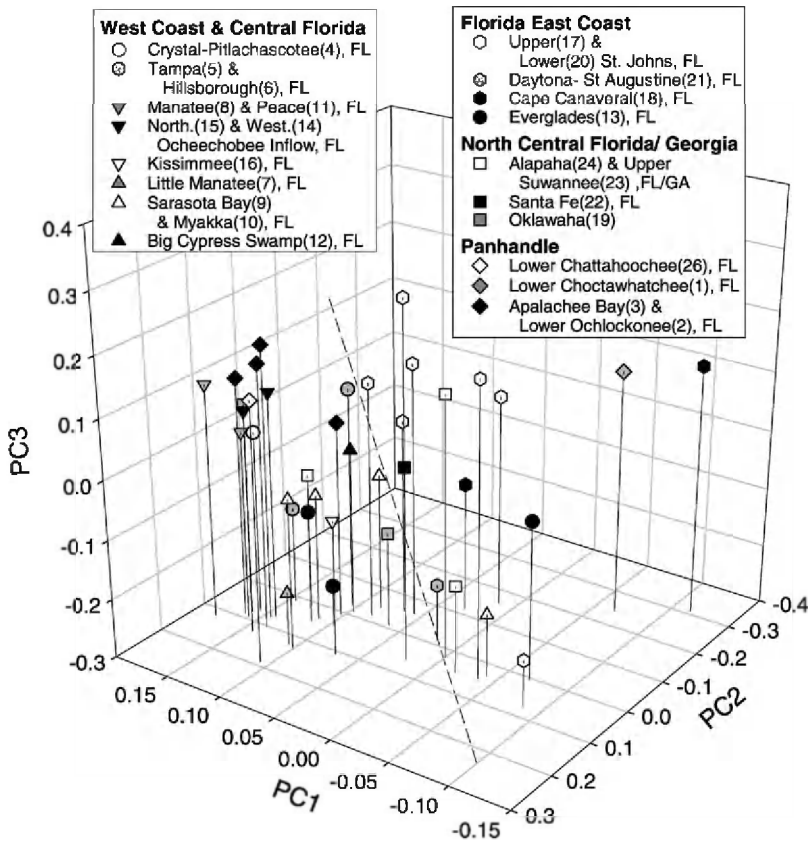
Fig. 3. Genetic relationships of *S. minima* samples from Florida and southern Georgia as analyzed using principle components analysis (PCA). Drainages are listed by symbol and by numbers referencing the map in Fig. 1. Drainage names are grouped into "arbitrary" geographic regions under bold subtitles.

branches indicating little significant structure among the samples. Samples from within and outside of Florida are interspersed throughout clusters and show little evidence of differing by region with the possible exception of the Mississippi (28).

Fig. 3 presents the principal components analysis (PCA) for samples taken from Florida and Georgia. The PCA accounts for 28.3% of the variation (eigenvalue 1: 11.2%, 2: 8.6%, 3: 7.3%) here presented in the first three ordinates. The samples are identified by drainages and grouped in the key under somewhat "artificial" geographic regions. PCA analysis provides some discrimination of samples. Note that samples from the Florida East Coast region occur primarily on the right hand side of the figure and are defined by lower values on PCA ordinates 1 and 2. Samples from the Everglades (13) appear in both the left and right sides of the figure and exhibit low PCA ordinate 3 values. On the left side of the figure samples from the West Coast & Central Florida region appear having higher values on PCA ordinates 1 and 2. An arbitrary line is drawn as a visual aid separating the Florida East Coast and the West Coast & Central Florida regions. Samples from the North Central Florida region
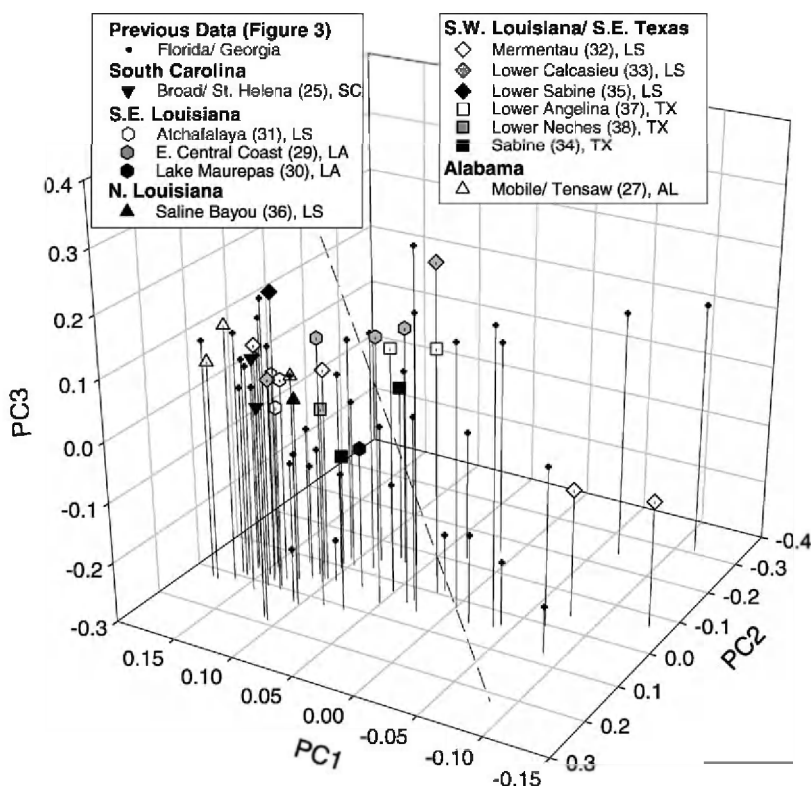
Fig. 4. Genetic relationships of *S. minima* samples from Alabama, Louisiana, Mississippi, S. Carolina and Texas as analyzed using principle components analysis (PCA). Drainages are listed by symbol and by numbers referencing the map in Fig. 1. Drainages are grouped under subtitles representing geographic regions of historical introduction. Genetic relationships are integrated with previous Florida and Georgia data.

straddle the East/West divide and appear intermediate in genetic composition. The samples from the Panhandle region show high PC1 and PC2 values and group with the West Coast & Central Florida samples with the exception of the Lower Choctawhatchee (1) sample which represents a late (1992) occurrence.

Fig. 4 presents the PCA analysis for the samples from Alabama, Louisiana, South Carolina and Texas. The outlying Mississippi samples are not presented in order to enlarge the plot of the remaining samples. The orientation in the PCA is identical to Fig. 3 and the Florida samples are presented as a backdrop. Overall, 68% of these samples appear on the West Coast & Central Florida side of the PCA. The Broad/ St Helena (25) infestation, which may derive from the adjacent and early (1936) Savannah, Georgia infestation, appears similar in PCA location to samples from the Manatee (8) infestation, which may derive from a 1937 introduction. The Mobile/Tensaw (27), Alabama infestation, first appearing in 1982, appears in the PCA in a similar region as most of the Florida Panhandle samples, where *S. minima* appeared in 1979. A high amount of variation is apparent in the S.W. Louisiana/S.E.

Texas region, especially in the Mermentau (32) samples where the four samples collected are spread across the PCA.

A different approach to variation among populations is the use of AMOVA. A simple and robust comparison can be made between regions of earlier infestations—Florida, Georgia, and South Carolina and the regions colonized later, Alabama, Mississippi, Louisiana and Texas. Results indicate 100% of the variance is within regions. Because the recent Mississippi introductions are outliers they skew the distribution. When the Mississippi samples are removed the analysis indicates 99.7% of the variance is within regions and 0.3% is between the regions.

Closer examination of population structure using AMOVA is presented in Table 1 including population sample sizes, drainages, PHIst (ϕ_{ST}), and probabilities. ϕ_{ST}, as presented here, represents the proportion of the total variance that is partitioned between the two populations. The probabilities in the upper matrix represent significance figures for the corresponding ϕ_{ST} estimate in the lower matrix. Values <0.01 may be considered highly significant, values from 0.01–0.05 significant, values 0.05–0.10 marginal, and values >0.10 not significant. Note that negative variance components (ϕ_{ST}) can occur in the absence of genetic structure because the true value is near zero, the sample sizes are small and, by chance, slightly negative or positive components occur. The "artificial" geographic regions used in the PCA figures are also used for the AMOVA analysis. The Everglades samples, which probably represent a separate introduction(s), are analyzed as a separate population from the other Florida East Coast samples. Also we use the Mississippi samples which were not presented in the PCA. The Everglades, Alabama, South Carolina and Mississippi regions have only 2–3 samples making statistical comparisons suspect. The other regions range from 6 to 14 individuals, still small numbers but probably providing some diagnostic value. The highest ϕ_{ST} values, from 0.258 to 0.571, occur when the Mississippi region is compared to other regions, all with significant or highly significant probabilities. The Alabama region appears significantly different from South Carolina, the Florida Everglades, and the Florida East Coast. The Florida Everglades population appears significantly different from S.E. Louisiana and marginally different from the Florida East Coast. Among larger sample comparisons (therefore those with more evidence) Florida East Coast versus West Coast & Central Florida appear significantly different ($P = 0.026$). North Central Florida appears significantly different ($P = 0.021$) from the Florida East Coast but not significantly different ($P = 0.576$) from the West Coast & Central Florida. The S.E. Louisiana introduction appears significantly different ($P = 0.000$) from North Central Florida but not the Florida East Coast ($P = 0.135$) or West Coast & Central Florida populations ($P = 0.176$). The Florida Panhandle appears marginally different ($P = 0.075$) from the West Coast & Central Florida as does the S.W. Louisiana/ S.W. Texas ($P = 0.071$). In summary, neighbor joining with bootstrap analysis shows little evidence of genetic structure. PCA analysis and AMOVA, however, do appear to show some partitioning of diversity between geographic regions.

4. Discussion

The primary goal of this survey was to determine whether genetic differences exist between different regions in Florida and other southern regional *S. minima* populations.

Table 1
Distance matrix ϕ_{ST} between populations of *S. minima* from the Southern United States

Population name		FEC	FEv	FWC	NCF	FP	SC	LT	SEL	AL	MS	Samples #	Drainages in population
Florida East Coast	FEC		0.073	0.026	0.021	0.165	0.119	0.152	0.135	0.048	0.000	10	Lower & Upper St. Johns, Cape Canaveral, Daytona- St. Augustine
Florida Everglades	FEv	0.114		0.137	0.519	0.156	0.864	0.539	0.028	0.000	0.000	3	Everglades
West Coast and Central FL	FWC	0.080	0.079		0.576	0.075	0.705	0.071	0.176	0.239	0.000	14	Sarasota Bay, Little Manatee, Manatee, Tampa, Crystal-Pithlachascotee, Peace R., Myakka, Hillsborough, Kissimmee, N. & W. Okeechobee Inflow, Big Cypress
North Central Florida	NCF	0.073	0.011	−0.006		0.126	0.878	0.340	0.000	0.498	0.030	7	Oklawaha, Upper Suwannee, Santa Fe, Alapaha
Florida Panhandle	FP	0.044	0.050	0.044	0.053		0.101	0.870	0.773	0.565	0.000	6	Apalachee Bay-St. Marks, Lower Ochlockonee, L. Choctawhatchee, L. Chattahoochee
South Carolina	SC	0.091	−0.129	−0.057	−0.086	0.101		0.704	0.748	0.000	0.000	2	Broad-St. Helena
S.W. Louisiana– S.E. Texas	LT	0.029	−0.007	0.038	0.018	−0.043	−0.045		0.238	0.390	0.000	12	Mermentau, Lower Calcasieu, Lower Sabine, Sabine Lake, Lower Neches, L. Angelina
S.E. Louisiana	SEL	0.044	0.125	0.032	0.081	−0.025	−0.077	0.022		0.220	0.000	8	Lake Maurepas, Atchafalaya, East Central Louisiana Coastal, Saline Bayou
Alabama	AL	0.122	0.165	0.055	0.015	−0.029	0.560	0.003	0.075		0.000	3	Mobile/Tensaw
Mississippi	MS	0.312	0.258	0.402	0.373	0.458	0.429	0.390	0.412	0.571		2	Upper Leaf

Drainages and number of samples within populations are presented. Lower matrix—ϕ_{ST}, defined as the proportion of the total phenotypic variance that is partitioned between two populations. Upper matrix—Probability, defined as the probability that the random ϕ_{ST} distance > observed ϕ_{ST} distance.

All forms of analysis show substantial overlap between the Florida samples and the later infestations treated as a group (Alabama, Louisiana and Texas). This overlap of genetic diversity has implications for the introduction of the biological control agent, *C. salviniae*, particularly for the small variant described in Florida (Calder and Sands, 1985). Jacono et al. (2001) describe the weevils' distribution in Florida. It is present throughout most of the drainages where *S. minima* is present, including in all of the Florida regions described in this study. Since the weevil appears to have been effective in reducing the virulence of *S. minima* infestations throughout Florida there is no reason to believe, given the genetic overlap between the southern US populations (excepting Mississippi) and Florida, that the biological control would fail for genetic reasons in those areas. Note however that RAPD samples the genome randomly so there is no absolute assurance that specific genetic differences do not exist that would affect *Cyrtobagous* establishment. Environmental factors (i.e. severe weather, flooding, cold climate) could also affect establishment and survival.

The genetic survey presented here does not constitute a true population study which would require larger sample sizes for each drainage. The PCO and AMOVA analysis indicate that some genetic structure exists and that it may reflect the introduction history presented by Jacono et al. (2001). The difference between the Florida East Coast and the West Coast & Central Florida regions may reflect the earliest populations identified in the St. Johns River in 1930 (Small, 1931) and in Sarasota Bay (Sarasota) and Manatee (Bradenton) in 1935 and 1937. AMOVA indicates that the North Central Florida region is significantly different ($P = 0.021$) from the Florida East Coast region despite the fact the Oklawaha is a tributary of the St. Johns. This may reflect the separate 1937 introduction to the Oklawaha drainage (Jacono et al., 2001). This region may also include a separate Alapaha (24) introduction since *Salvinia* was collected near Valdosta, Georgia in 1969 before it was collected in the geographically intermediate Santa Fe (22) and Upper Suwannee (23). The S.E. Louisiana and S.W. Louisiana/S.E. Texas regions are not significantly different ($P = 0.238$) nor are they significantly different from the Florida East Coast or West Coast & Central Florida region.

Large overlaps in population diversities may be explained by introductions from the same or related sources. Cultivation of *S. minima* has been documented in gardens and greenhouses in the U.S. since as early as the late 1880s (Weatherby, 1921; Weatherby, 1937; Fernald, 1950). Later introductions, probably after the 1960s, may also have occurred from tropical fish hobbyists who sold it as an aquarium plant. A large tropical fish industry grew up near Tampa, Florida. Introductions from this local material would therefore tend to be similar genetically to Florida West populations. It is often difficult to identify whether an introduction to a new drainage has occurred from regional dispersion or from a separate introduction. The spotty nature of dispersal which Jacono et al. (2001) present for Florida implies either numerous regional introductions from outside sources, spotty local movement that skipped intermediate drainages or incomplete collection data. Therefore, correlations made between the regional genetics presented here and early historical introductions, while worth considering, must be viewed cautiously.

The amount of diversity discovered by RAPDs seems surprisingly high at first glance for a plant both introduced and reproducing clonally. If the source material originated from multiple dealers with different geographic sources this would introduce diversity from the large native range. de la Sota and Cassá de Pazos (2001) indicate that *S. minima* appears

in at least two cytotypes ($2n = 4x = 36$ and $2n = 6x = 54$) and display figures of a regular metaphase. This indicates that sexual reproduction is possible. Reviews of clonally reproducing plants, where recruitment from sexually produced diaspores is supposedly rare, indicate genetic diversities comparable to other species (Ellstrand and Roose, 1987; Widén et al., 1994). Also, there are numerous avenues in polyploids for the establishment of genetic diversity including polysomic inheritance (in autopolyploids), fixed (non-segregating) heterozygosity because of the combination of divergent parental genomes (in allopolyploids), multiple (recurrent) origins, gene silencing, divergence of duplicate genes, chromosomal repatterning, and others (Soltis and Soltis, 1993). Another interesting source of variation is somatic mutation. Pineda-Krch and Fagerström (1999) present a model for evolutionary change through selection and genetic drift within meristematic cell lineages. In vegetatively reproducing plants somatic mutations can be fixed and passed on to the succeeding ramets. *S. minima* is also a prime candidate for the action of somatic mutation due to frequent direct exposure to the sun's radiation, rapid doubling times, and densely packed populations with high numbers of individuals. Finally, some of the diversity displayed may reflect "noise" from repeatability and homology problems inherent to the RAPDs technique.

In summary, *S. minima* in the southern United States shows a relatively large amount of genetic diversity as measured by RAPDs. As a group, populations established by more recent introductions to Alabama, Louisiana and Texas are not significantly different from the earlier ones of Florida, Georgia and South Carolina. There is evidence from PCO and AMOVA analysis that the Florida east coast populations may be somewhat different from southwestern and central Florida populations as well as from the later introductions to Louisiana and Texas. The more recent introductions in Alabama, Louisiana and Texas show a good deal of genetic diversity, on the same order as that of Florida. The most recent infestation found in 1999 in Mississippi is represented by only two samples but appears to differ genetically from any other sampled.

Acknowledgements

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