REPTILES & AMPHIBIANS

Multiple Records of the Introduced Parthenogenetic Smooth-scaled Tegulet, *Gymnophthalmus underwoodi* Grant 1958, in Puerto Rico

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Abstract.—The introduction of invasive species to islands can be devastating to local biota. While many invasive species' successful colonization of new habitats can be attributed to features like high fecundity or low mate selectivity, parthenogenetic species pose a unique threat in their ability to reproduce asexually at high rates. The Smooth-scaled Tegulet (*Gymnophthalmus underwoodi*), first described from Barbados in the 1950s, has since been documented as an introduced species across additional Lesser and Greater Antillean islands as well as northwestern Brazil and Venezuela. Herein we report multiple individuals from Puerto Rico, including the first genetic records, which indicate that the individuals on this island are most closely related to populations recorded from Brazil and Montserrat. Additional genetic information is necessary to determine the timing and migration of this species across the Caribbean. Regardless, the continued geographic expansion of this parthenogen across the Caribbean could present a threat to local fauna.

The introduction of invasive species to islands can be devastating to local biota (Blackburn et al. 2004; Medina et al. 2011). Invasives can present a variety of threats ranging from predation and habitat destruction to introduction of foreign pathogens (Mack et al. 2000; Bellard et al. 2016; Shiokawa et al. 2019). Islands in the Caribbean have been subjected to introductions of invasive species for centuries to the detriment of endemic island species (Case et al. 1992). Notably, the introduction of mammals like mongooses, rats, and cats have had a tremendous impact on island ecosystem health (Case et al. 1992; Myers et al. 2000). Reptilian and amphibian introductions, such as the Cuban Treefrog (*Osteopilus septentrionalis*; Rödder and Weinsheimer 2009), the Cane Toad (*Rhinella marina*; Mittan-Moreau et al. 2022), the Green Iguana (*Iguana iguana*; Burgos-Rodríguez et al. 2016), geckos (*Hemidactylus frenatus*, *Lepidodactylus lugubris*; Cole et al. 2005), and the Smooth-scaled Tegulet (*Gymnophthalmus underwoodi*; Snyder et al. 2017), also have impacted local communities by preying on, displacing, and in some cases outcompeting native species (Reynolds and Niemiller 2010; Alfonso and Hernandez 2017; Brown and Diotallevi 2019).

While many successful colonizations of new habitats by invasive species can be attributed to features like high fecundity or low mate selectivity, parthenogenetic species such as *L. lugubris* and *G. underwoodi* can reproduce asexually at prodigious rates and require only a single individual to start a new population. Since the description of *Gymnophthalmus underwoodi* from the Lesser Antilles (Barbados) in the 1950s, this species has been documented as native or introduced on several additional Antillean islands as well as northwestern Brazil and Venezuela, although its exact origins are not fully known (Cole et al. 1990; Powell et al. 2011; Alfonso et al. 2012; Snyder et al. 2017; Maciel et al. 2021). Individuals have been found in a variety of habitats, including dry forests, beaches, rocky slopes, and areas adjacent to urban growth (Recoder et al. 2018; van den Burg et al. 2021). More recently, this species has been introduced in Cuba (Alfonso et al. 2012; Alfonso and Hernandez 2017), Montserrat (Snyder et al. 2017), Saba (van den Burg et al. 2021), St. Eustatius (Thibaudier et al. 2023), Guadeloupe (Breuil and Serre-Collet 2012), St. Kitts (Orchard 2010), Saint-Barthélemy (Questel and Boggio

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2012), and Jamaica (GBIF 2023). Herein we document multiple records of this species in Puerto Rico and identify their closest relatives using genetic analysis.

Methods

Two deceased individuals identified as *Gymnophthalmus underwoodi* were collected opportunistically from the municipalities of Gurabo (on 14 March 2021) and Juana Díaz (on 15 January 2023) in Puerto Rico (Figs. 1 & 2). The specimens were reported to us by members of the community who encountered them while gardening. DNA was extracted using the DNeasy Qiagen Blood and Tissue Kit and a fragment of the 16S rRNA locus (523 base pairs) was amplified and sequenced at ElimBio (Hayward, California, USA). Additional 12S rRNA and 16S rRNA sequences from *G. underwoodi*, *G. cryptus*, *G. leucomystax*, *G. pleii*, *G. speciosus*, and *G. vanzoi* and an outgroup from *Iphisa elegans* were included from Genbank (individual localities, primer sequences, PCR profiles, and Genbank Accession numbers are in Table 1). Morphological vouchers were fixed in formalin and/or ethanol and were accessioned in the Herpetology Collection at the University of Puerto Rico at Mayagüez.

A Bayesian phylogeny was estimated using MrBayes 3.2.7, running the program for 20 million generations, sampling every 1,000 generations, and discarding the first 25% of generation as burnin (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003; Ronquist et al. 2012). We used Tracer v1.7 to assess adequate Markov chain mixing (effective sample sizes > 200) and summarized a 50% majority-rule consensus tree (Rambaut et al. 2018). A maximum-likelihood phylogeny was estimated using IQ-TREE v1.6.12 on the IQ-TREE web server, utilizing the built-in model selection tool ModelFinderPlus for each locus partition and implementing 1,000 ultrafast bootstraps (Nguyen et al. 2015; Trifinopoulos et al. 2016; Kalyaanamoorthy et

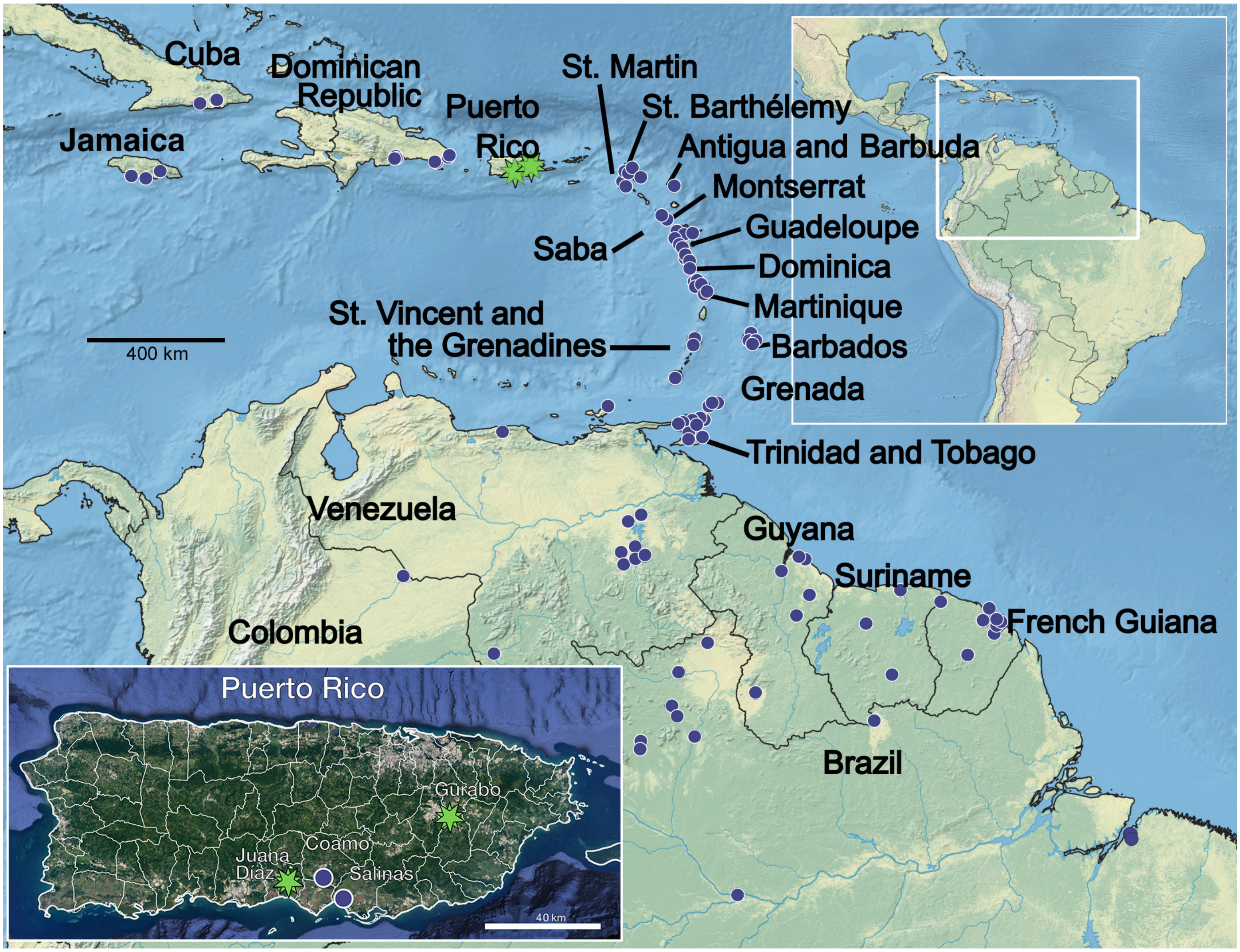

Figure 1. Map of the Caribbean and northern South America indicating locations where *Gymnophthalmus underwoodi* has been formally documented in the literature and on iNaturalist (Recoder et al. 2018; Maciel et al. 2021; GBIF 2023). Blue markers are records from the literature and green stars indicate the Puerto Rican samples from this study. Map Source: Natural Earth, free vector and raster map data at naturalearthdata.com, and 2023 Google: Imagery 2023 Airbus, CNES/Airbus, Data CSUMB SFML, CA OPC, Maxar Technologies, U.S.G.S., USDA/FPAC/GEO.

Figure 2. A Smooth-scaled Tegulet (*Gymnophthalmus underwoodi*) (not collected) from Gurabo Municipality, Puerto Rico, the same locality as UPRM-R1238. Photographs by B. Pinto-Rodríguez.

Table 1. Species, localities, and Genbank Accession numbers for the 16S rRNA gene. Primers: 16Sar: CGCCTGTTTAYCAAAAACAT and 16Sbr: CCGGTCTGAACTCAGATCACGT; profile: [94 °C (3:00); 94 °C (0:45); 55 °C (0:45); 72 °C (1:30) for 37 cycles; 72 °C (5:00)] (Whiting et al. 2003).

Species (Field/Coll. No.)	Locality	12S	16S	Reference
G. cryptus (AMNH-R-138374)	San Juan de Manapiare, Amazonas Rio Uraricoera, Alto Alegre, Venezuela	AF101362		Kizirian and Cole 1999
G. leucomystax (AMNH-R-139856)	Southern Rupununi Savanna, Guyana	AF101363		Kizirian and Cole 1999
G. leucomystax (AMNH-R-139857)	Southern Rupununi Savanna, Guyana	MH732666	MH732714	Recoder et al. 2018
G. leucomystax (LG337)	Roraima, Brazil	MH732664	MH732713	Recoder et al. 2018
G. pleii (AMNH-R-128428)	Martinique, West Indies	AF101364		Kizirian and Cole 1999
G. speciosus (MTR33465)	San José, Costa Rica	MH732660	MH732710	Recoder et al. 2018
G. underwoodi (APL21703)	Manaus, Amazonas, Brazil	MH732663	MH732712	Recoder et al. 2018
G. underwoodi (UPRM-R1237)	Calle Capa Prieto, Estancias del Sur, Juana Díaz Municipality, Puerto Rico	—	OR835200	This study
G. underwoodi (UPRM-R1238)	Camino de los Lirios, Gurabo Municipality, Puerto Rico	—	OR835201	This study
G. underwoodi (JC/FT7054)	Sam Lord's Castle, St. Phillip, Barbados	AF101369		Kizirian and Cole 1999
G. underwoodi (NYSMH6432)	Bottomless Ghaut, Montserrat	—	KX866265	Snyder et al. 2017
G. underwoodi (MTR946601)	Ilha de Maracá, Roraima, Brazil	MH732661	MH732711	Recoder et al. 2018
G. underwoodi (MTR946590)	Ilha de Maracá, Roraima, Brazil	MH732662	KT254406	Recoder et al. 2018
G. vanzoi (AMNH-R-128438)	Chacachacare Island, St. George, Trinidad and Tobago	MH732643	MH732709	Recoder et al. 2018
G. vanzoi (APSJ235)	Santarém, Pará, Brazil	MH732698	MH732646	Recoder et al. 2018

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G. vanzoi (LSUMZH-12396)	Boa Vista, Roraima, Brazil	MH732651	MH732702	Recoder et al. 2018
G. vanzoi (MPEG29841)	Mazagão, Amapá, Brazil	MZ544009	MZ544020	Maciel et al. 2021
G. vanzoi (MTR946639)	Fazenda Salvamento, Alto Alegre, Roraima, Brazil	AF420687	AF420743	Pellegrino et al. 2001
Iphisa elegans (LSUMZH-12697)	Reserva Faunistica Cuyabeno, Sucumbios Province, Ecuador	AF101370		Kizirian and Cole 1999

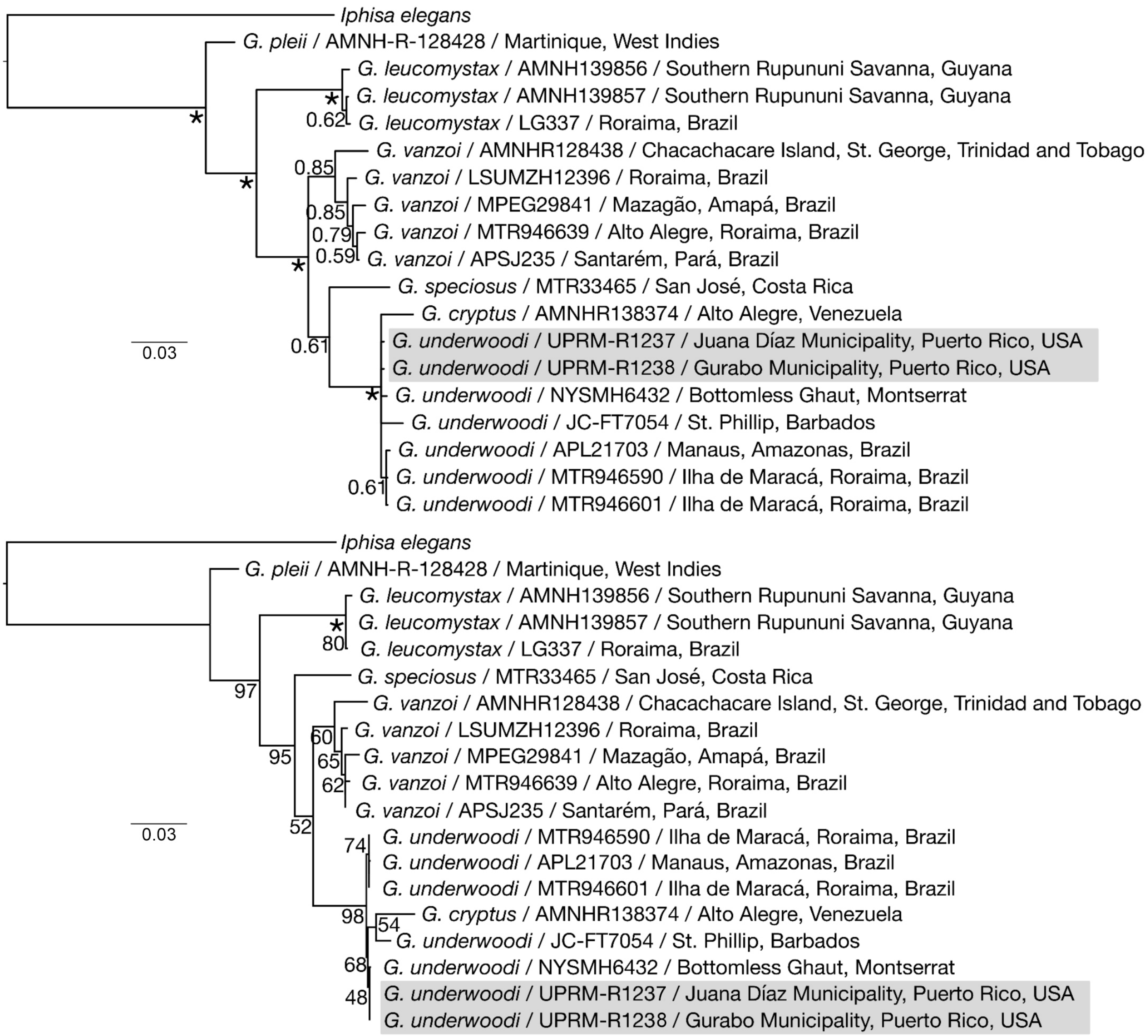

Figure 3. Bayesian (top) and Maximum Likelihood (bottom) phylogenies of species of *Gymnophthalmus*. Posterior probability (PP)/Bootstrap support (BS) is indicated on each branch and asterisks (*) indicate a PP/BS of 1.0/100. Samples highlighted in gray are the new records from Puerto Rico.

Table 2. Uncorrected pairwise distances between all samples of species in the genus *Gymnophthalmus* for the 16S rRNA locus. Values highlighted in gray are comparisons between individuals of *G. underwoodi* from Barbados, Brazil, and Montserrat, and the bolded value is the comparison between the two sequences in this study.

	G. pleii (AMNH-R-128428)	*G. cryptus* (AMNH-R-138374)	*G. speciosus* (ALM 5979)	*G. vanzoi* (ANMH-R-140975)	*G. vanzoi* (JC 4823)	*G. vanzoi* (ANMH-R-138055)	*G. vanzoi* (MTR 946639)	*G. speciosus* (MTR 33465)	*G. underwoodi* (UPRM-R1237)	*G. underwoodi* (UPRM-R1238)	*G. underwoodi* (JCFT 7054)	*G. underwoodi* (MTR 946590)	*G. underwoodi* (APL 21703)	*G. underwoodi* (MTR 946601)	*G. underwoodi* (NYSM H6432)	*G. leucomystax* (AMNH-R-139856)	*G. leucomystax* (MTR 946618)	*G. leucomystax* (LG 337)
G. pleii (AMNH-R-128428)	-																	
G. cryptus (AMNH-R-138374)	0.1	-																
G. speciosus (ALM5979)	0.09	0.06	-															
G. vanzoi (ANMH-R-140975)	0.09	0.05	0.05	-														
G. vanzoi (JC4823)	0.09	0.05	0.05	0	-													
G. vanzoi (ANMH-R-138055)	0.09	0.06	0.05	0.01	0.01	-												
G. vanzoi (MTR946639)	0.1	0.09	0.08	0.05	0.05	0.04	-											
G. speciosus (MTR33465)	0.09	0.06	0.08	0.06	0.07	0.07	0.04	-										
G. underwoodi (UPRM-R1237)	0.1	0.04	0.08	0.08	0.08	0.08	0.05	0.04	-									
G. underwoodi (UPRM-R1238)	0.1	0.04	0.08	0.08	0.08	0.08	0.05	0.04	**0**	-								
G. underwoodi (JC/FT7054)	0.1	0.01	0.06	0.05	0.05	0.05	0.08	0.07	0.04	0.04	-							
G. underwoodi (MTR946590)	0.1	0.04	0.08	0.08	0.08	0.08	0.05	0.04	0	0	0.04	-						
G. underwoodi (APL21703)	0.1	0.04	0.08	0.08	0.08	0.08	0.05	0.04	0	0	0.04	0	-					
G. underwoodi (MTR946601)	0.1	0.04	0.08	0.08	0.08	0.08	0.05	0.04	0	0	0.04	0	0	-				
G. underwoodi (NYSMH6432)	0.1	0.04	0.08	0.08	0.08	0.08	0.05	0.04	0	0	0.04	0	0	0	-			
G. leucomystax (AMNH-R-139856)	0.05	0.09	0.1	0.1	0.1	0.1	0.11	0.09	0.09	0.09	0.1	0.09	0.09	0.09	0.09	-		
G. leucomystax (MTR946618)	0.09	0.08	0.1	0.09	0.09	0.09	0.07	0.06	0.07	0.07	0.09	0.07	0.07	0.07	0.07	0.05	-	
G. leucomystax (LG337)	0.09	0.08	0.09	0.09	0.09	0.09	0.06	0.05	0.06	0.06	0.09	0.06	0.06	0.06	0.06	0.05	0.01	-

al. 2017; Hoang et al. 2018). We also calculated uncorrected pairwise distances for the 16S locus using the APE 5.0 package (Paradis and Schliep 2019) in R (R Core Team 2022).

Results and Discussion

Phylogenetic results indicate that the Puerto Rican samples are nested within a clade of *G. underwoodi* and *G. cryptus* (Fig. 3). The uncorrected pairwise distance matrix indicated that the Puerto Rican samples were identical to the sequence of *G. underwoodi* from Montserrat and nearly identical to those from the populations in Brazil (1–2 base pair differences; Table 2).

The expansive distribution of *G. underwoodi* across the Antilles has been attributed in part to passive overwater dispersal as well as accidental introductions as stowaways in exotic plants shipped across the region (Scantlebury et al. 2010; Recoder et al. 2018). The Puerto Rican individuals were found in urban areas in two different municipalities in eastern and southern Puerto Rico, and multiple individuals were reported by their collectors at each locality (Gurabo and Juana Díaz Municipalities). In Puerto Rico, additional reports document sightings of *G. underwoodi* from Salinas Municipality (https://www.facebook.com/groups/PRNatural) and Coamo Municipality (GBIF 2023) in the south. How long *G. underwoodi* has been on the island is unknown, but the species' occurrence in southern and eastern Puerto Rico and the fact that these individuals were initially misidentified (thought to have been Puerto Rican Skinks, *Spondylurus nitidus*) is suggestive of an even wider distribution of this species on the island. Establishment of *G. underwoodi* on Antigua and Montserrat has demonstrated this species' ability to rapidly expand its population from a few introduced individuals over the course of only a few years (Daltry 2007; Snyder et al. 2017). Additionally, the lack of available genetic data for lizards introduced across the Caribbean renders identification of the source population or migration patterns of this species challenging (Recoder et al. 2018). Previous genetic studies of this species indicated that populations from Barbados, Suriname, and Trinidad likely originated from a distinct hybridization event that led to the population in Roraima, Brazil (Cole et al. 1990; Kizirian and Cole 1999). Genetic data from the present study indicates that the Puerto Rican and Montserrat populations might have arisen from the same hybridization event as the Brazilian populations (Table 2). *Gymnophthalmus underwoodi* would benefit from a more thorough sampling across its current distribution to better understand the timing and magnitude of its spread, as well as sequencing of additional genetic markers to identify source populations and potential modes for the spread of this invasive species.

The spread of *G. underwoodi* across the Antilles in recent years has potential negative effects for local species, such as displacement of native lizards (Powell et al. 2011; Snyder et al. 2017), but these effects have not been studied for most islands that now host invasive populations. As a parthenogenetic species, *G. underwoodi* is able to colonize new areas with relative ease, requiring only one individual to establish a new population that could easily replace native lizard populations of similar ecologies (Cole et al. 1990; Powell et al. 2011). Native populations of *G. pleii*, which do not seem to be much affected by invasive Small Indian Mongoose (*Urva auropunctata*) predation, have decreased in the presence of *G. underwoodi* over the course of 10+ years in at least one location where they occur in sympatry (Breuil 2009). While assessments of invasive herpetofauna on endemic species are relatively sparse (Cole et al. 2005) the newfound presence of *G. underwoodi* on Puerto Rico could have an impact on native ground-dwelling lizards in the region; affected species include *Pholidoscelis exsul* (Common Puerto Rican Ameiva), *Spondylurus nitidus* (Puerto Rican Skink), four species of dwarf geckos in the genus *Sphaerodactylus*, *Diploglossus pleii* (Puerto Rican Galliwasp), and at least eight native species of anoles (Rivero 1998; Díaz-Lameiro et al. 2022). We recommend that future studies continue to monitor the distribution and demography of *G. underwoodi* across Puerto Rico and document the natural history of the species to better understand how it might negatively impact native herpetofauna and local ecosystems.

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