

SOURCE POPULATION, INTRODUCTION HISTORY, AND GENETIC DIVERSITY IN  
THE PANTROPICAL YAM *Dioscorea bulbifera* L.: AN INVASIVE VINE IN FLORIDA

By

MATTHEW DAVID CROXTON

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To my mom and dad--you chose me and have always believed in me.

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Abstract of Thesis Presented to the Graduate School  
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SOURCE POPULATION, INTRODUCTION HISTORY, AND GENETIC DIVERSITY IN  
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By

Matthew David Croxton

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The air potato vine, *Dioscorea bulbifera* L., was imported into Florida from a number of locations in the tropics near the beginning of the 20<sup>th</sup> century and became recognized as a pest weed within a short time. Established control methods (chemical treatment and manual removal) are expensive, and herbicides may have damaging off-target effects. Because it has a variable phenotype and high genetic diversity, analyses using plastid and genomic markers were conducted in order to determine the source population of the vine. Locating the source population is the accepted method for identifying the most closely co-adapted herbivores of the host plant, and may be essential to the implementation of a host-specific biological control strategy for this species.

I found no genetic diversity of *D. bulbifera* in Florida, affirming the clonal reproduction of the species in Florida through propagation from aerial bulbils, and suggesting that the source population may be localized to a relatively small geographic region once the source genotype is identified from the native range. Results from the source population study are emphatic that invasive *D. bulbifera* in Florida is not African in origin, and host specific biocontrols to the invasive are likely to be discovered from Southeast Asia or Oceania.

## CHAPTER 1 INTRODUCTION

### **Invasion Biology of Study Species**

Air potato, *Dioscorea bulbifera* L. is a twining, monocotyledonous vine that belongs to the taxonomically complex yam family, the Dioscoreaceae. Both conventional and molecular treatments of this species have ascribed or applied numerous varietal designations for this pantropical plant. In Florida, USA, *D. bulbifera* is considered a Category I exotic pest plant (FLEPPC, 2007). While this designation is given due to demonstrated ecological impact, rather than economic impact or geographical extent, others have also established that it is both widespread and costly to manage. Expense of manual removal and chemical control, along with significant off-target effects (especially of the latter), have galvanized research efforts to seek out host-specific biocontrols of this dioecious vine from its indigenous range in the tropics of the Old World (Wheeler *et al.*, 2007). I present the results of genetic analyses undertaken to determine the source population and genetic diversity in invasive Florida *D. bulbifera*.

A key weed risk assessment that has been adapted to Florida unequivocally identifies *D. bulbifera*, *post hoc*, as a risk for becoming a major invader (Gordon *et al.*, 2008). Florida and Alabama are states where it is formally recognized as a noxious weed with significant ecological impact, but it is also reported in the United States from Puerto Rico, and from the states of Hawaii (Hawaii, Maui, Molokai, Oahu, and Kauai Islands), Texas, Louisiana, Georgia, and Mississippi (Wester 1992; Zomlefer *et al.*, 2008; USDA, Natural Resources Conservation Service, 2009). It is also present (sometimes in cultivation) in the West Indies and Central America, but there is little data to verify ecological or economic impacts in those regions (Al-Shehbaz & Schubert, 1989).

Invasive plant species can exert significant negative effects on ecosystems where they encroach, translating into costly management expenses incurred in the course of control activities (Pimentel *et al.*, 2005). If left unchecked, invasive plants may change the trajectory of natural communities so significantly that native species are unable to maintain established functional roles, and are endangered with population declines if unable to adapt rapidly to new roles. Impacts of invasive species introductions often include: decrease in long-term biodiversity of native species, landscape fragmentation, abiotic modifications to soil moisture and chemistry, changes in fire regime, light availability, displacement of native species, vectoring pathogens of economically important species, and conversion of diverse habitats into monocultures. Anthropogenic, long distance dispersal is frequently cited as the primary mode for invasive plant introductions. Often, following anthropogenic introduction, invasive plants do not initially exhibit weedy tendencies. This 'lag phase' is long and well described for some invasive taxa, but may be very short in other cases.

Two prevalent hypotheses are often used to explain the success of invasive species outside their native range: enemy release and evolution of increased competitive ability (EICA). The first asserts that invasive plants thrive when introduced into new environments because reduced selective pressure from natural enemies allows them to thrive (Liu & Stilling, 2006), while the second states that invasive species may evolve upon introduction to a novel environment in ways that allow them to better compete against native species (Blossey & Notzold, 1995).

Classical weed biocontrol is premised upon introducing a plant's natural enemy into the invasive range where the plant lacks such natural enemies. Lack of natural enemies in the introduced range illustrates the importance of host specificity in preventing unchecked proliferation. Because no herbivore is adapted to the abundant resource, the plant continues to

spread, maximizing the amount of resources it can allocate to reproduction, dispersal, and growth, within the limits of the plant's vegetative architecture. Host specific natural enemies have maximized their success in phytophagy by adapting to their host over a significant time – long enough to co-evolve with the plant taxon and to diverge in concert with changes to the original host. When allopatry generates many unique host/herbivore relationships across a large geographic area, then locating the correct herbivore most closely co-evolved to the invasive is greatly enhanced by identifying the source population. Host specificity is the primary qualification for potential biocontrol agents, especially when native, non-target taxa are closely related to the invasive, and could be impacted by host switching of the agent (Goolsby *et al.*, 2006).

### **Management, Identification, and Invasive Ecology**

The costs of managing *D. bulbifera* can be high; in one example using multiple herbicides to treat an area affected by air potato and other invasive plants, more than \$1700 per ha/year is the expenditure recorded (Wheeler *et al.*, 2007). Existing management activities include chemical control and manual removal programs (Stern & DiMarco, 2002). These activities are not sufficient or cost-effective enough to give land managers the desired management impact, and exert significant off-target effects on native vegetation.

Air potato is readily identified in Florida by simple, alternate, cordate, net-veined leaves, sinistrorse twining habit, and namesake aerial bulbils. Only pistillate flowers, and just one observation of fruiting in Florida have been documented for this dioecious plant, suggesting the potential for rare sexual reproduction of the vine (Hammer, 1998). Hermaphrodite flowers were reported from *D. bulbifera* var. *sativa*, a variety of air potato prevalent in Oceania (Prain & Burkill, 1936), but no hermaphrodite flowers have been reported from Florida. Vegetative propagation from aerial bulbils is the only mode of reproduction that can be readily verified for

invasive *D. bulbifera*, and establishes the possibility that the entire Florida population may be clonal. However, two bulbil morphologies can be found in Florida: a smooth skinned bulbil having gilvous (faintly yellowish when viewed in bright light) periderm, and a warty, rough skinned bulbil with coffee-colored periderm. It is not established whether their presence indicates two or more genetically distinct varieties.

In addition to disturbed areas, air potato invades a variety of natural areas in Florida, including pinelands, mesic hardwood hammocks, and occasionally intruding into xeric uplands (Hutchinson & Menges, 2006; Morisawa, 1999). Although not salt tolerant, *D. bulbifera* can be found in the Florida Keys, as well as other locations closely abutting the coastline. The monocotyledonous stem of *D. bulbifera* has a near-obligate association with upright vegetation, on which it relies for support, protection from desiccation, and access to enriched organic soils. 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 (Odom *et al.*, 2008) and changes in function of the plant community. Once air potato invades an area, it is difficult to eliminate due to the prolific production of persistent aerial bulbils. In addition, invasiveness of *D. bulbifera* is facilitated by canopy disturbance events such as hurricanes (Horvitz *et al.*, 1998). Following disturbance events, the combination of rapid twining growth and sprouting from vegetative bulbils triggers preferential establishment of the species, displacing slower-growing woody vines that are dependent on bark adherence to advance into the canopy (Horvitz & Koop, 2001; Gordon, 1998).

## Taxonomy and Distribution

A family estimated by some to have as many as 900 species, the Dioscoreaceae are a diverse group of mostly tropical, climbing and herbaceous monocots belonging to the order Dioscoreales (Al-Shehbaz & Schubert, 1989). The dioecious genus, *Dioscorea* L., is the largest and most widely distributed, being comprised of about 450 species (Wilkin *et al.*, 2005). Prior estimates of diversity in the genus judged the number of species at 600 (Knuth, 1924), and have ranged as high as 850 (Al-Shehbaz & Schubert, 1989). A recent reconstruction of the molecular phylogeny for this group has done much to clarify intra-sectional relationships in the genus, but certain clades have yet to be completely resolved (Wilkin *et al.*, 2005).

High morphological variability, dioecy, and small flowers have been cited as reasons for the long-standing taxonomic difficulties surrounding members of *Dioscorea*; these mitigating factors continue to frustrate morphological treatments, all the way down to varietal classification. One taxon, as yet unresolved with genetic methods, is that of *Dioscorea bulbifera* L. (sampled from Madagascar), which is tentatively placed between a compound-leaved clade and a clade of Malagasy endemics in a combined plastid phylogeny (Wilkin *et al.*, 2005). *D. bulbifera* has been assigned to the section Opsophyton (Uline), a group of five or six Eastern Hemisphere species, of which, *D. bulbifera* is the sole representative in China (Zhizun & Gilbert, 2000).

Having the most extensive endemic range of any species in the family, and an even larger introduced range, *D. bulbifera* L. has long been studied, and under many different names. Prain and Burkill (1936) list more than 15 pre-Linnean names and references between 1684 and 1750, while more than 25 binomial synonyms appear in later works. This species is the only one in the family native to both Africa and Asia, with limits well defined by numerous authors and accounts (Burkill, 1960; Coursey, 1967). The native range in Africa extends from the West African coast, south of 10°N latitude, eastward to Ethiopia on the opposite end of the continent.

In locations where rainfall is sufficient, it extends through South and Central Africa almost to South Africa, at elevations between 200-1300 m (Wilkin, 2001). In Asia, air potato can be found from India and Nepal eastward to Southern Japan. It extends south through the Malay Archipelago and down to Queensland in Australia. The adventive range of this species is extensive and includes many outlying Pacific islands, Madagascar, portions of Central and South America, the West Indies, and Florida. Disjunction in the range of *D. bulbifera* between Africa and Asia was caused by drying climatic conditions during the Pliocene; the same environmental changes also disjoined the distributions of *Dioscorea* sections Lasiophyton and Enantiophyllum (except for *D. alata*), and the species of these sections are now considered endemic to either Africa or Asia, but not both (Burkill, 1960).

### **Introduction History**

Yams are among the earliest anthropogenic plant introductions to the New World by Europeans. Even earlier movements of *D. bulbifera* around the Old World and outlying areas are documented in association with cultivation. One example is the introduction of *D. bulbifera* to the islands of Hawaii by Polynesians as early as 1000 A.D. (Wester, 1992), and corroborated back to 1500 A.D. using radiocarbon dating at a well-preserved archaeological site (Burney *et al.*, 2001). An introduction history of invasive *D. bulbifera* to North America and adjacent areas is incomplete and hindered somewhat by taxonomic complexity that resulted in numerous synonyms, and later, numerous varieties.

Yam specialists have usually ascribed an African source for *D. bulbifera* in North America, rather than an Asian source (Coursey, 1967; Burkill, 1939, 1960; Prain & Burkill, 1936). The introduction of *D. bulbifera* var. *anthropophagorum* from Africa to America occurred as early as the 16th century. According to Prain and Burkill (1936), this variety was found throughout the West Indies and Central America during the 1930s (and various locations

in the Americas between latitudes 29°N and 35°S), and was grown as a curiosity in northern Florida. A 1601 description of the ‘Ycam’ introduced to the West Indies is likely the West African variety of *D. bulbifera*, placing the introduction of the species to the New World early in the slave trade (Coursey, 1967). Yam bulbils and tubers were transported across the Atlantic as durable foodstuffs during long sea voyages. Africans who were familiar with the cultivation and preparation of the yams planted them upon their arrival in the New World, and most abundantly in the West Indies. Burkill maintains that the names given to the West Indian cultivars of *D. bulbifera* validate their introduction from the “Elmina Lagos coast” (Burkill, 1939). Botanist William Bartram reported ‘Discorea [sic] bulbifera’ from a Mobile garden in his ‘*Travels*’ from 1777, but Bartram commentator Harper states that this species may not have been *D. bulbifera* L. (Harper, 1998).

Comprehensive treatments of the Florida floras have asserted that *D. bulbifera* is of Asian origin, and was originally planted for ornament, but cite no references to support this assertion (Clewell, 1985; Hall, 1993; Wunderlin & Hansen, 2003). Florida horticulturalist Nehrling, circa 1905, mentions the species in Florida, and was also the first to record the plant’s potential for weediness (Nehrling, 1933). Many yam species were introduced intentionally into Florida from Asia, Oceania, Africa, and the West Indies between 1840 and 1920. *D. alata* was introduced to Fort Myers during the 1840’s by the United States Department of Agriculture (USDA). The Office of Foreign Seed and Plant Introductions was also responsible for bringing ‘other yams’ into Florida and distributing them to plant experimenters (Young, 1923). In documenting the introductions of *D. bulbifera* presented in this study, I encountered many more accounts of USDA yam introductions, often unidentified as to the species. Many of these introductions presumably did not become established, however, because weak demand and lack of knowledge

about cultivation practices made them a commercial failure (Young, 1923). As shown in Table 1-1, early 20<sup>th</sup> century introductions of *D. bulbifera* into Florida included material from Africa, Polynesia, the West Indies, and Southeast Asia.

### **Previous Work**

Previous studies of intraspecific genetic diversity in *D. bulbifera* found high levels of differentiation between African and Asian types, and noted significant variation among plants in Asia and Oceania. An analysis of chloroplast restriction fragment length polymorphisms (RFLP) indicated that certain chloroplast genotypes were distributed over a wide geographic range (Thailand to Taiwan), while some locations had more than two types present in a small area (Taiwan). The same study concluded that Southeast Asia was the differentiation center of *D. bulbifera* and found only a small amount of genetic variation present within Africa, based on a total of three collections from Ethiopia, Tanzania, and Madagascar (Terauchi *et al.*, 1991). A random amplified polymorphic DNA (RAPD) analysis (Ramser *et al.*, 1996) added an additional three accessions from Ethiopia, but did not note significant intraspecific variation relative to the amount present in Asia and Oceania.

The first analysis of genetic diversity in *D. bulbifera* (Terauchi *et al.*, 1991) suggested that the amount of variation present within the genotypes of Asian and Oceanian accessions was equal to the amount of differentiation between the African and most closely related Asian samples. Subsequent analyses have bolstered the support for high diversity in Oceania (Ramser *et al.*, 1996), and in Asia (Ramser *et al.*, 1996; Zheng *et al.*, 2006), with the most recent study grouping a geographically diverse sample from mainland China into 5 clusters using inter-simple sequence repeat (ISSR) markers.

Uncertainty surrounding morphological treatments of varieties in *D. bulbifera*, and demonstrable, intraspecific genetic diversity structured across its broad geographical range, made

the decision straightforward to adopt a genetic methodology in identifying the source population of invasive *D. bulbifera* in Florida. To address the question of source population, I collected samples of the target species in Florida (extensively) and across the native range (opportunistically). I sampled widely in Florida in order to increase the likelihood of collecting from among the unique introductions documented. By sequencing intragenic chloroplast DNA markers that are shown to possess intraspecific variability across a variety of angiosperms (Shaw *et al.*, 2005), I sought to identify the haplotype or types most similar to those present in Florida. A second genetic method, microsatellite genotyping, was used to evaluate intraspecific variability in Florida by using markers previously developed for this purpose in yams (Tostain *et al.*, 2006).

Table 1-1. Introductions of *D. bulbifera* to Florida and adjacent areas: 1500's to 1919.

Author	Date <sup>a</sup>	Source	Destination	Notes	Reference
Clusius, C.	1500's	West Africa ("Elmina Lagos")	W. Indies; Americas	Introduced by Portugese ships trafficking African slaves	(Burkill, 1939)
Bartram, W.	1777	?	Mobile (Alabama)	Not Asiatic <i>D. bulbifera</i> , according to Harper	(Harper, 1998)
Nehrling, H.	1905	?	Gotha, Florida	Noted weedy behavior and propensity for escape	(Nehrling, 1933)
USDA #18656	1906, May	Mayaguez, Puerto Rico	Miami, Florida	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	Mayaguez, Puerto Rico	Florida, (?)	Received by R.A. Young; Aerial tubers better for food than ground tubers	(USDA, 1922a)
USDA #46218	1918, May	Honolulu, Hawaii	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)

<sup>a</sup> denotes either date of introduction or report of sighting

## CHAPTER 2 METHODS

### **Collection**

Volunteers through the Florida Cooperative Extension and Master Gardener programs collected accessions of *D. bulbifera* from July to November of 2006. Collectors were given species identification guidelines, in order to prevent the sampling of *D. alata*, the only yam in Florida with which *D. bulbifera* is likely to be confused on the basis of morphology. Collectors were also requested to note the presence of flowers or fruits, as well as to categorize the bulbil morphology as either warty/dark or smooth/light. Geospatial coordinates for sample locations were recorded using global positioning system (GPS) instruments, or approximated using either a street address or detailed descriptions. Leaves were desiccated in silica gel for preservation.

Most non-Florida accessions were collected between April and September of 2007. A few samples were obtained at dates outside this range; these include an accession from Puerto Rico (November, 2005) and accessions from China (July, 2004), Uganda (February, 2004 and September, 2006). The data gathered for all accessions is summarized in the supplementary data. Data about flowering, fruiting, and bulbil morphology were not requested from collectors of non-Florida samples; in addition to geositions by GPS, many also included photographs, and indicated whether the collection was from a wild or cultivated plant. As with Florida accessions, leaf material was preserved by desiccation in silica gel.

### **DNA Extraction**

DNA was extracted from dried leaf material (1 to 2 cm<sup>2</sup>) using a modified method of Kim *et al.* (1997). In some cases, extracts were further cleaned using Promega's Wizard DNA cleanup kit (Promega, USA). Extracts were subsequently used for both chloroplast and microsatellite analyses.

## Chloroplast DNA Sequencing

Two chloroplast DNA (cpDNA) intergenic regions, spanning psbM-trnD and ycf6-psbM (Shaw *et al.*, 2005), were amplified for 26 accessions using the following 10uL PCR reaction mix: 2.5mM MgCl<sub>2</sub>, 0.5mM primer, 200mM each dNTP, 0.2U Taq polymerase, 1uL of DNA. Reactions were run using either ABI 2720 or MJ Research PTC-200 thermalcyclers with the following conditions: 2 minutes at 94°C, followed by 30 cycles of [94°C for 30 seconds, 55°C for 30 seconds, 72°C for 1 minute], and a final extension of 72°C for 5 minutes. For troublesome samples, hot-start polymerase (Promega HotStart) was substituted for the standard polymerase enzyme. Unincorporated nucleotides and excess primers were removed from PCR products using ExoI and Antarctic Phosphatase (New England Biolabs) according to manufacturer protocols. A total of 1,690 bp were sequenced in both forward and reverse directions using BigDye Terminator Cycle Sequencing kit v3.1 and electrophoresed on either an ABI 3730xl or ABI 3130 genetic analyzers.

Sequences were trimmed and contiged in Sequencher v4.8 (Gene Codes Corp.) then aligned using ClustalW. Relationships among haplotypes were visualized using a 95% parsimony haplotype network constructed with TCS 1.21 using a total of 1690 bp (Clement *et al.*, 2000). Alignment gaps of more than one base that could represent a single insertion/deletion event were collapsed and treated as a fifth state. A neighbor-joining tree was also constructed using PAUP\* ver. 4.0b10 (Swofford, 2002). Accessions of *D. alata* from India (421, 428, and 205) were defined as outgroup and used to root the resulting tree. A 1000-replicate neighbor-joining (NJ) search was run using the distance criterion, followed by a 1000-replicate NJ bootstrap analysis on this tree. Bootstrap percentages were indicated at their respective nodes on the NJ tree.

### **Microsatellite Analysis**

Yam accessions (n=133) of Florida and non-Florida origin were tested for polymorphism using microsatellite markers *Da1A01* and *Da1F08* (Tostain *et al.*, 2006) fluorescently labeled with 6-FAM (Eurofins MWG Operon). The 20uL PCR reactions had final concentrations of 2mM MgCl<sub>2</sub>, 200mM each dNTPs, 0.6mM each primer pair, 2uL DNA, and 1uL Biolase enzyme (Bioline). PCR reactions for microsatellite markers were run on either a PTC-200 (MJ Research) or ABI 2720 model thermal cycler under the following conditions: 1 minute at 94°C, followed by 30 cycles each of [94°C for 30 seconds, 59°C for 30 seconds, and 72°C for 30 seconds]. Reaction products were separated by capillary electrophoresis using an ABI 3130 with the LIZ600 size standard. Genotypes were called using GeneMapper ver. 4.0 (ABI). GenAlEx ver. 6.1 was used to generate pairwise genotypic distances which were used to cluster genotypes in a principal coordinate analysis (Peakall & Smouse, 2006).

## CHAPTER 3 RESULTS

### **Sampling**

In all, 100 accessions of Florida *D. bulbifera* were used in the analyses, representing 93 unique geositions. Only one observation of flowering (pistillate plant) was reported from the collections in Florida (Accession 085). For 68 accessions from Florida, collectors recorded bulbil morphology: 39 reported the “Warty/Dark” morphotype, while 20 reported the “Smooth/Gray” morphotype. For seven accessions, a “Smooth/Gray/Warty” type was recorded, and for two additional accessions a “Gray/Warty” bulbil type was seen. These observations are summarized in Table A-1.

### **Chloroplast DNA Analysis**

There were seven haplotypes of *D. bulbifera* which were grouped into two distinct clusters. One cluster includes all the haplotypes of African origin and the other cluster groups haplotypes from Florida and Chinese samples (Figure 3-2). Accession data for the samples represented in this network are summarized (Table 3-1). The neighbor-joining tree also clearly groups the Florida samples with the Chinese samples separate from the African samples (Figure 3-3). Despite distinct genetic differences between the African and Chinese haplotypes observed, there was relatively little genetic diversity observed within each of the haplotype groups, when compared to the significant amount of divergence separating them. No intraspecific variation was observed within Florida.

Four African haplotypes (D, E, F, and G) differ at two loci on the DNA alignment. Single-base length polymorphism in a homonucleotide region differentiates African haplotypes D, E, and F from each other, while a two bp deletion in G differentiates it from haplotype F. Haplotypes A and B are separated by the same length polymorphism that also differentiates D

from E, and haplotype C is defined by a single base substitution from haplotype B. African and Florida/Asian haplotype groups were separated from each other by a total of eight changes – a combination of substitutions (four), insertions/deletions (two), and length polymorphisms (two).

A minimum of eight inferred mutations separate the two clusters representing haplotypes from Africa and those from Florida/China. Confidence in this separation is very high, and is corroborated by the bootstrap percentages indicated in the neighbor-joining tree. Bootstrap percentages indicate the frequency at which bifurcating branches of a node on the tree are resolved in the given topology, unlike other measures of confidence, more commonly encountered (such as the venerable 95% interval). In the NJ tree generated for this study, separation of the Florida/China samples from the African group is supported with a bootstrap value of 100%. It also differentiates between Florida and China samples with a 98% bootstrap percent. A 64% bootstrap value separates a Ghanaian accession (120) from the rest of the main African clade. This is consistent with the parsimony network, where 120 is the sole representative of haplotype D, and is closer by one inferred mutation to the Florida accessions than any other African haplotype. The NJ tree did not resolve (412), haplotype E from the parsimony network, and this shows up in the lowered bootstrap support for this group, at 51%.

### **Microsatellite Analysis**

For both microsatellite loci, the products for each locus were biallelic, with five unique allele patterns observed among accessions of *D. bulbifera* (Table 3-2). No genotype differentiated between cultivated and wild *D. bulbifera* in Africa. In many African samples, *DAIA01* failed to amplify, even after multiple amplification attempts, suggesting the presence of a null allele. All 100 Florida samples had identical genotypes. In just five samples from Florida, no product was recorded from one of the two loci. This occurrence was rare enough to suggest an experimental anomaly, rather than a null allele, was responsible for the results obtained in these

samples. In addition, five Florida samples that lacked a geoposition or unique location data were also excluded from the genetic distance calculation for the PCA. Principal coordinate analysis clustered 90 Florida, with one Puerto Rican, and one Chinese accession, while African accessions clustered into two separate groups (Figure 3-4). Principal coordinates one and two explain 74.85% and 21.60% of the variation in the data, respectively. Similar to the cpDNA, data from the nuclear microsatellite loci exhibited very low genetic diversity and most alleles across Africa differed by only 1 to 3 repeat units.

Table 3-1. Chloroplast DNA haplotypes, listed by locality; some localities are given with a more specific regional identifier in parentheses. Coordinates given in decimal degrees.

Haplotype	Locality	Latitude	Longitude
A	Florida (Palm Beach)	26.923036	-80.185042
	Florida (Hillsborough)	27.76258	-82.14865
	Florida (Martin)	27.1738	-80.27323
B	China (Yunnan)	21.894586	101.027001
C	China (Guangdong)	23.212917	113.421556
D	Ghana (Tuna)	9.801	-1.97
E	Benin (Serou)	9.663333	1.697222
F	Uganda	0.399123	33.01079
	Uganda	0.39787	33.01608
	Ghana (Tamale)	9.346	-0.823
	Ghana (Anyinamso)	6.65194	-1.89803
	Burundi (Kigwena)	-4.09835	29.50636
	Ghana (Ayinasu)	6.94795	-2.08802
	Ghana (Mfensi)	6.7834	-1.802
	Ghana (Pakyi)	6.53294	-1.66884
	Togo (Tove)	6.878055	0.651389
	Benin (Savalou)	7.855833	1.980556
	Uganda	0.39895	33.01087
	Uganda	0.39895	33.01087
	Togo (Misahohe)	6.950834	0.595556
	G	Ghana (Nkurakan)	6.107
Togo (Kuma Adame)		6.973611	0.595833
Uganda		0.39729	33.01719

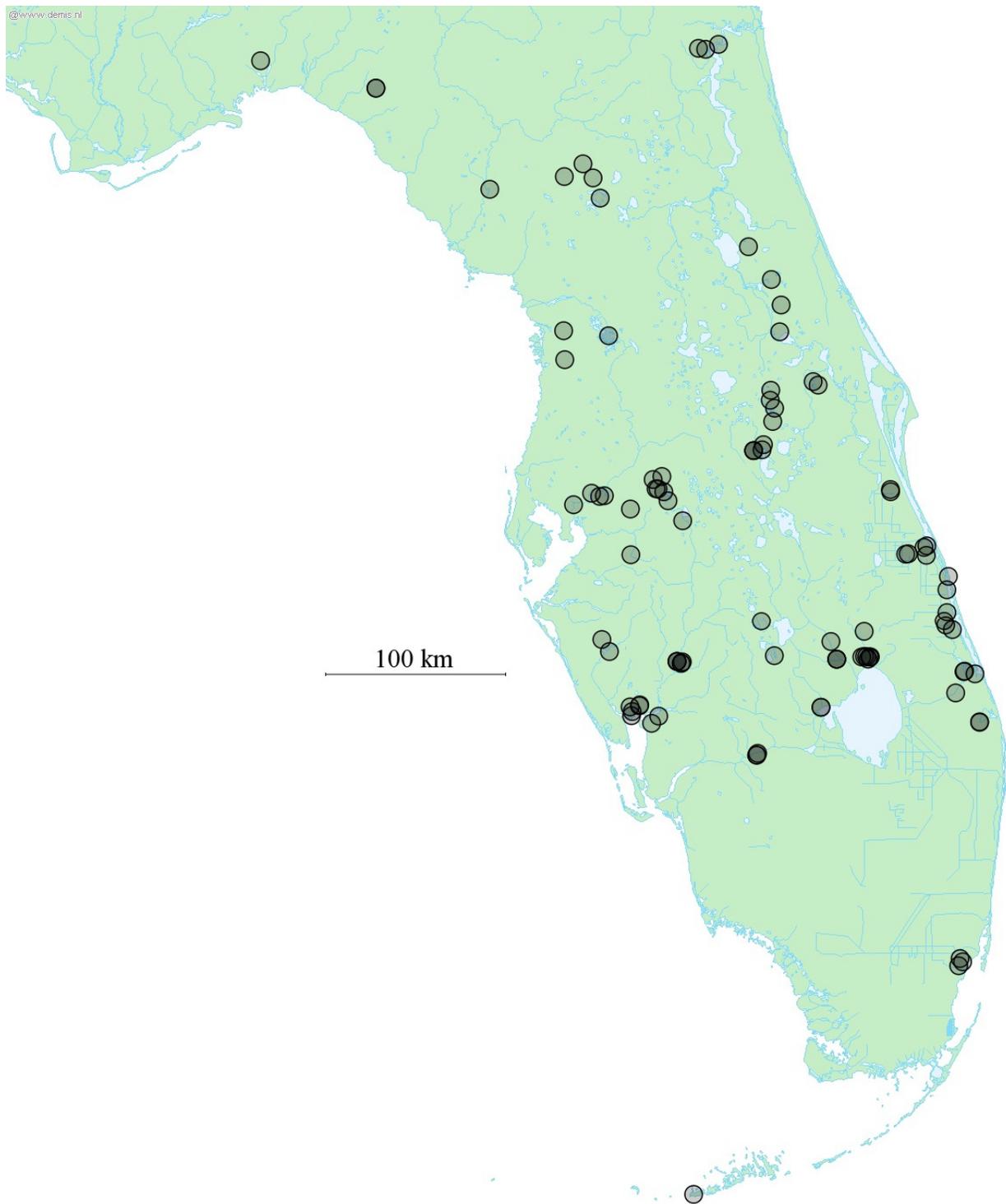


Figure 3-1. Map of 99 *D. bulbifera* L. accessions in Florida, USA. Out of 100 Florida accessions that were genotyped, 93 are represented as unique points on the map. Six accessions, recorded from the same point as another accession, represent collections from secondary or tertiary plants near the original place of collection, and for which a unique position was not recorded. One accession from Broward County had no other coordinate or distance/bearing information associated with it.

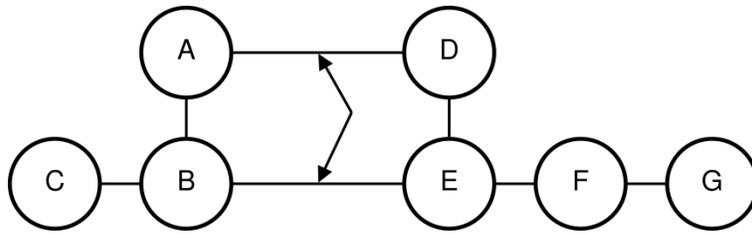


Figure 3-2. Parsimonious haplotype network from chloroplast DNA alignments. Lettered circles represent unique haplotypes, while lines connecting haplotypes each represent an inferred intermediate separated by a single mutation. The two lines indicated by arrows represent eight inferred mutations.

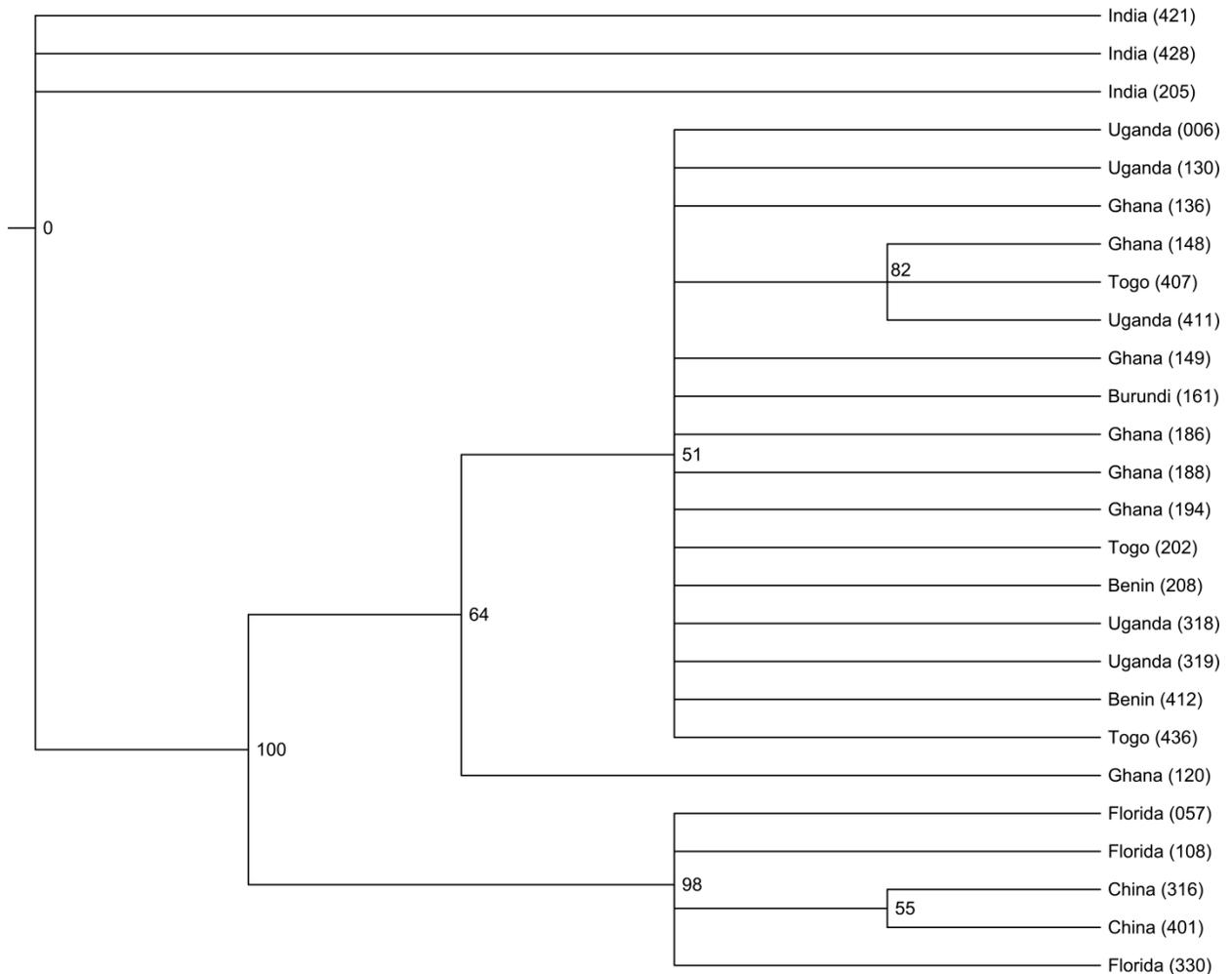


Figure 3-3. Neighbor-joining tree of chloroplast DNA alignments. Bootstrap percentages are given at each node. The country of origin is indicated at the terminus of each branch, along with the corresponding accession number. Sequences from accessions 421, 428, and 205 (all from India) were designated as an outgroup for this analysis.

Table 3-2. Microsatellite genotypes of *D. bulbifera* at loci *Da1F08* and *Da1A01*. Two alleles are listed for each locus by size of fragment in base pairs, and suspected null alleles are noted with zeros. The number of locations where the genotype was found, listed in the second column, represent unique geositions only, after the convention explained in Figure 3-1. If a genotype was unique to a single accession, then the accession number is noted parenthetically in the first column.

Number of Accessions with Genotype	Locations Found	Locus <i>Da1F08</i>		Locus <i>Da1A01</i>	
95	Florida (93), Puerto Rico (1), China (1)	152	154	205	205
1 (408)	China (1)	152	152	207	207
6	Uganda (4), Togo (2)	152	152	198	198
1 (186)	Ghana (1)	152	152	198	211
11	Benin (3), Ghana (6), Burundi (1), Togo (1)	152	152	0	0

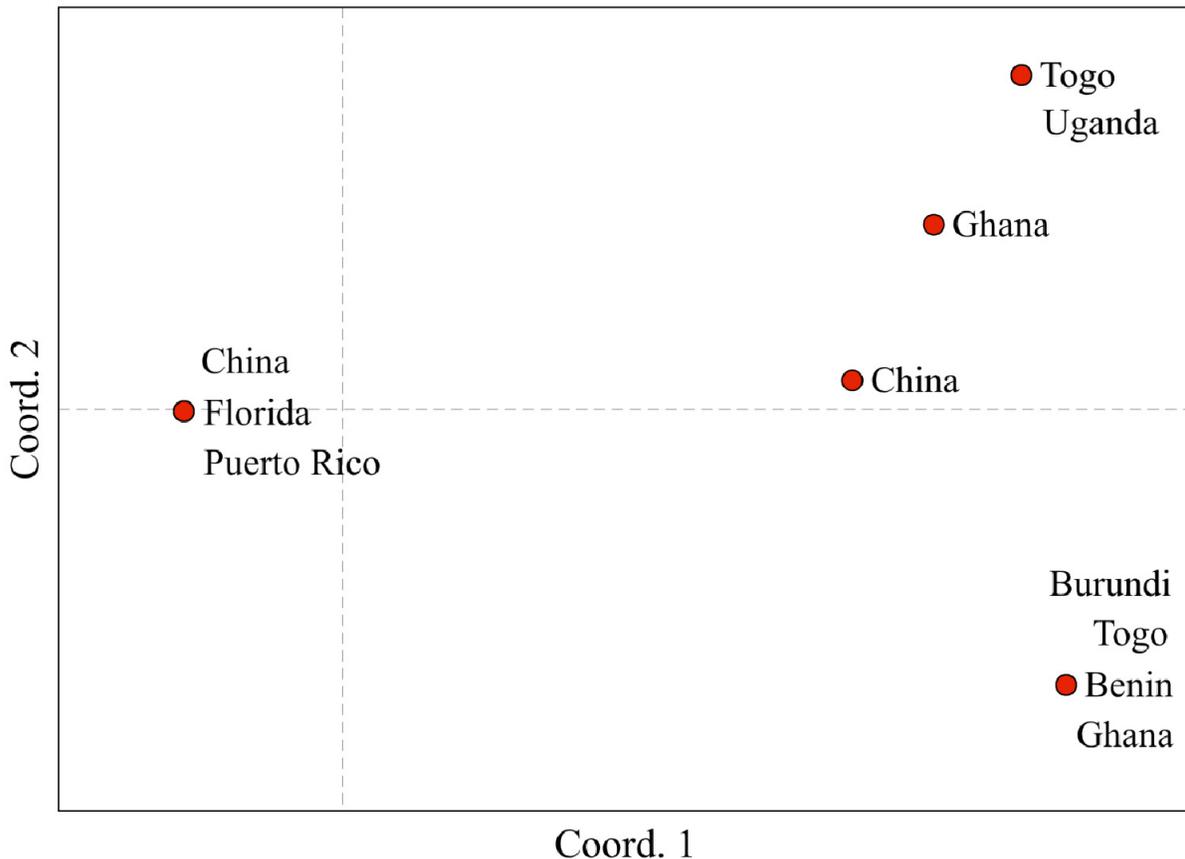


Figure 3-4. Principal coordinate analysis of codominant microsatellite markers *Da1A01* and *Da1F08* in *D. bulbifera*. Each point is labeled with the name that corresponds to the places where the samples were collected.

## CHAPTER 4 DISCUSSION AND FUTURE WORK

### **Interpretation of Results**

Both analyses showed that genetic variability is low in Africa. Low genetic diversity in Africa, with no trend between genotype/haplotype and cultivation status is somewhat surprising because these results represent the largest genetic survey (in geographic breadth and number of accessions) of *D. bulbifera* on this continent. West Africa, where sampling was most intensive, has been identified as the primary center for yam culture and cultivation. Ayensu and Coursey (1972) maintained that, “It is only in certain parts of West Africa that the yam is, or appears ever to have been, of importance as a food crop.” However, in support of low African diversity, no male flowers of *D. bulbifera* were confirmed from a survey of South-Central Africa (female specimens were found from Zambia, Zimbabwe, Malawi and Mozambique). Wilkin has suggested that it may not be native there, and also raises the question of how female plants in the region are able to set fruit (Wilkin, 2001). Also, during the course of extensive travels in Africa, Overholt reports seeing no fruit on *D. bulbifera* (William Overholt, personal communication, 2009). Although selection occurs during the process of cultivation, African yam cultivation is characterized by vegetative propagation, and the bulbils of *D. bulbifera* are optimized for this method of regeneration (Coursey, 1967).

Because there was no genetic diversity detected among the Florida accessions tested, I infer that low diversity is the result of a population bottleneck during the establishment period of *D. bulbifera* into Florida, and indicates that very low propagule pressure may have been necessary for establishment. No intraspecific variation was observed within Florida, suggesting that clonal reproduction and vegetative spread have distributed the species throughout Florida. Ecologists have often noted how very narrow population bottlenecks do not seem to adversely

affect the establishment of invasive species. One such study reports that 66% of the most invasive class of weeds in China reproduce clonally (Liu *et al.*, 2006). Given the results of this genetic analysis, and the scarcity of evidence for sexual reproduction among Florida *D. bulbifera*, it is reasonable to assert that multiple introductions from unique source populations did not play a role in the establishment of *D. bulbifera*. Rather, it would seem that clonal propagation of the invasive genotype was sufficient to avoid genetic barriers to establishment.

### **Varietal Classification in *D. bulbifera* L.**

Ten varieties of *D. bulbifera* were described by Prain and Burkill (1936), but are taxonomically insignificant or even invalid according to others (Al-Shehbaz & Schubert, 1989; Zhizun & Gilbert, 2000). In another case, Miège (*via* Terauchi *et al.*, 1991) named 12 varieties in Ivory Coast alone. Varietal designations continue to be amended (Yifeng *et al.*, 2008), underscoring the need for a better understanding of the intraspecific taxonomy of this yam. Phylogenetic treatments of *Dioscorea* have elucidated some of the relationships between yams (Wilkin *et al.*, 2005), and marker-based genetic diversity in *D. bulbifera* has often supported varietal designations, although minor discrepancies have been noted in each study (Zheng *et al.*, 2006; Terauchi *et al.*, 1991; Ramsler *et al.*, 1996).

The ten varieties of *D. bulbifera*, first treated by Knuth in ‘*Das Pflanzenreich*’ (*non-vid.*, 1924), were later reiterated with very minor modifications by Prain and Burkill (1936). Varieties mentioned in recent treatments that are not duplicated by Prain and Burkill's nomenclature include vars. *bulbifera*, *ramiflorus*, *albitubera*, and *pauciflorum*. These names are mentioned in a Chinese publication, and the proceedings from the conference in which it appears has only an abstract available (Proceedings of the 2006 Systematic & Evolution Botany Conference in China, p. 27; [http://www.syst-evol.cn/2006/syst-evol\\_ii.pdf](http://www.syst-evol.cn/2006/syst-evol_ii.pdf)). A very recent publication identifying a new variety of *D. bulbifera* (var. *albotuberosa*) from China is potentially relevant,

due to the genetic similarity demonstrated in the present study between samples from China and Florida (Yifeng *et al.*, 2008).

On the basis of existing varietal classifications (*viz.* Prain & Burkill's), African *D. bulbifera* could apparently be excluded as the source population of Florida air potato, because the Florida population lacks the highly angular bulbils that characterize *D. bulbifera* var. *anthropophagorum* (see also: Figure A-1). Hamon also describes the bulbils of wild, West African *D. bulbifera* as polyhedral, and only occasionally round (Hamon *et al.*, 1995); this is consistent with an image of African bulbils (plate 12) presented by Coursey (1967). Hamon does not note the morphology of bulbils on cultivated plants in this region.

It is difficult to apply the key to *D. bulbifera* varieties beyond bulbil morphology; documentation of the underground tuber morphology in Florida is lacking, descriptions of character sizes in the key are vague, and staminate flowers are not found in Florida. Also, Overholt *et al.* (2008) have hypothesized a biannual tuber regeneration phenology that, if proven, would seem to frustrate the diagnostic value of tuber characters (Prain & Burkill, 1936).

With the exception of some observations about differences between greatly divergent populations (*i.e.* Africa/Asia), I interpret the varietal key of Prain and Burkill as equivocal for determining the source population, or even the variety of Florida *D. bulbifera*. Rarity, ambiguity, or outright lack of these descriptive characters in the Florida population frustrates diagnosis using the key. When compared to the very recent key to the varieties of *D. bulbifera* in China, similar obstacles emerge, such as reliance on male flowers to differentiate some varieties (Yifeng *et al.*, 2008). I am inclined to agree with Prain and Burkill's (1936) assessment that *D. bulbifera*, herbarium material "rarely suffices for distinguishing varieties." However, even morphometric examination of wild plants may be unproductive for distinguishing among varieties. A

multivariate phenetic study on the variable, compound-leaved *D. quartiniana* evidenced continuous variation, providing no statistical support for intraspecific divisions, despite longstanding assertions of their validity (Wilkin, 1999).

No genetic study to date has thoroughly studied the genetic differentiation between East and West African *D. bulbifera*, so it is difficult to compare the amount of diversity found in the present study to the divergence between the African samples observed in previous works that examined only East African locations. Additional sampling of *D. bulbifera* in Asia will be necessary in order to determine if a more similar genotype to the Florida accessions can be found, and also to define the locations in Asia where this genotype is present. Genetic diversity of African *D. bulbifera* from this study reinforces the high degree of intraspecific divergence from Asian *D. bulbifera* formerly reported. The small number of Asian *D. bulbifera* accessions obtained for this study (n=3) do not provide enough context to make detailed comparisons of genetic diversity in this region with other studies that sampled this area much more thoroughly.

### **Biocontrol Implications**

The USDA/Agricultural Research Service (ARS) Invasive Plant Research Laboratory recently submitted a petition to the USDA/Animal and Plant Health Inspection Service (APHIS) Technical Advisory Group (TAG) for field release of *Lilioceris* sp. near *impressa* (Coleoptera: Chrysomelidae), a herbivore of *D. bulbifera* in Nepal ([http://www.aphis.usda.gov/plant\\_health/permits/tag/petitions.shtml](http://www.aphis.usda.gov/plant_health/permits/tag/petitions.shtml)). Results from the present study suggest that these beetles could have a higher likelihood of maintaining host specificity to the invasive population in Florida than do potential candidates from Africa. Host switching is of some concern, as the two native Florida yams, *D. floridana* and *D. villosa*, are likely to be the alternates, if such a switch ever occurred. Of less concern, due to their geographic isolation from

Florida over water, are members of the West Indies endemic genus, *Rajania*, soon to be subsumed in *Dioscorea* L. (Wheeler *et al.*, 2007).

The EICA hypothesis may not be relevant for *D. bulbifera* because reproduction within the invasive range is thought to be entirely vegetative, so that novel genetic configurations conferring a selective advantage remain in the somatic line and are not exchanged via sexual reproduction. Despite the apparently clonal nature of *D. bulbifera* in Florida, somatic mutations conferring herbicide resistance or other attributes associated with increased competitiveness could arise, as observed in other weed species that reproduce vegetatively, such as hydrilla (Michel *et al.*, 2004). Because I have established the occurrence of multiple introductions, likely of genetically distinct *D. bulbifera*, there may have been a period of selection between genotypes during the lag phase following introduction. A genetic bottleneck, due to selection between genotypes, would have reduced the genetic diversity below the level present at initial introductions. *D. bulbifera*'s success in Florida may be attributable to release from natural enemies, so finding suitable biological control agents may help reduce the negative ecological impacts of this invasive vine.

### **Future Needs and Concluding Thoughts**

Historical research into the extent and sources of intentional introductions of *D. bulbifera* in Florida will provide necessary context to the genetic work used to further characterize the geography of the source population. In addition, some of the important varietal keys and genetic studies of Asian *D. bulbifera* are not yet translated from Chinese into English; an international collaboration with Chinese researchers may be extremely productive for procuring both plant material and access to updated varietal treatments.

In conclusion, this collection of studies has documented geographically diverse introductions of *D. bulbifera* to Florida, synthesized the historical and taxonomic diversity

relevant to its identification and invasiveness in Florida ecosystems, and used a pair of genetic methodologies to overturn the paradigm that invasive *D. bulbifera* in Florida is an African introduction. Despite convincing evidence from other sources that the African variety may be differentiated from all others on the basis of bulbil morphology, extensive sampling there was worthwhile for a number of reasons. Historical data and noted yam experts have asserted the African origin of Florida *D. bulbifera*, introduction records established their import from West Africa, preliminary results suggested an African source (Overholt & Hughes, 2004), and the genetic diversity of West African *D. bulbifera* was unknown. This study also establishes that Florida *D. bulbifera* has a low level of genetic diversity: a finding that contributes to understanding the invasion dynamics of weeds, and can be applied in efforts to find and import effective biocontrols from the Asia/Pacific region.

APPENDIX A  
SUPPLEMENTAL DATA

Table A-1. Accession Data and Summary of Results. Coordinates are given in decimal degrees. Dashes indicate that data was not obtained, and the haplotype column heading is abbreviated. Italicized accessions indicate that the yam species examined was not *D. bulbifera*.

#	Date	Latitude	Longitude	Locality, County	Nation, State	Bulbil Type	<i>Da1F08</i>	<i>Da1A01</i>	Hap.	Status		
208	14-May-07	7.855833	1.980556	Savalou	Benin	–	152	152	F	Wild		
412	12-May-07	9.663333	1.697222	Serou	Benin	–	152	152	E	Wild		
446	07-May-07	9.661667	1.696944	Serou	Benin	–	152	152	–	Wild		
161	06-Apr-07	-4.09835	29.50636	Kigwena Forest	Burundi	–	152	152	F	Wild		
401	27-Jun-07	23.212917	113.421556		China, Guangdong	–	152	154	205	205	C	Wild
408	Mid-2007	24.100267	113.219414	Yingde City	China, Guangdong	–	152	152	207	207	–	Wild
316	06-Jul-04	21.894586	101.027001	Menglun	Yunnan	–	–	–	–	–	B	–
120	Sept. 2007	9.801	-1.97	Tuna	Ghana	–	152	152			D	Cult.
136	Sept. 2007	9.346	-0.823	Tamale	Ghana	–	–	–	–	–	F	Cult.
148	Sept. 2007	6.107	-2.2786	Nkurakan	Ghana	–	152	152			G	Cult.
149	Sept. 2007	6.65194	-1.89803	Anyinamso	Ghana	–	152	152			F	Wild
186	Sept. 2007	6.94795	-2.08802	Ayinasu	Ghana	–	152	152	198	211	F	Wild
188	Sept. 2007	6.7834	-1.802	Mfensi	Ghana	–	152	152			F	Wild
194	Sept. 2007	6.53294	-1.66884	Pakyi No.1	Ghana	–	152	152			F	Wild
198	Sept. 2007	7.63116	-0.13327	Adumadum	Ghana	–	152	152			–	Cult.
202	26-May-07	6.878055	0.651389	Tove	Togo	–	152	152	198	198	F	Wild
407	24-May-07	6.973611	0.595833	Kuma Adame	Togo	–	152	152			G	Wild
436	23-May-07	6.950834	0.595556	Misahohe	Togo	–	152	152	198	198	F	Wild
006	01-Feb-04	0.399123	33.01079	Mabira Forest	Uganda	W/D	152	152	198	198	F	Wild
130	28-Sep-07	0.39787	33.01608	Mabira Forest	Uganda	–	152	152	198	198	F	Wild
317	07-Sep-06	0.39895	33.01087	Mabira Forest	Uganda	–	152	152	198	198	–	Wild
318	07-Sep-06	0.39895	33.01087	Mabira Forest	Uganda	–	152	152	198	198	F	Wild
319	07-Sep-06	0.39895	33.01087	Mabira Forest	Uganda	–	152	152	198	198	F	Wild

Table A-1. Continued

#	Date	Latitude	Longitude	Locality, County	Nation, State	Bulbil Type	<i>DaIF08</i>	<i>DaIA01</i>	Hap.	Status		
411	28-Sep-07	0.39729	33.01719	Mabira Forest	Uganda	–	152	152	198	198	G	Wild
001	05-Aug-06	27.00324	-82.10045	Charlotte	USA, FL	W/D	152	154	205	205	–	–
002	11-Aug-06	27.37555	-80.83504	Okeechobee	USA, FL	W/D	152	154	205	205	–	–
003	11-Aug-06	27.24741	-80.84855	Okeechobee	USA, FL	–	152	154	205	205	–	–
005	18-Aug-06	28.28339	-81.45721	Osceola	USA, FL	W/D	152	154	205	205	–	–
007	05-Aug-06	26.95124	-81.99168	Charlotte	USA, FL	W/D	152	154	205	205	–	–
009	18-Aug-06	27.654417	-80.360833	Indian River	USA, FL	–	152	154	205	205	–	–
011	17-Aug-06	28.882972	-82.527072	Citrus	USA, FL	–	152	154	205	205	–	–
014	18-Aug-06	27.807194	-80.482306	Indian River	USA, FL	–	152	154	205	205	–	–
016	05-Aug-06	26.95369	-82.14495	Charlotte	USA, FL	W/D	152	154	205	205	–	–
017	17-Aug-06	28.738219	-82.520722	Citrus	USA, FL	–	152	154	205	205	–	–
018	19-Aug-06	28.28344	-81.45726	Osceola	USA, FL	W/D	152	154	205	205	–	–
021	05-Aug-06	26.97425	-82.14115	Charlotte	USA, FL	W/D			205	205	–	–
022	05-Aug-06	26.99754	-82.15312	Charlotte	USA, FL	W/D	152	154	205	205	–	–
023	11-Aug-06	27.2365	-80.81324	Okeechobee	USA, FL	W/D	152	154	205	205	–	–
024	03-Aug-06	26.915523	-82.031814	Charlotte	USA, FL	W/D	152	154	205	205	–	–
025	29-Aug-06	28.28741	-81.41007	Osceola	USA, FL	G/W	152	154	205	205	–	–
030	18-Aug-06	27.799167	-80.497972	Indian River	USA, FL	–	152	154	205	205	–	–
031	11-Aug-06	27.24864	-80.82101	Okeechobee	USA, FL	–	152	154	205	205	–	–
032	29-Aug-06	28.31311	-81.40195	Osceola	USA, FL	–	152	154	205	205	–	–
033	05-Aug-06	27.00748	-82.09835	Charlotte	USA, FL	W/D	152	154	205	205	–	–
035	29-Aug-06	28.28344	-81.45726	Osceola	USA, FL	–	152	154	205	205	–	–
036	11-Aug-06	27.25012	-80.83302	Okeechobee	USA, FL	W/D	152	154	205	205	–	–
038	11-Aug-06	27.32457	-81.02231	Okeechobee	USA, FL	S/G	152	154	205	205	–	–
043	11-Aug-06	27.24803	-80.80004	Okeechobee	USA, FL	S/G	152	154	205	205	–	–
049	18-Aug-06	27.759694	-80.484833	Indian River	USA, FL	–	152	154	205	205	–	–
051	10-Aug-06	26.99566	-81.0781	Glades	USA, FL	W/D	152	154	205	205	–	–
052	08-Aug-06	28.495436	-81.339258	Orange	USA, FL	S/G	152	154	205	205	–	–

Table A-1. Continued

#	Date	Latitude	Longitude	Locality, County	Nation, State	Bulbil Type	<i>DaIF08</i>	<i>DaIA01</i>	Hap.	Status
054	17-Aug-06	28.857861	-82.273992	Citrus	USA, FL	–	152 154	205 205	–	–
058	10-Aug-06	26.7564	-81.4388	Hendry	USA, FL	W/D	152 154	205 205	–	–
059	11-Aug-06	27.42936	-80.3847	St. Lucie	USA, FL	S/G	152 154	205 205	–	–
066	10-Aug-06	26.75637	-81.4412	Hendry	USA, FL	W/D	152 154	205 205	–	–
069	10-Nov-06	25.73913	-80.29503	Miami-Dade	USA, FL	W/D	152 154	205 205	–	–
070	10-Aug-06	26.99543	-81.0783	Okeechobee	USA, FL	W/D	152 154	205 205	–	–
072	11-Aug-06	27.40737	-80.3759	St. Lucie	USA, FL	W/D	152 154	205 205	–	–
073	01-Aug-06	29.5461	-82.3211	Alachua	USA, FL	W/D	152 154	205 205	–	–
074	11-Aug-06	27.38436	-80.3385	St. Lucie	USA, FL	W/D		205 205	–	–
077	15-Oct-06	25.72277	-80.28062	Miami-Dade	USA, FL	W/D	152 154	205 205	–	–
080	12-Aug-06	27.47337	-80.3692	St. Lucie	USA, FL	W/D	152 154	205 205	–	–
082	13-Aug-06	26.920383	-80.185125	Palm Beach	USA, FL	–	152 154	205 205	–	–
085	11-Aug-06	27.58733	-80.3693	Indian River	USA, FL	S/G	152 154	205 205	–	–
089	10-Aug-06	27.2465	-80.8039	Okeechobee	USA, FL	W/D	152 154	205 205	–	–
090	10-Aug-06	26.76524	-81.4348	Hendry	USA, FL	W/D	152 154	205 205	–	–
093	09-Aug-06	28.585247	-81.360867	Orange	USA, FL	W/D	152 154	205 205	–	–
095	17-Aug-06	28.429269	-81.349947	Orange	USA, FL	W/D	152 154	205 205	–	–
098	10-Nov-06	25.70318	-80.30293	Miami-Dade	USA, FL	W/D	152 154	205 205	–	–
099	18-Aug-06	24.559692	-81.795211	Monroe	USA, FL	W/D	152 154	205 205	–	–
101	03-Oct-06	30.314906	-81.655419	Duval	USA, FL	–	152 154	205 205	–	–
102	10-Aug-06	27.24621	-80.804	Okeechobee	USA, FL	W/D	152 154	205 205	–	–
104	10-Aug-06	28.535344	-81.363708	Orange	USA, FL	W/D	152 154	205 205	–	–
105	22-Jul-06	28.03248	-81.9395	Polk	USA, FL	–	152 154	205 205	–	–
107	12-Aug-06	28.012781	-82.471131	Hillsborough	USA, FL	–	152 154	205 205	–	–
108	09-Aug-06	27.76258	-82.14865	Hillsborough	USA, FL	–	152 154	205 205	A	–
109	14-Oct-06	30.095947	-83.58395	Taylor	USA, FL	S/G	152 154	205 205	–	–
110	14-Oct-06	30.233108	-84.233264	Wakulla	USA, FL	S/G	152 154	205 205	–	–
114	05-Oct-06	30.290286	-81.727608	Duval	USA, FL	–	152 154	205 205	–	–

Table A-1. Continued

#	Date	Latitude	Longitude	Locality, County	Nation, State	Bulbil Type	<i>DaIF08</i>	<i>DaIA01</i>	Hap.	Status
116	14-Oct-06	29.653953	-82.523161	Alachua	USA, FL	S/G	152 154	205 205	–	–
117	14-Oct-06	30.095947	-83.58395	Taylor	USA, FL	S/G	152 154	205 205	–	–
126	27-Sep-06	30.295189	-81.768047	Duval	USA, FL	–	152 154	205 205	–	–
127	05-Nov-06	27.99127	-82.15105	Hillsborough	USA, FL	S/G	152 154	205 205	–	–
131	22-Nov-06	29.647069	-82.361469	Alachua	USA, FL	S/G	152 154	205 205	–	–
137	09-Nov-06	27.42825	-81.41372	Highlands	USA, FL	W/D	152 154	205 205	–	–
140	24-Nov-06	28.13878	-82.02323	Polk	USA, FL	S/G	152 154	205 205	–	–
141	09-Nov-06	27.27438	-82.26928	Sarasota	USA, FL	W/D	152 154	205 205	–	–
145	2006/2007	27.0675	-80.31985	Martin	USA, FL	W/D		205 205	–	–
146	11-Nov-06	28.07032	-82.36972	Hillsborough	USA, FL	–	152 154	205 205	–	–
150	09-Nov-06	27.25386	-81.34084	Highlands	USA, FL	S/G	152 154	205 205	–	–
164	24-Nov-06	28.0922	-82.00665	Polk	USA, FL	S/G	152 154	205 205	–	–
165	11-Nov-06	28.05777	-82.29782	Hillsborough	USA, FL	S/G	152 154	205 205	–	–
166	24-Apr-07	–	–	Broward	USA, FL	–	152 154	205 205	–	–
171	11-Nov-06	28.0545	-82.32455	Hillsborough	USA, FL	S/G	152 154	205 205	–	–
180	24-Nov-06	28.09173	-81.99553	Polk	USA, FL	S/G	152 154	205 205	–	–
193	09-Nov-06	27.33471	-82.31184	Sarasota	USA, FL	S/G	152 154	205 205	–	–
321	20-Oct-06	27.22512866	-81.88877737	DeSoto	USA, FL	–	152 154	205 205	–	–
322	20-Oct-06	27.22512866	-81.88877737	DeSoto	USA, FL	–	152 154	205 205	–	–
323	20-Oct-06	27.22512866	-81.88877737	DeSoto	USA, FL	–	152 154	205 205	–	–
325	20-Oct-06	27.2158333	-81.86527778	DeSoto	USA, FL	–	152 154	205 205	–	–
326	20-Oct-06	27.2158333	-81.86527778	DeSoto	USA, FL	–	152 154	205 205	–	–
327	20-Oct-06	27.23611831	-80.98949571	Highlands	USA, FL	–	152 154	205 205	–	–
328	20-Oct-06	27.23611831	-80.98949571	Highlands	USA, FL	–	152 154		–	–
329	20-Oct-06	27.23611831	-80.98949571	Highlands	USA, FL	–	152 154	205 205	–	–
330	20-Oct-06	27.1738	-80.27323	Martin	USA, FL	S/G/W	152 154	205 205	A	–
331	20-Oct-06	27.17631	-80.26935	Martin	USA, FL	S/G/W	152 154	205 205	–	–
332	20-Oct-06	27.16284	-80.21102	Martin	USA, FL	S/G/W	152 154	205 205	–	–

Table A-1. Continued

#	Date	Latitude	Longitude	Locality, County	Nation, State	Bulbil Type	<i>DaIF08</i>	<i>DaIA01</i>	Hap.	Status		
333	22-Sep-06	29.71757	-82.41824	Alachua	USA, FL	W/D	152	154	205	205	–	–
335	26-Oct-06	27.76538	-80.60124	Indian River	USA, FL	G/W	152	154	205	205	–	–
336	26-Oct-06	28.0778	-80.68611	Brevard	USA, FL	W/D	152	154	205	205	–	–
337	26-Oct-06	28.08878	-80.68721	Brevard	USA, FL	W/D	152	154	205	205	–	–
338	26-Oct-06	28.61094	-81.09422	Orange	USA, FL	–	152	154			–	–
339	26-Oct-06	28.6285	-81.12235	Seminole	USA, FL	S/G	152	154	205	205	–	–
341	26-Oct-06	28.87751	-81.31046	Volusia	USA, FL	S/G/W	152	154	205	205	–	–
342	26-Oct-06	29.01163	-81.30248	Volusia	USA, FL	S/G/W	152	154	205	205	–	–
343	26-Oct-06	29.13915	-81.35762	Volusia	USA, FL	S/G/W	152	154	205	205	–	–
344	26-Oct-06	29.30308	-81.48654	Volusia	USA, FL	S/G/W	152	154	205	205	–	–
347	–	28.15408588	-81.97388657	Polk	USA, FL	–	152	154	205	205	–	–
348	–	28.07758927	-81.96321675	Polk	USA, FL	–	152	154	205	205	–	–
349	–	27.93274462	-81.85666331	Polk	USA, FL	–	152	154	205	205	–	–
351	09-Nov-06	27.22252	-81.85878	DeSoto	USA, FL	W/D	152	154	205	205	–	–
151	28-Nov-05	18.332183	-66.717767	Rio Abajo State Forest, Arecibo <i>Kigwena</i>	USA, Puerto Rico	–	152	154	205	205	–	–
138	06-Apr-07	-4.09835	29.50636	<i>Forest</i>	<i>Burundi</i>	–	161	161	211	211	–	<i>Wild</i>
413	Oct. 2007	22.438444	88.4005	<i>Kolkata</i>	<i>India</i>	–	172	174	198	214	–	–
415	Oct. 2007	22.438444	88.4005	<i>Kolkata</i>	<i>India</i>	–	172	174	198	214	–	–
421	Oct. 2007	22.438444	88.4005	<i>Kolkata</i>	<i>India</i>	–	172	174	198	214	–	–
433	Oct. 2007	22.438444	88.4005	<i>Kolkata</i>	<i>India</i>	–	172	174	198	214	–	–
439	Oct. 2007	22.438444	88.4005	<i>Kolkata</i>	<i>India</i>	–	172	174	198	214	–	–
444	Oct. 2007	22.438444	88.4005	<i>Kolkata</i>	<i>India</i>	–	172	174	198	214	–	–
500	Nov. 2007	-21.25	47.45	<i>Ranomafana,</i> <i>Ifanadiana</i>	<i>Madagascar</i>	–	172	174	198	214	–	–
187	23-May-07	6.950556	0.586667	<i>Misahohe</i>	<i>Togo</i>	–			197	197	–	<i>Wild</i>

<i>D. bulbifera</i> variety	Tuber/Bulbils Acrid and nauseous	Bulbils Angular	Tuber				Flowers Male sepals up to 4mm	Leaves			Locations recorded by Prain & Burkill (1936)	Locations recorded from Terauchi <i>et al.</i> , 1991; Ramser <i>et al.</i> , 1996	
			Undeveloped	Large Tuber				Elongated	Elongated	Triangular			Short
				White, smooth; gibbous bulbils	Bulbils dark, warted	Short-stalked; few rootlets							
<i>vera</i>	Yes	No	No				No	No	No	Yes	Malay Peninsula; Australia	Taiwan; Japan	
<i>deltoidea</i>	Yes	No	No				No	No	No	Yes	Hong Kong; Guangdong Province ("Kwang-tung"); Phillipines	–	
<i>heterophylla</i>	Yes	No	No				No	No	Yes		Malay Peninsula	Thailand	
<i>simbha</i>	Yes	No	No				No	Yes			Northern India – Himalayas	–	
<i>elongata</i>	Yes	No	No				Yes				Australia	Papua New Guinea	
<i>kacheo</i>	No	No	No	No	No	Yes					Sikkim Himalayas	–	
<i>suavior</i>	No	No	No	No	Yes						India; Java; Madoera; Australia; Fiji; Samoa; Tahiti	Taiwan; Australia	
<i>birmanica</i>	No	No	No	Yes							Burma (near borders w/ Thailand and China)	–	
<i>sativa</i>	No	No	Yes								Sri Lanka; India; Bangladesh; Japan; Andaman Islands; Singapore; Java; Phillipines	Tonga; Hawaii	
<i>anthropophagorum</i>	No	Yes									Africa	Africa	
Florida	Yes <sup>1</sup>	No <sup>1</sup>	No <sup>2</sup>	?	?	?	?	?	?	No <sup>2</sup>	No <sup>2</sup>		

Figure A-1. Morphological classification of varieties and geographic locations in *D. bulbifera* L. Morphology summarized from the key of Prain and Burkill (1936). <sup>1</sup>(Ward, 1977) <sup>2</sup>(Wheeler *et al.*, 2007)

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## BIOGRAPHICAL SKETCH

A native of Florida, Matthew has been fascinated with trees, and the things that grow on them, since early childhood. A terrific high school science faculty helped grow his wonderment for biology in unique directions, and first exposed him to the world of plant taxonomy. Field exercises in this subject on a *Magnolia virginiana* wetland adjacent to his school first brought him into direct contact with invasive *Dioscorea bulbifera*. During the course of air potato fights in this glade, he learned firsthand about the power and risks of long distance anthropogenic dispersal – lessons not easily replicated in the classroom. He also flung a few good ones himself. These experiences left a lasting (sometimes purplish) impression on him, and he credits them for his enduring fascination with this unique vine.

His mentors at Bryan College, where he earned his B. S. in biology, stoked his interest in biogeography, evolution, and molecular biology. Two internships administered by the Oak Ridge Institute for Science and Education helped him gain hands-on research experience. As a research associate in the DNA core facility of a cancer research center, he gained many of the skills needed to take on projects independently. Looking forward, he hopes to continue studies in biogeography.