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ORIGINAL PAPER

# Evaluating hybridization as a potential facilitator of successful cogongrass (*Imperata cylindrica*) invasion in Florida, USA

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**Abstract** Interspecific hybridization is cited as one potential mechanism for increased invasiveness, particularly among some grass species. In the southeastern United States, the successful invasion of cogongrass (*Imperata cylindrica*) has sometimes been attributed to hybridization with the previously naturalized *Imperata brasiliensis*. This research aimed to determine whether genetic signals are consistent with these two species having experienced interspecific hybridization in Florida (USA), where it has been proposed that such an event facilitated cogongrass invasion across the region. Individuals of invasive *I. cylindrica* populations (n = 66) were sampled broadly from the state, and *I. brasiliensis* (n = 63) individuals were sampled from expertly identified and vouchered populations in Miami-Dade County. Genetic analysis utilized amplified fragment length polymorphisms in sampled individuals, and failed to detect significant genetic differentiation between the two species. Analysis of molecular variance partitioned the majority of detected variation within populations (86 %), while only 8 % was significantly partitioned between *I. cylindrica* and *I. brasiliensis* ($F_{ST} = 0.135$, $P < 0.001$). Both STRUCTURE analysis and principal coordinates analysis strongly indicated the presence of a single genetic group across the sampled populations. Hybrid analysis furthermore failed to support interspecific hybridization. Florida populations thus are suggested to share genetic parent material(s) and/or have experienced substantial admixture across the state. Therefore, this study suggests *Imperata* populations in South Florida that are currently considered to be *I. brasiliensis* are not genetically distinct from *I. cylindrica*, and regional cogongrass invasion likely was not facilitated by previously postulated interspecific hybridization.

**Keywords** AFLP · Genetic diversity · Grasses · Hybridization · Invasive species · Poaceae

## Introduction

Hybridization has been demonstrated to facilitate invasion by some species (Ellstrand and Schierenbeck 2000) and has generated an increase in the study of the role and significance of hybridization during biological invasion (Schierenbeck and Ellstrand 2009). In biological invasions, species may be introduced to a region with genetic variation that provides opportunities for novel recombination and other processes that can facilitate—or limit—invasion success (Lee 2002;

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Ward et al. 2008; Whitney and Gabler 2008). Inter- or intraspecific recombination during invasions may give rise to new phenotypes with fitness benefits such as adaptive flexibility to novel conditions in the newly encountered environment (Lee 2002). Research on the well-studied *Spartina* system has demonstrated that hybridization between native and exotic congenerics contributed to declines in populations of the local native species; such as, *S. alterniflora* invasion in California and the global spread of *S. anglica* (Daehler and Strong 1997; Ainouche et al. 2004; Ayres et al. 2004; Salmon et al. 2005). Interspecific hybridization between congenerics also has contributed to invasion by the broadly distributed Johnsongrass (*Sorghum halepense*) (Paterson et al. 1995), and the cryptic invasion of *Phragmites australis* in North America (Saltonstall 2002) has been demonstrated to have involved both inter- and intraspecific hybridization (Meyerson et al. 2010; Lambertini et al. 2012; Meyerson et al. 2012).

In the case of the cogongrass (*Imperata cylindrica* (L.) Raeuschel) invasion in the southeastern United States (US), interspecific hybridization with Brazilian satintail, *Imperata brasiliensis* Trin, has been proposed to be a significant contributing factor in invasion and spread, via 'hybrid swarms' (Howard 2005; Capochichi et al. 2008; Vergara et al. 2008). Cogongrass is a known exotic, highly invasive, perennial C4 grass that was introduced into the southeastern US in the early twentieth century (Bryson and Carter 1993; MacDonald 2004). In addition to interspecific hybridization, multiple introductions from previously isolated parent material from East Asia (Tabor 1949, 1952) may also have contributed to its invasion success (Lucardi et al. 2014). The aggressive invasion by *I. cylindrica* has been difficult to manage and is both economically and ecologically costly (MacDonald 2004). However, populations of *I. cylindrica* observed in Florida (FL) during the mid-twentieth century were reported *not* to form dense, monotypic, impenetrable mats, unlike Old World populations (Hubbard et al. 1944). *Imperata brasiliensis*, although recently listed as a Federal Noxious Weed (USDA-APHIS, 2010), is characterized by a lack of invasive characters, such as more-or-less static, non-expanding populations and frequent occurrence in mixture with other plant species (Hall 1998; Vergara et al. 2008; Welker and Longhi-Wagner 2012; Keith Bradley, personal communication; D.W. Hall, personal communication).

*Imperata cylindrica* and *I. brasiliensis* overlap in biology and ecology sufficiently as to have been considered by some to be synonymous (Hall 1998). Alternatively, Keith Bradley (Institute for Regional Conservation; personal communication) identified all *Imperata* populations occurring in undisturbed natural areas and that exhibit a non-invasive ecology as *I. brasiliensis* (Table 1). Species identification of *I. cylindrica* and *I. brasiliensis* rely on a single floral morphological trait. Inflorescences possessing two stamens per flower diagnose *I. cylindrica*, whereas all other species within *Imperata* (including *I. brasiliensis*) possess flowers with only one stamen. Morphological assignment requires the availability and timely collection of intact inflorescences among populations, which can markedly differ in phenology (Burnell 2006; Howard 2005). Environmental conditions throughout Florida vary, such that flowering among *Imperata* populations are rarely in sync making morphological identification between co-occurring species unreliable and impractical (Lippincott 2000). In fact, Hall (1978) observed some FL *Imperata* populations in possession of both single and bi-staminate flowers, resulting in his synonymous taxonomic treatment. Others have also encountered broad phenotypic plasticity and morphological variability in *I. cylindrica* populations, further confounding taxonomic differentiation between the two species (Al-Jaboory and Hassawy 1980; Cheng and Chou 1997; Bryson et al. 2010).

Brazilian satintail's US distribution includes Puerto Rico and five southern states (Louisiana, Mississippi, Alabama, Florida, and South Carolina; USDA, NRCS. 2013. The PLANTS Database (http://plants.usda.gov, National Plant Data Team, Greensboro, NC 27401-4901 USA). The recognized native distribution of *I. brasiliensis* includes Brazil and Argentina (South America); however, naturalized populations have been reported to occur in South Florida, suggesting potential introduction from South America via the Caribbean (Hubbard et al. 1944; Hall 1978, 1998; Wiggins 1980; Bryson and Carter 1993; Gabel 2003; Welker and Longhi-Wagner 2012). With the exception of Puerto Rico, all US states with *I. brasiliensis* also possess populations of *I. cylindrica*. Morphological distinction between *I. brasiliensis* and *I. cylindrica* in areas where ranges overlap is problematic and misidentification of both species has likely occurred (Gabel 1982; Bryson and Carter 1993; Hall 1998;

**Table 1** Deposited *I. brasiliensis* accessions in Florida herbaria

| Catalog | Accession no. | Species | County | Year collected | Notes |
|---|---|---|---|---|---|
| University of Florida Herbarium | FLAS28226 | *I. brasiliensis* | Dade | 1905 | NL Britton; Determined by Gabel (1982) |
| | FLAS70524 | *I. brasiliensis* | Dade | 1955 | FC Craighead, Determined by LE Arnold and ML Gabel (1982) |
| | FLAS99979 | *I. brasiliensis* | Dade | 1967 | GN Avery; Determined by ML Gabel (1982) |
| | FLAS160172 | *I. brasiliensis* | Dade | 1983 | Herndon; Original ID |
| | **FLAS188997** | *I. brasiliensis* | **Dade** | **1995** | **CL Lippincott, from Thompson Park*, Collected with KA Bradley; Determined by GF Guala** |
| | FLAS211677 | *I. brasiliensis* | Dade | 1995 | EL Bridges from Deering Estate; Original ID |
| | FLAS187920 | *I. brasiliensis* | Dade | 1996 | CL Lippincott, original ID: *I. cylindrica*; Determined by CL Lippincott as *I. brasiliensis* 1997 |
| | FLAS187921 | *I. brasiliensis* | Dade | 1996 | CL Lippincott from Thompson Park*, Original ID: *I. cylindrica*; Determined by CL Lippincott as *I. brasiliensis* 1997 |
| Missouri Botanical Garden | MO-880369/ 791364 | *I. brasiliensis* | SW coast, FL | 1875 | Anonymous from Banks of Caloosa River; Determined by ML Gabel 1999 |
| | MO-880368/ 2970652 | *I. brasiliensis* | Hernando | 1901 | SM Tracy from Pine Island, FL. Determined by ML Gabel 1999 |
| | MO-880370/ 801557 | *I. brasiliensis* | Lee | 1916 | JP Standley; Determined by ML Gabel 1999 |
| | MO-317954/ 3689525 | *I. brasiliensis* | Baladwin Co., AL | 1970 | R Kral, Original ID |
| | MO-317955/ 2383245 | *I. brasiliensis* | Hillsborough | 1975 | Shuey; Determined by ML Gabel 1999 |
| | MO-880371/ 05014720 | *I. brasiliensis* | Lee | 1976 | R Kral, Original ID |
| Fairchild Tropical Botanic Garden | FTG33541 | *I. brasiliensis* | Dade | 1977 | GN Avery, original ID |
| | FTG11240 | *I. brasiliensis* | Dade | 1964 | FC Craighead, original ID: *Muhlenbergia ermersleyi* Vasey; Determined by GN Avery in 1979 |
| | FTG11128 | *I. brasiliensis* | Collier | 1964 | FC Craighead, original ID |
| | FTG13346 | *I. brasiliensis* | Collier | 1964 | FC Craighead, original ID |
| | FTG37732 | *I. brasiliensis* | Dade | 1979 | J Popenoe, original ID |
| | FTG328520 | *I. brasiliensis* | Collier | 1978 | J Popenoe, original ID |
| | FTG39466 | *I. brasiliensis* | Collier | 1979 | DW Black, original ID; Bear Island, Big Cypress Preserve |
| | FTG57717 | *I. brasiliensis* | Dade | 1983 | A Herndon, original ID |
| | **FTG81856** | *I. brasiliensis* | **Dade** | **1996** | **KA Bradley, original ID; Martinez Pineland*** |
| | **FTG81855** | *I. brasiliensis* | **Dade** | **1996** | **KA Bradley, original ID; Seminole Wayside Park*** |
| | **FTG82249/ 6914** | *I. brasiliensis* | **Dade** | **1998** | **KA Bradley, original ID; Thompson Park*** |

**Table 1** continued

| Catalog | Accession no. | Species | County | Year collected | Notes |
|---|---|---|---|---|---|
| | **FTG84686** | *I. brasiliensis* | **Dade** | **1998** | **KA Bradley, original ID; Goulds Pineland** |
| Robert K. Godfrey Herbarium (FSU) | 4537 | *I. brasiliensis* | Charlotte | 1956 | R Kral, Punta Gorda, Gulfside peninsular |
| | 4536 | *I. brasiliensis* | Escambia | 1995 | Burkhalter, University of West Florida Campus, Pensacola |
| | 86292 | *I. brasiliensis* | Wakulla | 2013 | St. Marks Nat'l Wildlife Refuge (Wakulla Unit) |
| Atlas of Florida, University of South Florida | 47314 | *I. brasiliensis* | Dade | 1961 | FC Craighead; Determined by ML Gabel (1982) |
| | 136274 | *I. brasiliensis* | Hendry | 1965 | LJ Brass; Determined as *I. cylindrica* by ML Gabel (1982) and as *I. Brasiliensis* (accepted) by RP Wunderlin 1982 |
| | 79695 | *I. brasiliensis* | Dade | 1967 | GN Avery, Determined by ML Gabel (1982) |
| | 255394 | *I. cylindrica* | Okeechobee | 1993 | SL Orzell, original ID: *I. brasiliensis*; Determined by BF Hansen 2010 |
| | 235596 | *I. cylindrica* | Dade | 1995 | EL Bridges, Deering Estate, Original ID: *I. brasiliensis*; Determined by BF Hansen 2007 |
| | 224862 | *I. brasiliensis* | Dade | 1997 | CL Lippincott, Charles Deering Park; Determined by GF Guala |

Accession numbers are unique to the corresponding database. Accessions in **bold** were collected/deposited by K. Bradley. Majority of vouchers were collected from Dade Co., FL. * Locations sampled for this research

Howard 2005). It is evident that botanical nomenclature for some voucher specimens has alternated between *I. brasiliensis* and *cylindrica* depending on the determining authority (Table 1).

It has been proposed that *I. cylindrica* invaded FL from the north and west (originating from Mississippi and Alabama), contacted and hybridized with naturalized populations of *I. brasiliensis* in FL, which increased genetic diversity in *I. cylindrica* to have facilitated invasion throughout the region (Howard 2005; Capo-chichi et al. 2008; Vergara et al. 2008). In this study, we sought to determine if both species occur in FL and if interspecific hybridization between these species has occurred in that state. To do so, we had to first locate *I. cylindrica* and *I. brasiliensis* populations. Cogongrass (*I. cylindrica*) is present as a weedy species throughout much of Florida (Hubbard et al. 1944; Hall 1978, 1998; MacDonald 2004). We sampled some sites where *I. cylindrica* was being actively managed. However, for *I. brasiliensis,* we were advised that the best location to sample in FL was "in South Peninsular Florida" (D.W. Hall, personal communication).

Herbarium records for *I. brasiliensis* were primarily collected from FL, especially from the now unified Miami-Dade County. Furthermore, the Grass Manual on the Web (http://herbarium.usu.edu/webmanual/; Gabel 2003) shows that *I. cylindrica* and *I. brasiliensis* do not overlap in Miami-Dade County. We consulted with local experts, and determined that sampling within Miami-Dade County would allow for direct access to already vouchered *I. brasiliensis* populations (several of our samples included populations vouchered by K. Bradley from Miami-Dade Municipal Parks; Table 1). Therefore, we sampled all *I. brasiliensis* tissues from Miami-Dade County based collectively on voucher locations (Table 1), on-site identification of populations (by K. Bradley), the best evidence available from botanical experts, and sampling data from previous studies (Hall 1978, 1998, personal communication; Vergara et al. 2008). All populations were preliminarily identified as

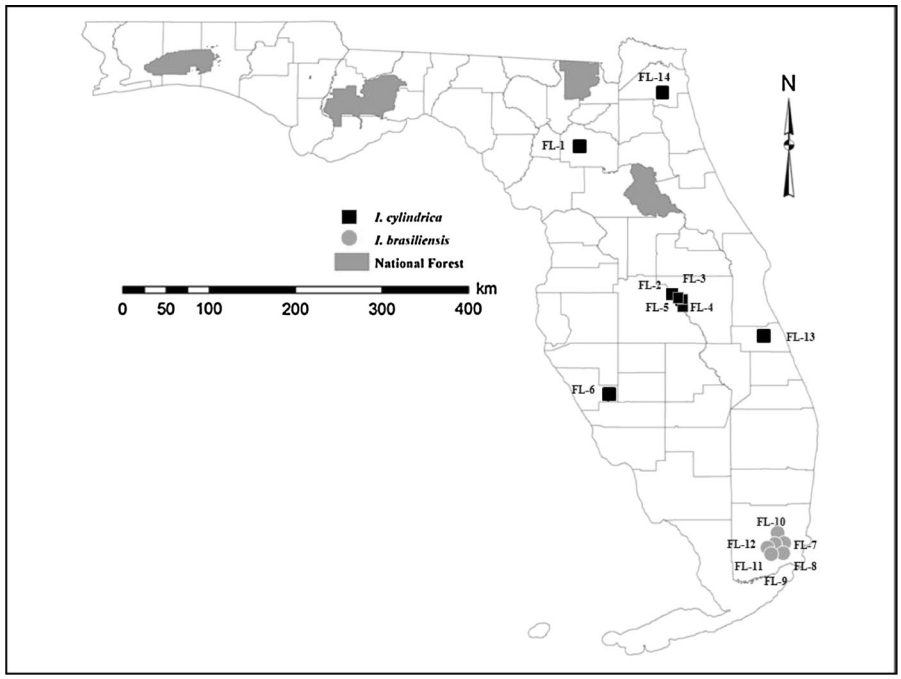


**Fig. 1** Map of population sites sampled in FL of *I. brasiliensis* (*circles*) or *I. cylindrica* (*squares*). *Gray shaded areas* represent National Forests acquisition boundaries

cogongrass (*I. cylindrica*) or Brazilian satintail (*I. brasiliensis*) upon sampling of tissues.

This research sought to determine if: (1) populations identified and vouchered as *I. brasiliensis* are genetically differentiated from *I. cylindrica* and, (2) if the presence or signature of interspecific hybridization is detectable from genetic data. To resolve this, we examined genetic variation within and among populations of FL *Imperata* using highly-reproducible genetic markers known as amplified fragment length polymorphisms (AFLPs). AFLPs are arbitrarily amplified dominant markers and were selected for several reasons: no a priori sequence information was necessary, the whole genome could be simultaneously sampled, and this technique is considered reproducible and practical (in cost and data generation) (Campbell et al. 2003; Bussell et al. 2005; Meudt and Clarke 2007). Recently, AFLPs were used in genetic analysis of *I. cylindrica* populations in other southeastern US states (Capo-chichi et al. 2008; Lucardi et al. in press). Because of shared morphological and ecological traits, we sampled populations of *I. brasiliensis* from only one county where we could obtain reliable evidence that the populations we sampled were botanically accepted *I. brasiliensis* (Table 1). We sampled *I. cylindrica* from across northern and central FL (Fig. 1). Based on previous molecular analyses to delimit species within the genus *Imperata* (e.g., Gabel 1982 and Vergara et al. 2008), we hypothesized that we would find clear evidence of genetic support for genetic groups consistent with *I. brasiliensis* (limited to South FL) and I. *cylindrica*, and potentially hybrids of intermediate ancestry. A lack of such genetic structure would indicate the presence of a genetically undifferentiated population of *Imperata* and would thus suggest hybridization may not have been responsible for invasion success.

## Methods

### Sampling

Live leaf tissues of *I. cylindrica* and *I. brasiliensis* were collected in Florida during the summer, 2009. While both species are currently federally listed noxious weeds, *I. brasiliensis* was listed only after sampling occurred. A permit was granted by the U.S. Dept. of Agriculture, Animal and Plant Health Inspection Service, Plant Pest Quarantine for *I. cylindrica* (Permit #: P526P-12-00211, P526-080721-005). In Osceola County, a collections agreement with The Nature Conservancy was obtained for sampling from invasive *I. cylindrica* populations

located within the Disney Wilderness Preserve (DWP). A permit to sample *I. brasiliensis* was granted by Miami-Dade County Parks and Recreation Department, Natural Areas Management (MDPR Permit #145). The Institute for Regional Conservation (Keith Bradley) located and graciously assisted with on-site identification of *I. brasiliensis* in Miami-Dade County Municipal Parks.

*Imperata cylindrica* tissues were collected from Alachua (Gainesville), Osceola (Kissimmee), Sarasota, Indian River (Vero Beach), and Duval (Jacksonville) counties, comprising eight populations (n = 66 individuals; Fig. 1). Six *I. brasiliensis* populations were sampled in municipal parks located within Miami-Dade County (n = 63 individuals; Fig. 1). These patches were small and non-expanding, relative to *I. cylindrica*, and occurred primarily in pine rockland habitats. Bradley identified these populations as *I. brasiliensis* (Table 1) and MDPR was not managing them as invasive (Possley et al. 2008; MDPR, personal communication). All populations were without flowers at the time of collection. Populations in Miami-Dade County have not been known or observed to flower within the last decade (Bradley, personal communication). *Imperata brasiliensis* patches generally do not flower unless stimulated by burning (Gabel 1982; Howard 2005; Bradley, personal communication).

Within each population, a tiller was assumed representative of an "individual," although we acknowledge that individual patches could have arisen from only one to a few genetically distinct propagules. Distances between individual tillers varied proportional to the size of the patch. For example, some *I. brasiliensis* sites were $<1$ m$^2$, and sampled tillers were sampled much closer together than from typically large *I. cylindrica* sites to achieve similar sample sizes. Tissues from 129 individuals were sampled from 14 populations (Fig. 1). Individual leaves were collected from throughout each patch to obtain a representative sampling of population-level diversity. Aboveground leaf tissues were stored in individually labeled plastic bags in a cooler on ice. Tissues were later (<36 h) dried by placing tissues in silica gel with color indicator, and stored dried until extractions.

## Tissue processing and molecular methods

DNA was extracted from leaf tissues using modified NucPrep® Chemistry: Isolation of Genomic DNA from Animal and Plant Tissue kit (Life Technologies, Carlsbad, CA, USA). Approximately 1 cm$^2$ of leaf tissue was aseptically transferred into a 2-ml microcentrifuge tube. Samples were fully disrupted utilizing a Retsch mixer mill and then processed. Purified DNA was transferred into sterile, individual tubes and kept frozen until analysis (−20 °C for short-term storage, −80 °C for long-term storage).

AFLP analysis utilized a modified protocol for capillary electrophoresis based on the technique and methodology developed by Vos et al. (1995). Extracted DNA underwent digestion by restriction enzymes, ligation of linking primers, pre-selective amplification to generate fragments of interest, and finally, selective amplification cycles generating fragment-based marker sets allowing for the detection of polymorphisms. Restriction digest of individual genomic DNA was achieved in 25 µl reactions incubated at 37 °C for 2 h in a thermal cycler, finalized by denaturing of enzymes by heating samples to 70 °C for 15 min. Ligation of Eco and Mse linkers were conducted in 20 µl reactions at 16 °C overnight or at 37 °C for 3 h. Individual tissue ligated reactions were stored at −80 °C to prevent degradation. Pre-selective amplifications were 20 µl polymerase chain reactions (PCR) with an initial denaturing step of 94 °C for 1 min, 30-cycles of 30 s at 94 °C, 1 min at 56 °C, and 1 min at 72 °C, and followed by final annealing for 2 min at 72 °C. For selective amplification, pre-selective amplification products were individually diluted 1:20 with sterile water. Each combination of selective Mse and fluorescent selective Eco primers comprised a separate PCR amplification. Selective amplification for all individuals were achieved in

**Table 2** Six AFLP selective amplification primer combinations utilized

| | *Mse*I primer | Fluorescent dye-labeled *Eco*RI primer |
|---|---|---|
| 1 | *Mse*I-CAT | *Eco*RI-ACT-FAM |
| 2 | *Mse*I-CTA | *Eco*RI-AGG-HEX |
| 3 | *Mse*I-CTG | *Eco*RI-AGC-NED |
| 4 | *Mse*I-CTT | *Eco*RI-ACT-FAM |
| 5 | *Mse*I-CTC | *Eco*RI-AGG-HEX |
| 6 | *Mse*I-CAC | *Eco*RI-AGC-NED |

Fluorescent dye-labeled selective primers are denoted by "EcoRIprimer-[Axx]-[dye]. Each fluorescent dye is visualized as a different color for fragment analysis: FAM (blue), HEX (green), NED (yellow or black)

20 µl reactions and underwent an initial denaturing step of 94 °C for 2 min, 10-cycles of 30 s at 94 °C, 30 s at 65 °C, and 1 min at 72 °C (reducing annealing temperature by 1 °C/cycle), 30-cycles of 30 s at 94 °C, 30 s at 56 °C, and 1 min at 72 °C, and finished with 30 s at 72 °C. Specific reagents and primer sequences utilized for this method can be found in Lucardi (2012).

Six selective primer sets were applied to each individual in this study (Table 2). Selective primers were fluorescently tagged such that products from multiple combinations of DNA primers could be analyzed simultaneously. Three different fluorescently tagged products (1.5 µl of each) were combined per well with single-stranded, fluorescent ROX-1000 size standard (0.25 µl; MapMarker (50–1000), BioVentures, Inc., Murfreesboro, TN, USA) and fixed with formamide (10 µl, Hi-Di™, Life Technologies, Carlsbad, CA, USA). Pooled fragment products were run on an ABI 3730 capillary sequencer at the Arizona State University DNA Lab (Tempe, AZ, USA). Positive control replicates accompanied each run to check reproducibility. Negative control replicates accompanied each run to check for cross-contamination. The standard error of positive control replicates (SE = 0.004; 95 % CI; <1 mismatch/individual/locus) suggests good reproducibility of this AFLP methodology.

## Data management and analysis

Fragment data were digitally visualized in GeneMarker® (SoftGenetics, LLC, State College, PA, USA), and were exported into general text format for input to Excel 2007 (Microsoft Corporation, Redmond, WA, USA.). Fragments were sorted based on migration size (basepairs) and auto-scored utilizing an independently developed procedure (Lucardi 2012; Lucardi and Walker, unpublished methodology) utilizing both Excel 2007 and PASW v.18.0 (SPSS, IBM Corporation, Armonk, NY, USA). Data matrices were generated from scored fragments and auto-populated over several steps between both software programs. Matrices were coded '0' for absence and '1' for presence of a fragment. Detected polymorphic loci less than 200 basepairs in length were removed from statistical analyses to avoid potential effects of fragment-size homoplasy (the result of co-migrating bands during electrophoresis not of the same physical locus in the genome), due to disproportionate numbers of smaller fragments produced by AFLPs (Koopman and Gort 2004; Bonin et al. 2007). Homoplasious biases may influence errors in allele frequency detection, generally toward overestimation, generating erroneous heterozygosity estimates and underrepresentation of genetic differentiation between subpopulations (Meudt and Clarke 2007; Caballero et al. 2008).

Data conversions of presence-absence matrices for input into population genetic software programs utilized *AFLPdat* R-package source script (Ehrich 2006). Within-population genetic diversity assessed the number of polymorphic and private bands, percentage of polymorphic loci, expected heterozygosity (biased, $H_e$ and unbiased, $UH_e$) based on Hardy–Weinberg expectations (Nei 1978), and Shannon's Information/Diversity Index (I), a coefficient of similarity (GenAlEx 6.3, Peakall and Smouse 2006). Because both species can clonally reproduce via below-ground rhizomes, clonal diversity was also estimated as the number of unique multilocus genotypes per population. We utilized the "*Clones*" function within *AFLPdat*. The function required a corresponding error parameter (Ehrich 2006). Standard error among all positive control replicates supplied the error parameter for clonal diversity analysis. The "*Clones*" function estimates genotype diversity (Nei 1987), effective number of genotypes (Parker 1979), and Nei's gene diversity (1987). Basic *t* tests were employed to determine if significant differences in clonal diversity between species existed ($\alpha = 0.05$). Paired Mantel Tests were performed (GenAlEx 6.5, Peakall and Smouse 2006) between tri-square linearized genetic distance matrix derived from AFLP data and straightline population-pairwise geographic distances (from GPS coordinates entered as decimal degrees).

Population pairwise $F_{ST}$ (Arlequin v.3.5; Excoffier and Lischer 2010) measured genetic differentiation between populations. STRUCTURE v.2.3.3 (Pritchard et al. 2000) inferred assignments of *Imperata* individuals into genetic clusters (*K*). Based on our a priori hypotheses, we expected to find two or three clusters ($K = 2$ or 3) to be consistent with the spatial distribution of two genetically different species and a potential hybrid cluster. We used the Evanno et al. (2005) method to objectively infer the most likely number of clusters, if greater than two. Several simulations of $K = 1$–7 were performed, with admixture ancestry model applied with a burn-in of 10,000 and 50,000 MCMC (Markov Chain Monte Carlo)

(Pritchard et al. 2000). The Evanno et al. (2005) method is unable to infer a $K = 1$, therefore additional analysis of population structure was assessed with principal coordinates analysis (PCA, GenAlEx v.6.3) of individual genetic covariance (with data standardization). Analysis of molecular variation (AMOVA, Excoffier et al. 1992) was performed in Arlequin v.3.5 (Excoffier and Lischer 2010), using a squared genetic distance matrix for binary haploid data. AMOVA groups were based on putative species identification to test genetic differentiation between them. The program NEWHYBRIDS (v1.1.beta) was employed for detection of individuals of mixed ancestry (Anderson and Thompson 2002). We utilized genotype frequency class distributions included with the program; no priors were applied. Posterior probabilities were assigned into genotype classes for all individuals: Pure groups (2), F1, F2, Backcross groups (2), to equal six frequency classes. The graphical interface component was used with 5,000 sweeps for both MCMC and since burn-in. Both Jeffrey's and Uniform priors were applied and observed patterns did not strongly influence probability assignments. Reported results from NEWHYBRIDS used Jeffrey's priors for both $\theta$ and $\pi$.

## Results

### Genetic diversity

The AFLP genome scan detected 668 polymorphic loci from eight populations of *I. cylindrica* (n = 66) and six populations of *I. brasiliensis* (n = 63, $N = 129$ individuals, Table 3). The number of polymorphic loci per population ranged from 41 to 221; number of private bands per population (polymorphic bands detected in only one population) ranged from 0 to 95. The average percentage of polymorphic loci per population was 16 % (SE ± 2 %, Table 3). Four *I. cylindrica* and two *I. brasiliensis* populations were above the mean; 35 % of loci were detected only in *I. cylindrica,* 37 % only in *I. brasiliensis*, and over 27 % were shared between the two. Heterozygosity ($H_e$) values ranged from 0.018 (FL-11) to 0.078 (FL-7), with a mean heterozygosity value of 0.041 (SE ± 0.001); unbiased heterozygosity ($UH_e$) ranged from 0.020 (FL-11) to 0.082 (FL-7), with a mean value of 0.047 (SE ± 0.001, Table 3). Average heterozygosity was 0.044 and 0.037 and average unbiased heterozygosity was 0.051 and 0.041, for *I. cylindrica* and *I. brasiliensis,* respectively. Irregularly large discrepancies were absent between $H_e$ and $UH_e$, indicating that heterozygosity estimates were not strongly affected by variation in population sample sizes. Shannon's Information Index (I) ranged from 0.029 to 0.134, with an average value of 0.067 (SE ± 0.002); average values were I = 0.070 for *I. cylindrica* and I = 0.062 for *I. brasiliensis.* Clonal diversity analysis found that genotype diversity values for all *I. cylindrica* populations were equal to one, meaning that the number of genets (or unique genotypes detected) was equal to the number of individuals sampled (Table 4). Genotypic diversity was reduced (< 1), ranging from 0.87 to 0.99 among *I. brasiliensis* populations. Reductions in the effective number of genotypes were observed for all *I. brasiliensis* populations, but not for *I. cylindrica*. However, among the clonal diversity measures, only genotype diversity was significantly different between *I. cylindrica* and *brasiliensis*; all other metrics were not significantly different from the mean ($P < 0.05$; Table 4).

### Among population differentiation and structure

Within-species pairwise population $F_{ST}$ values ranged from 0 to 0.204 for *I. cylindrica,* and 0.012 to 0.325 for *I. brasiliensis* (Table 5). Significant between species $F_{ST}$ values ranged from 0.059 to 0.292. Greater population genetic dissimilarity was observed within *I. brasiliensis,* than within *I. cylindrica* or between species. All *I. cylindrica* populations (FL-1, 2, 3, 4, 5, 6, 13, and 14) were genetically similar to *I. brasiliensis* populations FL-7, 8, and 12 with low pairwise $F_{ST}$ values between them. No significant ($P < 0.05$) genetic differentiation was found between one *I. brasiliensis* population (FL-12), and all other populations, with the exception of conspecific population, FL-9. Significant genetic similarity was also observed between Miami-Dade populations and *I. cylindrica* populations found on the eastern side of the state (e.g., FL-13 and 14 to FL-10, $F_{ST} = 0.060, 0.059$; $P < 0.05$; Table 5). Paired Mantel Test found a significant relationship between pairwise population genetic and geographic distances, however, the detected relationship is not strong ($R^2 = 0.158$, $P < 0.001$; Fig. 4).

Analysis in NEWHYBRIDS identified hybrid individuals among those sampled in FL. Data log likelihood (Data LogL) values increased rapidly

**Table 3** Genetic Diversity Indices with Population Information[a] for *I. cylindrica* ("*IC*") and *I. brasiliensis* ("*IB*"), with overall totals and averages in bold

| Species | County | Other information | Number of individuals (n) | Number of bands detected | Number of private bands | Percentage polymorphic loci | $H_e \pm SE$ | $UH_e \pm SE$ | Shannon's information index (I) |
|---|---|---|---|---|---|---|---|---|---|
| *IC* (FL-1) | Alachua | ROW | 10 | 151 | 41 | 25.70 | 0.074 ± 0.006 | 0.082 ± 0.007 | 0.116 ± 0.009 |
| *IC* (FL-2) | Osceola | Treated (IMP), burned | 10 | 112 | 24 | 19.19 | 0.049 ± 0.005 | 0.054 ± 0.005 | 0.079 ± 0.007 |
| *IC* (FL-3) | Osceola | Treated (GLY, IMP) | 5 | 54 | 6 | 7.75 | 0.028 ± 0.004 | 0.035 ± 0.005 | 0.042 ± 0.006 |
| *IC* (FL-4) | Osceola | Logged, treated (IMP), burned | 10 | 112 | 14 | 19.01 | 0.053 ± 0.005 | 0.059 ± 0.006 | 0.085 ± 0.008 |
| *IC* (FL-5) | Osceola | Treated (IMP), burned | 5 | 92 | 14 | 15.49 | 0.056 ± 0.006 | 0.070 ± 0.007 | 0.084 ± 0.008 |
| *IC* (FL-6) | Sarasota | ROW | 10 | 77 | 0 | 12.50 | 0.030 ± 0.004 | 0.033 ± 0.004 | 0.049 ± 0.006 |
| *IC* (FL-13) | Indian River | ROW, disturbed (construction) | 6 | 56 | 16 | 8.80 | 0.029 ± 0.004 | 0.035 ± 0.005 | 0.045 ± 0.006 |
| *IC* (FL-14) | Duval | ROW | 10 | 100 | 43 | 16.90 | 0.037 ± 0.004 | 0.042 ± 0.004 | 0.063 ± 0.006 |
| *IB* (FL-7) | Miami-Dade | Thompson Park* | 13 | 221 | 95 | 38.73 | 0.078 ± 0.005 | 0.084 ± 0.005 | 0.134 ± 0.008 |
| *IB* (FL-8) | Miami-Dade | Martinez Pineland* | 10 | 71 | 11 | 11.27 | 0.026 ± 0.003 | 0.029 ± 0.004 | 0.043 ± 0.005 |
| *IB* (FL-9) | Miami-Dade | Martinez Pineland* | 10 | 131 | 50 | 21.48 | 0.046 ± 0.004 | 0.051 ± 0.004 | 0.079 ± 0.007 |
| *IB* (FL-10) | Miami-Dade | Pine Shore Park | 10 | 73 | 20 | 11.44 | 0.029 ± 0.004 | 0.033 ± 0.004 | 0.048 ± 0.006 |
| *IB* (FL-11) | Miami-Dade | Ingram Pineland | 10 | 41 | 5 | 6.69 | 0.018 ± 0.003 | 0.020 ± 0.003 | 0.029 ± 0.005 |
| *IB* (FL-12) | Miami-Dade | Seminole Wayside Park* | 10 | 76 | 20 | 10.74 | 0.025 ± 0.003 | 0.028 ± 0.004 | 0.042 ± 0.005 |
| **Total/mean** | | | **129** | **668** | **359** | **16 ± 2** | **0.041 ± 0.001** | **0.047 ± 0.001** | **0.067 ± 0.002** |

*ROW* right-of-way, *IMP* imazapyr (herbicide), *GLY* glyphosate (herbicide)

[a] All *I. cylindrica* tissues sampled in Osceola Co., FL, were collected from the Disney Wilderness Preserve (The Nature Conservancy). Species assignment, location, habitat information, band data, and genetic diversity indices ($H_e$ = expected heterozygosity, $UH_e$ = unbiased expected heterozygosity, I = Shannon's Information index, with ±SE for 14 populations. * *I. brasiliensis* populations vouchered in Table 1

**Table 4** Clonal diversity analysis per population: number of individuals sampled (n), number of unique genotypes, genotype diversity, effective number of genotypes, and Nei's gene diversity

| Species | Population | Individuals sampled (n) | No. of genotypes | Genotype diversity | Effective no. of genotypes | Nei's gene diversity |
|---|---|---|---|---|---|---|
| *I. cylindrica* | FL-1 | 10 | 10 | 1 | 10 | 0.07 |
| | FL-2 | 10 | 10 | 1 | 10 | 0.05 |
| | FL-3 | 5 | 5 | 1 | 5 | 0.03 |
| | FL-4 | 10 | 10 | 1 | 10 | 0.05 |
| | FL-5 | 5 | 5 | 1 | 5 | 0.06 |
| | FL-6 | 10 | 10 | 1 | 10 | 0.03 |
| | FL-13 | 6 | 6 | 1 | 6 | 0.03 |
| | FL-14 | 10 | 10 | 1 | 10 | 0.05 |
| | **Total/mean** | **66** | **66** | **1** | **8.25** | **0.05** |
| | **SE±** | | **0.86** | **0** | **0.86** | **0.01** |
| *I. brasiliensis* | FL-7 | 13 | 12 | 0.99 | 11.27 | 0.08 |
| | FL-8 | 10 | 8 | 0.93 | 6.25 | 0.03 |
| | FL-9 | 10 | 9 | 0.98 | 8.33 | 0.05 |
| | FL-10 | 10 | 8 | 0.93 | 6.25 | 0.03 |
| | FL-11 | 10 | 9 | 0.98 | 8.33 | 0.02 |
| | FL-12 | 10 | 7 | 0.87 | 4.55 | 0.03 |
| | **Total/mean** | **63** | **53** | **0.95** | **7.50** | **0.04** |
| | **SE±** | | **0.70** | **0.02** | **0.96** | **0.01** |
| | ***P* value (<0.05)** | | 0.42 | **0.02** | 0.16 | 0.07 |

Significant differences in species clonal diversity values ($\alpha = 0.05$) are denoted in bold

**Table 5** Pairwise population matrix of $F_{ST}$ values[a] among 14 *Imperata* populations in Florida

| | | *I.cylindrica* | | | | | | | | *I. brasiliensis* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | FL-1 | FL-2 | FL-3 | FL-4 | FL-5 | FL-6 | FL-13 | FL-14 | FL-7 | FL-8 | FL-9 | FL-10 | FL-11 |
| *I.cylindrica* | FL-1 | * | | | | | | | | | | | | |
| | FL-2 | 0.144 | * | | | | | | | | | | | |
| | FL-3 | *0.173* | 0.000 | * | | | | | | | | | | |
| | FL-4 | 0.051 | 0.029 | *0.051* | * | | | | | | | | | |
| | FL-5 | 0.016 | 0.045 | 0.000 | 0.051 | * | | | | | | | | |
| | FL-6 | 0.137 | 0.052 | *0.077* | 0.000 | 0.065 | * | | | | | | | |
| | FL-13 | *0.204* | *0.107* | *0.080* | *0.114* | *0.134* | *0.092* | * | | | | | | |
| | FL-14 | *0.176* | *0.111* | *0.097* | *0.117* | *0.087* | *0.095* | 0.012 | * | | | | | |
| *I. brasiliensis* | FL-7 | 0.000 | *0.077* | *0.099* | 0.000 | 0.059 | 0.000 | *0.188* | *0.134* | * | | | | |
| | FL-8 | *0.104* | *0.083* | *0.082* | 0.001 | *0.128* | 0.007 | *0.180* | *0.150* | 0.082 | * | | | |
| | FL-9 | *0.292* | *0.254* | *0.198* | *0.242* | *0.243* | *0.244* | *0.155* | *0.060* | *0.290* | *0.325* | * | | |
| | FL-10 | *0.251* | *0.157* | *0.104* | *0.143* | *0.248* | *0.159* | *0.060* | *0.059* | *0.240* | *0.233* | *0.166* | * | |
| | FL-11 | *0.235* | *0.151* | *0.109* | *0.143* | *0.142* | *0.139* | 0.032 | 0.028 | *0.216* | *0.212* | *0.100* | *0.052* | * |
| | FL-12 | 0.067 | 0.012 | 0.037 | 0.019 | 0.023 | 0.000 | 0.008 | 0.015 | 0.012 | 0.037 | *0.093* | 0.037 | 0.042 |

[a] Significant pairwise population $F_{ST}$ values are shown in italics ($P < 0.05$)

(<100 iterations, average likelihood value −4,900). The majority of samples (105 out of 129) were placed in the pure *I. cylindrica* group with a posterior probability >98 %, 6 were assigned to the F1 group with >91 % probability, 11 to the backcross with *I. cylindrica* group with >92 % probability and 7 are

ambiguous (i.e., <90 % probability of being in a single group). Of the 24 individuals not assigned to the pure *I. cylindrica* class, 6 individuals were assigned to the F1 frequency class, all sampled from *I. cylindrica* populations (FL-1, 2, 5); 11 individuals were assigned to backcross with *I. cylindrica:* 7 *I. cylindrica* individuals (FL-1, 2, 3, 4, 5, 13) and 4 *I. brasiliensis* individuals (FL-7, 8, 9). Ambiguous individuals were both *I. cylindrica* (5 of 7; FL-1, 4, 6, 14) and *I. brasiliensis* (2 of 7; FL-7). Fewer *I. brasiliensis* individuals resulted in mixed or ambiguous ancestry (6 of 63 sampled); whereas 18 of 66 sampled *I. cylindrica* individuals were mixed.

The objectively inferred number of clusters resulted in a mode of $\Delta K$ at $K = 3$ for STRUCTURE analysis (mean LnP[D] = −11,934; Fig. 2). However, the partitioning of the inferred three clusters was not consistent with spatial distributions of any of the populations of *Imperata*. Both *I. cylindrica* and *I. brasiliensis* did not form separate clusters consistent with initial expectations; rather, the two less prevalent genetic clusters are embedded within one dominant cluster (mean $\alpha = 0.093$). Principal coordinates analysis (PCA, genetic covariance with data standardization) of individuals resulted in the first two axes accounting for 63 % of the variation in the dataset (Fig. 3). The majority of individuals formed a broad cluster in the top and bottom-left quadrants of the PCA. A few individuals belonging to both species (FL-7, 14 (*I. brasiliensis*) and FL-1, 2, 4, 8 (*I. cylindrica*) did not cluster within the main group. An analysis of molecular variance between putative species (AMOVA) resulted in a significant, but low $F_{ST}$ value of 0.135 ($P < 0.001$), indicating a low degree of genetic differentiation based on a two-group population structure between *I. cylindrica* and *brasiliensis* (Table 6).

## Discussion

### Genetic diversity

Within-population genetic diversity was not markedly different between species, and diversity estimates were comparable among populations of *I. cylindrica* and *I. brasiliensis*. Genetic diversity estimates within and among populations were consistent with species exhibiting the capacity to outcross and clonally reproduce (Ward et al. 2008). Two *I. brasiliensis* populations, one from Thompson Park (FL-7) and one of two populations sampled from Martinez Pineland (FL-9), resulted in diversity above the mean. The *I. brasiliensis* population located in Thompson Park (FL-7) resulted in the highest overall genetic variation and the population from Ingram Pineland resulted in the overall lowest genetic diversity (FL-11; Table 3). Populations sampled from Miami-Dade County were not managed or treated as invasive because *I. brasiliensis* is considered 'naturalized' (Possley et al. 2008; MDPR; K. Bradley, personal communication). Population FL-1, sampled from Gainesville (Alachua County), resulted in the greatest number of detected polymorphic bands (151) among *I. cylindrica* populations. The populations with the lowest genetic diversity include both *I. cylindrica* (FL-3, FL-13) and *I. brasiliensis* (FL-11, Ingram Pineland). Population FL-3 (*I. cylindrica*, Disney Wilderness Preserve, Osceola County) was collected from an area treated historically with glyphosate and imazapyr, whereas population FL-13 (*I. brasiliensis*, Indian River County, Atlantic coast) was collected from a disturbed right-of-way between the interstate and concurrent residential/retail construction.

### Among population differentiation and structure

Our analyses were not consistent with two genetically differentiated species, with one (*I. brasiliensis*) constrained geographically. No clear pattern of genetic dissimilarity was observed between *I. cylindrica* and *I. brasiliensis* populations. We observed high probabilities in hybrid analysis for the majority of individuals assigned to pure *I. cylindrica* (105/129 individuals, including 90 % of sampled Miami-Dade populations), strongly suggesting the presence of a single effective gene pool in the dataset. Remaining individuals were assigned to F1 and *I. cylindrica* backcross categories indicating historical admixture with something (e.g., *I. brasiliensis,* other *I. cylindrica* populations, or something else). A larger proportion of known *I. cylindrica* individuals (primarily North and Central FL populations) were assigned as mixed ancestry, whereas, most individuals from Miami-Dade populations were assigned as pure *I. cylindrica* with high posterior probabilities. Results from STRUCTURE (Fig. 2), PCA (Fig. 3), and NEWHYBRID analyses were consistent. The mean alpha value (when K = 3) was

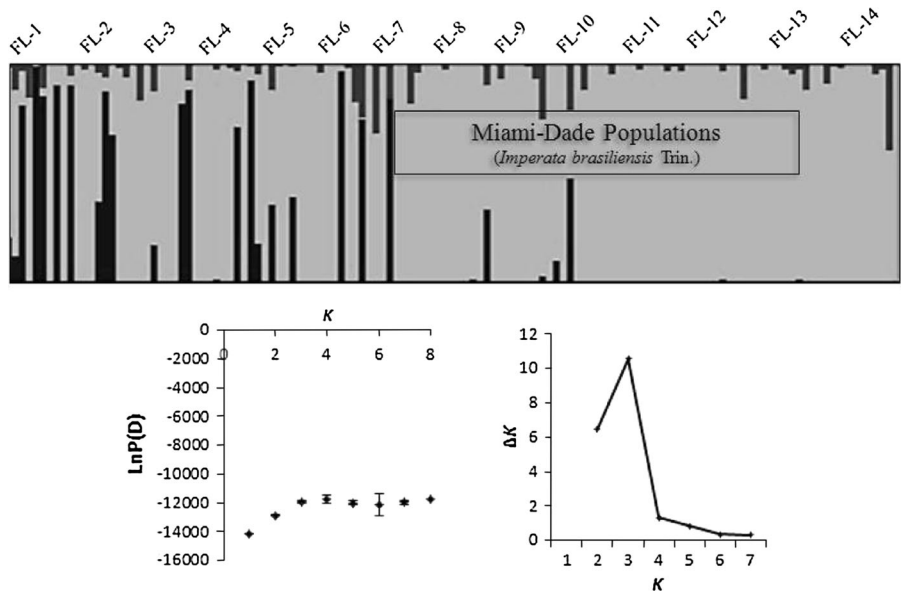


**Fig. 2** Bar plot from STRUCTURE analysis when three clusters assumed ($K = 3$). Population identifiers are labeled above the *plot* and each *bar* represents an individual; each cluster is represented by a different shade. An individual *bar* comprised of a *single color* is completely assigned to one cluster, whereas individual bars comprised of multiple shades indicate mixed ancestry. Miami-Dade individuals (n = 63) are located toward the *middle*, as indicated by the label, with all other individuals being *I. cylindrica* (n = 66). Graphs of associated mean log-likelihood probabilities for each $K$ (error bars represent SD) and $\Delta K$ located below *bar plot*

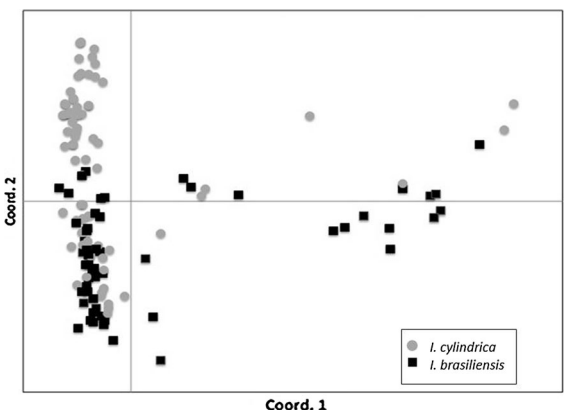


**Fig. 3** Principal coordinates analysis (PCA) of genetic covariance (with data standardization) of *Imperata* individuals identified as *I. cylindrica* (*circles*) or *I. brasiliensis* (*squares*) in FL (N = 129 individuals, 14 populations). These first two axes account for 63 % of the variation in this dataset

low ($\alpha = 0.093$), suggesting relatively low admixture within individuals. No distinct 'clusters' were observed between potential species to be consistent with our initial expectations for one widespread and one spatially limited species. The observed lack of strong genetic structure among populations and

**Table 6** Results from analysis of molecular variation (AMOVA) using $F_{ST}$ between *I. cylindrica* and *I. brasiliensis* ("Groups")

| Source of variation | *df* | Sum of squares | Percentage of variation | *P* value |
|---|---|---|---|---|
| Among groups | 1 | 114.30 | 8.15 % | <0.001 |
| Among populations within groups | 12 | 279.61 | 5.37 % | 0.003 |
| Within populations | 115 | 1,710.66 | 86.38 % | <0.001 |
| Total | 128 | 2,104.57 | | |

$F_{ST} = 0.135$ ($P < 0.001$), $F_{SC} = 0.058$ ($P < 0.001$), $F_{CT} = 0.081$ ($P < 0.001$)

absence of a strong signal of hybridization (Figs. 2 and 3) suggested a single, heterogeneous genetic assemblage.

Similar values of genetic dissimilarity were observed within and between species. Population-level differentiation was higher among populations of *I. brasiliensis* populations than among *I. cylindrica* populations, or between species, indicating an alternative explanation to interspecific hybridization. Isolation-by-distance relationships were found to be weak

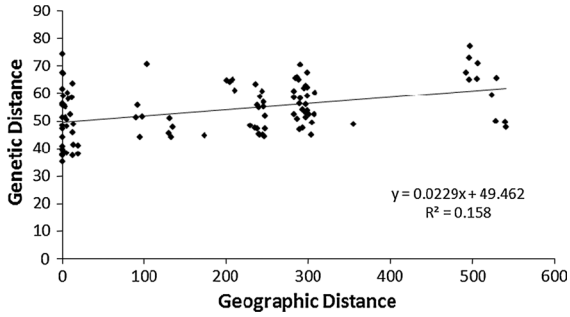


**Fig. 4** Paired Mantel Test between population pairwise genetic and geographic distances ($r = 0.397$; $P < 0.001$)

(Fig. 4). In addition, the $F_{ST}$ value between species was relatively low, not providing strong support for species-level genetic distinction (Table 5; Heywood 1991; Wei et al. 2005), where, $F_{ST}$ values can range from total panmixis ($F_{ST} = 0$) to complete genetic isolation ($F_{ST} = 1$) (Beaumont 2005). Observed pairwise $F_{ST}$ and AMOVA values, deduced from this research, are less than what would be expected between congeneric plant species that have hybridized (Wei et al. 2005; Szczepaniak et al. 2007). Other studies employing similar tests for evidence of hybridization (i.e., AFLP markers, pairwise $F_{ST}$, and AMOVA) generally detected stronger population structuring among populations, with higher mean and pairwise $F_{ST}$ values, and stronger clustering between tested groups (e.g., Wei et al. 2005; Szczepaniak et al. 2007; Song et al. 2010).

Thus, these analyses do not support *I. brasiliensis* in Miami-Dade being a genetically distinct species. These populations may have historically been a distinct species, but asymmetrical introgression may have swamped the genetic signal of separate species. At present, we do not consider populations of *Imperata* in Miami-Dade, FL to be a different species. Further genetic study with our Miami-Dade accessions alongside with *I. brasiliensis* from its native range (i.e., South America) and resolving the phylogeny of the genus may further elucidate relationships among congenerics and reported hybridization.

Considering the earliest known purposeful introduction of cogongrass into the USA occurred in 1921, followed by additional introductions of exotic parent material into FL, Miami-Dade *Imperata* populations may have arisen from multiple introductions (increasing propagule pressure) of *I. cylindrica* from foreign and domestic sources. With on-going invasion in surrounding states, on-going propagule rain increases genetic diversity and subsequently, increases and broadens phenotypic variation. Genetic evidence from this study did not support Miami-Dade populations identified as *I. brasiliensis* to be a genetically distinct species from *I. cylindrica* found throughout the state. These findings are consistent with previous molecular studies in *Imperata*, where *I. brasiliensis* was grouped closest to East Asian samples, suggesting that *Imperata* in South FL could have arisen from an earlier introduction of *I. cylindrica* from Japan (Gabel 1982; Vergara et al. 2008), rather than a separate introduction and naturalization of a genetically unique *Imperata* species.

The observed lack of 'invasive' expression in Miami-Dade may be due to ecological constraints. The pine rockland habitats from which Miami-Dade populations were sampled from may be considered sub-optimal habitats for *I. cylindrica* (Hubbard et al. 1944; Hall 1978; Gabel 1982): the presence of canopy cover, shallow to nonexistent organic substrate, temporary inundation events, and low nutrient availability (Snyder et al. 1990). Plant species richness in these habitats relies on fire events to release nutrients back into the soil. Therefore, limited nutrient availability due to fire suppression can affect plant growth, spread, outcrossing, and dispersal in these habitats (Possley et al. 2008). In addition, recent studies of *I. cylindrica* populations in Mississippi, suggested that certain soil nutrients and organic matter significantly influenced variation in leaf and inflorescence size, as well as seedling growth (Holly and Ervin 2007; Bryson et al. 2010).

## Conclusion

Data from this research were inconsistent with expectations of two separate *Imperata* species co-occurring in FL. As a result, observed patterns of genetic diversity and differentiation among sampled *Imperata* populations in Florida failed to support interspecific hybridization having occurred between *I. cylindrica* and previously naturalized *I. brasiliensis*. Consequently, the conclusion that hybridization facilitated cogongrass invasion throughout the region similarly cannot be supported by these data. It is unclear from the present work whether genetically distinct *I. brasiliensis* may have existed in Florida at one time or if current

*Imperata* populations there may simply be less robust ecotypes of *I. cylindrica*. Additional genetic comparisons with known *I. brasiliensis* populations from elsewhere would aid in clarifying this issue.

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