IRCF REPTILES & AMPHIBIANS
CONSERVATION AND NATURAL HISTORY

INTRODUCED SPECIES

# The Cuban Blue Anole, *Anolis allisoni* Barbour 1928 (Squamata: Dactyloidae), a New Nonnative Lizard Introduced in Florida

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Anoline lizards, *Anolis* (*sensu lato*; Family Dactyloidae), are one of the most species rich groups of terrestrial vertebrates with 399 currently recognized species (Nicholson et al. 2012; Uetz and Hošek 2015). Because anoles are highly variable in morphology (Conant and Collins 1998; Schettino 1999; Camposano 2011) and have the ability to change color in response to external stimuli (Hadley 1931; Weber 1983), diagnosing species can be difficult, especially when dealing with introduced populations.

The state of Florida, USA, has more introduced amphibians and reptiles than any other place in the world (Krysko et al. 2011a). Eleven species of anoles have been documented in Florida, only one of which is native (Green Anole, *A. carolinensis* [Voigt 1832]) and all but one (Jeremie Anole, *A. coelestinus* Cope 1862) of the nonnative taxa are established and reproducing (Krysko et al. 2011a, 2011b). In this paper, we document the eleventh introduced anole, the Cuban Blue Anole, *Anolis allisoni* Barbour 1928, in Florida. *Anolis allisoni* is native to Belize (Islas de la Bahia), Cuba, and Honduras (Islas de Barbareta, Guanaja, Morat, Roatán, Utila, the Cayos Cochinos in the Islas de la Bahıa), and also has been introduced into La Ceiba, Atlantida (on the northern Honduran coast), and in Quintana Roo, Mexico (McCranie and Köhler 2015). Juveniles and adult females are green in color, whereas adult males turn blue anteriorly (unique within *Anolis*; but males from Honduras have necks, limbs, and lower bodies that remain bright green) and have a reddish dewlap (Barbour 1928; Lee 2000). *Anolis allisoni* readily hybridizes with the Cuban Green Anole, *A. porcatus* Gray 1840, in Cuba (Schwartz and Henderson 1991; Glor et al. 2004), and *A. porcatus* is believed to hybridize with the native *A. carolinensis* in its introduced range in southern Florida (Kolbe et al. 2007). All three of these closely related species are assigned to the *Anolis carolinensis* series (Losos 2009).

**Fig. 1.** Adult male nonnative Cuban Blue Anole (*Anolis allisoni*) photographed in Tampa, Hillsborough County, Florida, on 5 July 2013. Photograph by Tony Henneke.

## Materials and Methods

**Specimen acquisition.—**On 5 July 2013 at 0901 h, a single adult male *Anolis allisoni* was observed on the back of a resident's house on Magdalene Manor Drive, Tampa, Hillsborough County, Florida (Fig. 1; 28.07457°N, 82.47916°W, Datum WGS84; 15 m elev.). A digital image (photographic voucher UF-Herpetology 170513) was deposited in the Division of Herpetology, Florida Museum of Natural History, University of Florida. Species identity was confirmed by Joseph. P. Burgess.

After additional sightings by the homeowners, KLK and CMK visited this site on 18 August 2013. Although no adult male *A. allisoni* was observed, five green-colored juvenile anoles were collected (Fig. 2; UF-Herpetology 170869–170873) from the same side of the house. The resident informed us that he observed the male *A. allisoni* the same day shortly after we departed the site, and that neighbors believe a similar unusually colored lizard was observed on their homes

as well. On 21 September 2013, KLK and CMK visited this site again, but after intensive searches did not observe a male *A. allisoni*.

**Laboratory techniques.—**We obtained DNA isolations from the five *Anolis* specimens using ZR Genomic DNA™ Tissue Microprep Kit (Zymo Research, LLC). Using total cellular DNA as a template and polymerase chain reaction (PCR) methodology (Saiki et al. 1988), DNA was amplified and sequenced following Glor et al. (2004) for nicotinamide adenine dinucleotide dehydrogenase subunit 2 (ND2) using primers L4437b (Macey et al. 1997) and H5730 (Glor et al. 2004). 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 94 °C for 3 min, followed by 35 cycles of amplification: denaturing at 94 °C for 1 min, annealing at 52 °C for 1 min, and extension at 72 °C for 1 min, fol-


**Fig. 2.** Green Anoles (*Anolis carolinensis*) collected on 18 August 2013 from the same site as the nonnative Cuban Blue Anole (*Anolis allisoni*) in Tampa, Hillsborough County, Florida. Note that A–D = UF-Herpetology 170869–170873, respectively. Photographs by Kenneth L. Krysko.

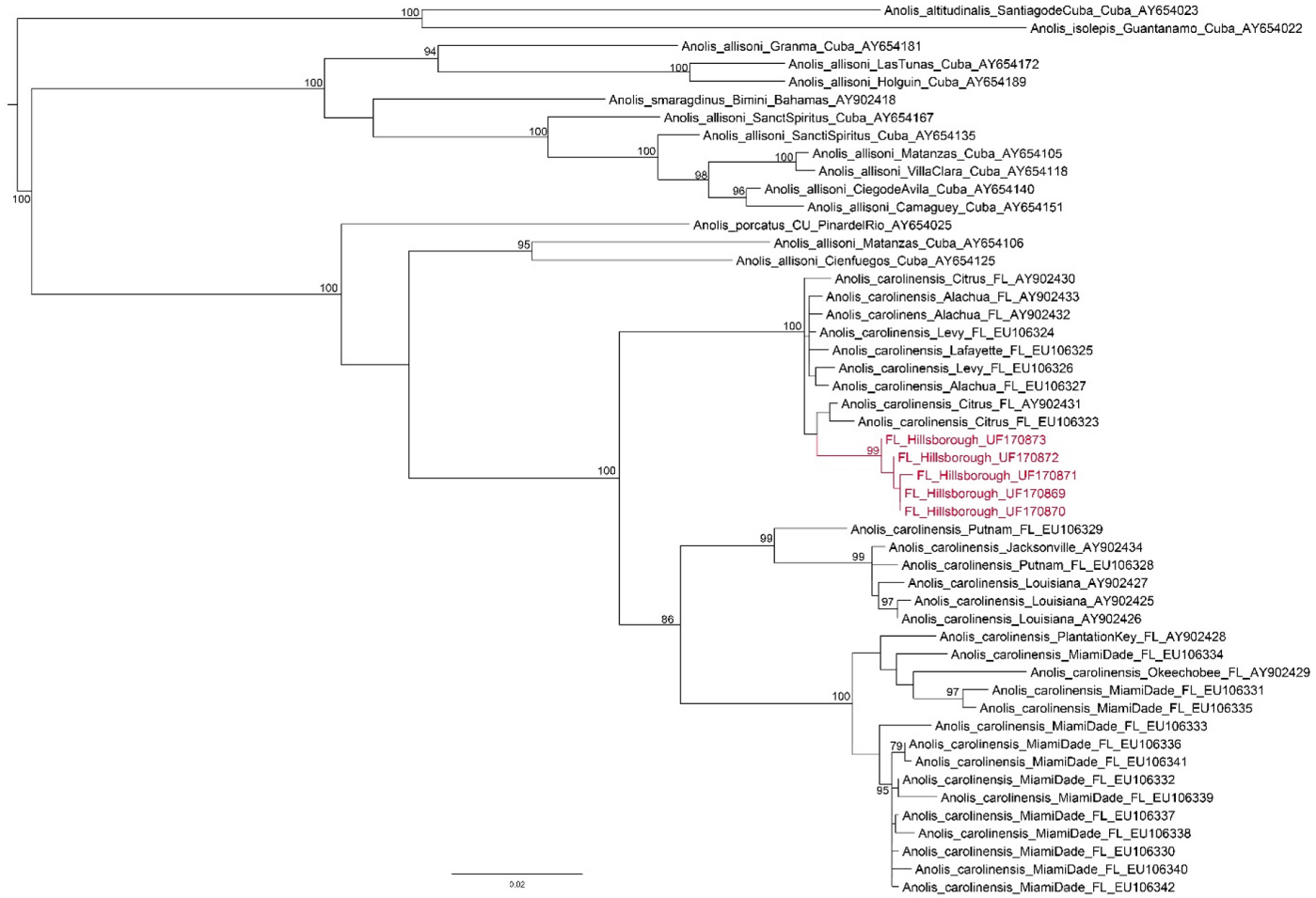


**Fig. 3.** Maximum Likelihood phylogeny for Green Anoles (*Anolis carolinensis*) (UF-Herpetology 170869–170873 highlighted in red) collected in Tampa, Hillsborough County, Florida, on 18 August 2013. Note that values (≥ 70%) above major nodes represent bootstrap support.

lowed by a final extension at 72 °C for 7 min. Three µl of each PCR product were electrophoresed on a 1% agarose gel, visualized with GelRed™ staining (Biotium Inc., Hayward, California, USA), and compared with a DNA standard. Sequence files received for our specimens from the automated sequencer (Genomics Division, Interdisciplinary Center for Biotechnology Research, University of Florida) were edited as necessary with Geneious software (ver. 6.1, created by Biomatters <http://www.geneious.com>). GenBank accession numbers for our specimens are KP174772–KP174776.

**Phylogenetic analyses.—**We downloaded DNA sequence data for ND2 from GenBank for 45 samples from the *Anolis carolinensis* series, including *A. allisoni*, *A. altitudinalis*, *A. carolinensis*, *A. isolepis A. porcatus*, and *A. smaragdinus* (Glor et al. 2004). Along with our five specimens, all sequences were assembled using the Muscle algorithm and manually edited as necessary with Geneious software.

A total of 1,038 base pairs (bp) of sequence data were analyzed. Relationships among haplotypes were estimated 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 1,000 nonparametric bootstrap replicates (Felsenstein 1985) to assess node support using MEGA version 6 (Tamura et al. 2013). The most credible support of phylogenetic relationships was confined to nodes where nonparametric bootstrap values ≥70% (Hillis and Bull 1993; Felsenstein 2004).

## Results

Our ML analysis (Fig. 3) produced a phylogeny similar to that of Glor et al. (2005). Of the five *Anolis* specimens we collected, four haplotypes were recovered and all were nested within the *A. carolinensis* clade. These samples are most closely related to the nearest sample locality in Citrus County, Florida, and all *A. carolinensis* are most closely related to *A. porcatus* from western Cuba.

## Discussion

In the past, introduced species have been reported in the literature by various means; some by improperly writing the species name without providing any evidence or means of

verification, or by properly vouchering a specimen (preferred) and/or a distinguishable photograph in a systematic collection with associated data (see Krysko et al. 2011a). In this paper, we follow the proper way of documenting a new introduced species by providing a clear photographic voucher, but also go a step further by attempting to determine its stage of introduction (i.e., if it is established) using molecular analysis. Although our voucher photograph clearly illustrates an adult male *Anolis allisoni*, our molecular data suggest that the five other green anoles collected at the same site have mtDNA of native *A. carolinensis* (Fig. 3), and therefore no current evidence supports an established population of *A. allisoni*. However, we cannot determine if *A. allisoni* hybridized in the wild with native *A. carolinensis* because our data consist of mtDNA, which is maternally inherited. Nonetheless, *A. allisoni* was either released or escaped from a nearby enclosure and currently represents a stage-2 introduction (after Colautti and MacIsaac 2004).

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