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MOLECULAR ANALYSES CONFIRMING THE INTRODUCTION OF NILE CROCODILES, *CROCODYLUS NILOTICUS* LAURENTI 1768 (CROCODYLIDAE), IN SOUTHERN FLORIDA, WITH AN ASSESSMENT OF POTENTIAL FOR ESTABLISHMENT, SPREAD, AND IMPACTS

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***Abstract.*—The state of Florida, USA, has more introduced herpetofauna than any other governmental region on Earth. Four species of nonnative crocodilians have been introduced to Florida (all since 1960), one of which is established. Between 2000–2014 we field-collected three nonnative crocodilians in Miami-Dade County, Florida, and one in Hendry County, Florida. We used DNA barcoding and molecular phylogenetics to determine species identification and native range origin. Also, we described diet, movement, and growth for one crocodile. Our molecular analyses illustrated that two of the crocodiles we collected are most closely related to Nile Crocodiles (*Crocodylus niloticus*) from South Africa, suggesting this region as a source population. We, thus, documented the first known introduction of *C. niloticus* in Florida. Two, and possibly three of the introduced crocodiles shared the same haplotype, suggesting they are likely from the same introduction pathway or source. One animal was captured, measured, marked, and released, then recaptured 2 y later allowing us to calculate growth rate (40.5 cm/y) and movement. The most likely route of travel by waterway (i.e., canal) illustrates that this animal traveled at least 29 km from its original capture site. One crocodile escaped from a facility in Hendry County, Florida, and survived in 1,012 ha of semi-wild habitat for three to four years, confirming that this species can survive in southern Florida.**

Key Words.—crocodile; DNA barcoding; growth; invasive species; management; nonnative; phylogenetics; reptile

INTRODUCTION

Florida has the most introduced (stages 2–5 following Colautti and MacIsaac 2004) amphibians and reptiles in the world (Krysko et al. 2011). Four species of nonnative crocodilians have been introduced to Florida since 1960: Slender-snouted Crocodile (*Mecistops cataphractus* [Cuvier 1824]), Cuvier's Dwarf Caiman (*Paleosuchus palpebrosus* [Cuvier 1807]), Schneider's Smooth-fronted Caiman (*Paleosuchus trigonatus* [Schneider 1801]), and Spectacled Caiman (*Caiman crocodilus* [Linnaeus 1758]; Ellis 1980; Krysko et al. 2011). *Caiman crocodilus* is the only established (stage 3 or higher) nonnative crocodilian (Ellis 1980), while the remaining three have only achieved stage 2 introductions (Krysko et al. 2011). Florida also has two native crocodilians: the American Alligator (*Alligator mississippiensis* Daudin 1802) and American Crocodile (*Crocodylus acutus* Cuvier 1807; Hornaday 1875; Clarke 1888; Reese 1907; Mazzotti et al. 2009), both of which are protected species.

The Nile Crocodile (*Crocodylus niloticus* Laurenti 1768) is a large species capable of reaching 6 m in length (Fergusson 2010). It is known to prey upon crustaceans, arachnids, insects, fishes, amphibians, reptiles, birds, and mammals including humans (Vansleb 1678; Cott 1961; Wallace and Leslie 2008). This species was considered to occupy most of sub-Saharan Africa and parts of the southern Mediterranean coast; however, recent phylogenetic analyses provided evidence for a revised taxonomy (Hekkala et al. 2011). Nestler (2012) found high variation in *C. niloticus* skulls using geometric morphometrics, supporting the notion of multiple lineages within *C. niloticus*. *Crocodylus niloticus sensu stricto* occurs mostly in the eastern half of the continent from South Africa northward, including Madagascar, to Egypt and, formerly, eastward to Israel (Vansleb 1678; Laurenti 1768; Pooley and Gans 1976; Glaw and Vences 2007; Hekkala et al. 2011), whereas the taxonomically resurrected West African Crocodile (*Crocodylus suchus* Geoffroy-Saint-Hillaire 1807) occurs from Senegal and Mauritania in western Africa southeastward to Uganda and the Democratic Republic of Congo (Schmitz et al. 2003; Hekkala et al. 2011; Shirley et al. 2015).

DNA barcoding is a molecular technique wherein a short fragment of DNA, typically mitochondrial cytochrome c oxidase subunit I (COI), is used to identify individuals to species (Hebert et al. 2003a, b). DNA barcoding has been demonstrated to be an effective technique for identifying species of the cryptic *Crocodylus niloticus* complex (Eaton et al. 2010; Shirley et al. 2015). We used DNA barcoding to confirm species identity of two presumed *Crocodylus niloticus* as stage 2 introduced species in southern Florida, USA. Additionally, we employed molecular phylogenetic methods to identify the likely native range origin of introduced individuals. Finally, we provided data on movement, growth, and foraging to comment on survival of *Crocodylus niloticus* in Florida.

MATERIALS AND METHODS

Specimen acquisition.—We responded to reports from private citizens of unusual looking crocodilians in southern Florida. We conducted subsequent daytime visual and nighttime spotlight surveys to find and capture potential nonnative crocodiles. We captured animals by hand or with a harpoon in combination with nets to corral an animal into a small section of man-made canal. Upon capture, we measured snout-vent length (SVL) from the anterior snout edge to posterior end of the cloaca, total length (TL) from anterior snout edge to the posterior tip of the tail, head length (HL) from anterior snout edge to the posterior edge of the cranial table, tail girth (TG) as circumference of tail immediately posterior to cloaca, and mass of each individual. We determined sex by manually probing the cloaca. We tail notched each individual for later identification and stored the clipped tail scutes in Drierite anhydrous calcium sulfate for later molecular analysis. We performed gastric lavage to obtain stomach contents (Fitzgerald 1989). For one crocodile that was captured, released, and recaptured due to permitting guidelines, we measured distance between primary and secondary capture sites using an image from Google Earth. We deposited all photographs and tissues as vouchers in the Division of Herpetology, Florida Museum of Natural History, University of Florida (UF-Herpetology).

Laboratory techniques.—We extracted DNA from scute tissue samples using ZR Genomic DNA™-Tissue Microprep Kit (Zymo Research, LLC, Irvine, California, USA). We used primers FishR2_t1 and FishF2_t1 (Ivanova et al. 2007; Eaton et al. 2010; Shirley et al. 2015) to amplify and sequence a 565 base pair (bp) fragment of the COI mtDNA gene for DNA barcoding. Following Hekkala et al. (2011), we used 4,137 bp for phylogenetic analyses, including the mtDNA 12S rRNA (421 bp), control region/d-loop (735 bp), NADH nicotinamide adenine dinucleotide dehydrogenase subunit 4 (ND4; 860 bp) region, and the nDNA recombination activating gene 1 (RAG1; 714 bp), ribosomal protein S6 (693 bp), and tropomyosin intron (714 bp; see Table 1 for primers). For all molecular markers, PCR was conducted in 25 μl reactions: 9.5 μl H_2O, 12.5 μl GoTaq® Master Mix (Promega Corp, Madison, Wisconsin, USA), 1.0 μl each primer (10 μM), and 1.0 μl DNA template. PCR parameters included initial denaturing at 95° C for 15 min, followed by 35 amplification cycles: denaturing at 94° C for 30 s, annealing at 57° C for 90 s, and extension at 72° C for 60 s, followed by a final extension at 72° C for 10 m (Shirley et al. 2015). We verified PCR products by visualization on a 1% agarose gel with GelRed™ staining (Biotium Inc., Hayward, California, USA). We assembled and edited sequence files generated by the automated sequencer (Genomics Division, Interdisciplinary Center for Biotechnology Research, University of Florida, Gainesville, Florida, USA) as necessary using Geneious (ver. 6.1, Biomatters Ltd., Auckland, New Zealand).

DNA barcoding.—We downloaded reference COI sequence data for *Crocodylus* from GenBank (Shirley et al. 2015; Table 1) and aligned them with sequences generated as part of this study using Geneious. We identified our introduced crocodiles to species by visually matching fixed, segregating nucleotide positions, and unique COI haplotypes to the reference sequences.

Phylogenetic analyses.—We downloaded reference DNA sequence data for 35 *Crocodylus niloticus* and *C. suchus*, and one each of *C. acutus*, *C. moreletii*, *C. rhombifer*, *Mecistops cataphractus*, and *Osteolaemus tetraspis* to be used as outgroups from the Dryad data repository (doi:10.5061/dryad.s1m9h; Table 2). We aligned these reference sequences with our sequences from introduced crocodiles generated in this study. Phylogenetic relationships were estimated using both maximum likelihood (ML) and Bayesian inference (BI) methods.

We implemented ML in the RAxML-HPC BlackBox (Stamatakis 2006; Stamatakis et al. 2008) on the CIPRES Science Gateway (Miller et al. 2010) using the General Time Reversible model of nucleotide evolution with gamma distributed rate heterogeneity (GTR + Γ). We implemented BI in BEAST (ver. 1.8; Drummond and Rambaut 2007) on the UF-HPC Galaxy

TABLE 1. Primers (5'–3' direction) used to sequence crocodilians for mitochondrial DNA (mtDNA) cytochrome oxidase *c* subunit I (COI), 12S ribosomal (12S), control region (d-loop), nicotinamide adenine dinucleotide dehydrogenase subunit 4 (ND4) region, and nuclear DNA (nDNA) recombination activating gene 1 (RAG1), ribosomal protein S6, and introns for tropomyosin.

Gene Region	DNA Marker	Primer Name	Primer Sequence	Source
COI	mtDNA	FishF2_t1	CGA-CTA-ATC-ATA-AAG-ATA-TCG-GCA-C	Ivanova et al. (2007)
COI	mtDNA	FishR2_t1	ACT-TCA-GGG-TGA-CCG-AAG-AAT-CAG-AA	Ivanova et al. (2007)
12S	mtDNA	12s183	TTG-CCC-TAA-GCA-GCC-TGT-AT	Hekkala et al. (2011)
12S	mtDNA	12s375	CCG-TCT-TTG-ACA-GTC-CTG-GT	Hekkala et al. (2011)
Control Region	mtDNA	L15463	CGC-TGG-CCT-TGT-AAG-ACA-GA	Hekkala (2004)
Control Region	mtDNA	H16258	CAC-TAA-AAT-TAC-AGA-AAA-GCC-G	Hekkala (2004)
ND4	mtDNA	F2	AAA-ACC-TAA-ACC-TGC-TMC-AAT-G	Hekkala et al. (2011)
ND4	mtDNA	Leu	CAT-TAC-TTT-TAC-TTG-GAT-TTG-CAC	Hekkala et al. (2011)
RAG1	nDNA	F	AGC-ACA-AAG-CTT-CTT-GCA-GTT	Hekkala et al. (2011)
RAG1	nDNA	R	GGA-CAG-AAG-GTG-TTG-TCT-TGG-T	Hekkala et al. (2011)
S6	nDNA	F	ATC-AGT-GGT-GGC-AAT-GAC-AA	Hekkala et al. (2011)
S6	nDNA	R	TCT-TGC-CCT-CTT-TGT-TCA-GG	Hekkala et al. (2011)
Tropomyosin	nDNA	F	GAG-TTG-GAT-CGS-GCT-CAG-GAG-CG	Friesen et al. (1999)
Tropomyosin	nDNA	R	CGG-TCA-GCC-TCY-TCM-GCA-ATG-TGC-T	Friesen et al. (1999)

server (http://hpc.ufl.edu; Giardine et al. 2005; Blankenberg et al. 2010; Goecks et al. 2010). We performed a mixed-model analysis to infer trees and assess nodal support using models incorporating evolutionary information specific to each gene. We selected the most likely models of nucleotide substation in jModelTest based on Akaike Information Criterion (AIC) scores (ver. 2.1.4; Guindon and Gascuel 2003; Darriba et al. 2012). The best fit models for each locus were: GTR + Γ for 12S, Hasegawa, Kishino and Yano with gamma distributed rate heterogeneity and proportion of invariant sites (HKY + I + Γ) for control region, GTR + I for ND4, and HKY for RAG1, S6, and tropomyosin.

We used an uncorrelated lognormal relaxed clock, constant population size, estimated base frequencies, randomly generated starting tree, and normal relaxed clock mean (ucld.mean) priors. We performed two independent Markov Chain Monte Carlo (MCMC) runs, each with three heated and one cold chain, for 40 million generations sampling every 1,000th generation. We independently analyzed both MCMC runs for posterior convergence using Tracer (ver. 1.6) where ESS values > 200 and split standard deviation less than 0.005 for -lnL tree values among chains indicated stationarity was achieved. We discarded all trees sampled in the first five million generations (i.e., prior to stationarity) as burn-in and combined the remaining trees from both runs using LogCombiner (ver. 1.8). We generated the maximum clade credibility (MCC) tree with mean heights using TreeAnnotator (ver. 1.8) and visualized the phylogenetic hypothesis with posterior probabilities using FigTree (ver. 1.4.2).

RESULTS

***Nonnative crocodiles*.—**The first crocodile (approximately 1.2–1.5 m TL; photographic voucher UF-Herpetology 175632) in this study escaped from its enclosure in 1996 or 1997 at Billie Swamp Safari, Seminole Reservation, Hendry County (26.330348°N, 81.055936°W; datum WGS84; Jonathan Vasquez, pers. comm.). This crocodile was somewhat contained by a perimeter fence within the 1,012 ha property. In 2000, it was finally recaptured by Billie Swamp Safari staff and measured 3 m TL. We were unable to secure a tissue sample from this crocodile.

The second crocodile (UF-Herpetology 175743), a hatchling, was captured by Robert Freer on 14 April 2009 on the house porch of a resident at 24800 SW 193 Avenue, Miami, Miami-Dade County (25.534833°N, 80.504653°W), and subsequently transported to Kliebert's Turtle and Alligator Farm in Hammond, Louisiana. In June 2015 we acquired a tissue sample from this crocodile. The third crocodile (UF-Herpetology 165995) was a female captured on 27 October 2011 at the Preston B. Bird/Mary Heinlein Fruit and Spice Park, Homestead, Miami-Dade County (25.53385°N 80.49348°W; datum WGS84; Fig. 1), and subsequently kept in captivity by JAW. It measured 57.7 cm SVL, 115.2 cm TL, 15.3 cm HL, and weighed 4.4 kg.

The fourth crocodile (UF-Herpetology 173082) was a female (86.3 cm TL) captured on 13 March 2012 in a canal in Homestead, Miami-Dade County (25.50632°N, 80.47343°W). This crocodile was released and recaptured 9 March 2014 in Everglades National Park

TABLE 2. Voucher and GenBank accession numbers for the two introduced Nile Crocodiles (*Crocodylus niloticus*) used in this study for molecular analyses. The comparative DNA sequences were taken from Hekkala et al. (2011). Individual UF 165995 was found at 24800 SW 187 Avenue, Miami, Miami-Dade County, Florida, USA, and UF 173082 was found at Everglades National Park, SW 237 Avenue, Miami, Miami-Dade County, Florida, USA.

Voucher	CO1	12S	D-loop	ND4	RAG1	S6	Tropomyosin
UF 165995	KP297880	KP297878	KP297882	KP297884	KP297886	KP297888	KP297876
UF 173082	KP297881	KP297879	KP297883	KP297885	KP297887	KP297889	KP297877

(ENP; 25.61707°N, 80.5753°W; U.S. National Park Service, unpubl. report), and measured 23.6 cm HL, 88.0 cm SVL, 167.9 cm TL, 43.6 cm TG, and weighed 17.0 kg. Its stomach contained remains of a Largemouth Bass (*Micropterus salmoides*). Straight-line distance over land between primary and secondary capture sites was 16 km; however, the most likely route of travel by canal was 29 km.

***Genetics and phylogenetic analyses*.—**We successfully sequenced DNA for all seven loci from the third and fourth nonnative crocodiles (UF-Herpetology 165995 and UF-Herpetology 173082). These two individuals shared the same haplotype for all loci. We successfully sequenced DNA for ND4, RAG1, S6, and Trop for the second crocodile (UF-Herpetology 175743), which shared the same haplotypes as the two crocodiles above; however, sequences for CO1, dloop, and 12S were unclean and too difficult to read after multiple DNA isolations and; thus, this individual was removed from subsequent analyses.

Nonnative crocodiles UF-Herpetology 165995 and UF-Herpetology 173082 exhibited *C. niloticus* haplotypes and shared diagnostic single nucleotide polymorphisms (SNP) at 7 sites unique to previously published COI sequences for this species. The ML and BI analyses resulted in a tree topology congruent with that of Hekkala et al. (2011; Fig. 2). Both UF-Herpetology 165995 and UF-Herpetology 173082 aligned most closely with an individual from South Africa.

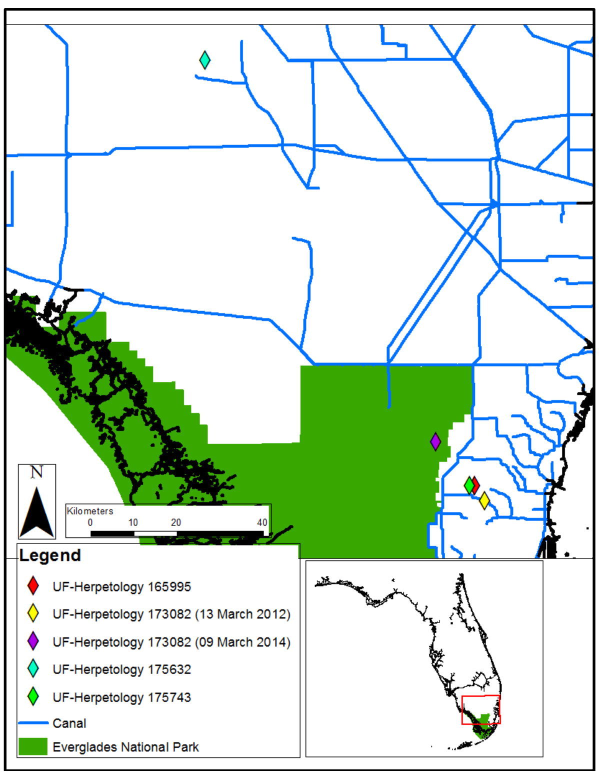

FIGURE 1. Capture locations of Nile Crocodiles (*Crocodylus niloticus*) in southern Florida, USA, 2000–2014.

DISCUSSION

This study provides the first verified vouchered records of introduced *Crocodylus niloticus* in Florida, USA. Our DNA barcoding confirms that two of our samples (UF-Herpetology 165995 and UF-Herpetology 173082) are *C. niloticus* and not the recently resurrected *C. suchus*. Our phylogenetic analyses suggest that these two samples are also most closely related to the native range origin of South Africa. Because these two samples are genetically identical for all loci sequenced in this study, it suggests that they likely came from the same introduction pathway source. Shirley et al. (2015) suggested that 95% of all sampled *C. niloticus sensu lato* in captivity in the USA, including captive collections in southern Florida, were *C. suchus*. Thus, our crocodiles either come from a different source or one of these sources had or have different animals than those that were sampled by Shirley et al. (2015). Over the last decade several large groups of *C. niloticus* have been imported from South Africa and Madagascar for both zoological display (e.g., Disney's Animal Kingdom) and the pet trade, with the latter being the most likely introduction pathway for these individuals. Nonetheless, our study reinforces the use of molecular data in positively identifying introduced species and determining their native range origin when a published reference data set is available. Both are critical pieces of information for the management of introduced, and potentially invasive, species.

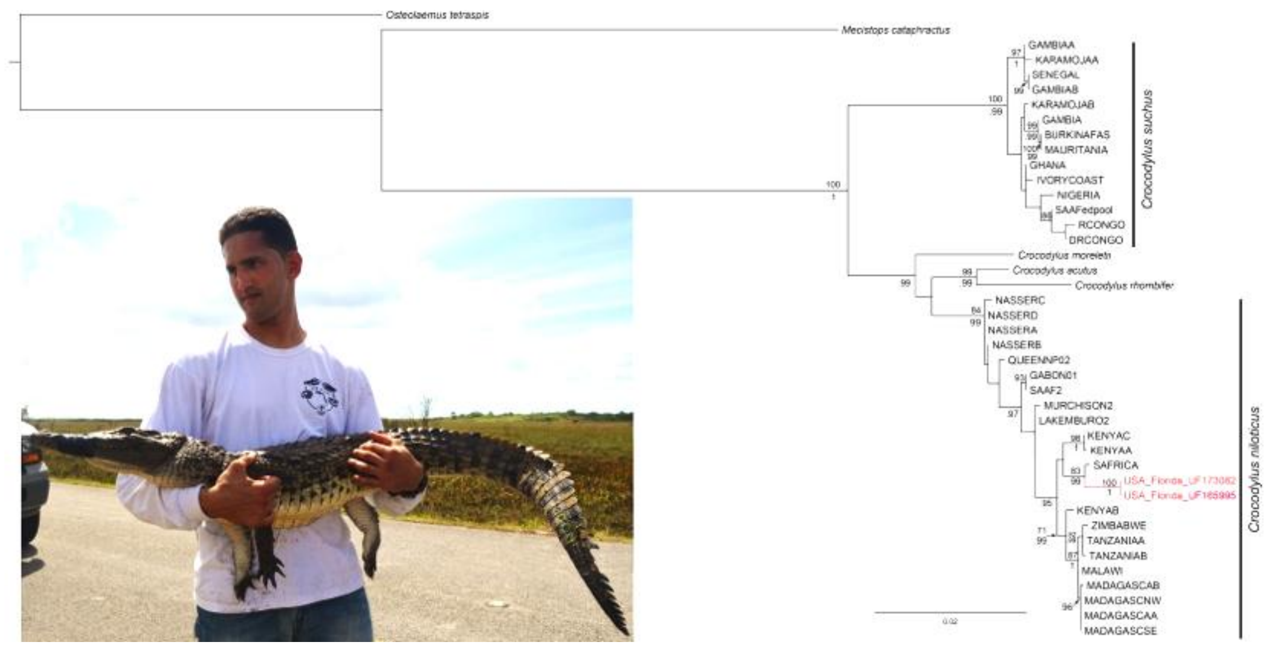

FIGURE 2. Maximum likelihood phylogeny for Nile (*Crocodylus niloticus*) and West African (*C. suchus*) crocodiles, including two specimens (UF-Herpetology 165995 and 173082; highlighted in red) introduced to Florida, USA. Note that values (≥ 70%) above major nodes represent bootstrap support, and values (≥ 95%) below major nodes represent posterior probabilities from the Bayesian inference phylogeny. Inset photograph of the late Rafael Crespo holding UF-Herpetology 173082 after its capture in Everglades National Park on 9 March 2014. (Inset photographed by Michael R. Rochford).

Our recapture data indicate that *Crocodylus niloticus* can survive in the wild in southern Florida for nearly two years. *Crocodylus niloticus sensu lato* (UF-Herpetology 175632) from Hendry County survived for 4–5 y in the wild, and confirmed *C. niloticus* UF-Herpetology 173082 from Miami-Dade County survived for 2 y. Closely related *Crocodylus suchus* at an attraction in Homestead, Miami-Dade County, are kept outdoors year-round without artificial heat sources, including during a record prolonged cold period (Mario Aldecoa, pers. comm.) that killed many native *C. acutus*, as well as manatees, fishes, and nonnative pythons (Hallac et al. 2010; Mazzotti et al. 2011).

Survival in *C. niloticus* is size-related (Hutton 1987) and UF-Herpetology 173082 was introduced and able to survive at a size when it was most vulnerable. This animal grew remarkably quickly, almost doubling in size in 2 y. Hatchlings from Ngezi, Zimbabwe, grew more quickly at a rate of 31.7 cm/y and reached 90 cm TL in 5 y (Hutton 1987). Our crocodile captured in ENP grew at a rate of 40.5 cm/y, 28% faster than wild *C. niloticus* hatchlings from certain parts of their native range (Hutton 1987).

Juvenile native *Crocodylus acutus* from Turkey Point Power Plant (TP), Homestead, Miami-Dade County, grew 40.15 cm/y, which is greater than growth in juvenile *C. acutus* from other areas in Florida (Mazzotti et al. 2007) and comparable to UF-Herpetology 173082 that did not benefit from the highly productive TP ecosystem. This was comparable to subadult *Alligator mississippiensis* growth rates from Shark Slough in ENP (Table 3) and was greater than growth rates found for subadult native *A. mississippiensis* from the Shark Valley region of Florida (Jacobsen and Kushlan 1989), northern Florida (Deitz 1979), South Carolina (Bara 1977), eastern Texas (Saalfeld et al. 2008), and Louisiana (Chabreck and Joanen 1979). The subadult ENP *C. niloticus* grew at a faster rate than some native Florida crocodilians and other crocodilians reported in the literature, with the exception of *C. acutus* from TP. Growth is closely related to temperature, salinity, population density, food quality, and food quantity (Hutton 1987; Mazzotti 1999), indicating the southern Florida environment and Everglades habitat provided sufficient prey and thermoregulatory opportunities for favorable growth for at least one of the introduced crocodiles in our study (UF-Herpetology 173082).

Putative, unverified *Crocodylus niloticus* have been introduced (stage 2) to Mississippi (Anonymous 1998) and Florida (Quinn 1994) but were recaptured quickly. While there is no current evidence of an established population of *C. niloticus* in Florida or Mississippi, much of the Atlantic and Gulf coasts could provide similar climatic conditions. In its native range, *C. niloticus* reaches latitudes of approximately 32 degrees, north and south of the equator, incorporating both

TABLE 3. Comparison of growth rates of *Alligator mississippiensis* and *Crocodylus acutus* in the USA and *Crocodylus niloticus* (UF-Herpetology 73082) in Zimbabwe and the USA.

Species	Location	Growth Rate (cm/y)	Source
Crocodilus niloticus	Miami-Dade County, Florida, USA	40.5	This paper
C. acutus	Turkey Point Power Plant, Miami-Dade County, Florida, USA	40.2	Mazzotti et al. 2007
Alligator mississippiensis	Eastern Texas, USA	35.0	Saalfeld et al. 2008
C. niloticus	Ngeze, Zimbabwe	31.7	Hutton 1987
A. mississippiensis	North of Shark Slough, Florida, USA	31.0	Hines et al. 1968
C. niloticus	Hwange National Park, Zimbabwe	28.6	Hutton 1987
A. mississippiensis	North Florida, USA	24.0	Deitz 1979
A. mississippiensis	South Carolina, USA	23.5	Bara 1977
A. mississippiensis	Louisiana, USA	22.0	Chabreck and Joanen 1979
A. mississippiensis	Shark Valley, Florida, USA	13.6	Jacobsen and Kushlan 1989

tropical and temperate zones. As a comparison, this latitude in the southeastern USA incorporates both of these climate zones and falls just south of Savannah, Georgia, USA. Thus, the Atlantic coast of Florida and the entire coastline of the Gulf of Mexico are within the natural climate zones and latitudinal boundaries of *C. niloticus.* Crocodiles at Lake Ngezi (Zimbabwe) were subjected to minimum air temperatures of 2° C and the nearby area of Kadoma has a mean minimum temp of 8.1° C in July, which is the coolest month in that area (Hutton 1987). Record low air temperature in Miami, Miami-Dade County, Florida, is -2.8° C (National Oceanic and Atmospheric Administration, Climatological Records for Miami, FL. Available from www.srh.noaa.gov/images/mfl/climate/ Daily%20Records%20-%20Miami.pdf [Accessed 3 February 2016]), which is similar to what *C. niloticus* experiences in its native range where temperatures have reached -1.0° C in Haifa, Israel (Wikipedia, Haifa. Available from https://en.wikipedia.org/wiki/Haifa [Accessed 03 February 2016]). Temperatures in coastal Georgia have dropped as low as -10° C (Dahlberg and Smith 1970), which may be intolerable and indicates *C. niloticus* would probably be unsuccessful colonizing this latitude in the western hemisphere.

If *C. niloticus* became established, it may threaten the native species of Florida through predation (documented herein) and competition, compounding the existing threat to native wildlife already impacted by human-induced habitat modification and introduced invasive species such as the Burmese Python (*Python bivittatus*), and Argentine Black and White Tegu (*Salvator merianae*; Dorcas et al. 2012; Mazzotti et al. 2014). Additionally, many crocodilian species are already known to hybridize in captivity and where their native ranges overlap in the wild (Weaver et al. 2008; Machkour-M'Rabet et al. 2009; Rodriguez et al. 2011; Srikulnath et al. 2012). Introducing *C. niloticus* to the native range of *C. acutus* may result in hybrids degrading the genetic integrity of *C. acutus*, a state and federally listed species.

There are both economic risks and risks to human health and safety presented by establishment of Nile Crocodiles in Florida. Throughout its native range, *Crocodylus niloticus* is responsible for significant loss of cattle (Aust 2009; Aust et al. 2009), and other domestic/farm animals, annually, which is a potential issue for the agricultural industry of Florida (Shrestha and Alavalapati 2004). *Crocodylus niloticus* was responsible for at least 493 attacks on people 2010–2014, 354 (71.8%), of which were fatal (CrocBITE. 2015. The Worldwide Attack Database. Available from http://www.crocodile-attack.info [Accessed 6 February 2015]). We recommend a scientific risk assessment to evaluate the potential for *C. niloticus* establishment, spread, and impact in Florida.

Native crocodilians play a vital role in our ecosystems, and concern over introduced *Crocodylus niloticus* should not lead to unwarranted fear or persecution of native species, which are protected by both state and federal laws. Removing introduced wildlife quickly is the recommended course of action to avoid ecological impacts (Kraus 2009). However, one of the captured *C. niloticus* had to be legally released shortly after its first capture due to legal considerations. The Florida Fish and Wildlife Conservation Commission considers *C. niloticus* Class 1 wildlife and requires extensive experience, a captive facility inspection, and lengthy application process before licensing an individual to possess such animals. The U.S. Fish and Wildlife Service (USFWS), through the Endangered Species Act, protects *C. niloticus* wherever it occurs, including outside its native range, and we had to obtain permission to capture or euthanize this species in Florida. Distinguishing exotic crocodilian species from native species is difficult and we recommend the USFWS require a clearly identifiable photograph of a nonnative crocodilian before authorization for lethal take is granted. We suggest that state and federal wildlife agencies coordinate policies regarding capture and transport of protected species outside their native range to facilitate rapid response efforts to remove introduced species.

Acknowledgments.—This paper is dedicated to the fond memory of one of our coworkers, the late Rafael Crespo, who helped capture one of these Nile crocodiles in ENP. We are indebted to Tom Rahill and the "Swamp Apes" for help locating UF-Herpetology 173082; and Jake Edwards, Jennifer Eckles, Tylan Dean, Christopher Smith, Barry Offenburger, Jacob Poley, and Scott Devore for assistance capturing UF-Herpetology 173082 on 9 March 2014; T. Mike from Kliebert's Turtle and Alligator Farm for voucher photographs and tissue of UF-Herpetology 175743; Jonathan Vasquez for information on UF-Herpetology 175632; Irvy R. Quitmyer for identifying fish remains found in UF-Herpetology 173082; and Amy J. Benson for providing important literature. The US Fish and Wildlife Service granted us permission to remove UF-Herpetology 173082.

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