



INTRODUCED SPECIES

Molecular Analysis Confirming the Introduction of the Western African Fan-footed Gecko, *Ptyodactylus togoensis* (Tornier 1901) (Sauria: Phyllodactylidae), in Florida

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Geckos of the family Phyllodactylidae are trans-Atlantic squamates commonly referred to as Leaf-toed Geckos (Dixon and Kroll 1974; Gamble 2008). The genus *Ptyodactylus* is comprised of rock-dwelling geckos native to northwestern Africa northeastward to Pakistan (Blanford 1876; Metallinou 2015). The majority of species in the genus have only recently been described or elevated to species status. For example, Metallinou (2015) recently elevated the former subspecies *P. hasselquistii togoensis*, which occurs in western Africa in Togo, Benin, Ghana, Burkina Faso, Niger, Mali, Mauritania, and the southernmost portion of Algeria, to species status, calling it *P. togoensis*. Morphological similarities within the genus make it difficult to confirm the identity of a species without knowing an individual's origin.

Because of the increasing number of introduced amphibians and reptiles in Florida (Krysko et al. 2016), identifying both species and introduction pathways is important. Herein, we document a new introduced gecko species by providing a physical specimen and a clear photographic voucher. We used molecular analysis to determine species identity and the native-range origin, and we comment on its introduction pathway and current invasion stage in Florida.

Materials and Methods

Specimen acquisition. On 23 June 2016, a neonate (3.9 cm SVL, 5.9 cm TL) *Ptyodactylus* was collected next to an importer's facility at 6450 Stirling Road, Hollywood, Broward County, Florida (26.04591°N, 80.21976°W, Datum WGS84). The specimen (UF-Herpetology 178182) and digital photographic vouchers of the live animal (Fig. 1) were deposited in the Division of Herpetology, Florida Museum of Natural History, University of Florida. Initial identification as *Ptyodactylus* was provided by Aaron M. Bauer and Tony Gamble. Visits to the site on 24 June and 17 July 2016 yielded no further individuals.

Laboratory techniques. A DNA isolation was obtained using ZR Genomic DNA™ Tissue Microprep Kit (Zymo Research, LLC, Irvine, California). DNA was amplified and sequenced for the 12S region using primers 12Se (Weins et al. 1999) and L1091 (Kocher et al. 1989). PCR was conducted in 25 µl reactions: 9.5 µl H₂O, 12.5 µl GoTaq® Master Mix (Promega Corp, Madison, Wisconsin), 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 45 cycles of amplification; denaturing at 94 °C for 1 min; annealing at 49 °C for 1 min; and extension at 72 °C for 1 min; followed 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), and compared with a DNA standard. Sequence files from the automated sequencer (Genomics Division, Interdisciplinary Center for Biotechnology Research, University of Florida) were edited as necessary with Geneious software (ver. 6.1, Biomatters, <http://www.geneious.com>). The GenBank accession number for our specimen is KX981451.



Fig. 1. Western African Fan-footed Gecko, *Ptyodactylus togoensis* (UF-Herpetology 178182), collected on 23 June 2016 in Broward County, Florida. Photograph by KLK.

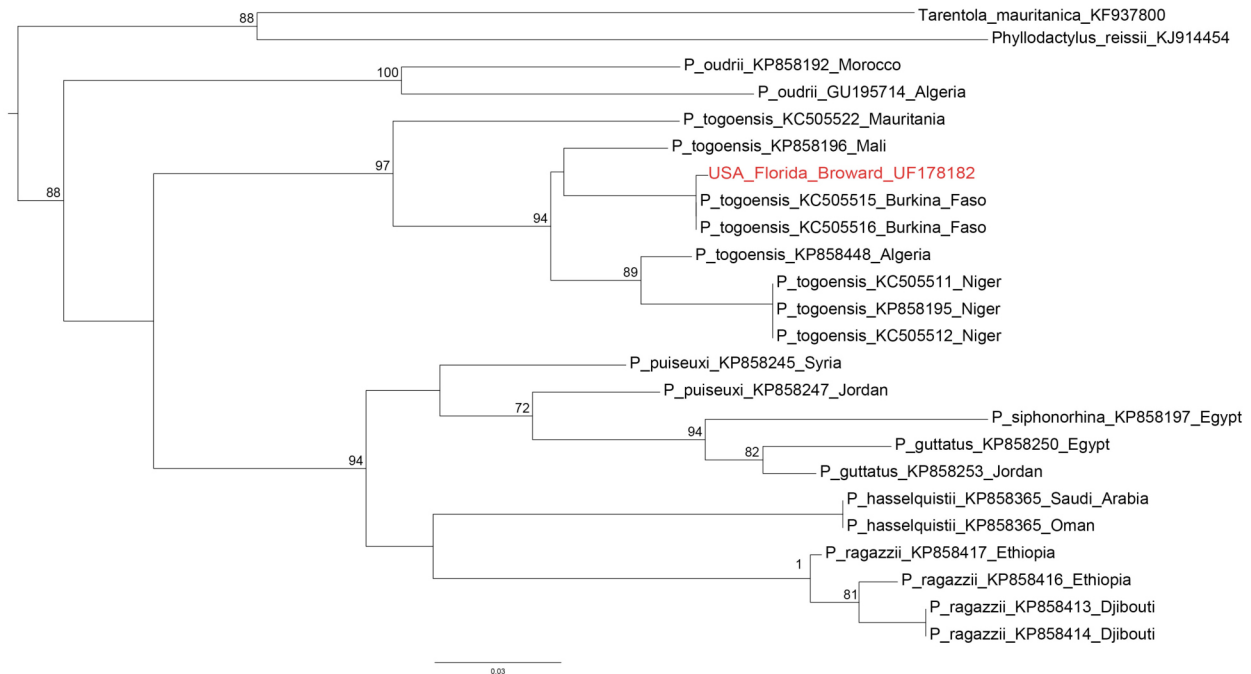


Fig. 2. Maximum likelihood phylogeny for Fan-footed Geckos, genus *Ptyodactylus*. Included outgroups have their genus included in their label. Note that our Florida sample is highlighted in red; values ($\geq 70\%$) above major nodes represent bootstrap support.

Phylogenetic analyses. 12S DNA sequence data for a total of 23 specimens were downloaded from GenBank. Included were two *Ptyodactylus oudrii*, eight *P. togoensis*, two *P. puiseuxi*, one *P. siphonorhina*, two *P. guttatus*, two *P. hasselquistii*, and four *P. ragazzii* (Metallinou et al. 2015), and one of each outgroup, *Tarentola mauritanica* (Mačát et al. 2014) and *Phyllodactylus reissi* (Torres-Carvajal et al. 2014). Along with our specimen, all sequences were assembled using MEGA6 software (ver. 6; Tamura et al. 2013).

We analyzed a total of 369 base pairs (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 2,000 nonparametric bootstrap replicates (Felsenstein 1985) to assess node support using MEGA. The most credible support of phylogenetic relationships were confined to nodes where nonparametric bootstrap values $\geq 70\%$ (Felsenstein 1985).

Results

Our molecular analysis produced a nearly identical phylogeny as Metallinou et al. (2015), with the exception of the inclusion of our sample and outgroups (Fig. 2). Our Florida sample is nested within the *P. togoensis* clade and is most closely related to individuals from Burkina Faso. Only 1 bp difference was found between our sample and the two samples from Burkina Faso.

Discussion

We identify our Florida specimen as *Ptyodactylus togoensis* with a native-range origin as Burkina Faso in western Africa. The animal importer in Hollywood, Florida, is the probable source for the introduction of this species. This importer has been implicated in the introduction of at least 46 (25.5%) of the 180 confirmed herpetofaunal taxa introduced in Florida (Krysko et al. 2016). We found this species, listed as the West African Fan-Footed Gecko (*Ptyodactylus hasselquistii*), on their old price lists dated from 14 October 2014 to 14 April 2015 (Fig. 3). Although the specimen we found was a neonate, we have no current evidence of reproduction and the establishment of this species.

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Gecko:		Qty. 1	6	12	25
African Fat Tail adult	Hemitheconyx caudicinctus	\$15.00	\$14.00	\$12.00	
Bibron's	Pachydactylus bibroni	\$5.00	\$4.00	\$3.50	
Cape Yellow Head	Lygodactylus capensis	\$7.00	\$6.00		
Flying	Ptychozoon sp	\$10.00	\$9.00		
Giant Day small / large	Phelsuma grandis	\$25.00	\$20.00		
Giant Halmehara		\$40.00			
Golden	Gekko ullkovskii	\$5.00	\$4.50	\$4.00	\$3.50
House	Hemidactylus	3.50	6/3.00	12/2.50	25/2.00
		100/1.00			50/1.50
Lined Day	Phelsuma lineata	\$22.50			
Marbled	Gekko grosmanni	\$5.00	\$4.50	\$4.00	\$3.50
Tokay adult	Gekko gekko	\$8.00	\$7.00	\$6.00	
West African Fan Footed	Ptyodactylus hasselquistii	\$10.00			
White Lined	Gecko vittatus	\$10.00	\$9.00	\$8.00	
White Spotted Gecko	Tarentola annularis	\$6.00	\$5.00	\$4.00	
Iguanids:		Qty. 1	6	12	25

Fig. 3. Animal importer's price list for 21 October 2014.

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