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Authors: Roberto, Igor Joventino, Fedler, Matthew T., Hrbek, Tomas, Farias, Izeni P., and Blackburn, David C.

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The Taxonomic Status of Florida Caiman: A Molecular Reappraisal

IGOR JOVENTINO ROBERTO,^{1,2,3} MATTHEW T. FEDLER,⁴ TOMAS HRBEK,¹ IZENI P. FARIAS,¹ AND DAVID C. BLACKBURN²

¹Laboratory of Animal Genetics and Evolution (LEGAL), Federal University of Amazonas (UFAM), Manaus, Amazonas, Brazil 69077-000

²Florida Museum of Natural History, University of Florida, Gainesville, Florida, 32601, USA

⁴Fish and Wildlife Research Institute, Florida Fish and Wildlife Conservation Commission, 1105 SW Williston Road, Gainesville, Florida, 32601, USA

ABSTRACT.—The state of Florida has the world's highest diversity of established nonnative reptiles, including snakes, lizards, turtles, and crocodilians, most of them introduced from the pet trade. The Spectacled Caiman (*Caiman crocodilus*) is the only nonnative crocodilian established in Florida, with the earliest date of introduction in 1950. Despite its likely origin being Colombia, the taxonomic identity of the subspecies occurring in Florida remains unknown. We report the first study that resolves the taxonomic status of the population of *Ca. crocodilus* in Florida based on molecular analysis of samples collected from the 1970s to 2013. We also investigate the probable origin of this population as well as the specimens of the Dwarf Caimans, *Paleosuchus palpebrosus* and *Paleosuchus trigonatus*, which have been collected in Florida. We identified only one lineage of *Ca. crocodilus* in Florida, corresponding to *Caiman crocodilus fuscus*, and our results indicate the Magdalena River basin in Colombia as the most likely area of origin. We also correct the identification of *Paleosuchus* recorded in Florida and identify the Guiana Shield, and specifically Guyana, as the most likely region from which these specimens were introduced.

Florida harbors the highest diversity of established nonindigenous reptile species in the world, with three turtles, 50 lizards, five snakes, and one crocodilian, most of which arrived through the commercial pet trade (Krysko et al., 2011, 2019). Over the last few decades, an increasing number of exotic crocodilian species has been registered in Florida, including *Caiman crocodilus*, *Crocodylus niloticus*, *Paleosuchus palpebrosus*, *Paleosuchus trigonatus*, and *Mecistops cataphractus* (Krysko et al., 2011; Rochford et al., 2016). However, only *Ca. crocodilus* is established in Florida (Ellis, 1980; Krysko et al., 2019). Additional cryptic species might have also been introduced, but their identification is often obfuscated by the lack of diagnostic morphological features. Identification of crocodilian taxa, especially closely related species or subspecies, has often been difficult because of similarities in external morphology and the lack of diagnostic characters distinguishing some taxa (Medem, 1983; Busack and Pandya, 2011).

In studies of exotic species, genetic tools have proven effective for identifying lineages and/or cryptic species and their geographic origin (Armstrong and Ball, 2005; Le Roux and Wieczorek, 2008; Frankham et al., 2010; Fedler et al., 2016; Cock et al., 2017; Harris et al., 2017). Recent molecular phylogenetic studies have revealed substantial cryptic lineage diversity within Crocodylia, some of which have been or will likely be recognized as distinct species within *Caiman* (Roberto et al., 2020), *Osteolaemus*, *Mecistops* (Shirley et al., 2014), *Crocodylus* (Hekkala et al., 2011), and *Paleosuchus* (Muniz et al., 2018; Bittencourt et al., 2019). Rochford et al. (2016) also employed similar tools to identify introduced individuals of *Crocodylus* in southern Florida as *Cr. niloticus*, most likely of South African origins, while previous to this study it was thought the introduced species was the morphologically similar *Crocodylus suchus*.

The only species of crocodilian established in Florida is *Ca. crocodilus*. The *Caiman crocodilus*/*yacare* species complex currently comprises *Ca. yacare* and three subspecies of *Ca. crocodilus*: *Caiman crocodilus crocodilus*, *Caiman crocodilus chiapasius*, and *Caiman crocodilus fuscus* (Balaguera-Reina et al., 2020).

Molecular phylogenetic analyses revealed substantial lineage diversity within this species complex (Venegas-Anaya et al., 2008; Roberto et al., 2020). Members of this complex can be difficult to distinguish using only external morphology (i.e., coloration and scalation; Busack and Pandya, 2011), which can lead to misidentification of some subspecies in both their native and introduced ranges.

The Florida population of *Ca. crocodilus* is suspected to have been introduced from Colombia via the pet trade in the 1950s (King and Krakauer, 1966). Despite the suspected origin, the identity of these Florida caiman within the *Ca. crocodilus* complex remains unclear. Some authors have attributed the Florida caiman to *Ca. c. crocodilus* (Velasco and Ayarzagüena, 2010), while others have attributed them to *Ca. c. fuscus* (Rochford et al., 2019), though in both cases without reference to specific evidence. To date, there have been no genetic analyses to verify the identity of the *Caiman* established in Florida. However, Roberto et al. (2020) found three distinct mitochondrial lineages of *Ca. crocodilus* in Colombia. Two lineages, one in the Orinoco and other in the Amazon drainages, are lineages of *Ca. c. crocodilus*, whereas the third lineage is *Ca. c. fuscus* found in the Magdalena River basin and along the Caribbean Coast. Based on the current understanding of the distribution of lineages in Colombia, it is possible that the Florida populations of *Ca. crocodilus* could contain any or all of these three lineages.

Two other alligatorid species have also been reported as introduced in the Everglades of southern Florida. However, there is no evidence that these two species, the Dwarf Caimans *P. palpebrosus* and *P. trigonatus*, are established (Krysko et al., 2011). The geographic origin of these individuals that were introduced via the pet trade also has yet to be explored.

Using phylogenetic analysis of the mitochondrial Cytochrome b (Cytb) gene, we evaluated the likely geographic origin of the Florida *Caiman* population and the two captured *Paleosuchus* specimens. We aimed to understand whether the *Ca. crocodilus* population that is established in Florida comprises one or more distinct lineages of *Ca. crocodilus* and to determine the geographic origin of the source populations. Similarly, we aimed to determine the taxonomic identity and likely origin of the *Paleosuchus* found in Florida.

³Corresponding Author. E-mail: igorjoventino@yahoo.com.br
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TABLE 1. *Caiman crocodilus fuscus* sequences sampled in Florida.

Species	Voucher number	Cytb base pairs	Locality	Latitude	Longitude	Year
<i>Caiman crocodilus fuscus</i>	UF-Herp-151733	1150	Everglades National Park, Frog City boat ramp	25.7598	–80.599	2007
<i>Ca. c. fuscus</i>	UF-Herp-152672	1150	Everglades National Park, Frog City boat ramp	25.7598	–80.599	2008
<i>Ca. c. fuscus</i>	UF-Herp-152673	1150	Everglades National Park, Frog City boat ramp	25.7598	–80.599	2008
<i>Ca. c. fuscus</i>	UF-Herp-171437	1150	Homestead, L-31E, 0.07 mi S C-102 canal	25.5182	–80.347	2013
<i>Ca. c. fuscus</i>	UF-Herp-171438	1150	Homestead, L-31E, 0.07 mi S C-102 canal	25.5182	–80.347	2013
<i>Ca. c. fuscus</i>	UF-Herp-154567	1150	Everglades National Park, Frog City boat ramp	25.7598	–80.599	2009
<i>Ca. c. fuscus</i>	UF-Herp-171436	1150	Homestead, L-31E, 0.07 mi S C-102 canal	25.5182	–80.347	2013
<i>Ca. c. fuscus</i>	UF-Herp-45439	520	Homestead, Homestead Air Force Base			1979
<i>Paleosuchus palpebrosus</i>	UF-Herp-175564	1090	near Black Point, Homestead, Miami-Dade County			1999
<i>Paleosuchus trigonatus</i>	UF-Herp-153469	1090	Everglades National Park			2008

MATERIALS AND METHODS

Tissue samples from introduced *Caiman crocodilus* and *Paleosuchus* in Florida were obtained from the Division of Herpetology at the Florida Museum of Natural History (FLMNH; Table 1). Tissues used included muscle and scute samples preserved in 95% ethanol and stored frozen in the FLMNH Genetic Resource Repository as well as dried skin removed from skulls stored in the herpetology collection. Genomic DNA was extracted from tissues using the Qiagen DNeasy Blood & Tissue Kit (Qiagen). Polymerase chain reaction (PCR) mixes contained 9.5 μ L H₂O, 12.5 μ L GoTaq[®] Master Mix (Promega Corp, Madison, Wisconsin, USA), 1.0 μ L each primer (10 μ M), and 1.0 μ L genomic DNA template. We amplified the mitochondrial region Cytb using two external primers, GluCRf.1 (5'-CAACCAAAACCTGAGGYCTGA-3') and ProCRr.1 (5'-ATTAGAAYGTCGGCTTTGGGG-3'), one internal primer CytbCRr.2 (5'-AAGATYAGGTGGGTATGAG-3'), which is paired with GluCRf.1, and sequencing primers CytbCRf.1 (5'-ATGACCCACCACTACGAAAA-3') and CytbCRf.3 (5'-CCATACATYGGAGACACCAT-3'; Hrbek et al., 2008). Primer CytbCRf.3 can also be paired with ProCRr.1 to amplify the second half of the Cytb if needed. PCRs were carried out by denaturing at 95°C for 5 min followed by 35 cycles of amplification, denaturing at 95°C for 30 sec, annealing at 52°C for 30 sec, and extension at 72°C for 60 sec followed by a final extension at 72°C for 5 min. Four microliters of each PCR product were electrophoresed on a 2% agarose gel and visualized with GelRed[™] stain (Biotium Inc., Hayward, California, USA). PCR products with a distinct band were sent to Genewiz, Inc. (South Plainfield, New Jersey, USA) for sequencing. Sequence files were assembled and verified in Geneious 9.1.8 (<https://www.geneious.com>; Kearse et al., 2012). These newly collected data were deposited in GenBank (MW660822–MW660831).

We added our eight samples of Florida *Ca. crocodilus* and two samples of Florida *Paleosuchus* to the Cytb dataset of Roberto et al. (2020), which is the most comprehensive survey of genetic diversity in these taxa to date. It includes all members of the *Ca. crocodilus*/*yacare* complex as well as *Caimon latirostris*, *Melanosuchus niger*, *P. palpebrosus*, and *P. trigonatus* lineages that have been previously delimited, including sampling from throughout the ranges of these species. Our final dataset included 296 haplotypes in the subfamily Caimaninae: 54 of *M. niger*, 22 *P. palpebrosus*, 37 *P. trigonatus*, 21 *Ca. latirostris*, 83 *Ca. c. crocodilus*, 52 *Ca. c. fuscus*, 3 *Ca. c. chiapasius*, and 23 *Ca. yacare*. We aligned the sequences in Geneious using MUSCLE (Edgar, 2004) and jModelTest (Posada, 2008), which determined the best nucleotide substitution model based to be TIM3+G. We used BEAST 2.5.1 (Bouckaert et al., 2014) to estimate the phylogenetic

relationships using Bayesian Inference with an uncorrelated lognormal relaxed clock and a coalescent constant population size. We ran three independent Markov Chain Monte Carlo (MCMC) runs for 50 million generations sampling every 5,000 generations. We checked the parameters on TRACER to ensure all ESS values were at least 200. We combined all runs with logcombiner and generated the maximum clade credibility tree (MCCT) using TreeAnnotator (Bouckaert et al., 2014). All analyses were done on the CIPRES gateway (Miller et al., 2010; <https://www.phylo.org>).

RESULTS

Resulting sequences of Cytb of *Caiman crocodilus* from Florida ranged from 520 to 1,150 base pairs (bp; Table 1) and represented the same haplotype. All individuals from Florida were the *Ca. c. fuscus* lineage (Figs. 1A, 2), a clade with individuals from Costa Rica through Panama to the northern Caribbean coast and Magdalena River basin of Colombia. The individuals from the established Florida population are most similar to the individuals of the Caribbean coast of Colombia in the Bolivar Department.

Sequences of individuals of *P. palpebrosus* and *P. trigonatus* from Florida were 1,090 bp. The *P. palpebrosus* collected in Florida (UF-Herp-175564) is from a clade of *P. palpebrosus* from the Brazilian Amazon, similar to the haplotypes of Guiana Shield in the Roraima state (Figs. 1B, 3). The *P. trigonatus* from Florida (UF-Herp-153469) also falls within a clade from the Guiana Shield.

DISCUSSION

The taxonomic status of the Florida *Ca. crocodilus* has been uncertain. Smith and Kohler (1977) considered the subspecies identity of *Ca. crocodilus* in Florida as undefined. Previously King and Krakauer (1966) considered the Florida *Caiman* only as the “spectacled caiman,” the common name applied to all subspecies of *Ca. crocodilus*. Wilson and Porras (1983) considered this to be the “Brown Caiman,” the common name of *Ca. c. fuscus*. Velasco and Ayarzagüena (2010) classified this population as *Ca. c. crocodilus*, while the last International Union for Conservation of Nature (IUCN) assessment (Balaguera-Reina and Velasco, 2019) only considered the Florida individuals as *Ca. crocodilus*, without considering the subspecies. Rochford et al. (2019) asserted that *Ca. c. fuscus* occurs in Florida, but provided no information on how they identified the Florida specimens to subspecies, as was also the case in other previous works (e.g., King and Krakauer, 1966; Smith and Kohler, 1977; Wilson and Porras, 1983; Velasco and Ayarzagüena, 2010).

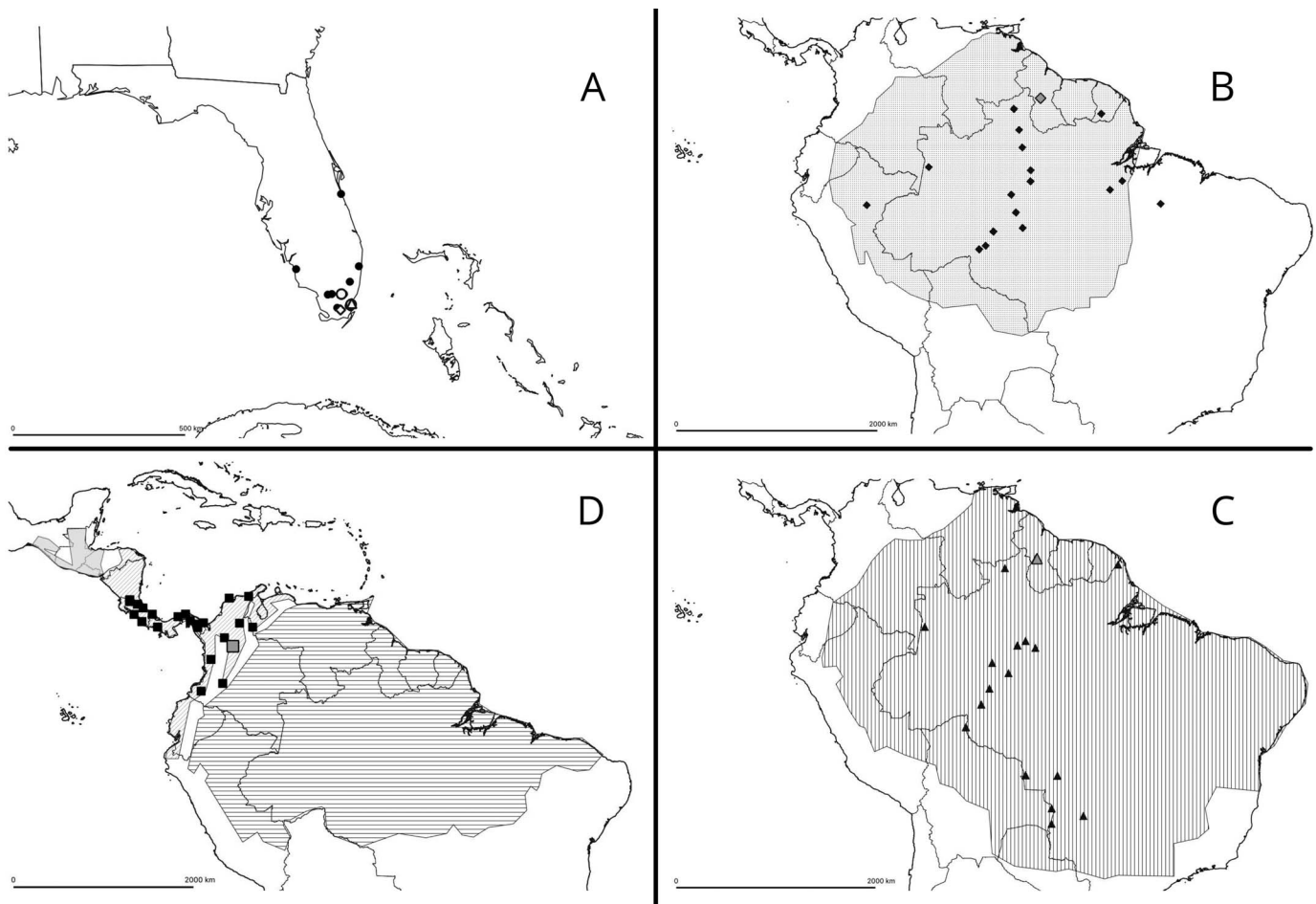


FIG. 1. Geographic distribution of *Caiman* sequences used in this study. (A) South Florida showing the localities of the exotic populations sampled. Black circles represent vouchered specimens *Caiman crocodilus fuscus*. Hollow circles (*Ca. c. fuscus*), triangle (*Paleosuchus palpebrosus*), and diamond (*Paleosuchus trigonatus*) represent nonnative specimens which were sequenced in this study. Native distributions of (B) *P. trigonatus*, (C) *P. palpebrosus* and (D) *Ca. c. fuscus* with black diamonds, triangles, or squares (respectively) representing sequenced specimens, and the gray diamond, triangle, or square representing the locality most likely to represent the origin population of the introduced specimens of the corresponding species in Florida. Estimated ranges of *P. trigonatus* and *P. palpebrosus* are outlined on maps B and C, following the range maps available from the IUCN (www.iucnredlist.org). Estimated ranges for the currently recognized subspecies of *Ca. crocodilus* are labeled on map D following Escobedo-Galván et al. (2011), with exception of *Caiman crocodilus apaporiensis* (see Balaguera-Reina et al., 2020) not shown on figure.

Using a molecular phylogenetic approach, our work clarifies, for the first time, the taxonomic status of the Florida Caiman, providing evidence that they are *Ca. c. fuscus*. Our results also corroborate that these populations originated from individuals that were likely collected from the Magdalena River and nearby coastal Colombia. Further, we found no evidence of more than one lineage of *Ca. crocodilus* in south Florida.

Our samples of *Ca. crocodilus* from Florida included individuals collected between 1979 and 2013. All individuals had the same haplotype as the Brown Caiman, *Ca. c. fuscus*, representing a single geographically circumscribed lineage occurring in the Caribbean coastal area of Colombia. The specimen UF-Herp-45439, collected in 1979, yielded only a short fragment of Cytb (520 bp) that was identical to the corresponding region of several haplotypes from the Colombian, Panamanian, and Costa Rican *Ca. c. fuscus*. This similarity resulted in this specimen being placed in an unsupported clade with other *Ca. c. fuscus* from northern Colombia, Panama, and Costa Rica rather than with the same clade as the Florida specimens, from which we obtained full sequences. Despite the uncertainty in placement of UF-Herp-45439, we believe this specimen shares the same origin as the more recently collected specimens.

The first report of *Ca. crocodilus* in Florida is from 1950 (King and Krakauer, 1966) and is believed to have resulted from the release of individuals from Colombia brought into the United States via the pet trade. However, established populations of *Ca. crocodilus* were not reported in Florida until the late 1970s, based on evidence of nesting, more individuals of different size classes, and high rates of encounter in south Florida (Ellis, 1980). These established populations were inferred by Ellis (1980) to derive from populations in Colombia because of importation records, indicating that during the 1970s nearly 250,000 live *Ca. crocodilus* were imported to the United States from Colombia (Busack, 1974). Medem (1971) reported that Barranquilla, at the mouth of the Magdalena River, was one of the principal export centers in Colombia for this species and, based on the distribution of *Ca. c. fuscus*, we can infer that the likely origin of the Florida population was from this river basin.

The records of *P. palpebrosus* and *P. trigonatus* from Florida also deserve clarification. The identification reported by Krysko et al. (2011) is incorrect. The specimen UF-Herp-153469 identified as *P. trigonatus* is actually *P. palpebrosus*, whereas UF-Herp-175564 (the same as photographic voucher UF-Herp-165484) is *P. trigonatus* but was originally identified as *P.*

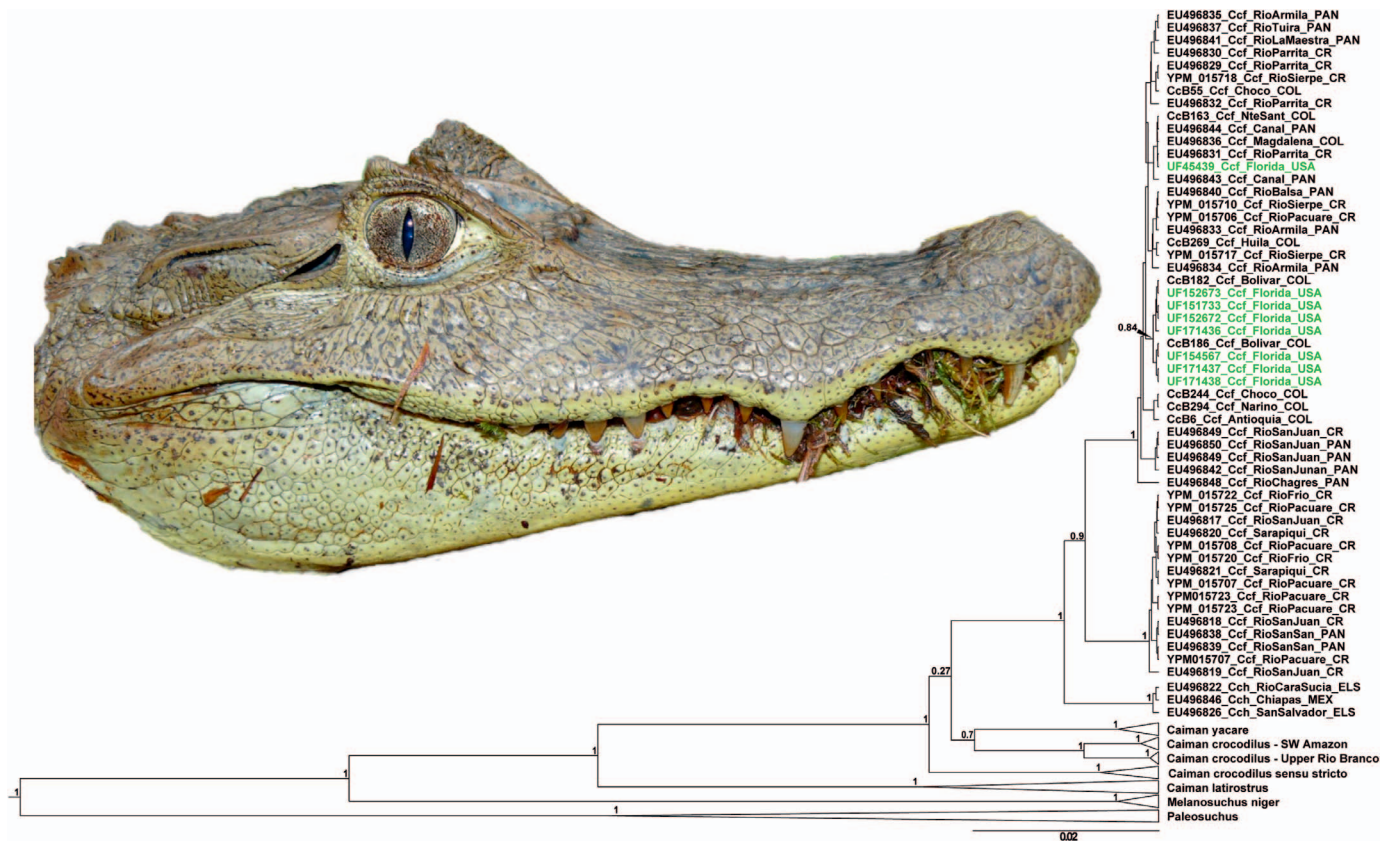


FIG. 2. Bayesian phylogenetic tree based on the Cytb gene of the genus *Caiman* (green text: *Caiman crocodilus fuscus*, samples from south Florida, USA). Values at nodes are Bayesian posterior probabilities.

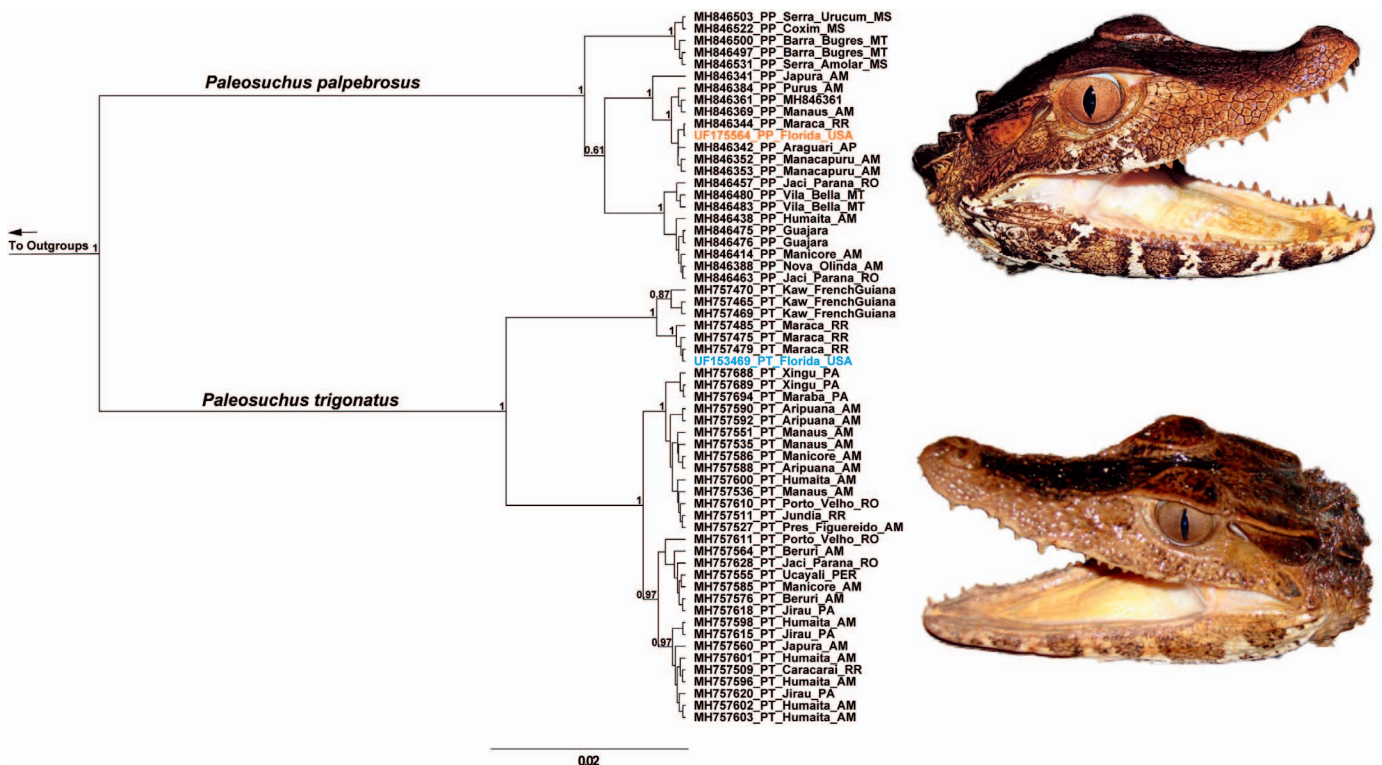


FIG. 3. Bayesian phylogenetic tree based on the Cytb gene of the genus *Paleosuchus* (orange text: *P. palpebrosus*, and blue *P. trigonatus*, samples from south Florida, USA). Values at nodes are Bayesian posterior probabilities.

palpebrosus. Our phylogenetic analyses of *P. palpebrosus* from Florida indicated it to be a member of the Amazon clade (Muniz et al., 2018), closely related to the Guiana Shield population. Our analyses also indicated that the *P. trigonatus* sample belongs to the Guiana+ coastal clade of Bittencourt et al. (2019). In both cases, the individuals of *Paleosuchus* from Florida are in clades found on the Guiana Shield of northeastern South America. Not surprisingly, Guyana is one of the major sources of *Paleosuchus* for the United States pet trade, with approximately 430 individuals being exported every year (Sinovas et al., 2017).

Molecular genetics remain an important tool for identifying and managing populations of crocodilians exploited for the pet trade. *Caiman crocodilus* is the most traded live crocodilian species exported to the United States. Between 1996 and 2010, 805,000 individuals were imported, a number that continues to rise and represents approximately 2.3% of the total reptile species individuals imported to the United States (Robinson et al., 2015). It is possible that different lineages of *Ca. crocodilus* are entering the United States, although our analyses provide evidence for only one lineage in Florida. However, new molecular analyses such as quadruplex PCR assays and multiplex real-time PCR protocols have proven to be cost effective for identifying commercially traded species (Magnussen et al., 2007; Cardenosa et al., 2018) and could be used to track and identify endangered or overexploited crocodilian taxa in the international trade. Quadruplex PCR assays and multiplex real-time PCR protocols could be particularly useful for deducing centers of geographic origin and lineage diversity within the morphologically similar members of crocodilian species complexes.

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