Biol Invasions
https://doi.org/10.1007/s10530-023-03028-0

ORIGINAL PAPER

What is *Salvinia molesta* (Salviniaceae)? Determining the maternal progenitor and genetic diversity of the clonal invasive fern giant salvinia

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Received: 14 September 2022 / Accepted: 7 February 2023

Abstract The aquatic fern *Salvinia molesta* D.S. Mitch. is an invasive species that can have devastating effects on the freshwater habitats it colonizes. Currently, a lack of clarity surrounding the genomic composition and genetic diversity of *S. molesta* impedes eradication efforts. *Salvinia molesta* is a polyploid hybrid with unknown and controversial parentage, first noted in Africa but morphologically similar to South American species. Giant salvinia is also thought to reproduce primarily, perhaps exclusively, through vegetative reproduction, raising the possibility that the global invasion comprises one or a few clonal genotypes. This research focuses on identifying the maternal genome donor of *S. molesta*, determining if this species consists of a single or multiple independently derived lineages, and evaluating invasive-range genotypic diversity. Whole chloroplast genome (plastome) sequencing from field-collected and herbarium specimens was used to quantify genetic diversity in *S. molesta* and the phylogenetic relationships among *Salvinia* species. Phylogenetic analysis revealed that *S. molesta* and *S. herzogii* share the same plastome, although *S. herzogii* is unlikely to be *S. molesta*'s maternal progenitor due to its own hybrid status and odd ploidy. Rather, we conclude that *S. molesta*'s maternal progenitor is either an undescribed or extinct species. The observed plastome diversity within *S. molesta* indicates the presence of multiple divergent genotypes which strongly suggest multiple origins of this hybrid. Additionally, this diversity clearly indicates that a single clone does not dominate the invasive range. This genomic diversity could have direct implications for the successful

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10530-023-03028-0.

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Published online: 01 April 2023

management of this invasive species, particularly for biological control.

Keywords Asexuality · Invasive species · Phylogeny · Polyploidy · Plastome

Introduction

Hybridization, polyploidy, and asexuality are commonly associated with plant invasions (Ellstrand and Schierenbeck 2000; Liu et al. 2006; Te Beest et al. 2012), creating novel, aggressive genotypes that can spread rapidly. These traits are all features of the aquatic fern giant salvinia, *Salvinia molesta* D.S. Mitch., (Salviniaceae). Giant salvinia has escaped its presumed native range in South America and has become an aggressive invader of standing freshwater habitats in Southern Africa, eastern/southern Asia, Australia, Europe, and the United States. Previous studies have shown that *S. molesta's* biomass can double in five days and that it can have an exponential growth rate while colonizing a new habitat (Mitchell and Tur 1975; Rani and Bhambie 1983). This growth potential and the ecosystem-altering effects of its establishment have led to *S. molesta* being ranked among the 100 worst invasive species worldwide (Luque et al. 2014). This invasion has been remarkably fast- *S. molesta* was first noted in the late 1950s in Zimbabwe (Mitchell 1972). The current U.S. invasion is thought to largely originate from populations first recorded from Louisiana and Texas in 1998 (Jacono 1999) and has since spread to twelve states (Coetzee and Hill 2020). Although the control of this species has been the subject of considerable research (e.g., Mitchell and Tur 1975; Room 1983; Jacono et al. 2001; Lal 2016; Coetzee and Hill 2020), a lack of clarity surrounding the origin and genetic diversity of *S. molesta* may be hindering current control and eradication efforts (Thomas and Room 1986). A better understanding of the biogeographic origin of giant salvinia could guide the search for biocontrol agents (Schaffner 2001), while knowledge of the genetic diversity of this species could help predict the specificity of such agents (Gaskin et al. 2011).

Chromosome pairing information suggests that giant salvinia is an allopentaploid (Kuriachan 1979). Its putative hybrid origin and odd-number of chromosome sets are consistent with many aspects of *S. molesta*'s invasive behavior, such as its high growth rate and non-viable spore production (Mitchell and Tur 1975; Nagalingum et al. 2008; Galam et al. 2015). Among the estimated 13 species in the genus *Salvinia*, the search for the progenitor species of *S. molesta* has focused on the *Salvinia auriculata* Aubl. species complex, a group of four to six primarily South American species possessing four apically united hairs on each papillae- so called "egg-beater" or "fouet-like" hairs (Mitchell and Thomas 1972; Jacono et al. 2001; Miranda and Schwartsburd 2019). Giant salvinia exhibits this hair type and is otherwise morphologically similar to several members of this complex. This suggests that despite being first noted in Africa, *S. molesta* has a South American origin. Specifically, it has been proposed that its likely parental species are *S. biloba* Raddi and *S. auriculata* or *S. biloba* and *S. herzogii* de la Sota (Mitchell 1972; Nagalingum et al. 2008). In addition to hair type, *S. molesta*, *S. biloba*, and *S. herzogii* are especially similar morphologically due to their bilobed floating leaves and sorophore display (Miranda and Schwartsburd 2019). A taxonomic analysis of *Salvinia* species occurring in Brazil shows that these proposed parental species have overlapping distributions (Miranda and Schwartsburd 2019). Identifying giant salvinia's maternal genome donor could be achieved through chloroplast genome (i.e., plastome) sequencing of *S. molesta* and other *Salvinia* species, as previous work supports fern plastomes as being maternally inherited (Wolf 2010). By sampling *S. molesta* across its invasive range, this approach also has the potential to identify distinct plastome haplotypes of *S. molesta* that reflect an evolutionary history of multiple, independent hybridization events (Beck et al. 2012b; Sigel et al. 2014; Dillenberger et al. 2018).

Many hybrid polyploids have evolved asexual reproduction (Whitton et al. 2008; Herben et al. 2017) and *S. molesta* is no exception. *Salvinia molesta* does not produce viable spores (Loyal and Grewal 1966), and it is commonly viewed as reproducing largely or perhaps exclusively through vegetative reproduction (Mitchell 1972; Jacono 1999; Mora-Olivo and Yatskievych 2009; Miranda and Schwartsburd 2016). This raises the possibility that the global *S. molesta* invasion may comprise relatively few, perhaps even one, widespread clonal genotypes. Indeed, multiple empirical studies support the notion that asexually reproducing invasive species exhibit low genotypic

diversity in their non-native range. Zhang et al. (2010) found that 80% of introduced *Eichhornia crassipes* Mart., populations were composed of a single clone and that a single genotype accounted for almost 75% of invasive individuals. Very low genetic variability was found across the invasive range of apomictic *Hieracium aurantiacum* L. in North America, with a single AFLP genotype documented in 46 out of the 48 sampled populations (Loomis and Fishman 2009). A study of the clonal invader giant reed, *Arundo donax* L., revealed that 98% of invasive individuals in the United States exhibited the same multilocus genotype (Ahmad et al. 2008). In the apomictic grass *Pennisetum setaceum* (Forssk.) Chiov., a single genotype was observed across three states in its invasive range (Poulin et al. 2005). The possibility of low genetic diversity has implications for both the adaptive potential of giant salvinia and for its biocontrol, including the potential for specific interactions between host and biological control genotypes (Gaskin et al. 2011; Darling 2015; Sun et al. 2020).

Taken together, previous research establishes several null hypotheses: (1) *Salvinia molesta*'s progenitor species are likely members of the South American *S. auriculata* complex rather than an eastern-hemisphere (Europe/Asia/Africa) species; (2) as an allopolyploid species, *S. molesta* is likely to be the result of multiple independent hybridization events; and (3) as an asexually reproducing species *S. molesta* is likely to display low genetic diversity in its invasive range. In order to evaluate these hypotheses we adopt phylogenetic and haplotype network approaches using whole plastome sequences from field and herbarium samples of *S. molesta* and other *Salvinia* species.

Materials and methods

Sampling

We obtained samples from 94 herbarium and freshly collected specimens, representing 14 *Salvinia* species (Online Resource 1). These specimens were collected between 1956 and 2020 (mean collection year = 2000). We emphasized sampling of members of the *S. auriculata* complex, especially to obtain a geographically and temporally diverse set of *S. molesta* individuals. Herbarium specimens were obtained from 13 herbaria (Online Resource 1) through loans, in-person visits, and samples sent from collaborators. A small amount of leaf tissue was removed from herbarium specimens and an annotation label was applied. Field collections focused on obtaining a broad contemporary sample of *S. molesta* from the Gulf Coastal Plain (AR, TX, LA, MS, AL). Potential sampling sites were identified using the iNaturalist platform (2020). Once a population was located, material suficient for a herbarium voucher was pressed, and a portion of leaf tissue was preserved in silica gel for DNA extraction.

DNA extraction, library preparation, and sequencing

DNA extractions were performed with a standard CTAB protocol modified for 96 well plates (Beck et al. 2012a). A Qubit fluorometer (Life Technologies) was used to establish DNA concentration for all extracts. Library preparations were performed using the NEBNext Ultra II DNA Library Prep Kit for Illumina with the NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 1) (New England Biolabs). Library preparation followed the detailed text and video protocols outlined in Saeidi et al. (2018), with 200 ng of input DNA. Samples with low library concentrations were re-amplified with universal Illumina primers prior to the hybridization reaction (Saeidi et al. 2018). Unenriched libraries were sequenced with 150 bp paired-end chemistry on an Illumina NextSeq 550 (Illumina) at the University of Kansas Genome Sequencing Core.

Sequence analysis

Following de-multiplexing, adapters and low-quality sequence reads were removed with Trimmomatic (Bolger et al. 2014). Trimmed sequences were aligned to a published *Salvinia* plastome (Li et al. 2018) using Geneious (Biomatters). Consensus sequences were extracted from each assembly with at least 30,000 reads using an ambiguity threshold of 60%. Sites with coverage of less than ten were considered ambiguous. After MAFFT (Katoh et al. 2002) alignment in Geneious, a maximum likelihood phylogeny was constructed using RAxML (Stamatakis 2014) with the GTRCAT model of sequence evolution and 1000 maximum-likelihood bootstrap (MLBS) replicates. The tree was rooted with an *Azolla filiculoides* Lam. plastome (GenBank accession # MF177094.1).

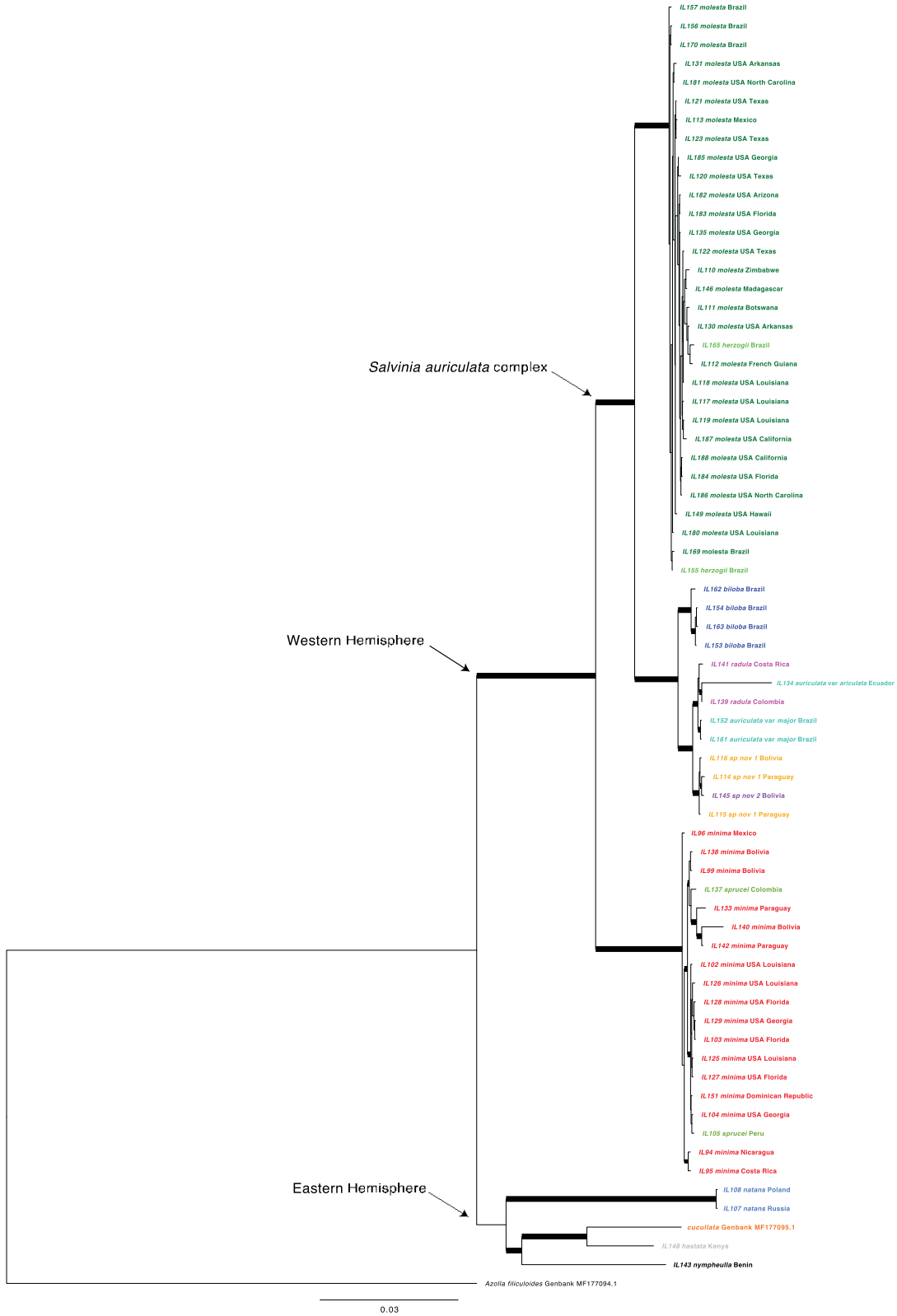
IL157 molesta Brazil
IL156 molesta Brazil
IL170 molesta Brazil
IL131 molesta USA Arkansas
IL181 molesta USA North Carolina
IL121 molesta USA Texas
IL113 molesta Mexico
IL123 molesta USA Texas
IL185 molesta USA Georgia
IL120 molesta USA Texas
IL182 molesta USA Arizona
IL183 molesta USA Florida
IL135 molesta USA Georgia
IL122 molesta USA Texas
IL110 molesta Zimbabwe
IL146 molesta Madagascar
IL111 molesta Botswana
IL130 molesta USA Arkansas
IL165 herzogii Brazil
IL112 molesta French Guiana
IL118 molesta USA Louisiana
IL117 molesta USA Louisiana
IL119 molesta USA Louisiana
IL187 molesta USA California
IL188 molesta USA California
IL184 molesta USA Florida
IL186 molesta USA North Carolina
IL149 molesta USA Hawaii
IL180 molesta USA Louisiana
IL169 molesta Brazil
IL155 herzogii Brazil
Salvinia auriculata complex
IL162 biloba Brazil
IL154 biloba Brazil
IL163 biloba Brazil
IL153 biloba Brazil
Western Hemisphere
IL141 radula Costa Rica
IL134 auriculata var ariculata Ecuador
IL139 radula Colombia
IL152 auriculata var major Brazil
IL161 auriculata var major Brazil
IL116 sp nov 1 Bolivia
IL114 sp nov 1 Paraguay
IL145 sp nov 2 Bolivia
IL115 sp nov 1 Paraguay
IL96 minima Mexico
IL138 minima Bolivia
IL99 minima Bolivia
IL137 sprucei Colombia
IL133 minima Paraguay
IL140 minima Bolivia
IL142 minima Paraguay
IL102 minima USA Louisiana
IL126 minima USA Louisiana
IL128 minima USA Florida
IL129 minima USA Georgia
IL103 minima USA Florida
IL125 minima USA Louisiana
IL127 minima USA Florida
IL151 minima Dominican Republic
IL104 minima USA Georgia
IL105 sprucei Peru
IL94 minima Nicaragua
IL95 minima Costa Rica
Eastern Hemisphere
IL108 natans Poland
IL107 natans Russia
cucullata Genbank MF177095.1
IL148 hastata Kenya
IL143 nympheulla Benin
Azolla filiculoides Genbank MF177094.1
0.03

Fig. 1 Maximum likelihood phylogeny resulting from analysis of *Salvinia* plastome sequence data. Branches supported with 100% maximum likelihood bootstrap support are shown with bold, thickened lines. Sample names correspond to those in Online Resource 1

The number and phylogenetic position of *S. molesta* plastomes was then used to identify the haplotype of the putative maternal progenitor species. A network approach was used to identify potential multiple origins of *S. molesta* and to estimate the minimum number of invasive *S. molesta* genotypes in the United States. Following alignment, all nucleotide positions exhibiting ambiguities, gaps, and identical bases were masked. A Templeton, Crandell and Sing network (TCS; Clement et al. 2002) was produced from the resulting masked alignment in PopArt (Population Analysis with Reticulate Trees) (Leigh and Bryant 2015).

Results

Sampling and sequencing

A total of 94 Illumina libraries were constructed from 79 herbarium and 15 field-collected extractions (Online Resource 1). The 79 herbarium specimens exhibited library DNA concentrations ranging from 0.00 to 33.9 ng/µl (mean 11.78634 ng/µl). There was an inverse relationship between the age of the herbarium specimen (ranging from 1956 to 2020; Online Resource 1) and DNA library concentration, with older specimens yielding lower-concentration libraries ($P = 0.005$). A subset of 84 libraries were chosen for sequencing, comprising 76 herbarium specimens and 8 field-collected samples. This subset of samples was chosen based on taxonomy, DNA library concentration, and geographic location to maximize the *Salvinia* species sampled and to include a diverse geographic representation of *S. molesta*. The 76 herbarium specimens chosen for sequencing yielded between 259,732–56,834,492 sequence reads (mean 15,862,965 ± 8,155,554). There is no relationship between the number of raw reads and the age of the herbarium specimen ($P = 0.085$). Sequencing coverage ranged from 0.463 to 643.7, (mean 106.3 ± 113.6). There was no clear relationship between the average nucleotide coverage and the age of the herbarium specimen ($P = 0.089$). For each specimen between 574–1,271,576 reads mapped to the reference *Salvinia* plastome (mean 149,397 ± 180,986). There was no significant relationship between the number of reads mapped to the reference plastome and the age of the herbarium specimen ($P = 0.394$). On average, 0.944% of total reads per herbarium sample mapped to the reference (0.003–4.58%, ± 0.0091%).

Phylogenetic and network analyses

Following sample selection based on read number, the maximum likelihood plastome phylogeny (Fig. 1) was reconstructed with 58 herbarium specimens, eight field-collected samples and two sequences previously accessioned on GenBank (accession #MF177094.1, #MF177095.1). These sequences span 13 *Salvinia* species, including all but one western hemisphere species, *Salvinia oblongifolia* Mart. Two main clades are apparent, one comprising plastomes from most eastern-hemisphere specimens (maximum likelihood bootstrap support, MLBS = 45%) and the other comprising plastomes from all western-hemisphere specimens, as well as *S. molesta* specimens from Africa and Madagascar, with complete support (MLBS = 100%). The western-hemisphere clade is further divided into two completely supported (MLBS = 100%) subclades, one comprising *S. minima* and *Salvina sprucei* Kuhn and the other comprising samples of species belonging to the *S. auriculata* complex. Within the *S. auriculata* complex, samples of *S. biloba, S. auriculata*, and *S. radula* Baker are united (MLBS = 100%) with four morphologically anomalous samples hypothesized to be previously undescribed species (Bond Schwartsburd pers. comm.). All *S. molesta* samples are united with two *S. herzogii* samples (MLBS = 100%).

The plastome TCS network of *S. molesta* and *S. herzogii* samples (Fig. 2) was created from 31 samples and resulted in three groups of similar haplotypes. The first group, separated by 13 substitutions from the rest of the network, includes all Brazilian samples (both *S. molesta* and *S. herzogii*). The second group comprises only samples of *S. molesta* from across its invasive range, including a common shared haplotype (13 samples from Mexico, Texas, Louisiana, California, Georgia, and North Carolina) and nine additional haplotypes from the U.S., French Guiana, and Madagascar invasions. The third group

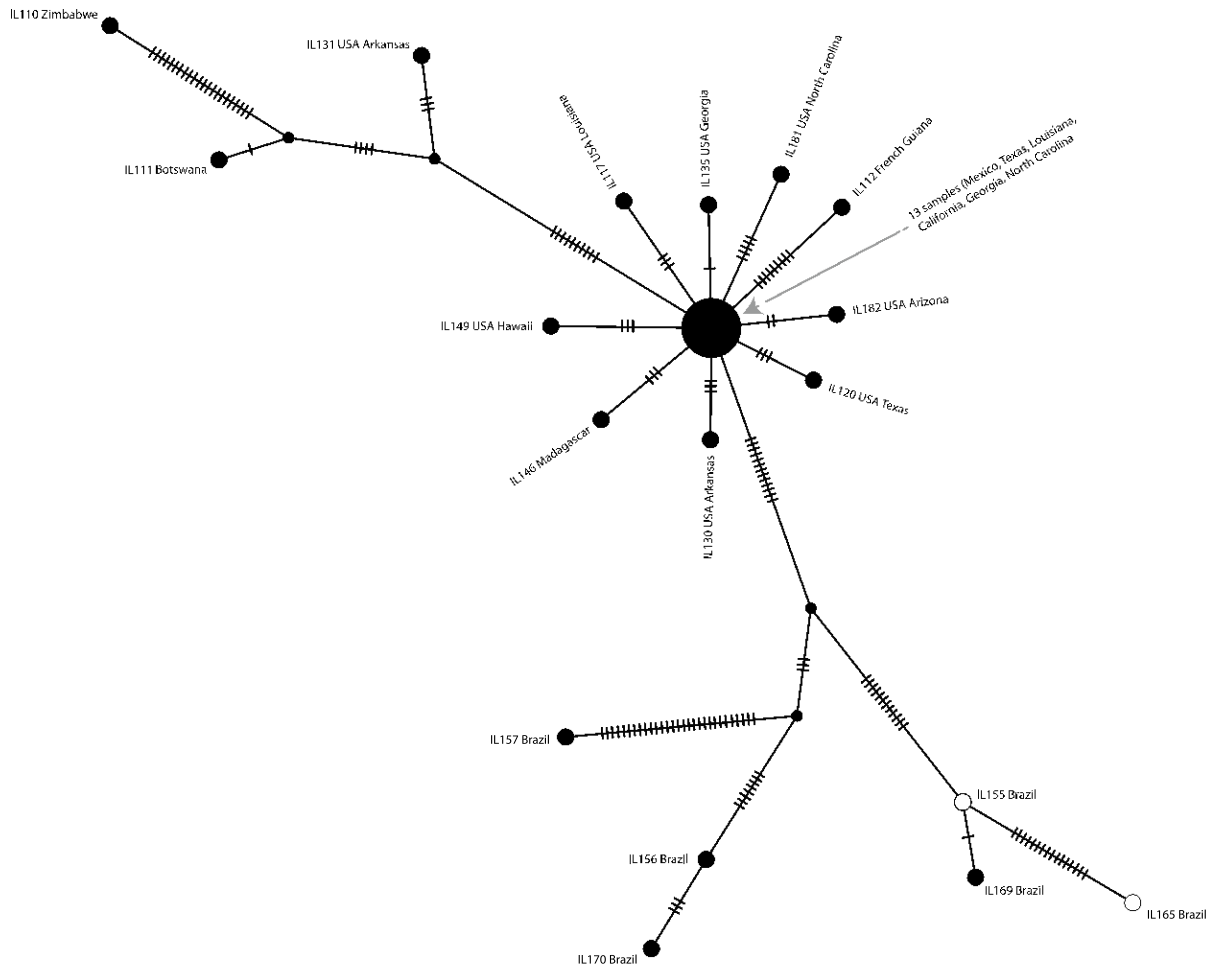

Fig. 2 TCS haplotype network constructed from *Salvinia molesta* and *Salvinia herzogii* plastome sequences. Filled circles denote *S. molesta* samples, open circles denote *S. herzogii* samples. Perpendicular hatch marks denote nucleotide substitutions separating haplotypes. Sample names correspond to those in Online Resource 1

is separated by ten substitutions from the rest of the network and includes a single haplotype of *S. molesta* from the U.S. (Arkansas) and two samples from Africa.

Discussion

Identifying *Salvinia molesta*'s maternal progenitor

The two primary clades in the ML plastome phylogeny representing species native to the eastern and western hemispheres are consistent with previous findings (Nagalingum et al. 2008), and establish a fundamental historical biogeographic split within the genus (Fig. 1). It also excludes the possibility of an eastern-hemisphere maternal donor of *S. molesta*. The paternal donor could be either an eastern- or western-hemisphere species, alternatives that carry different historical biogeographic implications. A western-hemisphere paternal donor would imply initial hybridization in that region and the subsequent transport of the hybrid to the various regions where *S. molesta* is invasive. An eastern-hemisphere paternal donor would imply the transport of the western-hemisphere maternal donor to the eastern hemisphere, subsequent hybrid origin of *S. molesta* there, followed by transport to the various regions where it is invasive. Within the western-hemisphere clade, giant salvinia samples are placed in a well-supported clade

encompassing all species of the *S. auriculata* complex. This is consistent with morphology and establishes that *S. molesta*'s maternal donor is part of this group. In his initial description of *S. molesta*, Mitchell (1972) suggested that *S. biloba* and *S. auriculata* or *S. herzogii* are *S. molesta*'s likely progenitors based on geographic proximity and morphological characteristics of extant populations. In a taxonomic treatment of *Salvinia* species occurring in Brazil, Miranda and Schwartsburd (2019) also suggest *S. biloba* and *S. herzogii* as its putative parents. However, *Salvinia auriculata* and *S. biloba* can be excluded as maternal donor given their strongly supported placements outside the well-supported *S. molesta* clade (Fig. 1).

Notably, both included specimens of *S. herzogii* are placed in this "molesta" clade, as is consistent with the previous findings of Machado et al. (2016) based on phylogenetic analysis of a single plastid gene. Although this suggests that *S. herzogii* could be the maternal progenitor of *S. molesta*, a prior study cast doubt on this scenario. Cytological evidence established that *S. herzogii* is itself a polyploid, in this case a heptaploid (Schneller 1980). It should be noted, however, that this is based on mitotic and meiotic counts from a single *S. herzogii* locality. If this count is reflective of the entire species, this excludes *S. herzogii* as a potential genome donor, since based on chromosome pairing behavior (Kuriachan 1979) the pentaploid *S. molesta* likely resulted from the union of reduced gametes from a tetraploid (diploid gamete) and a hexaploid (triploid gamete). Rather, the plastome data suggest the allopolyploids *S. molesta* and *S. herzogii* likely share as their maternal parent a species that is either unsampled, undescribed, or extinct. The only described western hemisphere *Salvinia* species not included in this phylogeny is *S. oblongifolia* Mart. Although this species also occurs in Brazil, it is currently not sympatric with *S. molesta* and is considered a poor morphological match due to its oblong laminae and spatulate (rather than fouet-like) hair system (Miranda and Schwartsburd 2019).

The phenomenon of a "missing" progenitor in ferns has been frequently reported (e.g., Hoot et al. 2004; Windham and Yatskievych 2005; Kim et al. 2008; Beck et al. 2010), with the genomic signature of an extinct or undetected progenitor persisting in the hybrid and/or polyploid taxon. The grouping of six *S. molesta* and *S. herzogii* haplotypes, all collected from Brazil, in the haplotype network (Fig. 2) suggests that the maternal progenitor of *S. herzogii* was genetically similar to extant Brazilian (presumably native) *S. molesta*. This makes biogeographical sense, as *S. herzogii* is currently sympatric with giant salvinia in southeastern Brazil. Since *S. molesta* and *S. herzogii* are quite similar (Miranda and Schwartsburd 2019), this suggest that their putative common maternal genome donor shares a similar haplotype, and likely, their same general morphology. The search for this missing parent should therefore start with examining *S. molesta* material from the shared range of *S. molesta* and *S. herzogii* in southeastern Brazil, specifically looking for specimens that exhibit well-formed spores that would suggest sexuality. More broadly, our inference of an unsampled giant salvinia maternal donor species combined with the observation of two putatively undescribed species highlight the need for a complete taxonomic revision of *Salvinia*. Additional cytological data is badly needed. Our knowledge of chromosome number and pairing behavior is based on very few counts, and a number of species remain completely unexamined. This is particularly critical given the frequency of polyploidy in this complex genus.

Genetic diversity of the *S. molesta* invasion

Although one *S. molesta* haplotype was particularly common in the United States and Mexico, a total of 13 unique invasive giant salvinia haplotypes are observed in the haplotype network separated by as many as 45 substitutions (i.e., IL112R from French Guiana vs. IL110R from Zimbabwe, Fig. 2). This observation of intraspecific *S. molesta* diversity applies to both the African invasion (three samples representing three unique haplotypes that are separated by as many as 39 substitutions) and U.S. invasion (21 samples representing nine unique haplotypes that are separated by as many as 18 substitutions). This diverse giant salvinia invasion is consistent with previous work on *S. molesta*. Galam et al. (2015) observed significant variation in the U.S. *S. molesta* invasion using nuclear *gapCp* gene sequence data and AFLP genotypes. More variation was observed within rather than between populations, and 180 unique *gapCp* haplotypes were observed in 240 total individuals from six populations in Louisiana and Texas. This invasive-range genetic diversity could be due to either multiple origins of *S. molesta*,

incorporating unique, genetically-variable maternal plastomes each time, or somatic mutation following a single origin. The somatic mutation scenario seems less likely given the very recent appearance and spread of this species, with the first (native) Brazilian collections from 1923 (Miranda and Schwartsburd 2019), first observed outside of Brazil at Lake Kariba in 1959 (Mitchell 1972), and not seen in the U.S. until 1998. This would therefore require the large amount of intraspecific plastome diversity seen in *S. molesta* to have arisen in a human lifetime. Therefore, we propose that the observed genetic diversity is the result of multiple independent origins that led to the formation of various independently derived lineages of *S. molesta* and its repeated long-distance dispersal and introduction throughout its invasive range. Ultimately, distinguishing between the two hypotheses for invasive-range diversity will require an expanded sampling of native-range *S. molesta*, in order to discover if the observed diversity of invasive-range haplotypes is present. Regardless of their cause, the presence of multiple *S. molesta* genotypes has potential implications for biocontrol, as differential interactions between invader and biocontrol genotypes have been consistently documented (Burdon et al. 1981; Lym et al. 1996; Garcia-Rossi et al. 2003; Manrique et al. 2008; Campanella et al. 2009; Boughton and Pemberton 2011; Goolsby et al. 2013; Harms et al. 2020).

A growing number of studies of asexual invasive plants also report high levels of genetic diversity. In the clonal invader *Pueraria lobata* (Willd.) Ohwi, 93% of loci were found to be polymorphic across 20 invasive populations (Pappert et al. 2000). In the vegetatively reproducing *Oxalis pes-caprae* L., 88% of ISSR bands were found to be polymorphic, with no band private to a given population (Rottenberg and Parker 2004). Both Geng et al. (2016) and Williams et al. (2020) reported genetic variation in the vegetatively reproducing *Alternanthera philoxeroides* (Mart.) Griseb., from both nuclear (61 unique multilocus genotypes observed among 179 individuals) and chloroplast (6 haplotypes among 375 individuals) datasets. Thum et al. (2020) analyzed nuclear microsatellite variation in *Myriophyllum spicatum* L., and found 24 unique multilocus genotypes in this vegetatively reproducing aquatic invasive across 103 sampled lakes. Finally, in the vegetatively reproducing aquatic invasive *Lemna minuta* Kunth, Paolacci et al. (2021) inferred four genetic clones from nine lakes in Ireland. Collectively these studies highlight the possibility for substantial genetic diversity within asexual plant invasions, consistent with multiple introductions of these species.

Herbarium-derived plastome data for understanding plant invasions

These results join numerous studies that have demonstrated successful plastome sequencing from herbarium specimens (Baaker et al. 2016; Zeng et al. 2018; Kates et al. 2021) and suggest that plastome genotyping from herbarium specimens holds promise for understanding the genetic diversity of plant invasions across both space and time. Plastome sequencing through low-coverage shotgun sequencing- so called “genome skimming” (Straub et al. 2012) is relatively inexpensive, is technically and analytically straightforward, does not require any prior knowledge of the genome, and avoids the complexity of homeologous gene copies when dealing with polyploids such as *Salvinia*. Limits of genome skimming relative to nuclear genome data include the fact that the plastome is effectively a single locus and is unable to provide biparental information. Genome skimming data from a temporally and geographically diverse set of herbarium specimens would potentially allow researchers to ask several basic questions: (1) How many genotypes form the invasion? (2) What is the geographic distribution of these genotypes? (3) Did these genotypes arrive relatively early in the invasion or are they recent arrivals? Although researchers have been applying “first generation” genetic tools (Sanger sequencing, microsatellites, etc.) to invasive species herbarium-derived DNAs for some time (Saltonstall 2002; Dormontt et al. 2014; Brandes et al. 2019), to our knowledge this is the first study to use whole plastome sequencing to investigate the genetic diversity of a plant invasion.

Acknowledgements The authors would like to thank Gar-rie Landry for help with fieldwork, and the curators of ARIZ, BRIT, F, FLAS, FLOR, FUEL, FURB, MBM, MBML, MO, NY, US and VIC for permission to sample from herbarium specimens.

Author contribution SH, JB and ES contributed to study conception and design. Fieldwork was conducted by SH, JB, ES, Brittany Sutherland, and Pedro Bond Schwartsburd. Data collection and analysis was conducted by SH and JB. The first draft of the manuscript was written by Stacy Holt and

James Beck and all authors commented on previous versions of the manuscript. All authors read and approved of the final manuscript.

Funding This work was supported by National Science Foundation award OIA 1920858 to EMS and JBB, by the Wichita State University Department of Biological Sciences and by the University of Louisiana at Lafayette Department of Biology.

Data availability All sequencing reads generated during this project are available on the NCBI Sequencing Read Archive (SRA) (BioProject ID #PRJNA925219).

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

References

Ahmad R, Liow PS, Spencer DF, Jasieniuk M (2008) Molecular evidence for a single genetic clone of invasive *Arundo donax* in the United States. Aquat Bot 88:113–120. https://doi.org/10.1016/j.aquabot.2007.08.015

Bakker FT, Lei D, Yu J, Mohammadin S, van de Wei Z, Gravendeel B, Nieuwenhuis M, Staats M, Alquezar-Planas DE, Holmer R (2016) Herbarium genomics: plastome sequence assembly from a range of herbarium specimens using an iterative organelle Genome Assembly pipeline. Biol J Linn Soc 117:33–43. https://doi.org/10.1111/bij.12642

Beck JB, Windham MD, Yatskievych G, Pryer KM (2010) A diploids-first approach to species delimitation and interpreting polyploid evolution in the fern genus *Astrolepis* (Pteridaceae). Syst Bot 35:223–234. https://doi.org/10.1600/036364410791638388

Beck JB, Alexander PJ, Allphin L, Al-Shehbaz IA, Rushworth C, Bailey CD, Windham MD (2012) Does hybridization drive the transition to asexuality in diploid *Boechera*? Evolution 66:985–995. https://doi.org/10.1111/j.1558-5646.2011.01507.x

Beck JB, Allison JR, Pryer KM, Windham MD (2012) Identifying multiple origins of polyploid taxa: a multilocus study of the hybrid cloak fern (*Astrolepis integerrima*; Pteridaceae). Am J Bot 99:1857–1865. https://doi.org/10.3732/ajb.1200199

Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Boughton AJ, Pemberton RW (2011) Limited field establishment of a weed biocontrol agent, *Floracarus perrepae* (Acariformes: Eriophyidae), against Old World climbing fern in Florida-a possible role of mite resistant plant genotypes. Environ Entomol 40:1448–1457. https://doi.org/10.1603/EN11030

Brandes U, Furevik BB, Nielsen LR, Kjær ED, Rosef L, Fjellheim S (2019) Introduction history and population genetics of intracontinental scotch broom (*Cytisus scoparius*) invasion. Divers Distrib 25:1773–1786. https://doi.org/10.1603/EN11030

Burdon JJ, Groves RH, Cullen JM (1981) The impact of biological control on the distribution and abundance of *Chondrilla juncea* in south-eastern Australia. J Appl Ecol 18:957–966. https://doi.org/10.2307/2402385

Campanella DM, McEvoy PB, Mundt CC (2009) Interaction effects of two biological control organisms on resistant and susceptible weed biotypes of *Chondrilla juncea* in western North America. Biol Control 50:50–59. https://doi.org/10.1016/j.biocontrol.2009.01.005

Clement M, Snell Q, Walker P, Posada D, Crandall K (2002) TCS: estimating gene genealogies. In: Proceedings of the 16th International parallel distributed processing symposium 2:184

Coetzee JA, Hill MP (2020) *Salvinia molesta* D. Mitch. (Salviniaceae): impact and control. CAB Rev 15:1–11. https://doi.org/10.1079/PAVSNNR202015033

Darling JA (2015) Genetic studies of aquatic biological invasions: closing the gap between research and management. Biol Invasions 17:951–971. https://doi.org/10.1007/s10530-014-0726-x

Dillenberger MS, Wei N, Tennessen JA, Ashman TL, Liston A (2018) Plastid genomes reveal recurrent formation of allopolyploid *Fragaria*. Am J Bot 105:862–874. https://doi.org/10.1002/ajb2.1085

Dormontt EE, Gardner MG, Breed MF, Rodger JG, Prentis PJ, Lowe AJ (2014) Genetic bottlenecks in time and space: reconstructing invasions from contemporary and historical collections. PLoS ONE 9:e106874. https://doi.org/10.1371/journal.pone.0106874

Ellstrand NC, Schierenbeck KA (2000) Hybridization as a stimulus for the evolution of invasiveness in plants? PNAS 97:7043–7050. https://doi.org/10.1073/pnas.97.13.7043

Galam D, Silva J, Sanders D, Oard JH (2015) Morphological and genetic survey of Giant Salvinia populations in Louisiana and Texas. Aquat Bot 127:20–25. https://doi.org/10.1016/j.aquabot.2015.07.005

Garcia-Rossi D, Rank N, Strong DR (2003) Potential for self-defeating biological control? Variation in herbivore vulnerability among invasive *Spartina* genotypes. Ecol Appl 13:1640–1649. https://doi.org/10.1890/01-5301

Gaskin JF, Bon MC, Cock MJ, Cristofaro M, De Biase A, De Clerck-Floate R, Ellison CA, Hinz HL, Hufbauer RA, Julien MH, Sforza R (2011) Applying molecular-based approaches to classical biological control of weeds. Biol Control 58:1–21. https://doi.org/10.1890/01-5301

Geng Y, van Klinken RD, Sosa A, Li B, Chen J, Xu CY (2016) The relative importance of genetic diversity and phenotypic plasticity in determining invasion success of a clonal weed in the USA and China. Front Plant Sci 7:213. https://doi.org/10.3389/fpls.2016.00213

Goolsby J, Cortés Mendoza E, Moran P, Adamczyk J, García M, Kirk A (2013) Evaluation of spanish Arundo scale *Rhizaspidiotus donacis* (Hemiptera; Diaspididae) survival and fecundity on three new world genotypes of *Arundo donax* (Poaceae; Arundinoideae). Biocontrol Sci

Technol 23:499–506. https://doi.org/10.1080/09583157.2013.772562
Harms N, Shearer J, Cronin JT, Gaskin JF (2020) Geographic and genetic variation in susceptibility of *Butomus umbellatus* to foliar fungal pathogens. Biol Invasions 22:535–548. https://doi.org/10.1007/s10530-019-02109-3
Herben T, Suda J, Klimešová J (2017) Polyploid species rely on vegetative reproduction more than diploids: a re-examination of the old hypothesis. Ann Bot 120:341–349. https://doi.org/10.1093/aob/mcx009
Hoot SB, Napier NS, Taylor WC (2004) Revealing unknown or extinct lineages within *Isoetes* (Isoetaceae) using DNA sequences from hybrids. Am J Bot 91:899–904. https://doi.org/10.3732/ajb.91.6.899
iNaturalist (2020) Available at https://www.inaturalist.org
Jacono CC (1999) *Salvinia molesta* (Salvineaceae) new to Texas and Louisiana. SIDA 18:927–928
Jacono CC, Davern TR, Center TD (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
Kates HR, Doby JR, Siniscalchi CM, LaFrance R, Soltis DE, Soltis PS, Guralnick RP, Folk RA (2021) The effects of herbarium specimen characteristics on short-read NGS sequencing success in nearly 8000 specimens: old, degraded samples have lower DNA yields but consistent sequencing success. Front Plant Sci. https://doi.org/10.3389/fpls.2021.669064
Katoh K, Misawa K, Kuma KI, Miyata T (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucl Acids Res 30:3059–3066. https://doi.org/10.1093/nar/gkf436
Kim S-T, Sultan SE, Donoghue MJ (2008) Allopolyploid speciation in *Persicaria* (Polygonaceae): insights from a low-copy nuclear region. PNAS 105:12370–12375. https://doi.org/10.1073/pnas.080514110
Kuriachan P (1979) Interspecific origin of *Salvinia molesta* Mitchell–evidence from karyotype. Indian J Bot 2:51–54
Lal A (2016) *Salvinia molesta*: an assessment of the effects and methods of eradication. M.S. Thesis, University of San Francisco
Leigh JW, Bryant D (2015) PopART: full-feature software for haplotype network construction. Methods Ecol Evol 6:1110–1116. https://doi.org/10.1111/2041-210X.12410
Li FW, Brouwer P, Carretero-Paulet L, Cheng S, De Vries J, Delaux PM et al (2018) Fern genomes elucidate land plant evolution and cyanobacterial symbioses. Nat Plants 4:460–472. https://doi.org/10.1038/s41477-018-0188-8
Liu J, Dong M, Miao SL, Li ZY, Song MH, Wang RQ (2006) Invasive alien plants in China: role of clonality and geographical origin. Biol Invasions 8:1461–1470. https://doi.org/10.1007/s10530-005-5838-x
Loomis ES, Fishman L (2009) A continent-wide clone: population genetic variation of the invasive plant *Hieracium aurantiacum* (Orange Hawkweed; Asteraceae) in North America. Int J Plant Sci 170:759–765. https://doi.org/10.1086/599241
Loyal DS, Grewal RK (1966) Cytological study on sterility in *Salvinia auriculata* Aublet with a bearing on its reproductive mechanism. Cytologia 31:330–338. https://doi.org/10.1508/cytologia.31.330
Luque GM, Bellard C, Bertelsmeier C, Bonnaud E, Genovesi P, Simberloff D, Courchamp F (2014) The 100th of the world's worst invasive alien species. Biol Invasions 16:981–985. https://doi.org/10.1007/s10530-013-0561-5
Lym RG, Nissen SJ, Rowe ML, Lee DJ, Masters RA (1996) Leafy spurge (*Euphorbia esula*) genotype affects gall midge (*Spurgia esulae*) establishment. Weed Sci 44:629–633. https://doi.org/10.1017/S0043174500094455
Machado SA, Oliveira AV, Fabrin TM, Prioli SM, Prioli AJ (2016) Molecular characterization of the species *Salvinia* (Salviniaceae) from the upper Paraná River floodplain. Genet Mol Res 15:1–11. https://doi.org/10.4238/gmr.15038575
Manrique V, Cuda JP, Overholt WA, Williams DA, Wheeler GS (2008) Effect of host-plant genotypes on the performance of three candidate biological control agents of *Schinus terebinthifolius* in Florida. Biol Control 47:167–171. https://doi.org/10.1016/j.biocontrol.2008.07.005
Miranda CV, Schwartsburd PB (2016) Aquatic ferns from Viçosa (MG, Brazil): Salviniales (Filicopsida; Tracheophyta). Braz J of Bot 39:935–942. https://doi.org/10.1007/s40415-016-0284-9
Miranda CV, Schwartsburd PB (2019) *Salvinia* (Salviniaceae) in southern and southeastern Brazil—including new taxa, new distribution records, and new morphological characters. Braz J Bot 42:171–188. https://doi.org/10.1007/s40415-019-00522-5
Mitchell D (1972) The kariba weed: *Salvinia molesta*. Br Fern Gaz 10:251–252
Mitchell DS, Thomas PA (1972) Ecology of water weeds in the neotropics. UNESCO, Paris
Mitchell DS, Tur NM (1975) The rate of growth of *Salvinia molesta* (*S. auric*ulata Auct.) In laboratory and natural conditions. J Appl Ecol 12:213–225. https://doi.org/10.2307/2401730
Mora-Olivo A, Yatskievych G (2009) *Salvinia molesta* in Mexico. Am Fern J 99:56–58
Nagalingum NS, Nowak MD, Pryer KM (2008) Assessing phylogenetic relationships in extant heterosporous ferns (Salviniales), with a focus on *Pilularia* and *Salvinia*. Bot J Linn Soc 157:673–685. https://doi.org/10.1111/j.1095-8339.2008.00806.x
Paolacci S, Bog M, Lautenschlager U, Bonfield R, Appenroth KJ, Oberprieler C, Jansen MA (2021) Clonal diversity amongst island populations of alien, invasive *Lemna minuta* Kunth. Biol Invasions 23:2649–2660. https://doi.org/10.1007/s10530-021-02530-7
Pappert RA, Hamrick JL, Donovan LA (2000) Genetic variation in *Pueraria lobata* (Fabaceae), an introduced, clonal, invasive plant of the southeastern United States. Am J Bot 87:1240–1245. https://doi.org/10.2307/2656716
Poulin J, Weller SG, Sakai AK (2005) Genetic diversity does not affect the invasiveness of fountain grass (*Pennisetum setaceum*) in Arizona, California and Hawaii. Divers Distrib 11:241–247. https://doi.org/10.1111/j.1366-9516.2005.00136.x
Rani VU, Bhambie S (1983) A study on the growth of *Salvinia molesta* Mitchell in relation to light and temperature.

Aquat Bot 17:119–124. https://doi.org/10.1016/0304-3770(83)90108-0

Room PM (1983) `Falling apart' as a lifestyle: the rhizome architecture and population growth of *Salvinia molesta*. J Ecol 71:349–365. https://doi.org/10.2307/2259719

Rottenberg A, Parker JS (2004) Asexual populations of the invasive weed *Oxalis pes–caprae* are genetically variable. Proc R Soc Lond Ser B 7:S206–S208. https://doi.org/10.1098/rsbl.2003.0135

Saeidi S, McKain MR, Kellogg EA (2018) Robust DNA isolation and high-throughput sequencing library construction for herbarium specimens. J Vis Exp JoVE. https://doi.org/10.3791/56837

Saltonstall K (2002) Cryptic invasion by a non-native genotype of the common reed, *Phragmites australis*, into North America. PNAS 99:2445–2449. https://doi.org/10.1073/pnas.03247799

Schaffner U (2001) Host range testing of insects for biological weed control: How can it be better interpreted? Bioscience 51:951–959

Schneller J (1980) Cytotaxonomic investigations of *Salvinia herzogii* de la Sota. Aquat Bot 9:279–283. https://doi.org/10.1016/0304-3770(80)90027-3

Sigel EM, Windham MD, Pryer KM (2014) Evidence for reciprocal origins in *Polypodium hesperium* (Polypodiaceae): a fern model system for investigating how multiple origins shape allopolyploid genomes. Am J Bot 101:1476–1485. https://doi.org/10.3732/ajb.1400190

Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30:1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Straub SC, Parks M, Weitemier K, Fishbein M, Cronn RC, Liston A (2012) Navigating the tip of the genomic iceberg: next-generation sequencing for plant systematics. Am J Bot 99:349–364. https://doi.org/10.3732/ajb.1100335

Sun Y, Beuchat C, Müller-Schärer H (2020) Is biocontrol efficacy rather driven by the plant or the antagonist genotypes? A conceptual bioassay approach. NeoBiota 7:81–101. https://doi.org/10.3897/neobiota.63.54962

Te Beest M, Le Roux JJ, Richardson DM, Brysting AK, Suda J, Kubešová M, Pyšek P (2012) The more the better? The role of polyploidy in facilitating plant invasions. Ann Bot 109:19–45. https://doi.org/10.1093/aob/mcr277

Thomas PA, Room AP (1986) Taxonomy and control of *Salvinia molesta*. Nature 320:581–584

Thum RA, Chorak GM, Newman RM, Eltawely JA, Latimore J, Elgin E, Parks S (2020) Genetic diversity and differentiation in populations of invasive eurasian (*Myriophyllum spicatum*) and hybrid (*Myriophyllum spicatum*× *Myriophyllum sibiricum*) watermilfoil. Invasive Plant Sci Manag 13:59–67. https://doi.org/10.1017/inp.2020.12

Whitton J, Sears CJ, Baack EJ, Otto SP (2008) The dynamic nature of apomixis in the angiosperms. Int J Plant Sci 169:169–182. https://doi.org/10.1086/523369

Williams DA, Harms NE, Knight IA, Grewell BJ, Futrell CJ, Pratt PD (2020) High genetic diversity in the clonal aquatic weed *Alternanthera philoxeroides* in the United States. Invasive Plant Sci Manag 13:217–225. https://doi.org/10.1017/inp.2020.32

Windham MD, Yatskievych G (2005) A novel hybrid *Polypodium* (Polypodiaceae) from Arizona. Am Fern J 95:57–67. https://doi.org/10.1640/0002-8444(2005)095[0057:ANHPPF]2.0.CO;2

Wolf PG, Roper JM, Duffy AM (2010) The evolution of chloroplast genome structure in ferns. Genome 53:731–738. https://doi.org/10.1139/G10-061

Zeng CX, Hollingsworth PM, Yang J, He ZS, Zhang ZR, Li DZ, Yang JB (2018) Genome skimming herbarium specimens for DNA barcoding and phylogenomics. Plant Methods 14:43. https://doi.org/10.1186/s13007-018-0300-0

Zhang YY, Zhang DY, Barrett SC (2010) Genetic uniform-ity characterizes the invasive spread of water hyacinth (*Eichhornia crassipes*), a clonal aquatic plant. Mol Ecol 19:1774–1786. https://doi.org/10.1111/j.1365-294X.2010.04609.x