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Insights into the introduction histories and population genetic dynamics of the Nile monitor (Varanus niloticus) and Argentine black and white Tegu (Salvator merianae) in Florida.

Jared Price Wood

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By

Jared Price Wood
B.S., Southeastern Oklahoma State University, 2010

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INSIGHTS INTO THE INTRODUCTION HISTORIES OF THE NILE MONITOR (*VARANUS NILOTICUS*) AND ARGENTINE BLACK AND WHITE TEGU (*SALVATOR MERIANAE*) IN FLORIDA VIA NEXT GENERATION SEQUENCING AND POPULATION GENETIC ANALYSIS

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Jared Price Wood
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A Dissertation Approved
18 March 2016

by the following Dissertation Committee

_____
Dissertation Director
Perri Eason

_____
Robert Page

_____
Todd Campbell

_____
Sarah Emery

_____
Gary Cobbs

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ABSTRACT

INSIGHTS INTO THE INTRODUCTION HISTORIES OF THE NILE MONITOR (*VARANUS NILOTICUS*) AND ARGENTINE BLACK AND WHITE TEGU (*SALVATOR MERIANAE*) IN FLORIDA VIA NEXT GENERATION SEQUENCING AND POPULATION GENETIC ANALYSIS

Jared P. Wood

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This dissertation examines the population genetic dynamics of two Florida invasives: the Nile monitor (*Varanus niloticus*) and Argentine black and white tegu (*Salvator merianae*). I also provide insights into the introduction histories of both species. This study was developed as part of a collaborative effort with the Florida Wildlife Commission to expand our knowledge of these highly detrimental, invasive lizards. All research activities involving animals and animal tissues were approved by the University of Louisville's Institutional Animal Care and Use Committee (IACUC Proposal #: 12024).

I start with a brief introduction into what makes invasive species successful from a conservation genetics perspective, and discuss how conservation biologists can use genetic data to manage invasive populations. The dissertation is then divided into four data chapters which are designed to stand as independent manuscripts. Chapters II-III

have been published in *Amphibia-Reptilia*, and Chapter IV has been accepted by the *Journal of Heredity*. Chapters II and III describe how novel microsatellite markers were developed for both species via 454 pyrosequencing. We successfully developed 17 polymorphic loci for *V. niloticus* and 10 polymorphic loci for *S. merianae.*

Chapter IV examines the population structure, degree of connectivity, and introduction history of three invasive *V. niloticus* populations in southern Florida. The results of these analyses demonstrate that all three populations have limited genetic diversity and are highly differentiated from one another. Our results also suggest that these populations resulted from independent introduction events that occurred within the past few decades. We conclude by advising wildlife managers to focus management efforts on containment of existing populations and intensification of monitoring efforts on potential migration corridors.

My final data chapter (V) focuses on the population structure, degree of connectivity between populations, and most likely introduction scenarios of two invasive *S. merianae* populations in Florida. The results of this study also demonstrate that *S. merianae* populations have limited genetic diversity and show significant levels of differentiation. Furthermore, we also found some evidence of migration between populations, and our introduction analyses suggest that both populations originated from an unknown ghost population. We recommend that managers focus on containment rather than eradication strategies, and increase monitoring efforts of the pet trade and potential migration corridors. I conclude this dissertation by summarizing my findings and proposing future directions in which I wish to examine this system further (chapter VI).

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INTRODUCTION

Invasive species are estimated to be second only to human-mediated habitat destruction and alteration as the major cause of global extinctions (Clavero and Garcia-Berthou 2005; Walker and Steffen 1997). In addition to impacting biodiversity, invasive species are also estimated to cost the United States $125 billion in economic damage per year (Pimentel et al. 2000). Thus, it is not surprising that the management and control of invasive species is a top priority for many biologists.

One major question that conservation biologists are concerned with is what makes invasive species successful. The initial introduction of an exotic species, especially in long-distance invasions, usually results from the direct or indirect activities of people (Sakai et al. 2001). One of the earliest documented vertebrate introductions can be dated back to 1538 when European settlers brought European pigs (*Sus scrofa*) to America as a food source (Hardin 2007). Founding populations usually consist of a small number of colonists (Allendorf and Lundquist 2003), and are thought to have much less genetic diversity than the native populations from which they are derived (Barrett and Kohn 1991). This reduction in genetic diversity should reduce the invasive capacity of a newly introduced population, thereby reducing invasion potential (Sakai et al.

2001). Population genetic theory predicts that populations with reduced genetic diversity should be at a disadvantage due to the detrimental effects of inbreeding, drift, and a limited ability to evolve (Fisher 1930). Inbreeding increases the probability that deleterious recessive mutations will be expressed due to increases in homozygous individuals (Lawson Handley et al. 2011). Therefore, inbreeding depression should reduce a population's ability to grow and lower the probability that a population will persist (Nieminen et al. 2001). Furthermore, although introduced species are likely to be pre-adapted to some aspects of new environments, many aspects of the environment may be novel (Sakai et al. 2001). Reduced genetic diversity should reduce the ability of the population to respond to these novel selective pressures (Goodnight 1988). However, despite experiencing reductions in genetic diversity, many introduced species remain successful, and in many cases, even outcompete native species (Allendorf and Lundquist 2003). Thus, conservation geneticists face two paradoxes: first, if population bottlenecks are harmful, why do introduced species remain so successful; second, if local adaption is important, how are introduced species able to outcompete and replace native species (Allendorf and Lundquist 2003)?

In recent years, increased attention has been placed on solving the invasion paradox. Multiple introductions have been proposed as one mechanism by which introduced species overcome the effects of limited genetic diversity. Multiple introductions are common in invasions (Novak and Mack 2005), and intraspecific hybridization (i.e. admixture) is capable of producing large amounts

of variation and novel genotypes (Facon et al. 2005). These novel genotypes may allow admixed individuals to outcompete their parental genotypes (Facon et al. 2005). Several recent studies have reported that admixture stemming from multiple introductions may be driving invasion success (Facon et al. 2005; Kolbe et al. 2008; Lavergne and Molofsky 2007).

A weak link between losses in molecular variation and losses in adaptive evolutionary potential may also play a role in explaining the invasion paradox. Although most studies examining the population genetics of invasive populations have looked at reductions in molecular diversity, Reed and Frankham (2001) found only a weak correlation between molecular genetic diversity and quantitative genetic diversity, which is more closely linked to traits associated with fitness. One reason for this weak link is due to the differential forces of selection and drift (Reed and Frankham 2001). Molecular genetic markers are generally neutral and dominant or epistatic and are therefore insensitive to the forces of selection (Dlugosch and Parker 2008). Thus, populations that have recently gone through a bottleneck may maintain levels of quantitative genetic diversity sufficient for local adaptation despite experiencing reductions in molecular genetic diversity due to drift (Reed and Frankham 2003). Furthermore, additive variation may even increase after a bottleneck due to frequency shifts at loci with nonadditive variation (Cheverud and Routman 1996; Turelli and Barton 2006; Willi et al. 2006). Finally, inbred populations may actually benefit from increases in adaptive potential, because neutral or deleterious alleles are most likely to be lost in small populations (Kimura 1983; Reed and Frankham 2003).

Lag times are a common phenomenon associated with invasions that occur between colonization and subsequent rapid increases in population growth (Kowarik 1995). These lag times are expected if evolutionary changes are an important component of the invasion process (Sakai et al. 2001). It is hypothesized that these periods allow for admixture to occur, new traits to evolve that increase invasive potential, and deleterious alleles to be purged (Sakai et al. 2001). Thus, it may be prudent for managers to target isolated introductions for eradication before they have the chance to adapt to their novel environments (Dlugosch and Parker 2008).

In addition to helping conservation biologists better understand what makes invasive species so successful, conservation genetics can also serve more of an applied role in the control and eradication of invasives. Eradication efforts are costly, both in terms of monetary costs and time. Due to these costs, it is crucial for managers to place considerable effort into plans that maximize eradication success (Myers et al. 2000). For example, attempting to eradicate only a fraction of a population, or a sink population within a source-sink metapopulation, would result in rapid recolonization and a waste of resources (Hanski 1999). Although neutral genetic markers, such as microsatellites, may only provide limited information about adaptive potential, these markers are a valuable means of identifying population structure and can be indicative of the degree of connectivity between spatially isolated populations (Robertson and Gemmell 2004). Significant levels of genetic differentiation are indicative of limited dispersal, while negligible genetic differentiation indicates that adjacent

populations are highly connected (Robertson and Gemmell 2004). The identification of distinct population units can assist eradication attempts by focusing efforts on identifying units with negligible immigration (Abdelkrim et al. 2005). If no genetically isolated units exist, then it may be necessary to eradicate clusters of populations at one time (Abdelkrim et al 2005), or limiting further growth and expansion may be more logistically feasible than complete eradication.

The State of Florida has been heavily impacted by the introduction of exotic species over the last few decades. The invasion of reptiles and amphibians in Florida has recently been described as "aggressive" and "a runaway train" (Engeman et al. 2011; Krysko et al. 2011). Southern Florida is particularly susceptible to invasion by reptiles because it has a subtropical climate, a highly altered natural environment that provides suitable habitat for invasive species, and a robust exotic industry (Pernas et al. 2012). Thus, it is not surprising that Florida has more nonnative species than any other U.S state (Butterfield et al. 1997).

Two nonnative lizard species of particular concern in Florida are the Nile monitor (*Varanus niloticus*) and Argentine black and white tegu (*Salvator merianae*). Both species were most likely introduced to Florida via the exotic pet trade, as they both could be readily found for inexpensive prices at most pet stores (Hardin 2007). However, both species grow to large sizes and have ill temperaments, and are often released by inexperienced pet owners once they become too difficult to care for (Enge et al. 2004). It is also believed that

breeders release individuals that have lost tails or incurred other injuries that reduced their resale value, or to start their own breeding stocks (Enge et al. 2004; Pernas et al. 2012). *V. niloticus* was first documented in the City of Cape Coral in 1990, and new breeding populations are now established in West Palm Beach and Homestead, Florida (Enge et al. 2004). *Salvator meriane* was first observed in Hillsborough County in 2006 (Hardin 2007). Another breeding population is currently established in southern Miami-Dade County (Pernas et al. 2012). Both of these species are generalist predators that have the potential to impact Florida's native species, including several sensitive species like the burrowing owl (*Athene cunicularia*), gopher tortoise (*Gopherus polyphemus*), and American crocodile (*Crocodylus acutus*) (Enge et al. 2004; Mazzotti et al. 2015).

Although the most likely introduction pathway for both species is the exotic pet trade, the population structure and degree of connectivity between regions in Florida is currently unknown. As discussed above, this information can be beneficial for managers seeking to develop efficient and cost-effective eradication or containment strategies, especially since the Florida Wildlife Commission is limited by a lack of funding and personnel (Hardin 2007).

In my dissertation, I develop the genetic resources (microsatellites) needed to analyze the genetic structure of both *V. niloticus* and *S. meriane* populations in Florida. In addition, I also investigate the degree of connectivity between populations for both species, and infer the most likely introduction scenarios. Finally, I use my results to make recommendations for management strategies aimed at eradication or containment.

CHAPTER II

CHARACTERIZATON OF 17 NOVEL MICROSATELLEITE LOCI IN THE NILE MONITORS (*VARANUS NILOTICUS*) VIA 454 PYROSEQUENCING

Introduction

Invasive species are one of the greatest threats to global biodiversity (Wilcove et al. 1998). Currently, the US state of Florida is home to more introduced species of herpetofauna than any other place on Earth—a fact that is largely due to Florida's subtropical climate and thriving exotic pet industry (Smith and Krysko 2007). Of the introduced herpetofauna in Florida, the Nile monitor (*Varanus niloticus*) is among those with considerable invasive potential. Native to Africa (Luxmoore et al. 1988), *V. niloticus* is believed to have been introduced to Cape Coral, Florida circa 1990 via the pet trade (Enge et al. 2004) and is still popular in the exotic pet industry due to its large size (up to 2.43 m total length and 8.1 kg body mass; Faust 2001; Faust and Bayless 1996) and inexpensive retail price (Enge et al. 2004). Of particular concern is that captive Nile monitors are frequently released when they outgrow their juvenile enclosures and/or become expensive to feed (Enge et al. 2004). Once released, *V. niloticus* poses a direct threat to Florida's sensitive, endemic fossorial wildlife because it is a highly mobile generalist predator with strong burrowing capabilities (Enge et al. 2004).

Since their initial introduction to Cape Coral, new populations have been established approximately 185 km and 200 km away in the cities of West Palm Beach and Homestead, respectively (Engeman et al. 2011; Jennifer Ketterlin Eckles, personal communication). To prevent further spread of *V. niloticus* throughout Florida, it is essential for managers to know if these more recently established populations are the result of dispersal or the consequence of secondary human-mediated introductions. Although the answers to these questions are currently unknown, microsatellites provide a cost-effective method for estimating levels of population differentiation and connectivity (Selkoe and Toonen 2006). To facilitate such endeavors, we developed 17 novel microsatellite markers from *V. niloticus* that will be used to identify how many genetically distinct groups of *V. niloticus* are in southern Florida.

Methods

DNA from a single *V. niloticus* captured in Cape Coral, Florida, USA (26°35'34.70"N, 82° 0'33.72"W) was submitted to the University of Georgia Genomics Facility (GGF), where this isolate was pooled with DNA from two other species that were differentiated by terminal barcodes (Meyer et al. 2007). Genomic DNA was obtained from muscle tissue using the Wizard Genomic DNA Purification Kit (Promega) according to the manufacturer's instructions. A library of single stranded template DNA fragments was then produced using the GS FLX Titanium General Library Preparation Kit (Roche). Initial sequencing employed the 454 GS FLX Titanium Sequencing Kit XLR70 (Roche) run on 25%

of a 70 x 75 mm picotiter plate and additional sequencing employed the 454 GS FLX Titanium Sequencing Kit XL+ (Roche) run on 50% of a 70 x 75 mm picotiter plate. The GGF also performed basic data processing, such as base calling and filtering.

These sequencing efforts yielded a total of 43,306,932 bp across 101,489 reads. Of these reads, 30,254 were generated using the XLR70 kit (mean length = 298.3 bp, std. dev. = 150.8 bp) and 71,235 were generated using the XL+ kit (mean length = 481.2 bp, std. dev. = 186.2 bp). MSATCOMMANDER 0.8.2 (Faircloth, 2008) was used to scan these pyrosequencing reads for dinucleotide microsatellites with ≥ eight tandem repeats and tri-pentanucleotide microsatellites with ≥ six tandem repeats. In total, MSATCOMMANDER identified 1040 presumptively non-redundant potentially amplifiable loci. We then used the PRIMER3 interface available through MSATCOMMANDER (Rozen and Skaletsky 2000) to design primers via batch processing of repeat containing 454 fragments.

Twelve dinucleotide, four trinucleotide, and four tetranucleotide loci whose corresponding 454 fragments contained at least ten, nine, and seven tandem repeats respectively were selected for marker development. An M13 (-21) sequence was fused to the 5′ end of either the forward or reverse primer of each primer pair in order to facilitate fluorescent labeling with 6-FAM via the nested PCR approach described by Schuelke (2000). These 20 loci were then screened for polymorphism and scoring reliability using DNA isolated from muscle tissue of 11 individuals sampled from Cape Coral, Florida. All reactions

had a final volume of 25 µl and contained 20-200 ng of template, 1x GoTaq colorless flexi buffer, 1.5 mM MgCl2, 0.2 mM of each dNTP, 0.8 µM of non-M13(-21)-twinned primer, 0.8 µM of 6-FAM labeled M13(-21) primer, 0.2 µM of M13(-21)-twinned primer, and 0.625 units of GoTaq polymerase (Promega). Reaction conditions were as follows: 2 min at 94° C followed by 25 cycles of (1) 94° C for 30 s, (2) 62° C for 30 s decreasing by 0.3° C per cycle, and (3) 72° C for 40 s, followed by eight cycles of (1) 94° C for 30 s, (2) 53° C for 30 s, and (3) 72° C for 40 s, followed by a final step of 30 min at 72° C.

Genotyping reaction products were visually inspected via agarose gel electrophoresis and products from successful reactions were shipped to the Arizona State University DNA lab, where fragment analysis was performed using an Applied Biosystems 3730 DNA Analyzer. Of the 20 loci that were screened, 17 were polymorphic and straightforward to score. Thus, we genotyped additional individuals at these 17 loci for a total of 40 individuals from Cape Coral. The locus-specific primers, melting temperatures, and summary statistics based on 40 *V. niloticus* genotypes are presented in Table 1. All loci were scored manually using PEAK SCANNER 1.0 (Applied Biosystems). Allelic bins were determined by graphically examining the rank-ordered fragment size distributions of each locus, so that we could identify breaks in the amplicon sizes (Guichoux et al. 2011). We then wrote functions in Microsoft EXCEL to bin the data from each locus into discrete classes that were defined by each allele's empirically determined size range.

Table 1. Characterization of 17 microsatellite loci developed for *Varanus niloticus*. Samples collected from Cape Coral, Florida, USA.

Locus	Repeat (number)	Primer Sequence (5′- 3′)	Size Range (bp)	T_M (°C)	*k*	*N*	H_O	H_E	F_{IS}	No. Effective Alleles	*M*	GenBank Accession No.
Mon1	AC(11)	F: GGCAGGATGGTTGGTTTCC*	294-316	59	3	33	0.73	0.59	-0.22	2.4	0.50	KT591094
		R: CAGTCCCAGGGCCATTAGG		60								
Mon2	AC(12)	F: TGTTTCTGACTGGATCTGGC	150-174	58	3	38	0.42	0.42	0.01	1.7	1.00	KT591095
		R: CCAACCATGCCTAAGCCTC*		59								
Mon3	GT(12)	F: TGATTCCAACATTGCTCTTCTAGG*	43-67	60	2	33	0.42	0.37	-0.14	1.6	0.40	KT591096
		R: CTTGCCTGGCCACTGTTTC		60								
Mon4	GT(11)	F: CCTTTCAGCCAAAGGGTAGC*	83-105	60	2	40	0.48	0.45	-0.05	1.8	0.67	KT591097
		R: CTGCCAAGAAATAGGGCTGTC		60								
Mon6	AG(11)	F: GTTCTTGAATATTGTTCCCTGTCC*	257-279	59	1	40	0.00	0.00	N/A	1.0	N/A	KT591098
		R: TTTCAAGCCAAGGTATCAAGTG		58								
Mon8	AC(10)	F: ACTTAGAATGCCCGTTCAGC	111-131	59	3	37	0.68	0.58	-0.16	2.4	0.38	KT591099
		R: GCATCTTTCTTAAATCTTGGTGCC*		60								
Mon9	GT(10)	F: GCTGGTGAAATGGTGCAGG*	162-182	60	3	39	0.67	0.56	-0.18	2.3	1.00	KT591100
		R: AGGGCTCACAGGGTCAAAG		60								
Mon10	CT(10)	F: CAACATCGAACTCGCTGGG	266-286	60	2	39	0.18	0.20	0.13	1.3	0.67	KT591101
		R: TCCCTACAGGTTGCTCAGG*		59								
Mon12	GT(10)	F: AGCCTGGAGGAAGGTTGTC	198-218	60	4	35	0.69	0.69	0.01	3.2	0.67	KT591102
		R: AGCCTTTACAGAGGGCTCC*		59								

Mon13	GGT(9)	F: CCCGGCTCAGTATATCAGGG	294-321	60	2	35	0.29	0.28	0.01	1.4	0.33	KT591103
		R: CTTCATCCTGTGCCCGTTTC*		60								
Mon14	ATC(9)	F: TTGCCAACCTTCTGGCTTG	126-153	59	3	36	0.56	0.57	0.03	2.3	0.50	KT591104
		R: CTTCTGTAGCCTTGGATTAACTTG*		58								
Mon15	AGG(8)	F: AAACCCAGCAGGTCATCCC*	184-208	60	1	35	0.00	0.00	N/A	1.0	N/A	KT591105
		R: GCTGACAAACAGGCACTGG		60								
Mon16	AAT(9)	F: AGAGCTAACAAACAGCTTATGGG*	77-104	60	4	35	0.66	0.55	-0.19	2.2	0.67	KT591106
		R: TGGCAGACAGTCCTCTTGAC		60								
Mon17	AAAT(7)	F: AGTTGGTCATAATCCACTGAAAGG*	178-206	60	3	34	0.71	0.54	-0.29	2.2	1.00	KT591107
		R: ACCCTGATTTGCCAGGGTC		60								
Mon18	GCCT(7)	F: ATGGCGAGTTCCGAGATCC	477-505	60	2	36	0.28	0.24	-0.15	1.3	1.00	KT591108
		R: CACAAGCAGTCTTGATGGAGG*		60								
Mon19	AAAT(7)	F: ATTATGGACCGAGTGCCTCC	137-165	60	2	38	0.61	0.50	-0.20	2.0	0.33	KT591109
		R: GGGAAGCCTAGTGCAGTACC*		61								
Mon20	GCCT(7)	F: CGAGCACATTCTGCAGTCG	551-579	60	2	38	0.53	0.50	-0.04	2.00	1.00	KT591110
		R: GCCTTGGACTAGGGCTGAC*		61								
Pop. Mean					2.5	36.53	0.46	0.41	-0.10	1.88	0.67	
Pop. SE					0.2	0.57	0.06	0.05	0.03	0.14	0.07	

k: number of alleles; T_M: melting temperature; *N*: number of individuals; H_O: observed heterozygosity; H_E: expected heterozygosity; F_{IS}: inbreeding coefficient *M*: k: allelic range in repeat units (Garza and Williamson 2001); N/A: not applicable.

*Denotes which primer in each primer pair had an M13(-21) tag appended to its 5′ end (sensu Schuelke, 2000).

We used GENALEX 6.5 (Peakall and Smouse 2012) to calculate several summary statistics including: number of alleles, effective number of alleles, observed heterozygosity, and expected heterozygosity. We also used GENEPOP 4.3 (Rousset 2008) to test for departures from Hardy-Weinberg proportions and genotypic equilibrium. GENEPOP 4.3 was also used to calculate the Weir and Cockerham (1984) estimator of F_{IS}, which describes the direction and magnitude of the correlation of alleles within individuals within populations. This estimator of the inbreeding coefficient is useful for small data sets because it does not make assumptions regarding numbers of populations or sample sizes (Weir and Cockerham 1984). *M*-ratios (Garza and Williamson 2001) were calculated in EXCEL using the output from GENALEX. *M* is defined as *k* (number of alleles) divided by *r* (allele size range in number repeat units) and is a useful summary statistic for detecting recent reductions in population size (Garza and Williamson 2001). MICRO-CHECKER 2.2.3 (Van Oosterhout et al. 2004) was used to examine each locus for evidence of null alleles, large allele dropout, and scoring errors (Table 1).

In order to give readers a feel for the level of sequence conservation in the genomic regions immediately surrounding each locus, we conducted BLASTn searches of NCBI's 'nucleotide collection (nr/nt)' database using the 454 fragments that primers were designed from as queries. These searches were performed using NCBI's default settings for BLASTn and a critical E-value of 10-7—a somewhat stringent threshold designed to filter out alignments that only or overwhelmingly correspond to microsatellite repeat regions.

Results and Discussion

We detected 1-4 alleles per locus (mean ± SE = 2.5 ± 0.2). *Mon6* and *Mon15* were both monomorphic in Cape Coral and therefore could not be subjected to tests for Hardy-Weinberg proportions and genotypic disequilibrium. However, we have included these loci in our report because preliminary genotyping in West Palm Beach and Homestead have shown that these loci are polymorphic in these populations. As such, current evidence suggests *Mon6* and *Mon15* will be useful for analyses of population differentiation. Observed and expected heterozygosities in Cape Coral ranged from 0.18 to 0.73 (mean ± SE = 0.46 ± 0.06) and 0.20 to 0.59 (mean ± SE = 0.41 ± 0.05), respectively. Upon performing Holm's (1979) correction for multiple testing there were no statistically significant departures from Hardy-Weinberg equilibrium, and MICRO-CHECKER did not detect any evidence of null alleles. However, *Mon1-Mon14* and *Mon3-Mon8* exhibited statistical departures from genotypic equilibrium. At present, the relative contributions of recent evolutionary phenomena, such as multiple introductions from different regions of the native range, and persistence of disequilibrium due to more temporally distant events and tight physical linkage are unclear. However, if these loci do turn out to be in disequilibrium in other populations, difficulties associated with non-independence can easily be avoided by dropping one of the loci from each of these respective pairs. Estimates of F_{IS} (mean ± SE = -0.10 ± 0.03) revealed mild heterozygote excess—a result that may reflect modest outbreeding. It is also noteworthy that the mean *M*-ratio (mean ± SE = 0.67 ± 0.07) is below the critical value of 0.68 suggested by Garza and Williamson (2001), which likely indicates that genetic diversity in the Cape Coral population is still recovering from the founding event presumed to have occurred in the

early 1990's.

The results of our BLASTn searches are presented in Table 2. These searches suggest that several of the loci we have identified should receive priority from researchers interested in extending these resources to other varanids. *Mon12* is especially noteworthy, as it shows a strong signal of homology with a previously identified microsatellite locus from *V. salvator*. *Mon17, Mon19*, and *Mon20* are also potentially of interest, as they all exhibit similarity to sequences from other reptilian genomes (in a phylogenetic sense, birds are reptiles).

Table 2. Results of BLASTn searches of NCBI's 'nucleotide collection (nr/nt)' database using microsatellite containing 454 fragments as queries.

Query	Locus	Best hit accession ID	Best hit description	Alignment length (bp)	% identity	Bit score	E-value
HN7TS9H02DQAES	*Mon1*	AC154274	*Mus musculus* BAC clone RP24-298J16 from 17, complete seq.	70	84.0	73.4	5.0×10^{-9}
HN7TS9H02ELQ4D	*Mon2*	No significant hits	N/A	N/A	N/A	N/A	N/A
HN7TS9H02DEI2X	*Mon3*	No significant hits	N/A	N/A	N/A	N/A	N/A
HMEZZP203GE0BW	*Mon4*	No significant hits	N/A	N/A	N/A	N/A	N/A
HN7TS9H03GN69L	*Mon6*	No significant hits	N/A	N/A	N/A	N/A	N/A
HMEZZP203FKADE	*Mon8*	No significant hits	N/A	N/A	N/A	N/A	N/A
HMEZZP203GSD5W	*Mon9*	No significant hits	N/A	N/A	N/A	N/A	N/A
HN7TS9H03GXHKZ	*Mon10*	No significant hits	N/A	N/A	N/A	N/A	N/A
HN7TS9H03GPPOC	*Mon12*	HQ896229	*Varanus salvator* clone JX14 microsatellite sequence	185	87.0	233.1	3.5×10^{-54}
HN7TS9H03HA3NZ	*Mon13*	No significant hits	N/A	N/A	N/A	N/A	N/A
HN7TS9H02EIY13	*Mon14*	No significant hits	N/A	N/A	N/A	N/A	N/A
HN7TS9H02DDL5H	*Mon15*	No significant hits	N/A	N/A	N/A	N/A	N/A
HN7TS9H02DPRRL	*Mon16*	CR394571*	Zebrafish DNA seq. from clone CH211-180M12 in link. group 21	43	100.0	78.8	8.6×10^{-11}
HN7TS9H02C6YMJ	*Mon17*	LK064835	*Apteryx australis mantelli* genome assem. AptMant0 scaffold 233	110	85.0	138.0	8.5×10^{-25}
HN7TS9H02DG8XH	*Mon18*	No significant hits	N/A	N/A	N/A	N/A	N/A
HN7TS9H02DDW8V	*Mon19*	JX038444	*Micrurus fulvius* clone FQ6DGU405F3RTD microsatellite seq.	79	87.0	91.5	2.1×10^{-14}
HN7TS9H02EVHLU	*Mon20*	XM_003216189	PREDICTED: *Anolis carolinensis* follistatin, transcript variant X1	127	74.0	88.0	3.5×10^{-11}

N/A = not applicable

* = Alignment nearly entirely corresponds to microsatellite repeat region proper and is therefore unlikely to be of biological significance

Conclusions

Herein, we have described the development of 17 novel microsatellite loci from *V. niloticus*. The resources we have developed will be used to gain insights into the introduction histories of *V. niloticus* populations in Florida and to examine the degree to which these populations are connected by gene flow. It is also possible, if not likely, that some of the markers we have characterized will prove useful in other varanid species.

CHAPTER III

CHARACTERIZATION OF 14 NOVEL MICROSATELLITE LOCI IN THE ARGENTINE BLACK AND WHITE TEGU (*SALVATOR MERIANAE*) VIA 454 PYROSEQUENCING

Introduction

After habitat destruction, invasive species are the next greatest threat to global biodiversity (Wilcove et al. 1998). Florida is especially susceptible to invasion by nonnative herpetofauna because of its numerous ports of entry, subtropical climate, and disturbed habitats (Mazzotti et al. 2015; Pernas et al. 2012). The Argentine black-and-white tegu (*Salvator merianae*) is one of the four largest non-native lizards currently breeding in Florida (Engeman et al. 2011). It is also one of the largest lizards in the New World, reaching sizes of up to 145 cm total length and 8 kg (Duarte Varela and Cabrera 2000; Lopes and Abe 1999). *Salvator merianae* is native to South America (Luxmoore et al. 1988). However, a breeding population of *S. merinae* was documented in portions of Hillsborough and Polk Counties in 2006 (Engeman et al. 2011) and the existence of this population has since been attributed to activities associated with the exotic pet industry (Engeman et al. 2011). *Salvator merinae* has already been documented depredating American alligator (*Alligator mississippiensis*) and red-bellied cooter (*Pseudemys nelson*) nests in Florida (Mazzotti et al. 2015). Thus, *S. merianae* is currently viewed as a direct

threat to Florida's sensitive fossorial wildlife (e.g., sea turtles, gopher tortoise (*Gopherus polyphemus*), eastern indigo snake (*Drymarchon couperi*), American crocodile (*Crocodylus acutus*), Cape Sable seaside sparrow (*Ammodramus maritimus mirabilis*), and Key Largo woodrat (*Neotoma floridana smalli*); Mazzotti et al., 2015).

Since *S. merinae's* initial introduction to Hillsborough and Polk Counties, a new breeding population has been documented approximately 330 km away in southern Miami-Dade County (Pernas et al., 2012). It is unclear whether this recent establishment is the result of dispersal or the consequence of secondary human-mediated introduction. However, to prevent further spread of *S. merianae* throughout Florida, it is essential for managers to know how this new population became established. Microsatellite-based population genetic approaches have considerable potential to provide perspective on this question, but as of now, such genetic resources are not available for *S. merinae*. To facilitate such endeavors, we developed 14 novel microsatellite markers from *S. merianae* that will be used to examine the introduction histories of and degree of differentiation and connectivity between Florida's invasive *S. merinae* populations.

Methods

DNA from a single *S. merianae* captured in Miami-Dade County, Florida, USA (25°26'0.70"N, 80°30'5.77"W) was submitted to the University of Georgia Genomics Facility (GGF), where this isolate was pooled with DNA from two other species that were differentiated by terminal barcodes (Meyer et al., 2007). Genomic DNA was obtained from liver tissue using the Wizard Genomic DNA Purification Kit (Promega) according to the manufacturer's instructions. A library of single stranded template DNA

fragments was then produced using the GS FLX Titanium General Library Preparation Kit (Roche). Initial sequencing employed the 454 GS FLX Titanium Sequencing Kit XLR70 (Roche) run on ¼ 70 x 75 mm picotiter plate, and additional sequencing employed the 454 GS FLX Titanium Sequencing Kit XL+ (Roche) run on ½ 70 x 75 mm picotiter plate. The GGF also performed basic data processing, such as base calling and filtering.

These sequencing efforts yielded a total of 127,343,751 bp across 300,675 reads. Of these reads, 90,457 were generated using the XLR70 kit (mean length = 275.8 bp, std. dev. = 155.5 bp) and 210,218 were generated using the XL+ kit (mean length = 487.1 bp, std. dev. = 199.1 bp). We then used MSATCOMMANDER 0.8.2 (Faircloth 2008) to scan these pyrosequencing reads for dinucleotide microsatellites with ≥ eight tandem repeats and tri-pentanucleotide microsatellites with ≥ six tandem repeats. In total, MSATCOMMANDER identified 3,154 presumptively non-redundant potentially amplifiable loci (PALs). Finally, we used PRIMER3 (Rozen and Skaletsky 2000) to design primers targeting these potentially amplifiable loci (PALs) via batch processing of repeat-containing 454 fragments.

Twelve dinucleotide, four trinucleotide, and four tetranucleotide loci whose corresponding 454 fragments contained at least ten, nine, and seven tandem repeats respectively were manually selected for marker development. An M13(-21) sequence was fused to the 5′ end of either the forward or reverse primer of each primer pair in order to facilitate fluorescent labeling with 6-FAM via the nested PCR approach described by Schuelke (2000). These 20 loci were then screened for polymorphism and scoring reliability using DNA isolated from muscle tissue of 11 individuals sampled from

Miami-Dade County. All reactions had a final volume of 25 µl and contained 2 µl of template (DNA concentration between 10 and 100 ng /µl), 5 µl of 5x buffer, 1.5 mM MgCl2, 0.2 mM of each dNTP, 0.8 µM of non-M13(-21)-twinned primer, 0.8 µM 0f 6-FAM labeled M13(-21) primer, 0.2 µM of M13(-21)-twinned primer, and 0.625 units of GoTaq polymerase (Promega). Reaction conditions were as follows: 2 min at 94° C followed by 25 cycles of (1) 94° C for 30 s, (2) 62° C for 30 s decreasing by 0.3° C per cycle, and (3) 72° C for 40 s, followed by eight cycles of (1) 94° C for 30 s, (2) 53° C for 30 s, and (3) 72° C for 40 s, followed by a final cleanup step of 30 min at 72° C. Genotyping reaction products were visually inspected by gel electrophoresis by loading 5 µl of PCR product in 2% agarose gels. Products from successful reactions were shipped to the Arizona State University DNA Lab, where fragment analysis was performed using an Applied Biosystems 3730. Of the 20 loci that were screened, 14 were polymorphic and straightforward to score. Thus, we genotyped additional individuals at these 14 loci for a total of 40 individuals from the Miami-Dade County population. Locus-specific primers, as well as their melting temperatures, size ranges, and summary statistics are presented in Table 1. All loci were scored manually using PEAK SCANNER 1.0 (Applied Biosystems). Allelic bins were determined by graphically examining the rank-ordered fragment size distributions of each locus, so that we could identify breaks in the amplicon sizes (Guichoux et al. 2011). We then wrote functions in Microsoft EXCEL to bin the data from each locus into discrete classes that were defined by each allele's empirically determined size range.

Table 1. Characterization of 14 microsatellite loci genotyped in *S. merianae*. Samples collected from Miami-Dade County, Florida, USA.

Locus	Repeat (number)	Primer Sequence (5′-3′)	Size Range (bp)	T_M (°C)	*k*	*N*	H_O	H_E	F_{IS}	No. Effective Alleles	*M*	GenBank Accession No.
Teg1	AC (12)	F: GCCAATCACAGCCAACCTC	75-99	60	4	40	0.63	0.56	-0.11	2.26	0.80	KT619111
		R: AAGCTTGAGCAGTCCAGGG*		60								
Teg2	AC (12)	F: CTGATTGCAGGCAGAGGAC	390-414	59	2	40	0.03	0.03	N/A	1.03	0.40	KT619112
		R: ACCAGCAGCCAAGAATTCAG*		59								
Teg4	AC (12)	F: TTTCCCACGCTACCGAGAC	440-464	60	2	40	0.00	0.26	1.00	1.34	1.00	KT619113
		R: TCATCAAGATTGGGCACTACTTTC*		60								
Teg5	GT (12)	F: GCTCTTAAGGGATTGACTCCAG*	280-304	59	3	36	0.42	0.60	0.32	2.48	0.60	KT619114
		R: CATGAAGGTGCCCATGCAG		60								
Teg6	GT (11)	F: AAAGTGCCACGCACGTATC*	357-379	60	2	40	0.25	0.22	-0.13	1.28	1.00	KT619115
		R: CAAGGCATTACCTGGGAGC		59								
Teg7	AC (11)	F: CAGCATCCATGAGACTTGCG	406-428	60	4	40	0.33	0.28	-0.14	1.39	0.57	KT619116
		R: GGATGCAGCTTATACCAGCC*		59								
Teg9	AG (10)	F: TTTGCAACATCCTCGGCAC	335-355	60	2	40	0.08	0.12	0.37	1.13	1.00	KT619117
		R: ACCCAGAGTTCTCACGCAG*		60								
Teg10	AC (10)	F: GAGGGCAGCAAGGTTGAAG*	281-301	59	4	39	0.59	0.53	-0.11	2.11	0.21	KT619118
		R: GCACAGGCTGAACTCGTTG		60								
Teg12	AC (10)	F: AGGTGCAACGCTGGAAATG*	143-163	60	2	38	0.08	0.08	-0.03	1.08	1.00	KT619119
		R: GTCGCCTGCGCTTTCTATG		60								
Teg13	GTT (9)	F: ATGGCCTTCCTCCCAACTC	412-439	60	2	38	0.47	0.45	-0.04	1.82	0.25	KT619120

		R: GCACAGCGGTAATCCAAGC*		60								
Teg14	AGC (9)	F: CCCTCCACGGTTTCAGAGG*	177-204	60	4	40	0.68	0.64	-0.04	2.79	0.40	KT619121
		R: AGGAGAACTGGGCATGCTG		60								
Teg17	ATCT (7)	F: ACCACGACAAGGGAATCGG*	296-324	60	2	40	0.88	0.49	-0.77	1.97	0.33	KT619122
		R: GACTTGTGCCAGGATGCAG		60								
Teg19	ATTT (7)	F: CTCTGTGTGGGCATTGCAG	330-358	60	3	38	0.32	0.53	0.41	2.11	1.00	KT619123
		R: ACCCACCCTGAAACCTTCG*		60								
Teg20	CATT (7)	F: AGATCCCTCAGTCTCATGTGG*	124-152	59	2	38	0.58	0.43	-0.33	1.76	1.00	KT619124
		R: TCTGAGAGCCTTCTGGCTG		59								
Pop. Mean					2.71	39.07	0.38	0.37	0.03	1.75	0.68	
Pop. SE					0.24	0.34	0.07	0.06	0.12	0.15	0.09	

k: number of alleles; T_M: melting temperature; *N*: number of individuals; H_O: observed heterozygosity; H_E: expected heterozygosity; F_{IS}: inbreeding coefficient *M*: k: allelic range in repeat units (Garza and Williamson 2001); SE: standard error; N/A: not applicable.

*Denotes which primer in each primer pair had an M13(-21) tag appended to its 5′ end (sensu Schuelke 2000).

We used GENALEX 6.5 (Peakall and Smouse 2012) to calculate several summary statistics including: number of alleles, effective number of alleles, observed heterozygosity, and expected heterozygosity. We used GENEPOP 4.3 (Rousset 2008) to test for departures from Hardy-Weinberg proportions, departures from genotypic equilibrium, and to calculate the Weir and Cockerham (1984) estimator of F_{IS}. *M*-ratios (Garza and Williamson 2001) were calculated in EXCEL using output from GENALEX. We also used MICRO-CHECKER 2.2.3 (Van Oosterhout et al., 2004) to examine each locus for evidence of null alleles, large allele dropout, and scoring errors (Table 1).

In order to give readers a feel for the level of sequence conservation in genomic regions immediately surrounding each locus, we conducted BLASTn searches of NCBI's 'nucleotide collection (nr/nt)' database using the 454 fragments that primers were designed from as queries. These searches were performed using NCBI's default settings for BLASTn and a critical E-value of 10-7—a somewhat stringent threshold designed to filter out alignments that only, or overwhelmingly, correspond to microsatellite repeat regions.

Results and Discussion

The number of alleles (*k*), number of genotypes (*N*), observed heterozygosity (H_O), expected heterozygosity (H_E), Weir & Cockerham estimator of F_{IS}, number of effective alleles, and *M*-ratio for each locus are given in Table 1. In addition, Table 1 gives the mean for each of these population genetic parameters across all 14 loci, as well as the standard error of the mean. Upon performing Holm's (1979) correction for multiple testing, four loci (*Teg4, Teg5, Teg17, Teg19*) showed significant deviations from

Hardy-Weinberg expectations, with *Teg4, Teg5*, and *Teg19* exhibiting homozygote excess (Table 1). Therefore, it was not surprising that MICRO-CHECKER detected evidence of null alleles at these three loci. After correcting for multiple testing (Holm 1979), there was also statistical evidence for genotypic disequilibrium between *Teg14 and Teg19*. The mean *M*-ratio across the 14 loci (mean ± SE = 0.68 ± 0.09) was very close to the critical value of 0.68 suggested by Garza and Williamson (2001). This result is not surprising given that the Miami-Dade population was recently established and is consistent with the notion that the founding event involved a limited number of individuals.

Conclusions

Herein, we have described the development of 14 novel microsatellite loci from *S. merianae*. The resources we have developed will serve to enable researchers to assess the degree of gene flow between the two invasive populations currently established in Florida and gather insights into their introduction histories. Although there is limited allelic richness across these 14 loci (38 alleles total), preliminary analyses are suggesting that differentiation between the Hillsborough-Polk and Miami-Dade populations is pronounced (G_{ST} = 0.170; G'_{ST} = 0.545). Thus, at present, it seems likely that these markers will provide sufficient resolution for obtaining a general understanding of *S. merinae* population genetic dynamics in Florida. Unfortunately, our BLASTn searches were largely non-informative. However, a portion of the 454 fragment that *Teg19* was identified from, including the repeat containing region, exhibited moderate sequence similarity with a microsatellite-containing region of the *Anolis carolinensis* genome

(Table 2). As such, *Teg19* should receive priority among researchers seeking to extend these resources to populations where amplification success may be an issue, such as within *S. merinae's* native range or in other teiid species.

Table 2. Results of the BLASTn searches of NCBI's 'nucleotide collection (nr/nt)' database using microsatellite-containing 454 fragments as queries.

Query	Locus	Best hit accession ID	Best hit Description	Alignment length (bp)	% identity	Bit score	E-value
HN7TS9H02D8IL5	*Teg1*	No significant hits	N/A	N/A	N/A	N/A	N/A
HN7TS9H02DPM21	*Teg2*	CU634003*	Zebrafish Clone CH1073-436C4 in linkage group 19	69	88.0	84.2	2.3×10^{-12}
HN7TS9H02EUDWN	*Teg4*	No significant hits	N/A	N/A	N/A	N/A	N/A
HN7TS9H02D5S53	*Teg5*	AC040927*	*Mus musculus* Chromosome 5 clone RP23-186A21	42	100.0	77.0	3.6×10^{-10}
HN7TS9H02D8VTH	*Teg6*	AL844881*	Mouse Chromosome 2 clone RP23-244B19	66	86.0	78.8	1.1×10^{-10}
HN7TS9H02EWK6Z	*Teg7*	AC117257*	*Mus musculus* BAC clone RP24-484F21 from Chromosome 17	43	95.0	69.8	5.9×10^{-8}
HN7TS9H02C2I6P	*Teg9*	No significant hits	N/A	N/A	N/A	N/A	N/A
HN7TS9H02DMS22	*Teg10*	No significant hits	N/A	N/A	N/A	N/A	N/A
HMEZZP203FU4HR	*Teg12*	AC015820*	*Homo sapiens* chromosome 11 clone RP11-108G3	50	90.0	68.0.0	6.5×10^{-8}
HN7TS9H03F99SN	*Teg13*	No significant hits	N/A	N/A	N/A	N/A	N/A
HN7TS9H02EJHVJ	*Teg14*	AF279246*	*Xenopus laevis* twisted gastrulation protein mRNA, complete cds	43	97.0	73.4	4.0×10^{-9}
HN7TS9H02CYF9X	*Teg17*	BX571803	Zebrafish clone DKEY-273G3 in linkage group 9	132	81.0	131.1	2.1×10^{-26}
HN7TS9H02DIGZ3	*Teg19*	BK006913	*Anolis carolinensis* protocadherin gene alpha subcluster, partial sequence	84	80.0	71.6	1.6×10^{-8}
HMEZZP203FY8ZX	*Teg20*	LM125528*	*Taenia asiatica* genome assembly, TASK_scaffold0000307	60	88.0	73.4	1.5×10^{-9}

N/A = not applicable

* = Alignment strongly corresponds to microsatellite repeat region proper and is therefore unlikely to be of biological significance

CHAPTER IV

INSIGHTS INTO THE INTRODUCTION HISTORY AND POPULATION GENETIC DYNAMICS OF THE NILE MONITOR (*VARANUS NILOTICUS*) IN FLORIDA

Introduction

Invasive species are the second largest threat to global biodiversity, exceeded only by human-mediated habitat destruction (Wilcove et al. 1998; Mooney and Cleland 2001). Introduced species can disrupt ecosystem function, decrease diversity of native species, and detrimentally impact local and regional economies (Mack et al. 2000). Florida is especially susceptible to invasion of herpetofauna because of its subtropical climate, number of ports of entry, extensive exotic pet industry, and exposure to hurricanes, which may facilitate the establishment of exotic species once released from captivity (Corn et al. 2002; Hardin 2007). Consequently, it is not surprising that the number of nonnative lizard species currently outnumbers native lizard species in Florida (Pernas et al. 2012).

The Nile monitor (*Varanus niloticus*) is native to sub-Saharan Africa and was first observed in the southwest region of Cape Coral, Lee County, Florida in 1990 (Enge et al. 2004; Luxmore et al. 1988). At present, there are documented breeding populations of this species in Cape Coral, West Palm Beach, and on the Homestead Air Reserve Base (Figure 1; Table 1) (Florida Wildlife Commission 2015). *V. niloticus* is of particular concern because it is highly mobile, capable of reaching sexual maturity at two years of age, has clutches of up to 60 eggs, and is capable of achieving high densities (de

Buffrénil 1992; de Buffrénil and Rimblot-Baly 1999). These large lizards are typically found in close proximity to water and, in Florida, seem to do particularly well in disturbed areas near canals (Campbell 2005; Faust 2001), which have similar habitat characteristics to the marsh edges and mangroves they inhabit in their native range (Lenz 1995). Dietary studies from Africa have shown that monitors are generalist predators that prey upon insects, mollusks, amphibians, birds, bird eggs, reptiles, reptile eggs, and small to moderately sized mammals (Bennett 2002; Losos and Greene 1988). Because Nile monitors are semiaquatic and adept at burrowing, it is probable that they will negatively impact endangered gopher tortoises (*Gopherus polyphemus*), American crocodiles (*Crocodylus acutus*), burrowing owls (*Athene cunicularia*), and other species that are endemic to Florida (Enge et al. 2004; Campbell 2005).

Currently, the introduction histories of Florida's *V. niloticus* populations are not known. However, because Nile monitors are inexpensive and commonly available via the North American pet trade, their establishment is usually attributed to release by reptile enthusiasts who became discouraged by their large size and aggressive temperament, or breeders who wanted to establish local populations (Enge et al. 2004). Despite what is known about the ecology and natural history of *V. niloticus*, very little is known about the genetics of wild, invasive populations. This is unfortunate because such information could inform management strategies that seek to eradicate these populations or prevent further introductions through identification of management units (Abdelkrim et al. 2005; Rollins et al. 2009). Management units are an important component of developing realistic and cost-effective management strategies (Abdelkrim et al. 2005) because isolated populations are generally easier to control than populations connected

by dispersal. Thus, complete eradication may be a viable option for small and moderately sized populations that exhibit marked genetic differentiation. However, when little genetic differentiation is present across the range of invasion, indicating potentially connected breeding populations, control may be a more realistic goal (Rollins et al. 2009). With respect to documented populations of *V. niloticus* in Florida (Figure 1; Table 1), it is currently unclear whether there is dispersal between populations in different regions of the state. In order to generate a better understanding of the introduction histories and the population genetic dynamics of *V. niloticus* in Florida, we used polymorphic microsatellite loci to conduct a variety of analyses to assess intra-population genetic diversity, the degree of gene flow between populations, and the most likely introduction scenario.

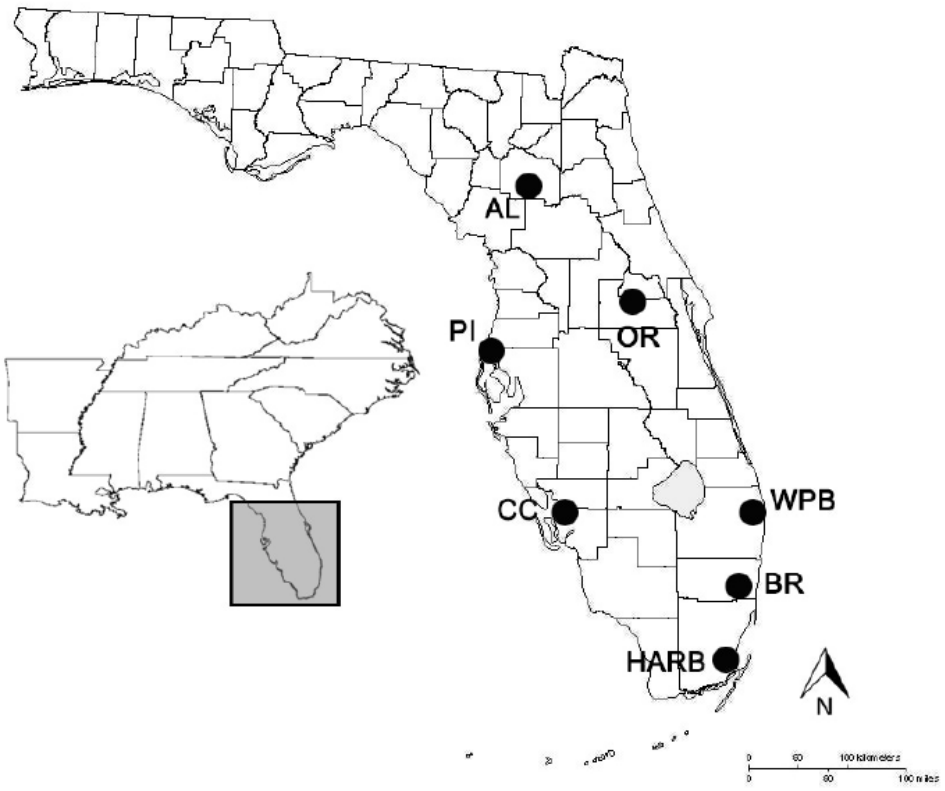

Figure 1. Map showing the location of the sampling sites in Southern Florida and the position of Florida within the Southeastern US (see Table 1 for key to labels).

Table 1. Location and number of *V. niloticus* specimens used for genetic analyses. Site abbreviations correspond to the abbreviations used in Figure 1. Table 1 also shows other locations in Florida where *V. niloticus* sightings have been confirmed and the year in which first sightings were reported for all locations. Information for *V. niloticus* sightings in locations other than Cape Coral, West Palm Beach, and Homestead Air Reserve Base are approximations based on data obtained from the Florida EddMaps webpage (https://www.eddmaps.org/distribution/List.cfm?sub=18353).

Site	Site Name	County	Confirmed Sightings	Year of First Sighting	Latitude	Longitude	No. of Samples
CC	Cape Coral	Lee	389	1990	26°35'34.70"N	82° 0'33.72"W	40
WPB	West Palm Beach	Palm Beach	80	2007	26°40'41.39"N	80° 8'48.80"W	17
HARB	Homestead Air Reserve Base	Miami-Dade	47	2008	25°28'46.86"N	80°24'0.19"W	10
BR	Broward	Broward	9	2007	26°10'36.77"N	80°22'43.58"W	N/A
OR	Orange	Orange	4	2009	28°35'10.59"N	81°15'4.56"W	N/A
AL	Alachua	Alachua	2	2011	29°33'40.79"N	82°19'54.77"W	N/A
PI	Pinellas	Pinellas	2	2014	28° 8'24.19"N	82°40'57.51"W	N/A

N/A: not available; no samples were obtained from these locations

Materials and methods

Field sites, sampling, and tissue collection

V. niloticus specimens were obtained from three locales in southern Florida: the City of Cape Coral, the C-51 canal in West Palm Beach, and the Homestead Air Reserve Base (Homestead; Figure 1; Table 1). In Cape Coral, *V. niloticus* inhabits most of the freshwater canals located in the southwestern region of the city, and this population is believed to be the largest in Florida (EddMaps: https://www.eddmaps.org/distribution/viewmap.cfm?sub=18353). Since 2004, one of us (TSC) has collected 420 specimens from this locale—a subset of which was used in this study. All of the tissues from this subset were obtained from lizards collected between 2006 and 2010 from a 63.73 km^2 area centered around approximately 26°35'34.70"N, 82° 0'33.72"W.

The purportedly largest population of *V. niloticus* on the Atlantic Coast of Florida occurs in West Palm Beach. Surprisingly, *V. niloticus* has only been documented along a 22.67 km long by 67.97 m wide stretch along the C-51 Canal between Flying Cow Road and Interstate 95 (26°40'41.39"N, 80° 8'48.80"W). The north bank along this stretch of the C-51 Canal is heavily vegetated and offers cover for *V. niloticus.* The south bank is maintained by the South Florida Water Management District as an open corridor, and *V. niloticus* often uses this bank as a basking site. Seventeen specimens from West Palm Beach were used in our study, which were collected by Florida Wildlife Commission personnel between 2011 and 2013.

V. niloticus samples from Homestead were collected by Environmental Flight of Homestead Air Reserve Base and USDA-APHIS personnel between 2010 and 2012. This population is believed to be the smallest of the three populations in Florida. Only ten specimens have been collected from this site to date, and tissues from all ten were used in our study.

DNA Isolation & PCR-based Genotyping

We obtained muscle tissue samples from a total of 67 lizards (Cape Coral: $N = 40$; West Palm Beach: $N = 17$; Homestead: $N = 10$), and extracted genomic DNA using the Wizard Genomic DNA Purification Kit (Promega) according to the manufacture's instructions. We examined 17 microsatellite loci developed from *V. niloticus*, nine of which have dinucleotide repeat motifs (*Mon1, Mon2, Mon3, Mon4, Mon6*, *Mon8, Mon9, Mon10, Mon12*) four of which have trinucleotide repeat motifs (*Mon13, Mon14, Mon15, Mon16)*, and four of which have tetranucleotide repeat motifs (*Mon17, Mon18, Mon19, Mon20*; Wood et al. 2016). When these loci were under development (Wood et al. 2016), we conducted initial screening using 11 samples from Cape Coral. During this phase of marker development, five independent PCRs were performed on these 11 samples for all 17 loci without disagreement in genotype among any of the replicate reactions for each respective sample by locus combination. All genotyping reactions followed the nested PCR approach described by Schuelke (2000), had final volumes of 25 µl and contained 2 µl of template (DNA concentration between 10 and 100 ng /µl), 1x buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.8 μ M of non-M13(-21)-twinned primer, 0.8 μ M 0f 6-

FAM labeled M13(-21) primer, 0.2 μ M of M13(-21)-twinned primer, and 0.625 units of GoTaq polymerase (Promega). Reaction conditions were as follows: 2 min at 94° C followed by 25 cycles of 94° C for 30 s, 30 s at 63° C decreasing by –0.3°C per cycle, and 72° C for 40 s, followed by eight cycles of 94° C for 30 s, 53° C for 30 s, and 72° C for 40 s, followed by a final extension step of 30 min at 72° C. Successful amplification was confirmed via electrophoresis using 2% agarose gels, and fragment analysis was performed using an Applied Biosystems 3730 and GENESCAN 600 as an internal sizing standard (Arizona State University). All loci were scored manually using PEAK SCANNER 1.0 (Applied Biosystems). Allelic bins were determined by graphically examining the rank-ordered fragment size distributions of each locus, so that we could identify breaks in the amplicon sizes (Guichoux et al. 2011). We then wrote functions in Microsoft EXCEL to bin the data from each locus into discrete classes that were defined by each allele's empirically determined size range.

Summary Statistics & Quality Control

MICRO-CHECKER 2.2.3 (Van Oosterhout et al., 2004) was used to examine each locus for evidence of null alleles, large allele dropout, and scoring errors. We used GENALEX 6.5 (Peakall and Smouse, 2012) to calculate several summary statistics including: number of alleles, effective number of alleles, observed heterozygosity, and expected heterozygosity. We also used GENEPOP 4.3 (Rousset, 2008) to test for departures from Hardy-Weinberg proportions and genotypic equilibrium. GENEPOP 4.3 was also used to calculate the Weir and Cockerham (1984) estimator of F_{IS}. Finally, we

used POPGENKIT (http://cran.r-project.org/web/packages/PopGenKit/index.html) to construct rarefaction curves (sampling interval = 1, number of replicates = 1000) and determine allelic richness (A_R; standardized to a sample size of 10).

Assessment of population structure

In order to determine the degree of genetic differentiation between the *V. niloticus* populations in Cape Coral, Homestead, and West Palm Beach, we used a variety of approaches. First, we used GENALEX 6.5 (Peakall and Smouse 2012) to calculate G_{ST} values based on Nei and Chesser's (1983) unbiased estimators of H_S (*i.e.,* the Hardy-Weinberg expected heterozygosity averaged across subpopulations) and H_T (*i.e.,* the Hardy-Weinberg expected heterozygosity in the total population ignoring subdivision), where $G_{ST} = (H_T - H_S)/H_T$. We also used GENALEX to calculate G''_{ST}, which is a modified version of Hedrick's G'_{ST} (a standardized G-statistic that is formulated to equal one when populations have non-overlapping allele sets irrespective of the level of genetic diversity) that corrects for the tendency G'_{ST} to underestimate the degree of subdivision when only a small number of populations have been sampled (Merimans and Hedrick 2011). All resampling tests conducted in GENALEX were based on 9,999 permutations. We also used ARLEQUIN 3.5.1.2 (Excoffier and Lischer 2010) to perform an AMOVA that partitioned genetic variation among populations, among individuals within populations, and within individuals. We further visualized the genetic patterns among the Florida populations by conducting a Principal Component Analysis (PCA) on individual genotypes using the gstudio package (Dyer 2012) in R 3.1 (R core Team 2014).

Finally, we used STRUCTURE 2.3.4 (Pritchard et al. 2000; Falush et al. 2003) to estimate the number of populations (*K*) and to assign individuals to populations (*i.e.,* clusters). Because one of us (SAD) is involved in ongoing work that suggests all three Florida populations are derived from a single evolutionary lineage in West Africa, we used the correlated allele frequencies model. In addition, we allowed for the possibility of admixture. We conducted 10 replicate STRUCTURE runs for K = 1-6 (burn-in period = 500,000, number of MCMC reps after burn-in = 500,000) and used STRUCTURE HARVESTER (Earl and Vonholdt 2012), CLUMPP (Jakobsson and Rosenberg 2007), and DISTRUCT (Rosenberg 2004) to visualize and interpret the results.

Among-population gene flow

To examine the possibility of post-introduction admixture among populations, we assessed the degree of recent gene flow with BayesAss 1.3 (Wilson and Rannala 2003). This method uses a coalescent approach to infer pairwise migration rates during recent generations. We performed 10^8 iterations, with a sampling frequency of 2,000 and a burn-in of 10^7. Convergence was assessed based on visual inspection of the likelihood scores as well as consistency of the results across three independent runs.

Because small, variable sample sizes may affect migration estimates with this method, we also used GENECLASS2 (Piry et al. 2004) to perform assignment tests via Paetkau's (1995) frequency-based criterion. For this analysis, the default frequency for missing alleles was 0.01, the Monte-Carlo resampling method was that of Paetkau et al. (2004), the number of simulated individuals used for the probability computations was

10,000, and the type I error rate was 0.01. We also used GENECLASS2 and Paetkau's (1995) likelihood computations to test for the presence of first-generation migrants. L_home is the likelihood that an individual's genotype originated from the population in which it was sampled (Piry et al. 2004). L_home/L_max is the ratio of L_home to the highest likelihood value observed in all sampled populations, including the population where the individual was sampled (Paetkau et al. 2004). This likelihood estimation is appropriate when all source populations are thought to have been sampled (Piry et al. 2004). Because the populations sampled in our study correspond to the only known *V. niloticus* populations in Florida, we initially used the 'L_home/L_max' likelihood estimation (Piry et al. 2004).

Effective population size and demographic changes

To investigate the probability of inbreeding in the introduced *V. niloticus* populations, we estimated the effective population size (N_e) of each population with NeESTIMATOR 2.0 (Do et al. 2014) using the one-sample methods including the linkage disequilibrium method (Waples and Do 2008) and heterozygote-excess method (Zhdanova and Pudovkin 2008). The linkage disequilibrium method takes advantage of the non-random association of alleles across loci that often develops in small populations, while the heterozygote-excess method is based on the observation that a small number of breeding individuals in a population often produces progeny with an excess of heterozygotes (Zhdanova and Pudovkin 2008). These results were also compared to N_e

estimates provided by the approximate Bayesian computation method of the web-based program ONeSAMP (Tallmon et al. 2008)

We tested for evidence of recent population bottlenecks in the Florida populations by examining deviations from expected heterozygosity using the program BOTTLENECK 1.2.02 (Piry et al. 1999). Deviations were assessed under the stepwise mutation model (SMM), infinite alleles model (IAM), and the two-phase model (TPM) with 70% SMM. The data were analyzed with 1,000 iterations, and the sign test, Wilcoxon signed-rank test, and mode-shift test implemented by BOTTLENECK were used to assess significance. We additionally performed the Mode-shift test in BOTTLENECK to examine whether the distribution of allele frequencies displayed a mode-shift distortion in which alleles in low-frequency classes become less abundant than alleles at intermediate frequencies, a characteristic sign of a recent population decline (Luikart et al. 1998). This is in contrast to the L-shaped distribution typically displayed by constant-sized populations. Finally, we calculated *M*-ratios (Garza and Williamson 2001) in EXCEL using the output from GENALEX. *M*-ratios are defined as the ratio of *k* (total number of alleles) to *r* (overall range in allele size), where low values are indicative of recent reductions in population size (Garza and Williamson 2001). All *M*-ratios were assessed against a critical value of 0.68 as suggested by Garza and Williamson (2001) on the basis of a survey they performed of putatively stable and unstable animal populations from a variety of taxa (also see Peery et al. 2012).

Introduction scenario testing

To distinguish among distinct introduction scenarios for the Florida *V. niloticus* populations, we used DIYABC 2.1.0 (Cornuet et al. 2014). The number of possible scenarios was narrowed down based on occurrence records, our gene flow and population bottleneck results (see results section), as well as previous data showing that all three Florida populations originated from the same source population in West Africa (Dowell 2015). A total of eight introduction scenarios were considered, with all scenarios hypothesizing a population bottleneck following each introduction event (Figure 2). Scenarios 1–4 describe the Florida populations originating from three independent introduction events. In scenario 1, all populations were introduced at the same time, while scenarios 2–4 hypothesize that the populations diverged from the ancestral source population at different time periods, indicating different introduction times. Serial introduction scenarios 5 and 6 involve the West Palm Beach and Homestead populations originating from the Cape Coral population, rather than independently from the source population. Lastly, scenarios 7 and 8 describe more complex serial introduction pathways, where the Cape Coral population originated from the source population, the next population originated from Cape Coral, and the third population originated from the second. Because *V. niloticus* individuals in Cape Coral were first observed over ten years prior to those in the other two locations, we did not consider scenarios where the West Palm Beach or Homestead populations were introduced first.

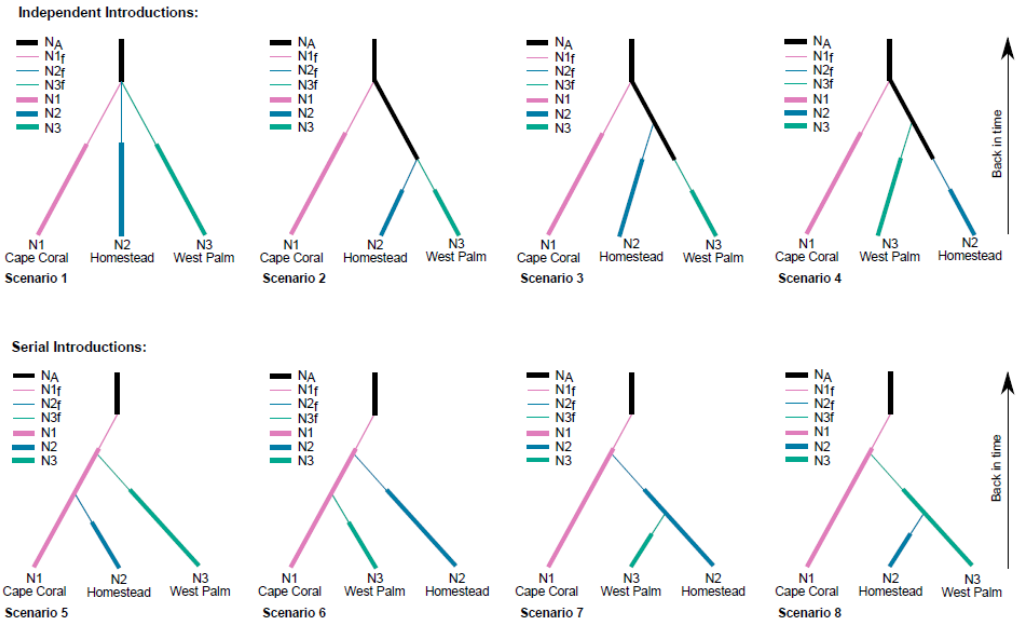

Figure 2. Graphical representation of the competing introduction scenarios for *Varanus niloticus* examined with the software DIYABC. In each scenario, thin lines represent bottlenecked populations following introduction events, while thick lines represent the current effective population size. The abbreviations used are as follows: *NA* = ancestral (source) effective population size; *N1* = effective population size for the Cape Coral population; *N2* = effective population size for the Homestead population; *N3* = effective population size for the West Palm Beach population; $N1–3_f$ = the effective number of founding individuals; *t* = time in generations.

For all analyses, prior distributions were uniform and defined as follows: $1 < N < 10{,}000$; $1 < NA < 50{,}000$; $1 < N_f < 100$; $1 < \text{db} < 20$; $1 < t_1 \leq t_2 \leq t_3 < 100$; where '$N$' denotes the current effective population size, 'NA' denotes the ancestral (source) effective population size, 'N_f' denotes the effective number of founding individuals, 'db' denotes the bottleneck duration in generations, and 't' the time in generations. To assess how the prior distributions affect the results, we performed a second analysis in which we modified the effective population size prior to a more realistic value ($1 < N < 100$), and kept the remaining priors the same. For the microsatellite mutation model, priors were set to default values which included the Generalized Stepwise Mutation model (Estoup et al. 2002), and a uniform prior distribution for both the mean mutation rate ($1E^{-4}$ to $1E^{-3}$) and the geometric distribution ($1E^{-1}$ to $3E^{-1}$). Summary statistics included the mean number of alleles, mean genic diversity, and mean size variance for both the one-sample and two-sample statistics. Additionally, we used the mean Garza-Williamson's M index (one-sample statistic) as well as pairwise F_{ST} values and the mean classification index (two-sample statistics). For each scenario, we simulated 1 million datasets, for a total of 8 million.

To distinguish among the various introduction scenarios, we performed two separate analyses: (A) comparing all eight scenarios, and (B) comparing scenario 1 to the serial introduction scenarios (5–8). For each analysis, the optimal scenario was selected based on posterior probabilities compared using the logistic regression analysis implemented in the program, using the 1% closest simulated data sets. To evaluate the confidence in our optimal scenario, we analyzed 100 simulated pseudo-observed data sets (pods) for each scenario, using parameter values drawn from the same prior distribution

as our previous analyses. The relative posterior probabilities of each scenario were calculated for every pod and then used to estimate type I and type II error rates. Posterior distributions of the parameters were computed for the most likely scenario, using the logit transformation. Confidence in the parameter estimations was assessed by calculating relative bias and relative root mean square error, using 500 test data sets and the mode as the point estimate.

Results

Summary Statistics & Quality Control

The summary statistics and genetic diversity estimates computed for the 17 loci we used for genotyping clearly show that all three populations of *V. niloticus* have limited diversity (Table 2). Upon performing Holm's (1979) correction for multiple testing via treating the tests associated with each population as a family of tests, there were no statistically significant departures from Hardy-Weinberg proportions in the Cape Coral or West Palm Beach populations. However, in the Homestead population, *Mon14* exhibited statistical evidence of homozygote excess. Not surprisingly, MICRO-CHECKER detected evidence for null alleles at *Mon14* in the Homestead population; however, MICRO-CHECKER did not detect any evidence for null alleles in the West Palm Beach or Cape Coral populations. Upon correcting for multiple testing (Holm 1979; see above), there was no statistical evidence for genotypic disequilibrium among any of the pairs of loci in the Homestead or West Palm Beach populations. However, there was statistical evidence for genotypic disequilibrium between *Mon1-Mon14* and *Mon3-Mon8* in the

Cape Coral Population. Because some loci were monomorphic in some populations, but not others, exact tests for Hardy-Weinberg proportions and pairwise genotypic disequilibrium could not be computed for all loci in all populations. As can be seen by examining the rarefaction curves shown in Figure 3, > 50% of the loci exhibit or approach asymptotic behavior in the three respective populations, meaning that most of the allelic variation was likely sampled despite substantial differences in sample sizes across populations.

Table 2. Summary statistics and diversity estimates for the 17 microsatellite loci that were used for comprehensive genotyping.

Locus/Pop.	N	No. Alleles	Obs. Het.	Exp. Het.	F_{IS}	$^{a}A_R$	No. Effective Alleles	No. Private Alleles	*M*
Cape Coral									
Mon1	33	3	0.727	0.588	-0.223	2.99	2.425	0	0.500
Mon2	38	3	0.421	0.419	0.008	2.27	1.720	0	1.000
Mon3	33	2	0.424	0.367	-0.140	2.00	1.581	0	0.400
Mon4	40	2	0.475	0.447	-0.050	2.00	1.809	1	0.667
Mon6	40	1	0.000	0.000	N/A	1.00	1.000	0	N/A
Mon8	37	3	0.676	0.575	-0.163	3.00	2.350	1	0.375
Mon9	39	3	0.667	0.558	-0.183	2.80	2.262	2	1.000
Mon10	39	2	0.179	0.204	0.134	1.93	1.257	0	0.667
Mon12	35	4	0.686	0.685	0.014	3.83	3.178	1	0.667
Mon13	35	2	0.286	0.284	0.009	1.99	1.397	0	0.333
Mon14	36	3	0.556	0.567	0.034	2.98	2.308	2	0.500
Mon15	35	1	0.000	0.000	N/A	1.00	1.000	0	N/A
Mon16	35	4	0.657	0.548	-0.185	3.18	2.213	1	0.667
Mon17	34	3	0.706	0.541	-0.291	2.66	2.179	1	1.000
Mon18	36	2	0.278	0.239	-0.148	1.98	1.314	1	1.000
Mon19	38	2	0.605	0.500	-0.199	2.00	1.999	1	0.333
Mon20	38	2	0.526	0.499	-0.042	2.00	1.994	1	1.000
Pop. Mean	36.529	2.471	0.463	0.413	-0.095	2.33	1.882	0.706	0.674
Pop. SEM	0.556	0.212	0.058	0.049	0.031	0.18	0.141	0.166	0.069
Homestead									
Mon1	10	3	0.600	0.540	-0.059	N/A	2.174	0	0.429
Mon2	10	3	0.700	0.565	-0.189	N/A	2.299	1	0.500
Mon3	8	2	0.375	0.430	0.192	N/A	1.753	0	0.500
Mon4	10	3	0.900	0.535	-0.653	N/A	2.151	2	0.429
Mon6	10	2	0.200	0.180	-0.059	N/A	1.220	0	0.667
Mon8	10	3	0.800	0.625	-0.231	N/A	2.667	0	0.375
Mon9	9	3	0.556	0.648	0.200	N/A	2.842	2	0.188
Mon10	9	4	0.556	0.574	0.091	N/A	2.348	2	1.000
Mon12	9	5	0.889	0.636	-0.347	N/A	2.746	1	1.000
Mon13	7	3	0.571	0.541	0.020	N/A	2.178	1	0.375

Mon14	10	3	0.000	0.460	1.000	N/A	1.852	0	1.000
Mon15	9	2	0.444	0.494	0.158	N/A	1.976	0	0.667
Mon16	9	4	0.778	0.673	-0.098	N/A	3.057	2	0.571
Mon17	7	2	0.286	0.408	0.368	N/A	1.690	0	1.000
Mon18	8	2	0.500	0.469	0.000	N/A	1.882	1	1.000
Mon19	7	1	0.000	0.000	N/A	N/A	1.000	0	N/A
Mon20	10	1	0.000	0.000	N/A	N/A	1.000	0	N/A
Pop. Mean	8.941	2.706	0.480	0.457	0.026	N/A	2.049	0.706	0.647
Pop. SEM	0.277	0.254	0.073	0.050	0.095	N/A	0.147	0.206	0.073
W. P. Beach									
Mon1	13	5	0.923	0.728	-0.231	4.75	3.674	2	0.556
Mon2	17	2	0.059	0.057	N/A	1.57	1.061	1	1.000
Mon3	17	3	0.706	0.642	-0.070	3.00	2.792	0	0.600
Mon4	17	1	0.000	0.000	N/A	1.00	1.000	0	N/A
Mon6	14	3	0.643	0.482	-0.300	3.00	1.931	1	0.750
Mon8	15	3	0.600	0.558	-0.041	2.98	2.261	0	0.375
Mon9	17	1	0.000	0.000	N/A	1.00	1.000	0	N/A
Mon10	14	3	0.286	0.500	0.458	2.72	2.000	0	1.000
Mon12	16	4	0.563	0.549	0.007	3.61	2.216	0	0.800
Mon13	13	3	0.923	0.660	-0.365	3.00	2.939	2	0.429
Mon14	16	2	0.375	0.305	-0.200	2.00	1.438	0	1.000
Mon15	14	2	0.643	0.436	-0.444	2.00	1.774	0	0.667
Mon16	12	2	0.667	0.486	-0.333	2.00	1.946	0	0.400
Mon17	12	2	0.333	0.444	0.290	2.00	1.800	0	1.000
Mon18	12	3	0.833	0.601	-0.350	3.00	2.504	2	0.750
Mon19	15	3	0.467	0.518	0.133	2.67	2.074	1	0.429
Mon20	14	1	0.000	0.000	N/A	1.00	1.000	0	N/A
Pop. Mean	14.58	2.529	0.472	0.410	-0.111	2.43	1.965	0.529	0.697
Pop. SEM	0.446	0.259	0.077	0.060	0.076	0.24	0.182	0.194	0.064

N: number of individuals, F_{IS}: inbreeding coefficient (Weir and Cockerham 1984), [a]A_R: allelic richness, *M*: *M*-ratios (Garza and Williamson 2001)

[a]Allelic richness values are not given for Homestead, as this population was sampled to the lowest depth and A_R values for Cape Coral and West Palm Beach were standardized to the Homestead sampling depth. The number of decimal places for A_R is fewer than in other columns because POPGENKIT only calculates A_R values to two decimal places.

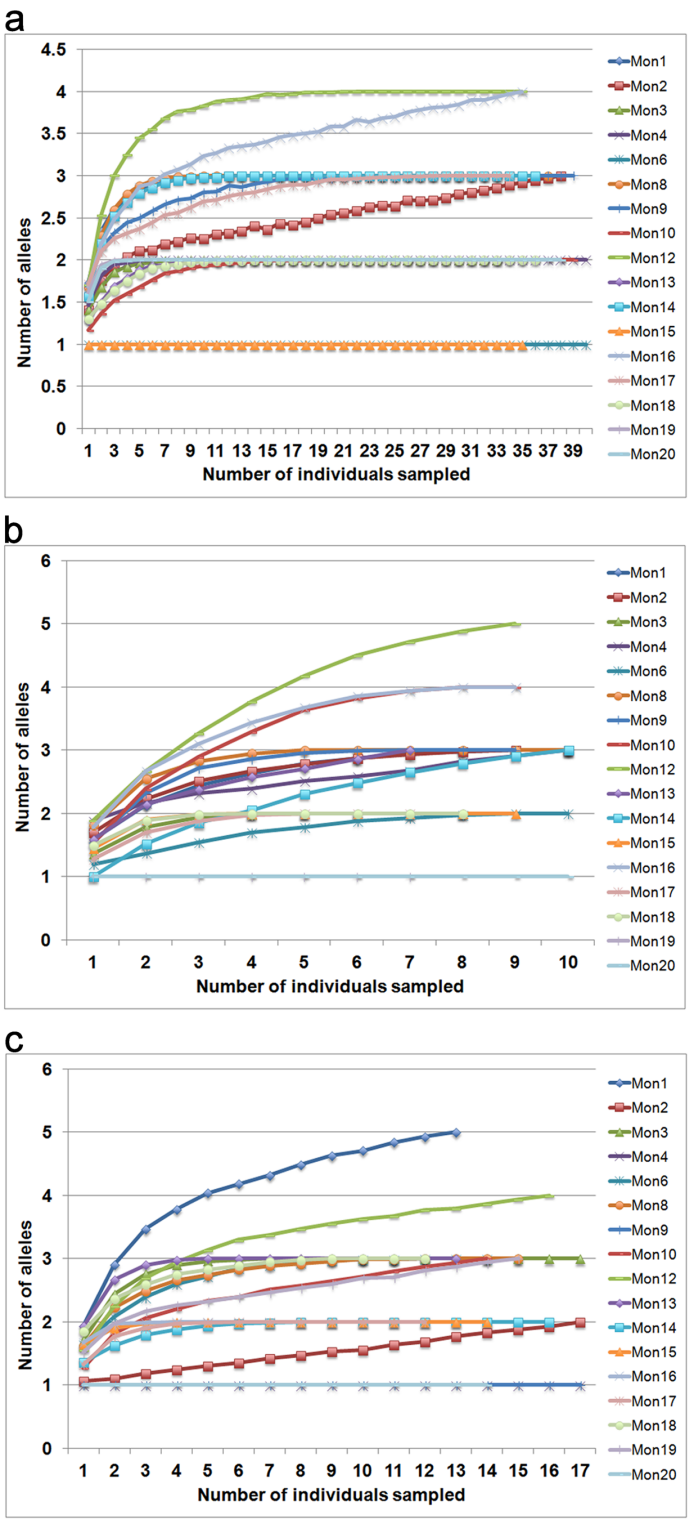

Figure 3. Rarefaction curves for the 17 microsatellite loci used for genotyping in Cape Coral (a), Homestead (b), and West Palm Beach (c)

Assessment of population differentiation

We excluded *Mon14* and *Mon3* prior to performing analyses in GENALEX, ARLEQUIN, STRUCTURE, and GENECLASS2, as the approaches we implemented in these software packages assume independence among loci. *Mon14* was dropped in place of *Mon1* because *Mon14* exhibited evidence of null alleles in the Homestead population, and *Mon3* was dropped in place of *Mon8* because *Mon8* exhibited higher levels of diversity in two of the three populations sampled (Table 2). Locus-specific G_{ST} estimates across all three populations (*i.e.,* 'global' estimates of differentiation) ranged from 0.079 to 0.490 and were, without exception, highly statistically significant (maximum P = 0.0013). Similarly, all locus-specific G''_{ST} estimates were highly statistically significant (maximum P = 0.0011), with values ranging from 0.286 to 0.912. The global G_{ST} estimate that resulted from combining information across all loci was 0.268 (SE = 0.037, P = 0.0001) and the global estimate for G''_{ST} was 0.628 (SE = 0.053, P = 0.0001). Similar estimates of G_{ST} and G''_{ST} were obtained from comparisons between pairs of populations (Cape Coral vs. Homestead: G_{ST} = 0.210, P = 0.0001, G''_{ST} = 0.626, P = 0.0001; Cape Coral vs. West Palm Beach: G_{ST} = 0.240, P = 0.0001, G''_{ST} = 0.658, P = 0.0001; Homestead vs. West Palm Beach: G_{ST} = 0.198, P = 0.0001 G''_{ST} = 0.601, P = 0.0001). Collectively, these G-statistics are indicative of pronounced genetic differentiation between the Cape Coral, West Palm Beach, and Homestead populations.

The AMOVA also suggested a high degree of genetic structure (Table 3). In addition, the AMOVA yielded a negative variance component, which, in turn, resulted in a negative estimate of F_{IS} (Table 3). While slightly negative variance components may

occur when the actual value of an estimated parameter is zero, the directionality of the F_{IS} estimate obtained via AMOVA is generally consistent with the population-specific, locus-by-locus estimates of F_{IS} obtained from GENEPOP (Table 2). In addition, the substantive, albeit lesser, magnitude of the within-population variance component relative to the among-population and within-individual variance components (Table 3) suggests that the negative within-population variance component may reflect the mild heterozygote excess observed in all three populations, which can occur in small populations and following population bottleneck events (Falconer 1989; Maruyama and Fuerst 1985; Rasmussen 1979; Robertson 1965).

Table 3. AMOVA results

Source of Variation	Degrees of Freedom	Sum of Squares	Variance Component	Fixation Index	*P*-value[a]
Among populations	2	117.386	1.52326	F_{ST} = 0.38053	0.00000[b]
Among individuals	64	118.726	-0.62469	F_{IS} = -0.25191	1.00000[c]
Within individuals	67	208.000	3.10448	F_{IT} = 0.22447	0.04040[d]
Total	133	444.112	4.00304	N/A	N/A

[a]All significance tests performed in ARLEQUIN are based on 10,100 permutations. [b]P(permuted $F_{ST} \geq$ to observed F_{ST}). [c]P(permuted $F_{IS} \geq$ observed F_{IS}), [d]P(permuted $F_{IT} \leq$ observed F_{IT}).

The genetic relationships among Florida's Nile monitor populations were visualized via PCA, with the first two principal components accounting for 32.04% of the variation in the data (Figure 4). Each population formed a discrete cluster, with no overlap among individuals. The general conclusion that all three populations exhibit pronounced differentiation was reinforced by the analyses we performed in STRUCTURE. As shown in Figure 5, the optimal value of *K* is three. Moreover, STRUCTURE recovered our sampling scheme by unambiguously assigning all 10 Homestead, all 17 West Palm Beach, and all 40 Cape Coral individuals to the three respective clusters **(**Figure 6). Collectively, these results reinforce the view that the three documented *V. niloticus* populations in Florida are the result of separate introduction events.

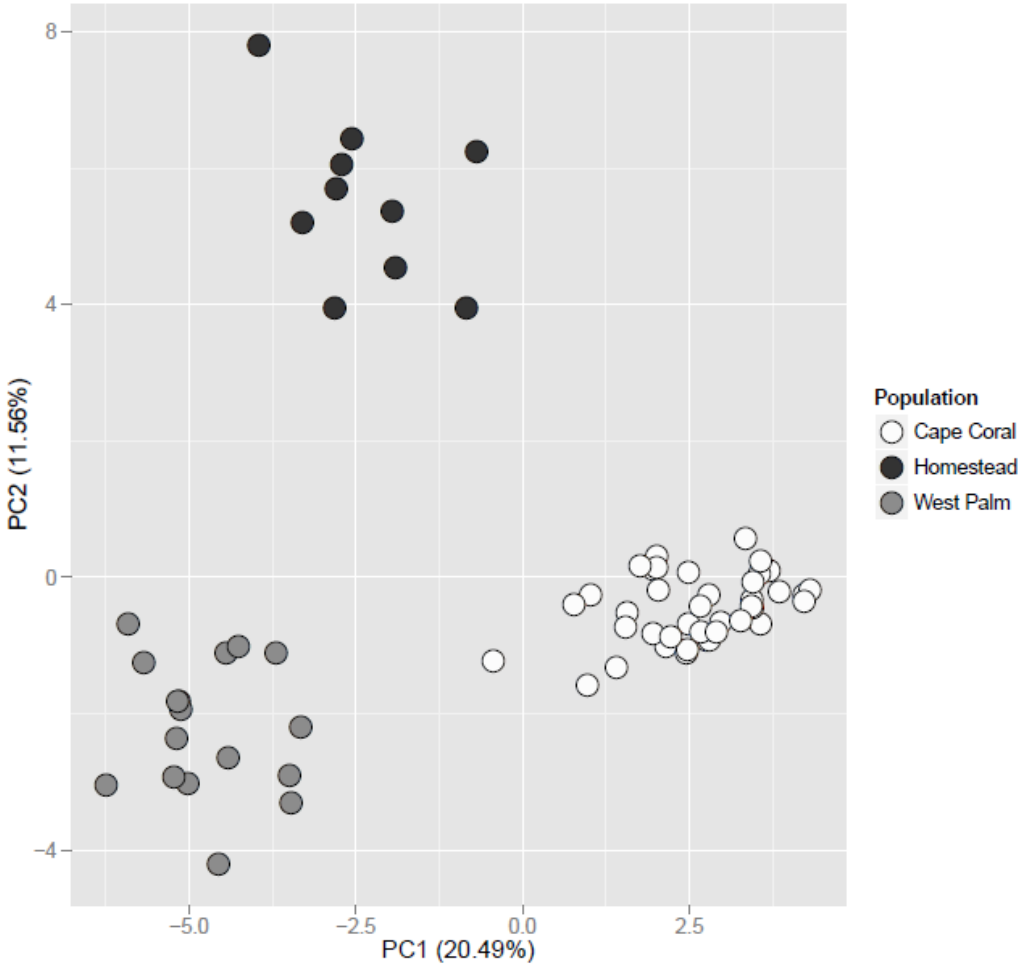

Figure 4. Principal component analysis (PCA) of *Varanus niloticus* individuals from the three Florida populations.

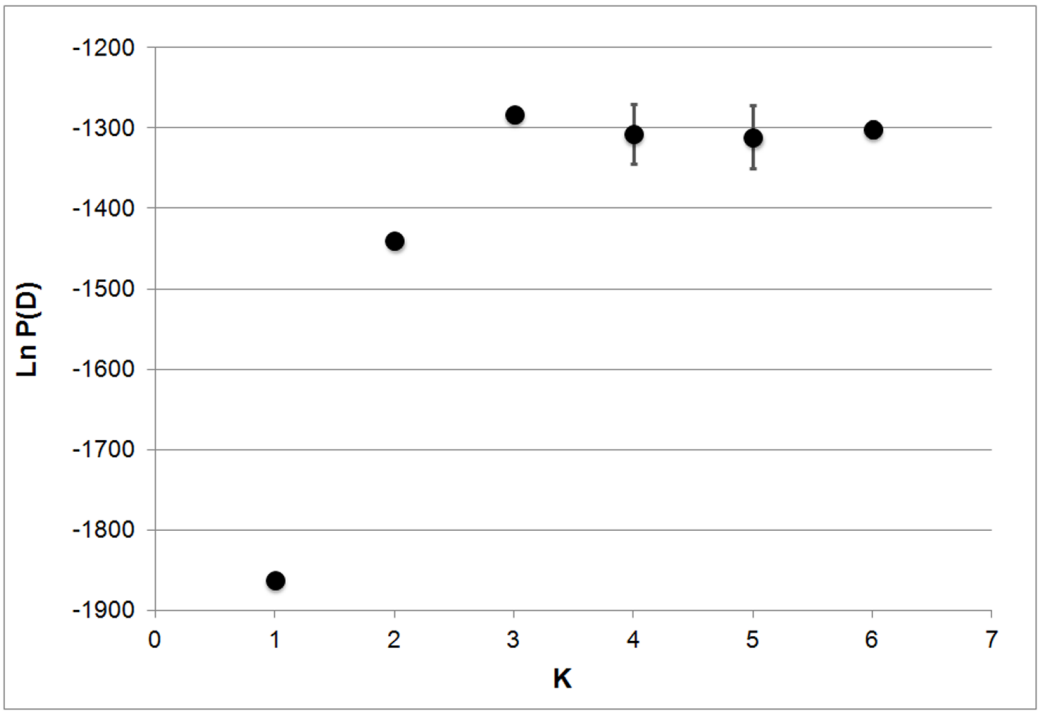

Figure 5. Results of ten replicate STRUCTURE runs for *K* = 1-6. Black circles represent means of the log probability of the data given *K* (Ln P(D)) ± one standard deviation.

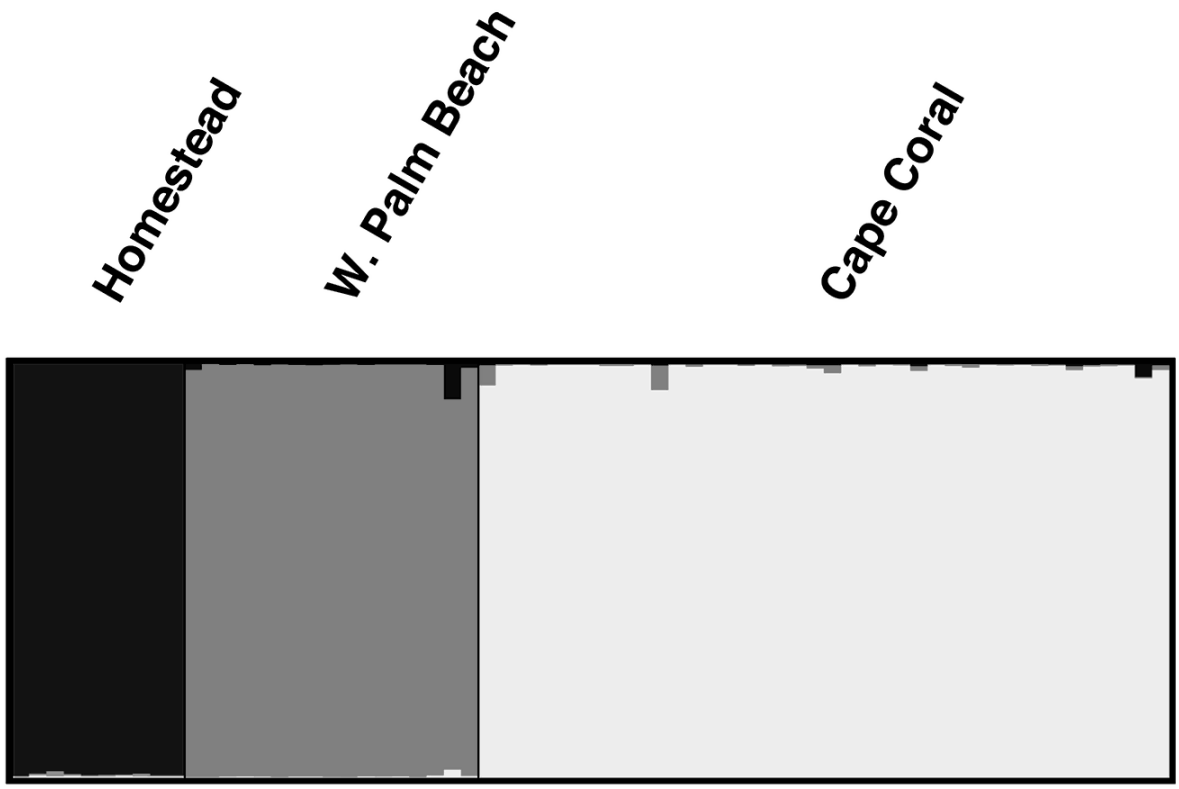

Figure 6. Results of the analysis performed in STRUCTURE when $K = 3$. Bars represent average cluster membership across 10 replicate runs that were aligned using CLUMPP.

Among-population gene flow

The overall pattern of pronounced genetic differentiation that we inferred from the analyses described above was corroborated by our assessments of gene flow. The results of BayesAss (Table 4) suggest that the proportion of migrants among all pairwise comparisons is very low compared to the degree of self-recruitment. Each population exhibited signatures of genetic isolation, showing high proportions of the genetic contribution (97–99%) originating from within the same population.

Table 4. Bayesian assessment of migration within and among Florida populations of *Varanus niloticus*. Columns represent migration sources, rows represent migration sinks, and bold values along the diagonal indicate the proportion of non-migrants. The confidence interval for each estimate is shown in parentheses.

Population	Homestead	West Palm	Cape Coral
Homestead	**0.972** (0.904–0.999)	0.014 (0.000–0.059)	0.014 (0.000–0.065)
West Palm	0.009 (0.000–0.040)	**0.982** (0.938–0.999)	0.009 (0.000–0.038)
Cape Coral	0.004 (0.000–0.018)	0.004 (0.000–0.018)	**0.992** (0.972–0.999)

The assignment-based analyses, performed in GENECLASS2, correctly assigned all 67 individuals to the locales from which they were sampled (Figure 7). Consequently, the 'L_home/L_max' statistics (see above) provided no evidence of first-generation migrants between any of the populations we sampled (all –log(L_home/L_max) = 0.0000, minimum *P*-value across all 67 samples = 0.5000). However, one individual from Homestead ($P_{Homestead}$ = 0.0013), one individual from West Palm Beach (P_{WPB} = 0.0036), and one individual from Cape Coral (P_{CC} = 0.0086) were below the threshold of the assignment analysis ($\alpha = 0.01$), raising the possibility that these individuals were introduced to these populations from unknown sources. We therefore repeated the migrant detection analysis in GENECLASS2 using the L_home likelihood estimation, which produces a more appropriate test statistic when all potential sources of migrants have not been sampled (Piry et al. 2004). Interestingly, the results of these tests suggest that the Homestead individual (-log(L_home) = 14.08, P = 0.0031), the West Palm Beach individual (-log(L_home) = 8.215, P = 0.0032), and the Cape Coral individual (-log(L_home) = 9.5470, *P* = 0.0081) are all first generation immigrants from unknown sources.

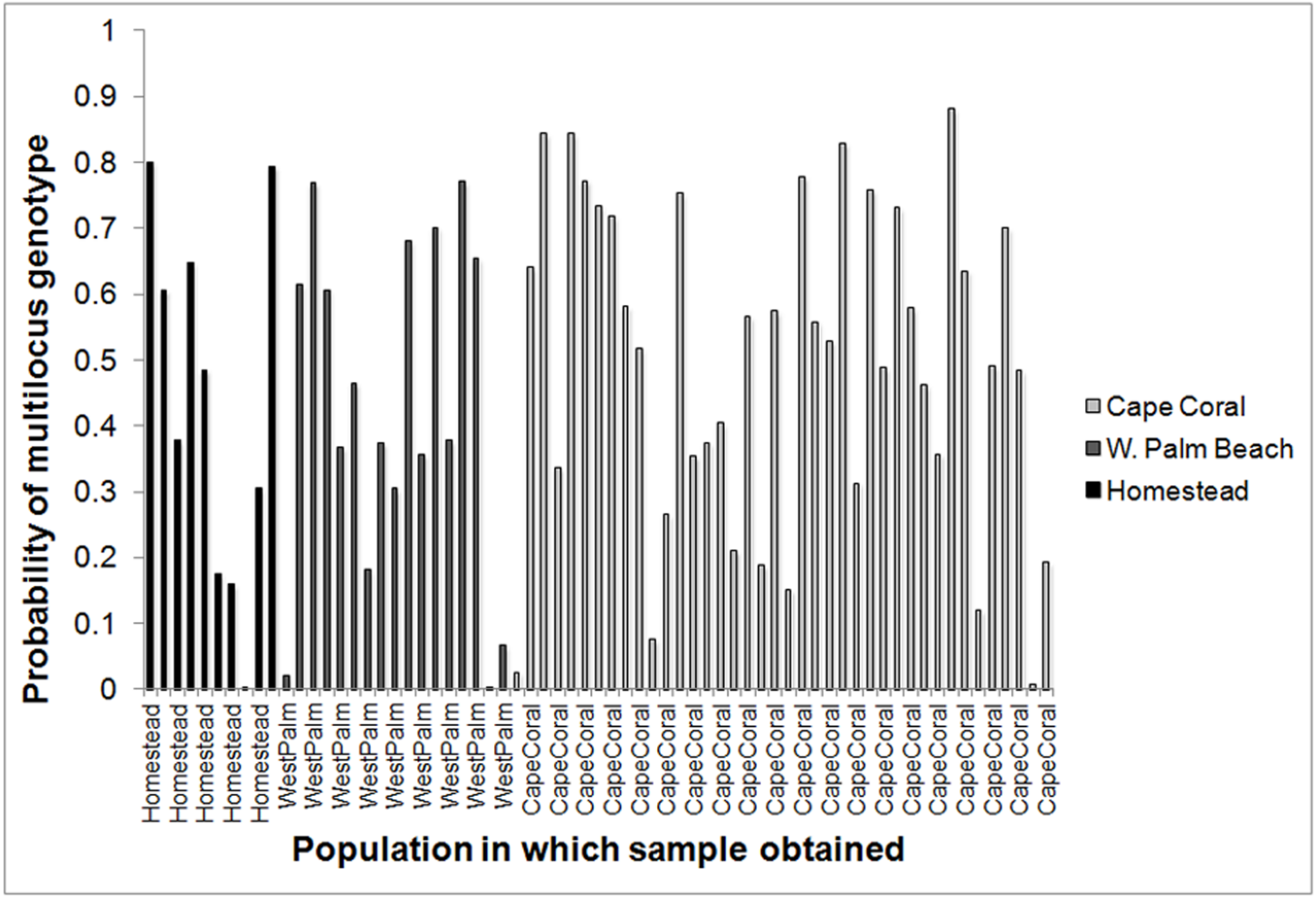

Figure 7. Stacked bar plots depicting the results of the assignment analysis performed in GENECLASS2. Each individual is represented by a bar that is presented over a label indicating the population in which that individual was sampled. For each individual, GENECLASS2 calculates the probability of that individual's multilocus genotype being

derived from Cape Coral (light gray), West Palm Beach (dark gray), and Homestead (black). Thus, each bar can consist of as many as three colors, with the height of each color indicating the relative strength of assignment to each of the three populations. The bars do not appear stacked because the relative strength of correct assignment (the likelihood that an individual originated from the population in which it was sampled) is extraordinarily high in all cases.

Effective population size and demographic changes

Across methods, the N_e for all Florida *V. niloticus* populations was estimated to be very low, ranging from 3.2–21.2 (Table 5). In general, the estimated N_e for the Cape Coral population was slightly higher than the Homestead and West Palm Beach populations.

BOTTLENECK detected significant heterozygosity excess in all Florida *V. niloticus* populations, indicating recent population declines (Table 6). Although admixture following separate introductions from differing source populations may also increase heterozygosity levels in introduced populations (Kolbe et al. 2007), the low overall genetic diversity of the introduced *V. niloticus* populations, in addition to the tight genetic clustering observed in the PCA, suggests that each population was derived from a single introduction event. Therefore, this excess of heterozygotes, relative to Hardy-Weinberg proportions, detected for each population likely resulted from reduced population sizes. The Wilcoxon test and Standardized Differences test all produced significant *P*-values across mutation models for every population (with the exception of the Standardized Differences test for Homestead under SMM). Additionally, the Sign test showed significant values for all populations under the IAM, and for the West Palm Beach and Cape Coral populations under the TPM. The Mode-shift test detected a distorted allele frequency distribution, indicative of population decline, in all Florida *V. niloticus* populations. Lastly, the calculated *M*-ratios for both the Homestead and Cape Coral populations were below, albeit within one SEM, of the critical value of 0.68, which is suggestive of population bottlenecks.

Table 5. Estimated effective population size (N_e) for Florida *Varanus niloticus* populations. The 95% confidence interval for each estimate is shown in parentheses and the symbol ∞ indicates that the program was unable to estimate N_e from the data. The linkage disequilibrium and heterozygosity excess methods were implemented in NeESTIMATOR, and the approximate Bayesian computation method was implemented in ONeSAMP.

Population	Linkage Disequilibrium	Heterozygosity Excess	Approximate Bayesian Computation
Homestead	7.2 (2.8–20.1)	∞ (4.4–∞)	13.8 (10.6–21.9)
West Palm	3.2 (2.1–9.0)	6.8 (2.9–∞)	12.1 (9.2–17.6)
Cape Coral	21.2 (9.5–66.2)	6.7 (4.1–24.1)	18.0 (13.2–26.8)

Table 6. Probability values for tests of bottleneck effects in Florida *Varanus niloticus* populations under the infinite alleles model (IAM), two-phase model (TPM), and stepwise mutation model (SMM). For the Wilcoxon test, probabilities for the one-tailed tests of heterozygote excess are shown. *M*-ratios were compared to the critical value of 0.68 to determine significance. Bold values denote significant *P*-values.

Population	Mutation Model	Sign Test	Standardized Differences Test	Wilcoxon Test	Mode-shift	*M*-ratio (SEM)
Homestead	IAM	**< 0.01**	**< 0.01**	**< 0.01**	Shifted	**0.647**
	TPM	0.15	**0.019**	**< 0.01**		(0.073)*
	SMM	0.15	0.11	**< 0.01**		
West Palm	IAM	**< 0.01**	**< 0.01**	**< 0.01**	Shifted	0.697
	TPM	**0.016**	**< 0.01**	**< 0.01**		(0.064)*
	SMM	0.073	**0.049**	**0.029**		
Cape Coral	IAM	**< 0.01**	**< 0.01**	**< 0.01**	Shifted	**0.674**
	TPM	**< 0.01**	**< 0.01**	**< 0.01**		(0.069)*
	SMM	0.096	**< 0.01**	**< 0.01**		

*Mean standard error (SEM) overlaps with critical *M*-value.

Introduction scenario testing

Introduction scenario testing revealed that hypothesizing independent introductions events (scenarios 1–4) produced higher posterior probabilities than hypotheses postulating other scenarios (Figure 8A, Table 7). Scenario 1, in which all three Florida populations originated independently from the source population around the same time, showed the highest likelihood (Figure 2). This was followed closely by scenario 2, in which the West Palm Beach and Homestead populations were introduced more recently than the Cape Coral population.

When analyzing all scenarios together (analysis A), the most likely scenario (scenario 1) showed relatively high error rates, indicating that it could not be unambiguously differentiated from the other independent introduction scenarios, which differed only by the timing of introduction (2–4; Table 7). However, when comparing scenario 1 only to the serial introduction scenarios (analysis B), the posterior probability and error rates significantly improved (Figure 8B; Table 7**)**. This indicates that the hypothesis of independent introduction events for the three *V. niloticus* population in Florida is supported over the serial introduction scenarios.

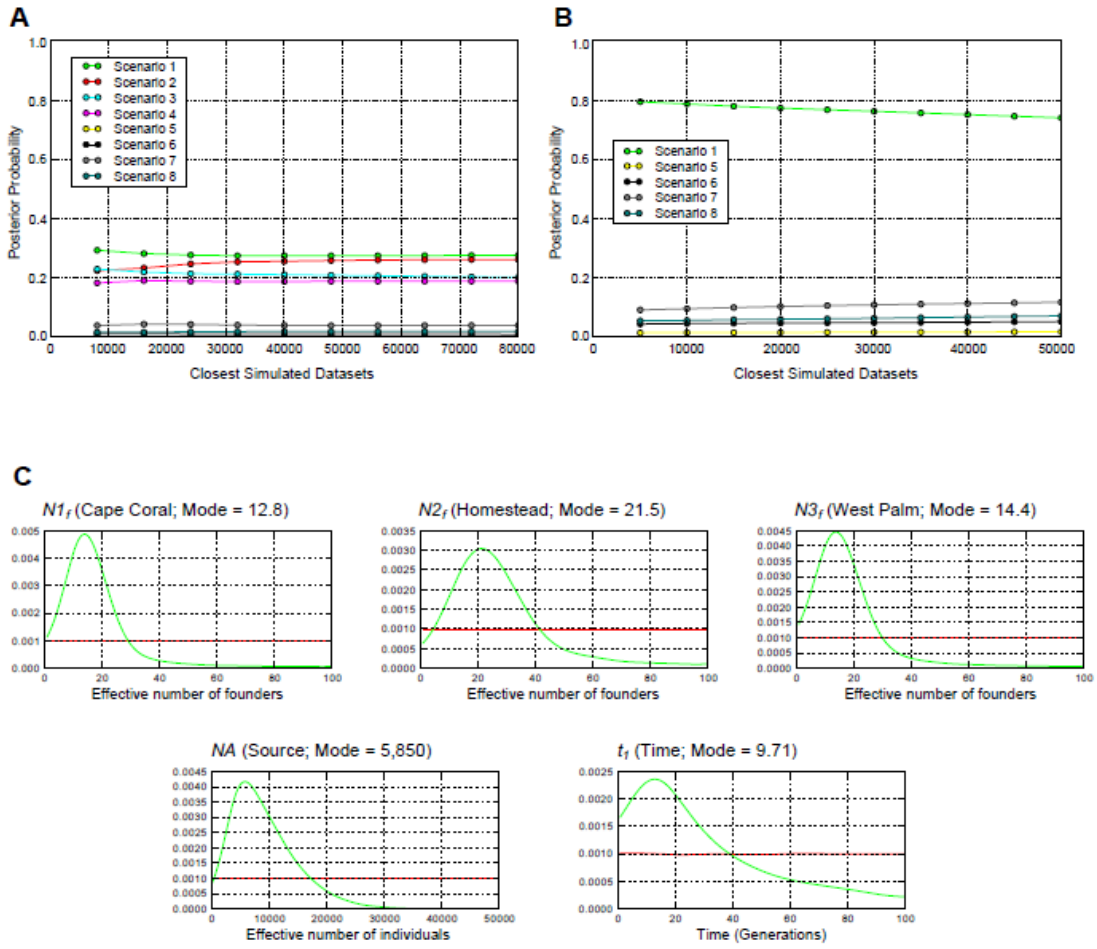

Figure 8. Logistic regression plots showing the posterior probability of (a) all eight *Varanus niloticus* introduction scenarios examined in the DIYABC analysis and (b) scenarios 1, 5, 6, 7, and 8. (c) Posterior distributions of parameters estimated from scenario 1, the most likely introduction scenario.

Table 7. Confidence in scenario selection by DIYABC for the introduction of *Varanus niloticus* into Florida. Analysis A compares all 8 scenarios, and analysis B compares scenarios 1, 5, 6, 7, and 8. See Figure 2 for a visual representation of the introduction scenarios.

Scenario	Analysis	Posterior probability	95% Credibility interval	Type I error	Type II error
* 1 – Independent introductions; Timing: Homestead (t_1) = West Palm (t_1) = Cape Coral (t_1)	A	0.2768	0.2602, 0.2935	0.58	0.097
	B	0.7399	0.7316, 0.7482	0.03	0.013
2 – Independent introductions; Timing: Homestead (t_1) = West Palm (t_1) < Cape Coral (t_2)	A	0.2620	0.2453, 0.2788	0.76	0.054
	B	N/A	N/A	N/A	N/A
3 – Independent introductions; Timing: West Palm (t_1) < Homestead (t_2) < Cape Coral (t_3)	A	0.2020	0.1882, 0.2158	0.56	0.081
	B	N/A	N/A	N/A	N/A
4 – Independent introductions; Timing: Homestead (t_1) = West Palm (t_2) < Cape Coral (t_3)	A	0.1889	0.1760, 0.2019	0.58	0.091
	B	N/A	N/A	N/A	N/A
5 – Serial introduction; Timing: Homestead from Cape Coral (t_1) < West Palm from Cape Coral (t_2) < Cape Coral introduced (t_3)	A	0.0014	0.0000, 0.0137	0.28	0.049
	B	0.0181	0.0162, 0.0201	0.38	0.063
6 – Serial introduction; Timing: West Palm from Cape Coral (t_1) < Homestead from Cape Coral (t_2) < Cape Coral introduced (t_3)	A	0.0093	0.0000, 0.0214	0.20	0.057
	B	0.0525	0.0484, 0.0565	0.23	0.12
7 – Serial introduction; Timing: West Palm from Homestead (t_1) < Homestead from Cape Coral (t_2) < Cape Coral introduced (t_3)	A	0.0402	0.0273, 0.0532	0.24	0.043
	B	0.1178	0.1119, 0.1237	0.28	0.050
8 – Serial introduction; Timing: Homestead from West Palm (t_1) < West Palm from Cape Coral (t_2) < Cape Coral introduced (t_3)	A	0.0193	0.0073, 0.0313	0.27	0.037
	B	0.0717	0.0674, 0.0760	0.29	0.063

* Most likely scenario - parameters presented for this scenario

Parameters estimated for scenario 1 showed that the effective number of founding individuals ranged from 12.8 to 21.5 (Figure 8C; Table 8); however, lack of a clear peak for the current N_e prevented accurate estimation of this parameter. The N_e for the source population was estimated to be around 5,850 individuals and the timing of the introductions likely occurred around 9.7 generations (approximately 19 years) ago. The posterior distributions for these parameters are shown in Figure 8C. The bias indices for each of the parameters are close to 0 (Table 8) indicating that the estimated parameters are robust. For all analyses, both sets of priors produced consistent outcomes, and the results of the second analysis (with prior distribution $1 < N < 100$) are presented in Tables 9-10.

Table 8. Posterior distribution statistics and bias estimates for parameters inferred from Scenario 1 of the approximate Bayesian computation analysis using the first prior set (1 < N < 10,000). $N1_f$ = Number of founders for Cape Coral population; $N2_f$ = Number of founders for Homestead population; $N3_f$ = Number of founders for West Palm Beach population; NA = Ancestral effective population size; t_1 = timing of introductions.

	$N1_f$	$N2_f$	$N3_f$	NA	t_1
Mean	19.0	28.2	19.3	9,310	31.4
Median	15.6	24.5	15.8	8,070	24.0
Mode	12.8	21.5	14.4	5,850	9.71
95% HPD	6.68–46.2	8.75–64.4	5.98–48.7	2,710–20,100	6.31–81.2
Mean Relative Bias:					
Mean	0.787 (2.885)	1.017 (1.296)	0.804 (2.603)	2.603 (3.265)	2.606 (4.048)
Median	0.626 (2.886)	0.943 (1.294)	0.630 (2.595)	2.370 (3.268)	1.965 (4.054)
Mode	0.5620	0.9507	0.5048	1.5803	0.4655
Median Relative Bias:					
Mean	0.771 (2.885)	1.003 (1.296)	0.776 (2.603)	2.608 (3.265)	2.623 (4.048)
Median	0.607 (2.923)	0.921 (1.272)	0.610 (2.571)	2.349 (3.266)	1.963 (4.100)
Mode	0.537	0.932	0.477	1.406	0.403
Square root of mean square error:					
Mean	0.823 (2.885)	1.061 (1.296)	0.848 (2.603)	2.731 (3.265)	2.671 (4.048)
Median	0.667 (2.887)	1.002 (1.294)	0.683 (2.595)	2.537 (3.268)	2.085 (4.054)
Mode	0.612	1.022	0.580	1.868	0.684

Table 9. Confidence in scenario selection by DIYABC for the introduction of *Varanus niloticus* into Florida using the secondary prior distribution of $1 < N < 100$. Analysis A compares all 8 scenarios, and analysis B compares scenarios 1, 5, 6, 7, and 8. See Figure 2 for a visual representation of the introduction scenarios.

Scenario	Analysis	Posterior probability	95% Credibility interval	Type I error	Type II error
* 1 – Independent introductions;	A	0.1935	0.1792, 0.2078	0.32	0.87
Timing: Homestead (t_1) = West Palm (t_1) = Cape Coral (t_1)	B	0.4289	0.3988,0.4591	0.09	0.13
2 – Independent introductions;	A	0.1914	0.1776, 0.2053	0.82	0.27
Timing: Homestead (t_1) = West Palm (t_1) < Cape Coral (t_2)	B	N/A	N/A	N/A	N/A
3 – Independent introductions;	A	0.1335	0.1221, 0.1449	0.5	0.55
Timing: West Palm (t_1) < Homestead (t_2) < Cape Coral (t_3)	B	N/A	N/A	N/A	N/A
4 – Independent introductions;	A	0.2068	0.1921, 0.2215	0.42	0.32
Timing: Homestead (t_1) = West Palm (t_2) < Cape Coral (t_3)	B	N/A	N/A	N/A	N/A
5 – Serial introduction; Timing:	A	0.0114	0.0012, 0.0215	0.18	0.30
Homestead from Cape Coral (t_1) <	B	0.0269	0.0118, 0.0420	0.18	0.26
West Palm from Cape Coral (t_2) < Cape Coral introduced (t_3)					
6 – Serial introduction; Timing: West	A	0.0287	0.0190, 0.0384	0.18	0.24
Palm from Cape Coral (t_1) <	B	0.0628	0.0477, 0.0779	0.12	0.24
Homestead from Cape Coral (t_2) < Cape Coral introduced (t_3)					
7 – Serial introduction; Timing: West	A	0.1372	0.1223, 0.1520	0.53	0.47
Palm from Homestead (t_1) <	B	0.2833	0.2576, 0.3090	0.48	0.38
Homestead from Cape Coral (t_2) < Cape Coral introduced (t_3)					
	A	0.0975	0.0859, 0.1091	0.54	0.47

8 – Serial introduction; Timing: Homestead from West Palm (t_1) < West Palm from Cape Coral (t_2) < Cape Coral introduced (t_3)	B	0.1980	0.1796, 0.2164	0.5	0.36

* Most likely scenario - parameters presented for this scenario

Table 10. Posterior distribution statistics and bias estimates for parameters inferred from Scenario 1 of the approximate Bayesian computation analysis using the secondary prior set ($1 < N < 100$). $N1_f$ = Number of founders for Cape Coral population; $N2_f$ = Number of founders for Homestead population; $N3_f$ = Number of founders for West Palm Beach population; NA = Ancestral effective population size; t_1 = timing of introductions.

	$N1_f$	$N2_f$	$N3_f$	NA	t_1
Mean	48.6	53.8	47.3	7,160	46.5
Median	45.8	52.7	43.6	5,740	45.3
Mode	20.2	33.8	18.1	2,740	44.0
95% HPD	11.1–93.6	14.2–95.1	9.96–93.1	1,880–16,900	20.5–76.9
Mean Relative Bias:					
Mean	1.28 (1.522)	0.688 (0.486)	1.558 (1.808)	2.135 (8.140)	0.365 (0.147)
Median	1.101 (1.521)	0.703 (0.482)	1.361 (1.808)	1.505 (8.136)	0.372 (0.147)
Mode	-0.293	1.238	-0.203	0.373	0.374
Median Relative Bias:					
Mean	1.281 (1.522)	0.690 (0.486)	1.557 (1.808)	2.037 (8.138)	0.376 (0.147)
Median	1.109 (1.500)	0.712 (0.470)	1.363 (1.833)	1.401 (8.136)	0.385 (0.136)
Mode	-0.349	1.796	-0.259	0.260	0.364
Square root of mean square error:					
Mean	1.289 (1.522)	0.695 (0.486)	1.567 (1.808)	2.422 (8.140)	0.391 (0.147)
Median	1.126 (1.521)	0.714 (0.482)	1.380 (1.808)	1.801 (8.136)	0.404 (0.147)
Mode	0.397	1.522	0.377	0.751	0.435

Discussion

Conceptual framework and intra-population patterns

The fact that invasion is a common biological phenomenon was once considered to be a genetic paradox (*e.g.,* Allendorf and Lundquist 2003; Frankham 2005; Lawson Handley et al. 2011). The first reason for this is that rates of adaptive evolution depend critically on additive genetic variation (Fisher 1958). Hence, recently founded populations with reduced genetic variation are expected to have limited capacities for adaptive evolution, as they struggle to become established in novel environments (Allendorf and Lundquist 2003). The second reason for an ostensible genetic paradox stems from the dynamics of small populations, in which loss of genetic diversity due to drift and elevated inbreeding (Frankham et al. 2010) is expected to act against would be invaders during the earliest phases of their establishment. Over the past decade, much progress has been made in understanding the genetic dynamics associated with invasion (reviewed by Lawson Handley et al. 2011). Importantly, a number of studies have shown that phenomena such as multiple introductions followed by admixture (*e.g.,* Kolbe et al. 2004; 2008; Facon et al. 2008) and a lack of correlation between molecular and quantitative genetic diversity (*e.g.,* Reed and Frankham 2001; Dlugosch and Parker 2008) may resolve the 'genetic paradox of invasion biology'. Indeed, invasion is now typically conceptualized as a multistage process that entails a lag phase, during which adaptations that facilitate invasiveness arise, followed by rapid range expansion (Keller and Taylor 2008). As such, catching potentially problematic populations early during the invasion process is of critical importance from a management perspective.

Although definitive conclusions about reductions to genetic diversity would require comparisons to populations in the native range (*sensu* Dlugosch and Parker 2008), our results do suggest that *V. niloticus* populations in Southern Florida are in the process of recovering from recent bottlenecks. Assessments of heterozygosity excess (BOTTLENECK) and allele distributions (*M*-ratio) both provided evidence of recent population declines in all three Florida *V. niloticus* populations. The view that these populations are still recovering from founder effects is additionally supported by our estimates of genetic richness (mean number of alleles per locus between two and three in all three populations), which are low when compared to estimates from microsatellite surveys of native, non-threatened varanid populations (Fitch et al. 2005; Fu et al. 2011) as well as native *V. niloticus* populations under harvest pressures (Dowell et al. 2015). Furthermore, the current N_e estimated for all three Florida populations was low compared to assessments of native *V. niloticus* populations (Dowell et al. 2015). Nevertheless, because none of the Florida populations of *V. niloticus* are inbred (see below) and most loci had more than one allele present at appreciable frequencies, heterozygosity-based measures of diversity were more substantial ($0.410 < \text{mean } H_e < 0.460$ in all three populations). Indeed, the degree of similarity in genetic richness and diversity among the three Florida populations (see Table 2) is rather remarkable given that these populations are generally assumed to be quite different in size (Cape Coral >> West Palm Beach >> Homestead) and time since establishment (by ca. 1990, 2000, and 2004 respectively; Enge et al. 2004; Campbell 2005). Perhaps most surprising is that the large, comparatively old, and deeply sampled Cape Coral population had the lowest diversity among the three populations, raising the possibility that this population was established

by a smaller and/or less diverse group of founders than the Homestead and West Palm Beach populations (see below).

A recent study by Dowell et al. (2015) examined the fine-scale genetic patterns of *V. niloticus* populations in West Africa under varying levels of exploitation pressure, and represents the only population-level assessment of native *V. niloticus* populations. For the four discrete populations that were inferred from microsatellite data, both genetic diversity and effective population size estimates were larger than for the introduced populations examined here, displaying H_e values between 0.328 – 0.429, and N_e estimates ranging from 10.9 – 1,327.27, depending on the population and method of analysis (Dowell et al. 2015). However, this study does not provide information on unharvested populations, and thus the results may not be representative of native *V. niloticus* populations across their full distributions. Additionally, because the previous study utilized different microsatellite markers than our present investigation, we were unable to make direct comparisons between these parameters.

Genetic structure and introduction scenario

We assessed the degree of genetic structure among the Cape Coral, Homestead, and West Palm Beach populations via several independent analyses that are based on a variety of conceptual and computational frameworks. In all cases, the results suggest there is marked genetic differentiation among South Florida's documented *V. niloticus* populations. Interestingly, the pair-wise G statistics that we calculated revealed that all three populations exhibit similar levels of differentiation (see above), lending credence to

the preliminary results of ongoing work suggesting that Florida's documented Nile monitor populations are all derived from a single evolutionary lineage in West Africa (Dowell et al. unpublished data). One of the approaches to assignment that we used (GENECLASS2) explicitly failed to detect migrants among the three populations and the other approaches explicitly indicated that there is little evidence for admixture.

Approximate Bayesian computation (ABC) has been widely used to differentiate complex models (reviewed in Beaumont 2010), including large numbers of complex introduction scenarios for invasive species (Auger-Rozenberg et al. 2012; Benazzo et al. 2015; Boissin et al. 2012; Boubou et al. 2012; Konečný et al. 2013). Upon introduction, populations may undergo stochastic processes, such as genetic drift and admixture, producing complicated genetic signatures that are undetectable by most genetic analysis methods (Guillemaud et al. 2010). The model-based approaches underlying ABC analyses are superior to other methods, including maximum-likelihood, for identifying complex demographic scenarios (Beaumont 2010; Guillemaud et al. 2010). While our introduction scenario analysis could not differentiate among hypotheses differing in the timing of introduction events, we found strong support for independent introductions over serial introduction hypotheses. The inferred timing of introduction (approximately 19 years ago) roughly corresponds to when the first *V. niloticus* individuals were observed in Florida. Additionally, this analysis suggests that the Cape Coral population was founded by fewer individuals than the other populations, which is reflected in the lower genetic diversity estimates for Cape Coral (see above). Collectively, these results strongly support the view that the *V. niloticus* populations in Cape Coral, Homestead, and West

Palm Beach resulted from independent introduction events and that these populations are not connected by substantive gene flow.

Although these findings are encouraging in terms of management plans aimed at control and/or eradication, the possibility of additional populations and/or releases raised by our analysis in GENECLASS2 is cause for concern. While it is true that none of the *P*-values associated with the -log(L_home) tests for first generation migrants would pass a multiple-testing correction that adjusted across all individuals (0.05/67 ~ 0.0007 and minimum $P = 0.0031$), numerous unverified sightings of *V. niloticus* have been reported in five counties that have no confirmed breeding populations (Florida Wildlife Commission 2015). As such, the identification of putative migrants from unknown sources in all three populations is not particularly surprising.

Conclusion and management recommendations

In this paper, we present data that are consistent with the idea that Southern Florida's *V. niloticus* populations are still in the relatively early stages of the invasion process. All three populations that we sampled exhibit limited genetic diversity and show signs of drift-mutation disequilibrium. In addition, anecdotal information on area occupied and yield as a function of trapping effort suggest that the West Palm Beach and Homestead populations are still relatively small. Our data also strongly suggest that *V. niloticus* has been introduced to Southern Florida on at least three separate occasions, as the Cape Coral, Homestead, and West Palm Beach populations are all well differentiated from one another genetically. Given the roles that multiple introductions, admixture, and heterosis may play in the invasion process (Facon et al. 2008; 2010), this result is simultaneously encouraging and cause for concern. In contrast to our findings, multiple

introduction events followed by admixture have made many invasive brown anole (*Anolis sagrei*) populations in Florida more diverse than the native Cuban populations from which they are derived (Kolbe et al. 2004). Moreover, analyses of seven additional invasive *Anolis* species in Florida and the Dominican Republic led Kolbe et al. (2007) to hypothesize that admixture between independently introduced individuals of varied genetic background may be a common mechanism by which genetic variation in invasive populations becomes elevated after the initial bottlenecks associated with founding events. Thus, it is imperative that wildlife managers focus on containment strategies aimed at preventing inter-regional admixture, which could enhance the invasiveness of *V. niloticus* in Florida. Given Florida's extensive network of canals, the high mobility of *V. niloticus*, and the number of confirmed sightings (Figure 1; Table 1) in regions removed from the three documented populations examined in this study, it is possible, if not likely, that intra-regional dispersal is already occurring. Indeed, the existence of metapopulations and hierarchical population structure is a potential explanation of the genetic evidence we present for migrants from unknown sources. As such, concerted follow-ups on credible sightings are warranted.

It is noteworthy to mention that *V. niloticus* has been listed as a conditional species by the Florida Wildlife Commission since 2010. Therefore, only breeders, public exhibitioners, researchers, and nuisance trappers that have obtained a permit, for which they must maintain records for each animal they possess, can keep and/or transport *V. niloticus* (http://myfwc.com/wildlifehabitats/nonnatives/regulations/snakes-and-lizards/). Consequently, it is unlikely that the pet trade is still contributing to ongoing introductions in Florida. At present, treating the regions around Cape Coral, West Palm Beach, and

Homestead as separate management units appears to be a sensible management strategy. However, the situation should continue to be monitored for evidence of gene flow and admixture.

CHAPTER V

INSIGHTS INTO THE INTRODUCTION HISTORY AND POPULATION GENETIC DYNAMICS OF THE ARGENTINE BLACK AND WHITE TEGU (*SALVATOR MERIANAE*) IN FLORIDA

Introduction

The second greatest threat to global biodiversity is the spread of invasive species (Wilcove et al. 1998). Invasive species can negatively impact native species either directly through competition, predation, and disease or indirectly through alteration of ecosystem structure and function (Klug et al. 2015; Mooney and Cleland 2001) The spread of invasive species has accelerated over the last few centuries due to increases in international trade and transport (Abdelkrim et al. 2005; Di Castri 1989; Mack et al. 2000), and port-rich coastal regions have frequently served as points of entry. Florida is especially susceptible to the proliferation of invasive reptiles largely due to three factors: (1) a subtropical climate; (2) the presence of altered habitats (ponds, canals, levees) that provide suitable migration corridors for invasive species; and (3) an extensive exotic pet industry that imports and/or produces potentially invasive organisms (Mazzotti et al. 2015; Smith 2006). Consequently, in Florida, there are more nonnative lizards than native lizard species (Krysko et al. 2011; Pernas et al. 2012). One of the nonnative species that is of particular concern is the Argentine black and white tegu (*Salvator merianae*) (Klug

et al. 2015). *S. merianae* was first observed in Hillsborough County, Florida in 2006 on the Balm Boyette Nature Preserve (Enge 2007). Purportedly, individuals to be observed were introduced by a dealer that illegally released specimens with broken tails or other defects that diminished their market value (Enge 2007). In addition to the Hillsborough population, there is also a self-perpetuating *S. merianae* population approximately 300 km away in Miami-Dade County near Florida City (Pernas et al. 2012).

Salvator merianae is a large lizard with a broad, omnivorous diet that consists of vegetation, fruit, seeds, snails, arthropods, fish, birds, bird eggs, small mammals, amphibians, reptiles, reptile eggs, and carrion (Galetti et al. 2009; Kiefer and Sazima 2002; Mercolli and Yanosky 1994). Due to *S. merianae's* propensity for depredating nests, this species poses a direct threat to Florida's sensitive, ground-nesting species such as American crocodiles (*Crocodylus acutus*), Eastern indigo snakes (*Drymarchon couperi*), Cape Sable seaside sparrow (*Ammodramus maritimus mirabilis*), and gopher tortoises (*Gopherus polyphemus*) (Mazzotti et al. 2015). *S. merianae* is native to southeastern Brazil, Uruguay, eastern Paraguay, and northern Argentina (Luxmoore et al. 1988). Within their native range *S. merianae* occupy open habitats such as forest clearings, secondary forests, and other disturbed areas across a broad range of tropical, subtropical, and temperate climates (Cardozo et al. 2012; Chamut et al. 2012; Embert et al. 2010; Fitzgerald 1994; Winck and Cechin 2008). *S. merianae* also exhibits dormancy in response to winter temperatures and periods of drought (Abe 1983). Based on these distributional and ecological characteristics, Lanfri et al. (2013) suggested that *S. merianae* could spread as far north as West Virginia.

Preventing the spread of harmful species, such as *S. merianae,* is necessary for effective management planning. However, the control of invasive species is often hindered by a lack of information about the history and origins of the population in question and the level of connectivity between groups of individuals (Rollins et al. 2009). It is generally assumed that isolated populations are easier to eradicate than populations that are connected by migration and gene flow, because connected populations may require simultaneous eradication to prevent recolonization by migrants from neighboring areas (Abdelkrim et al. 2005; Rollins et al. 2009). As such, when populations are connected, management strategies focused on containment may be most feasible (Rollins et al. 2009).

Currently, the introduction histories of Florida's *S. merianae* populations are not known. Furthermore, it is unclear whether there is migration between the Hillsborough and Miami-Dade populations. Examination of genetic structure across the range of an introduced species can provide insight into these issues and enable wildlife managers to avoid arbitrary decisions and/or labor intensive field methods such as radio telemetry (Abdelkrim et al. 2009). To this end, we used microsatellite markers to examine intra-population genetic diversity, genetic structure, and possible introduction scenarios in Florida's documented *S. merianae* populations.

Materials and Methods

Field sites, sampling, and tissue collection

Salvator merianae specimens were collected from Hillsborough and Miami-Dade counties, Florida (Figure 1). In Hillsborough County, *S. merianae* specimens are primarily found in ruderal habitats near Balm Boyette Scrub Preserve located between the cities of Riverview and Lithia. At the time of this study, 38 specimens had been collected from this locale—all of which were used in this study. These samples were collected between 2012 and 2013 by one of us (TSC) from a 43.5 km^2 area centered around approximately 27°47’55”N, 82°11’56”W.

In Miami-Dade County, *S. merianae* are primarily found in the southeastern portion of the County near Florida City (25°23’02”N, 80°30’44”W). To date, nearly 600 specimens (Klug et al. 2015) have been removed from this area — a subset ($N = 40$) of which was used in this study. *S. merianae* specimens in Miami-Dade County are primarily removed from disturbed areas such as ditches, canal levees, and historical wetlands that are comprised of late successional grasslands that are being replaced by shrubs and grasslands (Klug et al. 2015). The *S. merianae* specimens from Miami-Dade County that were used in this study were captured between 2009 and 2011. The Florida Wildlife Commission provided these samples.

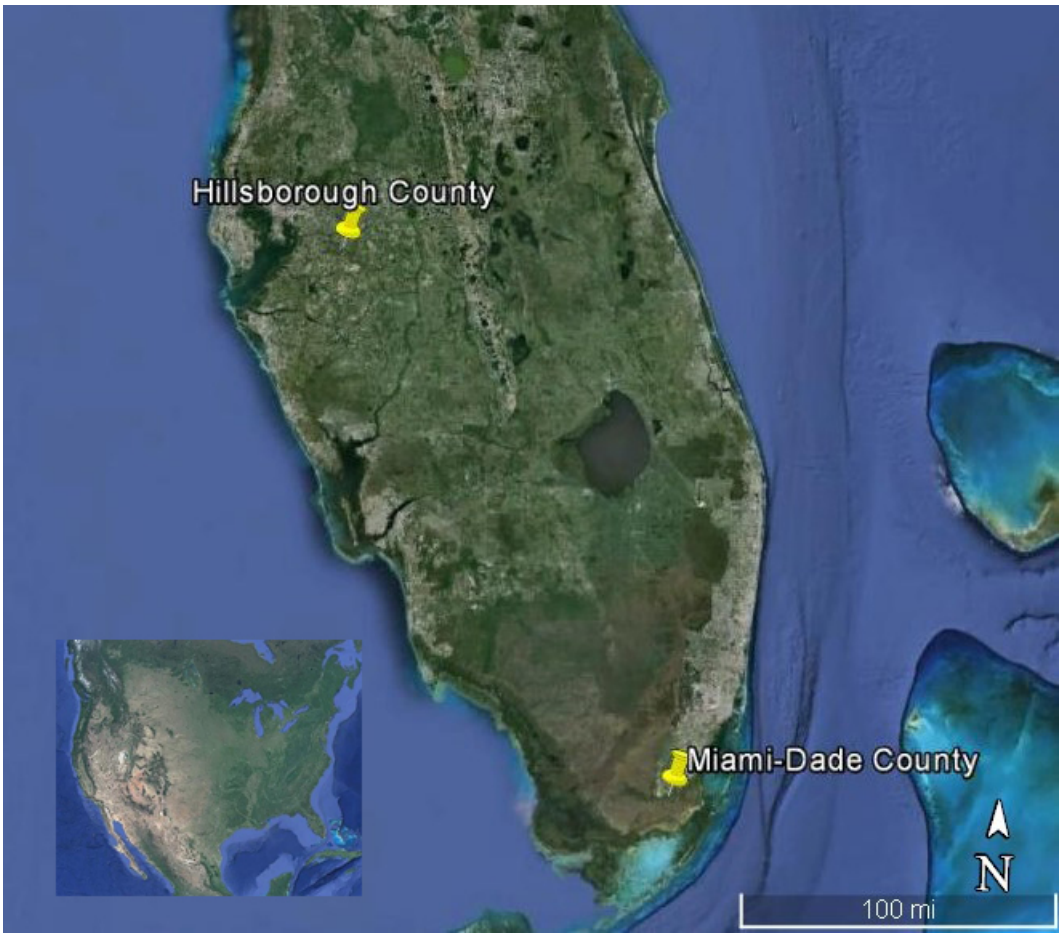

Figure 1. Map showing the location of the sampling sites of *S. merianae* in Southern Florida and the position of Florida within the Southeastern US.

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DNA isolation and PCR-based genotyping

We extracted Genomic DNA from muscle and liver samples obtained from a total of 78 tegus (Hillsborough: $N = 38$; Miami-Dade County: $N = 40$) using the Wizard Genomic DNA Purification Kit (Promega) according to the manufacture's instructions. We examined 14 microsatellite loci developed using *S. merianae* samples from the Miami-Dade population (Wood et al. 2015). All PCRs had a final volume of 25 µl and contained 2 µl of template (DNA concentration between 10 and 100 ng /µl), 1x buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.8 µM of non-M13(-21)-twinned primer, 0.8 µM 0f 6-FAM labeled M13(-21) primer, 0.2 µM of M13(-21)-twinned primer, and 0.625 units of GoTaq polymerase (Promega). Reaction conditions were as follows: 2 min at 94° C followed by 25 cycles of 94° C for 30 s, 30 s at 62° C decreasing by –0.3°C per cycle, and 72° C for 40 s, followed by eight cycles of 94° C for 30 s, 53° C for 30 s, and 72° C for 40 s, followed by a final cleanup step of 30 min at 72° C. Agarose gel electrophoresis (2% gels) was used to confirm successful amplification, and fragment analysis was performed at the Arizona State University DNA Lab using an Applied Biosystems 3730. PEAK SCANNER 1.0 (Applied Biosystems) was used to manually score all loci. In order to identify breaks in the amplicon sizes, allelic bins were determined by graphically examining the rank-ordered fragment size distributions of each locus (Guichoux et al. 2011). Finally, Microsoft EXCEL was used to bin the data from each locus into discrete classes that were defined by each allele's empirically determined size range.

Summary statistics and quality control

We used MICRO-CHECKER 2.2.3 (Van Oosterhout et al., 2004) to examine each locus for evidence of null alleles, large allele dropout, and scoring errors. GENALEX 6.5 (Peakall and Smouse, 2012) was used to calculate summary statistics including number of alleles, effective number of alleles, observed heterozygosity, and expected heterozygosity. GENEPOP 4.3 (Rousset, 2008) was also used to test for departures from Hardy-Weinberg proportions and genotypic equilibrium. Finally, GENEPOP 4.3 was used to calculate the Weir and Cockerham (1984) estimator of F_{IS}.

Assessment of population structure

Several approaches were used to determine the degree of genetic differentiation between the *S. meriane* populations in Hillsborough and Miami-Dade Counties. GENALEX 6.5 (Peakall and Smouse 2012) was used to calculate G_{ST} values based on Nei and Chesser's (1983) unbiased estimators of H_S and H_T and to calculate Hedrick's further standardized G_{ST} (G''_{ST}; Meirmans and Hedrick 2011). All resampling tests conducted in GENALEX were based on 9,999 permutations. We also performed an AMOVA that partitioned genetic variation among populations, among individuals within populations, and within individuals using ARLEQUIN 3.5.1.2 (Excoffier and Lischer 2010).

STRUCTURE 2.3.4 (Pritchard et al. 2000; Falush et al. 2003) was used to estimate the number of populations (*K*) and to assign individuals to populations (i.e.,

clusters). We also used STRUCTURE HARVESTER (Earl et al. 2012) to compute the optimal *K* based on ΔK (Evanno et al. 2005). We used the correlated allele frequencies model to allow for the possibility that both populations originated from a common source and allowed for the possibility of admixture. We conducted 10 replicate STRUCTURE runs for K = 1-6 with a burn-in period of 500,000, followed by 500,000 MCMC steps. CLUMPP (Jakobsson and Rosenberg 2007) was used to align cluster assignment across replicate runs and STRUCTURE PLOT (Ramasamy 2014) was used to visualize and interpret the results of the summarization across runs produced by CLUMPP.

Because introduced populations may not exhibit Hardy-Weinberg or linkage equilibrium, the major assumptions of STRUCTURE (Pritchard et al. 2000), it is also important to examine the genetic partitioning of these populations using alternate approaches. Therefore, we performed a Principal Component Analysis (PCA) on raw genotypes with the gstudio package (Dyer 2012) in R 3.1 (R Core Team 2014) and plotted the results with ggplot2 (Wickham 2009).

Among-population gene flow

We assessed the degree of recent gene flow between the Hillsborough and Miami-Dade populations with BAYESASS 1.3 (Wilson and Rannala 2003). This method infers pairwise migrations rates during recent generations by utilizing a coalescent-based approach. We performed 10^8 iterations, sampling every 2,000 iterations, with a burn-in of 10^7. To determine if the runs had reached convergence, we plotted likelihood scores over time and examined the consistency of results across independent runs.

In addition, we used GENECLASS2 (Piry et al. 2004) to perform assignment tests via Paetkau's (1995) frequency-based criterion. We used a default frequency of 0.01 for missing alleles and the Monte-Carlo resampling method described by Paetkau et al. (2004). Probability computations were based on 10,000 simulated individuals, and the type I error rate was 0.01. GENECLASS2 and Paetkau's (1995) frequency-based criterion were also used to test for the presence of first-generation migrants. Since the Hillsborough and Miami-Dade populations represent the only known *S. merianae* populations in Florida, we used the 'L_home/L_max' test statistic because it is most appropriate when all source populations have been sampled (Piry et al. 2004).

Effective population size and demographic history

To further examine the possibility of inbreeding within the introduced *S. merianae* populations, we estimated their effective population sizes (N_e) with NeESTIMATOR 2.0 (Do et al. 2014). These estimates were inferred using the linkage disequilibrium (LD) method, which is based on the frequent occurrence of non-random associations of alleles across independent loci in small populations (Waples and Do 2008). For comparison, we additionally estimated N_e using the heterozygote excess method, which is based on the observation that a small number of breeding individuals in a population frequently results in an excess of heterozygotes in the next generation (Zhdanova and Pudovkin 2008), as well as the molecular coancestry method, based on allele sharing (Nomura 2008).

We tested for evidence of recent population declines using the program BOTTLENECK 1.2.02 (Piry et al. 1999). This method assesses deviations from expected heterozygosity, indicative of population decline (heterozygote excess) and expansion (heterozygote deficiency), as well as examines the distribution of allele frequencies, which are typically skewed following bottleneck events (Piry et al. 1999). We tested for deviations under the stepwise mutation model (SMM), infinite alleles model (IAM), and the two-phase model (TPM) with 70% SMM. We performed 1,000,000 iterations and tested for significance with the sign test, standardized differences test, Wilcoxon signed-rank test, and mode-shift test, all implemented by BOTTLENECK. We additionally tested for genetic signatures of population expansion by performing a within-locus *k* test and an interlocus *g* test with the program KGTESTS (Bilgin 2007). The *k* test is based on the observation that the typical allele distribution at a locus has several modes in a constant-sized population due to a small number of historic splitting events in the genealogy (Reich and Goldstein 1998, Reich et al. 1999). Conversely, an expanding population shows a more peaked allele distribution with a single mode due to many recent splitting events occurring near the time of the expansion (Reich and Goldstein 1998, Reich et al. 1999). Furthermore, expanding populations typically show lower levels of variance in the widths of allele distributions across loci than do constant-sized populations (Reich and Goldstein 1998). Therefore, the *g* test measures the variance in the allele distribution at each locus as well as the variance of these variances across loci to determine if a population shows evidence of expansion (Reich et al. 1999). Finally, we calculated *M*-ratios (Garza and Williamson 2001) in EXCEL using the output from GENALEX. *M*-ratios are defined as the ratio of *k* (total number of alleles) to *r* (overall

range in allele size in number of repeat units). These ratios can be indicative of recent bottlenecks when they are less than the critical value of 0.68 defined by Garza and Williamson (2001).

Introduction scenario testing

To infer the introduction history of the Florida *S. merianae* populations, we tested six competing scenarios with DIYABC 2.1.0 (Cornuet et al. 2014). These scenarios test various hypotheses of the *S. merianae* introduction, including two independent introduction events from South America (scenario 1) and serial introduction pathways, where the second introduced population originated from the first introduced population, rather than separately from the native source population (scenarios 2 and 3). Additionally, we tested for the possibility of a 'ghost' population, i.e. a population that is contributing to the introduced populations but has yet to be genetically sampled. Scenario 4 describes a situation where a single introduction event occurred resulting in an undetected population, and the two sampled populations subsequently emerged from this original population. Lastly, scenarios 5 and 6 hypothesize a combination of independent introductions and the presence of an unsampled population.

For all analyses, we used uniform prior distributions defined as follows: : $1 < N < 10{,}000$; $1 < NG < 10{,}000$; $1 < NA < 50{,}000$; $1 < N_f < 100$; $1 < \text{db} < 20$; $1 < t_1 < t_2 < t_3 < 100$; where '*N*' denotes the current effective population size, '*NA*' denotes the ancestral (source) effective population size, '*NG*' denotes the unsampled (ghost) effective population size, 'N_f' denotes the effective number of founding individuals, 'db' denotes

the bottleneck duration in generations, and '*t*' the time in generations. Priors for the microsatellite mutation model were set to default values, including the Generalized Stepwise Mutation model (Estoup et al. 2002), and a uniform prior distribution for both the mean mutation rate (1E-4 to 1E-3) and the geometric distribution (1E-1 to 3E-1). Summary statistics included the mean number of alleles, mean genic diversity, and mean size variance for both the one-sample and two-sample statistics. Additionally, we used the mean Garza-Williamson's M index (one-sample statistic) as well as pairwise F_{ST} values and the mean classification index (two-sample statistics). We simulated 1 million datasets for each scenario, for a total of 6 million, and evaluated the scenario and parameters priors by performing a PCA, as implemented in the program.

We determined the optimal scenario based on posterior probabilities compared using the logistic regression analysis implemented in DIYABC, using the 1% closest simulated data sets. For comparison, we additionally performed a pre-processing step (Linear Discriminant Analysis) on the summary statistics prior to computing the logistic regression. To further evaluate the power of our ABC method in distinguishing among the various competing scenarios, we analyzed 100 simulated pseudo-observed data sets (pods) for each scenario, using parameter values drawn from the same prior distribution as our previous analyses. The relative posterior probabilities of each scenario, estimated for each pod, were then used to calculate the likelihood of excluding the focal scenario when it is actually the true scenario (type I error rate), as well as the likelihood of selecting the focal scenario when it is not the true scenario (type II error rate).

We computed the posterior distributions of the parameters under the most likely scenario, using the logit transformation on the 1% closest simulated data sets. Confidence

in the parameter estimations was assessed by calculating relative bias and relative root mean square error, based on 5,000 pods drawn from the posterior distributions.

Results

Summary statistics and quality control

In total, we genotyped 78 individuals at 14 microsatellite loci. While all 14 loci were polymorphic, the summary statistics presented in Table 1 suggest that the Hillsborough and Miami-Dade populations both have limited genetic diversity. Upon performing Holm's (1979) correction for multiple testing via treating the tests associated with each population as a family of tests, we detected significant departures from Hardy-Weinberg proportions for *Teg4*, *Teg*5, *Teg14*, *Teg17*, and *Teg*19. In addition, *Teg4*, *Teg5,* and *Teg19* exhibited homozygote excess. Not surprisingly, MICRO-CHECKER detected evidence of null alleles for *Teg4*, *Teg5,* and *Teg19*. Upon performing Holm's (1979) correction for multiple testing, there was evidence for genotypic disequilibrium between *Teg14-Teg19* in the Miami-Dade population. Due to the aforementioned quality control issues, we removed *Teg4, Teg5, Teg17*, and *Teg19* from all further analyses. Thus, all analyses performed in GENALEX, ARLEQUIN, STRUCTURE, BAYESASS, GENECLASS2, NeESTIMATOR, KGTESTS, ONeSAMP, BOTTLENECK, and DIYABC were based on the 10 remaining loci.

Table 1. Summary statistics and diversity estimates for the 14 loci that were used for genotyping.

Locus/Pop.	*N*	*k*	H_O	H_E	F_{IS}	No. Effective Alleles	No. Private Alleles	*M*
Hillsborough								
Teg1	27	4	0.74	0.66	-0.10	2.96	0	0.80
Teg2	34	3	0.62	0.49	-0.25	1.96	1	0.60
Teg4	37	3	0.00	0.10	1.00*†	1.12	2	0.60
Teg5	27	4	0.52	0.70	0.28†	3.32	1	0.50
Teg6	33	2	0.55	0.49	-0.10	1.96	1	0.67
Teg7	32	4	0.53	0.61	0.15	2.58	0	0.57
Teg9	30	3	0.63	0.64	0.03	2.78	1	0.50
Teg10	31	3	0.61	0.66	0.09	2.98	0	0.16
Teg12	33	3	0.61	0.63	0.05	2.71	1	0.75
Teg13	35	2	0.63	0.50	-0.24	2.00	1	0.29
Teg14	34	5	0.68	0.59	-0.13*	2.45	2	0.50
Teg17	34	2	0.88	0.49	-0.78*	1.97	0	0.33
Teg19	32	1	0.00	0.00	N/A	1.00	0	1.00
Teg20	27	4	0.67	0.63	-0.04	2.71	2	1.00
Pop. Mean	31.86	3.07	0.55	0.51	0.00	2.32	0.86	0.59
Pop. SEM	0.84	0.29	0.07	0.06	0.11	0.18	0.21	0.07
Miami-Dade								
Teg1	40	4	0.63	0.56	-0.11	2.26	0	0.80
Teg2	40	2	0.03	0.02	N/A	1.03	0	0.40
Teg4	40	2	0.00	0.26	1.00*†	1.34	1	1.00
Teg5	36	3	0.42	0.60	0.31*†	2.48	0	0.60
Teg6	40	2	0.25	0.22	-0.13	1.28	1	1.00
Teg7	40	4	0.33	0.28	-0.14	1.39	0	0.57
Teg9	40	2	0.08	0.12	0.37	1.13	0	1.00
Teg10	39	4	0.59	0.53	-0.11	2.11	1	0.21
Teg12	38	2	0.08	0.08	-0.03	1.08	0	1.00

Teg13	38	2	0.47	0.45	-0.04	1.82	1	0.25
Teg14	40	4	0.68	0.64	-0.04	2.79	1	0.40
Teg17	40	2	0.88	0.49	-0.77*	1.97	0	0.33
Teg19	38	3	0.32	0.52	0.41*†	2.10	2	1.00
Teg20	38	2	0.58	0.43	-0.33	1.76	0	1.00
Pop. Mean	39.07	2.71	0.38	0.37	0.03	1.75	0.50	0.68
Pop. SEM	0.34	0.24	0.07	0.05	0.12	0.15	0.17	0.09

* Significantly deviated from Hardy-Weinberg equilibrium; † Evidence of null alleles

Assessment of population differentiation

Locus-specific G_{ST} estimates ranged from 0.028 to 0.312 and were statistically significant (maximum $P = 0.011$, minimum $P = 0.001$). Locus-specific estimates G''_{ST} were also statistically significant (maximum $P = 0.009$, minimum $P = 0.001$), with values ranging from 0.119 to 0.893. The global G_{ST} estimate that resulted from averaging information across all loci was 0.170 ($SE = 0.025$, $P = 0.0001$). Similarly, the global estimate for G''_{ST} was 0.545 ($SE = 0.060$, $P = 0.0001$). The AMOVA results computed in ARLEQUIN are also indicative of a high degree of genetic differentiation between the Hillsborough and Miami-Date populations (Table 2) and suggested moderate heterozygote excess (i.e., produced a negative F_{IS} estimate). While this may seem contrary to the Weir and Cockerham estimators of F_{IS} in Table 1, when the locus with consistently high F_{IS} estimates is excluded (*Teg4*; $F_{IS} = 1$ in Hillsborough and Miami-Dade), the means of the Weir and Cockerham estimators are -0.0867 and -0.0508 for Hillsborough and Miami-Dade respectively.

Table 2. AMOVA results.

Source of Variation	Degrees of Freedom	Sum of Squares	Variance Component	Fixation Index	*P*-value[a]
Among populations	1	67.85	0.85	$F_{ST} = 0.32$	0.00[b]
Among individuals	76	111.62	-0.36	$F_{IS} = -0.20$	1.00[c]
Within individuals	78	171.00	2.19	$F_{IT} = 0.18$	0.22[d]
Total	155	350.47	2.68	N/A	N/A

[a]All significance tests performed in ARLEQUIN are based on 10,100 permutations. [b]P(permuted $F_{ST} \geq$ to observed F_{ST}). [c]P(permuted $F_{IS} \geq$ observed F_{IS}), [d]P(permuted $F_{IT} \leq$ observed F_{IT}).

In the PCA generated from the raw genotypic data, the first two principle components accounted for 35.57% of the overall genetic variation (Figure 2). The plot (Figure 2) produced separate clusters for the Hillsborough and Miami-Dade *S. merianae* populations, with only two intermediate individuals. In addition, one member of the Hillsborough population showed a large discrepancy in principal component 2 and did not cluster with the remaining individuals. As shown in Figure 3, STRUCTURE also inferred two clusters; however, one individual assigned to the Miami-Dade cluster had a substantial proportion of its genome derived from the Hillsborough cluster (Figure 4). Conversely, a second individual that was assigned to the Hillsborough cluster had a substantial proportion of its genome derived from the Miami-Dade cluster (Figure 4).

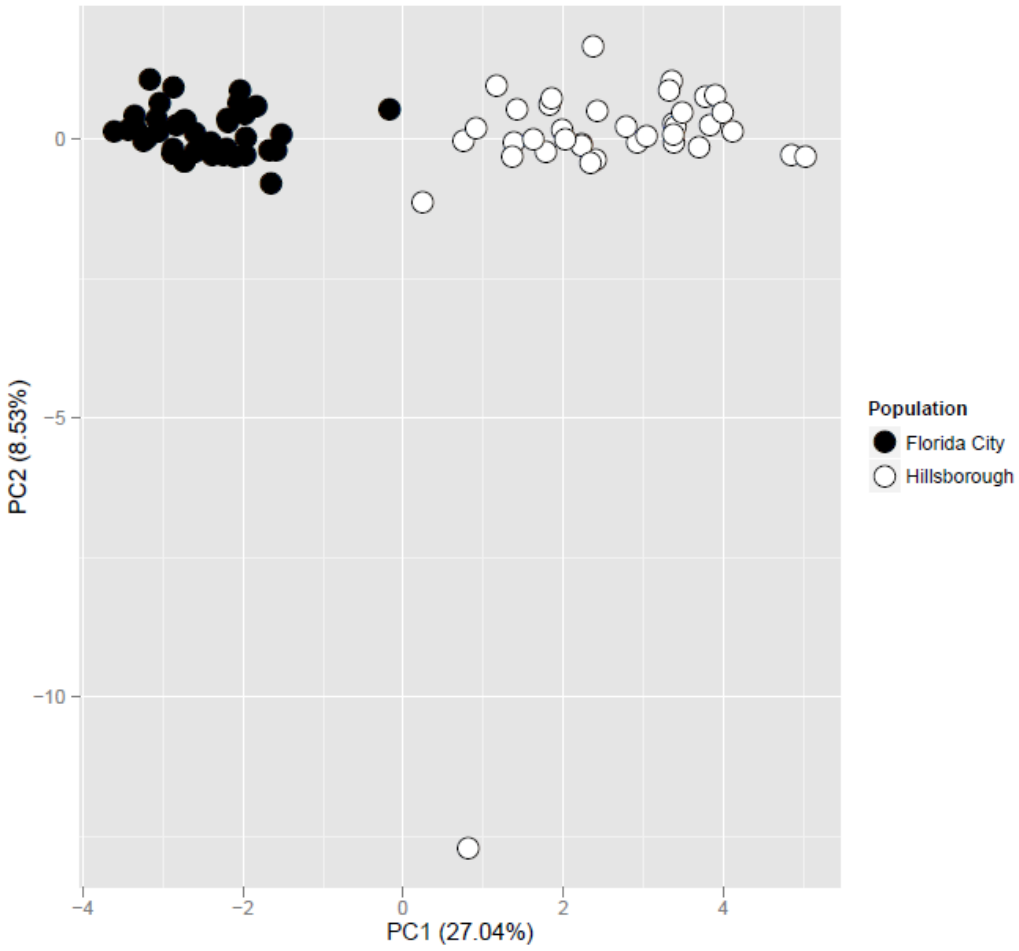


Figure 2. Principal component analysis based on raw genotypes of introduced *Salvator merianae* populations in Florida.

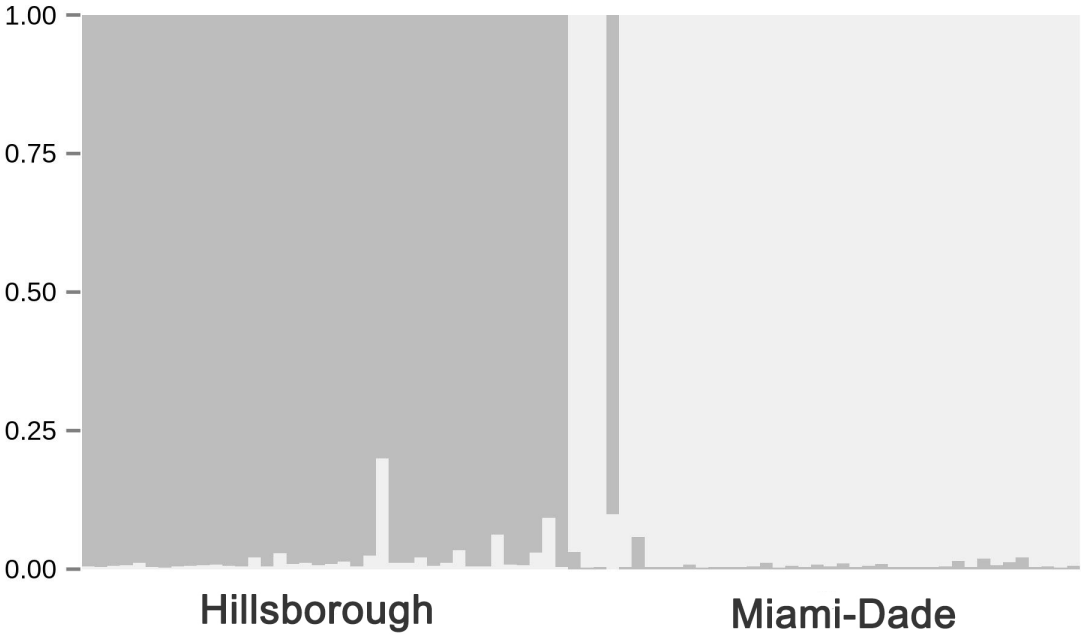
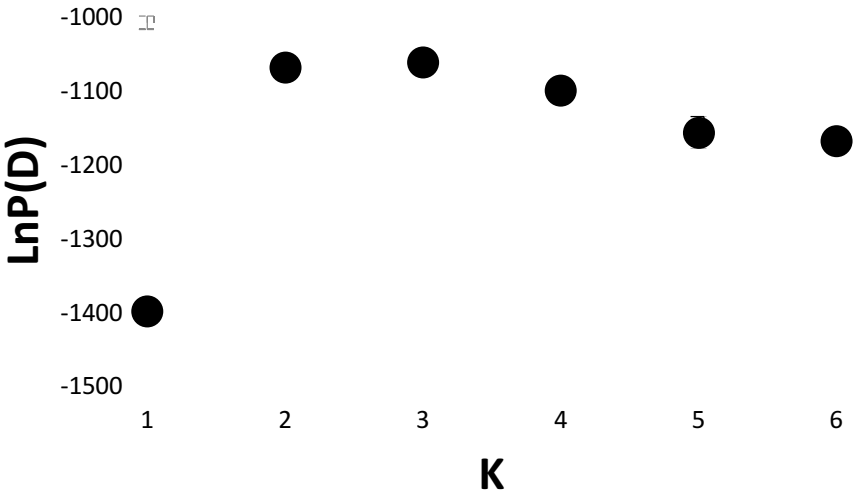


Figure 3. Results of the analysis performed in STRUCTURE when $K = 2$. Bars represent average cluster membership across 10 replicate runs that were aligned using CLUMPP.

A



B

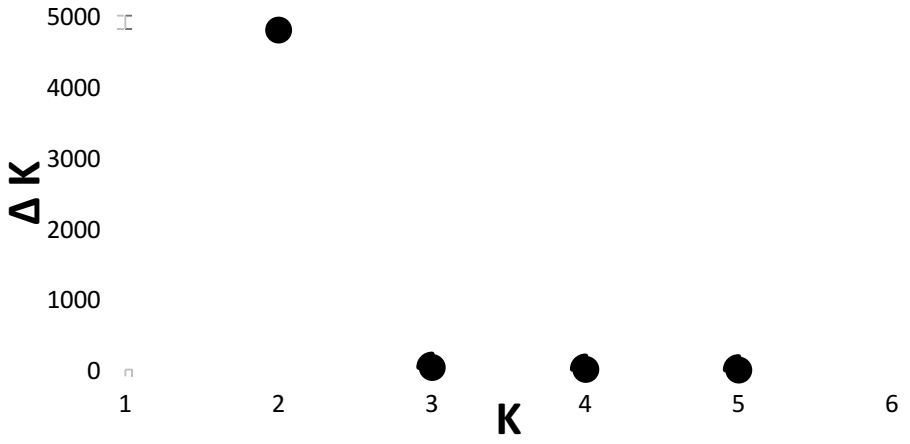


Figure 4. Evanno et al. (2005) plots for detecting the number of *K* groups that best fit the data. (A) Mean log likelihood (Ln P(D)) plus or minus one standard deviation over 10 replicate runs. (B) The modal value of delta *K* (*ΔK*) is the true *K* or the uppermost level of structure.

Among-population gene flow

Although the analysis performed in STRUCTURE provided evidence of admixture (Figure 3), our analysis of recent migration rates in BAYESASS suggests that gene flow between Hillsborough and Miami-Dade is rare (Table 3), as 98-99% of both populations' genetic contribution originated from within the same population.

Table 3. Bayesian assessment of migration within and among Florida populations of *Salvator merianae*. Columns represent migration sources, rows represent migration sinks, and values along the diagonal indicate the proportion of non-migrants. The confidence interval for each estimate is shown in parentheses.

Population	Hillsborough	Miami-Dade
Hillsborough	0.989 (0.963–1.000)	0.011 (0.000–0.037)
Miami-Dade	0.015 (0.002–0.040)	0.985 (0.960–0.998)

The assignment analyses, performed in GENECLASS2, correctly assigned 77 of 78 individuals to the locales from which they were sampled (Figure 5). One individual sampled in Miami-Dade County was assigned to the Hillsborough population. Not surprisingly, GENECLASS2 found evidence that this individual from the Miami-Dade population was a first-generation migrant from Hillsborough (log(L_home/L_max) = 2.295, $P = 0.0001$). Because the analyses we performed in DIABC suggested the presence of a 'ghost population' (see below) we, repeated the migrant detection analysis in GENECLASS2 using the L_home likelihood estimation, which produces a more appropriate test statistic when there are populations that have not been sampled (Piry et al. 2004). The results of this analysis suggested that that same Miami-Dade individual (-log(L_home) = 10.721, P = 0.0001) and an individual from Hillsborough County (-log(L_home) = 11.663, P = 0.0001) were both first-generation migrants.

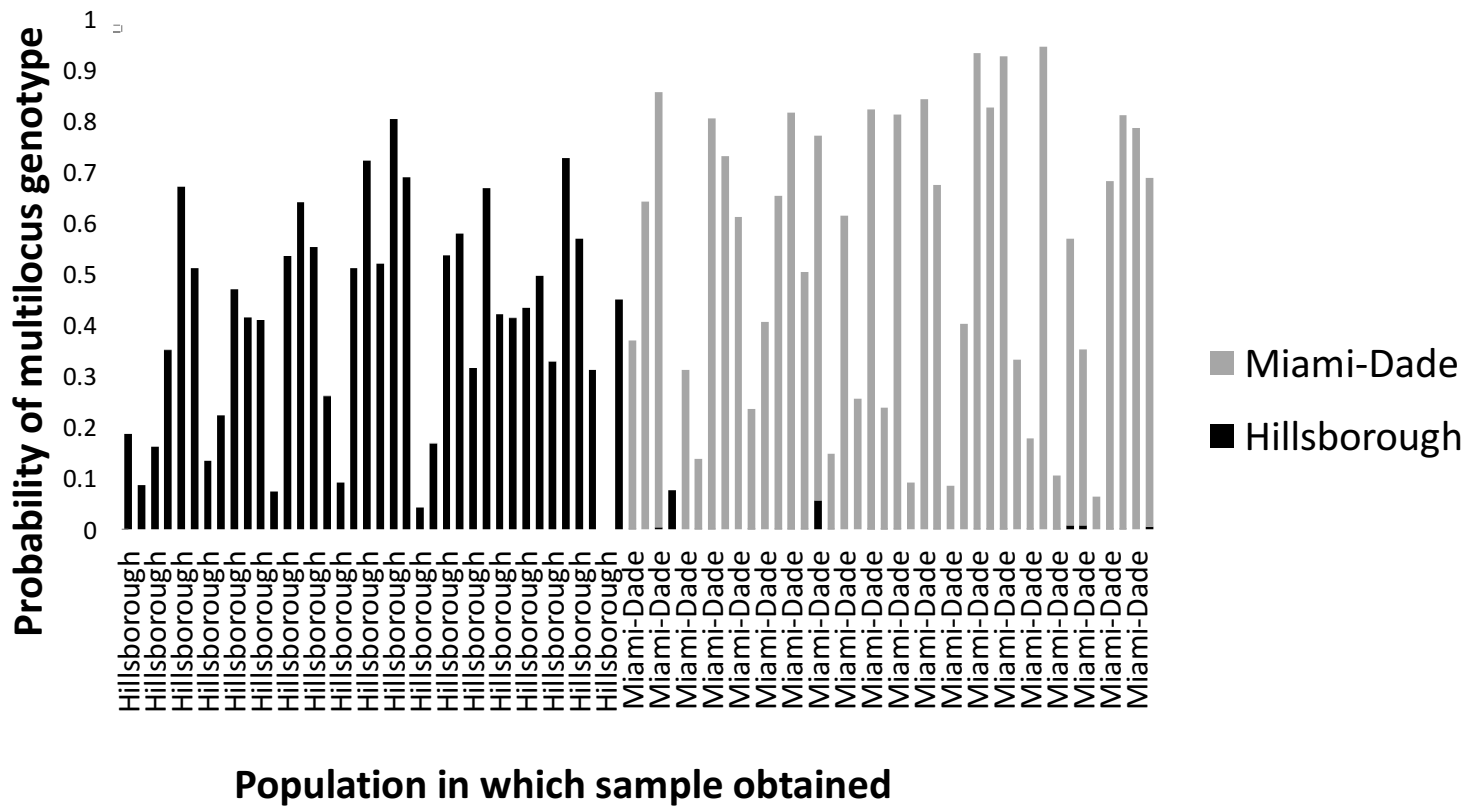


Figure 5. Stacked bar plots depicting the results of the assignment analysis performed in GENECLASS2. Each individual is represented by a bar that is presented over a label indicating the population in which that individual was sampled. For each individual, GENECLASS2 calculates the probability of that individual's multilocus genotype being derived from Hillsborough (black) and Miami-Dade (light gray). Thus, each bar can consist of as many as two colors, with the height of each color indicating the relative strength of assignment to each of the three populations.

Effective population size and demographic history

The N_e estimated for the introduced *S. merianae* populations varied considerably across methods (Table 4). The linkage disequilibrium method estimated the Miami-Dade population to have a larger N_e than the Hillsborough population, while the molecular coancestry method produced the opposite pattern, and the heterozygote excess method showed both populations to be similar in size.

The analyses performed in BOTTLENECK suggested that the Hillsborough *S. merianae* population has undergone a recent population bottleneck (Table 5). However, the opposite was true for the Miami-Dade population—heterozygosity excess was not detected for any of the tests or mutation models. Additionally, we found no evidence of population expansion for either population based on the *k* test (Hillsborough: $P = 0.93$; Miami-Dade: $P = 0.15$) and the *g* test (Hillsborough: $g = 1.89$; Miami-Dade: $g = 2.88$). Lastly, the calculated *M*-ratios for both Hillsborough and Miami-Dade populations were both equal to or below the critical value of 0.68 (Table 1). It is noteworthy to mention that the *M*-ratio for the Hillsborough population was lower than for the Miami-Dade population ($0.59 < 0.68$), indicating that the Hillsborough population has undergone a more intense bottleneck event.

Table 4. Estimated effective population size (N_e) for Florida *Salvator merianae* populations, estimated using the linkage disequilibrium (LD), heterozygote excess, and molecular coancestry methods in NeEstimator. For the first two methods, the lowest allele frequency used was set to 0.02. The 95% confidence interval for each estimate is shown in parentheses and the symbol ∞ indicates that the program was unable to estimate N_e from the data.

Population	LD	Heterozygote Excess	Molecular Coancestry
Hillsborough	10.8 (5.2–23.3)	∞ (4.9–∞)	22.0 (0–110.4)
Miami-Dade	∞ (47.1–∞)	9.9 (4.4–∞)	3.0 (1.6–4.8)

Table 5. Probability values for tests of bottleneck effects in Florida *Salvator merianae* populations under the infinite alleles model (IAM), two-phase model (TPM), and stepwise mutation model (SMM). For the Wilcoxon test, probabilities for the one-tailed tests of heterozygote excess are shown. Bold values denote significant *P*-values.

Population	Mutation Model	Sign Test	Standardized Differences Test	Wilcoxon Test	Mode-shift
Hillsborough	IAM	**0.001**	**< 0.001**	**< 0.001**	Shifted
	TPM	**0.024**	**< 0.001**	**< 0.001**	
	SMM	**0.031**	**0.008**	**0.004**	
Miami-Dade	IAM	0.304	0.219	0.246	Normal L-shaped distribution
	TPM	0.391	0.475	0.461	
	SMM	0.141	0.118	0.813	

Inference of introduction history

The scenario testing revealed that both the Hillsborough and Miami-Dade *S. merianae* populations most likely originated via introductions from a "ghost" population (Scenario 4; Figure 6). This introduction scenario had the highest posterior probability (Table 6), and was supported over other hypotheses, including independent introductions from the native ancestral population and serial introduction pathways. Power analyses revealed that the type I errors (i.e. false positives) were low, indicating a low probability of falsely rejecting a scenario that was actually true (Table 6). However, the type II errors (i.e. false negatives) were higher (0.28–0.44), suggesting a higher probability of falsely selecting an untrue scenario. Further examination of the selected scenario via posterior model checking with all available summary statistics showed that none of the proportions (simulated < observed) fell outside the 0.05–0.95 range. Therefore, we concluded that scenario 4 correctly explained the observed dataset, based on Cornuet et al. (2010).

Finally, we inferred the posterior distributions of demographic parameters based on scenario 4. The effective number of founders for each of these populations, including the un-sampled population, ranged from 19 to 57 (based on the mode), and appeared to be robust, producing small bias indices (Table 7).

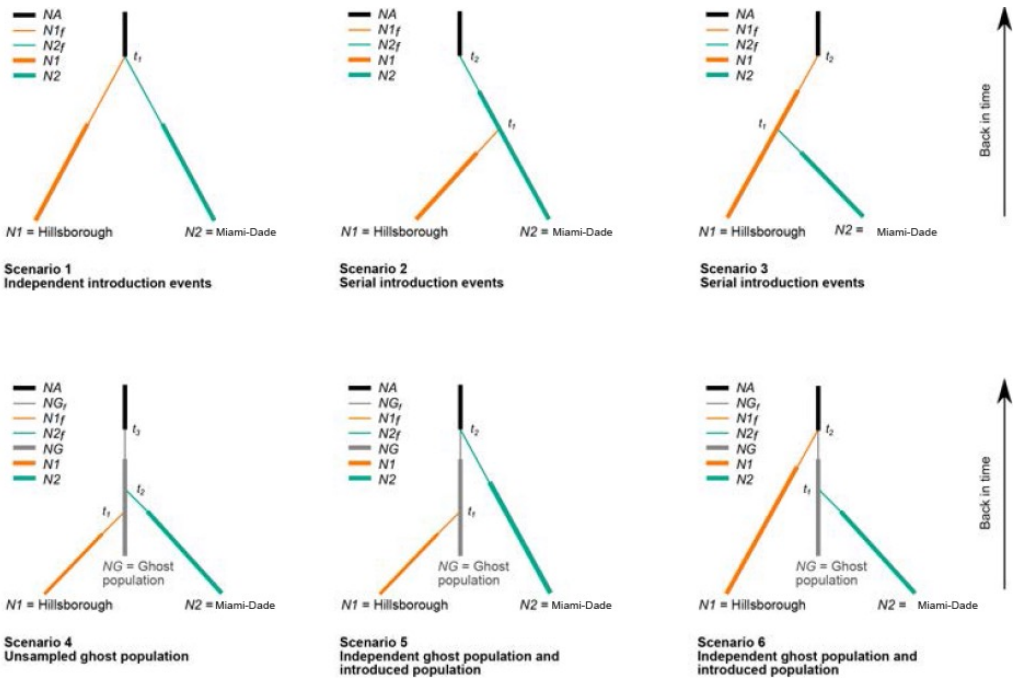


Figure 6. Graphical representation of the competing introduction scenarios for *Salvator merianae* compared with the software DIYABC. In each scenario, thin lines represent bottlenecked populations following introduction events, while thick lines represent the current effective population size. The abbreviations used are as follows: *NA* = ancestral (source) effective population size; *N1* = effective population size for the Hillsborough population; *N2* = effective population size for the Miami-Dade population; *NG* = effective population size for unsampled (ghost) population; N_f = the effective number of founding individuals; *t* = time in generations.

Table 6. Confidence in scenario selection by DIYABC for the introduction of *Salvator merianae* into Florida. The bold scenario number indicates most likely introduction history.

Scenario	Posterior probability	95% Credibility interval	Type I error	Type II error
1 – Independent introductions	0.0156	0.0111,0.0200	0.126	0.41
2 – Serial introduction (Miami-Dade → Hillsborough)	0.1531	0.1363,0.1699	0.048	0.28
3 – Serial introduction (Hillsborough → Miami-Dade)	0.1023	0.0936,0.1110	0.036	0.37
4 – Unsampled population as source for Hillsborough and Miami-Dade	0.6615	0.6431,0.6799	0.090	0.29
5 – Unsampled population as source for Hillsborough; Independent introduction for Miami-Dade	0.0218	0.0174,0.0262	0.080	0.48
6 – Unsampled population as source for Miami-Dade; Independent introduction for Hillsborough	0.0457	0.0371,0.0544	0.074	0.44

Table 7. Posterior distribution statistics and bias estimates for *Salvator merianae* parameters inferred from Scenario 4 of the Approximate Bayesian Computation. $N1_f$ = Number of founders for Hillsborough population; $N2_f$ = Number of founders for Miami-Dade population; NG_f = Number of founders for unsampled ghost population; NA = Ancestral effective population size.

	$N1_f$	$N2_f$	NG_f	NA
Mean	57.2	32.0	37.8	32,300
Median	56.8	24.9	33.6	27,100
Mode	57.2	18.8	24.9	12,700
95% HPD	24.5; 92.5	8.82; 85.2	9.24; 8.26	6,750; 75,300
Mean relative bias:				
Mean	0.0480	0.335	0.365	0.3510
Median	0.0204	0.0335	0.2296	0.1519
Mode	-0.0198	-0.2553	0.0395	-0.2505
Relative root mean square error:				
Mean	0.473	0.947	1.213	1.097
Median	0.462	0.673	1.140	0.925
Mode	0.482	0.562	1.281	0.745

Discussion

Conceptual framework and genetic diversity

Population genetic theory predicts that small, isolated populations have limited capacity for adaptive evolution due to reduced levels of additive genetic variation (Fisher 1958; Frankham and Ralls 1998). In addition, loss of genetic variation is expected to increase the extinction risk of small populations by limiting population growth through the effects of inbreeding depression and drift (Allendorf and Lundquist 2003; Dlugosch and Parker 2007). However, despite recent founder effects, population viability for invaders often remains high, and in many cases, invasive species outcompete their native counterparts (Allendorf and Lundquist 2003). This phenomenon was once considered to be a genetic paradox (Allendorf and Lundquist 2003; Frankham 2005; Handley et al. 2011). In recent years, new evidence has been generated that may solve this "paradox." Multiple introductions followed by admixture may be one mechanism by which genetic variation rebounds to increase an invasive population's adaptive capacity (Kolbe et al. 2004; 2008; Facon et al. 2008). Additionally, most studies that examine the dynamics of founder events use neutral molecular markers that are irrelevant to adaptive potential (Reed and Frankham 2003). Although these molecular measures have been used as surrogates for quantitative variation, Reed and Frankham (2001) showed that they are poorly linked to ecologically important quantitative traits. Invasion is now often conceptualized as a multistage process that includes a lag phase, during which mutation and/or admixture produce(s) novel phenotypes that improve invasiveness (Reznick and Ghalambor 2001), followed by rapid range expansion (Keller and Taylor 2008).

Therefore, it is critical for managers to identify potentially problematic populations during the early phases of invasion, as this is when control efforts are most likely to be successful (Frankham 2005).

Our tests for genetic signatures associated with recent genetic bottlenecks revealed that only the Hillsborough *S. merianae* population showed unequivocal evidence of a recent founder effect. This result is surprising considering that both populations were likely founded by a small number of individuals and our introduction scenario analyses suggested that the Miami-Dade population founded from fewer individuals than the Hillsborough population. Overall, the most likely explanation of these results is that our failure to detect a bottleneck in the Miami-Dade populations is a Type II statistical error. This lack of power associated with bottleneck tests has been described by Peery et al. (2012), who found limited power to detect 10- to 1000-fold population declines with heterozygosity-excess tests and 10-fold declines with M-ratios. Therefore, we used allelic diversity as an additional measure of bottleneck detection. During a sudden bottleneck event, individuals are expected to lose allelic diversity at a higher rate than heterozygosity (Luikart and Cornuet 1998). Unsurprisingly, both *S. merianae* populations had low levels of allelic diversity (range: 2 - 4), while heterozygosity estimates remained substantial (mean H_e: 0.44). Although definitive conclusions about reductions in genetic diversity would require comparisons to populations in the native range of *S. merianae*, these estimates are consistent with low levels of allelic diversity estimates reported for other invasive reptiles in Florida (Short and Petren 2001; Wood et al. In Press). Furthermore, N_e estimates were comparable to assessments of N_e in *V. niloticus*—another ecologically similar large, lizard that is invasive to Florida (Wood et al. In Press).

Additionally, estimates of N_e for *S. merianae* were also substantially lower than N_e estimates observed in invasive populations of *Boa constrictor imperator* on Cozumel Island (Vazquez-Dominguez et al. 2012).

Gene flow and introduction scenarios

We used several independent analyses to analyze the degree of genetic structure between the Hillsborough and Miami-Dade populations of *S. merianae*. Although most of our results suggested that there is marked genetic differentiation between the two populations, STRUTURE and PCA detected evidence that two *S. merianae* specimens (tegu 24 collected in Hillsborough County and tegu 42 collected in Miami-Dade County) had admixed genotypes. Our PCA analysis also showed that one member of the Hillsborough population did not cluster with any of the individuals from the Miami-Dade or Hillsborough populations, indicating that this individual could have originated from an unknown source population. Furthermore, tegu 42 was assigned to the Hillsborough population by GENECLASS2. The L_home tests for first generation migrants performed in GENECLASS2 also detected evidence that two individuals, one from each population, are migrants. This result is troubling given that we only sampled ~40 individuals in each population and found evidence of gene flow in both. However, it is worth noting that BAYESASS suggested that gene flow between these two populations is limited.

Our introduction scenario analyses found that both of the *S. merianae* populations likely resulted from an undetected 'ghost' population. This result can be interpreted two different ways. One explanation is that a separate undetected population of *S. merianae*

exists in Florida, and served as a source for the Hillsborough and Miami-Dade populations. This type of introduction scenario has been termed the 'invasive bridgehead effect', whereby secondary invasions stem from a successfully established population (Estoup and Guillemaud 2010). In terms of evolutionary shifts conferring advantages in the non-native habitat, the invasive bridgehead scenario is more parsimonious than scenarios involving independent introductions (and thus independent evolutionary changes) from the native source population (Estoup and Guillemaud 2010). This introduction scenario has been documented in the widespread Asian lady beetle (*Harmonia axyridis*) (Lombaert et al. 2010); however, few other examples have been confirmed.

The alternative, and possibly more plausible, explanation is that both *S. merianae* populations independently originated from the same captive-bred population. In the United States, *S. merianae* is one of the most commonly bred tegu species (Bartlett and Bartlett 1996). Additionally, the number of reported *S. merianae* imported into the United States is relatively low, compared to other reptiles in the pet trade, with an average of 500 live individuals per year (http://trade.cites.org/). However, there has been a noticeable decline in imports during recent years, with only around 100 live *S. merianae* individuals imported in 2013 (http://trade.cites.org/). This trend could be a reflection of the predominance of captive-bred individuals in the pet market, which might suggest a higher likelihood of the introduced individuals resulting from a captive population. Future studies comparing the genetic patterns of native and captive-bred *S. merianae* populations to those in Florida could further distinguish between these two scenarios.

Conclusion and management recommendation

Our findings have important implications for tegu control strategies in Florida. Collectively, our results suggest that both *S. merianae* populations in Florida are still in the early stages of the invasion process, and according to our *g* and *k* tests, are not expanding. In addition, our results show a high degree of differentiation between the Miami-Dade and Hillsborough populations. Based on these findings, we propose that the two Florida populations be viewed as two separate management units. Given the current low level of gene flow between populations, the likelihood that recolonization would serve as an obstacle to successful eradication attempts is low. However, even under moderate to high levels of harvest in their native range, *S. merianae* populations appear to be quite resilient (Fitzgerald 1994). Therefore, we recommend that managers focus on containment rather than eradication strategies, thereby reducing the chances of further range expansion and inter-regional admixture, which could enhance the future invasiveness of *S. merianae*. It is also noteworthy to mention that we found direct evidence of migration between populations. Given Florida's extensive network of canals and levees and the mobility of tegus, it is possible that individuals could migrate between populations (Klug et al. 2015). According to Florida EddMaps (http://www.eddmaps.org/florida/distribution/viewmap.cfm?sub=18346), verified *S. merianae* specimens have already been documented via photograph near the cities of Port Charlotte, Naples, and Port St. Lucie—the Naples and Port St. Lucie specimens both being over 150 km from the nearest breeding population. In addition to the possibility of direct dispersal between populations, there may be passive dispersal, potentially by a

community of breeding enthusiasts that transport tegus between Hillsborough and Miami-Dade Counties. As such, we also emphasize the importance of concerted follow-ups on credible sightings. Finally, our results strongly suggest that Florida's *S. merianae* populations both originated from a common, unsampled source population. Although, it is possible that an unknown wild breeding population exists in Florida, it is more likely that this unknown source is a captive population. Therefore, it is imperative that the Florida Wildlife Commission continues to closely monitor the exotic pet trade, as it seems to be primarily responsible for the introduction and establishment of *S. merianae,* and may still be a contributing factor.

CHAPTER VI

SUMMARY AND FUTURE DIRECTIONS

Summary

To my knowledge, my dissertation is the first study that has examined the population genetics of large, predatory, invasive lizards. In my second chapter, I discuss the development of 17 polymorphic microsatellite loci for *V. niloticus* using 454 pyrosequencing. These microsatellite markers are the first to be developed for *V. niloticus* and will be be useful for the continued monitoring of *V. niloticus* populations in Florida. These markers should also be beneficial to scientists studying native *V. niloticus* populations. Moreover, our BLASTn search found evidence that many of the loci we developed have the potential to cross-amplify in other varanid species. In the third chapter, I discuss the development of 14 polymorphic microsatellite loci for the tegu species, *S. merianae*. These microsatellite markers are the first to be developed for *S. merianae*. Efforts are already underway to test their usefulness in Brazilian *S. merianae* populations. In chapter four, I discuss a diverse approach to using genetic techniques to examine the population genetics of three *V. niloticus* populations in southern Florida and to infer the most likely introduction scenario. Our findings reveal that all three populations have limited genetic diversity, indicating that these populations were all founded from a small number of colonists. Furthermore, our findings showed that all three populations are highly differentiated from one another, and that each population

originated from independent introduction events. However, despite a strong degree of genetic differentiation among populations, we did detect limited evidence for an unknown source population in Florida as well as some possible migration. In chapter five, we found similarly low levels of genetic diversity in invasive *S. merianae* populations. Although we only found limited evidence for gene flow among *V. niloticus* populations, our analyses revealed strong evidence for migration between the two tegu populations in Florida. Unexpectedly, our scenario testing revealed that both *S. merianae* populations most likely originated from a common unknown source population. This result can most likely be attributed to both tegu populations originating from the same captive stock.

Future directions

As discussed in the first chapter, the success of invasive species despite the typical significant reductions in their genetic diversity is a genetic paradox. Recent studies suggest that multiple introductions and admixture most likely play a crucial role in invasive populations overcoming the detrimental effects of inbreeding depression and drift (Facon et al. 2008; Keller and Taylor 2010; Kolbe et al. 2004). We, however, detected no significant evidence for admixture in either monitor or tegu populations. Therefore, we cannot conclude that multiple introductions have contributed to the success of invasive monitors and tegus in Florida. Although we are not implying that multiple introductions and admixture are irrelevant to invasion success, our data do support the hypothesis that they are are not an indispensable force for successful invasion (Dlugosch

and Parker 2008; Rollins et al. 2013). Some other invasions have succeeded with low numbers of founders or low genetic diversity. For example, invasive American bullfrogs (*Rana catesbeiana*) successfully invaded Europe despite having a founding population that consisted of only six individuals (Ficetola et al. 2008). Similarly, allelic diversity estimates were low (mean number of alleles = 4) for boa constrictors (*Boa constrictor*) in their successful invasion of Puerto Rico (Reynolds et al. 2013). Furthermore, both of these studies also reported moderate to substantial levels of heterozygosity, supporting the idea that allelic diversity decreases faster than heterozygosity during a population bottleneck (Allendorf 1986). Collectively, these patterns are congruent with the hypothesis that molecular genetic markers are poor predictors of losses in quantitative variation, which are more closely linked to ecologically important traits (Reed and Frankham 2001). Moreover, any increase in adaptive potential that results from increased genetic variation may only be essential in extreme ecological conditions (Allendorf and Lundquist 2003). Accordingly, it is likely that *V. niloticus* and *S. merianae* populations do not suffer from a competitive disadvantage due to reduced molecular variation, since Florida's environment is optimal for both species and both lack natural predators and competitors (Allendorf and Lundquist 2003; Callaway & Aschehoug 2000).

Very few studies have examined the relationship between quantitative genetic variation and invasion success. Koskinen et al. (2002) found that despite losing 50% of molecular variation during an initial introduction, life-history traits for grayling fish (*Thymallus thymallus*) showed no decline in additive variation. Lindholm et al. (2005) also found no evidence for substantial losses in additive variation despite the presence of a strong genetic bottleneck in the invasion of Australia by guppies *(Poecilia reticulata)*.

A greater number of studies have examined how quantitative genetic variation is affected by bottlenecks, but most of these have been conducted in laboratory settings on insects and plants (Reviewed in Saccheri et al. 2001). Since invasions by large predators are becoming increasingly common, future studies are needed to further our understanding of how molecular and quantitative genetic variation influence the invasion success of highly impactful species. Furthermore, it would be interesting to see if differing degrees of molecular and quantitative genetic variation between invasive *V. niloticus* and *S. merianae* populations and native populations affect life history traits related to fitness such as population size, growth rates, body size, fecundity, and survival (Reed and Frankham 2003).

In conclusion, we recommend that Florida wildlife managers concentrate control strategies on containment rather than eradication. Given the resiliency of both of these species to harvesting pressures (de Buffrénil abd Rimblot-Baly 1999: Fitzgerald 1994), it is unlikely that complete eradication is feasible. Furthermore, even if only a few females remain during eradications, a very high risk of a new invasion exists given the ability of both lizard species to overcome substantial population bottlenecks. Finally, we suggest that managers monitor potential migration corridors. Although admixture may not play a critical role in the immediate colonization and expansion of invasive *V. niloticus* and *S. meriane* populations in Florida, admixture may increase long-term invasive potential.

REFERENCES

Abe AS. 1983. Observation on dormancy in tegu lizards—*Tupinambis teguixin* (Reptilia, Teiidae). Naturalia 8: 135-139.

Abdelkrim J, Pascal M, Calmet C, Samadis S. 2005. Importance of Assessing Population Genetic Structure before Eradication of Invasive Species: Examples from Insular Norway Rat Populations. Conserv Biol 19 (5): 1509-1518.

Abdelkrim J, Pascal M, Samadi S. 2009. Genetic structure and functioning of alien ship rat populations from a Corsican micro-insular complex. Biol Invasions 11(3):473-482.

Allendorf FW. 1986. *Evolutionary Biology,* 2nd ed. Sinauer Associates, Inc. Sunderland, MA.

Allendorf FW, Lundquist LL. 2003. Introduction: population biology, evolution, and control of invasive species. Conserv Biol 17: 24–30.

Auger-Rozenberg M-A, Boivin T, Magnoux E, Courtin C, Roques A, Kerdelhué C. 2012. Inferences on population history of a seed chalcid wasp: invasion success despite a severe founder effect from an unexpected source population. Molec Ecol 21:6086-6103.

Barrett SCH, Kohn JR. 1991. Genetic and evolutionary consequences of small population size in plants: implications for conservation. Pp 3-30 in DA Falk and KE Holsinger, editors. Genetics and conservation of rare plants. Oxford University Press, New York.

Bartlett RD, Bartlett P. 1996. Monitors, tegus, and related lizards: everything about selection, care, nutrition, diseases, breeding, and behavior. Barron's Educational Series, Inc, Hauppauge, NY.

Beaumont MA. 2010. Approximate Bayesian computation in evolution and ecology. Annu Rev Ecol Evol Syst 41:379-406.

Benazzo A, Ghirotto S, Vilaça ST, Hoban S. 2015. Using ABC and microsatellite data to detect multiple introductions of invasive species from a single source. Heredity 115:262-272.

Bennett D. 2002. Diet of Juvenile *Varanus niloticus* (Sauria: Varanidae) on the Black Volta River in Ghana. J Herpetol 36(1): 116-117.

Bilgin R. 2007. kgtests: a simple Excel Macro program to detect signatures of population expansion using microsatellites. Mol Ecol Notes 7:416-417.

Boissin E, Hurley B, Wingfield M, Vasaitis R, Stenlid J, Davis C, de Groot P, Ahumada R, Carnegie A, Goldarazena A, Klasmer P, Wermelinger B, Slippers B. 2012. Retracing the routes of introduction of invasive species: the case of the *Sirex noctilio* woodwasp. Mol Ecol 21:5728-5744.

Boubou A, Migeon A, Roderick GK, Auger P, Cornuet J-M, Magalhães S, Navajas M. 2012. Test of colonisation scenarios reveals complex invasion history of the red tomato spider mite *Tetranychus evansi*. PLoS ONE 7:e35601.

Butterfield BP, Meshaka Jr, Guyer C. 1997. Nonindigenous amphibians and reptiles. Pp. 123-128, *In* Simberloff D, Schmitz DC, Brown TC. Strangers in paradise: impact and management of nonindigenous species in Florida. Island Press, Covelo, CA. 467 pp.

Callaway RM, Aschehoug ET. 2000. Invasive plants versus their new and old neighbors: a mechanism for exotic invasion. Science 290:521-523.

Campbell TS. 2005. Eradication of introduced carnivorous lizards from the Cape Coral area. Final Report to the Charlotte Harbor National Estuary Program, Fort Meyers, FL. Florida Fish and Wildlife Conservation Commission.

Cardozo G, Naretto S, Zak M, Chiaravigilio M. 2012. The role of landscape in contact zones o sister species of lizards. In: Tiefenbacher J (ed) Perspectives on nature conservation—patterns, pressures and prospects. James and Marilyn Lovell Center for Environmental Geography and Hazards Research, Department of Geography Texas State University, San Marcos, pp 161-176.

Chamut S, Jahn GA, Arce OEA, Manes ME. 2012. Testosterone and reproductive activity in the male tegu lizard, *Tupinambis merianae*. Herpetol Conserv Biol 7(3) 299-305.

Cheverud JM, Routman EJ. 1996. Epistasis as a source of increased additive genetic variance at a population bottlenecks. Evol 50:1042-1051.

Clavero M, Garcia-Berthou E. 2005. Invasive species are the leading cause of animal extinctions. Trends in Ecol and Evol 20 (3) 110.

Corn ML, Buck EH, Rawson J, Segarra A, Fischer E. 2002. Invasive Non-Native Species: Background and Issues for Congress. Washington, DC: Congress Res Serv and Libr Congr.

Cornuet J-M, Pudlo P, Veyssier J, Dehne-Garcia A, Gautier M, Leblois R, Marin J-M, Estoup A. 2014. DIYABC v2. 0: a software to make approximate Bayesian computation inferences about population history using single nucleotide polymorphism, DNA sequence and microsatellite data. Bioinformatics 30: 1187-1189.

Cornuet JM, Ravigne V, Estoup A. 2010. Inference on population history and model checking using DNA sequence and microsatellite data with the software DIYABC (v1.0). BMC BioInform 11:401.

de Buffrénil V. 1992. La Peche et l'exploitation du varan du Nil dans la region du lac Tchad. Bull Herpetol Soc Fr 62(2):47–56.

de Buffrénil V, Rimblot-Baly F. 1999. Female reproductive output in exploited Nile Monitor Lizard (*Varanus niloticus* L.) populations in Sahelian Africa. Can J Zool 77:1530 1539.

Di Castri F. 1989. Hisotry of biological invasions with special emphasis on the Old World. In JA Drake, editor. Biological Invasions: a global perspective. John Wiley & Sons, New York.

Dlugosch KM, Parker IM. 2008. Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. Mol Ecol 17:431-449.

Dlugosch KM, Parker IM. 2007. Molecular and quantitative trait variation across the native range of the invasive species *Hypericum canariense*: evidence for ancient patterns of colonization via pre-adaptation? Mol Ecol 16: 4268-4282.

Do C, Waples RS, Peel D, Macbeth G, Tillett BJ, Ovenden JR. 2014. NeEstimator v2: re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. Molecular Ecology Resources 14: 209-214.

Dowell SA. 2015. Spatial and temporal patterns of genetic variation in native and introduced populations of the Nile monitor (*Varanus niloticus*). Doctoral Dissertation. ETD Collection for Fordham University. Paper AAI3728437.

Dowell SA, de Buffrénil V, Kolokotronis S-O, Hekkala ER. 2015. Fine-scale genetic analysis of the exploited Nile monitor (*Varanus niloticus*) in Sahelian Africa. BMC Genetics 16: 32.

Duarte Varela, C.F., Cabrera, M.R. 2000. Testing skeletochronology in black tegu lizards (*Tupinambis merianae*) of known ages. Herpetol. Rev. 31: 224-226.

Dyer RJ. 2012. The gstudio package. Virginia: Virginia Commonwealth University.

Earl DA, Vonholdt BM. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv Genet Resour 4:359-361.

Embert D, Fitzgerald L, Waldez F. 2010. *Salvator merianae*. IUCN 2013. IUCN Red List of of Threatened Species. Version 2013.2.

Enge KM. 2007. FWC bioprofile for the Argentine black and white tegu (*Tupinambis merianae*). Florida Fish and Wildlife Conservation Commission Report, Tallahassee, FL 27 pp.

Enge KM, Krysko KL, Hankins KR, Campbell TS, King FW. 2004. Status of the Nile monitor *Varanus niloticus* in southwestern Florida. Southeast Nat 3: 571–582.

Engeman, R., Jacobson, E., Avery, M.L., Meshaka, W.E. Jr. 2011. The aggressive invasion of exotic reptiles in Florida with a focus on prominent species: A review. Curr. Zool. **57(5)**: 599−612.

Estoup A, Guillemaud T. 2010. Reconstructing routes of invasion using genetic data: why, how and so what? Mol Ecol 19: 4113-4130.

Estoup A, Jarne P, Cornuet JM. 2002. Homoplasy and mutation model at microsatellite loci and their consequences for population genetics analysis. Mol Ecol 11: 1591-1604.

Evanno G, Regnaut S, Goudet J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14: 2611-2620.

Excoffier L, Lischer HEL. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetic analyses under Linux and Windows. Mol Ecol Resour 10:564-567.

Facon B, Crespin L, Loiseau A, Lombaert E, Magro A, Estoup A. 2010. Can things get worse when an invasive species hybridizes? The harlequin ladybird *Harmonia axyridis* in France as a case study. Evol Appl 4:71-88.

Facon B, Jarne P, Pointier JP, David P.2005. Hybridization and invasiveness in the freshwater snail *Melanoides tuberculata*: hybrid vigor is more important than increase in genetic variance. J Evol Biol 18:524-535.

Facon B, Pointier JP, Jarne P, Sarda V, David P. 2008. High genetic variance in life-history strategies within invasive populations by way of multiple introductions. Curr Biol 18:363-367.

Faircloth, B.C. 2008. MSATCOMMANDER: detection of microsatellite repeat arrays and automated, locus-specific primer design. Mol. Ecol. Resour. 8:92-94.

Falconer, D. 1989. Introduction to quantitative genetics. 3rd edition. Longman Scientific & Technical with Wiley & Sons Inc., New York.

Falush D, Stephens M, Pritchard JK. 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics 164:1567-1587.

Faust RJ. 2001. Nile Monitors: Everything about History, Care, Nutrition, Handling, and Behavior. Barron's Educational Series, Hauppauge, NY pg. 95.

Faust, R.J., Bayless, M.K. 1996. Nile monitors: ecology, morphology, and husbandry. Reptiles Mag. 4(11):68-63.

Ficetola GF, Bonin A, Miaud C. 2008. Population genetics reveals origin and number of founders in a biological invasion. Mol Ecol 17: 773-782.

Fisher RA.1930. The genetical theory of natural selection. Clarendon, Oxford.

Fisher RA. 1958. The genetical theory of natural selection. Dover, New York.

Fitch AJ, Goodman AE, Donnellan SC. 2005. Isolation and characterization of microsatellite marikers for the Australian monitor lizard, *Varanus acanthurus* (Squamata: Varanidae) and their utility in other selected varanid species. Mol Ecol Notes 5:521-523.

Fitzgerald LA. 1994. The interplay between life history and environmental stochasticity: implication for the management of exploited lizard populations. Am Zool 34: 371-381.

Frankham R. 2005. Resolving the genetic paradox in invasive species. Heredity 94:385.

Frankham R, Ballou JD, Briscoe DA. 2010. Introduction to conservation genetics, 2nd edition. Cambridge University Press, New York.

Frankham R, Ralls K. 1998. Conservation biology: inbreeding leads to extinction. Nature 392:441-442.

Fu M, Yu D, Peng J, Wang Y, Gao S, Wang L, Liu X, Hu S, Gao L. 2011. Isolation and characterization of novel microsatellite markers in water monitor (*Varanus salvator*). Conservat Genet Resour 3:777-779.

Florida Wildlife Commission. 2015. Nile monitor (*Varanus niloticus*) [cited 2015 October 8]. Available from: http://www.myfwc.com/wildlifehabitats/nonnatives/reptiles/nile-monitor/.

Galetti M, Bovendorp RS, Fadini RF, Gussoni COA, Rodrigues M, Alvarez AD, Guimaraes PR, Alves K. 2009. Hyper-abundant mesopredators and bird extinction in an Atlantic forest island. Zoolog 26:288-298.

Garza JC, Williamson EG. 2001. Detection of reduction in population size using data from microsatellite loci. Mol Ecol 10:305-318.

Goodnight CJ. 1988. Epistasis and the effect of founder events on the additive genetic variance. Evol 41:80-91.

Guillemaud T, Beaumont MA, Ciosi M, Cornuet J-M, Estoup A. 2010. Inferring introduction routes of invasive species using approximate Bayesian computation on microsatellite data. Heredity 104:88-99.

Guichoux E, Lagache L, Wagner S, Chaumeil P, Leger P, Lepais O, Lepoittevin C, Malausa T, Revardel E, Salin F, Petit RJ. 2011. current trends in microsatellite genotyping. Mol Ecol Resour 11:591-611.

Hanski I. 1999. Metapopulation Ecology. Oxford University Press, Oxford, UK.

Hardin S. 2007. Managing non-native wildlife in Florida: State perspective, policy and practice. In: Witmer G, Pitt W, Fagerstone K ed. Managing Vertebrate Invasive Species: Proc Int Symp. Fort Collins, CO: USDA/APHIS/WS, National Wildlife Research Center, 43–52.

Holm S. 1979. A simple sequentially rejective multiple test procedure. Scand J Stat 6:65-70.

Jakobsson M, Rosenberg NA. 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics 23:1801-1806.

Keller SR, Taylor DR. 2008. History, chance and adaptation during biological invasion: separating stochastic phenotypic evolution from response to selection. Ecol Lett 11:852-866.

Keller SR, Taylor DR. 2010. Genomic admixture increases fitness during a biological invasion. J Evol Biol 23:1720-1731.

Kiefer MC, Sazima I.2002. Diet of juvenile tegu lizard *Tupinambis merianae* (Teiidae) in southeastern Brazil. Amphib-Reptil 23:105-108.

Kimura M. 1983. The neutral theory of molecular evolution. Cambridge University Press, Cambridge, United Kingdom.

Klug PE, Reed RN, Mazzotti FJ, McEachern MA, Vinci JJ, Craven KK, Yackel Adams AA. 2015. The influence of disturbed habitat on the spatial ecology of Argentine black and white tegu (*Tupinambis merianae*), a recent invader in the Everglades ecosystem (Florida, USA). Biol Invasion 17: 1785-1797.

Kolbe JJ, Glor RE, Schettino LR, Lara AC, Larson A, Losos JB. 2004. Genetic variation increases during biological invasion by a Cuban lizard. Nature 431:177-181.

Kolbe JJ, Glor RE, Schettino LR, Lara AC, Larson A, Losos JB. 2007. Multiple sources, admixture, and genetic variation in introduced *Anolis* lizard populations. Conserv Biol 21:1612-1625.

Kolbe JJ, Larson A, Losos JB, de Queiroz K. 2008. Admixture determines genetic diversity and population differentiation in the biological invasion of lizard species. Biol Lett 4:434-437.

Konečný A, Estoup A, Duplantier JM, Bryja J, Bâ K, Galan M, Tatard C, Cosson JF. 2013. Invasion genetics of the introduced black rat (*Rattus rattus*) in Senegal, West Africa. Mol Ecol 22:286-300.

Koskinen MT, Nilsson J, Veselov AJ, Potutkin AG, Ranta E, Primmer CR. (2002). Microsatellite data resolve phylogeographic patterns in European grayling, *Thymallus thymallus*, Salmonidae. Hered 88:391-401.

Kowarik I. 1995. Time lags in biological invasions with regard to the success and failure of alien species. In Pysek P, Prach K, Rejmanek M, Wade M (eds), *Plant invasions – general aspects and special problems*, pp 15-38. SPB Academic Publ, Amsterdam.

Krysko KL, Burgess JP, Rochford MR, Gillete CR, Cueva D, Enge KM, Somma LA, Stabile JL, Smith DC, Wasilewski JA, Kieckhefer GN III, Granatosky MC, Nielson SV. 2011. Verified non-indigenous amphbians and reptiles in Florida from 1983 through 2010: outlining the invasion process and identifying invasion pathways and stages. Zootax 3028:1-64.

Lanfri S, Di Cola V, Naretto S, Chiaraviglio M, Cardozo G. 2013. Understanding the ecological niche to elucidate spatial strategie of the southernmost *Tupinambis* lizards. Amphib-Reptil 34:551-565.

Lavergne S, Molofsky J.2007. Increased genetic variation and evolutionary potential drive the success of an invasive grass. Proc Natl Acad Sci USA 104:3883-3888.

Lawson Handley LJ, Estoup A, Evans DM, Thomas CE, Lombaert E, Facon B, Aebi A, Roy HE. 2011. Ecological genetics of invasive alien species. Biocontrol 56:409-428.

Lenz S. 1995. Zur biologie und ökologie des Nilwarans, *Varanus niloticus* (Linnaeus 1766) in Gambia, Westafrika. Mertensiella 5:1–256.

Lindholm AK, Breden F, Alexander HJ, Chan WK, Thakurta SG, Brooks R. 2005). Invasion success and genetic diversity of introduced population of guppies *Poecilia reticulate* in Australia. Mol Ecol 14:3671-3682.

Lombaert E, Guillemaud T, Cornuet JM, Malausa T, Facon B, Estoup A. 2010. Bridgehead effect in the worldwide invasion of the biocontrol harlequin ladybird. PLoS One 5:e9743.

Lopes, H.R., Abe, A.S. 1999. Biologia reprodutivo e comportamento do teiú, *Tupinambis merianae*, em cativeiro (Reptilia, Teiidae). In: Fang TG, Montenegro OL, Bodmer RE ed.. Manejo y Conservación de Fauna Silvestre en América Latina.La Paz, Bolivia: Instituto de Ecología, 259–272.

Losos JB, Greene HW. 1988. Ecological and evolutionary implications of diet in monitor lizards. Biol J Linnean Soc 35:379–407.

Luikart, G, Allendorf FW, Cornuet JM, Sherwin WB. 1998. Distortion of allele frequency distributions provides a test for recent population bottlenecks. J Hered 89(3): 238-247.

Luikart G, Cornuet JM. 1998. Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. Conserv Biol 12:228-237.

Luxmoore R, Groombridge B, Broads S. 1988. Significant Trade in Wildlife: A Review of Selected Species in CITES Appendix II. Volume 2: Reptiles and Invertebrates. IUCN Conservation Monitoring Centre, Cambridge, UK 306 pp.

Mack RN, Simberloff D, Lonsdale WM, Evans H, Clout M, Bazzaz FA. 2000. Biotic invasions: causes, epidemiology, global consequences, and control. Ecol Appl 10:689-710.

Maruyama T, Fuerst PA. 1985. Population bottlenecks and nonequilibrium models in population genetics. II. Number of alleles in a small population that was formed by a recent bottleneck. Genetics 111: 675-689.

Mazzotti, F.J., McEachern, M., Rochford, M., Reed, R.N., Ketterlin Eckles, J., Vinci, J., Edwards, J., Wasilewski, J. 2015. *Tupinambis merianae* as nest predators of crocodilians and turtles in Florida, USA. Biol. Invasions. 17: 47-50.

Meirmans P, Hedrick PW. 2011. Assessing population structure: F_{ST} and related measures. Mol Ecol Resour 11:5-18.

Mercolli C, Yanosky A.1994. The diet of adult *Tupinambis teguixin* (Sauria: Teiidae) in the eastern chaco of Argentina. Herpetol J 4:15-19.

Meyer, M., Stenzel, U., Myles, S., Prufer, K., Hofreiter, M. 2007. Targeted high-throughput sequencing of tagged nucleic acid samples. Nucleic Acids Res. 35:e97.

Mooney HA, Cleland EE. 2001. The evolutionary impact of invasive species. PNAS 98(10): 5446-5451.

Myers JH, Simberloff D, Kuris AM, Carey JR. 2000. Eradicatiotion revisited: dealing with exotic species. Trends in Ecol Evol 15:316-320.

Nei M, Chesser RK. 1983. Estimation of fixation indices and gene diversities. Ann Hum Genet 47:253-259.

Nieminen M, Singer MC, Fortelius W, Schops K, Hanski I. 2001. Experimental confirmation that inbreeding depression increases extinction risk in butterfly populations. Am Nat 157:237-244.

Nomura T. 2008. Estimation of effective number of breeders from molecular coancestry of single cohort sample. Evol Appl 1:462-474.

Novak SJ, Mack RN. 2005. Genetic bottlenecks in alien plant speices: influeences of mating systems and introduction dynamics. In *Species Invasions: Insights into Ecology, Evolution, and Biogeography* (eds Sax DF, Stachowicz JJ, Gaines SD) pp 201-228. Sinauer Associates, Sunderland, MA.

Paetkau D, Calvert W, Stirling I, Strobeck C. 1995. Microsatellite analysis of population structure in Canadian polar bears. Mol Ecol 4:347-354.

Paetkau D, Slade R, Burden M, Estoup. 2004. Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power. Mol Ecol 13:55-65.

Peakall R, Smouse PE. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. Bioinformatics 28:2537-2539.

Peery MZ, Kirby R, Reid BN, Stoelting R, Doucet-Bëer E, Robinson S, Vásquez-Carrillo C, Pauli JN, Palsbøll PJ. 2012. Reliability of genetic bottleneck tests for detecting recent population declines. Mol Ecol 14: 3403-3418.

Pernas T, Giardina DJ, McKinley A, Parns A, Mazzotti FJ. 2012. First observations of nesting by the Argentine black and white tegu, *Tupinambis merianae*, in South Florida. Southeast Nat 11(4): 765-770.

Pimentel D, Lach L, Zuniga R, Morrison D. 2000. Environmental and economic costs of nonindigenous species in the United States. BioSci 50:53-65.

Piry S, Luikart G, Cornuet J-M. 1999. BOTTLENECK: a program for detecting recent effective population size reductions from allele data frequencies. Montpellier, France.

Piry S, Alapetite A, Cornuet JM, Paetkau D, Baudouin L, Estoup A. 2004. GENECLASS2: a software for genetic assignment and first-generation migrant detection. J Hered 95:536-539.

Pritchard JK, Stephens M, Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945-959.

R Core Team. 2014. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing.

Ramasamy RK, Ramasamy S, Bindroo BB, Naik VG. 2014. STUCTURE PLOT: a program for drawing elegant STRUCTURE bar plots in user friendly interface. Springer 3:431.

Rasmussen, DI. 1979. Sibling clusters and genotypic frequencies. Am Nat 113: 948-951.

Reed DH, Frankham R. 2001. How closely correlated are molecular and quantitative measures of genetic variation? A meta-analysis. Evolution 55:1095-1103.

Reed DH, Frankham R. 2003. Correlation between fitness and genetic diversity. Conserv Biol 17: 230-237.

Reich DE, Feldman MW, Goldstein DB. 1999. Statistical properties of two tests that use multilocus data sets to detect population expansion. Mol Biol Evol 16(4): 453-466.

Reich DE, Goldstein DB. 1998. Genetic evidence for a Paleolithic human population expansion in Africa. Proc Natl Acad Sci 95:8119-8123.

Reynolds RG, Puente-Rolon AR, Reed RN, Revell LJ. 2013. Genetic analysis of of a novel invasion of Puerto Rico by an exotic constricting snake. Biol Invasions 15: 953-959.

Reznick DN, Ghalambor CK. 2001. The population ecology of contemporary adaptations: what empirical studies reveal about the conditions that promote adaptive evolution. Genetica 112: 183-198.

Robertson BC, Gemmell NJ. 2004. Defining eradication units to control invasive pests. J Appl Ecol 41: 1042-1048.

Robertson, A. 1965. The interpretation of genotypic ratios in domestic animal populations. Animal Production 7: 319-324.

Rollins et al. 2013. High genetic diversity is not essential for successful introduction. Ecol Evol 3(13):4501-4517.

Rollins LA, Woolnough AP, Wilton AN, Sinclair, Sherwin WB. 2009. Invasive species can't cover their tracks: using microsatellites to assist management of starling (*Sturnus vulgaris*) populations in Western Australia. Mol Ecol 18 1560–1573.

Rosenberg NA. 2004. DISTRUCT: a program for the graphical display of population structure. Mol Ecol Notes 4:137-138.

Rousset F. 2008. GENEPOP 007: a complete re-implementation of the GENEPOP software for Windows and Linux. Mol Ecol Resour 8:103-106.

Rozen, S., Skaletsky, H. 2000. Primer3 on the WWW for general users and biologist programmers. Methods Mol. Biol. 132:365-386.

Saccheri IJ, Richard AN, Brakefield PM. 2001. Effects of bottlenecks on quantitative genetic variation in the butterfly *Bicyclus anynana*. Genet Res Camb 77:167-181.

Sakai AK, Allendorf FW, Holt JS, Lodge DM, Molofsky J, With KA, Baughman S, Cabin RJ, Cohen JE, Ellstrand NC, McCauley DE, O'Neil P, Parker LM, Thompson JN, Weller SG.2001. The population biology of invasive species. Ann Rev Ecol Syst 32:305-332.

Schuelke, M. 2000. An economic method for the fluorescent labeling of PCR fragments. Nat Biotechnol 18:233-234.

Selkoe, K.A., Toonen, R.J. 2006. Microsatellites for ecologists: A practical guide to using and evaluating microsatellite markers. Ecol. Lett. 5: 615-629.

Short KH, Petren K. 2011. Fine-scale genetic structure arises during range expansion of an invasive gecko. PLOS One 6(10): 1-9.

Smith KG. 2006. Patterns of nonindigenous herpetofaunal richness and biotic homogenization among Florida counties. Biol Conserv 127:327-335.

Smith, C.A., Krysko, K.L. 2007. Distributional comments on the teiid lizards (Squamata: Teiidae) of Florida with a key to species. Caribb. J. Sci. 43:260–265.

Tallmon DA, Koyuk A, Luikart G, Beaumont MA. 2008. ONeSAMP: a program to estimate effective population size using approximate Bayesian computation. Molecular Ecology Resources 8: 299-301.

Turelli M, Barton NH. 2006. Will population bottlenecks and multilocus epistasis increase additive genetic variance? Evol 60:1763-1776.

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535-538.

Vazquez-Dominguez E, Suarez-Atilano M, Booth W, Gonzalez-Baca C, Cuaron A. 2012. Genetic evidence of a recent successful colonization of introduced species on islands: *Boa constrictor imperator* on Cozumel Island. Biol Invasions 14:2101-2116.

Walker B, Steffen W. 1997. An overview of the implications of global change for natural land managed terrestrial ecosystems. Conserv Ecol 1(2): http://www.consecol.org/vol1/iss2/art2.

Waples RS, Do C. 2008. LDNE: a program for estimating effective population size from data on linkage disequilibrium. Molecular Ecology Resources 8: 753-756.

Weir BS, Cockerham CC. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38:1358-1370.

Wickham H. 2009. ggplot2: elegant graphics for data analysis. Springer, New York.

Wilcove DS, Rothstein D, Dubow J, Phillips A, Losos E. 1998. Assessing the relative importance of habitat destruction, alien species, pollution, over-exploitation, and disease. BioScience 48:607–616.

Willi Y, Van Buskirk J, Hoffman AA. 2006. Limits to the adaptive potential of small populations. Ann Rev Ecol Evol Sys 37:433-458.

Wilson GA Rannala B. 2003. Bayesian inference of recent migration rates using multilocus genotypes. Genetics 163:1177-1191.

Winck GR, Cechin SZ. 2008. Hibernation and emergence pattern of *Tupinambis merianae* (Squamata: Teiidae) in the Taim Ecological Station, southern Brazil. J Nat Hist 42:239-247.

Wood JW, Campbell TS, Page RB. 2015. Characterization of 14 novel microsatellite loci in the Argentine black and white tegu (*Salvator merianae*) via 454 pyrosequencing. Amphib-Reptil 36:444-449.

Wood JW, Campbell TS, Page RB. 2016. Characterization of 17 novel microsatellite loci in the Nile monitor (*Varanus niloticus*) via 454 pyrosequencing. Amphib-Reptil 37:121-125.

Wood JW, Dowell SA, Campbell TS, Page RB. In Press. Insights into the introduction history and population genetic dynamics of the Nile monitor (*Varanus niloticus*) in Florida. J Hered.

Zhdanova OL, Pudovkin AI. 2008. Nb_HetEx: a program to estimate the effective number of breeders. Journal of Heredity 99:694-695.

CURRICULUM VITAE
Jared Price Wood

CONTACT INFORMATION

400 SW Gordon St., Apt. 512
Burleson, TX 76028

Phone: (580) 677-1608
Email: j.wood@swau.edu

PROFESSIONAL EXPERIENCE

July 2015-present, Assistant Professor of Biology, Southwestern Adventist University
Jan 2011-July 2015, Graduate Teaching Assistant, University of Louisville
Aug 2008-Dec 2010, Research Assistant, Southeastern Oklahoma State University

EDUCATION

Ph.D., Biology, University of Louisville (Jan. 2011-March 2016; Co-Advisers: Drs. Robert Page and Perri Eason)

B.S., Fisheries and Wildlife Conservation, Southeastern Oklahoma State University (Summa Cum Laude, December 2010; Adviser: Dr. Tim Patton)

PUBLICATIONS

Wood JP, Dowell SA, Campbell TS, Page RB. In Press. Insights into the introduction history and population genetic dynamics of the Nile monitor (*Varanus niloticus*) in Florida. *Journal of Heredity*.

Wood JP, Campbell TS, Page RB. 2016. Characterization of 17 novel microsatellite loci in the Nile monitor (*Varanus niloticus*) via 454 pyrosequencing. *Amphibia-Reptilia* 37:121-125.

Wood JP, Campbell TS, Page RB. 2015. Characterization of 14 novel microsatellite loci in the Argentine black and white tegu (*Salvator merianae*) via 454 pyrosequencing. *Amphibia-Reptilia* 36: 444-449.

Dowell SA, Wood JP, Campbell TS, Kolokotronis SO, Hekkala ER. In press. Combining genetic and distributional approaches to sourcing introduced species: a case study on the Nile monitor (*Varanus niloticus*) in Florida. *Royal Science Open Society*.

Wood JP, Dowell SA, Campbell TS, Page RB. Insights into the introduction history and population genetic dynamics of the Argentine black and white tegu (*Salvator merianae*) in Florida. In preparation.

Wood JP, and TM Patton. Movement and overwinter survival of captive-raised juvenile American alligators (*Alligator mississipiensis*) in southeastern Oklahoma. Submitted to *Southeastern Naturalist.*

Patton TM, and JP Wood. 2010. A herpetofaunal survey of the Boehler Seeps Preserve, with reports of new county records and recommendations for conservation efforts. *Proceedings of the Oklahoma Academy of Science* 89:67.

REPORTS

Wood JP. 2012. Final report for SUP B12-030: Nile monitor survey of the Arthur R. Marshal Loxahatchee National Wildlife Refuge.

Wood JP. 2011. Alligator snapping turtle survey of the Clarks River National Wildlife Refuge. Submitted to the Clarks River National Wildlife Refuge.

Patton TM, JP Wood, and C Cheek. 2009. Final Report for SUP 21650-09-10: Aquatic Turtle Survey of the Tishomingo National Wildlife Refuge. Submitted to the Tishomingo National Wildlife Refuge.

Patton TM, JP Wood, and C Cheek. 2009. Final Report for SUP 21680-9-015: Aquatic Turtle Survey of the Little River National Wildlife Refuge. Submitted to the Little River National Wildlife Refuge.

Patton TM, and JP Wood. 2009. A Survey of Amphibians and Reptiles of the Boehler Seeps Preserve, Atoka County, Oklahoma. Final Report on Grant # OKTNC-22308, Submitted to The Oklahoma Nature Conservancy.

GRANT PROPOSALS

Awarded

Kentuckiana Safari Club Conservation Fund: Submitted Oct. 2013 ($8,000)
Proposal Title: Top-down regulation and interspecific competition exerted on Kentucky ecosystems by top predators: *Canis latrans* and *Lynx rufus*
Principal Investigator: Gavin Bradley, University of Louisville
Co-principal investigator: Jared Wood
Primary Scientific Sponsor: Perri Eason

Columbus Zoo Conservation Fund: Submitted May 2012 ($3,240)
Proposal Title: Feeding ecology of two Florida invasives: The Nile monitor (*Varanus niloticus)* and Argentine black-and-white tegu (*Tupinambis merianae*)
Principle Investigator: Jared Wood
Primary Scientific Sponsor: Perri Eason

Sigma Xi Grants-in-Aid of Research Program: Submitted Mar. 2012 ($500)
Proposal Title: Feeding ecology of two Florida invasives: The Nile monitor (*Varanus niloticus)* and Argentine black-and-white tegu (*Tupinambis merianae*)
Principal Investigator: Jared Wood
Primary Scientific Sponsor: Perri Eason

The Nature Conservancy, Oklahoma Chapter, Conservation Grant: Submitted Oct. 2010 ($5,000; deferred grant to Missouri State)
Proposal Title: Western Chicken Turtle (*Deirochelys reticularia miaria*) Demography, Reproductive Biology, Habitat Use, and Terrestrial Movements along the Muddy Boggy Drainage in Southeastern Oklahoma
Principal Investigator: Jared Wood
Primary Scientific Sponsor: Day Ligon, Missouri State University

Declined
National Geographic Young Explorers Grant: Submitted Jun. 2012 ($5,000)
Proposal Title: Feeding ecology of two Florida invasives: The Nile monitor (*Varanus niloticus)* and Argentine black-and-white tegu (*Tupinambis merianae*)
Principal Investigator: Jared Wood
Primary Scientific Sponsor: Perri Eason

PRESENTATIONS

Wood JP, TS Campbell, and RB Page. 2014. Insights Into the introduction histories of the Nile monitor (*Varanus niloticus*) and Argentine black-and-white tegu (*Tupinambis merianae*) in Florida via next generation sequencing and population genetic analysis. Joint Meeting of Ichthyologists and Herpetologists, Chattanooga, TN. Contributed Oral Paper.

Patton TM, and JP Wood. 2011. Movement and overwinter survival of captive-raised juvenile American alligators in southeastern Oklahoma. Oklahoma Academy of Science Technical Meeting, East Central University, Ada, OK. Contributed Oral Paper.

Wood JP, and TM Patton. 2010. A herpetofaunal survey of the Boehler Seeps Preserve, with reports of new county records and recommendations for conservation efforts. LS-OKAMP, Oklahoma State University, Stillwater, OK. Poster.

Wood JP, and TM Patton. 2010. The influence of incorporating a variety of survey protocols and a broad temporal component in the design of an amphibian and reptile survey. Southwestern Association of Naturalists, Junction, Texas. Oral Presentation.

Wood JP, and TM Patton. 2008. A herpetofaunal Survey of the Boehler Seeps Preserve, with reports of new county records and recommendations for conservation efforts. Oklahoma Academy of Science Technical Meeting, Southern Nazarene University, Bethany, OK. Contributed Oral Paper.

Wood JP, and TM Patton. 2008. A herpetofaunal survey of the Boehler Seeps Preserve, with reports of new county records and recommendations for conservation efforts. Oklahoma Research Day, Tulsa Community College, Tulsa, OK. Contributed Oral Paper.

McAllister J, JP Wood, and TM Patton. 2008. Natural disturbances and impacts on rare species: When do we let nature take its course? Oklahoma Research Day, Tulsa Community College, Tulsa, OK. Oral Presentation.

COURSES TAUGHT

Assistant Professor (Institution: Southwestern Adventist University)
Course: Biology 112, General Biology II
Format: Lecture and Lab

Course: Biology 230, Ecology
Format: Lecture and Lab
Course: Biology 443, Comparative Vertebrate Anatomy
Format: Lecture and Lab

Course: Biology 491, Herpetology
Format: Lecture and Lab

Graduate Teaching Assistant (Institution: University of Louisville)
Course: Biology 104, Introduction to Biology
Format: Lab

Course: Biology 244, Principles of Biology
Format: Lab

Course: Biology 347, Comparative Vertebrate Anatomy
Format: Lab

Undergraduate Teaching Assistant (Institution: Southeastern Oklahoma State University)
Course: Biology 1404, Principles of Biology I
Format: Lab

Course: Biology 2114, Zoology
Format: Lab

Course: Conservation 2224, Fundamentals of Soil Science
Format: Lab

Course: Biology 3414, Ecology
Format: Lab

Course: Conservation 4224, Techniques in Fisheries and Wildlife Management
Format: Lab

Course: Conservation 4524, Herpetology
Format: Lab

Course: Conservation 4971, Coastal Ecology
Format: Lab

PROFESSIONAL DEVELOPMENT

Memberships
Society for the Study of Amphibians and Reptiles (December 2011-present)
Herpetologists' League (July 2012-present)
Safari Club International (January 2014-present)

Workshops
Sponsored participant in the Safari Club's American Wilderness Leadership School, Jackson, WY, August 2014.
Participant in Grant Writing Workshop at the Southwestern Association of Naturalists Meeting, Junction, TX, April 2010.

Outreach
Co-adviser for conservation outreach and research programs for the Kentuckiana Safari Club Chapter (Jan. 2014-Jun. 2015)
Guest presenter for 'Conservation Awareness' at Louisville Adventist Academy, Louisville, KY (Aug. 2011, 2013)
Guest presenter for 'Reptiles and Amphibians of Oklahoma' at the Arbuckle-Simpson Nature Festival, Tishomingo, OK (May 2009, 2010)
Guest presenter for 'Reptiles and Amphibians of Oklahoma' at the Coal County Outdoor Classroom, Coalgate, OK (May 2009)
Guest presenter for 'Introduction to Reptiles and Amphibians' at Northwest Heights Elementary, Durant, OK (Aug. 2009)
Guest presenter for 'Reptiles and Amphibians of Oklahoma' at the Atoka County Outdoor Classroom, Atoka, OK (May 2008)

HONORS AND AWARDS

University of Louisville Faculty Favorite Award (2011-2012, 2012-2013)
William Clay Conservation Award (2012)
Alpha Chi Honor Society (2009-Present)
Outstanding Presentation in the Field of Conservation at the Oklahoma Academy of Science Technical Meeting, "A herpetofaunal survey of the Boehler Seeps Preserve, with reports of new county Records and recommendations for conservation efforts," (2008)