

CONFIRMATION OF INTRODUCED *AGAMA PICTICAUDA* IN FLORIDA BASED ON MOLECULAR ANALYSES

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ABSTRACT

As of 2010, Florida had the largest number of introduced and established non-indigenous herpetofaunal species in the world. With the advent of molecular methodologies, researchers now are able to test hypotheses regarding introduction pathways, species identity, and native range origins. African Agamas, *Agama* Daudin 1802, found in Florida are hypothesized to be the African Rainbow Lizard, *A. agama africana* Hallowell 1844, based on color patterns and hypothesized native range origins (i.e., Benin, Ghana, and Togo) of imported specimens for the pet trade. However, recent systematic studies within the native range of the *Agama* complex have resulted in multiple taxonomic revisions, which calls into question the species identity of introduced populations in Florida. The purpose of this study is to determine the species identity of African agamas within Florida, as well as the native range origins of Florida populations. We conducted a comparative maximum likelihood analysis between individuals from Florida and individuals from the native range. Based on our results we determined that the species found in Florida is *Agama picticauda* from western Africa.

Key words: invasive species, Florida, native range origins, species identity, phylogenetics, *Agama*.

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INTRODUCTION

Florida has the largest number of established non-indigenous amphibian and reptile species in the United States in part due to its subtropical/tropical climate (Myers and Ewel, 1990; Simberloff, 1997), taxonomic similarities amongst established species (Lockwood, 1999), major ports of entry, popularity of certain species in the pet trade and zoos, and an inadequate enforcement of laws preventing biological invasions (Krysko et al., 2011a). New species are included to this list of non-indigenous species as research, particularly phylogenetics, splits existing recognized single species into multiple species, or elevates subspecies to full species status (Fujita et al., 2012; Krysko et al., 2011b; Hamner et al., 2007). Taxonomic similarities among established species sometimes make correct identification difficult (Lockwood, 1999).

African agamas, *Agama* Daudin 1802, are indigenous throughout the continent of Africa (Wagner et al., 2009). Lizards in this genus were first introduced in Florida circa 1976 via the pet trade in Miami, Miami-Dade County (Wilson and Porras, 1983; Krysko et al., 2011a). The known Florida population was tentatively identified as the Common Agama, *Agama agama* (Linnaeus 1758) (Wilson and Porras, 1983). *Agama* sp. now occurs in numerous localities throughout Florida, including Duval County in the northern peninsula, Hillsborough and Seminole counties in the central peninsula, Charlotte and Lee counties in the southwestern peninsula, Saint Lucie, Martin, Palm Beach, Broward, and Miami-Dade counties in the southeastern peninsula, and Key Largo and Long Key in the Florida Keys, Monroe County (Campbell et al., 2008; Enge et al., 2004; Krysko et al., 2011a, 2011b; Connor et al., 2013). Enge et al. (2004) identified many of these Florida populations as the African Rainbow Lizard, *A. agama africana* Hallowell 1844, relying on known color patterns and localities of imported specimens for the pet trade. Based on interactions with pet importers they hypothesized that the native range origins of *A. agama africana* in Florida were Benin, Ghana, and Togo in western Africa.

Recent systematic revisions have resulted

in taxonomic changes within the *Agama* complex (Mediannikov et al., 2012; Leaché et al., 2014), thus, it is uncertain which lineages are associated with introduced Florida populations. Mediannikov et al. (2012) revised the genus using a single mitochondrial (mtDNA) gene (16S; 570 base pairs [bp]) and elevated *A. africana* to species status. Subsequently, Leaché et al. (2014) revised the genus using two mtDNA genes (16S and ND4 region; 1207 bp) and four nuclear genes (NT3, CMOS, PNN, and R35; 2793 bp), and suggested that certain *Agama* lineages found by Mediannikov et al. (2012) deserved different taxonomic names.

These recent taxonomic overhauls obfuscate species identifications of *Agama* introduced to Florida. Identity and native range origins of introduced populations are important to identify locality-level and species-level characteristics such as life histories and ecological traits, which may contribute to future management efforts (Cassey, 2002; Lockwood et al., 2001), and assess risks of similar species being introduced or the repeated introductions of the current species. This study tested the species identity and native range origins hypothesis proposed by Enge et al. (2004).

MATERIAL AND METHODS

We conducted comparative DNA analyses of 14 Florida individuals held in the collections of the Florida Museum of Natural History, University of Florida (UF-Herpetology) (Table 1). We obtained DNA isolations using ZR Genomic DNA™-Tissue Microprep Kit (Zymo Research, LLC). Total cellular DNA templates for polymerase chain reaction (PCR) followed the methodology of Saiki et al. (1988). We amplified DNA and sequenced the 16S mitochondrial (mtDNA) gene (570 base pairs [bp]; primers A2290, B2860, Aga-diffF, and Aga-diffR (Table 2; Mediannikov et al. 2012). PCR was conducted in 25 µl reactions: 9.5 µl H₂O, 12.5 µl GoTaq® Master Mix (Promega Corp, Madison, Wisconsin, USA), 1.0 µl each primer (10 µM), and 1.0 µl DNA template.

Sequence trace files from the automated sequencer (Genomics Division, Interdisciplinary Center for Biotechnology Research, University of Florida) were assembled and edited as necessary

Table 1. Species, mitochondrial DNA (mtDNA) haplotype, voucher/sample ID, locality, and GenBank accession numbers for *Agama* from Florida used in molecular analyses.

Species	mtDNA Haplotype	Voucher/Sample	Locality	GenBank 16S
<i>Agama picticauda</i>	A	UF 137046/AA11	U.S.A., Florida, Charlotte Co., Punta Gorda	KP191662
<i>Agama picticauda</i>	A	UF 137048/AA12	U.S.A., Florida, Charlotte Co., Punta Gorda	KP191661
<i>Agama picticauda</i>	A	UF 137052/AA13	U.S.A., Florida, Charlotte Co., Punta Gorda	KP191660
<i>Agama picticauda</i>	A	UF 132696/AA2	U.S.A., Florida, Miami-Dade Co., Homestead, Redland Middle School	KP191665
<i>Agama picticauda</i>	A	UF 132699/AA5	U.S.A., Florida, Miami-Dade Co., Homestead, Redland Middle School	KP191666
<i>Agama picticauda</i>	A	UF 141218/AA14	U.S.A., Florida, Miami-Dade Co., Homestead, Redland Middle School	KP191664
<i>Agama picticauda</i>	A	UF 141219/AA15	U.S.A., Florida, Miami-Dade Co., Homestead, Redland Middle School	KP191663
<i>Agama picticauda</i>	A	UF 174515/AA17	U.S.A., Florida, Miami-Dade Co., Homestead, Coral Castle	KU664597
<i>Agama picticauda</i>	A	UF 175601/AA19	U.S.A., Florida, Monroe Co., Key Largo	KU664596
<i>Agama picticauda</i>	B	UF 173222/AA16	U.S.A., Florida, Brevard Co., Palm Bay	KP191669
<i>Agama picticauda</i>	B	UF 136983/AA8	U.S.A., Florida, Seminole Co., Sanford	KP191668
<i>Agama picticauda</i>	B	UF 136984/AA9	U.S.A., Florida, Seminole Co., Sanford	KP191667
<i>Agama picticauda</i>	C	UF 174561/AA18	U.S.A., Florida, Collier Co., Naples	KU664595
<i>Agama picticauda</i>	D	UF 176424/AA20	U.S.A., Florida, Hillsborough Co., Apollo Beach	KU664594

Table 2. Primers (5'–3' direction) used to sequence 16S ribosomal DNA in *Agama*.

Gene Region	DNA Marker	Primer Name	Primer Sequence	Source
16S	mtDNA	A2290	CGC-CTG-TTT-ACC-AAA-AAC-AT	Kochen et al. 1989
16S	mtDNA	B2860	CCG-GTC-TGA-ACT-CAG-ATC-ACG-T	Kochen et al. 1989
16S	mtDNA	AGA-difF	AGG-TAC-CGC-CTG-CCC-AGT-GA	Mediannikov et al. 2012
16S	mtDNA	AGA-difR	ATC-GTT-GAA-CAA-ACG-AAC-C	Mediannikov et al. 2012

with Geneious software (ver. 9.0.1, <http://www.geneious.com>, Kearse et al., 2012). Sequences were aligned using Clustal with MEGA software (ver. 6, Tamura et al. 2013) and edited manually when necessary using SeaView (ver. 4.2.5, Gouy et al., 2010). We compared Florida specimens to three different data sets; 68 samples downloaded from GenBank incorporating the data set of Mediannikov et al. (2012), 111 samples downloaded from GenBank incorporating the data set of Leaché et al. (2014), and 200 unpublished donated samples (Leaché, pers. comm.).

We analyzed 472 bp of sequence data. We estimated relationships among haplotypes using Maximum Likelihood (ML) methodology with the Tamura-Nei model, complete deletion mechanism, nucleotide substitution, nearest-neighbor interchange heuristic method, very strong branch swap filter, and 1000 nonparametric bootstrap replicates (Felsenstein, 1985) to assess node support using MEGA (ver. 6, Tamura et al., 2013). The most credible support of phylogenetic relationships was confined to nodes with nonparametric bootstrap values $\geq 70\%$ (Hillis and Bull, 1993; Felsenstein, 2004).

RESULTS

We found four haplotypes of *Agama* in Florida. One haplotype (A) was found in Charlotte, Miami-

Dade, and Monroe counties; one (B) from Brevard and Seminole counties, one (C) in Collier County, and one (D) in Hillsborough County (Fig. 1). Our molecular data, when compared to the dataset from Mediannikov et al. (2012), illustrate that Florida samples are most closely related to individuals identified as *Agama agama* (Fig. 2). However, when Florida samples were compared to the dataset from Leaché et al. (2014), they were most closely related to individuals identified as *A. picticauda* Peters, 1877 (Fig. 3).

The ML analyses illustrate that Florida samples are most closely related to individuals from western Africa, although none of the Florida samples had haplotypes identical to those in the native range. Specimens from Brevard and Seminole counties are most closely related to samples from Ghana, Ivory Coast, and Liberia (Figs. 2–3; Mediannikov et al., 2012; Leaché et al., 2014; A. Leaché pers. comm.). Specimens from Charlotte, Miami-Dade, and Monroe counties are most closely related to samples from Benin (Fig. 2, Mediannikov et al., 2012) and Cameroon, Democratic Republic of the Congo, and Gabon (Fig. 3, Leaché et al., 2014; A. Leaché, pers. comm.). Specimens from Collier and Hillsborough counties are most closely related to samples from Benin, Niger, and Togo (Fig. 2, Mediannikov et al., 2012) and Niger (Fig. 3, Leaché et al., 2014).

DISCUSSION

Our molecular data support the native range origin hypothesized by Enge et al. (2004), where introduced *Agama* in Florida originated from Benin, Ghana, and Togo. However, we identified additional source localities: Liberia, Ivory Coast, Niger, Cameroon, Democratic Republic of the Congo, and Gabon. Multiple haplotypes of *Agama* throughout Florida indicate multiple independent introduction events from different origins in Africa.

Because of recent taxonomic revisions, our data do not support the species identification hypothesis of Enge et al. (2004) where introduced individuals in Florida were identified as *Agama agama africana*. Mediannikov et al. (2012) arbitrarily labeled all specimen samples from western

Africa as *A. agama* (Leaché et al., 2014). However, because there is no known holotype locality for the nominate species *A. agama*, this species name has been applied to populations in central Africa. The name *A. picticauda* was resurrected and used to represent populations in western Africa (Wagner et al., 2009; Leaché et al., 2014). Thus, we designate Florida populations as *A. picticauda*.

It should be noted that we used only 16s for this study because it was the only gene sequenced by Mediannikov et al. (2012). Nevertheless, an expansion of this study could include the nuclear genes used by Leaché et al. (2014), which might yield new information on the native range origins of *Agama picticauda* in Florida.

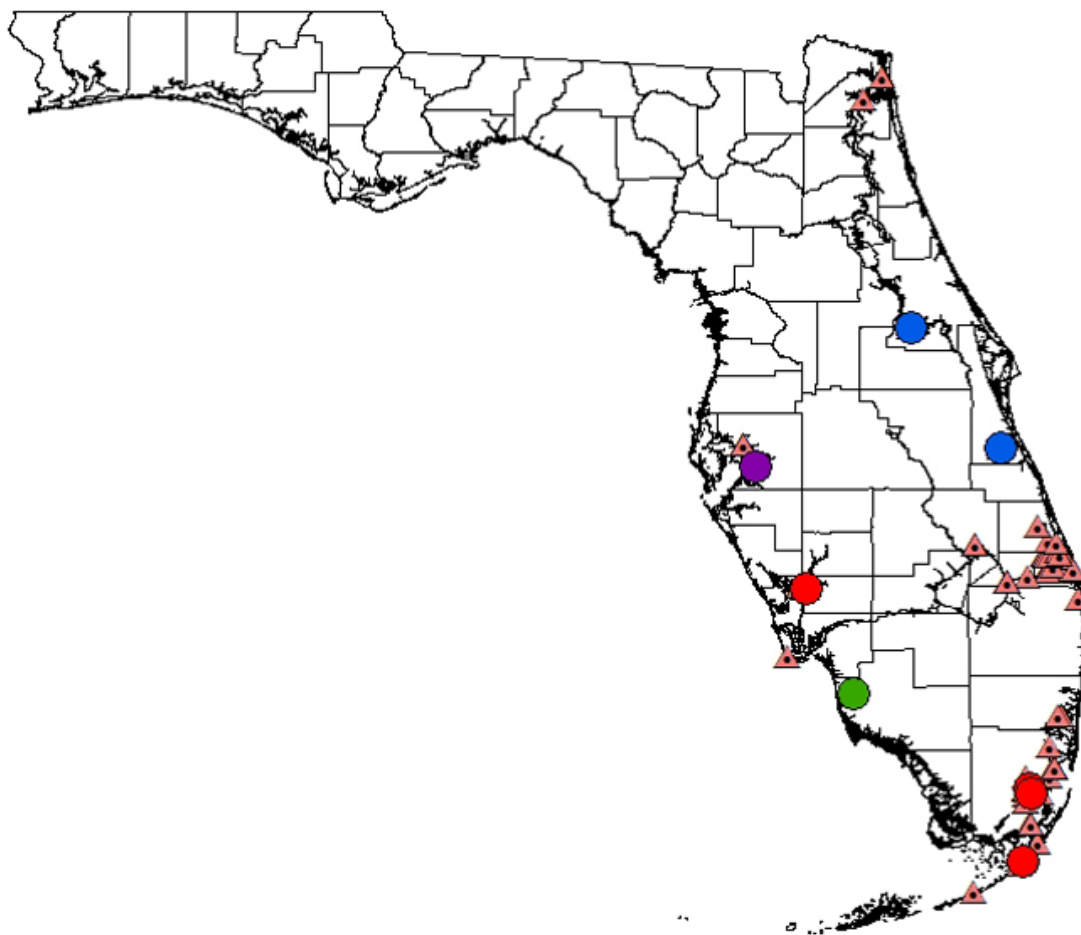


Figure 1. Map of Florida showing the distribution of introduced *Agama* based on vouchered records from UF-Herpetology. Coral-colored triangles with central dot indicate all known vouchered records, red circles indicate haplotype A, blue circles indicate haplotype B, green circles indicate haplotype C, and purple circles indicate haplotype D.

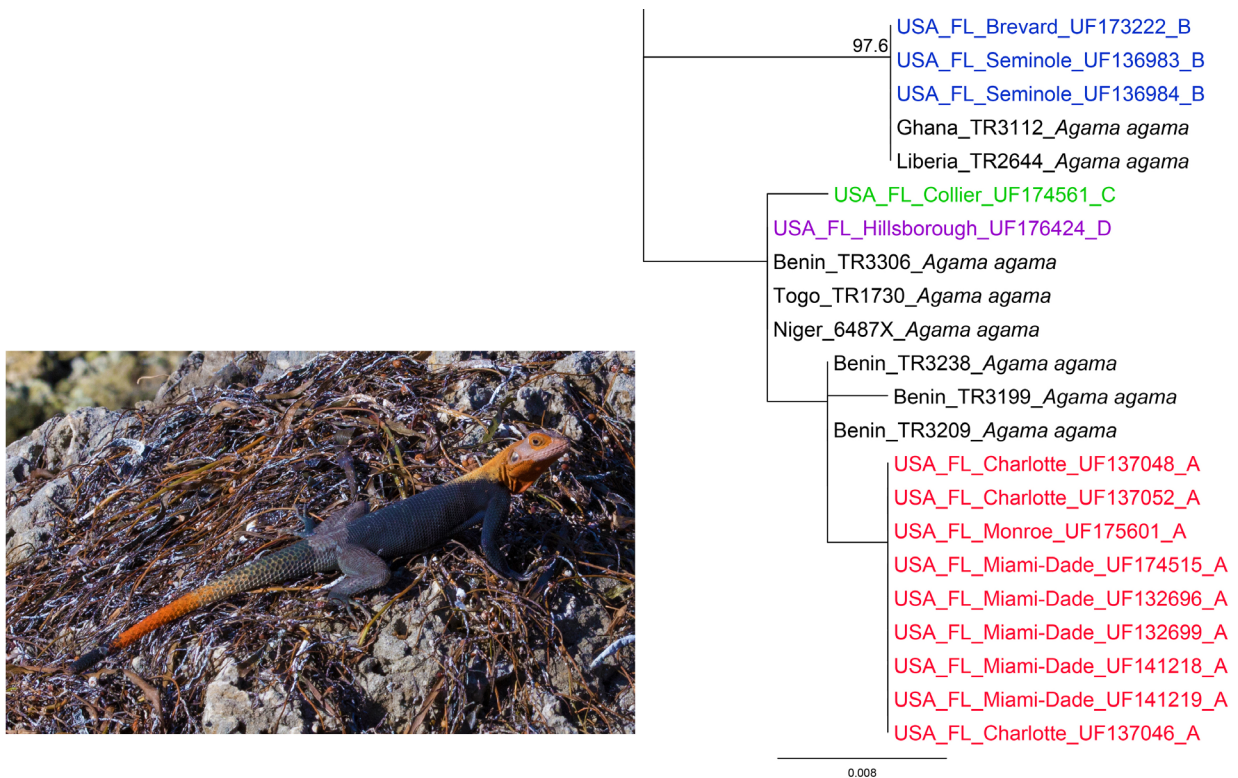


Figure 2. Enlarged area of introduced populations of *Agama* in Florida using Maximum Likelihood analysis and data set of Mediannikov et al. (2012). Note that values above major nodes represent bootstrap support $\geq 70\%$, and samples highlighted in red, blue, green, and purple represent different haplotypes for specimens collected from introduced populations in Florida. Photograph of *Agama picticauda* (UF-Herpetology 175601) from Key Largo, Monroe County, Florida, by Claudia MacKenzie-Krysko.

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Figure 3. Enlarged area of introduced populations of *Agama* in Florida using Maximum Likelihood analysis and data set of Leaché et al. (2014). Note that values above major nodes represent bootstrap support $\geq 70\%$; and samples highlighted in red, blue, green, and purple represent different haplotypes for specimens collected from introduced populations in Florida.

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