Origin and establishment of the introduced Cuban Blue Anole, *Anolis allisoni*, in Florida

Dolores G. Morris¹, Kathleen Morris², Christopher J. Thawley³*, Jason J. Kolbe³, and Sozos N. Michaelides³,⁴*

¹Department of Philosophy, University of South Florida, Tampa, FL 33620, USA
²Tampa, Florida, USA
³Department of Biological Sciences, University of Rhode Island, Kingston, RI 02881, USA
⁴Department of Biology, Concordia University, Montreal H4B 1R6, QC, Canada

*Corresponding author (cthawley@gmail.com, msozos@gmail.com)


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Abstract

In the state of Florida, USA, lizards of the genus *Anolis* are well represented with at least nine established non-native species and a single native species, *A. carolinensis*. The most recently introduced species is *A. allisoni*, a close relative to both the native *A. carolinensis* and one of the introduced species (*A. porcatus*). *Anolis allisoni* is thought to have been present in two locations in Florida since at least 2013 based on photographic evidence. Here, we analyzed mitochondrial DNA (mtDNA) sequences from these three closely related *Anolis* species to infer the most likely region of origin in the native range and confirm the establishment of the recent invader in Tampa, Florida. We found a single haplotype belonging to *A. allisoni*, which was closely related to native sequences from east-central Cuba. The most likely geographic origin is a tourist destination in the province of Sancti Spiritus, suggesting the potential for human-mediated introduction of *A. allisoni* to Florida. Given the evidence of hybridization within the *carolinensis* subgroup, the presence and establishment of the phylogenetically related and ecomorphologically similar *A. allisoni* may create novel opportunities for interspecific genetic exchange.

Keywords: *Anolis* lizards, Florida, invasions, mtDNA, Cuba

Introduction

The state of Florida in the southeastern USA is a center for non-native species introductions of reptiles and amphibians (Krysko et al. 2011). Among lizards, the genus *Anolis* is the most represented with nine established species in addition to the sole native species, *A. carolinensis*, which is found throughout Florida and the southeastern USA (Kolbe et al. 2007; Krysko et al. 2011). When introduced, closely related taxa with similar habitat use and ecology to the native species have the potential to interact strongly with and even drive evolutionary change in affected native species. For example, the invasion of the Cuban brown anole (*A. sagrei*) in Florida has forced *A. carolinensis* to move to higher perches, leading to the evolution of larger toepads in just 20 generations (Stuart et al. 2014). In another example, the introduction of the Cuban green anole, *A. porcatus*, has led to hybridization with the native species followed by genetic distinctiveness of the hybrid population in South Miami (Wegener et al. 2019). In the latter case, both species (*A. carolinensis* and *A. porcatus*) are members of the *carolinensis* subgroup, which includes nine species of canopy-dwelling anoles with similar coloration and morphology (Glor et al. 2005). Despite considerable divergence time between these two species, reproductive isolation between *A. carolinensis* and *A. porcatus* appears to be weak.

The latest introduction of an *Anolis* species in the continental USA is *A. allisoni* (Krysko et al. 2015). The Cuban Blue Anole, native to Cuba and islands off the coast of Honduras, Belize, and Mexico (Schwartz et al. 1991), is another member of the *carolinensis* subgroup. In Florida, the species is known from two locations (Fig. 1a). In 2013,
a photographic voucher of a single male *A. allisoni* (UF-Herpetology 170513) was obtained in Tampa (TAM), Florida (Krysko et al. 2015). Mature male *A. allisoni* are readily distinguished by the distinctive blue coloration of the front half of their bodies, and *A. allisoni* may also be distinguished from *A. carolinensis* by the teardrop-like shape of their ear opening. However, subsequent surveys in Tampa by Krysko et al. (2015) failed to find other individuals of

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**Figure 1.** Sampling locations and mtDNA haplotype network. The inset map of North America shows Florida (a) and Cuba (b) shaded in red. (a) Map of Florida showing the two known introduced populations of *A. allisoni* in Tampa (TAM) and Naples (NAP) in red circles. Open circles represent sampling locations of mtDNA sequences for *A. carolinensis*, and the yellow square in South Miami (MIA) denotes a hybrid population derived from introduced WC *A. porcatus* and native *A. carolinensis*. (b) Map of Cuba showing sampling locations (filled circles) for native *A. allisoni*. Green circles represent locations of *A. allisoni* sequences used in the network analysis. The dotted pink-shaded area around location 25 indicates the likely geographic origin in Cuba of the *A. allisoni* introduced to Tampa. Open circles represent sampling locations of mtDNA sequences of *A. porcatus*, and the two yellow-shaded regions denote parts in eastern and western Cuba where introgression between *A. porcatus* and *A. allisoni* was previously detected. Location numbers follow Glor et al. (2005) and Cadiz et al. (2018). (c) Close-up aerial image of a portion of Tampa showing the location of the first description of *A. allisoni* in 2013 (red square) and the new location in 2019 (red circle) along with photos of a male and female *A. allisoni* (photos taken by Dolores Morris). (d) Median-joining network of 37 *A. allisoni* sequences belonging to the east-central subclade. The size of each circle corresponds to the number of individuals sharing that haplotype. Dashes denote mutation steps. The sequences from *A. allisoni* collected in Tampa are identical (TAM-H1, red circle) and most closely related to haplotypes from location 25 in Cuba.
A. allisoni. Mitochondrial DNA (mtDNA) analyses of juvenile green anoles in the area, which are difficult to assign to species using morphology, were ascribed to A. carolinensis (Krysko et al. 2015). As such, it has been unclear whether an established population of A. allisoni has persisted in the area. A separate reproducing population, which has been present since at least 2014, is restricted to an outdoor courtyard in Naples (NAP; Fig. 1c), Florida (Donini et al. 2017). Nevertheless, neither of these previous studies characterized the details of the introduction(s) of A. allisoni in Florida. For instance, we do not know the geographic source(s) of introductions, likely introduction pathway, and whether sightings were due to single or multiple introductions. Genetic tools are particularly useful to address these questions which are important for management, but also for the detection and characterization of hybridization between native and introduced species (Fitzpatrick et al. 2012). In this study, we verified ongoing presence and used a mtDNA marker to confirm the phylogenetic identity and infer the most likely native-range source location(s) of A. allisoni in Tampa.

Materials and Methods

Sampling and genetic analyses. We caught three male lizards with A. allisoni phenotype from the Lake Teakwood Estates neighborhood in Tampa in 2019 (Fig. 1c). This population is ≈1 km from the sighting reported in Krysko et al. (2015). We extracted DNA from tail tips stored in 95% ethanol using the Isolate II Genomic DNA Kit (Bioline, USA) with overnight lysis. We sequenced approximately 800 base pairs (bp) of the mtDNA ND2 gene with the forward primer H5730 (5’- AGCGAATRGAAGCCCGCTGG-3’, Glor et al. 2004) and the reverse L4437a (5’- AAGCTTTCGGGCTCCATACC-3’, Macey et al. 1997). Amplifications were carried out in a total volume of 30 μl consisting of 15 μl of MyTaq HS Mix (Bioline), 1.2 μl (0.4 mM) of each primer, 10.6 μl PCR grade H₂O and 2 μl template DNA (20 ng). PCR conditions were as follows: an initial denaturation step at 95°C for 1 min, followed by 30 cycles at 95°C for 1 min, 53°C for 35 sec and 72°C for 80 sec, and a final extension step at 72°C for 5 min. PCR products were purified using the Isolate II PCR Kit (Bioline), and sequencing reactions were performed on the ABI 3130xl genetic analyzer at the University of Rhode Island (URI) Genomics and Sequencing Center.

Table 1. Mean pairwise mtDNA (ND2 gene) divergence (Tamura-Nei, %) between native A. carolinensis and each introduced Anolis species in Florida with available genetic data. Ecomorph category is from Losos (2009). Introduced A. porcatus originated from western Cuban (WC) populations and A. allisoni from east-central Cuban (ECC) populations.

<table>
<thead>
<tr>
<th>Species</th>
<th>Ecomorph</th>
<th>mtDNA divergence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WC A. porcatus</td>
<td>Trunk-crown</td>
<td>9.6</td>
</tr>
<tr>
<td>ECC A. allisoni</td>
<td>Trunk-crown</td>
<td>16.2</td>
</tr>
<tr>
<td>A. equestris</td>
<td>Crown-giant</td>
<td>28.8</td>
</tr>
<tr>
<td>A. chlorocyanus</td>
<td>Trunk-crown</td>
<td>30.0</td>
</tr>
<tr>
<td>A. cristatellus</td>
<td>Trunk-ground</td>
<td>30.9</td>
</tr>
<tr>
<td>A. garmani</td>
<td>Crown-giant</td>
<td>31.0</td>
</tr>
<tr>
<td>A. sagrei</td>
<td>Trunk-ground</td>
<td>33.4</td>
</tr>
<tr>
<td>A. cybotes</td>
<td>Trunk-ground</td>
<td>34.5</td>
</tr>
<tr>
<td>A. distichus</td>
<td>Trunk</td>
<td>35.3</td>
</tr>
</tbody>
</table>

Mitochondrial DNA sequences from both directions were corrected by eye and aligned to obtain a consensus sequence. All sequences were then aligned using MAFFT (Kato et al. 2002) implemented in Geneious 8 (Kearse et al. 2012) and trimmed to a uniform length of 798 bp. A unique sequence was submitted to GenBank under the accession number MZ475900.

Phylogenetic analyses. We used phylogenetic analyses to reconstruct relationships among haplotypes and to determine the genetic origin of the introduced haplotypes. We combined our sequences with 348 sequences (of varying lengths) obtained from GenBank representing native range A. allisoni from Cuba (Glor et al. 2004; Cádiz et al. 2018), A. porcatus from Cuba (Glor et al. 2004), A. allisoni x A. porcatus hybrids from Cuba (Glor et al. 2004),...
native *A. carolinensis* from Florida (Kolbe et al. 2007; Tollis et al. 2012; Campbell-Staton et al. 2012), *A. carolinensis* x *A. porcatus* hybrids from South Miami, FL (Wegener et al. 2019), and sequences of green anoles previously sampled from Tampa, FL and ascribed to *A. carolinensis* (Krysko et al. 2015). We also obtained from GenBank three sequences belonging to the Cuban species *A. oporinus*, *A. isolepis* and *A. altitudinalis* (Glor et al. 2004) to use as outgroups.

We constructed the phylogenetic tree using a Bayesian inference (BI) with the add-on plugin of MrBayes (Huelsenbeck et al. 2001), implemented in Geneious 8 (Kearse et al. 2012). The GTR + G + I substitution model was selected based on the lowest BIC criterion in MEGA X (Stecher et al. 2020), and the BI analysis was run with four chains of 2,000,000 generations sampling every 500 trees. We discarded (burn-in-length) the first 10% of trees after checking for convergence of the chains (trace viewer within Geneious 8), and posterior probability branch support was estimated from the 50% majority-rule consensus tree. To assess and visualize better the phylogenetic relationships among haplotypes, we also constructed a median-joining network in NETWORK v10 (Bandelt et al. 1999) using a subset of sequences (n = 37) belonging to the east-central *A. allisoni* subclade (see Results). This method uses median vectors as a hypothetical ancestral sequence required to connect existing sequences within the network with maximum parsimony. For this analysis, all sequences were trimmed to 798 bp to match the newly-generated sequence lengths.

In addition, we gathered unique sequences (introduced haplotypes of the ND2 gene only) from established non-native anoles in Florida from GenBank (Kolbe et al. 2007) and calculated mean pairwise mtDNA divergence between these and the native haplotypes of *A. carolinensis* using MEGA X (Stecher et al. 2020). Combined with ecomorphological categorization (i.e., habitat specialist group, Losos 2009), we discuss how human-mediated introductions may create opportunities for hybridization between the native (*A. carolinensis*) and the introduced *Anolis* species as well as among introduced *Anolis* species in Florida.

### Results and Discussion

Genetic samples from all three presumptive *A. allisoni* individuals sampled from Tampa were nested in the *A. allisoni* clade, providing conclusive evidence that they are indeed *A. allisoni* or hybrids with *A. allisoni* maternal mtDNA. More than 50 adult *A. allisoni* of both sexes were observed in our focal area (DM and KM personal observation) covering several residences and landscaped vegetation over an area of approximately 0.02 km². We observed individuals conducting dewlap displays, engaging in combat, and mating, supporting their establishment success. Anecdotal interviews with local residents and photographic evidence indicate that *A. allisoni* has been in the area since at least 2017. It seems likely that the previous sighting in this area (=1 km distance, Krysko et al. 2015) is from the same population. This population has also survived through multiple winters (DM and KM personal observation, spring 2021), suggesting that cold temperatures and potential associated mortality have not impacted its establishment.

All three individuals sampled in Tampa shared the same haplotype (TAM-H1). The overall topology of our phylogenetic tree (not shown) is similar to the one described by Glor et al. (2004) supporting two subclades for *A. allisoni* (western and east-central), which are sister to *A. porcatus* from eastern Cuba. The haplotype sampled in

<table>
<thead>
<tr>
<th>Species pair</th>
<th>mtDNA divergence (%)</th>
<th>Study</th>
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<tbody>
<tr>
<td><em>A. carolinensis</em> x WC <em>A. porcatus</em></td>
<td>9.6</td>
<td>Wegener et al. 2019</td>
</tr>
<tr>
<td>EC <em>A. allisoni</em> x EC <em>A. porcatus</em></td>
<td>10.0</td>
<td>Glor et al. 2004; 2005</td>
</tr>
<tr>
<td>WC <em>A. sagrei</em> x <em>A. quadriocellifer</em></td>
<td>10.2</td>
<td>Kolbe et al. 2004;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Reynolds et al. 2020)</td>
</tr>
<tr>
<td>WC <em>A. allisoni</em> x WC <em>A. porcatus</em></td>
<td>14.3</td>
<td>Glor et al. 2004; 2005</td>
</tr>
<tr>
<td><em>A. kragi</em> x <em>A. pulchellus</em></td>
<td>15.4</td>
<td>Jezkova et al. 2013</td>
</tr>
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</table>

Table 2. Mean pairwise mtDNA (ND2 gene) divergence between pairs of *Anolis* species with genetic evidence of hybridization and introgression. The abbreviations denote sequences sampled from western (WC) and eastern (EC) Cuban populations.
this study clusters within the east-central *A. allisoni* subclade, and the median-joining network (Fig. 1d) shows that the TAM-H1 haplotype is genetically similar (three nucleotides different) to haplotypes from the province of Sancti Spiritus in Cuba (location 25 in Fig. 1b as described in Glor et al. 2004).

Our finding is the first verification of the genetic Cuban origin of *A. allisoni* in Florida. The fact that this species was observed in 2013 (Krysko et al. 2015) and that we recently collected it, provides evidence that the species is reproducing, established, and likely dispersing across the urban landscape of Tampa. The province of Sancti Spiritus, the likely geographic origin in east-central Cuba, has many attractions for tourists (e.g., UNESCO World Heritage sites), suggesting a human-mediated introduction. Whether the introduction was deliberate, such as via the pet-trade, or accidental, is unknown. The species has also been introduced to Quintana Roo, Mexico as well as the island of Utila and city of La Ceiba, both in Honduras (McCranie et al. 2015). In the latter case, both locations (Utila and La Ceiba) are connected via ferry to the island of Roatan, where *A. allisoni* is native (McCranie et al. 2015), suggesting a nearby source for introductions.

When introduced and native species are phylogenetically related with similar habitat use and ecology, the native species may face both ecological and evolutionary pressure through competitive exclusion, niche displacement, hybridization, and introgression (Blackburn et al. 2014). Three introduced *Anolis* species in Florida (*A. porcatus*, *A. allisoni* and *A. chlorocyamus*) occupy the same structural habitat as the native *A. carolinensis* (all belong to the trunk-crown ecomorph, Table 1) and are similar in morphology and behavior (Losos 2009). Furthermore, two of these species, *A. porcatus* and *A. allisoni*, are closely related phylogenetically to the native anole with 9.6% (western Cuban haplotypes) and 16.2% mtDNA sequence divergence, respectively (Table 1). Specifically, within the carolinensis subgroup, hybridization events suggest that species boundaries and reproductive isolation might be weak. In western and eastern parts of Cuba where *A. allisoni* and *A. porcatus* occur sympatrically and have a mean pairwise mtDNA divergence of 10.0–14.3% (Glor et al. 2005), introgression is bidirectional (Figure 1; Glor et al. 2004). In Florida, introduced western Cuban (WC) *A. porcatus* in South Miami hybridizes with *A. carolinensis*; these taxa have a mean pairwise mtDNA divergence of 9.6% (Wegener et al. 2019). In comparison, the mean pairwise mtDNA divergence between *A. carolinensis* and eastern Cuban (EC) *A. porcatus* is 15.9% (Glor et al. 2005). Thus, given the phylogenetic and ecological similarity of *A. allisoni* to the native green anole in Florida (Table 1), the potential for hybridization exists.

Human-mediated introductions often create opportunities for intra- and inter-specific hybridization by removing spatial barriers between previously allopatric taxa (e.g. Kolbe et al. 2004; Michaelides et al. 2013; Stephens et al. 2020). For the non-native species, such opportunities may facilitate establishment, adaptation, and range expansion through increased diversity, hybrid vigor, and the generation of novel genotypes (Ellstrand et al. 2000; Rius et al. 2014). For the native species, however, interspecific hybridization with a non-native species could lead to genetic swamping and potentially extinction of local lineages (Todesco et al. 2016; Ottenburghs 2021). However, anthropogenic hybridization can also provide opportunities for exchange of adaptive genetic variation and speciation (see review by Ottenburghs 2021). The 16.2% mtDNA sequence divergence between the recently introduced *A. allisoni* and the native *A. carolinensis* is only slightly more than the divergence between successfully hybridizing species in Puerto Rico (Table 2). Hybridization between native *A. carolinensis* and all other introduced *Anolis* species in Florida (besides *A. porcatus*) is much less likely given the greater mtDNA sequence divergence with these species, all 28.8% or more (Table 1). Among the introduced species, opportunities for hybridization might occur for *A. porcatus* and *A. allisoni*, however, both are currently established in different locations in Florida. The closest *A. allisoni* population to South Miami, where WC *A. porcatus* was introduced, is in Naples (200 km to the west). The phylogenetic identity of this population is still unknown, but if this introduction has a WC *A. allisoni* origin then hybridization with WC *A. porcatus* is very likely upon future secondary contact.

In conclusion, photographic, observational, and genetic evidence suggest that *A. allisoni* is well-established in this Tampa neighborhood, having likely dispersed at least 1 km through suburban habitats. Knowledge of the geographic origin of this introduced species in east-central Cuba could guide future sampling (both in the
native and introduced range) and use of bi-parentally inherited nuclear markers to investigate whether hybridization is occurring and the potential consequences thereof.

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Author Contributions
DM and KM initiated the research, collected photographs and specimens, and revised the manuscript; CT coordinated the research, drafted and revised the manuscript; SM conducted the laboratory work, analyses and co-wrote the manuscript; JK provided funding, contributed to the interpretation of data and revised the manuscript. All authors approved the final version.

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