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Author(s): Matthew D. Croxton, Michael A. Andreu, Dean A. Williams, William A. Overholt, and Jason A. Smith
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Matthew D. Croxton, Michael A. Andreu, Dean A. Williams, William A. Overholt, and Jason A. Smith*

In Florida, air-potato is an invasive weed with high management priority, which may soon be targeted using classical biological control. This yam was introduced during the early 20th century by the United States Department of Agriculture (USDA) from areas throughout its extensive range. Our objectives were to characterize the genetic diversity of the invasive population in Florida and to identify the source regions of introduction. Authorities have often asserted the African provenance of the species in Florida, but our analyses, conducted using chloroplast markers, indicate that Florida air-potato is more similar to specimens examined from China than to those from Africa. Low intraspecific genetic diversity in Florida indicates that the invasive population was the result of at least two introductions becoming established in Florida.

Nomenclature: Air-potato, *Dioscorea bulbifera* L.

Key words: Pantropical distribution, biological control, invasive yam, chloroplast DNA.

Ecologically relevant questions about the introduction pathway of an exotic invasive plant species, its rate of spread, and appropriate management strategies may depend on determining its provenance (Goolsby et al. 2006). Knowledge of a source location provides context for deriving supporting data such as genetic diversity, enables comparisons between the environmental conditions in native and invasive ranges, and helps identify factors that contribute to invasiveness. It can also allow assessment of genetic consequences for different patterns of introduction (Müller-Schärer et al. 2004). Most important, for biological control of invasive plants, identifying the source of the population may lead to the discovery of biological control agents most closely adapted to the invader. Because host-specificity is a prerequisite for biocontrol efficacy and safety, identification of an invader's source may lead to the rapid identification of high-quality biocontrol candidates (Goolsby et al. 2006).

Air-potato (*Dioscorea bulbifera* L.) is a climbing yam that has a nearly pantropical distribution and is native to both Africa and Asia (Govaerts and Wilkin 2009). This vine is not native in Florida, where it invades a variety of ecosystems including hardwood hammocks, pinelands, and especially, disturbed areas (Horvitz and Koop 1998). It rapidly grows to the tops of tree canopies and forms a vine mat that weighs down and shades out native vegetation during the growing season. Recruitment and maintenance of native, late-successional growth is decreased in areas that are overtopped and shaded out by *D. bulbifera*, with a preliminary study suggesting that this leads to decreases in canopy height diversity and changes in function of the plant community (Odom et al. 2008). Once air-potato invades an area, it is difficult to eliminate because of the prolific production of persistent aerial bulbils. Vegetative propagation from aerial bulbils is the only mode of reproduction that can be readily verified for invasive *D. bulbifera*, and suggests the entire Florida population may be clonal, if established by a single introduction (Wheeler et al. 2007). The vine is found in most of the 67 Florida counties, from the Panhandle in northern Florida to Key West in the extreme south. The northern range of the plant in the southeastern United States is temperature-limited because freezing prevents bulbil sprouting and recruitment into areas that experience recurrent hard-freeze conditions (Jameson 2001).

Management of the species in Florida is accomplished with modest success through a combination of mechanical, manual, and herbicide treatments (Overholt et al. 2008). However, a multiyear regimen is necessary to ensure that aerial bulbils at a given site are eradicated and will not allow juvenile plants to reestablish after treatment is ceased.

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* Research Assistant, Gulf Coast Research and Education Center, University of Florida, 1200 North Park Road, Plant City, FL 33563; Assistant Professor, Texas Christian University, 2800 South University Drive, Fort Worth, TX 76129; Assistant Professor, Indian River Research and Education Center, University of Florida, 2199 South Rock Road, Fort Pierce, FL 34945; Assistant Professor, School of Forest Resources and Conservation, University of Florida, 118 Newins-Ziegler Hall, Gainesville, FL 32611. Corresponding author's E-mail: croxton@ufl.edu

Interpretive Summary

Two distinct, but genetically similar, chloroplast haplotypes were identified in the Florida population of the invasive yam vine, air-potato. Assuming no contribution of somatic mutation in the invasive population, these data are consistent with a minimum of two introductions into Florida. This study contributes baseline information regarding the diversity of air-potato before implementation of biological control in Florida. In the course of examining plant material to locate a putative source population, we characterized the genetic diversity of African air-potato, sampling six haplotypes on that continent. Three of the most prevalent African haplotypes were also geographically widespread, being found in both East Africa and West Africa. These three are also the most genetically distant from Florida and Chinese haplotypes, relative to other African haplotypes, as determined by parsimonious network analysis. A neighbor-joining analysis supported the relationships inferred in a statistical parsimony network. Our study did not find evidence that haplotypes were geographically structured in Africa. Despite a small number of microsatellite markers employed, this methodology confirmed the similarity of Chinese and Florida samples. Future work using a greater number of markers is expected to reveal greater intraspecific diversity, whereas further sampling in Asia and Oceania may localize Florida's source population to a regional, rather than a continental, level. Integrative taxonomic approaches are needed to synthesize morphological treatments of this species with more recent molecular studies, including the present work.

Because of its widespread distribution in Florida, high survivorship of propagules dispersed across the landscape, and labor-intensive management requirements, no combination of methods currently employed will be sufficient to restore all areas affected by this species without a major escalation in resources or treatment efficacy. Biological control is potentially the most cost-effective and direct way to obtain greater control of *D. bulbifera* on a statewide scale in Florida (Wheeler et al. 2007). If host specificity of a biocontrol agent is positively correlated with the extent of geographic association between host and biological control agent, then selection of an agent from the source population may play an important role in the maintenance of host specificity or in the extent of damage to the invader (Roderick and Navajas 2003).

Many botanists and yam authorities have asserted that *D. bulbifera* was introduced to North America and the West Indies as a result of the slave trade from West Africa (Al-Shehbaz and Schubert 1989; Burkill 1939; Coursey 1967; Prain and Burkill 1919). Preliminary findings based on chloroplast restriction patterns appeared to confirm an African origin (Overholt and Hughes 2004). Records indicate that the United States Department of Agriculture (USDA) brought *D. bulbifera* to Florida from West Africa, Asia, Polynesia, and the West Indies during the early part of the 20th century as part of a comprehensive plant introduction effort between 1902 and 1925 (Barrett 1933)(Table 1). Nehrling (1933) reported that *D. bulbifera* introduced to Gotha, Florida, in 1905 became extremely weedy, but the source of this introduction is unknown. Reasons for introduction of *D. bulbifera* to Florida range from medical research to agricultural and ornamental uses, with authors of Florida floras asserting that it was introduced as an ornamental from Asia (Clewell 1985; Wunderlin and Hansen 2003), whereas one yam specialist, who asserted an African origin, suggested that it

Table 1. Summary of air-potato (*Dioscorea bulbifera* L.) introduction events.[a]

Author	Date[b]	Source	Destination	Notes	Reference
C. Clusius	1500s	West Africa (Elmina Lagos)	West Indies; Americas	Introduced by Portugese slave traffickers	Burkill 1939
W. Bartram	1777	?	Mobile, AL	Not Asiatic *D. bulbifera*, according to Harper	Harper 1998
H. Nehrling	1905	?	Gotha, FL	Noted weedy behavior and propensity for escape	Nehrling 1933
USDA 18656	1906, May	Mayagüez, PR	Miami, FL	Gunda cultivar; large, irregular-shaped axillary bulbils	USDA 1907
USDA 21775	1908, January	French Guinea	Florida	Sent by M. A. Chevalier	USDA 1909
USDA 45994	1918, April	Mayagüez, PR	Florida	Received by R. A. Young; aerial tubers better for food than ground tubers	USDA 1922a
USDA 46218	1918, May	Honolulu, HI	Florida	Sent by J. E. Higgins, Hawaii Agricultural Station	USDA 1922a
USDA 47493	1919, April	Singapore, Straits settlements	Florida	Sent by I. H. Burkill; Specimens from either Singapore, India, or Bangladesh	USDA 1922b

[a] Abbreviation: USDA, U.S. Department of Agriculture.

[b] Denotes either date of introduction or the date of a report of sighting.

was first introduced as a food crop (Coursey 1967). Air-potato is not considered an important food anywhere in the Western Hemisphere (Martin 1974).

Morphological treatments of *D. bulbifera* varieties have long asserted that significant differences are present between African and Asian varieties, leading some authors to suggest they are actually separate species (Chevalier and Bentham via Miège 1982). The foremost character distinguishing African *D. bulbifera* from the Asian form is the presence of highly angular bulbils in the African plants. (Burkill 1960; Coursey 1967; Hamon et al. 1995; Martin 1974; Milne-Redhead 1975). Bulbil polymorphism in Florida is evident, and two morphotypes can be discriminated, one with smooth skin that is light in color, and the other is warty and dark. No plants recorded from Florida possess the angular acuteness ascribed to some cultivated African bulbils. Wild African bulbils are ovoid/subglobose and are consistent with the bulbil shapes present in Florida (Hamon et al. 1995; Milne-Redhead 1975).

Another characteristic said to differentiate the African and Asian forms of the plant is the presence of hairs on the leaf epidermis of cultivated African varieties, which are lacking in Asian and uncultivated African varieties (Burkill 1960; Hamon et al. 1995; Miège 1982). Cursory examination of live plant material from Florida under a dissecting microscope revealed only glabrous leaf surfaces (M. Croxton, personal observation). Using a combination of chloroplast and microsatellite DNA markers, we test the hypothesis that invasive *D. bulbifera* in Florida is of African origin. We also examine whether there are genetically distinct populations within Florida, providing evidence of establishment from multiple geographic sources, as suggested by historical records. This work expands on previous genetic diversity studies of *D. bulbifera* in Africa and Asia and is the first, to our knowledge, to examine relationships among these native populations in comparison with the nonnative Florida population (Ramser et al. 1996; Terauchi et al. 1991; Zheng et al. 2006).

Materials and Methods

Collection. Volunteers from the Florida Cooperative Extension Service and Master Gardener program collected specimens of *D. bulbifera* from July to November of 2006. Collectors were given species identification guidelines to prevent the sampling of winged yam (*Dioscorea alata* L.), the only yam in Florida with which *D. bulbifera* is likely to be confused on the basis of morphology. A single specimen of *Dioscorea alata* was collected in Florida for use as an outgroup taxon in the genetic analyses. Geospatial coordinates for sample locations were recorded using global positioning system (GPS) instruments or were approximated using either a street address or detailed descriptions (Figure 1).

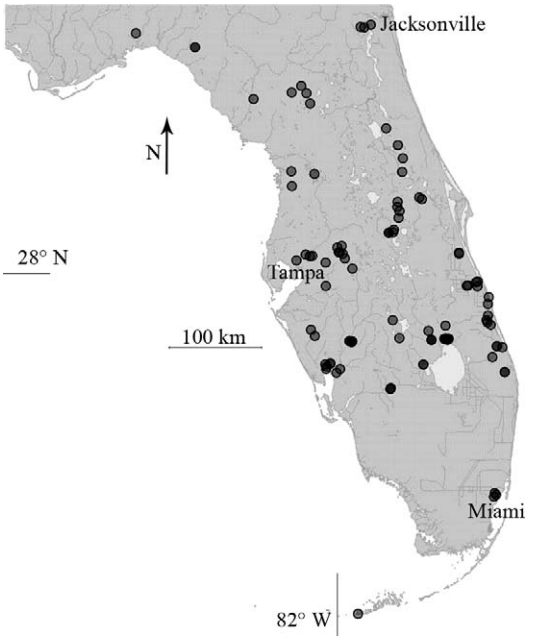

Figure 1. A map of Florida showing the sampling locations of plant material analyzed using microsatellite markers.

Most non-Florida samples were collected between April and September of 2007. Some samples were obtained on dates outside this range, including: an accession from Puerto Rico (November 2005), from China (July 2004), and from Uganda (February 2004 and September 2006). All leaf material was preserved by desiccation in silica gel.

Chloroplast DNA Analysis. DNA was extracted from leaf tissue using a variation on the method of Kim et al. (1997). Two chloroplast DNA intergenic regions, spanning psbM to trnD and ycf6 to psbM (Shaw et al. 2005), were amplified for 40 samples using the following 10 µl polymerase chain reaction (PCR) mix: 2.5 mM $MgCl_2$, 0.5 mM primer, 200 mM each deoxynucleotide triphosphate (dNTP), 0.2 U Taq polymerase, and 1 µl of template DNA. Reactions were run in thermal cyclers[1] at the following conditions: 2 min at 94 C, followed by 30 cycles of 94 C (201.2 F) for 30 s, 55 C for 30 s, 72 C for 1 min, and a final extension of 72 C for 5 min. For templates recalcitrant to amplification, a ''hot start'' polymerase[2] was substituted for the standard polymerase enzyme. Unincorporated nucleotides and excess primers were removed from PCR products following manufacturer[3] protocols. Products were sequenced in both forward and reverse directions using cycle-sequencing chemistry[4] and separated on capillary-based genetic analyzers.[5]

Sequences were aligned, trimmed, and contiged,[6] spanning 1,740 base pairs (bp) when products of both chloroplast regions were combined. Relationships among *D. bulbifera* haplotypes were visualized by constructing a

statistical parsimony network at a 95% confidence limit with TCS 1.21 (Clement et al. 2000), using alignments concatenated from the two chloroplast regions sequenced. Gaps of more than one base, which could represent a single insertion/deletion event, were collapsed and treated as a fifth state, leaving a 1,352-bp span.

Relationships among 11 chloroplast haplotypes were also evaluated using the neighbor-joining method of Saitou and Nei (1987). The bootstrap consensus tree, inferred from 1,000 replicates, is taken to represent the relationships of the samples analyzed (Felsenstein 1985). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates were collapsed. Distances were computed using the maximum composite-likelihood method (Tamura et al. 2004). All positions containing gaps and missing data were eliminated (complete deletion option), leaving a total of 1,514 positions in the final data set. Analyses were conducted using MEGA4 (Tamura et al. 2007). A specimen of *D. alata* from Florida served as the outgroup in the resulting tree. Pair-wise genetic distances (PhiPT) for African samples were calculated using GenAlEx (Peakall and Smouse 2006).

Microsatellite DNA Markers. We screened five microsatellite loci originally developed for *D. alata* (Tostain et al. 2006). Two of these loci (*Da1A01* and *Da1F08*) were polymorphic across worldwide samples and had consistent profiles that could be scored unambiguously. Both loci were fluorescently labeled with 6-FAM.[7] DNA extracts for individuals ($n = 133$) of Florida and non-Florida origin were genotyped at these two loci. The 20-μl PCR reactions had final concentrations of 2 mM $MgCl_2$, 200 mM of each dNTPs, 0.6 μM of each primer pair; to each reaction were added 2 μl DNA, and 1 μl Biolase enzyme.[8] PCR reactions for microsatellite markers were run on thermal cyclers[1] under the following conditions: 1 min at 94 C, followed by 30 cycles each of 94 C for 30 s, 59 C for 30 s, and 72 C for 30 s. Reaction products were separated by capillary electrophoresis[9] with the LIZ600 size standard. Genotypes were called using the instrument manufacturer's software.[10]

Results and Discussion

Chloroplast DNA Haplotypes. We inferred the presence of 10 haplotypes in sequences examined from 39 *D. bulbifera* individuals (Table 2). Haplotype A comprises 11 specimens from Florida and single specimens from both Puerto Rico and China. Haplotype B comprises four Florida samples, which differ from A by a single nucleotide in a mononucleotide repeat region. Haplotypes C and D are each represented by a single Chinese specimen and differ from A and B by a single nucleotide each in a mononucleotide repeat and by a single substitution in D. Single specimens comprise African haplotypes E (Burundi), F (Ghana), and G (Benin). Haplotype H is represented by 12 samples from a variety of African localities (Ghana, Uganda, Burundi, Benin, and Togo), whereas haplotype I comprises three samples, one each from Ghana, Togo, and Uganda. Finally, haplotype J is represented by two samples, one each from Ghana and Uganda. Most of these haplotypes also differ from each other by one to three nucleotides and are found both in West Africa and in East Africa (Figure 2; Table 2).

A neighbor-joining tree produced a pattern similar to the haplotype network (Figure 3). Florida, Puerto Rico, and Chinese samples were grouped into a single clade, separated from the nearest sister, a Burundian sample (haplotype E in Figure 2). Whereas most African haplotypes sampled in this study confirm prior observations of genetic divergence from Asian varieties, at least one African haplotype (E) seemed to occupy an intermediate position between haplotypes of African and Asian origin. A large clade representing samples of African origin was undifferentiated and comprised samples that correspond to haplotypes F, G, H, and I. Sister to the larger African branch are two African samples that correspond to haplotype J from the parsimony network. Using chloroplast haplotypes to calculate PhiPT yielded a result of zero between East and West African samples. Haplotype diversity values were −0.71 within East Africa and 0.63 for West Africa.

Microsatellite Allelic Variability. At both microsatellite loci *Da1F08* and *Da1A01*, products were biallelic and yielded a total of five unique genotypes for samples of *D. bulbifera* (Table 3). No exact correspondence was observed between African and non-African genotypes, even though some loci shared at least one allele. The dominant African genotype ($n = 11$) indicates the presence of a null allele at locus *Da1A01* because repeated attempts to amplify these samples at this locus failed. All Florida individuals genotyped ($n = 93$) had identical profiles.

Historical records indicate repeated introductions of *D. bulbifera* to Florida from locations throughout the native and introduced ranges. Allelic variability was not present among the microsatellite markers tested from Florida, which could incorrectly lead to the conclusion that the invasive population is due to establishment of individuals from a single introduction event. Taking a minimum number of introductions approach and its assumptions (Ross and Shoemaker 2008), our chloroplast data confirmed the presence of more than one haplotype in the Florida population. Even though genetic diversity of *D. bulbifera* in Florida is very low, assuming that somatic mutations have not contributed to diversity in the invasive range, haplotype data are consistent with a minimum of two separate introductions into Florida. This may be an underestimate, and multiple introductions of the same haplotype to Florida would not be evidenced with this

Table 2. The 10 haplotypes in the sequences examined from 39 air-potato (*Dioscorea bulbifera* L.) individuals. Where available, a specific county, region, or province is indicated parenthetically.

Haplotype	Locality	Latitude	Longitude	GenBank accessions
A	Florida (Osceola)	28°17′15″N	81°24′36″W	HM775158 HM775169
	Florida (Palm Beach)	26°55′23″N	80°11′6″W	
	Florida (Miami-Dade)	25°44′21″N	80°15′1″W	
	Florida (Duval)	30°18′54″N	81°39′20″W	
	Florida (Polk)	28°1′57″N	81°56′22″W	
	Florida (Hillsborough)	27°45′45″N	82°8′55″W	
	Florida (Wakulla)	30°13′59″N	84°13′60″W	
	Florida (Duval)	30°17′25″N	81°43′39″W	
	Florida (DeSoto)	27°13′30″N	81°53′20″W	
	Florida (Duval)	30°20′28″N	81°42′16″W	
	Florida (Martin)	27°10′26″N	80°16′24″W	
	Puerto Rico (Arecibo)	18°19′56″N	66°43′4″W	
	China (Guangdong)	24°6′1″N	113°13′10″E	
B	Florida (Osceola)	28°17′0″N	81°27′26″W	HM775159 HM775170
	Florida (Indian River)	27°47′57″N	80°29′53″W	
	Florida (Monroe)	24°33′35″N	81°47′43″W	
	Florida (Martin)	27°4′3″N	80°19′11″W	
C	China (Yunnan)	21°53′41″N	101°1′37″E	HM775160 HM775171
D	China (Guangdong)	23°12′47″N	113°25′18″E	HM775161 HM775172
E	Burundi	4°5′57″S	29°30′27″E	HM775162 HM775173
F	Ghana (Tuna)	9°48′4″N	1°58′12″W	HM775163 HM775174
G	Benin (Serou)	9°39′48″N	1°41′50″E	HM775164 HM775175
H	Uganda	0°23′52″N	33°0′58″E	HM775165 HM775176
	Uganda	0°23′57″N	33°0′39″E	
	Ghana (Tamale)	9°20′46″N	0°49′23″W	
	Ghana (Anyinamso)	6°39′7″N	1°53′53″W	
	Burundi	4°5′54″S	29°30′23″E	
	Ghana (Ayinasu)	6°56′53″N	2°5′17″W	
	Ghana (Mfensi)	6°46′59″N	1°48′7″W	
	Ghana (Pakyi No. 1)	6°31′49″N	1°4′8″W	
	Togo (Tove)	6°52′41″N	0°39′5″E	
	Benin (Savalou)	7°51′21″N	1°58′50″E	
	Uganda	0°23′56″N	33°0′39″E	
	Togo (Misahohe)	6°57′3″N	0°35′44″E	
I	Ghana (Nkurakan)	6°6′25″N	2°16′43″W	HM775166 HM775177
	Togo (Kuma Adame)	6°58′25″N	0°35′45″E	
	Uganda	0°23′50″N	33°1′2″E	
J	Ghana (Adumadum)	7°37′52″N	0°7′60″W	HM775167 HM775178
	Uganda	0°23′45″N	33°0′39″E	
Dioscorea alata	Florida (Hendry)	26°45′55″N	81°26′5″W	HM775168 HM775179

methodology. Although two bulbil morphotypes were observed among Florida specimens, we did not note a correlation between this character and haplotype.

Many recent studies of invasive species have suggested that multiple introductions from unique source populations and subsequent hybridization among them facilitates the development of invasiveness by increasing genetic diversity or by creating novel genetic combinations (e.g., Lavergne and Molofsky 2007; Novak and Mack 2005; Roman and Darling 2007). There are also examples of invasive, asexually reproducing species that nonetheless have high genetic diversity as a result of somatic mutation, multiple introductions, or variable ploidy levels in their introduced range (Chapman et al. 2004; Rottenberg and Parker 2004). The use of more polymorphic markers, such as amplification fragment length polymorphisms or *D. bulbifera*–specific microsatellite loci, could reveal more genetic variation than we found in this study, or this

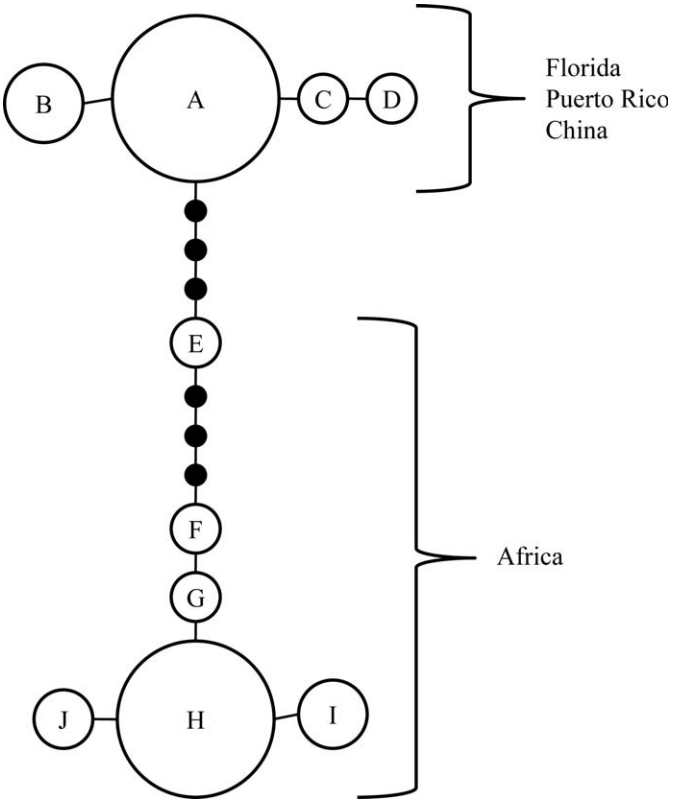

Figure 2. Statistical parsimony network of air-potato (*Dioscorea bulbifera* L.) chloroplast haplotypes at a 95% confidence interval. Displayed are *D. bulbifera* samples from Florida (haplotypes A and B), Puerto Rico (A), Africa (E, F, G, H, I, and J), and China (A, C, D). Lettered nodes represent observed haplotypes, whereas black nodes represent inferred intermediates.

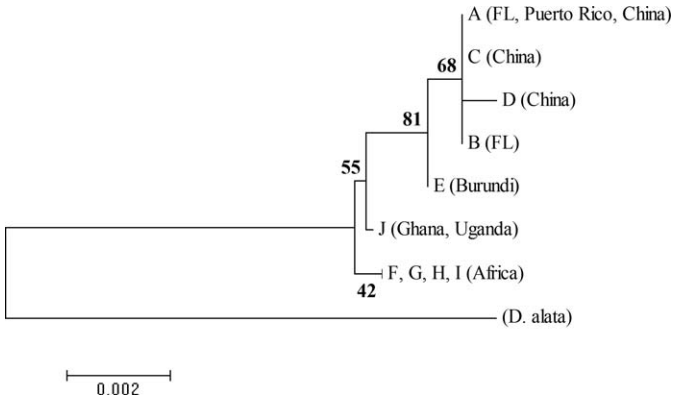

Figure 3. A consensus tree, showing the results of a neighbor-joining analysis, depicts relationships inferred among air-potato (*Dioscorea bulbifera* L.) haplotypes from Florida, Puerto Rico, Africa, and Asia. A sample of *Dioscorea alata* is the outgroup in this analysis. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) is listed at the corresponding node on the tree. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances (base substitutions per site) used to infer the tree.

population may be invasive in spite of having low genetic diversity, which has been the case in some invasive species (Loomis and Fishman 2009; Poulin et al. 2005; Wang et al. 2005).

Genetic studies of *D. bulbifera* that used chloroplast-restriction patterns have noted a clear separation between African and Asian plants (Ramser et al. 1996; Terauchi et al. 1991). To date, the genetic diversity of *D. bulbifera* has been studied most thoroughly in China (Zheng et al. 2006), with only limited assessments of plant material in West Africa, where the species is most frequently cultivated for food. The present study complements the efforts of Terauchi et al. (1991) and Ramser et al. (1996) by sampling the species much more widely in Africa and reinforces the notion that significant genetic divergence exists between specimens of Asia and Africa. Morphological evaluations of intraspecific diversity in continental Africa have indicated significant diversity there. One account identified more than 11 varieties in Africa, based on bulbil and leaf morphology (Miège 1982), vs. nine Asian varieties (Prain and Burkill 1936). Although we found six chloroplast haplotypes in Africa, genetic divergence between them was generally very low (1–3 bp differences). Haplotypes did not show evidence of significant geographical structure between East Africa and West Africa, even though the East African samples examined are separated by more than 3,000 km from the West African samples. Within regions (East/West), African haplotype diversity was significant. This diversity may reflect the long history of cultivation and human movement of the species in Africa.

Muirhead et al. (2008) established that many studies have failed to sample sufficiently in the source range when attempting to ascertain the origin of an invasive population. In this study, sampling of the source range is not sufficient to assert that China, or a particular region of China, is the source of Florida air-potato. Despite the presence of a chloroplast haplotype from Guangdong, China, which exactly matches the predominant haplotype found in Florida, the data do not conclusively identify China as the source location. Further sampling in Asia, Oceania, and the many geographically isolated populations where this plant is found will allow identification of the source region to a higher degree of certainty. These locations could be targeted in future collection efforts if they are thought to benefit the management strategy or understanding of the phylogeography in this species.

A greater understanding of genetic differences among the 600+ *Dioscorea* species and some of their varieties could help with identification of these plants, many of which can be difficult to distinguish morphologically. DNA barcoding of members of this genus, as part of an integrative

Table 3. Microsatellite genotypes by place of origin and genetic locus.

No. of samples	Locations found (*n*)	Locus *Da1F08*		Locus *Da1A01*	
95	Florida (93), Puerto Rico (1), China (1)	152	154	205	205
1	China (1)	152	152	207	207
6	Uganda (4), Togo (2)	152	152	198	198
1	Ghana (1)	152	152	198	211
11	Benin (3), Ghana (6), Burundi (1), Togo (1)	152	152	0	0

taxonomic analysis, would be especially useful because some of these species are economically important as food crops, whereas others have become invasive (e.g., Newmaster and Ragupathy 2009; Van De Wiel et al. 2009).

A leaf beetle (*Lilioceris* sp.; Coleoptera: Chrysomelidae) from Nepal has undergone host range testing as a biological control candidate of *D. bulbifera*, and a petition for its release has been submitted to USDA Animal and Plant Health Inspection Service (Pemberton 2009). Our results support an Asian origin for *D. bulbifera* in Florida, but whether any agent will be host specific and effective for management remains to be seen. If both agents from Africa and Asia successfully clear the screening process and are approved for release, it may be possible to evaluate the management success of both insects both independently and in combination to further test whether the insects from Asia actually provide better control of the plant.

The possibility of an Asian variety of *D. bulbifera* predominating in Florida, and possibly more widely throughout the West Indies as suggested by the similarity of a Puerto Rican plant to those in Florida, raises some interesting questions. Historical accounts are clear regarding the African provenance of *D. bulbifera* in America, so any meaningful attempt to explain why no genotypes of African origin have yet been confirmed must explain their apparent absence. *Dioscorea bulbifera* is an invasive species in other New World locations (e.g., the southern United States, Hawaii, and the Caribbean) that were not sampled during the course of this study. If *D. bulbifera* in those locations are the same as the Florida genotypes, it may indicate that a single or a few introductions occurred over a broad geographic area or that a single genotype or variety may be more invasive than others.

Sources of Materials

[1] ABI 2720 thermal cycler, Applied Biosystems, now Life Technologies, 5791 Van Allen Way, Carlsbad, CA 92008; or PTC-200 thermal cycler, MJ Research, now Bio-Rad, 2000 Alfred Nobel Drive, Hercules, CA 94547.

[2] HotStart polymerase, Promega, 2800 Woods Hollow Road Madison, WI 53711.

[3] *ExoI* and Antarctic phosphatase, New England Biolabs, 240 County Road Ipswich, MA 01938-2723.

[4] BigDye Terminator Cycle Sequencing kit v3.1, Applied Biosystems, now Life Technologies, 5791 Van Allen Way, Carlsbad, CA 92008.

[5] ABI 3730xl or ABI 3130 genetic analyzers, Applied Biosystems, now Life Technologies, 5791 Van Allen Way, Carlsbad, CA 92008.

[6] Sequencher v4.8, Gene Codes Corporation, 775 Technology Drive, Suite 100A, Ann Arbor, MI 48108.

[7] Primers fluorescently labeled with 6-FAM, Eurofins MWG Operon, 2211 Seminole Drive, Huntsville, AL 35805.

[8] Biolase enzyme, Bioline, 16 The Edge Business Centre, Humber Road, London NW2 6EW, United Kingdom.

[9] ABI 3130 genetic analyzer, Applied Biosystems, now Life Technologies, 5791 Van Allen Way, Carlsbad, CA 92008.

[10] GeneMapper ver. 4.0, Applied Biosystems,, now Life Technologies, 5791 Van Allen Way, Carlsbad, CA 92008.

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