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ORIGINAL PAPER

Provenance and genetic diversity of the non-native geckos *Phelsuma grandis* Gray 1870 and *Gekko gecko* (Linnaeus 1758) in southern Florida, USA

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Abstract Accurately characterizing the provenance and genetic diversity of non-native populations aids the management of biotic invasions because population-level genetic diversity is potentially correlated with invasive success. We sequenced the mitochondrial DNA of non-native *Phelsuma grandis* Gray 1870 (Cyt-*b*, 610 bp) and *Gekko gecko* (Linnaeus 1758) (ND2, 751 bp) specimens collected from southern Florida, and compared these sequences to those of georeferenced native-range conspecifics. Multiple mtDNA clades were identified in southern Florida populations of both species. In the case of *G. gecko* these were traceable to geographically distinct native-range locales, confirming that southern Florida's population ultimately derives from multiple native-range regions. The majority of the *P. grandis* sequenced were closely allied with a specimen from the far north of Madagascar, while a minority clustered in a well-supported clade with *P. grandis* derived from northeastern Madagascar. Sympatry of individuals belonging to multiple mtDNA clades was confirmed for both species, and uncorrected pairwise distances as high as 11.41% were detected in sympatric *G. gecko*, highlighting the potential for interpopulation—and perhaps even interspecific—diversity to be transformed into intrapopulation diversity during invasion events. Our findings provide further evidence that introduced squamate populations are frequently highly heterogeneous and derived from multiple, distinct native-range lineages.

Keywords Day gecko · Gekkonidae · Invasion genetics · Multiple source populations · Non-native squamates · Tokay gecko

Introduction

Determining the provenance, identity, and diversity of non-native organisms and populations is an important first step in characterizing any biotic invasion (Collins et al. 2002). The study of the provenance of non-native taxa is of great importance for its power to reveal whether non-native populations comprise individuals descended from multiple, genetically distinct lineages. Thus phenomenon serves to transform interpopulation diversity—and sometimes even overlooked or cryptic interspecific diversity (e.g., Hunter et al. 2018)—into

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intrapopulation diversity within the non-native range, resulting in elevated levels of genetic diversity and thereby potentially facilitating the adaptation, establishment, spread, and persistence of non-native organisms (Crawford and Whitney 2010; Gillis et al. 2009; Kolbe et al. 2004, 2007; Lavergne and Molofsky 2007; Smyser et al. 2020; Wagner et al. 2017). Furthermore, traits pertinent to invasive success often show spatial variation, including behaviors (Lycett et al. 2009), bioclimatic tolerances (Kolbe et al. 2013a), parasite assemblages (Kmentová et al. 2019), resistance to disease (Perrin et al. 2010), and degree of genetic admixture (Wielstra et al. 2017). Finally, provenance analysis can be used to pinpoint sources of introductions, as exemplified by the case of accidental introductions of organisms via cargo in ports accepting shipments from various locales (Júnior 2015; Kraus 2008; Krysko et al. 2016; Nania et al. 2020).

The state of Florida, USA is home to more non-native reptile and amphibian species than anywhere else on Earth (Krysko et al. 2016). Several studies have been conducted to establish the provenance, identity, and diversity of non-native squamate reptiles in the state. Kolbe et al. (2004) found evidence of at least eight separate introductions of *Anolis sagrei* to Florida, and Kolbe et al. (2007) conducted similar research on seven additional non-native Florida *Anolis* species, identifying mitochondrial (mtDNA) haplotypes derived from multiple native-range areas in all but one case. In a study of introduced anoles in Florida, the authors noted that the mean pairwise sequence divergences among haplotypes in introduced populations exceeded those of native populations in seven of Florida's eight (at that time) non-native *Anolis* species, and speculated that highly heterogeneous populations derived from multiple native-range areas may be the norm in this group (Kolbe et al. 2007). More recently, Nuñez (2016) documented the occurrence of multiple introductions of both *Ctenosaura similis* and *Agama picticauda* to Florida. Hunter et al. (2018) revealed that Florida's purported *Python bivittatus* population also comprises *P. molurus* as well as *P. bivittatus* × *P. molurus* hybrids, and thus clearly derives from multiple founding regions. Dowell et al. (2016) showed that the three extant Florida *Varanus niloticus* populations derive from three distinct regions of West Africa, but are apparently spatially and genetically isolated from one another. Similar studies did not yield evidence of multiple source populations for Florida's *Pituophis ruthveni* (Krysko et al. 2014), *Hemidactylus garnotii*, or *H. mabouia* (Carranza and Arnold 2006) populations, but in each case only two specimens were assayed for a mitochondrial gene. Non-native squamate populations comprising multiple native-range sources have also been reported from other locales, such as *Hemidactylus* spp. in the Maldives (Agarwal et al. 2019), *Podarcis muralis* in England (Michaelides et al. 2013), and *Anolis carolinensis* on the Pacific Islands (Michaelides et al. 2018), confirming that this phenomenon is not unique to Florida. Over 85% of amphibian and reptile introductions to Florida are associated with the pet trade pathway (Krysko et al. 2016). Taxa introduced via this pathway might be especially likely to derive from multiple, distinct sources, given that novelty is often a driver in the pet trade (e.g., Nekaris and Bergin 2016; Nijman et al. 2019), and that this desire for novelty may drive the availability—and ultimately the escape or release—of multiple varieties or morphs of a given taxon (Kolbe et al. 2013b).

The Madagascar giant day gecko *Phelsuma grandis* Gray 1870 and the tokay gecko *Gekko gecko* (Linnaeus 1758) are two of the 16 non-native gecko species currently established in Florida (Krysko et al. 2019). *Phelsuma grandis* is native to northern Madagascar (Sanchez and Probst 2014) and was first recorded in Florida in the 1990s (Bartlett and Bartlett 1999). The species is currently confined to southern Florida, where it is established in Miami (Thawley and Stroud 2017) and Homestead (Fieldsend and Krysko 2019a) in Miami-Dade County, and on at least 14 of the Florida Keys (Fieldsend and Krysko 2019b), Monroe County. *Gekko gecko* has a wide native range spanning from Nepal to the Aru Islands, Indonesia (Rösler et al. 2011). This species was first reported in Florida in the 1960s (King and Krakauer 1966), and has since been recorded from Leon county in the Panhandle (Means 1996) southward to Key West, Monroe County (Meshaka et al. 2004). Both species were introduced to southern Florida via the pet trade (Krysko et al. 2016), and continue to be intentionally released in the State (Krysko et al. 2019). In the case of *P. grandis*, breeding populations are undoubtedly established with the intention of harvesting for the pet trade (Krysko et al. 2019); however, data are not available on the number of *P. grandis* and *G. gecko* in the pet trade that are imported versus captured domestically.

Both *P. grandis* and *G. gecko* pose a potential threat to Florida's native biodiversity by virtue of being generalist predators (Dervin et al. 2013; Krysko et al. 2019; Meshaka Jr et al. 1997; Sanchez and Probst 2014). Both species consume gastropods (Meshaka Jr et al. 1997; Sanchez and Probst 2014), which is concerning given that Florida is home to native tree snails of the genera *Liguus*, *Orthalicus*, and *Drymaeus* (Kay 1995). *Gekko gecko* is particularly voracious, and has been documented feeding on a wide range of vertebrates including frogs, lizards, snakes, and birds (Krysko et al. 2019); it has also been observed preying on rodents in its native range (Bucol and Alcala 2013) and bats in its introduced range (Breuil et al. 2009), implying a threat to the threatened and endemic Florida bonneted bat *Eumops floridanus*, Key Largo woodrat *Neotoma floridana smalli*, Key Largo cotton mouse *Peromyscus gossypinus allapaticola*, and silver rice rat *Oryzomys palustris natator*, all of which apparently occur in sympatry with *G. gecko* (Beckmann 2011; Crouse 2007; de Torrez et al. 2018; Krysko and Daniels 2005; Krysko et al. 2019; Potts 2011).

The primary aim of this study was to establish the provenance and genetic diversity of southern Florida's *P. grandis* and *G. gecko* populations. In particular, we were interested in determining whether these non-native squamate populations show evidence of genetic contributions from multiple, genetically distinct native-range lineages. This phenomenon has been reported for several other non-native squamates in Florida, thus potentially challenging the classical view that non-native populations are genetically homogenous and depauperate compared to native-range populations (Kolbe et al. 2007). Species in the family Gekkonidae have been shown to generally exhibit high levels of intraspecific mtDNA variation, making these geckos excellent candidates for studies in invasion genetics (Barrett et al. 2016; Harris 2002).

A secondary aim of this research was to confirm that these non-native gekkonids have been correctly identified. Establishing the identity of non-native taxa is not always straightforward, and non-native organisms are sometimes initially misidentified (e.g., Hunter et al. 2018; Nuñez et al. 2016), which can be problematic since the life histories of morphologically similar taxa can differ substantially (Collins et al. 2002; Hunter et al. 2018; Raxworthy et al. 2007; Zhang et al. 2014). *Phelsuma grandis* was elevated from its subspecific classification as *P. madagascariensis grandis* to specific rank by Raxworthy et al. (2007) along with *P. kochi* Mertens 1954 and *P. madagascariensis* Gray 1831, which were previously classified as *P. m. kochi* and *P. m. madagascariensis*, respectively. Two subspecies of the red-spotted tokay *G. gecko* are currently recognized: *G. g. azhari*, which is endemic to Bangladesh (Mahony and Reza 2008; Mertens 1955; Rösler et al. 2011), and the nominate taxon *G. g. gecko*, which occurs throughout the rest of the native range (Rösler et al. 2011) and is the only *G. gecko* ssp. identified in Florida thus far (Krysko et al. 2019). A second species of tokay gecko sensu lato, the "black-spotted tokay" *G. reevesii* (Gray 1831), was revalidated by Rösler et al. (2011), having long been treated as a distinctive morph of *G. gecko* (e.g., Peng et al. 2011). Importantly, neither *P. grandis* nor *G. gecko* was recognized by the scientific community as a distinct species from its morphologically similar congeners at the time of its original introduction to Florida. Consequently, it is entirely possible that *P. kochi*, *P. madagascariensis*, and *G. reevesii* were legitimately imported into the United States under the listing *P. madagascariensis* or *G. gecko*, and subsequently introduced to Florida. For example, "Chinese tokay geckos"—listed as *G. gecko*—were availalable for import from Hong Kong during the 1980s (Murphy and McCloud 2010), but *G. reevesii* is now known to occur in both Hong Kong (Chan et al. 2006) and mainland China (Rösler et al. 2011). *Phelsuma grandis* and *G. gecko* display very little overlap in bioclimatic niche with these congeners (Raxworthy et al. 2007; Rösler et al. 2011; Zhang et al. 2014), and so could differ markedly in their ability to colonize and spread through Florida, highlighting the importance of ensuring that these non-native gekkonids are accurately identified.

Methods

Thirty-four *Gekko* sp. and 27 *Phelsuma* sp. were captured via hand, noose pole, baited hook (Krysko 2000), or the fishing/glue trap technique (Fieldsend and Krysko 2020) from various sites in southern Florida (Fig. 1) between 24 May 2018 and 31 July 2020. Upon capture, lizards were euthanized via intracoelomic injection of MS222 (tricaine methanesulfonate) following Conroy et al. (2009), transported

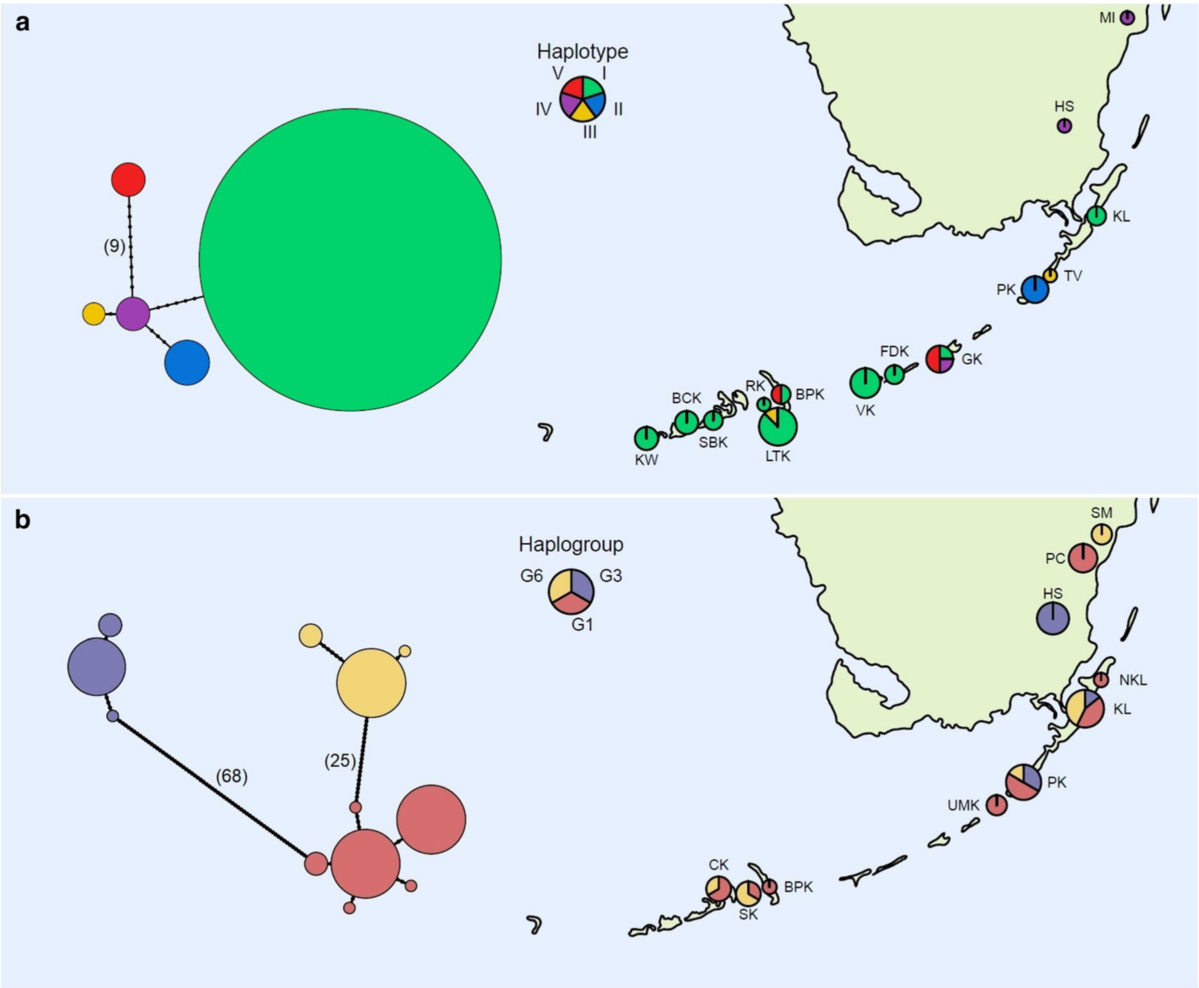


Fig. 1 Locations and genetic affiliations of southern Florida *Phelsuma grandis* (**a**) and *Gekko gecko* (**b**) populations included in this study. Embedded mtDNA haplotype networks were generated using 610 bp of Cyt-*b* (*P. grandis*) and 751 bp of ND2 (*G. gecko*). Haplotype network circle size corresponds to haplotype frequency (n = 1–27). Black dots represent the number of mutations separating haplotypes, and numbers in parentheses denote the number of mutations separating major mtDNA clades/subclades from one another. Map circle sizes correspond to population sample sizes (n = 1–8), with circle segment coloration representing the proportion of individuals from the population found to either possess a given haplotype (*P. grandis*) or belong to a given haplogroup (*G. gecko*). Site abbreviations are as follows: BCK – Big Coppitt Key; BPK—Big Pine Key; CK—Cudjoe Key; FDK—Fat Deer Key; GK—Grassy Key; HS—Homestead; KL—Key Largo; KW—Key West; LTK—Little Torch Key; MI—Miami; NKL—North Key Largo; PC—Pinecrest; PK—Plantation Key; RK—Ramrod Key; SBK—Saddlebunch Keys; SK—Summerland Key; SM—South Miami; TV—Tavernier; UMK—Upper Matecumbe Key; VK—Vaca Key

on dry ice, and stored at − 80 °C under Florida International University IACUC protocol # IACUC-17-019. Additionally, 12 *Phelsuma* sp. tissue samples were taken from specimens held in collections of the Florida Museum of Natural History, resulting in a total *P. grandis* sample size of 39. Full details of the specimens used in this study are given in Online Resource 1.

DNA was extracted from tissue samples using cetyltrimethylammonium bromide (CTAB) as per the protocol of Saghai-Maroof et al. (1984). The cytochrome b (Cyt-*b*) and NADH dehydrogenase 2 (ND2) mitochondrial genes were chosen for analysis of *Phelsuma* and *Gekko* respectively, in order to maximize the number of native-range sequences available for comparison on GenBank. Double-stranded DNA amplification for *Phelsuma* samples was achieved via Polymerase Chain Reaction (PCR) using an initial hot-start step of 10 m at 57 °C, followed by 37 cycles of 30 s of 94 °C denaturation/45 s of 57 °C annealing/45 s of 72 °C extension, followed by 60 s at 49 °C, followed by a final 15 m extension period at 72 °C. The PCR parameters for *Gekko* samples were as follows: 10 m at 57 °C, followed by 37 or 39 cycles of 30 s of 94 °C denaturation/45 s of either 53 °C or 57 °C annealing/45 s of 72 °C extension, followed by 60 s at 49 °C, followed by a final 15 m extension period at 72 °C. PCRs were performed in 50 µl reactions containing 1-3 µl of total DNA extract of variable concentration (average ~ 31 ng/µl), 27.5–29.5 µl ddH$_2$O, 10 µl of 5X Green GoTaq® Flexi Buffer (Promega, Madison, WI, USA), 3 µl of MgCl$_2$ (25 mM), 2.5 µl each of 10 µM forward and reverse primer (Online Resource 2), 1 µl of dNTP solution (10 mM with respect to each dNTP), and 0.5 µl Taq polymerase (5U/µl). Double-stranded PCR products were then cleaned with ExoSap-IT (Thermo Fisher Scientific, Waltham, MA, USA), and cycle sequencing was performed using BigDye$^{\text{TM}}$ Terminator v3.1 Cycle Sequencing Kit as per the manufacturer's instructions, but using 1/2 the reaction volume and 1/8 the amount of BigDye Terminator v3.1 (Thermo Fisher Scientific, Waltham, MA, USA). Sequences were read on both strands using an Applied Biosystems 3130XL Genetic Analyzer.

Sequence alignments comprised a combination of sequences generated for this study and sequences retrieved from GenBank (Online Resources 3 and 4). When multiple identical sequences were available on GenBank for a native-range locale, we included a single representative sequence in our alignment in order to reduce redundancy. Sequence alignments were conducted with MAFFT (Katoh and Standley 2013) in Mesquite (Maddison and Maddison 2019) and produced alignments with no internal gaps. Alignments were translated to amino acids with the vertebrate mitochondrial genetic code to verify in-frame reading through the sequences with no stop codons.

Phylogenetic analyses were undertaken using Bayesian inference as employed in MrBayes (v3.2.6, Ronquist et al. 2012) using the GTR + I + G model on each of three partitions by codon position, allowing parameters to vary independently for each partition, which differ in patterns of base compositional bias, selective constraints, and proportion of invariant sites. Default priors were used in all analyses. Posterior probabilities of phylogenetic trees were based on running 10 million generations of Metropolis coupled Markov Chain Monte Carlo (MC^3), which included two simultaneous runs of four chains, starting with a random tree and sampling every 100 generations. Preliminary runs were carried out to ensure stationarity of the dataset, with the potential scale reduction factor approaching 1 for all parameters, and the standard deviation of split frequencies less than 0.01. Twenty-five percent of trees were discarded as burnin; the remaining trees were used to determine the 50% majority-rule consensus tree and estimate Bayesian posterior probabilities.

Haplotype networks (Fig. 1) were generated in R version 3.5.3 (R Core Team 2019) using the *haploNet* function in the 'pegas' package (Paradis 2010).

Results

The primers used for *Phelsuma grandis* amplified a mean product of 651 base pairs (bp) of the Cyt-*b* mitochondrial gene, 610 bp of which were used for analysis. The equivalent values for the *Gekko gecko* ND2 mitochondrial gene were 1262 bp and 751 bp respectively, the latter value corresponding to the length of the majority of GenBank sequences available for comparison (Saijuntha et al. 2019). All sequences were deposited into GenBank (Online Resource 1).

All 39 *Phelsuma* sp. mtDNA haplotypes from southern Florida were reliably assigned to *P. grandis*, rather than its morphologically similar congeners *P. kochi* and *P. madagascariensis* (Fig. 2). Five distinct mitochondrial haplotypes were identified in the southern Florida *P. grandis* populations (Figs. 1, 2). Haplotype I was present in 27 of the 39 sequenced specimens (69.2%), and was the only haplotype identified in the Key Largo, Fat Deer Key, Vaca Key, Ramrod Key, Big Coppitt Key, and Key West populations. This haplotype was also detected on Grassy Key (1 of 4 specimens), Big Pine Key (1 of 2 specimens), and Little Torch Key (7 of 8 specimens). Haplotype II was confined to Plantation Key, where it was the sole haplotype detected in the four sequenced specimens. Haplotype III was present on Tavernier (1 of 1 specimens) and Little Torch Key (1 of 8 specimens), whilst Haplotype IV was detected in

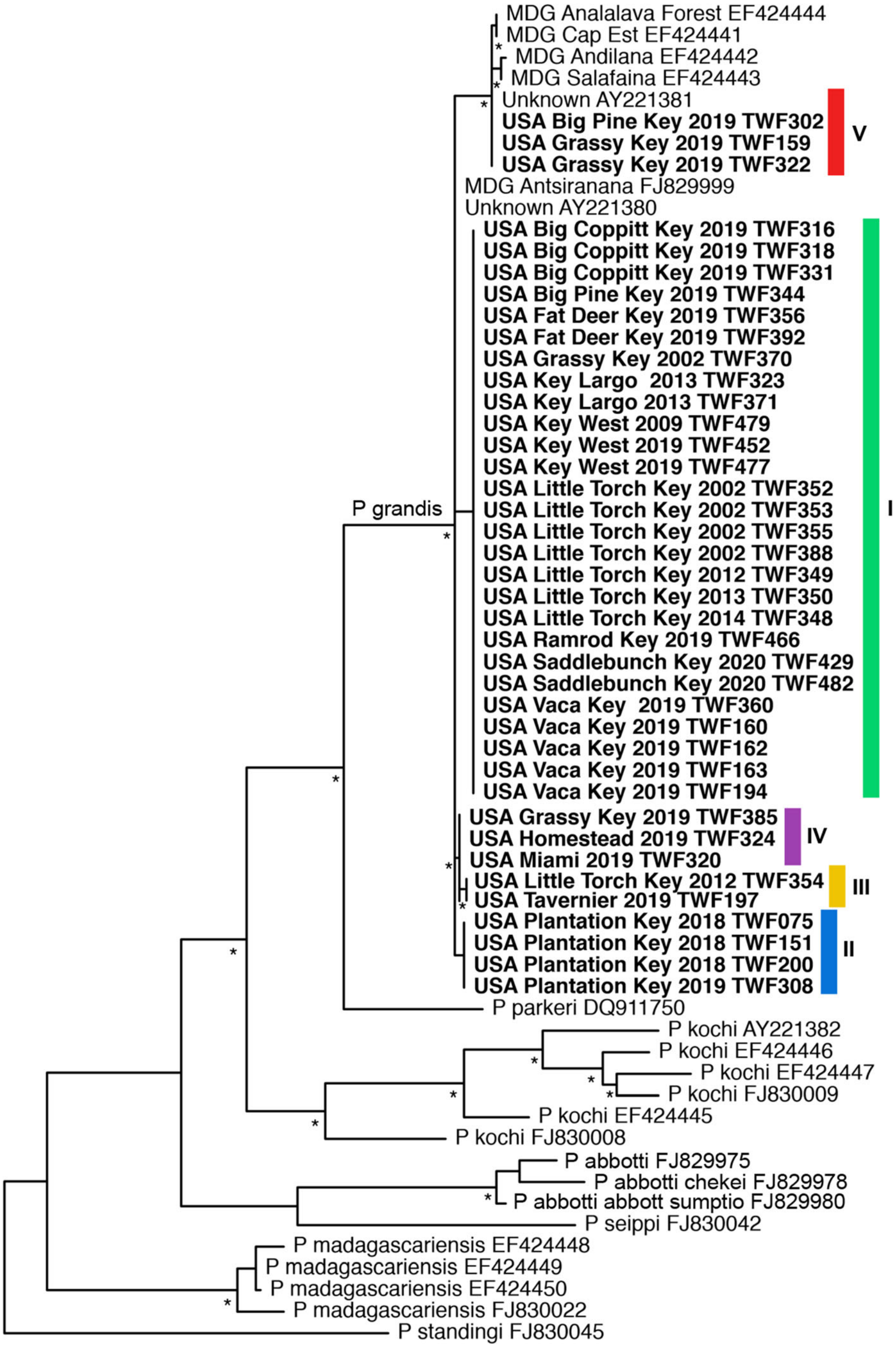


Fig. 2 *Phelsuma* 50% majority-rule consensus tree (Cyt-*b*, 610 bp). The year, if given, denotes the year in which the specimen was captured. Asterisks (*) denote nodes with Bayesian posterior probabilities ≥ 0.95. Haplotype (I-V) classifications follow Fig. 1. GenBank Accession Numbers are given for sequences that were not generated as part of this study. Countries of origin are identified by their ISO 3166-1 alpha-3 codes: Madagascar—MDG; United States of America—USA. AY221380 and AY221381 are captive specimens from Mauritius and the United Kingdom respectively

Miami (1 of 1 specimens) and Homestead (1 of 1 specimens), and on Grassy Key (1 of 4 specimens). Haplotype V was found on Grassy Key (2 of 4 specimens) and Big Pine Key (1 of 2 specimens).

Thirty-six of the 39 southern Florida *P. grandis* did not cluster with any native-range sequences in our phylogenetic analysis, but were found to be very closely allied (606–609 out of 610 bp identical, 99.34%–99.84% similarity) with a specimen from Antsiranana, Madagascar, which is located at the northern extreme of the species' native range. The remaining three sequences clustered in a well-supported clade containing native-range specimens from Analalava Forest, Andilana, Cap Est, and Salafaina. All four of these sites are located on Madagascar's northeastern coast, with Cap Est located on the Masoala peninsula at the southern edge of the species' native distribution, some 351 km south-east of Antsiranana (Raxworthy et al. 2007, Supplementary data).

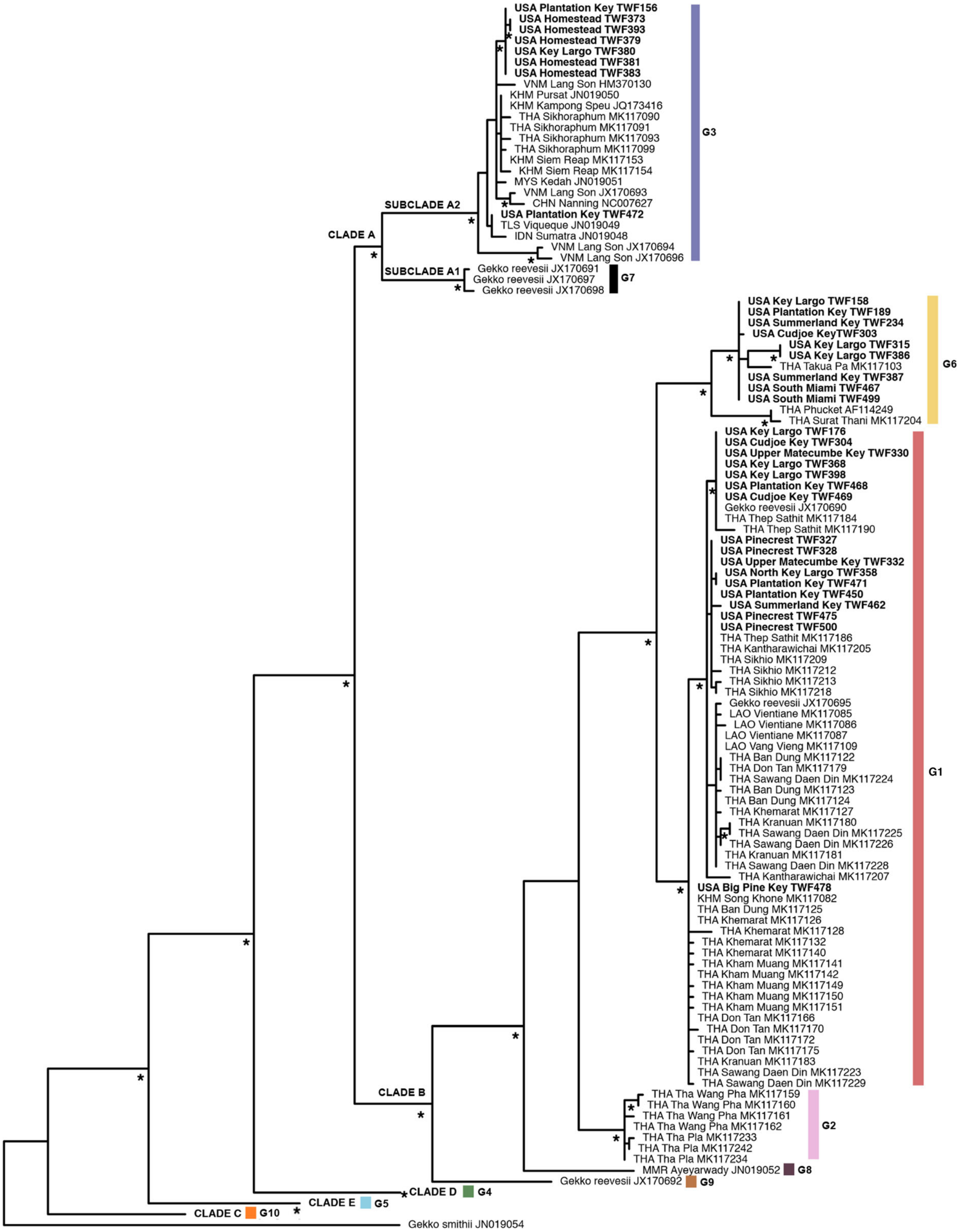
No *Gekko* sp. ND2 haplotypes from southern Florida could reliably be assigned to *G. reevesii*. Indeed, our analysis found *G. reevesii* to be both polyphyletic and deeply nested within *G. g. gecko*, a finding corroborated by previous mtDNA studies (Saijuntha et al. 2019; Wang et al. 2013). The subspecies *G. g. azhari* was not included in this analysis; however, the high support for the placement of the southern Florida *Gekko* specimens within well-characterized native-range *G. g. gecko* clades apparently spatially disjunct from *G. g. azhari*'s known range in Bangladesh (Mahony and Reza 2008; Mertens 1955; Rösler et al. 2011; Saijuntha et al. 2019) makes it likely that no *G. g. azhari* haplotypes were detected. Saijuntha et al. (2019) demonstrated the existence of five, distinct native-range *G. gecko* mtDNA clades (Clades A-E), which between them contain 10 discrete "haplogroups" (G1-G10) (Fig. 3). A total of 12 mitochondrial haplotypes were detected in the 34 assayed southern Florida *G. gecko* specimens (Figs. 1, 3), all of which clustered within two of the five native-range clades, with three of the 10 haplogroups represented. Eight of the southern Florida *G. gecko* (23.5%)—including specimens from Homestead, Key Largo, and Plantation Key—clustered within Clade A, Haplogroup G3 from Cambodia, South China, peninsular Malaysia, Sumatra (Indonesia), northeastern Thailand, Timor-Leste, and northern Vietnam. The remaining 26 southern Florida *G. gecko* clustered within two well-supported subclades of native-range Clade B. Nine (26.5%)—including individuals from South Miami, Key Largo, Plantation Key, Summerland Key, and Cudjoe Key—placed within the Clade B, Haplogroup G6 subclade, which contains native-range specimens from peninsular Thailand. The remaining 17 specimens (50%) from Pinecrest, North Key Largo, Key Largo, Plantation Key, Upper Matecumbe Key, Big Pine Key, Summerland Key, and Cudjoe Key fell within Clade B, Haplogroup G1, which includes native-range specimens from Cambodia, Laos, and northeastern Thailand, as well as two specimens identified by Wang et al. (2013) as *G. reevesii*.

Mean uncorrected pairwise distance for all southern Florida *P. grandis* ($n = 39$) was 0.60%, while the equivalent value for *G. gecko* ($n = 34$) was 5.39%. Maximum uncorrected pairwise distances were 2.00% for *P. grandis* and 11.77% for *G. gecko* (Online Resources 3 and 4).

Discussion

This study identifies the presence of multiple, distinct mitochondrial clades in southern Florida's non-native *Phelsuma grandis* and *Gekko gecko* populations. In doing so, it provides further evidence that genetically heterogeneous populations (Hunter et al. 2018; Kolbe et al. 2004, 2007; Nuñez 2016) may be the rule rather than the exception in the non-native squamate fauna of Florida. Similar research conducted outside of Florida (e.g., Agarwal et al. 2019; Michaelides et al. 2013, 2018) indicates that this may be a general phenomenon in non-native squamates, in contrast to the general pattern of reduced genetic diversity exhibited in introduced populations of most taxa (Dlugosch and Parker, 2008). While some of Florida's highly diverse non-native squamate populations clearly derive from accidental importation via cargo (e.g., *Anolis sagrei*, Kolbe et al. 2004; Krysko et al. 2016), > 85% of introductions of reptiles and amphibians to the state can ultimately be traced to the pet trade (Krysko et al. 2016). The relationship between invasion pathway and genetic diversity should thus be considered a pressing issue within the field of invasion biology, given that high-or-heightened population-level genetic diversity has now been implicated as a driver of invasive success in several case studies (Crawford and Whitney 2010; Lavergne and Molofsky 2007; Smyser et al. 2020; Wagner et al. 2017).

The uncorrected mean pairwise distance value for southern Florida *G. gecko* (5.39%) was found to be much higher than the equivalent value (0.60%) for


USA Plantation Key TWF156
USA Homestead TWF373
USA Homestead TWF393
USA Homestead TWF379
USA Key Largo TWF380
USA Homestead TWF381
USA Homestead TWF383
VNM Lang Son HM370130
KHM Pursat JN019050
KHM Kampong Speu JQ173416
THA Sikhoraphum MK117090
THA Sikhoraphum MK117091
THA Sikhoraphum MK117093
THA Sikhoraphum MK117099
KHM Siem Reap MK117153
KHM Siem Reap MK117154
MYS Kedah JN019051
VNM Lang Son JX170693
CHN Nanning NC007627
USA Plantation Key TWF472
TLS Viqueque JN019049
IDN Sumatra JN019048
VNM Lang Son JX170694
VNM Lang Son JX170696
G3
SUBCLADE A2
CLADE A
SUBCLADE A1
Gekko reevesii JX170691
Gekko reevesii JX170697
Gekko reevesii JX170698
G7
USA Key Largo TWF158
USA Plantation Key TWF189
USA Summerland Key TWF234
USA Cudjoe KeyTWF303
USA Key Largo TWF315
USA Key Largo TWF386
THA Takua Pa MK117103
USA Summerland Key TWF387
USA South Miami TWF467
USA South Miami TWF499
THA Phucket AF114249
THA Surat Thani MK117204
G6
USA Key Largo TWF176
USA Cudjoe Key TWF304
USA Upper Matecumbe Key TWF330
USA Key Largo TWF368
USA Key Largo TWF398
USA Plantation Key TWF468
USA Cudjoe Key TWF469
Gekko reevesii JX170690
THA Thep Sathit MK117184
THA Thep Sathit MK117190
USA Pinecrest TWF327
USA Pinecrest TWF328
USA Upper Matecumbe Key TWF332
USA North Key Largo TWF358
USA Plantation Key TWF471
USA Plantation Key TWF450
USA Summerland Key TWF462
USA Pinecrest TWF475
USA Pinecrest TWF500
THA Thep Sathit MK117186
THA Kantharawichai MK117205
THA Sikhio MK117209
THA Sikhio MK117212
THA Sikhio MK117213
THA Sikhio MK117218
Gekko reevesii JX170695
LAO Vientiane MK117085
LAO Vientiane MK117086
LAO Vientiane MK117087
LAO Vang Vieng MK117109
THA Ban Dung MK117122
THA Don Tan MK117179
THA Sawang Daen Din MK117224
THA Ban Dung MK117123
THA Ban Dung MK117124
THA Khemarat MK117127
THA Kranuan MK117180
THA Sawang Daen Din MK117225
THA Sawang Daen Din MK117226
THA Kranuan MK117181
THA Sawang Daen Din MK117228
THA Kantharawichai MK117207
USA Big Pine Key TWF478
KHM Song Khone MK117082
THA Ban Dung MK117125
THA Khemarat MK117126
THA Khemarat MK117128
THA Khemarat MK117132
THA Khemarat MK117140
THA Kham Muang MK117141
THA Kham Muang MK117142
THA Kham Muang MK117149
THA Kham Muang MK117150
THA Kham Muang MK117151
THA Don Tan MK117166
THA Don Tan MK117170
THA Don Tan MK117172
THA Don Tan MK117175
THA Kranuan MK117183
THA Sawang Daen Din MK117223
THA Sawang Daen Din MK117229
G1
THA Tha Wang Pha MK117159
THA Tha Wang Pha MK117160
THA Tha Wang Pha MK117161
THA Tha Wang Pha MK117162
THA Tha Pla MK117233
THA Tha Pla MK117242
THA Tha Pla MK117234
G2
MMR Ayeyarwady JN019052
G8
Gekko reevesii JX170692
G9
CLADE B
CLADE D
G4
CLADE E
G5
CLADE C
G10
Gekko smithii JN019054
*

◀**Fig. 3** *Gekko* 50% majority-rule consensus tree (ND2, 751 bp). Asterisks (*) denote nodes with Bayesian posterior probabilities ≥ 0.95. Clade, subclade, and haplogroup (G1–G10) classifications follow Saijuntha et al. (2019) with some modification. Clades C, D, and E (Saijuntha et al. 2019) have been collapsed to better visualize the clades containing sequences from southern Florida *G. gecko*. GenBank Accession Numbers are given for sequences which were not generated as part of this study. Countries of origin are identified by their ISO 3166-1 alpha-3 codes: Cambodia—KHM; China—CHN; Indonesia—IDN; Laos—LAO; Malaysia—MYS; Myanmar—MMR; Thailand—THA; Timor-Leste—TLS; United States of America—USA; Vietnam—VNM

P. grandis. An important consideration is that different mitochondrial genes were assayed for each species: analysis of *P. grandis* was conducted using the Cyt-*b* gene, while ND2 was used for analysis of *G. gecko*. These pairwise distance values thus relate to different regions of the mitochondrial genome, across which substitution rates can differ substantially (Pesole et al. 1999), and care must therefore be taken when drawing inferences. To check that this discrepancy was not merely an artifact of differing substitution rates between these two mitochondrial genes, we first downloaded 12 *Gekko* spp. complete mitochondrial genomes from GenBank (Hao et al. 2016a, 2016b; Kim et al. 2016; Kumazawa 2007; Li et al. 2013; Zhou et al. 2006). We then calculated the mean uncorrected pairwise distance at both the 751 bp ND2 region used in our analysis of *G. gecko*, and the 610 bp Cyt-*b* region equivalent to that used for analysis of *P. grandis*. We found that the ND2 region was only slightly more variable than the Cyt-*b* region (mean uncorrected pairwise distances 28.94% vs 25.71%, Online Resource 5). We were unable to perform an equivalent analysis with *Phelsuma* spp. due to a lack of available GenBank data. Nevertheless, given that the mean uncorrected pairwise distance for southern Florida's *G. gecko* population was found to be nearly nine times greater than the equivalent for *P. grandis*, analysis of different mitochondrial regions for the two species seems unlikely to be the major cause of this difference. It should however be noted that the two species are rather distantly related to one another within the Gekkonidae (Gamble et al. 2012), and differ substantially in various aspects of their natural history (e.g., size and behavior, Krysko et al. 2019).

Phylogenetic analysis (Fig. 2) revealed the existence of multiple *P. grandis* mtDNA clades in southern Florida, with northeastern Madagascar identified as the probable source of Haplotype V. Haplotypes I-IV proved to be more similar to that of a specimen from the city of Antsiranana in Madagascar's far north, which would be consistent with its position at the center of the *P. grandis* trade (CJ Raxworthy, pers. comm.); nevertheless, the paucity of georeferenced native-range sequences precludes confident inference of the provenance of these four haplotypes. Interestingly however, two individuals from Big Pine Key—captured on the same day from adjacent buildings (Online Resource 1)—were found to possess Haplotypes I and V respectively (Figs. 1, 2), implying the possibility of admixture between these distinct clades. Given the small sample sizes in our study, deeper genetic sampling is required in order to address population-level questions. This is particularly true of the Big Pine Key, Little Torch Key, Grassy Key, and Plantation Key populations, as these represent the oldest extant *P. grandis* populations in southern Florida (Krysko et al. 2003). As such, knowledge of their genetic composition would likely shed light on the colonization history of *P. grandis* in southern Florida since its initial introduction in the 1990s (Bartlett and Bartlett 1999). Deeper sampling would also help to confirm whether Haplotype II is a private haplotype of the Plantation Key Population, and whether the low prevalence of Haplotype I in the Upper Keys and mainland (2 of 9 specimens) versus the Lower Keys (25 of 30 specimens) (χ^2 (1, $N = 39$) = 12.14, $p \leq 0.0005$) is indicative of genuine population structure, or merely an artifact of small sample sizes and/or sampling bias.

Our study revealed the presence of at least three, deeply divergent mtDNA lineages in southern Florida's *G. gecko* population (Figs. 1, 3). Kongbuntad et al. (2016) postulate that *G. g. gecko* may actually represent a species complex. The maximum ND2 uncorrected pairwise distance of 18.44% detected between a *G. g. gecko* specimen from Ayeyarwady, Myanmar (GenBank Accession No. JN019052) and two *G. g. gecko* from northern Thailand (GenBank Accession Nos. MK117114 and MK117200) (Online Resource 3; Rösler et al. 2011; Saijuntha et al. 2019) provides some support for this this assertion, given that it exceeds the greatest ND2 uncorrected pairwise distance (18.2%) reported by Agarwal et al. (2019) in their study of the unresolved "*Hemidactylus frenatus*" gekkonid species complex. Nevertheless, this maximum uncorrected pairwise distance still falls short of the analogous *Gekko* interspecific uncorrected pairwise distances detailed in Online Resource 5, which range from 23.06% (*G. japonicus* and *G. swinhonis*) to 41.21% (*G. gecko* and *G. vittatus*). It must however be noted that the analysis is by no means exhaustive, and includes only six of the ~ 45 known *Gekko* species (Rösler et al. 2011). Interestingly, all three pairwise mtDNA haplogroup combinations (i.e., G1/G3, G1/G6, and G3/G6) were found in sympatry in southern Florida (Fig. 1; Online Resource 1). Of particular note, two specimens collected from the same private residence in Key Largo were found to belong to the G3 and G6 haplogroups respectively, and exhibited an uncorrected pairwise distance of 11.41% (Online Resource 3). In keeping with contemporary taxonomic nomenclature, southern Florida's tokay gecko population is currently classified as one subspecies, i.e., *G. g. gecko* (Krysko et al. 2019). Nevertheless, the depth of genetic divergence between the three mtDNA lineages—particularly between Haplogroup G3 (Clade A) and the other two haplogroups (Clade B)—suggests at least the possibility that multiple, reproductively isolated tokay gecko lineages are present in southern Florida. Intriguingly, Clade A and Clade B show close phylogeographical correspondence with the distribution of the morphologically distinct "nominal" and "central mainland" forms of tokay gecko described by Rösler (2005) respectively, providing further evidence of their distinctiveness. If admixture does indeed occur between these lineages, it appears likely that it is serving to transform interpopulation—and perhaps even interspecific—native-range diversity into intrapopulation diversity in southern Florida, potentially resulting in a highly genetically diverse population. Such admixture could conceivably increase invasive potential (Crawford and Whitney 2010; Lavergne and Molofsky 2007; Smyser et al. 2020; Wagner et al. 2017), although it should be noted that it could also have the opposite effect (e.g., outbreeding depression, Barker et al. 2019; Pantoja et al. 2018). Conversely, reproductive isolation between these lineages in southern Florida would provide very strong support for the argument that *G. g. gecko* is actually a species complex. This study system may thus present an unusual opportunity to use an introduction event to help resolve a species complex (e.g., Wegener et al. 2019). Since mitochondrial DNA is generally inherited uniparentally (Avise et al. 1979)—and is thus a poor indicator of hybridization and admixture—our future research will combine mtDNA data with nuclear markers such as microsatellites or nuclear genes (Hunter et al. 2018; Kurita et al. 2018; Pinto et al. 2019; Vuillaume et al. 2015), in order to check for cytonuclear discordance indicative of these phenomena (Toews and Brelsford 2012).

The work of Rösler et al. (2011), Wang et al. (2013), and Saijuntha et al. (2019) has made available for comparison ~180 georeferenced *G. gecko* ND2 sequences. Sequencing effort has thus far focused predominantly on Thailand, China, Vietnam, and neighboring countries, resulting in relatively high sampling density for China (excluding Hong Kong, Chan et al. 2006) and most of mainland Southeast Asia. Nevertheless, the wide native range of this species (Rösler et al. 2011) means that our phylogeographic knowledge of *G. gecko* remains incomplete, thereby lowering the confidence with which native-range provenance can be inferred. *Gekko gecko* ND2 sequence data are unavailable for Brunei (UMMZ 201955-201957), Philippines (Bucol and Alcala 2013), Singapore (Tan et al. 1978), and the Subcontinental nations of Bangladesh, Bhutan, India, and Nepal (Rösler et al. 2011), meaning that genetic sampling of specimens from these countries should be a top priority. Myanmar is another country for which sampling should be prioritized. Several gekkonid species exhibit deep intraspecific genetic divergence in Myanmar (Carranza and Arnold 2006), and the two Myanmar *G. gecko* specimens sequenced thus far have an ND2 uncorrected pairwise difference of 17.93% (Online Resource 3) despite having been collected from locations ca. 400 km apart (Rösler et al. 2011), suggesting that the nation could harbor considerable *G. gecko* diversity. Deeper sampling of Indonesia and Malaysia would also be welcomed, given their prominence in the international *G. gecko* trade (Caillabet 2013; Nijman and Shepherd 2015) and the low number of ND2 sequences from these countries presently available for comparative analysis. Another priority for future research is the taxonomic status of the black-spotted tokay, *G. reevesii*, as our analysis found specimens identified as *G. reevesii* to be both polyphyletic and clustered deep within *G. g. gecko*

(Fig. 3), and thus does not support its current taxonomic status as a separate species.

Our study adds to the growing body of evidence that non-native squamate populations in Florida and elsewhere often ultimately derive from multiple, distinct genetic lineages, and are frequently highly heterogeneous as a result. Such heterogeneity can enhance the fitness (Smyser et al. 2020), adaptive potential (Lavergne and Molofsky 2007), and invasive success (Crawford and Whitney 2010) of non-native organisms, illustrating both the importance of this genetic approach to invasion biology, and the need to understand how widespread this phenomenon is in other taxonomic groups.

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Availability of data and materials (data transparency) The datasets generated and analyzed during the current study are available in the GenBank repository, under the Accession Numbers MT554153-MT554166 and MW244758-MW244816. Specimen voucher numbers are listed in Online Resource 1.

Code availability (software application or custom code) N/A.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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